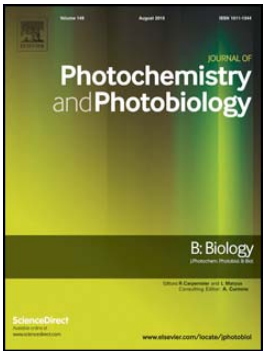

Green and ecofriendly synthesis of silver nanoparticles: Characterization, biocompatibility studies and gel formulation for treatment of infections in burns

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PII: S1011-1344(15)30162-7
DOI: doi: 10.1016/j.jphotobiol.2016.01.002
Reference: JPB 10220

To appear in:

Received date: 19 November 2015
Revised date: 20 December 2015
Accepted date: 4 January 2016

Please cite this article as: Kiran Jadhav, Dinesh Dhamecha, Debdutta Bhattacharya, Mrityunjaya Patil, Green and ecofriendly synthesis of silver nanoparticles: Characterization, biocompatibility studies and gel formulation for treatment of infections in burns, (2016), doi: 10.1016/j.jphotobiol.2016.01.002

Green and ecofriendly synthesis of silver nanoparticles: Characterization, Biocompatibility studies and gel formulation for treatment of infections in burns

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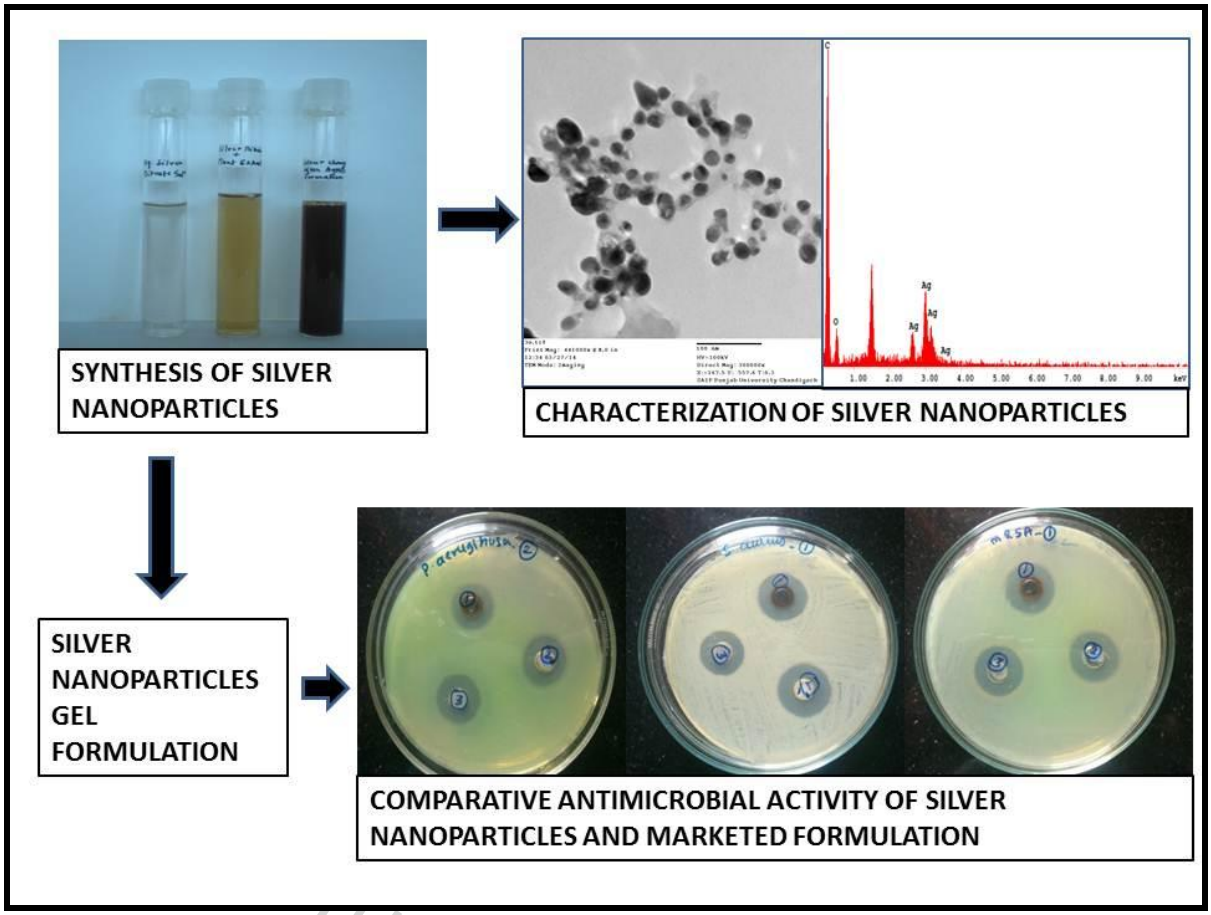
Graphical Abstract



