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Biosynthesis,Characterization and Antibacterial activity of Silver nanoparticles of *Excoecaria agallocha* L. fruit extract

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Abstract

In this present study, *Excoecaria agallocha fruit* aqueous extract was used to synthesize Silver Nano Particles (Ag NPs/SNPs) which has proven as eco-friendly, nontoxic, less time consuming and energy saving. The synthesized SNPs were characterized by UV-Visible spectroscopy, FTIR and SEM studies. The SNPs were checked for the antibacterial activity against both Gram positive and Gram negative bacteria. The characterization studies clearly revealed the formation and synthesis of SNPs which also showed the inhibitory activity on the tested bacteria. SNPs of *Excoecaria agallocha* fruit showed higher zone of inhibition against *Micrococcus luteus*, *Arthrobacter protophormiae*, *Rhodococcus rhodochrous*, *Bacillus subtilis*, *Alcaligens faecalis*, *Enterobacter aerogenes*, *Proteus mirabilis* and *Salmonella enterica* when compared to that of standard antibiotic, Streptomycin.

Keywords: *Excoecaria agallocha*, fruit, silver nanoparticle, FTIR, SEM, antibacterial activity.

Introduction

Nanoparticles can be synthesized using various approaches including chemical, physical, and biological. Though large quantities of nanoparticles can be synthesized by chemical method within short period of time, it requires capping agents for size stabilization of the nanoparticles. The development of green processes for the synthesis of nanoparticles is transforming into a predominant branch of nanotechnology. The advancement of green synthesis over chemical and physical methods is environment friendly, cost effective and easily scaled up for large scale synthesis of nanoparticles, furthermore there is no need of using high temperature, pressure, energy and toxic chemicals [1]. Recently, phytonanotechnology has provided new avenues for the synthesis of nanoparticles. Thus, plant derived nanoparticles synthesized by using readily available plant materials and the nontoxic nature of plants are suitable for fulfilling the high demand for nanoparticles with applications in the biomedical and environmental areas [2]. Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being. The name “mangrove” originated from a combination of the Portuguese word “Mangue” means tree and an English word “grove” means orchard or garden. Mangrove plants have been used in medicinal fields and their extracts have proven to possess inhibitory activity against human, animal, and plant pathogens. These specialized plants are known to tolerate extreme environmental conditions. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plant produces these chemicals to protect itself [3]. *Excoecaria agallocha* L. is a small mangrove tree found extensively in the tidal forests and swamps of The Krishna-Godavari area of Andhra Pradesh, India. This plant is also well-distributed in a number of other countries of temperate and tropical Asia. *Excoecaria agallocha* is commonly known as milky mangrove and its vernacular name is Tilla. The milky sap of this plant can cause temporary blindness if it enters into eyes. The latex is used as a caustic for removing obstinate ulcers. Fruits of this plant are small, round and clustered. The aim of present study is to synthesize SNPs (Silver nanoparticles) by Green synthesis method using *E. agallocha* fruits and characterization of SNPs and also to evaluate the antibacterial activity.

Materials and Methods

Collection of plant material

Excoecaria agallocha fruits were collected from nearby place of Corangi Reserve Forest, Kakinada, and Andhra Pradesh, India.

Preparation of plant extract

Collected fruits were shade dried at room temperature and the dried material was ground by using mechanical grinder. To 15 grams of this powder, 100 ml of double distilled water was added and heated at 60ºC for 15 minutes. After cooling to room temperature, the solution was filtered through what man No-1 filter paper and the obtained solution was stored at 4º C.

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Figure-1: *Excoecaria agallocha* fruits

Synthesis of SNPs

Twenty milliliters of plant extract was added to 80 ml of 1mM silver nitrate solution and heated up to 60^{o} C for 15 minutes and then placed in dark condition [4].

Characterization of synthesized SNPs

UV-Visible spectral analysis

The bioreduction of the Ag^{+} to Ag^{o} was monitored by using Thermo scientific evolution 201 spectrophotometer at room temperature under scanning range of 200 - 700nm.

Fourier Transform–infrared spectral analysis

The solution was centrifuged at 20,000 rpm for 30 minutes and allowed SNPs to settle at bottom. The resulted SNPs were washed thrice with double distilled water. The resulted SNPs were dried in an oven at 60^{o} C. Finally the dried SNPs were analyzed by Thermo Nicolet Nexus 670 model for FTIR.

