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Antimicrobial and Anticancer Activity of Spherical Silver Nanoparticles Synthesized using the Aqueous Leaf Extract of Ricinus Communis (Castor Plant)

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Abstract

Silver nanoparticles (AgNPs) synthesized using aqueous leaf extract of Ricinus communis (Castor plant) were examined for their antimicrobial and anticancer activities. Antimicrobial activity against the Escherichia coli, Pseudomonas aeruginosa (Gram negative), Bacillus subtilis, Staphylococcus aureus (Gram positive) and Rhodobactor sphaeroides (Purple bacteria) and anticancer activity was examined against MCF-7 cells (human breast cancer cell line). The AgNPs were characterized by the UV-Visible spectroscopy, Scanning electron microscopy (SEM), Energy-disruptive X-ray spectroscopy (EDX) and Transmission electron microscopy (TEM). Antimicrobial and anticancer activities of AgNPs were studied well diffusion method and SRB assay. Synthesis of AgNPs was confirmed by analyzing the spectra at 445 nm using UV-vis spectrophotometer. The TEM and SEM analysis showed an average 8.96 nm size aggregates of spherical shaped AgNPs. EDX showed the complete chemical composition of synthesized AgNPs. The maximum zone of inhibition was observed in R. sphaeroides (13, 14, 15, and 16 mm) than the other bacterial species (E. coli, P. aeruginosa, B. subtilis, and S.). The AgNPs tested for anticancer activity showed the high toxicity towards the MCF-7 cells (human breast cancer cell line) (LC50 14.95 µg/mL). We can conclude that, it is a novel, cost effective, eco-friendly, green approach towards antimicrobial and anticancerous activities. In future, R. communis synthesized AgNPs could be used in pharmaceutical field to develop antimicrobial and anticancerous products

Keywords: Ricinus communis, Silver nanoparticles, Antimicrobial, Anticancer

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Introduction:

Nanoparticles play an important role in pharmaceutical, industrial, biomedical and biotechnological applications. In particular, silver nanoparticles are proved to have potential antibacterial, antifungal, and anticancer properties[1-3]. Silver nanoparticles synthesized using plant aqueous extract have been tested for antimicrobial and anticancer activities[4-7].

Recently, antimicrobial and anticancer potential of silver nanoparticles synthesized using the various medicinal plants and silver-selenium nanoparticles by phytochemicals (quercetin and gallic acid) has been reported[8]. The antimicrobial, antioxidant and anticancer activity of biogenic silver nanoparticles have been studied[9-11].

In the present study, antimicrobial and anticancer activities of silver nanoparticles (AgNPs) synthesized using aqueous leaf extract of R. communis were assessed. It is a novel, cost effective, eco-friendly, green approach towards antimicrobial and anticancerous activities. In future, R. communis synthesized AgNPs could be used in pharmaceutical field to develop antimicrobial and anticancerous products.

Materials and Methods

Collection and preparation of castor leaf extract

The fresh and green leaves of the Ricinus communis (castor plant) were collected around the area of Yamuna bank, Delhi, India. Leaves were thoroughly rinsed with tap water followed using distilled water to remove dust and other particles. The aqueous extract of leaves was prepared using the method as described previously with some modification[12]. The rinsed leaves were then air dried for 1-2 h. Then 25 g (approximately) leaves were cut into fine pieces and placed into a 250 mL conical flask containing 100 mL distilled water and shake for 1 h in an orbital flask shaker. After 1 h the extract was filtered through the whatman-1 filter paper and store for the experiment.

Synthesis of AgNPs

For the synthesis of AgNPs, 30 mL leaves extract was added to 70 mL of 1 mM silver nitrate ($AgNO_3$) aqueous solution and kept at room temperature in an incubator. After 24 h, a change in color was observed from light yellow to dark brown, indicating the formation of

AgNPs (Fig. 1a). The AgNPs were further used for characterization using the different microscopic techniques.

