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Research Article

MICROWAVE IRRADIATION ASSISTED SYNTHESIS OF SILVER NANOPARTICLE USING LEAF EXTRACT OF *Baliospermum montanum* AND EVALUATION OF ITS ANTIMICROBIAL, ANTICANCER POTENTIAL ACTIVITY

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ABSTRACT

Nanoparticles are sized between 10-100nm and are of great scientific interest because of their unique properties. The unexpected and interesting unique properties of the nanoparticle are due to the large surface area and small size of the material. In this study, silver nanoparticle was synthesized via biological method using the leaf extract of *Baliospermum montanum* as a reducing agent under microwave irradiation condition. Then the silver nanoparticle was characterized by UV analysis, SEM-EDAX. The particle size was studied by SEM analysis and were found to be in the range of 10-60nm. The antibacterial and anticancer potentials of the silver nanoparticle were studied. The silver nanoparticle has shown maximum activity against *E.coli* among the tested bacteria. The cytotoxicity effect of silver nanoparticle was studied by MTT assay against Vero cell line and Hep2 cell line.

Keywords: Silver nanoparticle, Extract, microwave irradiation, characterization, potential activity.

INTRODUCTION

Nanotechnology deals with synthesis of nanoparticles and nanomaterials (generally range from 1-100nm) of variable size, shapes and their application in various fields. Nanoparticles have been studied widely because of their unique physicochemcial properties like catalytic activity (1), optical properties (2), electronic properties, antibacterial properties (3) and magnetic properties. The unique property could be attributed to their small sizes and large surface area.

Metal nanoparticle such as gold, silver, zinc, and platinum, are extensively used in products that directly come in contact with the human body, such as shampoos, soaps, detergent, shoes, cosmetic products, and toothpaste, also medical and pharmaceutical applications. Metal nanoparticles with unique properties; have been synthesized by chemical (4, 5) and biological methods. In chemical methods ecologically toxic chemicals have been used. This negative aspect can be overcome by biological method. In case of biological method, bio components such as microorganism, plant extract or enzymes are used for the nanoparticle synthesis. Biological synthesis of silver nanoparticle using plant extract of *Cissus quadrangularis* (6), coriander leaves (7), sundried *Cinnamomum camphora* leaves (8), phyllanthin extract (9), and purified apiin compound extracted from henna leaves (10) have been reported. Using microorganism like fungi (11, 12), actinomycets and bacteria have also been reported (13).

In this study, *Baliospermum montanum* leaf extract was used as a reducing agent to synthesis silver nanoparticle. *Baliospermum montanum* is a medicinal plant belonging to the family Euphorbiaceae. This plant is distributed throughout the India. The plant contains steroids, terpoids and flavanoids (14) and plant leaves contains 8-sitosterol 8-D-glucoside and hexacosanol. Leaves can cure asthma and bronchitis and also used for dropsy (15). The reduced silver nanoparticle was characterized by UV-visible spectrometer, SEM-EDAX analysis and their potential anticancer and antibacterial activity was assessed.

MATERIALS AND METHOD

Plant material and preparation of the aqueous extract

Baliospermum montanum leaves were collected from Tamil Nadu, India and used for the preparation of the aqueous extract. 25g of green tender leaves were thoroughly washed, cut into fine pieces, were crushed with 100 ml sterile distilled water and filtered through Whattman No.1 filter paper (42µm) to get an extract. The aqueous leaf extract was used as a reducing agent for further nanoparticle synthesis.

Synthesis of silver nanoparticle

Microwave irradiation method was followed for the synthesis of silver nanoparticle. In microwave method 100ml of 1mM silver nitrate containing 5 ml of plant extract was treated under microwave irradiation till the color changes from watery to yellowish-brown (6). Color change (brown color change) indicates the reduction of nanoparticle (16).

Characterization of synthesized silver nanoparticle

The synthesized nanoparticle was characterized by UV-Vis spectra analysis, SEM, EDAX analysis.

UV-Vis Spectra analysis

The reduction of pure Ag+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium and the absorption spectra were recorded over the range of 200-800 nm using UV-Vis spectrophotometer (VARIAN CARY EL06023680).

SEM and EDAX analysis of silver nanoparticles

Scanning Electron Microscopic (SEM) and EDAX analysis were done in IIT madras using FEI Quanta FEG 200 high resolution scanning electron microscope machine. In order to view the particle, sample was coated on the conductive carbon tape and the sample was viewed under microscope.

