Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(8):112-117

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Biogenic synthesis of silver nano particles using endophytic fungi *Penicillium nodositatum* and its antibacterial activity

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ABSTRACT

Present study was aimed at synthesizing the eco-friendly silver nanoparticles (AgNps) using endophytic fungi, Penicillium nodositatum isolated from the leaves of R. dumetorium .The AgNps were synthesized after the reduction of silver nitrate solution by the endophytic fungi Formation of AgNps was confirmed by UV-Vis spectroscopy. AgNps were also analyzed by scanning electron microscope (SEM). The antibacterial activity of the synthesized silver nanoparticles was tested against pathogenic bacteria such as Escherichia coli, Streptococcus, Salmonella and Pseudomonas species. AgNps were showing significant antibacterial activity. The AgNps synthesized using the endophyte, P. nodositatum can serve as major therapeutic agent against pathogenic bacteria.

Keywords: Endophytes, nanoparticles, *Randia dumetorium*, *Penicillium nodositatum*, SEM.

INTRODUCTION

Nanoparticles are gaining the attentions of many researchers due to their wide applications in industries and medicine. Silver nanoparticles (AgNps) are synthesized by physical, chemical or biological methods. Physical and chemical methods are less preferred over biological process as the former two methods use either expensive techniques or involve toxic chemicals. Synthesis of AgNps by green methods using plants or micro organisms is alternate and feasible procedures that engage cost effective techniques without any side effects [1-2].

Various natural materials viz., plants, bacteria, fungi and yeast were used for synthesizing gold and silver nanoparticles in the recent past [3-8].Synthesis of nanoparticles of varied shapes and sizes with potential antibacterial activity has been achieved with the aid of certain fungi and bacteria. Endophytes are one such organism that are underexplored and can be exploited in the biogenic synthesis of AgNps. Endophytes are defined in different ways by many scientists. According to Bacon et al. “endophytes are microbes that colonize living internal tissues of plants without causing any immediate, overt negative effects”. Only few reports are available about the use of endophytes in green synthesis of AgNps [9].

In this study, we have synthesized AgNps using endophytic fungi, *P. nodositatum* isolated from *R. dumetorium* for reduction of Ag^+ ions to Ag^0 nanoparticles from silver nitrate solution at room temperature. It was also shown that the average size of AgNps can be controlled by varying the volume of leaf extract. Further, biosynthesized AgNps are found to be effective against bacteria.

EXPERIMENTAL SECTION

Materials

Silver nitrate used for the synthesis of AgNps was procured from E. Merck, Germany. Dehydrated Luria broth (LB), nutrient agar (NA) and potato dextrose agar (PDA) media used for bacterial growth study were the products of Hi

Media, India. Cultures of *Escherichia coli*, *Streptococcus*, *Salmonella* and *Pseudomonas* species were collected from the Department of Microbiology, Mangalore University.

Isolation of Endophytic Fungi

Healthy leaves of *R. dumetorium* were collected from the Shimoga district, Karnataka, India and was identified and authenticated by Department of Botany, Kuvempu University, Shimoga. The leaves were washed several times under running tap water and cut into small pieces. These pieces were surface sterilized by sequential rinsing in 70% ethanol for 30 sec, 0.01% mercuric chloride for 5min, 0.5% sodium hypochlorite, and 2-3 minutes with sterile distilled water and then dried under sterile conditions. The sterilized pieces were placed on Petri dish containing potato dextrose agar(PDA) supplemented with streptomycin sulfate (250 μg/mL), incubated at room temperature and monitored every day for the growth of endophytic fungal colony [10]. The fungi which grew out from leaf segment were isolated and sub cultured in plates containing PDA. The fungal isolate was identified as *Penicillium nodositatum* based on its morphological and reproductive characters using standard identification manual.

Synthesis of AgNps using endophytic fungi

The isolated endophytic fungus, *P. nodositatum*, was grown in 250mL Erlenmeyer flask for 72 h at room temperature. The mycelial biomass grown for 72 h was separated by filtration and then extensively washed with distilled water to remove the traces of media components. This biomass was transferred to flasks containing 100 mL distilled water and incubated at the same position for 48 h. The suspension was filtered with Whatman filter paper number 1. The Fungal filtrate was mixed with aqueous silver nitrate solution (1 mM) for reduction. The nanoparticles were separated out from the mixture by centrifugation (10,000 rpm for 15 min).The AgNps pelleted at the bottom of the centrifuge tube were dispersed in a 10 mL of deionized water.