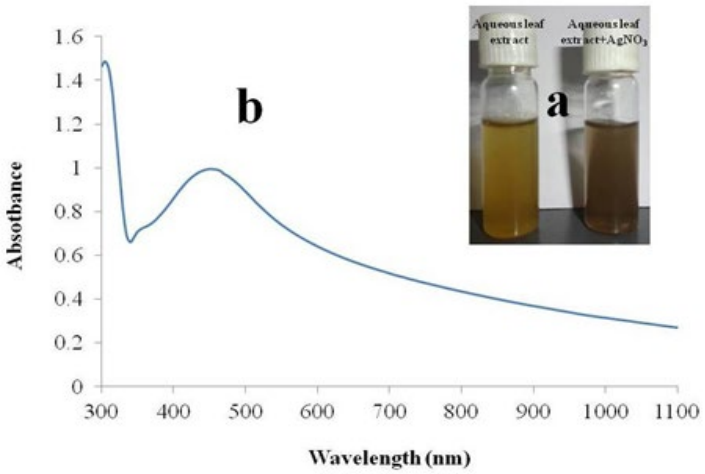

Fig. 1: (a) Aqueous leaf extract of R. communis without $AgNO_3$ and with 1 mM $AgNO_3$ solution. (b) UV-Visible spectra of synthesized AgNPs.

Characterization of AgNPs

The presence of AgNPs in the resultant colloidal solution was established through UV-Vis spectroscopy. The production of AgNPs was initially confirmed by the UV-Vis spectrum of the resultant colored colloidal solution. The spectrum was recorded using HALO DB-20 at the wavelength of 300-1100 nm, 10 nm resolution. After reduction, AgNPs were precipitated at the bottom of the conical flask. This precipitate was centrifuged at 5,000 rpm for 10 min and washed out twice with distilled water and then converted in to powder. For electron microscopic studies, the samples of AgNPs were prepared by placing a drop of reaction mixture on carbon coated copper TEM grid and allowing the water to evaporate. The micrographs of the AgNPs were obtained using Tecnai G2 transmission electron microscope and scanning electron microscope. Elemental analysis on single particle was carried out by EDX analysis.

Antimicrobial assay

The antimicrobial activity of AgNPs was evaluated against the E. coli, P. aeruginosa (Gram negative), B. subtilis, S. aureus (Gram positive) and Rh. sphaeroides (Purple bacteria). The wells were prepared on plates with Muller-Hinton agar (MHA) plates. Then, the plates were seeded with different bacterial strains using sterile swab. Four well were prepared in each plate, using gel puncture. Each well was loaded with 40 µL of different concentrations of AgNPs (50, 100, 200 and 500 ppm). Then, the plates were incubated at 350C for 24 h and zone of inhibition was observed.

Anticancer assay

Anticancer activity was evaluated using the Sulforhodamine B (SRB) assay against the MCF-7 human breast cells. MCF-7 cells (human breast cancer cell line) were seeded on to 96 well plates at a cell density of 5×10^3 cells/well in 100 µL of complete medium and incubated at 37°C for 24 h. A plate containing untreated cells was processed to evaluate a time zero absorbance as described below. Separately, cells were treated with an increasing concentrations of OR-1868- PM-AgNPs (0.5, 1, 5, 10 50 µg/ml) for 48 h followed by fixing with 40 µL of 20% TCA, incubated at 4°C for 1 h and subsequently washed with deionized water for five times. Plates were then air dried for 24 h and stained with 0.4% of SRB (40 µL) prepared in 1% acetic acid solution followed by incubation at room temperature in dark for 20 min. Then cells were washed with 1% acetic acid solution thrice after removing SRB and plates were dried for 4 h. Tris base (100 µL) was added to each well to solubilise the bound SRB and absorbance was measured at 510 nm using multimode plate reader[13]. Growth inhibition was estimated with reference to cells without drug treatment and the time zero control. GI50, TGI and LC50 were calculated using the following formulae as described earlier[14].