Invitro toxicity study:

Antibacterial activity of synthesized nanoparticle

All microorganisms used for this study were purchased from the National Chemical Laboratory (NCL), Pune, India and were maintained at 4^0C on nutrient agar. The antibacterial activity of the nanoparticle was studied by disc diffusion method against the following bacteria via, *E.coli, Enterococcus faecalis, Bacillus subtilis, Klebsiella pneumonia, Staphylococcus aureus, Salmonella typhi, Vibrio cholerae.*

Cytotoxicity assay

Cell line maintenance and growth conditions

The cytotoxicity potential of silver nanoparticle was studied against Hep2 and Vero cell line. Cell lines were purchased from NCCS (National centre for cell sciences), Pune, India. Cells were subcultured and were maintained at 37^0C at 5% CO_2 in CO_2 incubator. Cultures were continuously observed under an inverted microscope to assess the degree of confluency and to confirm the absence of bacterial and fungal contaminants.

Cytotoxicity test

Preliminary cytotoxicity effect of silver nanoparticle was assessed by MTT assay. Cell lines were subcultured and 200µl of media (containing 1000cells) were transferred into 96 well plates and incubated for 24 hr. The spent media was removed and 100µl fresh media was added. Synthesized nanoparticle was added at different concentration (20-140 µg) and then final volume was made to 200µl with the media and incubated for 4 hr. After incubation media containing drug was removed. 20µl of MTT reagent (5mg/ml) was added to each well containing media and incubated for 3.5 hr at 37 °C under an atmosphere of 5% CO_2 until a purple precipitate was visible. Media was removed carefully (Do not disturb cells and do not rinse with PBS). 150µl DMSO (MTT solvent) was added to dissolve the purple precipitate (17). Absorbance was read at 570 nm with a reference filter of 630 nm. Percentage cytotoxicity was calculated and used for finding the IC50 value of nanoparticle.

RESULTS AND DISCUSSION

Biosynthesis of silver nanoparticle

The silver nanoparticles were synthesized by microwave irradiation method. The advantage of using microwave irradiation is that it provides uniform heating around the nanoparticles and can assist the digestive ripening of such particle without aggregation. The microwave irradiation heats up a material through its dielectric loss, which converts the radiation energy into thermal energy (18). As the *Baliospermum montanum* leaf extract was added to the aqueous solution of the silver ion complex and microwave irradiation was passed, the solution color was started to change from watery to yellowish brown due to reduction of silver ion (Fig.1); which indicated formation of silver nanoparticles (16, 17). Reduction of silver ions could be easily followed by color change. Due to excitation of surface Plasmon vibrations in nanoparticle, it exhibits different color than the molecular scale particle. The silver nanoparticle solution exhibits yellow color (19). The color change was observed after 5 min exposure of silver ions solution containing leaves extract under microwave irradiation.

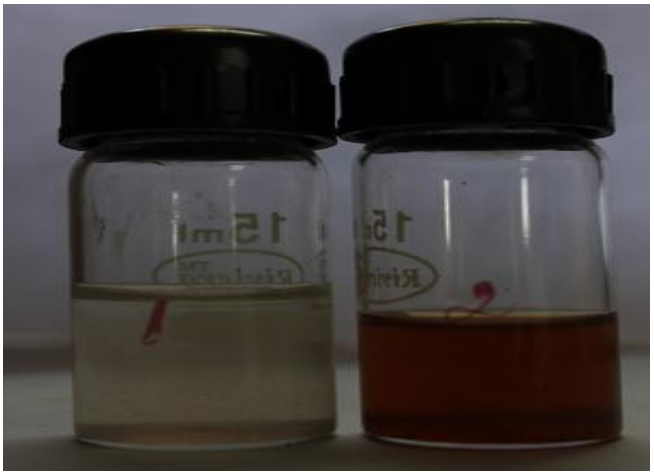


Figure 1: 1-1mM Silver nitrate solution, 2-the color changes to brown after the microwave irradiation

UV-Vis Spectra analysis

The formation of silver nanoparticle by reduction of the aqueous silver ions during exposure of *Baliospermum montanum* extract may be easily followed by UV-Vis spectroscopy. Silver nanoparticle exhibit brown color in aqueous solution due to the surface Plasmon resonance phenomenon (20, 21). In this study, the surface Plasmon resonance band of the silver nanoparticle was observed at 415nm (fig2).