UV-Vis Spectra analysis

The reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 hours after diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer UV-2450 (Shimadzu).

SEM analysis of silver nanoparticles

Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

Effect of biomass

$AgNO_3$(1mM) solution was mixed with2.5 ml, 5 ml, 7.5ml *P. nodositatum* extract and incubated for 1 hr to observe the effect of biomass of fungi on the formation of nanoparticles.

Effect of pH

Different pH ranging 9.0 to 11.0 was used with the difference of 1.0 to study the influence of pH on AgNps production from endophytic fungus, *P. nodositatum*.

Effect of temperature

Optimization studies with respect to temperature were carried out with hour incubation at 0◦, 4^0, 37◦, 100◦C.on endophytic fungus, *P. nodositatum* for AgNps production.

Antibacterial activity

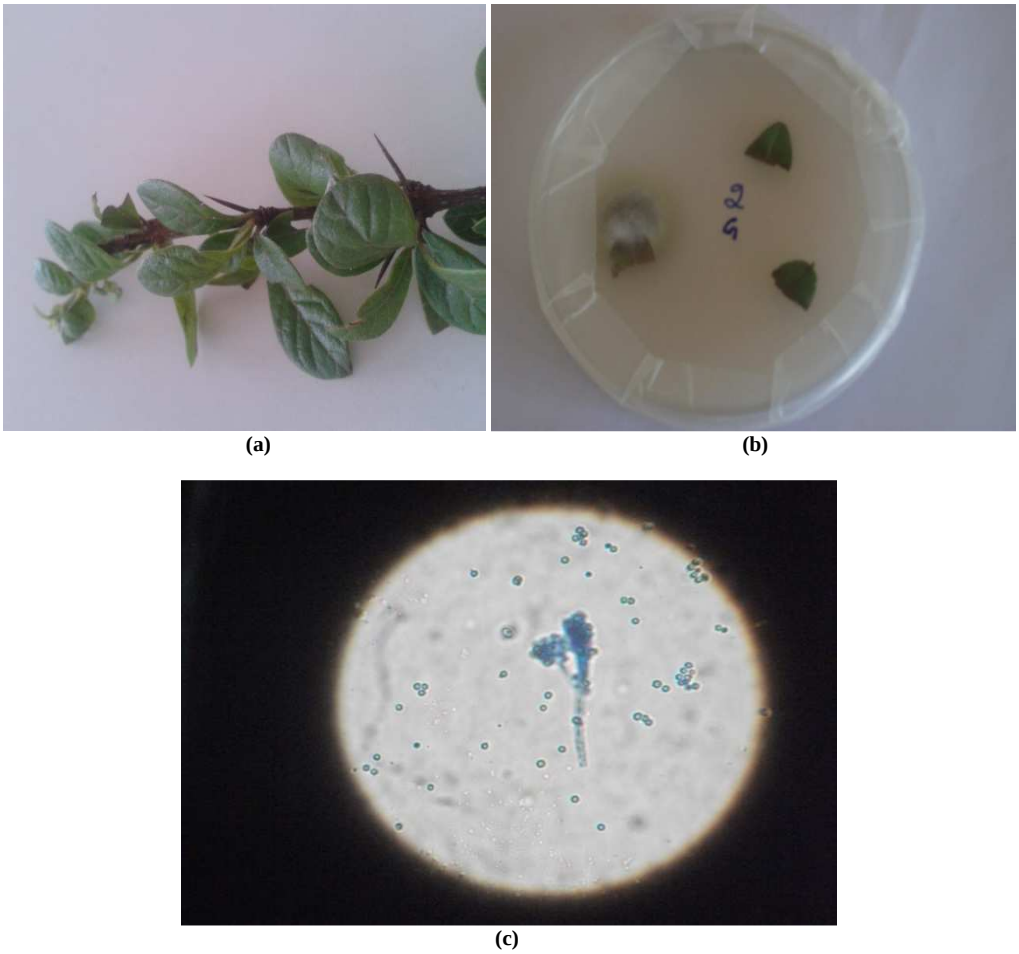
Antibacterial activity was assayed by using standard well diffusion method against pathogenic bacteria like *Escherichia coli, Streptococcus, salmonella and Pseudomonas* species. Nutrient agar was used for cultivation of the bacteria. 100µl of cultures of the bacteria were spread on Petri plates containing nutrient agar. Wells were punched in the nutrient agar with the aid of sterile borer. 50µl of the plant extract, solution containing AgNps and standard antibiotic drug (ampicillin) was inoculated in the wells and the plates were incubated at 37^0c overnight. The zone of inhibition was measured after 24hrs.

