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ORIGINAL ARTICLE

Green Synthesis of Silver Nanoparticles Using Leaf Extract of Common Arrowhead Houseplant and Its Anticandidal Activity

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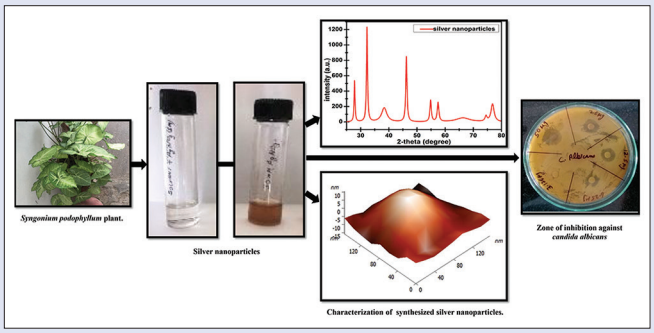
ABSTRACT

Background: Silver nanoparticles have excellent medical and nonmedical properties and application compared with other metallic nanoparticles. In the present study, fresh leaves of *Syngonium podophyllum* have been used for synthesis of silver nanoparticles. **Objectives:** In this study, we evaluated the anticandidal activity of *S. podophyllum* and the synthesized nanoparticles. **Materials and Methods:** In this study, simple and economical procedure was adopted for silver nanoparticles synthesis. *S. podophyllum* leaf was processed to obtain aqueous extract as a biological material for nanoparticles production. Synthesized nanoparticles were characterized by ultraviolet (UV) spectroscopy, X-ray diffraction (XRD), and atomic force microscopy. **Results:** The progress of silver nanoparticles biosynthesis from leaf extract of *S. podophyllum* was observed by UV-visible spectroscopy. The peaks maxima were observed at 455 nm for silver nanoparticles synthesized from the leaf extracts of *S. podophyllum*. XRD diffractogram showing Bragg peaks of face-centered cubic crystalline elemental silver confirming the formation of silver nanoparticles. The minimal inhibitory concentration values of aqueous extracts of *S. podophyllum* leaf were estimated by broth dilution method and found that the extracts exhibited antifungal activity against *Candida albicans*. The antifungal activity was also determined using disk diffusion method by measuring the diameter for zone of inhibition. **Conclusion:** *S. podophyllum* leaf extract shows strong antifungal activity against *C. albicans*. *S. podophyllum* could be applied in the fields of medical and pharmaceuticals for formulation of new drugs.

Key words: Anticandidal activity, atomic force microscopy, nanoparticles, spectroscopy, *Syngonium podophyllum*

SUMMARY

- The synthesis, characterization, and antifungal activities of silver nanoparticle from Common arrowhead house plant.
- The silver nanoparticles were confirmed to be spherical in shape.
- The antifungal activities of the confirmed their therapeutic potential.



Abbreviation used: AgNO3: Silver nitrate, MIC: Minimum inhibitory concentration, MTCC: Microbial type culture collection, SPR: Surface plasmon resonance, UV: Ultraviolet, XRD: X-ray diffraction

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INTRODUCTION

Diseases are treated using medicinal plants traditionally. Infectious skin diseases are specially caused by dermatophytes and *Candida* spp. Mycoses is a serious public health problem ranging from superficial to deep infections. Folklore information was reported for antimicrobial properties on the pathogenic fungi.[1]

Silver is extensively used in nanosystems and employed in various biomedical purposes. Silver nanoparticles have excellent medical and nonmedical properties and applications when compared with other metal nanoparticles.[2] The green approach of nanoparticles synthesis possesses reduced or no toxicity and number of plants and herbal extracts has been reported to be involved in such synthesis.[3] Plant extracts contain number of secondary metabolite which plays a critical role during the nanoparticle synthesis by acting as reducing or capping agents.[4] Studies have shown that silver nanoparticles are highly stable and toxic to bacteria, fungus, and viruses.

Syngonium podophyllum (Araceae) is commonly a houseplant, a parasitic vine with arrowhead leaf. *S. podophyllum* leaf is used against sore, dry skin, fungal infection, itching, rashes, and bruises.[5]

Various unorganized parts of the plants have been utilized for the synthesis of silver nanoparticles. In the present study, the fresh leaves of *S. podophyllum* have been used for the synthesis of silver nanoparticles. We evaluated the anticandidal activity of *S. podophyllum* and the synthesized nanoparticles.

