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Probing Antibacterial Properties of Sida cardifolia Sponsored Silver Nanoparticles

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***Abstract*—This study aims to investigate antibacterial activity of silver nanoparticles prepared by green synthesis method using S. cardifolia. Silver nitrate (AgNO3) was taken as the metal precursor. The antibacterial activity of nanosilver was investigated against Gram (+) [Staphylococcus aureus- MTCC-9442, Staphylococcus epidermidis- MTCC- 2639, Bacillus cereus- MTCC-9017] and Gram (-) bacteria [Escherichia coli- MTCC-9721, Proteus vulgaris- MTCC-7299, Klebsiella pneumonia- MTCC-9751] by agar-well diffusion method. Formation of Silver nanoparticles (AgNPs) was determined by UV–vis spectroscopy where surface plasmon absorption maxima can be observed at 442 nm from the UV–vis spectrum. The synthesized nanoparticles were also characterized by X-ray diffraction (XRD). The peaks in the XRD pattern confirmed as the AgNPs possessed a face-centered cubic and obtained peaks confirmed absence of contamination. Further, characterization by transmission electron microscopy (TEM) and fourier transform infrared (FTIR) spectrum suggested the complexity present between bioactive compound of extract of S. cardifolia and nanoparticles synthesized by green chemistry were in spherical shape. This study confirmed that S. cardifolia capped AgNPs possess effective antibacterial activity against all tested bacteria and may be employed as impressive antibacterial agent in biomedical field.**

***Keywords*— Silver nanoparticles, Green chemistry, Antibacterial activity, S. cardifolia.**

I. INTRODUCTION

The materials with particle size, ranging from 1 to 100 nanometers (nm) may deal with conundrums of environmental and technological challenges in numerous fields as the areas of catalysis, solar energy conversion, biology, water treatment and biomedical science. Nanoparticles retain substantial high surface to volume ratios as having such imperative quality may be employed in the science and Technological fields depending on surface area\volume ratios and some nanoparticles have actually proven to be good catalysts in the catalytic industry [1]. Notably, the assorted nanoparticles have been acknowledged for its inhibitory and bactericidal properties in the past decades whereas silver nanoparticles proved to inherit antibacterial activity significantly as compared with other metallic nanoparticles and studied widely for their applicability in electronics, optics and medicine due to their unique physical, chemical, antibacterial and biological properties [2–7]. Moreover, silver nanoparticles have different important applications: for example, they might be used as spectrally selective coatings for solar energy absorption, as an intercalation material for electrical batteries, and as optical receptors for biolabeling [8–12]. Certainly, chemical and physical methods have been employed to produce pure, well-defined nanoparticles, but unfavorably these techniques are energy consuming, potentially toxic to the environment and more expensive. Interestingly, preparation of metallic nanoparticles by green synthesis approach has advantages over physical and chemical approaches as it is environmental friendly, cost effective and the most significant advantage is that conditions of high temperature, pressure, energy and toxic chemicals are not required in the synthesis protocol. In this paper, an environmentally friendly technique executed to produce silver nanoparticles (AgNPs) using aqueous extract of S. cardifolia. The S. cardifolia (linn) syn. Country Mallow of Malvaceae family is widely distributed along with other species are common throughout the tropical and subtropical plains all over India and Srilanka growing wild along the roadside. It grows as wasteland weed. It is also known as the "Bala" or "Khareti" in Sanskrit and Hindi S. cardifolia has been used since ancient times for medicinal porpoises in Ayurveda. Further, AgNPs were subjected for characterization and their inhibitory effect against Gram-negative and Gram-positive bacteria.

II. METHODS MATERIALS

All reagents of analytical grade were used as received without further purification. AgNO3 (99.98%) was used as a silver precursor, and was provided by Merck, Germany. All solutions were freshly prepared using double distilled water and kept in the dark to avoid any photochemical reactions. All glassware used in experimental procedures were cleaned in a fresh solution of HNO3 /HCl (3:1, v/v), washed thoroughly with double distilled water prior to use.