ABSTRACT

The current study summarizes a unique green process for the synthesis of silver nanoparticles (AgNPs) by simple treatment of silver nitrate with aqueous extract of *Ammania baccifera*. Phytosynthesised AgNPs were characterized by various advanced analytical methods and studied for its use against infections associated with burns. Formation of AgNPs was observed by visual color change from colourless to dark brown and confirmed by UV-Visible characteristic peak at 436 nm. Zeta potential, particle size and polydispersity index of nano-silver were found to be -33.1±1.12, 112.6±6.8 nm and 0.3±0.06 respectively. XRD spectra revealed crystalline nature of AgNPs whereas TEM confirmed the presence of mixed morphology of AgNPs. The overall approach designated in the present research investigation for the synthesis of AgNPs is based on all 12 principles of green chemistry, in which no man-made chemical other than the silver nitrate was used. Synthesised nano-silver colloidal dispersion was initially tested for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against a panel of organisms involved in infections associated with burns (*Pseudomonas aeruginosa* (PA), *Staphylococcus aureus*(SA) *and methicillin resistant staphylococcus aureus*(MRSA)). MIC and MBC was found to be in range of 0.992 to 7.93 and 7.93 to 31.75 µg/ml respectively. MBC was used for formulation of AgNPs gel and tested for its efficacy using agar well diffusion method against PA, SA and MRSA. Comparative bactericidal efficacy of formulated gel (0.03% w/w) and marked formulation Silverex™ ionic (silver nitrate gel 0.2 % w/w) showed equal zone of inhibition against all pathogenic bacteria. Formulated AgNPs gel consisting of 95% lesser concentration of silver compared to marketed formulation was found to be equally effective against all organisms. Hence, the formulated AgNPs gel could serve as a better alternative with least toxicity towards the treatment presently available for infections in burns.

Key words: Silver nanoparticles, *Ammania baccifera,* characterization, antibacterial activity, antibacterial gel.

1. INTRODUCTION

Burns are damage to the skin caused by a variety of non-mechanical sources including chemicals, electricity, heat, sunlight or nuclear radiation [1]. Burn wound infection is challenging because it delays healing, encourages scarring and may result in bacteremia, sepsis or multiple-organ dysfunction syndrome whereby organs from several systems are unable to maintain homeostasis on their own, requiring immediate medical attention. Literature states that *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* are mainly responsible for infections in burns [2, 3]. Inspite of serious symptoms and pain associated with infections in burns; there are very limited options for physician for treatment of burns.

For a long time, silver-containing compounds are used in the treatment of burn wound infection and inflammation. These compounds are available in different forms, including creams, ointments and bandages [4, 5] where commonly silver nitrate and silver sulphadiazine in the form of gel are used for treatment in infections associated with burns [6, 7]. Use of silver nitrate as an antibacterial is still considered as controversial because of the associated moiety -nitrate which is known to be toxic to tissues and delays wound healing. Nitrate moiety acts as an antagonist to silver during tissue regeneration which impairs wound re-epithelialization and subsequently counterpoising the benefits of silver [8]. Although silver sulphadiazine remains the gold standard for treatment in topical bacterial infections in burns, latest studies demonstrate delay in wound healing on treatment with silver sulphadiazine [9] and bacterial resistance to the sulphadiazine element [10]. However, in the early twenty-first century it was realized that silver compounds can be toxic to certain host cells leading to dip in popularity [11]. Hence, it is important to have a lower, non-toxic but effective concentration of silver which could subsequently give desired therapeutic effect with minimal cytotoxicity. Modification of silver to nanoscale could act as a fruitful approach as nanoparticles exhibit increased chemical activity due to their nanosize range, large surface to volume ratios, unique surface plasmon resonance and crystallographic surface structure [12]. Literature also reveals that AgNPs causes production of reactive oxygen species (toxic radicals) such as superoxide anions, hydrogen peroxide and hydroxyl radicals which have potent bactericidal activity [13]