SEM analysis

Scanning electron microscope (Model-SEM Hitachi - S520, Japan) was used to study the surface morphology of synthesized SNPs.

Screening for antibacterial activity

The antibacterial activity was carried out by using agar well diffusion method [5]. Antibacterial activity was tested against some Gram positive bacteria viz, *Micrococcus luteus* MTCC 106, *Enterococcus faecalis* MTCC 439, *Bacillus subtilis* MTCC 441, *Arthrobacter protophormiae* MTCC 2682, *Rhodococcus rhodochrous* MTCC 265 and *Streptococcus mutans* MTCC 497 and Gram negative bacteria namely *Proteus vulgaris* MTCC 426, *Alcaligens faecalis* MTCC 126, *Salmonella enterica* MTCC 3858, *Enterobacter aerogenes* MTCC 10208, *Proteus mirabilis* MTCC 425 and *Pseudomonas aeruginosa* MTCC 1688. Three wells were bored in agar plate using sterile cork borer of 6 mm diameter and100 µl of sample prepared by dispersing 100 µg of nanoparticle material in 1 ml of dimethyl sulfoxide (DMSO) was placed in each well. In a separate well, DMSO was placed to maintain the control. After 24 hours of incubation (at 37^{o} C), the diameter of the clear zone was measured.

Results and Discussion

UV-Visible spectral analysis

The bioreduction of Ag^{+} to Ag^{o} continued slowly after the addition of *E. agallocha* fruit extract to silver nitrate solution. After bioreduction the Ag^{o} developed dark brown color Figure-2(B). The results of the UV–vis spectra of silver nanoparticles synthesized displayed (Figure-3) a strong broad peak between 460 nm which gives the confirmation of the synthesis SNPs.

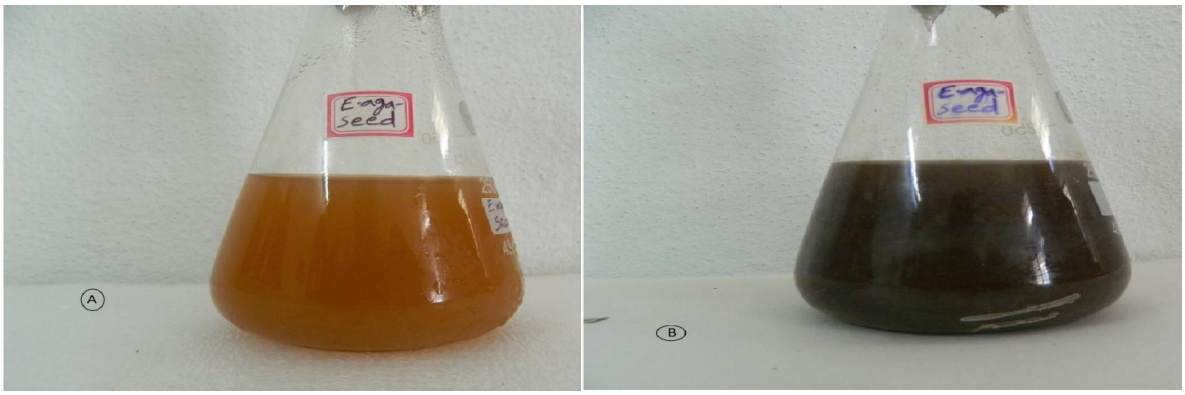

Figure-2 A) Plant extract with $AgNO_3$ Solution B) Brown solution of silver nanoparticles formed due to the bioreduction