III. SYNTHESIS OF AGNPS BY USING GREEN METHOD

The seeds of S cardifolia used in this experiment were fresh and purchased from the local market Jaipur, Rajasthan, India, and washed dried, powdered and stored in air tight container at -0.4°C. Aqueous extract of S. cardifolia was prepared by using 3 gm of ultra-fine powder of seeds of S. cardifolia in 100 ml of double distilled water. The mixture is boiled for 15 min and filtered through pal funnel using Whatman filter paper No 1. The resulted filtrate is used as aqueous extract of S. cardifolia

In a typical synthesis, a 2ml of aqueous extract of S. cardifoliaa was added drop wise to 100 ml of 1.0 M solution of AgNO3 at temperature controlled magnetic steerer. Throughout the reduction process, solution was kept at a constant temperature of 45°C in the dark to avoid any photochemical reactions. All solution components were purged with nitrogen gas prior to use. Subsequently, reduction proceeded in the presence of nitrogen to eliminate oxygen. The obtained colloidal suspension was then centrifuged at 20000 rpm for 15 min and washed four times to remove silver ion residue. The precipitated nanoparticles were then dried overnight at 40°C under vacuum to obtain the AgNPs.

IV. CHARACTERIZATION METHODS AND INSTRUMENTS

The prepared AgNPs were characterized by using the X–ray diffraction (XRD), transmission electron microscopy (TEM), ultraviolet–visible spectroscopy (UV-Vis) and scanning electron microscopy (SEM). The XRD patterns were recorded at a scan speed of 2° min–1 by XRD- 6000 instrument of Shimadzu. Meanwhile, the structures of the produced AgNPs were examined using high resultant TEM of Techni G2, S-twin 200KV. Moreover, For SEM micrograph, the solid samples were sprinkled on the adhesive carbon tape which is supported on a metallic disk on the Carl zeiss EVO-18, 30KV. (scanning electron microscope), whereas the confirmation of formation of AgNPs were determined using UV–visible spectroscopy 1800 of Shimadzu, Kyoto, Japan over the range of 300 to 800 nm.

V. EVOLUTION OF ANTIBACTERIAL ACTIVITY

In vitro, antibacterial activity of the samples were evaluated by utilizing the agar well diffusion method on Mueller–Hinton Agar (MHA) plates with determination of inhibition zones in millimeter (mm), which conform with recommended standards of the National Committee for Clinical Laboratory Standards (NCCLS; now renamed as Clinical and Laboratory Standards Institute, CLSI, 2000). The Gram negative E. coli-MTCC-9721, P. vulgaris- MTCC-7299, K. pneumonia-MTCC-9751 and Gram positive i.e. S. aureus- MTCC-9442, S. epidermidis- MTCC- 2639, B. cereus- MTCC-9017 bacteria were used for the antibacterial effect assay. The bacterial suspension was prepared by making a saline suspension of isolated colonies selected from 18 to 24 h incubated tryptic soy agar plate. The suspension was adjusted to match the tube of 0.5 McFarland turbidity standard using the spectrophotometer of 600 nm, which equals to 1.5 × 108 colony forming units (CFU)/ml. The surface of MHA was completely inoculated using a sterile swab, which steeped in the prepared bacterial suspension. Finally, the 6 mm of well bored (6 mm) on MHA plates for introducing assorted concentration of AgNPs solution like 0.0025 mmol/ml, 0.005 mmol/mL, 0.01 mmol/ml and 0.02 mmol/ml and were left to incubate at 37°C for 24 h [13,14]. After incubation, the diameter of the growth inhibition zones was measured. Vancomycin (10 µg) was used as the positive standards in order to control the sensitivity of the bacteria. All tests were done in triplicate.

VI. RESULTS AND DISCUSSION

In this research, the aqueous extract of S. cardifolia executed title role appropriately as a stabilizer, capping and reducing agent. The preparation of AgNPs by the aqueous extract of S. cardifolia is instantly progressive phenomenon as overall proses accomplished within few minutes. As a result, the colorless solution turned to dark brown which indicates the formation of AgNPs (Figure 1) [15].

Figure 1: Photograph of AgNPs prepared by aqueous extract of S. cardifolia. (A) AgNO3 solution, (B) aqueous extract of S. cardifolia and (C) AgNPs solution.

UV–visible spectroscopy

The formation of AgNPs in the aqueous extract of S. cardifolia was further determined by using the UV–visible spectroscopy, which was shown on the surface plasmon resonance (SPR) bands. Figure 2 shows that AgNPs started forming as aqueous extract of S cardifolia injected at a moderate temperature. However, previous studies have shown that the spherical AgNPs contribute to the absorption bands at around 400 nm in the UV–visible spectra. From this research, the SPR band characteristics of AgNPs detected around 442 nm (Figure 2), which strongly suggests that the AgNPs were spherical in shape and have been confirmed by the TEM results of this study. Thus, there is a normal case in this situation for the SPR absorption band for the particles, which agreed with the TEM results.