GI50= 100 x (T-To) / (C-To)

TGI = 100 x (T-To)/ (C-T)

LC50= 100 x (T-To) / To

Where T is the absorbance of the test well after 48 hours of drug exposure;

To is the absorbance at time zero;

C is the absorbance of the control.

Results and Discussion

UV-visible, TEM, SEM and EDX analysis

As mentioned above after visible analysis, AgNPs formation was confirmed by the UV-Vis spectroscopy. Figure 1b shows the distinct peak of UV-Vis spectra of AgNPs at ~445 nm. The similar result was observed[15].

Presence of AgNPs was further established by TEM analysis. The AgNPs were spherical in shape and 8.96 nm in size (Figure 2a).

Scanning electron microscope pictures show the AgNPs synthesized using R. communis (Figure 2b). The SEM images distinctly show the high density of synthesized AgNPs using R. communis confirming the development of Ag nanostructures.

The EDX attachment with the SEM is known to provide information on the chemical analysis of the field that are being investigated or the composition at specific location (spot EDX). The representative profile of the spot EDX analysis was obtained by focusing on the AgNPs (Figure 2c).

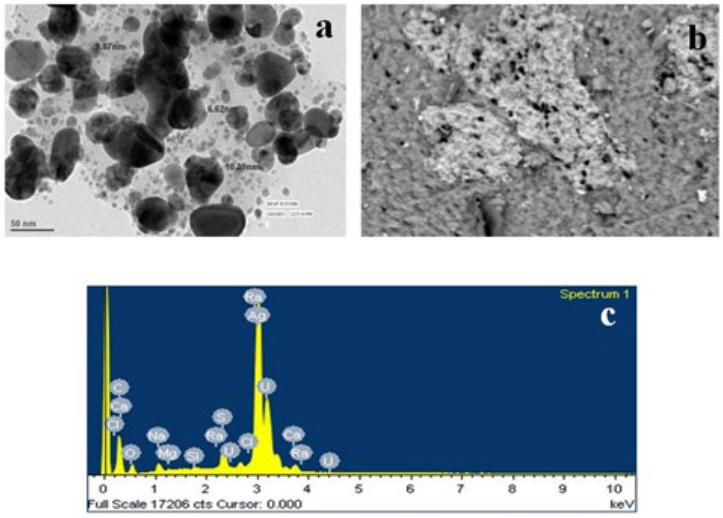

Fig. 2: (a) TEM (b) SEM and (c) EDX images of synthesized AgNPs

Antimicrobial activity

Well diffusion method was used to provide the evidence and validate the antimicrobial activity of AgNPs against E. coli, P. aeruginosa (Gram negative), B. subtilis, S. aureus (Gram positive) and R. sphaeroides (Purple bacteria). The antimicrobial activity of the AgNPs was indicated by the formation of the zone. The diameter of the inhibition zone was measured in millimetre (mm). The maximum zone of inhibition was observed in R. sphaeroides (13, 14, 15, and 16 mm) as compared to the other species (Table 1, Figure 3). The higher zone of inhibition of synthesized AgNPs occurred at a concentration 500 ppm. Silver nanoparticles exhibited antibacterial properties against bacterial pathogens with close attachment of the nanoparticles themselves with the microbial cells. Phytosynthesized silver nanoparticles have been tested for antibacterial and antifungal activities in a previous study also, using disc diffusion method against Proteus, Pseudomonas, Klebsiella, Bacillus and E. coli species of bacteria and Aspergillus, Fusarium, Curvularia and Rhizopus species of fungal[16]. Antibacterial activity of silver nanoparticles has been assessed by using disc diffusion method against B. subtilis, S. aureus, E. coli and K. pneumonia[17]. Similar, results were reported in the previous studies[18-22].