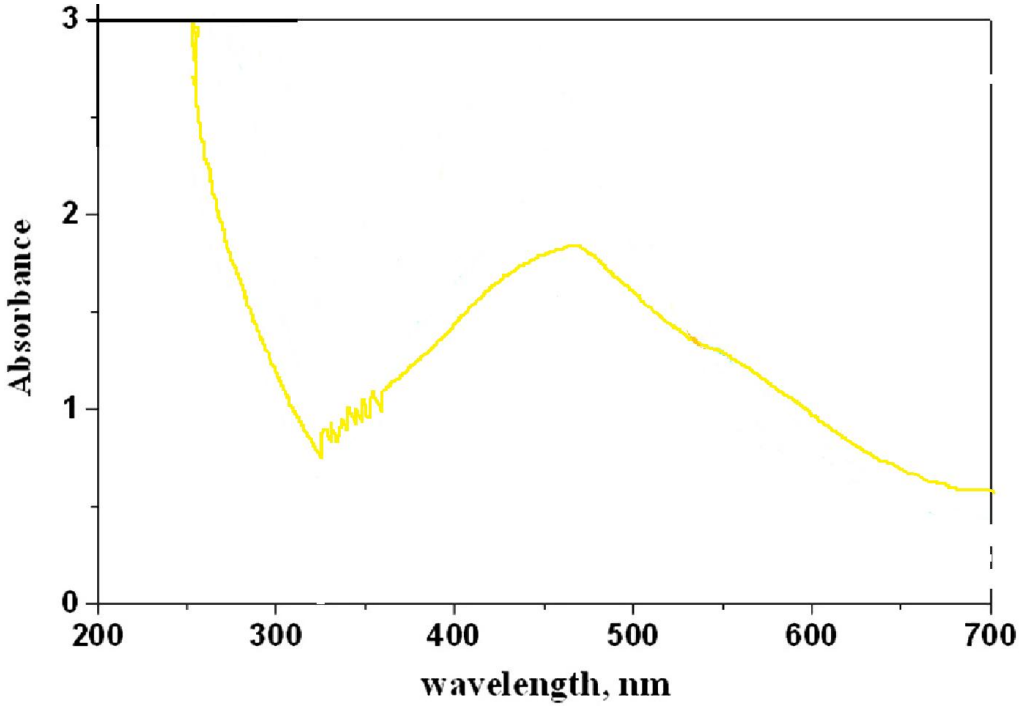

Figure-3: UV–Vis absorption spectra for synthesized silver nanoparticles

Fourier Transform–infrared spectral analysis

FTIR analysis was carried out to identify the possible biomolecules responsible for the reduction; capping and stabilization of the SNPs synthesized using *E. agallocha* fruits. FTIR spectral (Figure-4) results revealed that the spectra showed absorption band at 3178.28 cm^{-1}corresponds to –OH stretching due to the phenolic compound present in the fruit extract of *E. agallocha*. The peak at 2923.40cm^{-1}corresponds to alkane C–H stretching vibration. The peaks at 1692.61$cm^{-1}$and 1616.44 cm^{-1}were attributed to C=C stretching vibration of aromatic rings. The spectral bands appeared at 1580.81 cm^{-1} and 1446.58 cm^{-1}assigned to amide I and amide II which arise due to carbonyl (C=O) and amine (-NH) stretching vibrations in the amide linkages of the proteins, respectively. The band at 1,053.25 cm-1 corresponded to C–N stretching vibrations of amine. The peak at 1509.95 cm^{-1} was attributed to –NH stretching. The peak at 1335.51 cm^{-1} corresponds to C-O. The peak at 1390.41 cm^{-1} assigned to C–H alkenes stretch. Though the exact mechanism and the constituents responsible for plant mediated synthetic nanoparticles is not certainly elucidated, it has been proposed that proteins, amino acids, organic acid, vitamins, as well

as secondary metabolites, such as flavonoids, alkaloids, polyphenols, terpenoids, heterocyclic compounds, and polysaccharides, have significant roles in metal salt reduction and, furthermore, act as capping and stabilizing agents for synthesized nanoparticles [6].