MATERIALS AND METHODS

Materials

Silver nitrate was purchased from MERK India Ltd., Mueller–Hinton Media and Sabouraud dextrose agar media were procured from HIMEDIA India Ltd., and *Candida albicans* MTCC 183 was obtained from the Institute of Microbial Technology, Chandigarh, India, and all other materials used were of analytical grade.

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Preparation of plant leaf extracts

The leaf of *S. podophyllum* was collected from the campus of Maulana Azad National Institute of Technology, Bhopal, and washed with deionized water [Figure 1d]. The fresh leaves of *S. podophyllum* (4 g) were crushed with the help of pestle mortar, mixed in 200 ml of deionized water, boiled in a water bath for 30 min, and allowed to cool. The extracts were filtered using Whatman filter paper after filtration equal amount of ethanol is added to precipitate the mucilage present in the leaf extract; further, the extract was centrifuged at 7000 rpm for 10 min to make it mucilage free. The supernatant was collected and kept at 4°C until used.

Biosynthesis and characterization of silver nanoparticles

The nanoparticles were biosynthesized by adding 6.0 ml of silver nitrate solution (1 M) with 40 ml of extract and 154 ml of deionized water. The reaction mixture was observed for color change depending on parameter studied such as time, silver nitrate, and extract concentration at 80°C. The resultant reddish brown-colored reaction mixture was then centrifuged at 12,000 rpm for 10 min (REMI, India). The pellet obtained was washed thrice with deionized water and finally with acetone. The resultant pellet was dried and stored for further characterizations. The preparation of silver nanoparticles was characterized by ultraviolet-visible (UV-Vis) spectrophotometer (Themoscientific SpectraScan) in the wavelength range of 340–900 nm. The surface morphology and particle size distribution of the *S. podophyllum* nanoparticles were examined using an atomic force microscope (AFM, NDMDT) with a conducting *P* (n)-doped silicon tip under normal atmospheric condition in semicontact mode. The X-ray diffraction (XRD) patterns of silver nanoparticles were obtained using X-ray diffractometer (PANalytical Empyrean XRD) with diffraction angle in the range of 20°–80°.

Screening of anticandidal activity

The *C. albicans* was subjected to incubation at 35°C for 48 h and maintained on sabouraud dextrose agar. The working suspension of *C. albicans* was prepared as previously described by Ahmad and Beg.[6]

The minimum inhibitory concentration was determined by broth dilution method. *S. podophyllum* leaf extract and *S. podophyllum* nanoparticles was dissolved in dimethyl sulfoxide and dilutions were prepared at concentration range from 5 to 1000 μg/ml. The medium containing different concentration of plant extract was inoculated with 0.1 ml of fungal culture and incubated at 35°C for 48 h. The results were compared with those of the noninoculated broth and with media inoculum without extract. Antifungal susceptibility testing was performed by disk diffusion method using Mueller–Hinton agar + 2% glucose and 0.5 μg/ml methylene blue dye[7] concentration ranging from 3.125 to 50 μg.

RESULTS

Biosynthesis of iron nanoparticles

S. podophyllum silver nanoparticles synthesis were measured by observing surface plasmon resonance (SPR) by UV spectroscopy. Figure 1a shows the progress of biosynthesis when the reaction mixture was incubated at different $AgNO_3$ concentration. After confirming the $AgNO_3$ concentration, the amount of extract required for nanoparticle synthesis was determined [Figure 1b]. Time required for the nanoparticle synthesis was also determined [Figure 1c]. The SPR peaks were observed at 455 nm for the synthesized silver nanoparticles.

X-ray diffraction

We observed various Bragg peaks (angle 2 θ), sets of lattice planes which may be indexed to the (111), (200), (220), and (311) [Figure 2a]. Average crystallite size of nanoparticles was found to be 10.41 nm using Debye–Scherrer formula.

Debye–Scherrer formula: $D = K\lambda/\beta \cos\theta$.