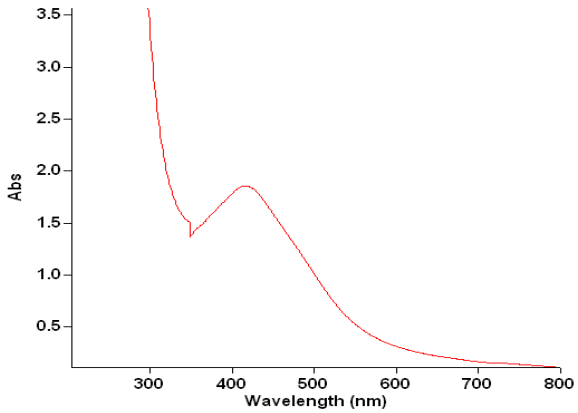


Figure 2: UV-Vis spectra analysis silver nanoparticle synthesized using *Baliospermum montanum* extract

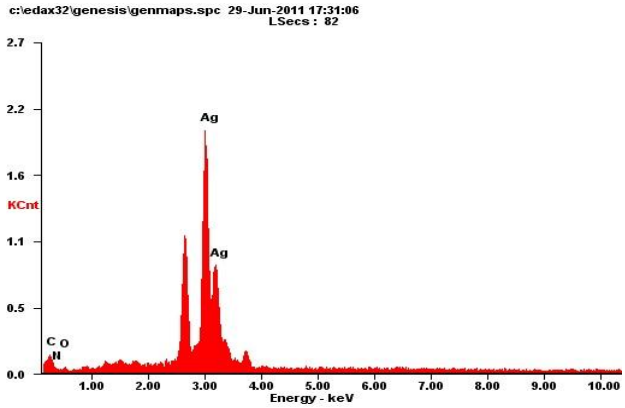


Figure 3: EDAX image of silver nanoparticles synthesized using *Baliospermum montanum*

SEM and EDAX analysis

Analysis through Energy dispersive X-ray (EDX) spectrometers confirmed the presence of elemental silver signal of silver nanoparticles (Fig. 3) the vertical axis displays the number of X-ray counts whilst the horizontal axis displays energy in K eV. Identification lines for the major emission energies for silver (Ag) are displayed and these correspond with peaks in the spectrum, thus giving confidence that silver has been correctly identified. The SEM report (Figure 4) of the synthesized silver nanoparticle showed that the particle were of the size 10 – 60nm.

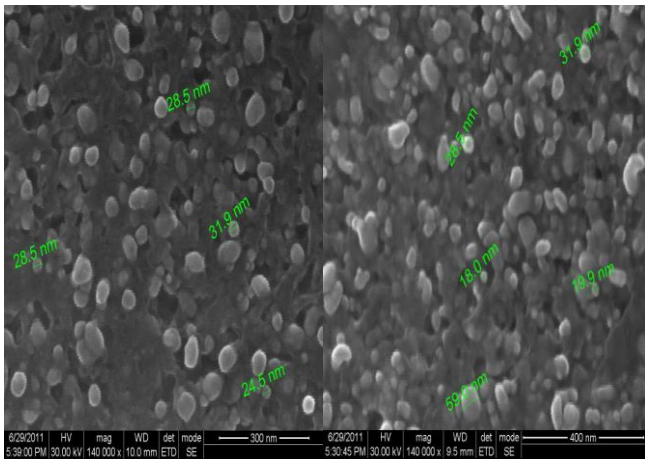


Figure: 4 SEM image of silver nanoparticle synthesized using *Baliospermum montanum* extract.

Antibacterial activity

Antibacterial activity of silver nanoparticle was evaluated by disc diffusion method against the following microorganism: *E.coli, Enterococcus faecalis, Bacillus subtilis, Klebsiella pneumonia, Staphylococcus aureus, Salmonella typhi, Vibrio cholerae* and the results were tabulated below.

The silver nanoparticle has shown antibacterial against all tested microorganism and maximum activity was found against *E.coli.* The second maximum activity was observed against *Staphylococcus aureus* and *Salmonella typhi* and least activity was found against *Vibrio cholerae, Klebsiella pneumonia* and *Bacillus subtilis.* The silver nanoparticle's activity was compared with the plant extract, silver nitrate solution and standard disc (streptomycin). Nanoparticle has shown maximum activity than silver nitrate solution, plant extract. Silver nanoparticle has maximum zone of inhibition than the standard disc against *E.coli,* whereas silver nanoparticle has shown same zone of inhibition of streptomycin against *staphylococcus aureus.* From table it was clear that silver nanoparticle has shown more activity than the silver nitrate solution. The mechanism involved in antibacterial activity of silver nanoparticle is not well known. It may be due to the attachment of silver nanoparticle to the surface of the cell membrane and disquieting the power function of bacteria such as permeability and respiration (5). Since nanoparticle has large surface area and small size than, the nanoparticle binds and interacts to the cell more than the large particle. It may be a reason the silver nanoparticle has shown more activity than the silver ions.

