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Endophytic synthesis of silver chloride nanoparticles from *Penicillium* sp. of *Calophyllum apetalum*

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Abstract

In the present study, *Penicillium* species extract isolated from *Calophyllum apetalum* was used for the synthesis of silver nanoparticles and it was confirmed by changing the color of the silver nitrate UV–Vis spectrum. The synthesized nanoparticles have been characterized by biophysical techniques such as scanning electron microscopy and x-ray diffraction.

Keywords: endophyte, *Penicillium* sp., *Calophyllum apetalum*, silver chloride nanoparticles
Classification numbers: 4.02, 5.08

1. Introduction

Biosafety, economical and synthetic biology are the current suggestive concepts to synthesize metallic nanoparticles. Due to the toxic effects of reactants, reaction intermediates and byproducts in chemical modes of nanoparticle synthesis, synthetic biology has got more prominence. The biogenic synthesis of metallic nanoparticles is surely a bioredox mechanism through cellular enzymes, secondary metabolites and other cellular components. The biogenic systems include algae, fungi, yeast, bacteria, actinomycetes and plants. In advancement these biogenic systems may be termed as bio-nano factories. Apart from other industrial applications, silver nanoparticles were mostly chosen because of their specific physicochemical properties, having high electrical and thermal conductivity, surface-enhanced Raman scattering, surface plasmon resonance, chemical stability, catalytic activity and non linear optical behavior [1]. These properties of silver nanoparticles have great advantages over the classical drugs, diagnostic agents and other herbal products. These nanoparticles are beneficial in tracing their biological activities analytically and technically in biological systems. Nanoparticles were alternate and efficient agents in biological sensing and imaging [2], formulation of dental resin composites, bone cement, ion exchange fibers and coatings for medical devices [3]. Silver nanoparticles can be synthesized by reduction in solutions, thermal decomposition, microwave assisted, laser mediated biological reduction methods [4–8], radiation [1], photochemical [2] and electro-spinning methods [3]. Biologically nanoparticles were synthesized by the constituents of plants, fungi, bacteria and actinomycetes.

Plants used for synthesizing silver and silver conjugated nanoparticles are known. In this context the beneficial rare plants were concentrated to isolate the effective strains of fungal endophytes in synthesizing nanoparticles effectively. Endophytes are the ubiquitous, emerging, endosymbiont microbes having inheritance characters of the respective host plants with potential application in agriculture, medicine and the food industry [9, 10]. *Calophyllum apetalum (Calophyllaceae)* is a folkloric herb known for its medicinal value commonly called *Alexandrian laurel. Calophyllum apetalum* (*C. apetalum*) seed oil is used commonly for treating rheumatism and leprosy. Xanthonoids and apetalinones were

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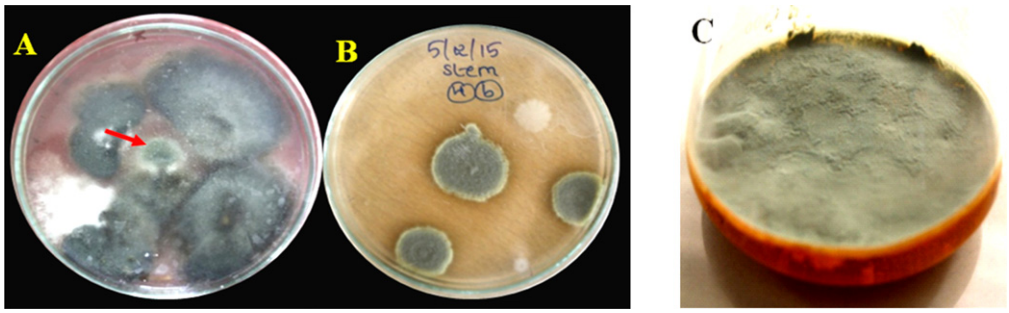


Figure 1. (A) Emerging endophytes from plant parts of *Calophyllum apetalum,* (B) pure culture of isolated endophyte Penicillium species and (C) mass culture of *Penicillium* species.

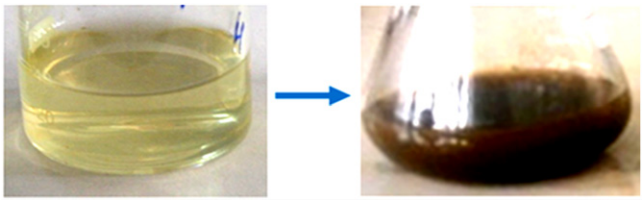


Figure 2. Endophyte *Penicillium* extract, before and after (24 h) treating with silver nitrate.

