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Research Article

BIO-SYNTHESIS OF SILVER NANO CUBES FROM ACTIVE COMPOUND QUERCETIN-3-O-β-D-GALACTOPYRANOSIDE CONTAINING PLANT EXTRACT AND ITS ANTIFUNGAL APPLICATION

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ABSTRACT

In this study, the biosynthesis of silver nano cubes was carried out using leaf extract of *Peltophorum pterocarphum* (*P. pterocarphum*) containing Quercetin-3-O-β-D-Galactopyranoside compound. Simple organic compound extraction and reaction with silver nitrate was carried out. Synthesized nano cubes were characterized by various techniques like UV-Visible spectroscopy, Scanning Electron Microscopy (SEM), Fourier Transform Infra Red (FTIR) spectroscopy and X-Ray Diffractometer (XRD). The antifungal assay of silver nano cubes was performed, which shows potential effect on plant pathogenic fungi *Rhizoctonia solani* (*R. solani*). It shows increasing inhibitory action when compared to commercially obtainable anti fungal agent fluconazole. Thus for first time it was revealed that the active compound extracted helps in synthesis of silver nano cubes from the plant source and its application as an antimicrobial agent against plant morbific fungi.

Keywords: Silver nano cubes; Characterization; Quercetin-3-O-β-D-Galactopyranoside; P. pterocarphum; Antifungal activity.

INTRODUCTION

Nanotechnology, a multidisciplinary scientific undertaking, involves creation and utilization of materials, devices or systems on the nanometer scale. The field of nanotechnology is currently undergoing explosive development on many fronts [1]. This technology is expected to create innovations and play a critical role in various applications. In recent years, researchers in the field of nanotechnology found metal nanoparticles have all kinds of previously unexpected benefits. Among them silver nano particles being most exploited and newer methods for synthesis of highly mono-disperse particles were under progress [2]. These methods are efficient in terms of synthesis rate as well as energy usage.

Commonly, nano materials are synthesized using either chemical or a physical method that includes sol-gel process, micelle, chemical precipitation, hydrothermal method, pyrolysis, and chemical vapor deposition [3]. The green synthesis of metallic nanoparticles has attracted tremendous attention in recent years due to lower cost and more eco-friendly. Silver nanoparticles have been intensively focused of owing to their wide range of applications in catalysis, optics, antimicrobials and biomaterial [4-6].

Many researches reported plant mediated silver nanoparticles synthesis from *Lantana camara,* pine, persimmon, ginkgo, magnolia, platanus leaves and *Trianthema decandra* [7-9]. The reducing components present in plants, which can reduce silver nitrate to silver nano particles, are flavonoids, saponins, tannins, polyphenols, sterols, citrate, etc.

In this article, we report a simple and eco-friendly method for the synthesis of silver nano particles using an aqueous solution of *P. pterocarphum* plant extract as a bio-reductant. *P. pterocarphum* is an abundantly available medicinal plant and its leaf extract have been used as an antifungal agent [10-12]. The extract has active bio-reductant compound Quercetin-3-O-β-D-Galactopyranoside. Silver nanoparticles are used as an alternative to chemically manufactured antimicrobial agent [13]. Hence, it was attempted to combine the inherent antimicrobial activities of silver and *P. pterocarphum* extract for enhanced antifungal activity.

Silver shows many modes of inhibitory action against fungi. Therefore, it is relatively safer to control various plant pathogens when compared to inorganic synthetic fungicides. Number of studies on antifungal activity of silver against plant pathogens have been carried out and reviewed. *R. solani* is an important root pathogen, which causes diseases to *Triticum aestivum, Hordeum vulgare* and *Pisum sativum* etc. This fungus was one of the sclerotia forming microorganism is pandemic in the world and causes root rot, seedling damping-off and crown rot in a wide host range of plants. Sheath blight caused by *R. solani* is one of the destructive diseases of rice *(Oryza sativa)*, causing significant yield losses in all rice growing countries predominantly India [14]. The antifungal activity of this bio-synthesized silver nano particles on this plant pathogenic fungus was studied and it is compared with commercially available antifungal agent fluconazole.