Table 1: antibacterial activity of silver nanoparticle

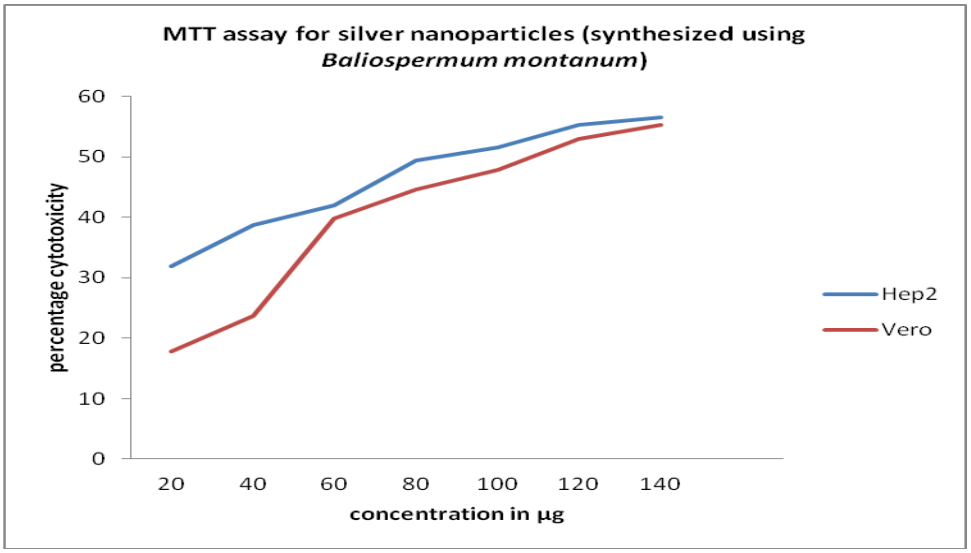
Microorganism	**zone of inhibition in mm**			
	10µl of 1mM $AgNO_3$	**10 µl of plant extract**	**10µl of Ag nanoparticle**	**Standard disc (streptomycin)**
E.coli	7	Nil	15	13
Enterococcus faecalis	8	6	11	12
Bacillus subtilis	Nil	Nil	10	12
Klebsiella pneumonia	5	6	10	11
Staphylococcus aureus	Nil	Nil	12	12

Salmonella typhi	8	Nil	12	16
Vibrio cholerae	5	5	10	21

Cytotoxicity (MTT) assay

In-vitro cytotoxicity effect of silver nanoaprticle was studied against Hep2 cell and Vero cell line at different concentration (20, 40, 60, 80, 100, 120, 140µg). The concentration required for 50% cell death (IC50) for HEP2 and Vero cell line were found to be 86µg and 107µg respectively. IC50 value was found to be less for the Hep2 cell line than Vero cell line (when compared to vero cell line nanoparticle was found to be more toxic to Hep2 cell line).

Graph 1: Cytotoxicity effect of silver NPs on Hep-2 and Vero cell line



CONCLUSION

There is an increasing commercial demand for nanoparticles due to their wide applicability in various areas. Metallic nanoparticles are traditionally synthesized by wet chemical techniques, where the chemicals used for quite often toxic and flammable. In this work an effective and environmental friendly technique for the synthesis of nanoparticles from 1 mM $AgNO_3$ solution using leaf extract of *Baliospermum montanum* was carried out. Synthesised nanoparticles were characterized using UV-Visible spectroscopy and SEM-EDAX. Characterization study confirmed that the particles were silver nanoaprticle, were in the size range of 10-60nm. Finally the synthesized nanoparticles were subjected to toxicological studies. Silver nanoparticle had showed antibacterial activity against the tested microorganisms. Thus, this particle can be further analysised for the bacterial growth inhibition and can be used to treat certain microbial diseases. Silver nanoparticle has shown the cytotoxicity effect on Vero and Hep2 cell line. Further study is required for evaluation its anticancer activity in order to bring out an effective anticancer drug.

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