Silver nanoparticles (AgNPs) in the size range of 50-500 nm has wide range of biomedical applications like antibacterial [14], antifungal [15], anti plasmodial [16] and in medical devices, including bone cement, surgical instruments, surgical mask [17] and anti-wounds activity [18]. AgNPs can be synthesized by the reduction of silver salt with different chemical reducing agent like sodium borohydride etc., but use of chemical reducing agents could incorporate hurdles in its biomedical application as an end product due to its associated toxicity [19]. Hence to reduce the toxicity associated with reducing agents used in synthesis of AgNPs, green chemistry method which is proved to be an easy and eco-friendly alternative was used. Literature suggests that various plant like *Salacia chinensis* [20], *Jatropha carcass* [21] etc. have been used for green synthesis of silver nanoparticles. Selection of plant for green synthesis of silver nanoparticles depends on presence of antioxidants (polyphenols, carbohydrates, flavonoids, tannins) and proteins. It has been proved that these phytochemicals are responsible for reduction of silver salt to AgNPs. Based on these facts, *Ammania baccifera* which is already reported to have high antioxidant content [22] was selected as the green source for synthesis of AgNPs.

Ammania baccifera belonging to the family Lythraceae is a erect herb, commonly originate as weed in rice fields and marshy quarters throughout India. The leaves are mainly used in the treatment of rheumatic pain, rubifacient, antihypertensive, antimicrobial and as an antiurolithiatic agent. It mainly consists of steroid, triterpenes, coumarins, flavonol and tannins which are mainly responsible for its antioxidant activities [22].

Although several independent studies have shown claims of AgNPs in various fields, but research relating their impact on counteracting bacterial infections in burns are limited. The present research work initially describes green synthesis of AgNPs and its characterization followed by antibacterial activity against a panel of pathogens like *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923) and methicillin resistant *Staphylococcus aureus* mainly responsible for infections in burns. Further, nano-silver colloidal dispersion was transformed to gel formulation and its efficacy was compared with marketed formulation Silverex™ ionic (0.2% silver nitrate).

2. Materials

Ammania baccifera was collected in the month of September from Kankumbi (situated in the framework of the Western Ghats) - a village in Belgaum district of Karnataka near the north-eastern part of the Goa-Karnataka border, India and authenticated (Accession number: RMRC-923) by botanist, Regional Medical Research Centre (Indian Council of Medical Research) Belgaum-590010, Karnataka, India. Silver nitrate was procured from Sigma Aldrich, India. Normal mouse fibroblasts used in the study were procured from National Centre of Cell Science, Pune, India. Dulbecco's modified eagle medium (DMEM), Fetal bovine serum (FBS), Pen Strep (mixture of penicillin and streptomycin) was procured from Gibco Life technologies, Auckland, New Zealand, Gentamycin (4mg/ml), Amphotericin (5mg/ml) and Muller Hinton agar and broth media were procured from Himedia Pvt. Ltd., Mumbai, India.

3. Experimental

3.1 Synthesis of AgNPs

Plant extract was prepared by boiling 10g of air dried powdered plant material (whole plant) with 100 ml of deionized water for 10 minutes. It was filtered using Whatmann filter paper no. #41 and filtrate was directly used as a green source for synthesis of AgNPs. AgNPs were prepared by addition of varying volume ratios (10:1, 10:2, and 10:3) of 1mM silver nitrate solution to quantity of plant extract [13]. Synthesized AgNPs were purified by refrigerated high speed centrifuge (Kubota 6500, Japan) at 17000 rpm (using rotor model AG-506R-36,873 relative centrifugal force (RCF)) maintained at 4ºC for 20 minutes. After centrifugation, AgNPs pellets were resuspended in deioned water and centrifuged thrice in the same manner to ensure complete removal of extraneous matter. Finally, purified nano-silver colloidal dispersion was collected, sterilized (filtration through 0.22 µm syringe filter), lyophilized and stored for further characterization.

