GREEN TECHNOLOGY MEDIATED SYNTHESIS OF SILVER NANOPARTICLES FROM MOMORDICA CHARANTIA FRUIT EXTRACT AND ITS BACTERICIDAL ACTIVITY

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INTRODUCTION

Nanotechnology involves constructing and designing of matter at atomic level from bulk magnitude size depends on the comprehension, use and control of matter of bulk magnitude [1]. Nanoparticles range from 1 to 100 nm with unique physicochemical properties that make them different from those of the bulk particles [2]. Nanoparticles are extensively explored and investigated for antimicrobial [3]. One such metal nanoparticle with prominent antimicrobial activity is silver nanoparticles. Numbers of products, such as toothpaste, deodorants, bedding, washers, water purification systems, and shampoo, contain silver nanoparticles [2]. Silver nanoparticles can be synthesized by employing chemical and physical method [4,5]. Both the methods involve certain disadvantages such as toxicity, costly chemicals, require large space, and energy. Complete data are still lacking about exposure of these chemically or physically synthesized silver nanoparticles on environment and human being [6].

Deployment of biosynthetic technique applying plant extracts has established substantial forefront in recent times. Large scale production, easiness, economically feasible nature, and eco-friendliness equaled to the other prevailing approaches such as using fungi and bacteria which would not be possible with the physical and chemical ways for the production of metal nanoparticles.

In this study, the silver nanoparticles were synthesized from fruit extract of the plant Momordica charantia. The biosynthesized nanoparticles were characterized, optimized, and its antimicrobial activity against Bacillus cereus and Staphylococcus epidermidis was studied.

METHODS

The M. charantia fruits were collected from the market of Vaishali Nagar, Jaipur. Silver nitrate was purchased from Merck.

Preparation from of fruit extract
The fruit was washed thoroughly, wiped and chopped. The chopped fruit was then dried in oven. The dried chopped fruit was ground well to make powder. 5 g of powder was weighed and dissolved in 50 ml of distilled water. The solution was then boiled for 15 minutes at 50°C. The solution was filtered, centrifuged at 9,000 rpm for 15 minutes and made up volume to 50 ml. The extract was stored in the refrigerator for further use.

Biosynthesis of silver nanoparticles
The biosynthesis of silver nanoparticles was done by adding 10 ml of aqueous plant extract in 50 ml of AgNO₃ (5 mM) solution. The mixture was stirred on the magnetic stirrer for 45 minutes. The solution was then centrifuged 3 times at 9,000 rpm by washing the pallet each time by distilled water to remove the unbound ligands from the pallet. The final pallet is then dissolved in the distilled water and dried in the oven at 50°C. The dried powder obtained was black in color.

Charaterization of nanoparticles
The silver nanoparticles which were synthesized from fruit extract of M. charantia were characterized using U.V spectrophotometer, Fourier transform infrared spectroscopy (FTIR), dynamic light scattering (DLS), scanning electron microscopy (SEM), and atomic force microscope (AFM).
After mixing plant extract and AgNO₃ solution, ultraviolet-visible (UV-VIS) spectra (Visiscan 167) were carried out by scanning the solution in range of 300 nm to 800 nm where distil water was used as reference. Silver nanoparticles and plant powder both were mixed with KBr to determine functional group using FTIR spectroscopy (Shimadzu 8400 S). The size distribution profiles of nanoparticles were analyzed using DLS (Zetasizer and Malvern), and SEM (Zeiss).

Antibacterial assay

Staphylococcus epidermidis (MTCC 26394) and B. cereus (MTCC 9017) are the pure strains purchased from IMTECH Chandigarh. Nutrient agar (Hi Media, India) and King’s medium were used for the growth at 30°C and 28°C and subcultured before use. The antibacterial assay was done by disc diffusion method [7] by taking three types of the concentration of biosynthesized Ag NPs 25 µg/ml, 35 µg/ml, and 45 µg/ml.