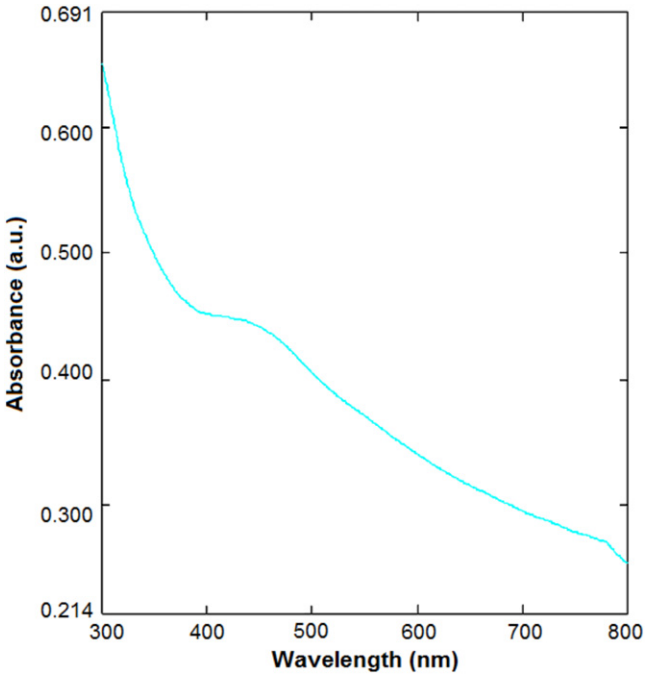


Figure 3. Strong broad peak at 420 nm of silver chloride nanoparticles synthesized by endophytic *Penicillium* extract.

isolated from the roots and stem barks of *C. apetalum* apart from known compounds like calozeyloxanthone and zeyloxanthonone [11]. Coumarins were rich in this *Callophyllaceae* species. Dipyranocoumarin, α-hydroxytomentolide were isolated from the leaves of *C. apetalum* together with the known compounds friedelin, apetalactone, inophyllum and canophyllol [12]. Presently there are reports only on the synthesis of silver nanoparticles by wild fungi and bacteria apart from plants. The application of endophytic extracts in synthesis of silver chloride nanoparticles would be an essential work.

The content of this work is collection and identification of plant species, isolation and identification of fungal endophytes, biosynthesis of silver chloride nanoparticles and their characterization by UV-visible spectroscopy, scanning electron microscope (SEM), and x-ray diffraction (XRD) studies.

2. Materials and methods

2.1. Identification and collection of Calophyllum apetalum

The plant *Calophyllum apetalum* was collected in the month of January, 2015 from the Agumbe ghats, Udupi district, Karnataka, India and was identified and authenticated by Dr S G K Bhat, Taxonomist, Udupi District, Karnataka. Freshly collected plant materials were washed thoroughly under running tap water followed by sterile distilled water to remove the adhered debris. Stem was subjected for surface sterilization under aseptic condition in sequential steps by immersing in mercuric chloride (1 mg ml^{-1}) for 10 min and 70% ethanol for another minute followed by washing finally with distilled water.

2.2. Inoculation of implants (Calophyllum apetalum)

After successive surface sterilization of stem of *Calophyllum apetalum,* it was cut into small pads (0.5 $\times$ 0.5 cm^2) and placed 5–6 pieces on solidified sterile potato dextrose agar (PDA) media. The inoculated plant implants were incubated till the growth of distinguishable fungal endophytes had been observed.

2.3. Identification of endophytes

Colonies emerging out of the surface sterilized stem, endophyte *Penicillium* species (*Penicillium* sp.) were selected based on the morphological characteristics, colony growth, hyphae and conidia [13, 14].

2.4. Isolation and mass culture of Penicillium species

The *Penicillium* species were sub cultured on PDA plates and mass cultured in conical flasks containing potato dextrose broth (PDB). After 15–20 days of incubation, the fungal mycelia mat was collected.

