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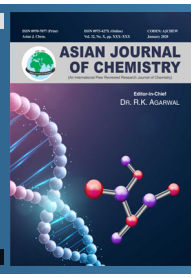

Synthesis and Biological Activity of Silver Nanoparticles from Medicinal Palm Tree *Bismarckia nobilis* Seeds

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Silver nanoparticles (AgNPs) have received significant attention due to their distinctive antimicrobial, anticancer, catalytic and photochemical activity. The objective of this work is to amalgamate silver nanoparticles from the aqueous extract of *Bismarckia nobilis* seeds using green method, characterization using UV-visible spectroscopy, X-ray diffraction, transmission electron microscopy and FTIR spectroscopy. Further, its antimicrobial and anticancer activities were evaluated. The results displayed the characteristic UV peak, cubic phase with crystalline nature, spherical in shape having average size 14 nm, prominent peaks of bio-functional groups, good antimicrobial and anticancer activities.

Keywords: *Bismarckia nobillis*, Silver nanoparticles, Anticancer activity.

INTRODUCTION

Since ancient times, plants are used for medicines and useful in the cure of various kind of diseases. The medicines synthesized by plants have a widespread popularity due to less side effects. It is estimated that 80% of world population is dependent on herbal medicines [1]. The natural products obtained in pure form are further used in new applications in using modern science. The nanotechnology is a modern technology used in the various field of science, medicines and other areas [2,3]. Nanomedicines has a great application of nanotechnology which is very useful to cure of various kind of diseases [4]. Plants mediated nanomedicines and techniques also helpful to enhance the scope of green chemistry. Nowadays, biological and green methods are used for the production of nanoparticles by using medicinal plants, fungi and bacteria [5-8]. Physico-chemical methods required lots of hazardous and toxic chemicals and are highly expensive and time-consuming process. The new green approach is developed to overcome this kind of problems by the researchers. Plants extract of medicinal plants acts as a reducing as well as stabilization agents for the synthesis of metallic nanoparticles. The plant extract contains various types of secondary metabolites having reduced as well as capping agent's properties for the products [9].

Due to global warming and safe environmental aspects, green technology offer grants to states in the efforts to reduce waste sources [10,11]. One of the most important objectives of green chemistry is to avoid waste materials than to treat wastes after it has been produced. In the field of nanotechnology silver nanoparticles also used as topical creams to prevent wound infection, used as antimicrobial agents in wound dressing and as anticancer agents. In present study, the palm tree seeds used as a reducing as well as stabilizing agent for the synthesis of nanoparticles. *Bismarckia nobilis* (Family: Arecaceae) is a monotypic genus of flowering plant in the palm family endemic to western and northern Madagascar, where they grow in open grassland [12,13]. This study is an attempt to explore the green

synthesis of silver nanoparticles from medicinal plant (*Bismarckia nobilis*), where the biological molecules undergo highly controlled assembly for making them suitable for the synthesis of silver nanoparticles.

EXPERIMENTAL

Analytical grade chemicals were procured from Sigma-Aldrich company. Deionized distilled water was used throughout the experiments.

Bacterial strains: The bacterial strains (*Salmonella typhi,* MTCC-734), *Klebsiella pneumoniae*, MTCC-39), *Staphylococcus aureus* (MTCC-737) and *Pseudomonas aeruginosa*, MTCC-741) were collected from IMTECH, Chandigarh, India. The microorganisms were sub-cultured in nutrient broth and incubated at 37 ºC for 24 h prior to the experiment.

Human cervical cancer cell line SiHa, human lung cancer cell line A-549 and human embryonic kidney KEK-293 cells were procured from National Centre for Cell Sciences (NCCS) Pune, India. The MTT (3-(4,5-dimethyl-2-yl)-2,5-diphenyl tetrazolium bromide), Dulbecco's modified Eagle's medium (DMEM), 0.25% trypsin, 0.02% EDTA mixture were purchased from HiMedia (Mumbai, India) while fetal bovine serum (FBS) was obtained from Gibco (Grand Island, NY).