RESULTS AND DISCUSSION

Isolation of Endophytic Fungi

The leaf sections of *R. dumetorium* were surface sterilized and placed on PDA plates and incubated at room temperature for seven days. The endophytic fungi grown from the leaf segment was pure cultured on to PDA plates.

Microscopic observations and the morphological studies revealed that the isolated endophytic fungus is *P. nodositatum*



(a) **(b)**

(c)

Figure 1: (a) Image of *R. dumetorium* plant (b) *P. nodositatum* grown from the sterilized leaf segment of *R. dumetorium* on PDA. (c) Microscopic image of endophytic fungi, *P. nodositatum*.

Visual inspection of AgNps

The color of the reaction mixture was turned to yellowish brown and then to dark brown after mixing of silver nitrate (1mM) with aqueous extract of *P. nodositatum*. The color change in the mixture confirmed the formation of AgNPs. Our observation is in agreement with the other studies reported earlier [11]. Surface plasmon vibrations in AgNps are believed to be responsible for the dark brown color.The intensity of the colour was increased with the increase in the period of incubation.

UV-Vis Spectral analysis

It is generally recognized that, UV–Vis spectroscopy could be used to examine the size and shape of nanoparticles in aqueous suspensions [12]. Figure 3 shows the UV-Vis Absorption spectra of AgNps formed in the reaction media. Absorbance peak at 420 nm confirmed the formation of AgNps form the endophytic fungal extract. Broadening of peak indicated that the particles are polydispersed. The monodespersity of AgNPs in solution can be achieved by optimizing various factors such as substrate concentration, electron donor, reaction or incubation time, pH, temperature, buffer strength, mixing speed and light [11].

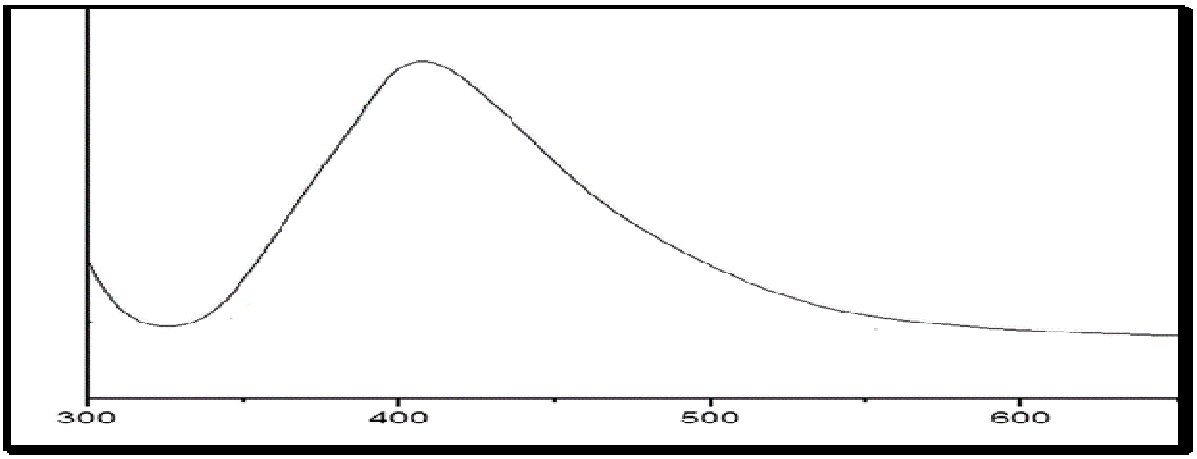


Figure 3: UV-Visible spectral analysis of AgNps synthesized from *P. nodositatum.*

Scanning Electron Microscopy (SEM) analysis

SEM measurements were carried out to determine the morphology and shape of AgNps. SEM micrograph (Figure 4) revealed that, the AgNps were ellipse shaped and well dispersed without agglomeration. The particle sizes of AgNps synthesized by endophytic fungi *P.nodositatum* were within 100nm.