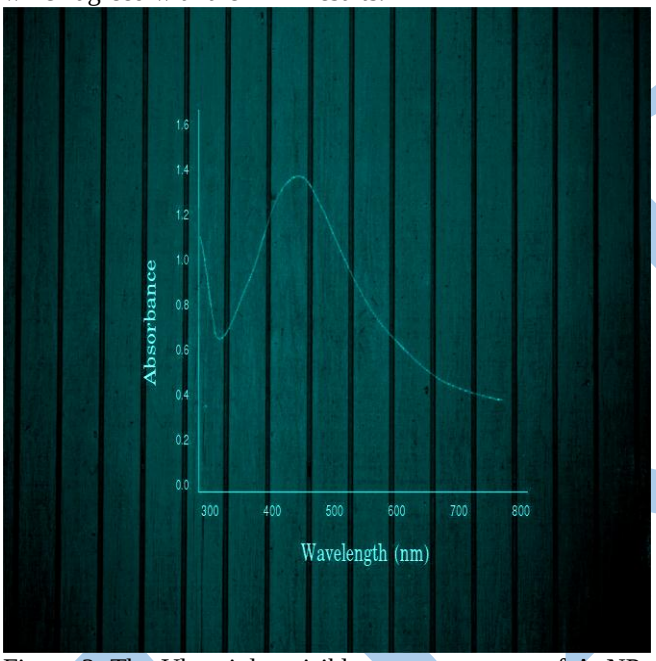

Figure 2: The Ultraviolet–visible spectrum curve of AgNPs prepared by aqueous extract of S. cardifolia.

X–ray diffraction

Figure 3 shows the XRD patterns of nanoparticles indicates the formation of the silver crystalline structure. The XRD peaks in the wide angle range of 2θ (30° < 2θ < 80°) determined that the peaks in 38.04°, 44.08°, 64.36° and 77.22° can be refered to the 111, 200, 220, and 311 crystalline particles of the face centered cubic (fcc) metallic silver respectively (XRD Ref. No.(Ag) 00–004–0783). The intensities of 111, 200, 220 and 311 reflections due to the AgNPs phase were also found to increase along with the increased AgNPs capped with bioactive compound of S. cardifolia. The peaks showed that the main composition of nanoparticles was silver and clearly no evidence of other peaks presence as impurities were not found in the XRD patterns. Therefore, this gives clear indication for the only presence of AgNPs.

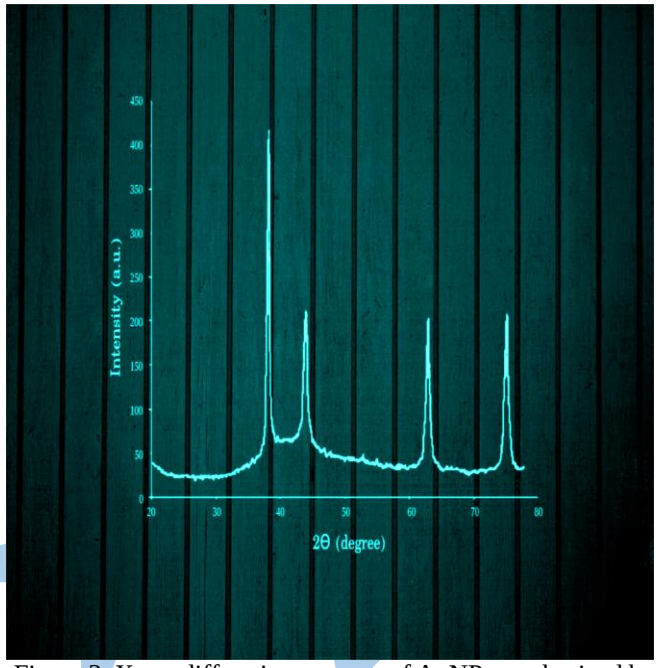

Figure 3: X-ray diffraction patterns of AgNPs synthesized by aqueous extract of S. cardifolia.

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM)

Surface morphology and size distribution of the particles were observed using Scanning Electron Microscope. The sample surface images were taken at different magnifications using the JEOL (SU 1510) operated at an accelerating voltage of 5kV and magnification x10k. The micrograph reveals that the particles found in form of aggregation may be due to capping with biomaterial of S. cardifolia or due to the Vander Waals forces and magnetic interactions among the particles (Figure 4). In figure 4B, the TEM images show that the AgNPs were well dispersed in colloidal solution with spherical particles of <25 nm and were not found in agglomerated form.