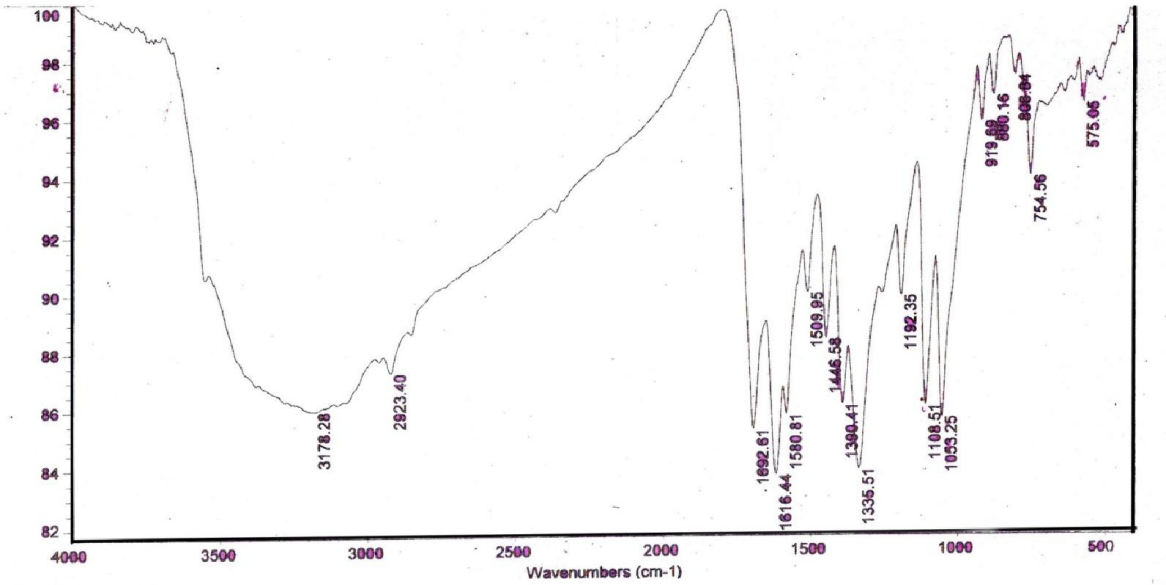

Figure-4: FTIR spectrum of synthesized silver nanoparticles

Scanning electron microscope observation of SNPs

The SEM image of the SNPs was shown in Figure-5. The SEM image shows the presence of approximately spherical shaped SNPs. This may be due to availability of different quantity and nature of capping agents present in the fruit extract. This is also supported by the shifts and difference in areas of the peaks obtained in the FTIR analysis.

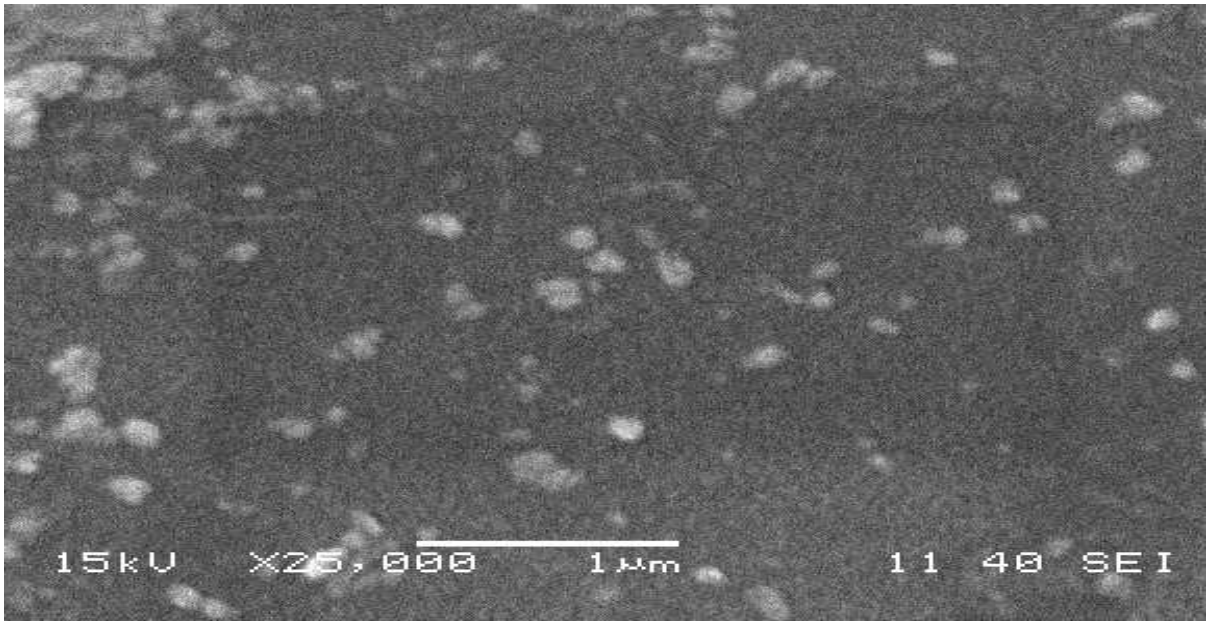

Figure-5: Scanning electron microscopic analysis of SNPs synthesized using *E. agallocha* fruit