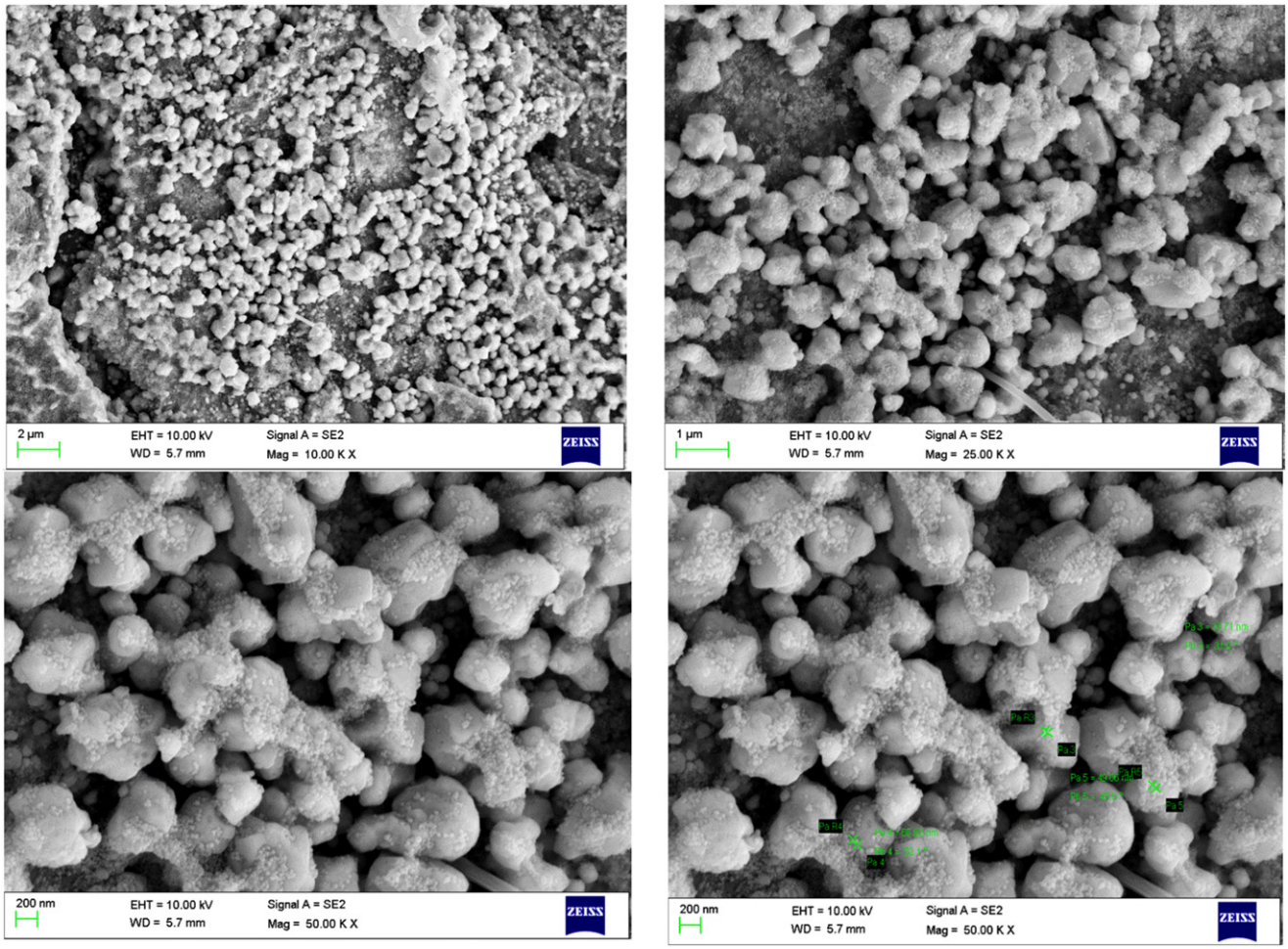


Figure 4. SEM images of silver chloride nanoparticles synthesized from endophytic *Penicillium* extract of *Calophyllum apetalum* at 10 000, 25 000 and 50 000× magnifications.