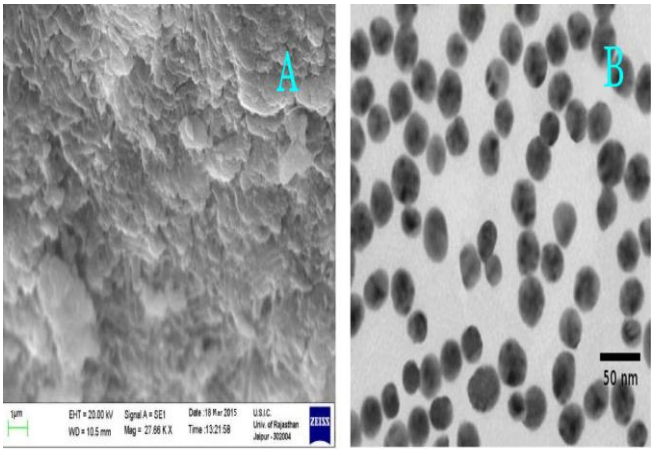

Figure 4: Scanning electron microscopy and transmission electron microscopy image of AgNPs, phytosynthesized by aqueous extract of S. cardifolia.

Antibacterial activity

Inhibition zone values were obtained against Gram negative E. coli- MTCC-9721, P. vulgaris- MTCC-7299, K. pneumonia- MTCC-9751 and Gram positive i.e. S. aureus- MTCC-9442, S. epidermidis- MTCC- 2639, B. cereus- MTCC-9017 bacteria and results and images of inhibition zones are presented as the average values in table 1 and figure 5, respectively. The outcomes pointed strong dose-dependent activities of AgNPs against both gram negative and gram positive microorganisms as growth of these bacteria affected with the alteration in concentration of AgNPs (Figure 5). he aqueous extract of S cardifolia did not show any activities against test microorganisms. The findings confirmed as 0.02 mmol/mL colloid solution of AgNPs showed 27 mm clear inhibitory zone against E. Coli after incubation for 24 h followed by S. aureus (26 mm), then 24 mm for S. epidermidis. The K. pneumonia and B. cereus exhibited same ZOI as 23 mm whereas P. vulgaris (21 mm) showed minimum Inhibition zone value proposing that AgNPs prepared by extract of S. cardifolia may be effectively used against Gram (+) than Gram (-) Bacteria (Table 1)

Silver nanoparticle Concentration (mmol/mL)	**Bacterial Sp (zone of inhibition mm)**					
	B. cereus	***S. aureus***	***S. epidermidis***	***E. coli***	***K. pneumoniae***	***P. vulgaris***
	11	13	12	11		11
	14	16	16	16		14
	19	21	19	22		17
	23	26	24	27		21
	11	11	19	10		11

Table 1: Antibacterial activity of Ag nanoparticles synthesized by extract of S. cardifolia.

Table 1 shows that the AgNPs suspension gave high antibacterial activity against Gram-negative and Gram-positive bacteria. As of their size, AgNPs can easily reach the nuclear content of bacteria and they present the large and impressive surface area; thus, the contacts with bacteria may be at great magnitude [16, 17]. This could be the reason behind their excellent antibacterial effect. In previous studies, some researcher argue that silver ions released from the surface of AgNPs are responsible for their antibacterial activity [18-20]. The diameters of inhibition zone in the agar plate are given in mm and tests were replicated three times for each treated samples.

Figure 5: The inhibition zone test between Gram-negative and Gram-positive bacteria (E. coli and S. aureus)

VII. CONCLUSIONS

In summary, present study confirmed a simple and green method of synthesis of nano-colloidal suspension of AgNPs using phyto reducing agents which requires no special physical conditions. AgNPs were successfully synthesized under moderate temperature (45°C). The formation of AgNPs was confirmed in the UV-visible absorption spectra, which showed the SPR band characteristics of AgNPs in the range of 442 nm. The XRD results confirmed that the AgNPs possessed a face-centered cubic crystal structure (fcc). In addition, this also revealed that AgNPs were the main composition present in the nanocomposites without any contamination peaks. The TEM images showed that the AgNPs were in spherical shape with size of <25 nm and these nano sized particles remunerated substantially effective against the Gram-positive and Gram-negative bacteria. Needless to say, further studies are required to investigate the biological effects of S cardifolia capped AgNPs on the types of bacteria for potential widening of this subject area.