MATERIALS AND METHODS

Silver nitrate and filter paper were purchased from Fischer Scientific, Mumbai, India. The chemical obtained was in the highest purity and used directly without further purification. Double distilled water required for the process was prepared in lab. *R. solani* strains used were obtained from International Type Culture Centre, Division of plant pathology, IARI, New Delhi, India. Fresh liquid cultures were prepared by inoculation in a Potato Dextrose Agar (PDA) (M096, Himedia Laboratories, Mumbai, India). The leaves of *P. pterocarphum* plants were collected from Anna University campus, Chennai, India and the samples were identified and authenticated at Centre for Advanced Studies in Botany, University of Madras, Chennai, India.

Extraction of active compound

The aqueous extraction of Quercetin-3-O-β-D-Galactopyranoside compound was done by following procedure prescribed by Manaharan et al. [10]. Initially, leaves were thoroughly washed with distilled water and allowed to dry at room temperature for 2 to 3 h. Further, the leaves were dried completely in an oven at 40 °C. The dried leaves were powdered in a mixer grinder. About 10 g of leaf powder was weighed and added to 100 mL of deionized water. The mixture was kept under shaking for 24 h. The obtained extract was centrifuged at 8000 rpm for 15 min. The debris and other unwanted products sedimented were removed and supernatant was filtered through Whatman filter paper No. 1.

Synthesis of silver nanoparticles

Silver nitrate solution of 100 mL having 2 mM strength was prepared. To this solution, 20 mL of plant extract was added. The solution was stirred using magnetic stirrer for 3 h under room temperature. Then the resultant colloidal solution was centrifuged at 15,000 rpm for 15 min. The supernatant was removed and settled particles were washed several times using deionized water by centrifugation.

Characterization

The reduction of silver ions was monitored by measuring the UV-Visible spectrum. It was done by diluting a small aliquot of the sample into distilled water using a Lambda 25 UV-Visible spectrophotometer (Perkin Elmer, USA) in the range of 300-600 nm. FTIR-2000 spectroscopy (Perkin Elmer, USA) was used to analyze the functional group present in the *P. pterocarphum* leaf extract. The synthesized silver nanoparticle was confirmed by changes occurred in the FTIR spectrum after synthesis. This study was undertaken to know the approximate size range and shape of the silver nanoparticles biosynthesized. The air dried nanoparticles were coated onto XRD grid and analyzed for the formation of silver nanoparticle by Philips X-Ray Diffractometer with Philips PW 1830 X-Ray generator operated at a voltage of 40 kV and a current of 30 mA with Copper Potassium alpha radiation. The diffracted intensities were recorded from 10° to 80° for 2θ values. SEM analysis was done using FEI Quanta 200 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. The extra solution was removed carefully using a blotting paper. Then the thin film on the SEM grid was allowed to dry and the images of nanoparticles were taken.

Antifungal activity

Silver nanoparticles were screened for antifungal activity by agar well diffusion method with sterile cork borer of size 5 mm. Seventy two hours old cultures grown on PDA were used for inoculation of fungal strain on PDA plates. Five wells were made by a cork borer. An aliquot (1 mL) inoculum of *R. solani* was introduced into the center well. After solidification, the appropriate wells were made on agar plate by using cork borer. In agar well diffusion method, 100 µL of silver nanoparticle solution and fluconazole solution were introduced to the corresponding wells. It was then incubation for a period of 48-72 h at 25 °C for observation of antifungal activity. The antifungal activity was evaluated by measuring zones of inhibition of fungal growth around the plate.

RESULTS AND DISCUSSION

Chemical constituents of extract

Phytochemical analysis of *P. pterocarphum* leaf extract reveals that the aqueous extract contains carbohydrates, glycosides and flavonoids. The Ag^+ reduction was based on these three molecules. The large amount of active compound Quercetin-3-O-β-D-Galactopyranoside present in aqueous leaf extract is responsible to control the shape and size of nanoparticles produced apart from being an antimicrobial agent. The compound extracted was further used for the reduction of Ag^+ into Ag^0 as shown in Figure 1.