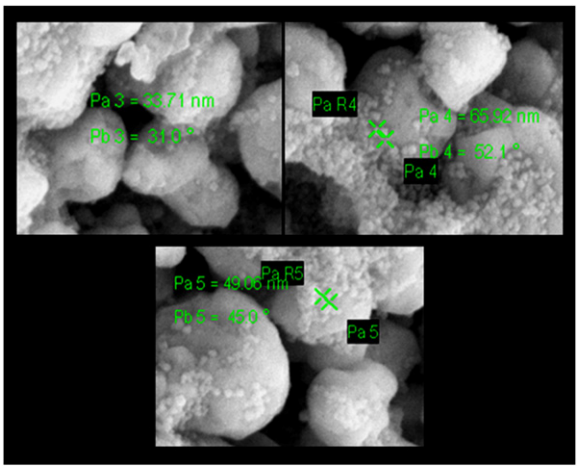


Figure 5. Magnified images of SEM (50 000×) of silver chloride nanoparticles synthesized by an endophyte, *Penicillium* sp. from stem of *Calophyllum apetalum.*

2.5. Preparation of endophytic extract

Aqueous extract of *Penicillium* sp. was prepared by grinding the mycelia mat with double distilled water and filtered through the Whatman filter paper no 1. The filtrate was centrifuged at 6000 rpm for 10 min and the supernatant was collected for further experiments.

2.6. Synthesis of silver chloride nanoparticles

10 ml of concentrated *Penicillium* sp. extract was added to 25 ml of freshly prepared 5 mM silver nitrate contained in an Erlenmeyer flask and kept in an orbital shaker in dark conditions for incubation for 24 h at 37 °C. The extract alone (without silver nitrate) and pure silver nitrate solution (without extract) were used as positive and negative controls, respectively. A silver nitrate treated sample was centrifuged at 10 000 rpm for 10 min. Supernatant was discarded and the pellate was washed thrice with deionized water to remove unreacted $AgNO_3$ and endophytic extract. The pure pellate was collected, air dried and preserved for further characterization.

2.7. Characterization of silver chloride nanoparticles

An aliquot of this pellate containing silver nanoparticles was used for UV–Vis spectroscopy (Shimadzu company model-UV3600) and SEM (Ultra 55 Model-II, Carl Zeiss SEM machine). For XRD studies, dried silver nanoparticles were coated on an XRD grid and the spectra were recorded by

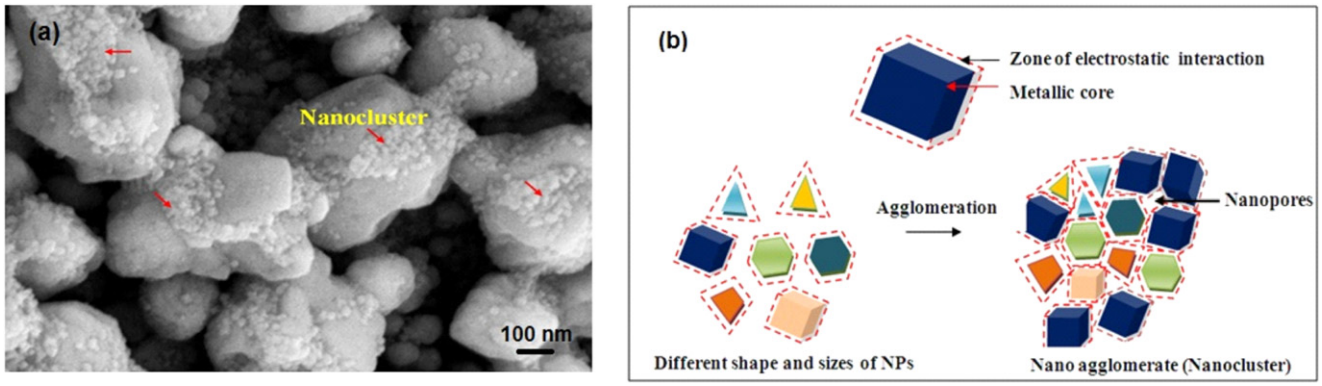


Figure 6. Magnified images of SEM (50 000×) of silver chloride nanoclusters (a) and an over view of nanocluster formation (b).

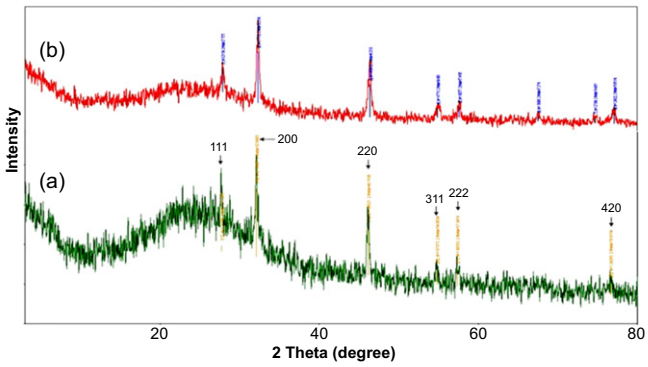


Figure 7. X-ray diffraction crystallographic studies of silver chloride nanoparticles synthesized by an endophyte *Penicillium* sp. (a) from stem of *Calophyllum apetalum* (b).

using a Rigaku diffractometer at a voltage of 40 keV and a current of 30 mA with Cu-Kα radiation with a wavelength of 1.5418 Å.