3.2 Characterization of AgNPs

Synthesised AgNPs were diluted with deionized water and scanned in wavelength range of 200-700 nm using UV-visible spectrophotometer (Hitachi - Inkarp 2300 SICAN, Inkarp Instruments Pvt. Ltd., Japan) to confirm the formation of AgNPs and study surface plasmon resonance (SPR)

effect [20]. Zeta potential, particle size and particle size distribution of AgNPs colloidal dispersion were recorded using zetasizer (Malvern Instruments, UK). X-ray diffraction (Philips PRO expert diffractometer, Netherland) spectrum of lyophilized AgNPs was recorded at room temperature using nickel filtered Cu Kα radiations which were operated at 40 Kv voltage, 30 mA Current and 7° to 70° (2θ) range. Atomic force microscopy (Nanosurf AG, Switzerland) analysis was performed under room temperature condition using a scanning probe microscope in non-contact mode with silicon nitride cantilevers and images were retrieved using Nanosurf easyscan-2 software program Gwyddion (Nečas and Klapetek, 2012) to make colour contrast adjustments, background subtraction for raw AFM images, surface normalization, cross-sectional analysis and to get three dimensional (3D) images. TEM (Hitachi H-7500, Japan) images were recorded by spotting sample onto a carbon-coated copper grid by solvent evaporation and scanned through 1,50,000x to 3,00,000x magnification to get clear images. To confirm the purity of silver nanoparticles, an EDAX (FEI, USA) spectrum of silver colloidal dispersion was recorded by initially spotting the sample in electron microscope followed by estimation of its elemental composition.

3.3 Silver content estimation-Inductive coupled plasma-atomic emission spectroscopy (ICP-AES) of AgNPs

ICP-AES (SPECTRO ARCOS, Germany) was employed for the qualitative and quantitative estimation of silver content at ppm level. The calibration curve of known silver was obtained by using standard silver (Centipur® - ICP multi-element standard solution IV, Catalog no: 111355, Merck Millipore corporation, Germany) concentrations of 0.1, 1, 10 and 100 ppm at 328.06 nm. Prior to analysis, AgNPs samples (3 ml) were given pretreated with 3 ml concentrated nitric acid and heated to 70°C for 15 minutes until it turns colourless. Samples were then filtered, volume was made upto 10 ml and analysed for silver content. Instrument was controlled by a computer and the operating system was programmed using visual basic software. Experiments were performed in triplicate and results were expressed in mean (µg/ml) ± standard deviation.

3.4 Antibacterial Activity

Minimum inhibitory concentration **(**MIC) and minimum bactericidal concentration **(**MBC) were determined by serial dilution technique [23]. Accordingly, the lowest concentration of AgNPs showing growth inhibition (visual observation) was considered as the MIC whereas the lowest concentration of AgNPs that showed zero growth on Mueller Hinton (MH) agar plates after spot inoculation and incubation for 24 hours was recorded as MBC. Assay was performed in triplicate with appropriate controls (medium without inoculum and medium without AgNPs) [24].

3.5 Cytotoxicity evaluation of AgNPs

Normal mouse fibroblast cell lines (L929) were seeded in the 96 flat bottom well plates at the densities of $1x10^4$ cells/well/0.1 mL medium and allowed to adhere by incubating for a period of 24 h in CO_2 incubator (Eppendorf, New Brunswick, Galaxy 170R, Germany) maintained with at 37°C and 95% humidity. The medium was discarded, replaced with fresh medium (0.1 mL) containing different concentrations of AgNPs and incubated at 37°C. At the end of 24 hours the medium in each well was discarded and 20µL of MTT (3-(4, 5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) solution (5 mg/ml in phosphate buffer saline) was added to each well and the plate was incubated at 37°C. On completion of 4 hours, DMSO (100 µL) was added to each well to dissolve formed formazan crystals and absorbance was recorded at 492 nm filter using ELISA plate reader (Lisa plus, India) and calculated for percent cell viability.

3.6 Formulation and Evaluation of AgNPs gel

Synthesised AgNPs were formulated into gel using varying concentration (0.5, 1, 1.5 and 2 %) of carbopol 934 as gelling agent. Weighed quantity of carbopol was slowly added to colloidal suspension of AgNPs along with stirring followed by addition of a drop of triethanolamine. Gel formulations were evaluated for their physicochemical properties like pH by using pre-calibrated pH meter (Equiptronics EQ-610 pH meter, India) and clarity by visual observation. Rheological property of formulated AgNPs gel was determined by using viscometer (Brookfield DV-III Ultra Brookfield Engineering Laboratories, Middleboro, MA) at 10 ± 1^oC.