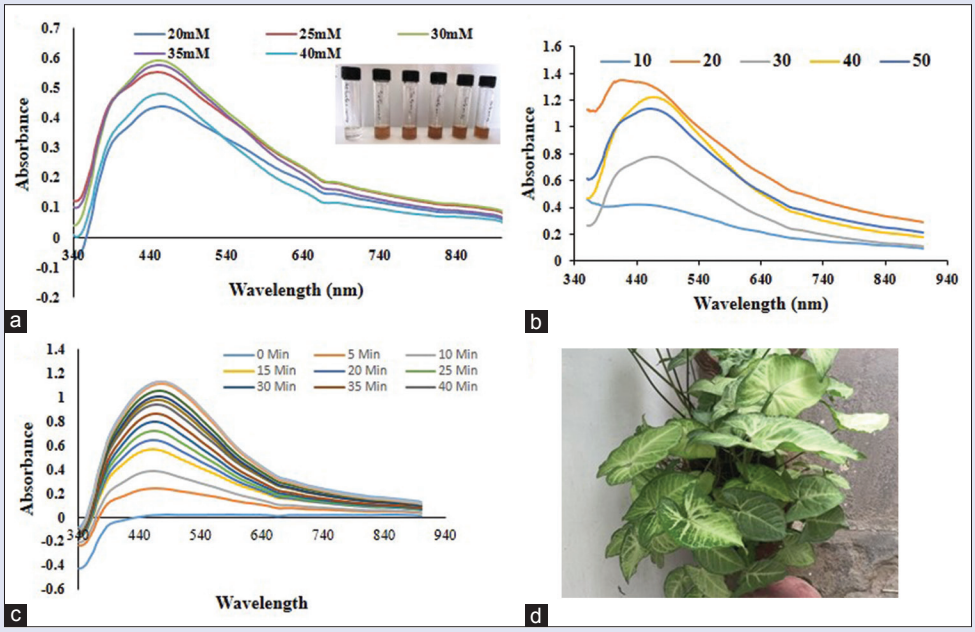

Figure 1: (a) Ultraviolet-visible absorption spectra of silver nanoparticles synthesized at 80°C from 40 ml extract with different concentrations of silver nitrate solutions. Inset showing color change of reaction mixture. (b) Ultraviolet-visible absorption spectra of silver nanoparticles synthesized at 80°C by treating 30 mM silver nitrate solution with different extract volume (ml). (c) Ultraviolet-visible absorption spectra of silver nanoparticles synthesized at 80°C by treating 30 mM silver nitrate with 40 ml extract solution at different time intervals. (d) *Syngonium podophyllum* plant