Synthesis of silver nanoparticles: The extract of *Bismarckia nobilis* seeds (10 mL) was added to 90 mL of $AgNO_3$ (2 mM) solution and was kept for 48 h in dark condition for the formation of AgNPs. The colour changes of solution indicated the formation of AgNPs. Then, solution was centrifuged at 10,000 rpm for 15 min and nanomaterial was collected followed by redispersion in deionized water to get rid of any unwanted impurity.

Characterization: Elite-UV-visible spectrophotometer was employed for screening for AgNPs from 400 to 500 nm range against aqueous leaf extract (as blank). Nanosilver powder was subjected to XRD analysis (X'PERT-PRO Diffractometer, PANalytical; Cu*K*α radiation, λ_{max} = 1.54 Å). Morphology was determined by transmission electron microscope (JEOL JEM 1011, 100 kVA). FT-IR analysis was conducted using Perkin-Elmer spectrophotometer Model RZX for confirming the presence of bio-functional groups.

Antimicrobial activity of silver nanoparticles: Antimicrobial activity of silver nanoparticles was assessed by well diffusion method [14,15]. The turbidity of sub-cultured microorganisms was adjusted with sterile distilled water using 0.5 McFarland as standard (~1.5×10^8 cells/mL). Mueller Hinton Agar (HiMedia) was prepared by dissolving readymade agar powder in distilled water. Agar plates were prepared and inoculated with the test microorganisms by spread plate method. The plates were left undisturbed for 30 min at room temperature. The powdered nanoparticles were weighed and dissolved in DMSO and used in triplicates. Then, the solution was added in the wells in a constant concentration of 50 µL/well. The standard of antibiotic chloramphenicol (HiMedia) was also prepared as a positive control. Then, the plates were incubated at 37 ºC for 24h in upright position. After incubation, the zone of inhibition was measured and compared with the standard antibiotic zone.

Minimum inhibitory concentration: The MIC assay was determined by microdilution method. The well plates were prepared by dispensing 100 µL of nutrient broth into each well. Stock solution (100 µL) of tested nanoparticles (concentration of 50 mg/mL) and added into the first well of the plate. Then, two-fold serial dilutions were performed by using a micropipette. The obtained concentration range was from 50 mg/mL and then added 50 µL of inoculum to each well except negative control. The positive control of antibiotic (chloramphenicol), negative control (nutrient broth), broth alone and the inoculum alone were also examined. The test plates were incubated at 37 ºC for 24 h. The lowest sample concentration showing clear well and inhibited complete growth were taken as MIC value.

Anticancer activity: The cytotoxicity of compounds was screened against different cell lines, namely HEK-293, A-549 and Siha cell line for 48 h treatment at concentration range 10-200 µg/mL. Initially, 2×10^4 cells/well was seeded into flat bottom 96-well plates (150 µL/well) in triplicates, allowed to attach and grow. The cells were incubated for 24 h and subsequently treated with varying concentrations of the compounds ranging from 25 to 400 µg/mL. After 48 h of treatment the medium was removed and cells were incubated with 20 µL of MTT (5 mg/mL in PBS) in fresh medium for 4 h at 37 ºC. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) is metabolic substrate which is reduced by the mitochondrial succinate dehydrogenase enzyme of mitochondria in physiologically active cells and forms formazan crystal. Formazan crystals, formed by mitochondrial reduction of MTT, were solubilized in DMSO (150 µL/well) and the quantification was performed by reading the absorbance at 540 nm after an incubation period of 15 min on the iMark Microplate Reader (Bio-Rad). The relative absorbance of treated *versus* control (untreated) cells was used in determining percentage viability [16].

RESULTS AND DISCUSSION

UV-Vis analysis: The formation of AgNPs was screened from 400 to 500 nm range against aqueous leaf extract (as blank) [17-20] using Elite-UV-visible spectrophotometer. The Ag SPR maximum absorption bands was observed at 420 nm of *Bismarckia nobilis* seed extract mediated AgNPs sample solutions at fixed intervals of time (Fig. 1).