Optimization of biosynthesized silver nanoparticles

Three parameters were selected and varied to optimize the biosynthesis of AgNPs are temperature, pH, and solvent. The temperature of the AgNO₃ (5 mM) solution was set at 10°C, 15°C, 20°C, 50°C, 60°C, and 75°C in six different flasks, respectively. 10 ml of plant extract was added in six different AgNO₃ (5 mM) solutions, and the same procedure was followed as for the biosynthesis of Ag NPs. Another parameter for optimization involves the pH of the AgNO₃ (5 mM) solution was set at 3, 5, 9, and 11 pH in four different flasks respectively. 10 ml of plant extract was added to each AgNO₃ (5 mM) solutions, and the same procedure was followed as for the biosynthesis of Ag NPs. Methanolic extract of fruit was prepared by using Soxhlet apparatus. The 20 g fruit powder was dissolved in 300 ml of methanol and extract was prepared in soxhlet apparatus for 36 hrs.

RESULT AND DISCUSSION

Nowadays, nanomaterials are at the most important platform of rapidly evolving nanotechnology phase. Nanomaterials are aiding contemporary technology to pact with nanosized matters, their irreplaceable properties specifically size dependent styles them greater materials and necessary in different human deeds. At present, nanomaterials are now being used in medical fields for example tissue engineering, strong antibacterial agent, detection for pathogens/proteins and drug carriers, etc. Size specificity and novelty have made silver nanoparticles popular in number of consumer products [2,8,9]. Biosynthesis of nanoparticles using plant material is becoming popular nowadays [10].

UV-VIS absorption spectrum was observed by Visiscan 167 UV-VIS spectrophotometer for bio reduction of silver ions to AgNPs mediated by M. charantia fruit extracts were observed by recording the absorption spectra, and show absorbance at between 420 and 430 nm (Fig. 1) which was due to excitation in surface plasmon vibration. This is the most important feature of metal nanoparticles, which is due to the electron oscillations that collectively gather around the surface of metal particles. In similar studies, it was found that the absorbance spectra showed the peak at 400-450 nm [11-13].

The silver nanoparticles formed by bioreduction process are stabilized by several capping ligands, which were characterized by FTIR analysis [14]. The FTIR measurements were carried out to identify the possible biomolecules responsible for the reduction of the silver ions into silver nanoparticles.

The peaks at 3650 cm⁻¹ indicate the presence of O-H stretching, the peaks at 3350-3300 cm⁻¹ that of N-H stretching 1650 cm⁻¹ due to C=O stretching and the peak at 1513 cm⁻¹ may be due to the presence of N=O asymmetric nitro compounds in the plant extract (Fig. 2). Whereas in case of silver nanoparticles, the peaks at 3450 cm⁻¹ indicate the presence of O-H stretching, the peaks at 3350-3300 cm⁻¹ that of N-H stretching, 1650 cm⁻¹ due to C=O stretching and the peak at 1550 cm⁻¹ may be due to the presence of N-O asymmetric nitro compounds in silver nanoparticle (Fig. 2). Another study of FTIR spectrum analysis showed peaks between 1000 and 2000 cm⁻¹ which confirmed the presence of proteins and other ligands required for the synthesis and stabilization of silver nanoparticles [15,16].

Light scattering (Zetasizer, Malvern) technique was used to determine the size distribution profile of nanoparticles in suspension. The average mean size of silver nanoparticles comes out to 20-150 nm as shown in Fig. 3. This measurement depends on the size of the particle core, the size of surface structures, particle concentration, and the type of ions in the medium. When light hits small particles, the light scatters in all directions (Rayleigh scattering) as long as the particles are small compared to the wavelength (below 250 nm). The other studies revealed that the average size of the silver nanoparticle synthesized from Pimenta dioica was 10-150 nm [17].