3.7 Antibacterial activity of AgNPs gel

Antibacterial activity was carried out via agar well diffusion assay (antibiotic susceptibility testing) to compare the effect AgNPs gel and marketed gel formulation against *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923) and methicillin resistant *Staphylococcus aureus*. Sterilized MHA (Mueller-Hinton agar) plates were prepared using biological safety cabinet level II (Esco Technologies, Inc., USA) and incubated at 37°C in bacterial incubator (Thermo Fisher Scientific, USA) overnight prior to initiation of experiments to ensure contamination free plates. Agar plates were dried 15 min prior to experimentation and seeded with suspensions (100 mcl) of test bacteria (standardized by using 0.5 McFarland standard which equals to 1.5 X 108 CFU/mL) to make a uniform lawn of bacteria. AgNPs colloidal dispersion (0.03%), AgNPs gel (0.03%) and marketed gel containing 0.2% silver nitrate (Silverex™ ionic) were placed in wells and incubated at 37°C for 24 h. All experimentations were carried out in triplicate and results were expressed in mean zone of inhibition (mm) ± standard deviation [25].

4. RESULT AND DISCUSSION

4.1 Synthesis of AgNPs

On addition of plant extract in varying ratios to silver nitrate solution, there was a visual change in colour from colourless to dark brown confirming the synthesis of AgNPs (figure 1). UV-Visible spectroscopic analysis of diluted silver colloidal dispersion showed peak at 436 nm (characteristic peak of AgNPs) (figure 2) confirming the synthesis of AgNPs. All ratios of plant extract to silver nitrate solution (10:1. 10:2 and 10:3) showed colour change from colourless to dark brown within 2 h. Hence, 10:1 ratio was finalized where least amount of extract was used for synthesis of AgNPs. The reason for formation of AgNPs was attributed to presence of flavonoids and polyphenols in plant extract for reducing silver nitrate to AgNPs and proteins for stability of AgNPs [26, 27]. Resultant AgNPs obtained from optimized ratio (10:1) was purified by centrifugation and subjected for further characterisation

Figure 1: Color changes after formation of AgNPs from yellow to dark brown A) 1mM silver nitrate B) after addition of plant extract to silver nitrate solution C) Color change after 1 hour

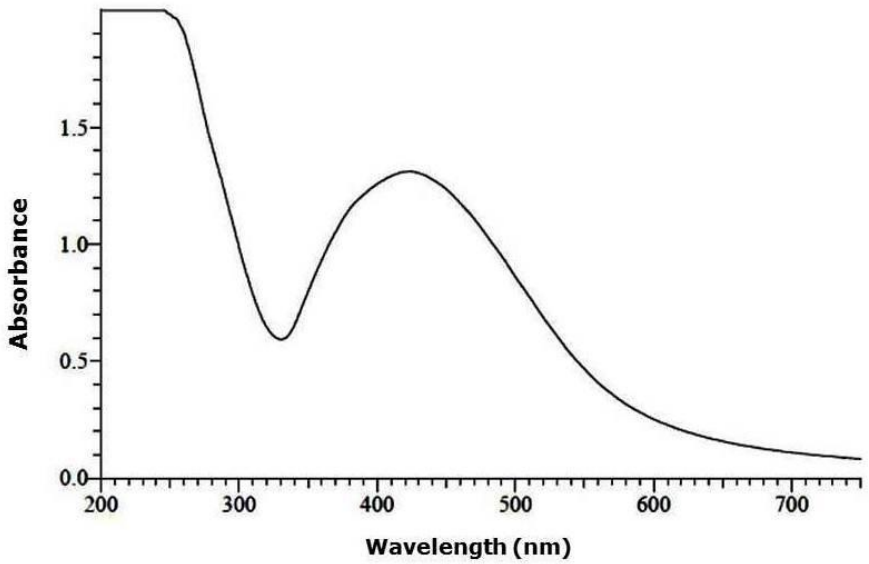


Figure 2: UV-Visible spectrum showing characteristic peak of silver nanoparticles.