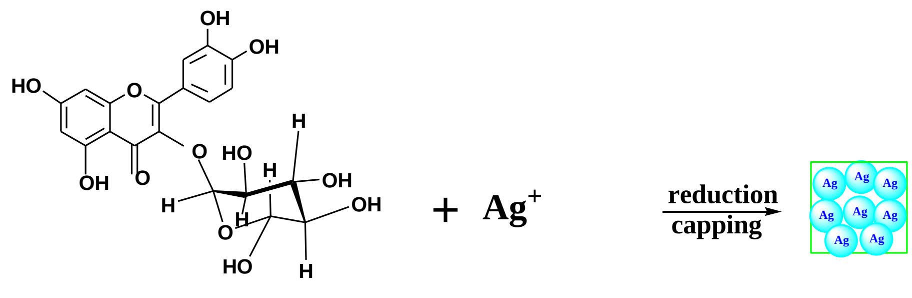


Fig. 1: Path way for the synthesis of silver nano particles

UV-Visible and FTIR analysis

The aqueous silver nitrate solution turned to brown color with the addition of plant leaf extract after keeping them at room temperature for 3 h. This characteristic color variation was due to the excitation of the surface plasmon resonance in the metal nanoparticles. Noble metal particles are ideal candidates for study with UV-Visible spectroscopy, since these silver particles exhibit strong surface plasmon resonance absorption in the visible region and are highly sensitive to the surface modification. Flavon-3-ols present in the extract was shown Figure 2, at 280 nm conforms the extract containing Quercetin-3-O-β-D-Galactopyranoside [15]. Silver nanoparticle have free electrons, which give rise to a surface plasmon resonance absorption band, due to combined vibration of electrons of Ag nanoparticles in resonance with the light wave, which extends from 390 to 490 nm. Natural flavonal mediated synthesized silver shows symmetric peaks in the visible region of the electromagnetic spectrum at 420 nm.

The FTIR spectrum in Figure 3 shows peaks between 2000 cm^{-1} to 3500 cm^{-1} represent OH group of water molecules of plant extract. Peaks at 1635 cm^{-1} corresponds to C=C aromatic ring stretching for steroil glycosides and 1018 cm^{-1} confirm the C=O stretching. Strong absorbance peaks at 1051 cm^{-1} and 1018 cm^{-1} are due to CO moiety. From Figure 3, broad peaks in ranging from 1000 cm^{-1} to 400 cm^{-1} and all broad peaks were almost disappearing after synthesis of silver. The absorbance result shows the reduction of Ag^+ to Ag^0 and oxidation of reducible alcoholic group to carboxylic group to stabilize the silver particles [16].