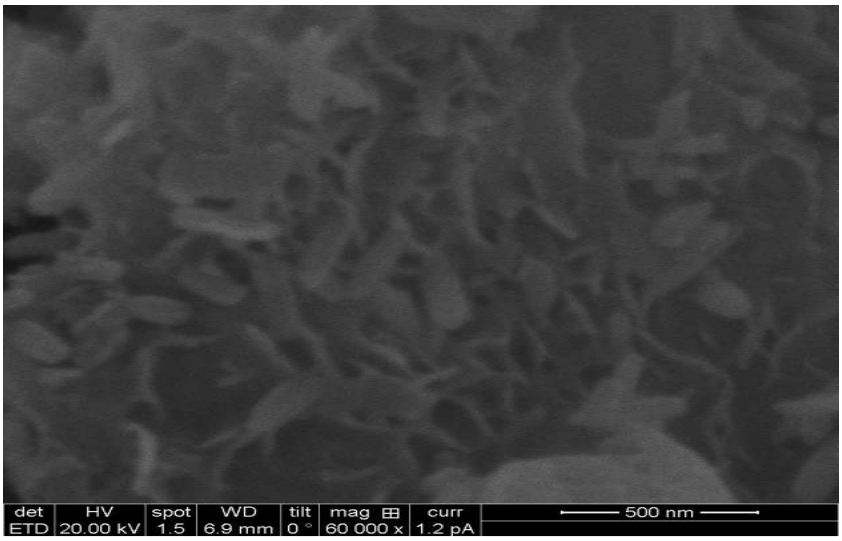


Figure 4: SEM image of AgNps synthesized using *P. nodositatum* extract

Optimization Studies for AgNps synthesis

Fungal metabolism and growth is immensely influenced by the various environmental conditions, such as, culture conditions, temperature, pH etc [13]. These factors directly affect the growth as well as the yield. The rate of AgNPs synthesis can be enhanced by optimizing the pH, temperature, inoculums size; as these factors influence the activity of enzymes [7]. The effect of volume of culture, pH and temperature, is shown in figure 2. Endophytic extract showed nanoparticle synthesis even at lower biomass. The AgNps synthesis rate was enhanced with increase in the biomass of the endophytic fungal extract. The synthesis of nanoparticle was high at alkaline pH and maximum synthesis was observed at pH 11.Many researchers have reported about the enhanced AgNps synthesis at alkaline pH [14]. The stability of AgNps increased in alkaline pH of the medium than in the acidic pH. However, regarding the optimum pH is concerned; many reports are contradictory to each other. According to Castro et al [15], optimum pH for the synthesis of AgNps is 10 but, Dubey and his associates [16] have reported the optimum pH as 11. These variations in the optimum pH may depend on the source of biomaterial used for the synthesis of AgNps.