3. Results

The extract of *Penicillium* species was prepared and used as a reducing agent for the synthesis of AgNPs (figure 1).

3.1. Synthesis of silver nanoparticles

The biosynthesis of AgNPs was characterized by the color change reactions from pale yellow to dark brown color of endophytic extract and $AgNO^3$ complex after 24 h of treatment (figure 2).

3.2. Characterization of silver nanoparticles

The strong absorption peak at 420 nm by UV–Vis spectroscopic studies confirmed the synthesis of AgNPs which was further confirmed by XRD and SEM studies. Due to the polydispersed nanoparticles, a broad peak was observed (figure 3). The results of SEM clearly indicated that cubic AgCl NPs of size ranging from 8.63–30.91 nm were synthesized. From the SEM micrographs it was assured that AgCl NPs of range 33.71–65.92 nm were synthesized and associated with each other as nanoclusters. Since the observed nanoparticles are below 100 nm size, it is considered to be effective in both medical and industrial applications (figures 4–6). The results of XRD studies were noteworthy in confirming the biosynthesis of silver nanoparticles (AgNPs) from endophytic *Penicillium* extract of *Calophyllum apetalum.* Cubic crystalline silver nanoparticles were generated by the bioreductive process. The distinct pattern of XRD peaks at 2θ diffraction has given six confirmative peaks. Lattice planes of face centered cubic (fcc) crystal at 2θ values were indexed with respective degrees, 27.726° (111), 32.0983° (200), 46.1538° (220), 54.8034° (311), 57.3419° (222) and 76.6154° (420) and compared with the data of JCPDS (Joint Committee on Powder Diffraction Standards, file No. 87-0720). The average particle sizes were calculated according to the Debye–Scherrer equation [15] (figure 7).

4. Discussion

Endophytic synthesis of silver chloride nanoparticles from *Penicillium* sp. of *Calophyllum apetalum* has given excellent results from UV–Vis spectroscopy, SEM and XRD studies. The strong absorption peak at 420 nm by UV, tracing of 33.71–65.92 nm range of nanoparticles by SEM and detection of cubic face centered silver chloride nanoparticles with average size of 40.797 nm was felt worth. The results of UV, SEM and XRD of silver chloride nanoparticles of *Calophyllum apetalum* endophyte *Penicillium* sp. were compared and confirmed with the existing recent works in the respective areas [16–18].

Nanoparticles of Pt, Zr, Ag, Au, Cd, Pb and Ti from *Fusarium oxysporum* [19–22], stable nanoparticles of Au, Pt and Pd from bacteria *Desulfovibrio* sp. [23], silver nanoparticles from *Penicillium* sp. of soil sample [24, 25] were the earlier works carried out in wild types of microbes by means of extracellular reduction. Several secondary metabolites from plants and fungi i.e., fungal naphthoquinones [26–28] and anthraquinones [29] from Fusarium oxysporum were found to have excellent redox properties, assumed to act as electron shuttle in metal reductions [30, 31]. These works strengthen the research carried out by endophytic synthesis of silver chloride nanoparticles from *Penicillium* sp. SEM views confirm the

formation of nanoclusters and indicate their potential kinetic energy. Formation of nanoclusters is due to their surface plasmon resonance which can be disassociated easily in media. Metal nanoclusters can be interfaced with biomolecules and conjugations with proteins, peptides, and DNA to form a new class of effective biomolecule-nanocluster composites. Bionanoconjugates having characteristic synergistic, physicochemical and physiological properties [32] were beneficial in agricultural, industrial and medicinal applications.

5. Conclusion

The endophytic synthesis of silver chloride nanoparticles by the aqueous extract of *Penicillium* sp. from *Calophyllum apetalum* was efficient. The UV, SEM and XRD results were authenticated in this regard. Further studies are needed in optimization of effective endophytic synthesis with different media, species and physicochemical parameters. It is also an effective method to regulate and manipulate genetically for large scale production. Surely, it can be assured that endophytic synthesis of nanoparticles is an effective and advantageous method.

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