Antibacterial activity

The synthesized SNPs showed efficient antibacterial activity against both Grams positive and Gram negative bacteria tested (Figure-6). The SNPs synthesized by using *E. agallocha* fruit extract displayed maximum zone of inhibition against *Micrococcus luteus*, *Arthrobacter protophormiae*, *Rhodococcus rhodochrous*, *Bacillus subtilis*, *Alcaligens faecalis*, *Enterobacter aero genes*, *Proteus mirabilis* and *Salmonella enterica* than the standard antibiotic, Streptomycin. *Enterococcus faecalis*, *Streptococcus mutans* and *Proteus vulgar is* were found moderately susceptible to SNPs with relatively smaller zone of inhibition when compared to streptomycin. The effect of SNPs and Streptomycin was similar against *Pseudomonas aeruginosa* with same size of inhibition zone. The mechanism of efficient antibacterial activity by silver nanoparticles against various pathogenic bacteria may vary and it depends on the cell constituents of the organism. SNPs might have been attached to the surface of the cell membrane of microorganisms, leading to the disturbance of its functions like permeability and respiration. It is obvious, therefore, that the binding of particles to the microorganism depends on the surface area available for interaction. In general, small nanoparticles have a larger surface area for interaction with bacteria, as compared to that of bigger particles, due to greater antibacterial activity [7, 8]. Other mechanisms involving interaction of silver molecules with biological macromolecules such as enzymes and DNA through an electron-release mechanism or free radical production [9, 10]. SNPs could destabilize the outer membrane and rupture the plasma membrane, thereby depleting intracellular ATP. Silver has a greater affinity to react with sulfur or phosphorus-containing biomolecules in the cell; therefore, sulfur-containing proteins in the membrane or within the cells and phosphorus containing elements like DNA are likely to be the preferential sites for binding of SNPs [11].

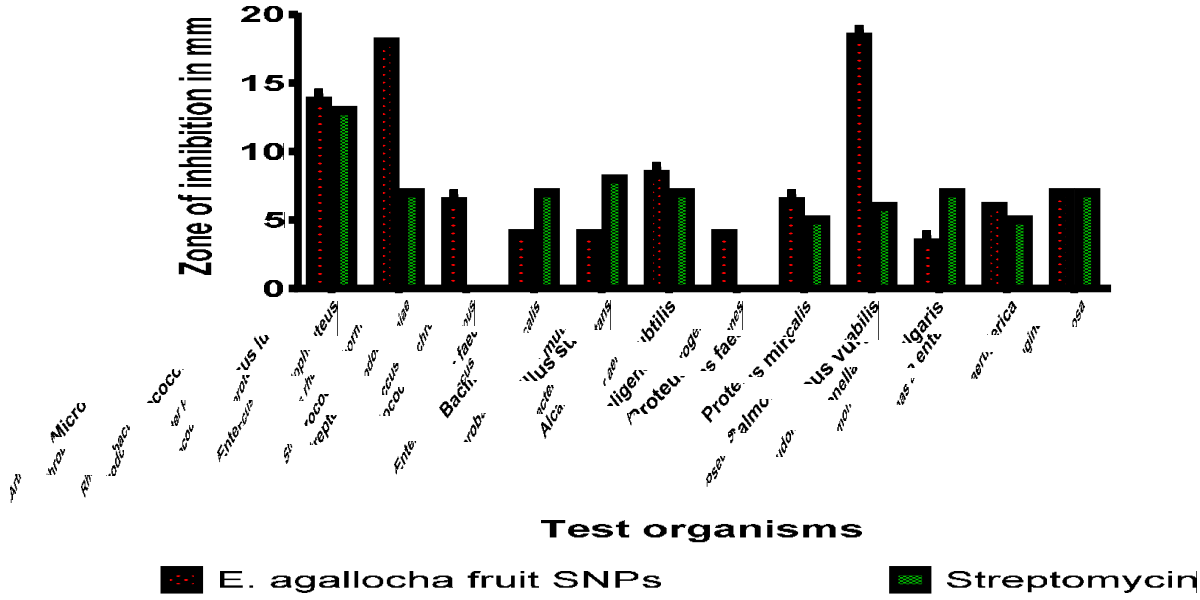

Figure-6: Antibacterial activity of SNPs synthesized using *E. agallocha* fruit extract

Conclusion

A critical need in the field of nanotechnology is the development of a reliable and eco-friendly process for synthesis of metallic nanoparticles. Silver nanoparticles synthesized by the green chemistry approach reported in this study using *E. agallocha* fruit extract could have potent applications in biomedical and pharmaceutical applications. In the present study, we have synthesized SNPs using *E. agallocha* fruit extract by green synthesis method and also successfully characterized the synthesized SNPs. Finally, the green synthesis of silver nanoparticles using plant material was found to be the most eco–friendly and conventional method, in comparison to chemical and physical synthesis methods.

Acknowledgements

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