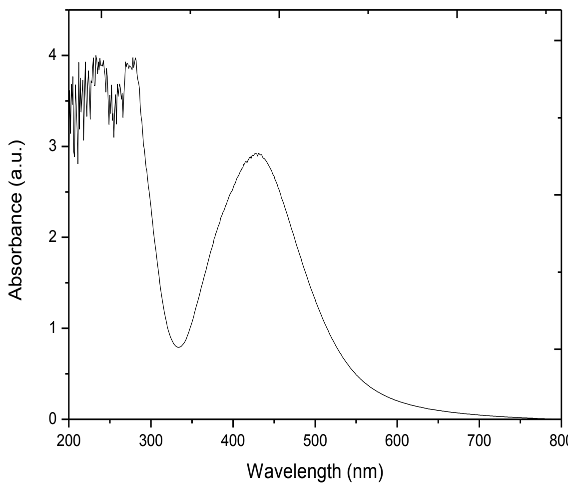


Fig. 2: UV-Visible spectrum

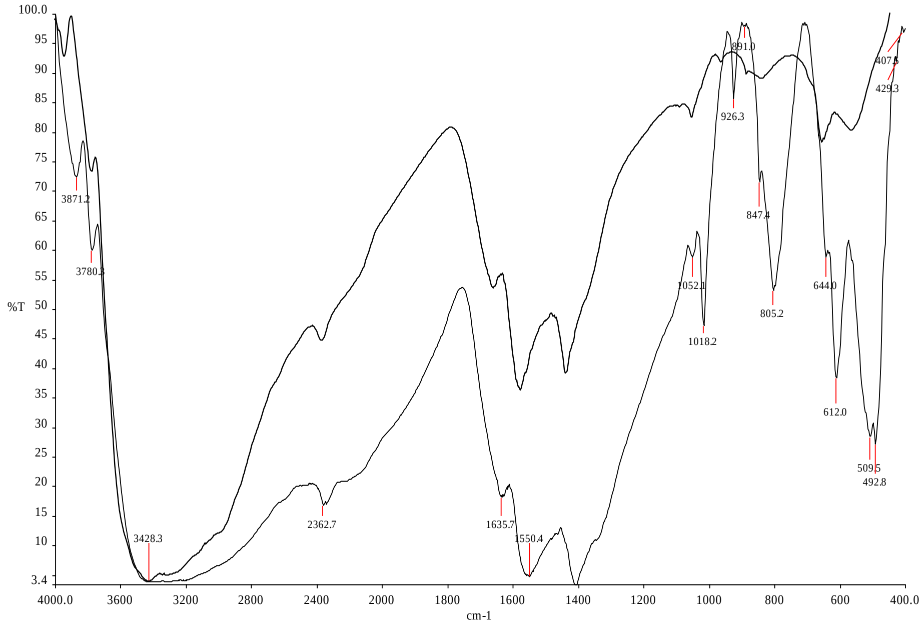


Fig. 3: FTIR spectrum

XRD and SEM

Structural characterization has been performed using XRD analysis. The typical XRD pattern for the synthesized silver particles were in nano cubes was shown in Figure 4. Three distinct diffraction peaks at 37.6°, 44.7° and 76.3° were found due to reflections from (111), (200) and (311) planes of silver in the face centered cubic lattice. The calculated particle size at d111, d200 and d311 were 320 nm, 220 nm and 210 nm, respectively. The morphology and the size of the nanoparticles were subjected to characterize by SEM analysis as shown in Figure 5. The shapes of the silver nanoparticles are mostly cubic and the diameters of the nanoparticles were lies between the range of 450 to 570 nm.

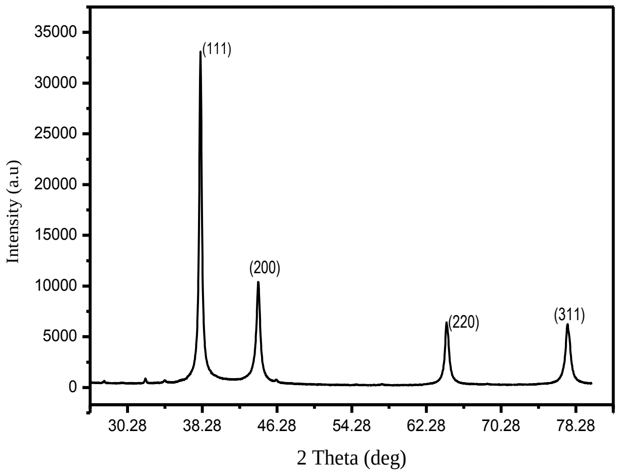
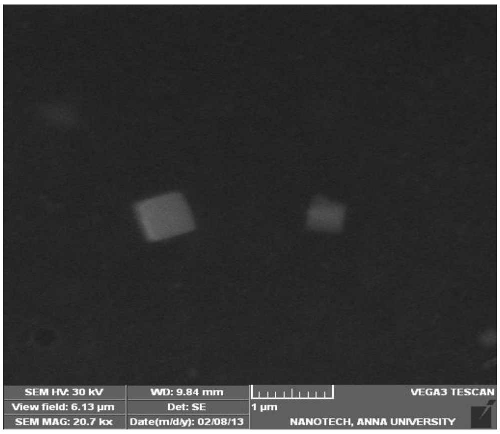