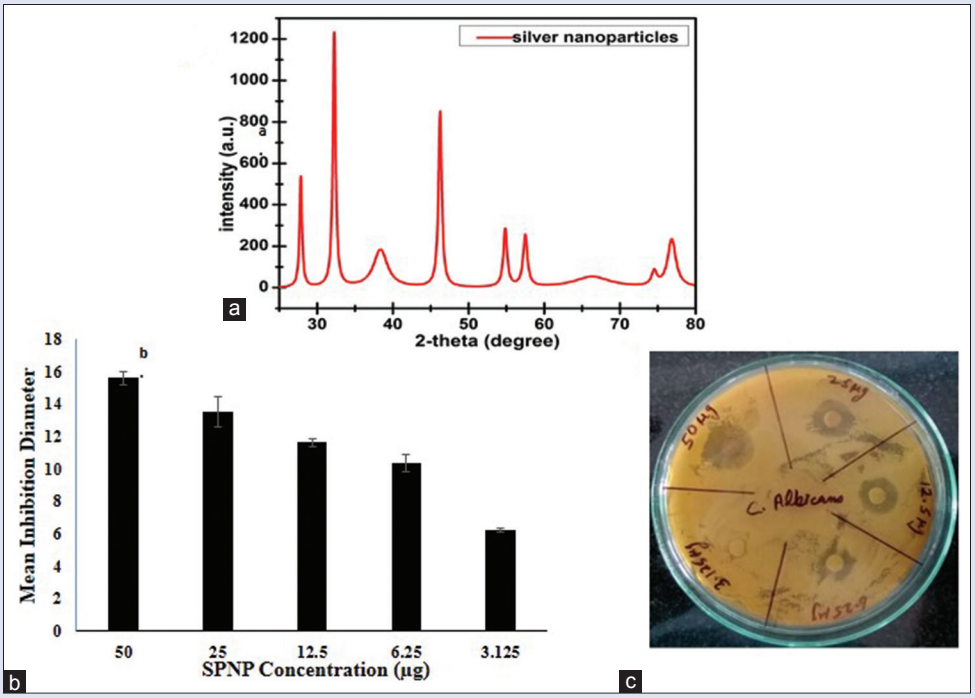

Figure 2: (a) X-ray diffraction patterns of synthesized AgNPs using leaf extract of *Syngonium podophyllum*. (b) Effect of silver nanoparticles of *Syngonium podophyllum* on *Candida albicans* at different concentration. (c) Zone of inhibition against *Candida albicans* at different concentration of *Syngonium podophyllum* nanoparticles

D = mean diameter of nanoparticles

β = the full width at half-maximum value of XRD diffraction line

λ = the wavelength of X-ray radiation source 0.15405 nm

θ = the half diffraction angle–Bragg angle

K = the Scherrer constant with the value 0.9.

Atomic force microscopy

The three-dimensional (3D) surface morphology and size analysis were obtained from AFM, shape and size distribution of the nanoparticles were done using MNOVA software using the line analysis measurement in semi contact mode [Figure 3]. Particles with 40 nm size were found to be present in maximum quantity and the shapes of the particles are spherical.

Anticandidal activity of *Syngonium podophyllum* and its nanoparticles

The *S. podophyllum* leaf extract and its nanoparticles were tested against *C. albicans*, a causative agent of cutaneous candidiasis by standard broth dilution method and well-diffusion assay.

The minimal inhibitory concentrations of aqueous extracts of *S. podophyllum* leaf were estimated by broth dilution method and were found that the extracts exhibited antifungal activity against *C. albicans* at a concentration of 1.8 µg whereas that of *S. podophyllum* silver nanoparticles is 0.2 µg, respectively. The antifungal activity of *S. podophyllum* silver nanoparticles was again determined using the disk diffusion method by measuring the diameter of zone of inhibition [Figure 2b].

DISCUSSION

The progress of silver nanoparticles biosynthesis from the leaf extracts of *S. podophyllum* was observed by UV-Vis spectroscopy. The formation of nanosilver from silver nitrate was observed by the occurrence of brown color change which was measured spectrophomertically. The SPR property is responsible for the occurrence of the absorption peak.[8]

Various parameters have been optimization for the biosynthesis of nanoparticles such as the extract concentration, $AgNO_3$ concentration, and incubation time. The biosynthesis at different $AgNO_3$ concentration, namely, 20, 25, 30, 35, and 40 mM and incubation time, namely, 0–70 min was shown in Figure 1a. After confirming the $AgNO_3$ concentration, i.e., 30 mM required for nanoparticles synthesis, the amount of extract required for nanoparticle synthesis was determined.

The response mixture was incubated at 80°C and the absorbance was measured at exclusive time intervals till the prevalence of a wide SPR was observed. The absorption peaks have been determined at 455 nm for the silver nanoparticles synthesized from response mixture consisting 40 mL leaf extracts of *S. podophyllum* at incubation time of 70 min. The crystalline structure of nanoparticles was identified by XRD technique showing sets of lattice planes indexed to the (111), (200), (220), and (311), respectively.[9] Average crystallite size of nanoparticles was found to be 10.41 nm using Debye–Scherrer formula [Table 1]. The 3D surface morphology and size evaluation have been obtained from AFM, shape, and size measurement of the nanoparticles had been done by utilizing MNOVA AFM software (NT-MDT Spectrum Instruments, Russia) making use of the line analysis mode. Particles with 40 nm size were found to be present in maximum quantity and the shapes of the particles are spherical.

The aqueous extract exhibited strong antifungal activity against *C. albicans*. The antifungal ability was again determined making use of the disk diffusion protocol with the aid of measuring the zone of inhibition [Figure 2c]. Maximum diameter of 15.60 mm was observed at concentration of 50 µg silver nanoparticles.