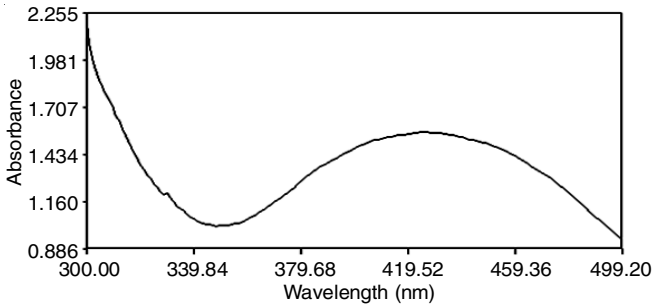

Fig. 1. UV-visible spectrum of *Bismarckia nobilis* seeds extract mediated synthesized AgNPs

XRD analysis: Silver nanoparticles were further subjected to XRD analysis (Fig. 2). The XRD pattern showed five distinct

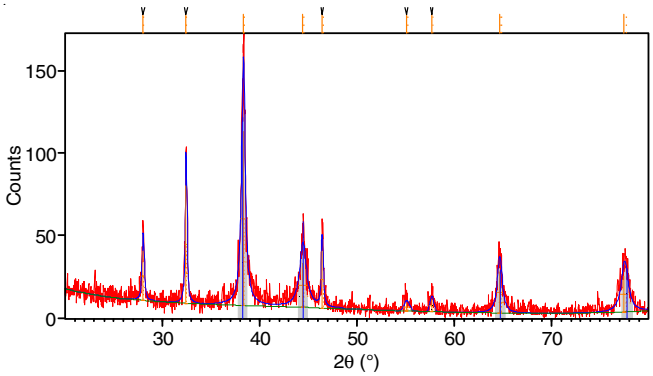

Fig. 2. XRD peak of silver nanoparticles

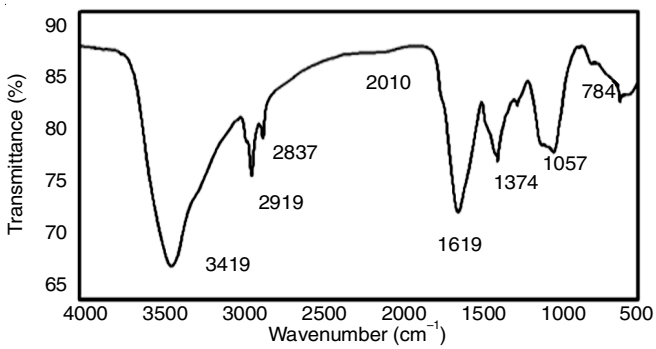

Fig. 4. FTIR data of functional groups present in synthesized silver nanoparticles

2θ (°) values with (hkl) lattice planes at 38.26º (111), 44.47º (200), 64.71º (220), 77.74º (311) and 81.90º (222). This kind of pattern represents the formation of face-centered cubic phase AgNPs.

TEM analysis: TEM images of silver nanoparticles on nanometric scale (Fig. 3) was clearly observed that synthesized AgNPs were spherical in shape with a diameter of about 10 to 18 nm. The average size of AgNPs was found to be 14 nm.