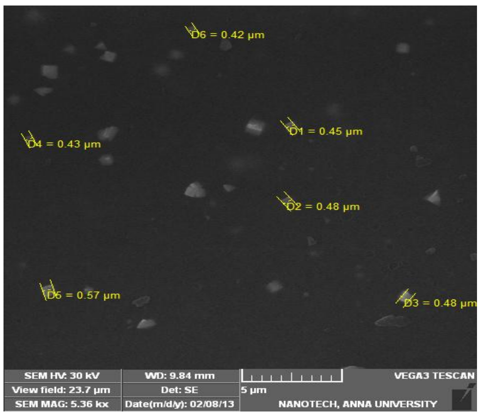
Fig. 4: XRD pattern for silver nano cubes

Antifungal activity

Concentrated silver nano cubes were subjected for observing the antifungal activity as shown in Figure 6. Silver nano cubes showed enhanced inhibitory action when compared to commercially available fungal drug fluconazole against *R. solani* (Table 1). Leaf extract of Quercetin-3-O-β-D-Galactopyranoside, which was reacted with silver nitrate enhanced the antifungal property by forming silver nano cubes.



(a)

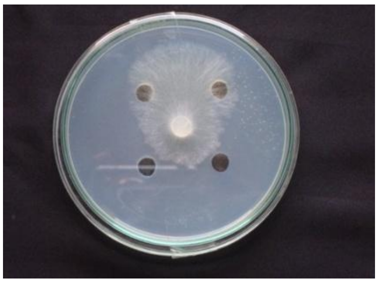


(b)

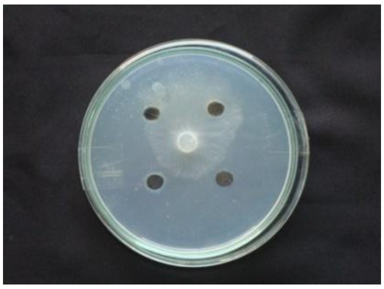
Fig. 5: SEM images of nano cubes at the resolution (a) 1 μm and (b) 5 μm

Table 1: Inhibitory activity of antifungal drug, silver nano cubes and control against *R. solani* at 5 mg mL^{-1} concentration

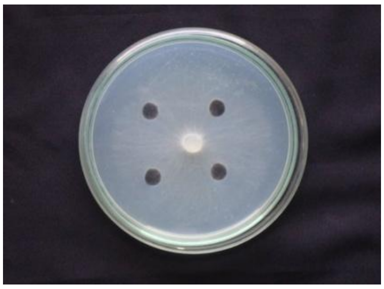
S. No	Concentration	Zone of inhibition (mm)	
		Fluconazole	Silver nano cubes
1	100 µL	12 ± 1	13 ±1
2	200 µL	7 ± 1	8 ± 1
3	Control	-	-



(a)



(b)



(c)

Fig. 6: Antifungal activity of silver nano cubes (a) 100 µL (b) 200 µL and (c) Control

CONCLUSION

The biosynthesis of silver nano cubes from the plant leaves of *P. pterocarphum* has been reported for the first time. It was concluded as an effective and eco friendly method. The synthesized nano cubes were completely characterized. The antimicrobial activity of the synthesized silver nano cubes against the fungal species *R. solani* was studied. It was concluded from the zone of inhibition, silver nano cubes have superior activity than commercial antifungal agent fluconazole. Increasing concentration of colloidal solution, the inhibitory effect against plant pathogens enhances. Thus, it can be effectively used against plant phyto-pathogenic fungi to protect various crop plants, instead of using the commercially available synthetic fungicides, which show higher toxicity to environment.

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