Anticancer activity

The cytotoxic effect of AgNPs was evaluated by SRB assay using MCF-7 cells as shown in Table 2. The bio based synthesis of nanoparticles which showed significant activities on cancer studies can be developed into potential anticancer agent and active pharma molecule. The anticancer activity of SNPs assessed in vitro by MTT assay on human cancer cell lines of colon (HCT-116), breast (MCF-7), liver (Hep-G2) and intestine ($Caco_2$) showed good anticancer activity which was negligible for the aqueous plant extracts.[23] The cytotoxic effect of biosynthesized silver nanoparticles has been reported by MTT assay against breast cancer cells (MCF-7 cell line)[24-26].

Species	Zone of inhibition (mm)			
	50(ppm)	100(ppm)	200(pm)	500(ppm)
E. coli	8±0.612	10±0.654	12±0.712	14±0.801
P. aeruginosa	10±0.654	11±0.678	13±0.789	14±0.801
B. subtilis	11±0.678	13±0.789	14±0.801	15±0.891
S. aureus	6±0.532	8±0.612	10±0.654	12±0.712
R. sphaeroides	13±0.789	14±0.801	15±0.891	16±0.991

Table 1: Antimicrobial activities of AgNPs at various concentrations against E. coli, P. aeruginosa (Gram negative), B. subtilis, S. aureus (Gram positive) and R. sphaeroides (Purple bacteria).

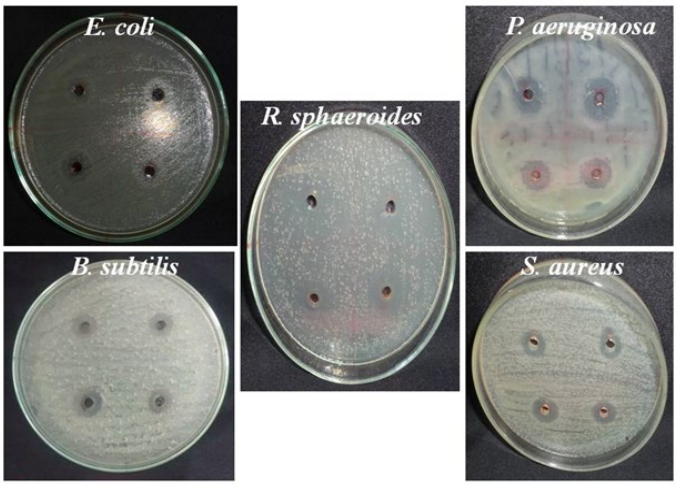

Fig. 3: Antimicrobial activities of synthesized AgNPs at different concentrations against the E. coli, P. aeruginosa (Gram negative), B. subtilis, S. aureus (Gram positive) and R. sphaeroides (Purple bacteria).

MCF-7	GI50 (µg/mL) Mean ± SEM	TGI (µg/mL) Mean ± SEM	LC50 (µg/mL) Mean ± SEM
OR-1868-PM-AgNPs	8.83 ± 0.08	11.45 ± 0.25	14.95 ± 0.75

Table 2: Anticancer activity of AgNPs against human breast cell MCF-7 at LC50 concentration

Conclusion

R. communis possesses the medicinal properties. Hence, R. communis extract has the reducing power agent which can be used in nanoparticles synthesis. Due to the medicinal properties and reducing power activity, in the present study, silver nanoparticles (AgNPs) synthesized using aqueous leaf extract of R. communis (castor plant) were examined for antimicrobial and anticancer activities against E. coli, P. aeruginosa (Gram negative), B. subtilis, S. aureus (Gram positive) and R. sphaeroides (Purple bacteria) and MCF-7 cells (human breast cancer cell line). We can conclude that, it is a novel, cost effective, eco-friendly, green approach towards the improvement of antimicrobial and anticancerous activities. In future, the R. communis synthesized AgNPs could be used in pharmaceutical field to develop antimicrobial and anticancerous products.

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