The bioactivity of plant extracts was due to the presence of secondary metabolites. New antimicrobial medicinal molecules are now

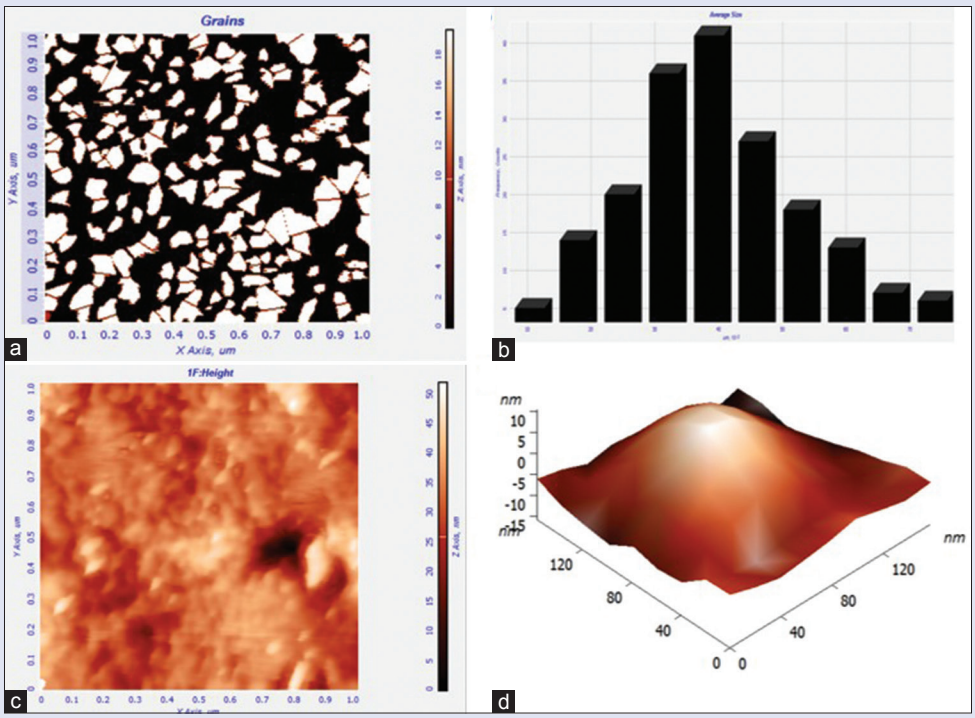

Figure 3: (a) Grains of nanoparticles observed by atomic force microscope for particles size distribution analysis. (b) Particle size distribution pattern of synthesized nanoparticles (c and d) Shape of synthesized silver nanoparticles observed using atomic force microscopy

Table 1: Average crystallite size of synthesized nanoparticles using Debye-Scherrer formula

2θ	FWHM (β)	D (nm)
27.83724	0.38717	10.14801
32.25496	0.4352	
38.36658	2.2108	
46.24043	0.51608	
54.85061	0.59034	
57.49236	0.66995	
66.33219	5.91183	
74.52319	0.81468	
76.84351	1.30552	

Debye-Scherrer formula - D=0.9 λ/βCosθ; FWHM (β): Full width half maximum; D: Average crystallite size

required and future optimization of these may permit the progress of applicable antimicrobial agent.[10] Approximately twenty different species of *Candida* are reported, of which *C. albicans* is responsible for many infections ranging from severe to mild candidiasis.[11] Silver nanoparticles synthesized through biosynthesis approach have been reported to have activity against pathogenic microorganisms. The exact mechanisms of silver nanoparticles antimicrobial activity are still under investigation. It is reported that nucleic acids and Ag+ ions form complexes which may interact with the nucleosides/proteins or silver ions may accumulate inside the membrane and penetrate into the cells causing damage to cell membranes. Silver ions can also interact with the purine and pyrimidine base pairs thereby disrupting the hydrogen bonding resulting in the denaturing of the DNA molecule.[12]

CONCLUSION

To conclude, *S. podophyllum* leaf extract has antifungal activity against *C. albicans*. Further, we have used low cost, ecofriendly, and quick method for the synthesis of silver nanoparticles using *S. podophyllum*. These bioinspired nanoparticles displayed very potent antifungal activity toward pathogenic fungi. Thus, *S. podophyllum* an ornamental plant has been effectively used for the synthesis of silver nanoparticles, and it could be applied in the fields of medical healthcare for the designing of newer drugs.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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