The silver nanoparticles extracted from M. charantia extracts were analyzed using AFM. Fig. 4 contains the micrographs obtained from the AFM measurements of the silver nanoparticles obtained using M. charantia extracts. Fig. 4 illustrates an AFM micrograph of biosynthesized silver nanoparticles scanned in an area of 50 µm x 49.5 µm. The figure contains the average lengths (measured along the longer axis of nanoparticles), widths (measured along the shorter axis of nanoparticles), and areas calculated for the biosynthesized silver nanoparticles. It is clear from the AFM micrograph that the average size of the biosynthesized Ag NPs was measured 150 nm. The other results showed that the AFM analysis of silver nanoparticles synthesized from Acalypha indica leaf extract was of average size 100-200 nm [15].

SEM analysis shows high-density AgNPs synthesized by M. charantia fruit extract (Fig. 5). It was shown that relatively spherical and uniform AgNPs were formed with diameter of 76.6 to 136.6 nm. The SEM image of silver nanoparticles was due to interactions of hydrogen bond and electrostatic interactions between the bioorganic capping molecules bound to the AgNPs. The nanoparticles were not in direct contact even within the aggregates (gold coating), indicating stabilization of the nanoparticles by a capping agent [15]. The SEM micrograph confirmed the synthesis of spherical silver nanoparticles in the size range of 60-180 nm in another study of biosynthesized Ag NPs from Carica papaya [15].

Optimization of silver nanoparticles synthesis

To determine the optimized conditions of synthesis of silver nanoparticles the temperature of the silver nitrate solution was varied (Fig. 6). The amount of silver nanoparticles synthesized at hot temperature was 44 mg and at cold temperature was 122 mg.

pH induce the reactivity of extract with silver ions during biosynthesis of silver nanoparticles. Therefore, change in pH would bring significant change in yield. In this study influence of pH on nanoparticles yield was assessed under different pH condition of the reaction mixture of extract. The pH of silver nitrate solution was also varied to optimize
release of enough concentration of Ag ions from the metal form. This kind of limitation can be overcome by using silver nanoparticles. However, to use silver against microorganisms, it is essential to prepare it with cost-effective and environment-friendly methods.

The antibacterial effects of the bioreduced silver nanoparticles from *M. charantia* were investigated against two bacterial strains, *B. cereus*/MTCC-9017 (Gram-positive) and *S. epidermidis*/MTCC-2639 (Gram-positive) using the disc diffusion method. The biosynthesized AgNPs were found to be effective at 45 µg/ml against *S. epidermidis* and *B. cereus*, respectively, whereas at concentration of 35 µg/ml of silver nanoparticles shows intermediate effectiveness and at 25 µg/ml AgNPs were found to insensitive against both *S. epidermidis* and *B. cereus*. Table 1 and 2 shows the concentration of silver nanoparticles and diameters of the inhibition zones against *S. epidermidis* and *B. cereus*. Thus, silver nanoparticles synthesized using fruit extract of *M. charantia* have potential antibacterial activity against *S. epidermidis* and *B. cereus* (Fig. 9).

**CONCLUSION**

In this study, a simple methodology was endeavored to achieve a green eco-friendly approach for the synthesis of silver nanoparticles using *M. charantia* fruit extracts. The silver ions in an aqueous solution reacted with the *M. charantia* fruit extracts, the biosynthesis of AgNPs were specified by the rapid color change of plant extract. The usual benevolent AgNPs were confirmed further by using UV-VIS spectroscopy. Alcoholic,
nitro and lactam groups were present in the fruit, and they serve as an effective reducing agent. AgNPs biosynthesized from *M. charantia* fruit extracts also exhibit great antibacterial activities against sample bacteria cultures. These biosynthesis silver nanoparticles can potentially be used for different medical applications. The cold temperature of the aqueous solution of 5 mM of AgNO$_3$, alkaline pH and
Table 1: Result showing measurement of zone of inhibition formed by AgNPs against *S. epidermidis*

| Disk potency (µg/ml) | Zone of inhibition (mm) | Inference      |
|----------------------|-------------------------|----------------|
| 25                   | 11                      | Resistant      |
| 35                   | 13                      | Intermediate   |
| 45                   | 16                      | Sensitive      |