4.2 Characterization of AgNPs

UV-Visible spectrophotometer gives the idea about the shape and size of particle which mainly governs optical and electronic properties of AgNPs and consequently affects its biomedical applications [28]. In the present research, it was used to confirm the formation and stability of AgNPs in aqueous colloidal dispersion. Change in color from colourless to dark brown color was observed due to surface plasmon vibrations in AgNPs [29] which was indicated by UV-Visible peak at 436 nm (typical band of AgNPs). Broadening of UV-Visible peak suggests that synthesized AgNPs were polydisperse in nature [30]. The stability of synthesized AgNPs was confirmed by measuring shift in wavelength at an interval of one week for 90 days. There was no significant change in the wavelength of AgNPs on storage, which suggests that the AgNPs did not aggregated and were stable during this period. The reason for stability was attributed to capping of AgNPs with phytochemicals used for synthesis of AgNPs. Soluble phytochemicals like flavonoids, polyphenols, tannins, and proteins encapsulate AgNPs generating negative charge on surface. Nano-silver bearing negative charge exhibit Brownian moments and are well dispersed in dispersion medium leading to formation of stable silver colloid [31].

Particle size, zeta potential and polydispersity index (PDI) of AgNPs were 112.6 ± 6.8 nm, -33.1 ± 1.12 and 0.3 ± 0.06 respectively. Particle size of AgNPs (112.6 ± 6.8 nm) measured by zeta sizer corresponds to hydrodynamic diameter of particles which is not the actual diameter

of nanoparticles but is always greater than the actual diameter of nanoparticles. Hydrodynamic diameter is the diameter of the particle along with the coated phytochemicals which are mainly responsible for stability of nanoparticles whereas actual diameter of the particle is represented by TEM. Due to coating of phytochemicals like flavonoids and polyphenols onto AgNPs, hydroxyl groups of these phytochemicals generate negative charge onto these nanoparticles which was confirmed by negative zeta potential value (-33.1 ± 1.12). Stability of the AgNPs is directly proportional to the magnitude of zeta potential. PDI of synthesized AgNPs (0.3 ± 0.06) was below 0.4 which suggests that the nanoparticles were almost monodisperse in nature.

XRD was used to confirm the crystalline or amorphous nature of particles [25]. In the present study, XRD spectrum showed presence of peaks as per Bragg's reflection from (111) and (200) planes of face center cubic (FCC) crystal structure corresponding to the 2θ value of 32.26, and 46.22 which was in line with the standard values of JCPDS No.: 04-0783 for silver (Figure 3). Assessment of XRD spectrum of synthesized AgNPs with standard values confirmed that AgNPs were nanocrystals in nature. In addition to the typical Bragg's peak representative of FCC AgNPs, additional peaks were also observed suggesting that the crystallization of bioorganic phase (phytoconstituents) occurred on the surface of the AgNPs.

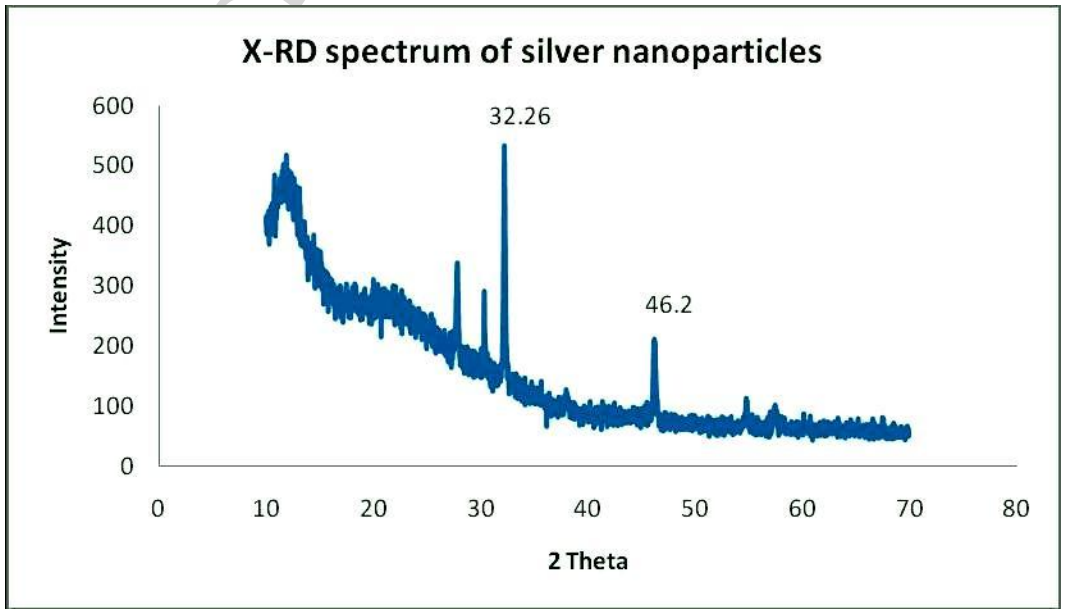


Figure 3: X-RD spectrum of synthesized AgNPs.