REFERENCE

[1]. Aguilar Z (2012) Nanomaterials for Medical Applications, USA

[2]. Lok CN, Ho CM, Chen R, He QY, Yu WY, et al. (2007) Silver nanoparticles: partial oxidation and antibacterial activities. J Biol Inorg Chem 12: 527-534.

[3]. De Jong WH, Borm PJ (2008) Drug delivery and nanoparticles:applications and hazards. Int J Nanomedicine 3: 133-149.

[4]. Alizadeha H, Saloutib M, Shapourib R (2013) Intramacrophage antimicrobial effect of silver nanoparticles against Brucella melitensis 16M. Scientia Iranica 20:1035-1038.

[5]. Cao Y, Jin R, Mirkin CA (2001) DNA-modified core-shell Ag/Au nanoparticles. J Am Chem Soc 123: 7961-7962.

[6]. Alizadeh H, Salouti M, Shapouri M (2014) Bactericidal Effect of Silver Nanoparticles on Intramacrophage Brucella abortus 544. Jundishapur J Microbiol 7:e9039.

[7]. Huang ZY, Mills G, Hajek B (1993) Spontaneous formation of silver particles in basic 2-propanol. J Phys Chem 97: 11542–11550.

[8]. Saha B, Bhattacharya J, Mukherjee M, Ghosh AK, Santra CR, et al. (2007) In Vitro Structural and Functional Evaluation of Gold Nanoparticles Conjugated Antibiotics. Nanoscale Res Lett 2: 614.

[9]. Danilczuk M, Lund A, Sadlo J, Yamada H, Michalik J (2006) Conduction electron spin resonance of small silver particles. Spectrochim Acta A Mol Biomol Spectrosc 63: 189-191.

[10]. Hamouda T, Myc A, Donovan B, Shih A, Reuter JD, et al. (2000) A novel surfactant nanoemulsion with a unique non-irritant topical antimicrobial activity against bacteria, enveloped viruses and fungi. Microbiol Res 156: 1-7.

[11]. Dibrov P, Dzioba J, Gosink KK, Häse CC (2002) Chemiosmotic mechanism of antimicrobial activity of Ag(+) in Vibrio cholerae. Antimicrob Agents Chemother 46: 2668-2670.

[12]. Shiraishi Y, Toshima N (2000) Oxidation of ethylene catalyzed by colloidal dispersions of poly(sodium acrylate)-protected silver nanoclusters. Colloids Surf A Physicochem Eng Asp 169: 59-66.11.

[13]. Sharma G, Jasuja ND, Rajgovind, Singhal P, Joshi SC (2014) Synthesis, Characterization and Antimicrobial Activity of Abelia grandiflora Assisted AgNPs. J Microb Biochem Technol 6: 274-278

[14]. Holt JG, Krieg RN, Sneath PHA, Staley JT, Williams ST(1994) Bergey's manual of determinative bacteriology, 9th ed. Williams and Wilkins, Baltimore, pp. 2451-2508.

[15]. Rajgovind, Sharma G, Deepak Gupta Kr, Jasuja ND, Suresh Joshi C (2015) Pterocarpus marsupium Derived Phyto-Synthesis of Copper Oxide Nanoparticles and their Antimicrobial Activities. J Microb Biochem Technol 7: 140-144.

[16]. Dragieva I, Stoeva S, Stoimenov P, Pavlikianov E, Klabunde K (1999) Complex formation in solutions for chemical synthesis of nanoscaled particles prepared by borohydride reduction process. Nanostruct Mater 12: 267-270.

[17]. Sondi I, Salopek-Sondi B (2004) Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. J Colloid Interface Sci 275: 177-182.

[18]. Gordon O, Slenters TV, Brunetto PS, Villaruz AE, Sturdevant DE, et al. (2010) Silver coordination polymers for prevention of implant infection: thiol interaction, impact on respiratory chain enzymes, and hydroxyl radical induction. Antimicrob Agents Chemother 54: 4208-4218. 29.

[19]. Ross ZM, O'Gara EA, Hill DJ, Sleightholme HV, Maslin DJ (2001) Antimicrobial properties of garlic oil against human enteric bacteria: evaluation of methodologies and comparisons with garlic oil sulfides and garlic powder. Appl Environ Microbiol 67: 475-480.