*S. epidermidis*: Staphylococcus epidermidis

Table 2: Result showing measurement of zone of inhibition formed by AgNPs against *B. cereus*

| Disk potency (µg/ml) | Zone of inhibition (mm) | Inference |
|----------------------|-------------------------|-----------|
| 25                   | 12                      | Resistant |
| 35                   | 13                      | Intermediate |
| 45                   | 18                      | Sensitive |

*B. cereus*: Bacillus cereus

methanolic extract was proved as the best condition for biosynthesis of AgNPs from *M. charantia* fruit extracts.

REFERENCES

1. Feynman RP. There’s plenty of room at the bottom. Science 1991;254(5036):1300-1.
2. Joshi SC, Kaushik U. Nanoparticles and reproductive toxicity: An overview. Res J Pharm Biol Chem Sci 2013;4(2):1396-410.
3. Ravishankar RV, Bui AJ. Nanoparticles and their potential application as antimicrobials. Science against microbial pathogens: Communicating current research and technological advances. In: Méndez-Vilas A, editor. Formatex Microbiology Series No. 3. VOL. 1. Spain: Formatex; 2011.
4. Senapati S. Biosynthesis and immobilization of nanoparticles and their applications. India: University of Pune; 2005.
5. Klaus-Joerger T, Joerger R, Olsson E, Granqvist C. Bacteria as workers in the living factory: Metal-accumulating bacteria and their potential for materials science. Trends Biotechnol 2001;19(1):15-20.
6. Kaushik U, Joshi SC. Effects of silver nanoparticles on liver functions of male albino rat. Int J Curr Res 2016;8(7):34748-52.
7. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by standardized single disc method. Am J Clin Pathol 1996;45(4):493-6.
8. Kaushik U, Joshi SC. Assessment of size based oral toxicity of silver nanoparticles on serum lipid profile, liver and kidney function of male Wistar rats. World J Pharm Res 2016;5(10):786-803.
9. Kaushik U, Joshi SC. Silver nanoparticles: Green synthesis, optical properties, antimicrobial activity and its mechanism using *Citrus sinesis*. Asian J Pharm Clin Res 2015;8(6):179-84.
10. Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P, Kalaiichelvan PT, Mohan N. Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens. Colloids Surf B Biointerfaces 2010;76(1):50-6.
11. Ahmad N, Sharma S, Singh VN, Shamsi SF, Fatma A, Mehta BR. Biosynthesis of Silver Nanoparticles from *Desmodium triflorum*: A novel approach towards weed utilization. Biotechnol Res Int 2011;2011:454090.
12. Ahmad N, Sharma S. Green synthesis of silver nanoparticles using extracts of *Ananas comosus*. Green Sustain Chem 2012;2(4):141-7.
13. Ravindra BK, Rajasab AH. A comparative study on biosynthesis of silver nanoparticles using four different fungal species. Int J Pharm Sci 2014;6(1):372-6.
14. Selvi KV, Sivakumar T. Isolation and characterization of silver nanoparticles from from *Fusarium oxysporum*. Int J Curr Microbiol Appl Sci 2012;1(1):56-62.
15. Mude N, Ingle A, Gade A, Rai M. Synthesis of silver nanoparticles using callus extract of *Carica papaya* – A first report. J Plant Biochem Biotechnol 2008;18(1):83-6.
16. Bobbu P, Netala VR, Aishwarya S, Reddy IR, Kotakadi SV, Tarte V. Rapid synthesis of silver nanoparticles using aqueous leaf extract of *Achyranthes aspera* and study of their antimicrobial and free radical scavenging activities Int J Pharm Pharm Sci 2016;8(5):341-6.
17. Geethalakshmi R, Sarada D. Synthesis of plant-mediated silver nanoparticles using *Trianthma decandra* extract and evaluation of their anti-microbial activities. Int J Eng Sci Technol 2010;2(5):970-5.