Surface morphology study by AFM evidently demonstrations well dispersed AgNPs with size in the range of 50-100 nm (figure 4). These results were in line with TEM analysis in which the particle size was found to be 60-80 nm. Synthesised AgNPs were co-ordinated with hydroxyl groups of phytochemicals averting large scale aggregation. This supports the claim that AgNPs were capped with phytochemicals leading to stabilization of nanoparticles.

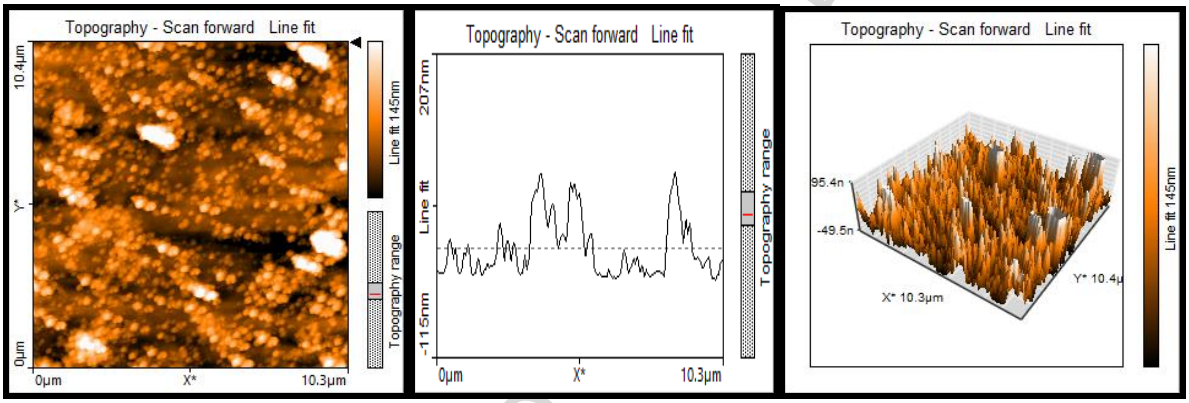
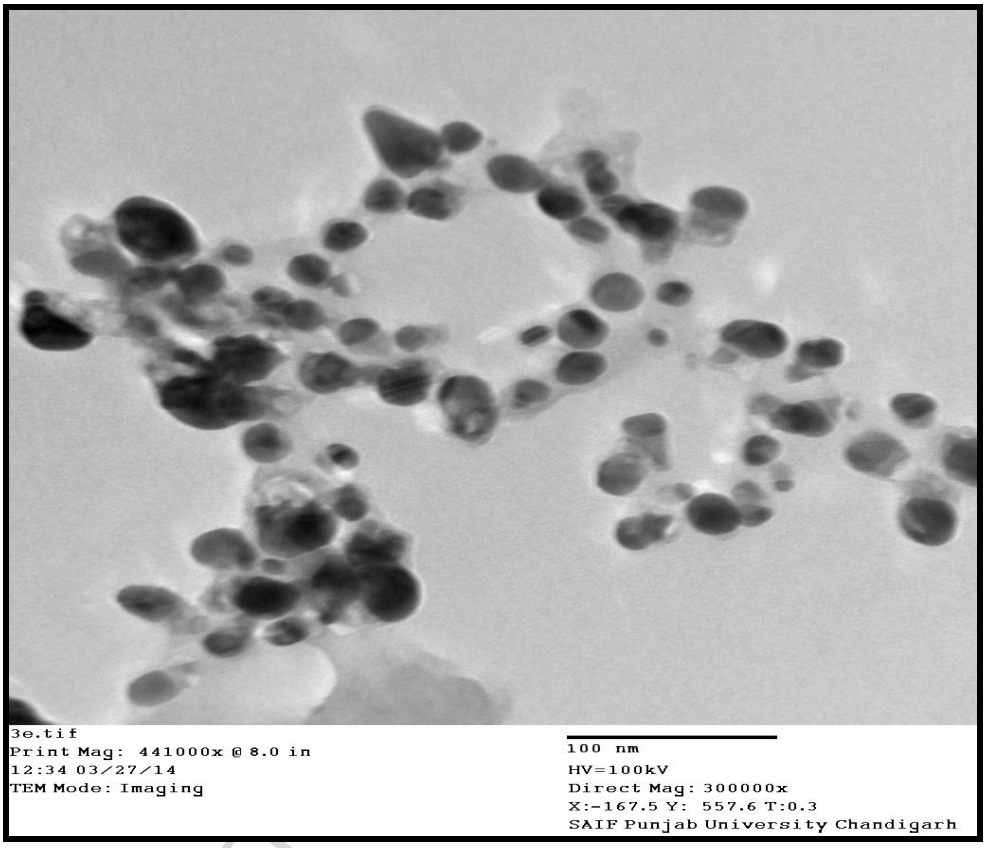