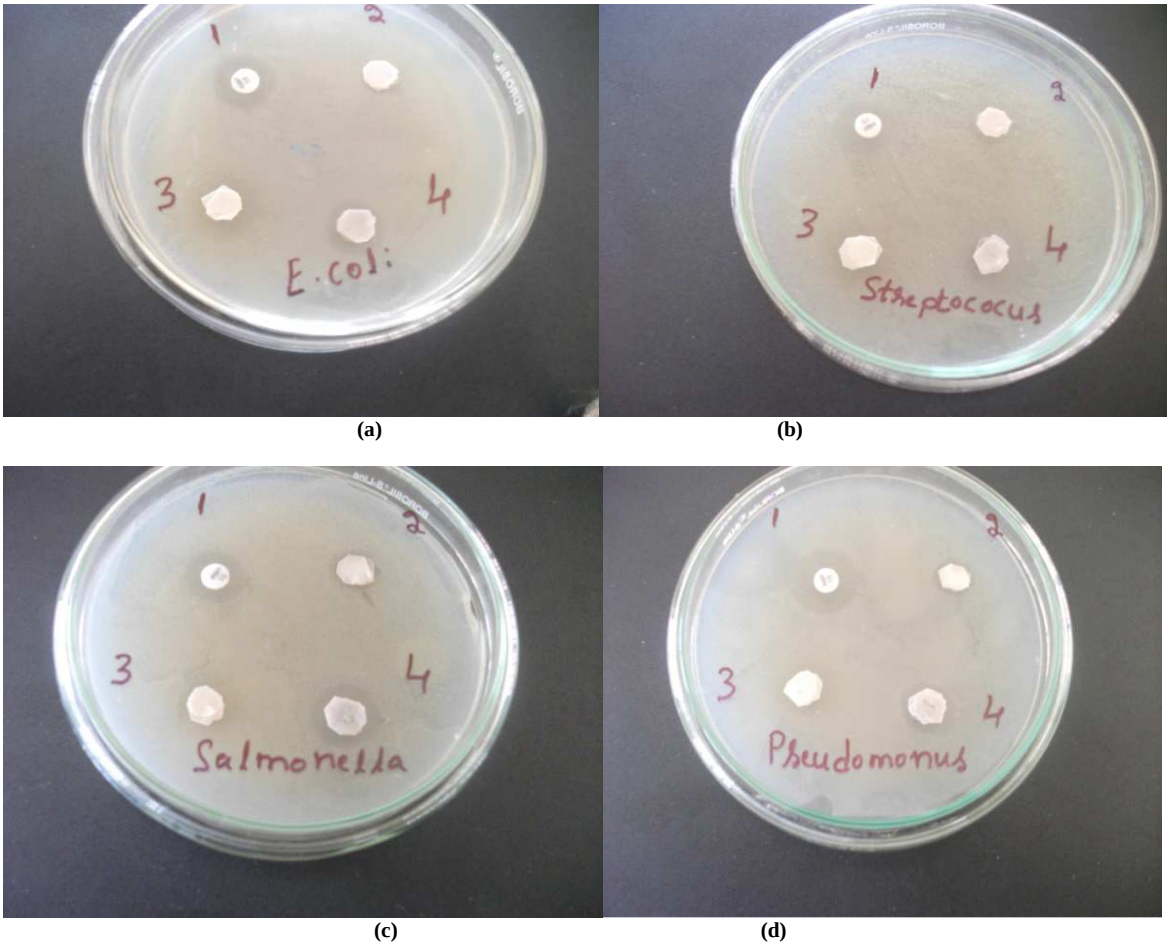
There was a significant increase in the number AgNps, as the temperature was raised from -20 to 100 °C. Our result is in agreement with the results reported previously in other endophytic fungi. As stated by Dubey et al, the increase in the absorbance peak sharpness with elevation of temperature may be due to the size of AgNps. As the temperature increases, size of AgNps particle becomes smaller and hence affects the absorbance peak [16].

(a) **(b)** **(c)**

Figure 2: Effect of (a) biomass of *P. nodositatum* (b) pH and (c) incubation temperature on green synthesis of AgNps.

Antibacterial activity of AgNps

The AgNPs synthesized from aqueous extract of *P. nodositatum* was assessed for their bactericidal property. AgNPs were tested against *E.coli*, *Streptococcus, Salmonella* and *Pseudomonas* species(Table 1) .The AgNPs synthesized using plant extract have been reported be highly toxic against gram positive as well as gram negative bacteria.



(a) **(b)**

(c) **(d)**

Figure 5: Effect of AgNps on (a) *E.Coli* (b) *Streptococcus* (c) *Salmonella* and (d) *Pseudomonas* species.

Table 1: Antibacterial activity of AgNps synthesized from the aqueous extract of *P. nodositatum*

Sl.No	Organism	Zone of inhibition (mm)			
		Amphicillin (10µg/ml)	Endophyte extract (20 µl)	Nano particle (20 µl)	Nano particle (40 µl)
1	*E. coli*	15.0	-	8.0	12.0
2	*Streptococcus sp*	13.0	7.0	9.0	10.0
3	*Salmonella sp*	13.0	6.0	8.0	11.0
4	*Pseudomonas sp*	17.0	6.0	7.0	10.0

Singh et al [6] have shown the antibacterial activity in AgNPs synthesized using endophyte isolated from *curcuma longa*. Similar results were obtained even in our study. The antibacterial potential of AgNPs synthesized from *P. nodositatum* extract was comparable to the standard drug, ampicillin. Our study has clearly established the antibacterial activity of AgNPs synthesized from *P. nodositatum*.

CONCLUSION

In the present study, we have synthesized AgNps using aqueous extract of *P. nodositatum* and confirmed the AgNps formation by UV-Vis spectroscope and SEM analysis. The AgNPs synthesized were within 100nm size and ellipsoidal in shape. The antibacterial activity of biologically synthesized nanoparticles was evaluated against *E. coli*, *Streptococcus*, *Salmonella* and *Pseudomonas* species. AgNps showed bactericidal activity. Present study showed a possibility of synthesizing therapeutic AgNps from endophyting fungi *P. nodositatum* with a simple, rapid and economical route.

Acknowledgements
The authors are grateful to Department of Biochemistry, Mangalore University for providing the necessary facility for the present study.

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