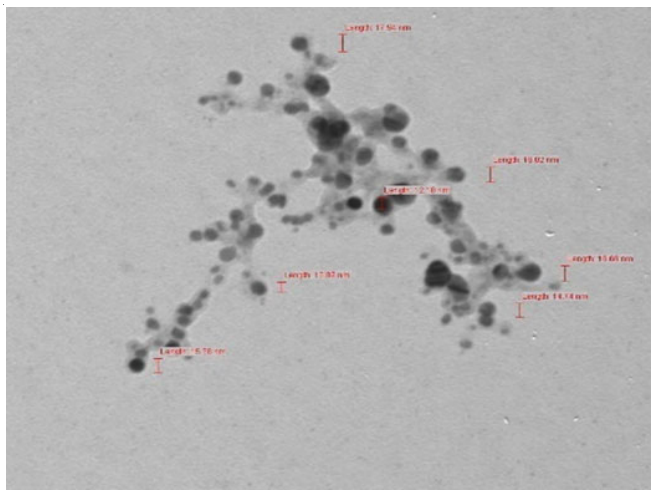

Fig. 3. TEM images of AgNPs

FTIR analysis: FTIR analysis were carried out to identify the biomolecules for capping and efficient stabilization of the silver nanoparticles synthesized by *Bismarckia nobilis* seeds extract. FTIR spectrum (Fig. 4) of the roots extract shows peaks at about 3419, 2919, 2837, 1619, 1374 and 1057 cm^{-1}. Peak at 3419 cm^{-1} arises due to N-H stretching of an amino group is a indicative of O-H group due to the presence of alcohols, phenols [21]. Peak at 2919 cm^{-1} indicates the presence of C-H bond stretching of the alkyl group, while peaks at 2837 and 1619 cm^{-1} are associated with N-H bond in amino acid. Peak at 1374 cm^{-1} represents C-N stretch vibration as well as amide I bands of proteins in the roots extract and C-O stretching assigned to alcohols represented by peak at 1057 cm^{-1}.

Biological activity: Silver nanoparticles were investigated for the antimicrobial activity against the bacterial species mentioned by the measurement of zone of inhibition. The antibacterial activity of AgNPs against the human pathogens showed varied levels of inhibition as shown in Table-1 and the present study revealed that AgNPs showed potential antibacterial activity against *S. typhi* (16.3 ± 1.0) and *P. aeruginosa* (14.0 ± 1.3). The nanoparticles showed maximum zone of inhibition against *S. typhi*. Microdilution method was used to determine the lowest concentration of nanoparticles that was inhibiting the growth of the bacteria [22] and found effective in the evaluation of best activity.

Anticancer activity: The cytotoxic effect of synthesized silver nanoparticles on human cervical cancer cell line SiHa, human lung cancer cell line A-549 and human embryonic kidney HEK-293 at concentration range 10-200 µg/mL was assessed by using MTT calorimetric assay. There was a change cell viability in control and AgNPs treated cells; a gradual increase in cytotoxicity was observed. The cytotoxic effect of silver nanoparticles was found significant on human lung cancer cell line A549 with an IC_{50} value of 41.2 µg/mL followed by human cervical cancer cell line SiHa, with an IC_{50} value of 49 µg/mL. With such a potential cytotoxic effect on cancerous cells, these nanoparticles were found to be least toxic for non-cancerous cells, when tested on human embryonic kidney cell line HEK293 even at a higher concentration of to 200 µg/mL (Fig. 5). These values are suggesting a potential anticancer effect of bio-generated AgNPs.

Conclusion

In this work, the synthesis of silver nanoparticles was carried out by using aqueous extract of seeds of *Bismarckia nobilis*

TABLE-1
ANTIBACTERIAL EFFECT OF NANOPARTICLES AGAINST SEVERAL BACTERIAL STRAINS

Sample	Zones of inhibition (mm)			
	S. typhi	*S. aureus*	*P. aeruginosa*	*K. pneumonia*
Bismarckia nobilis	16.3 ± 1.0	Nil	14.0 ± 1.3	Nil
	MIC (mg/mL)			
	3.2	Nil	12.5	Nil

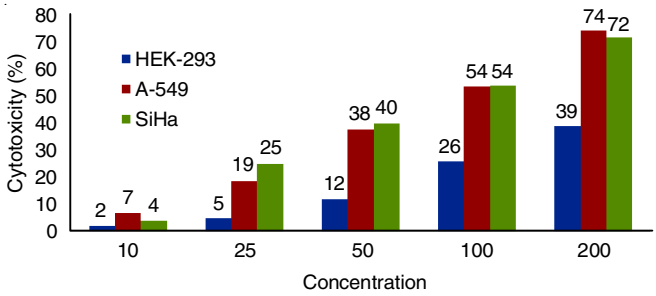

Fig. 5. Cytotoxicity of *Bismarckia nobilis* seed extract mediated synthesized AgNPs against KEK293, SiHa and A-549 cell line

medicinal plant as bioreducing agents. The UV-visible absorption spectra displayed the characteristic peaks of silver nanoparticles. The XRD patterns of silver nanoparticles correspond to the cubic phase orientation attached to silver nanocrystals. It also confirmed that synthesized nanoparticles were crystalline in nature. TEM images proved that silver nanoparticles were spherical in shape and having average size 14 nm. The synthesized AgNPs exhibited a good antimicrobial activity and significant cytotoxic effect on human cervical cancer cell line SiHa and Human lung cancer cell line A-549 and AgNPs were found to lie in the biological dosage range.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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