Figure 4: AFM image and analysis of synthesized AgNPs

A TEM image depicting the morphology of AgNPs at 300000x magnification suggests that AgNPs were found to be polymorphic showing triangular, hexagonal, deformed spherical and rod shaped morphologies (figure 5). From TEM images, it could be inferred that phytochemicals which are used as a green source for synthesis of AgNPs have control on particle size and morphology. Coated AgNPs were found to have narrow distribution of particle size in the range of 105-125 nm. TEM images revealed the presence of both the hydrodynamic diameter and the actual diameter of AgNPs which were found to be in line with zeta sizer analysis. At the same time, images show that the small percentage of particles were far from the range which could be due to incomplete or weaker reaction of the phytochemicals (polyphenols, flavonoids, proteins) with silver leading to formation of some larger particles [32].

EDAX spectrum (figure 5) displays clear identification peaks of major energies of silver confirming the presence of silver in AgNPs. Signal of aluminum is due to the background from supporting aluminum grid used as the sample cell. Energy signals of Carbon (C) and oxygen (O) represents the presence of organic hydroxyl groups from water soluble antioxidants (polyphenols, flavonoids, proteins capping AgNPs) used for the synthesis of AgNPs. This

validates that the phytochemicals of *Ammania baccifera* plays a key role in capping and stabilization of AgNPs.



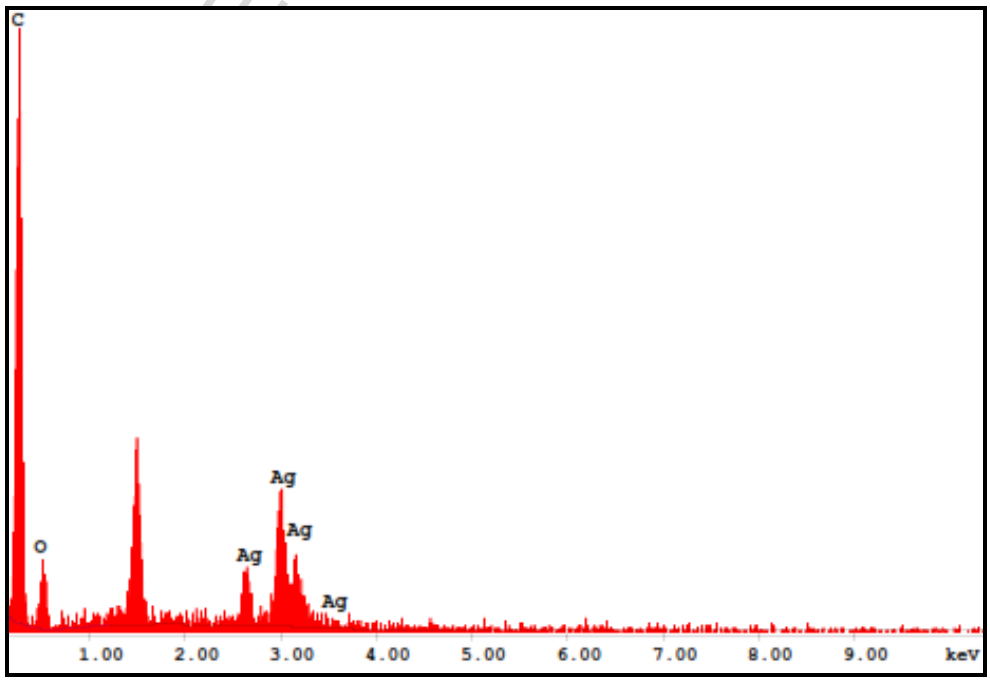


Figure 5: TEM image and EDAX spectra of synthesized AgNPs

4.3 Silver content estimation-Inductive coupled plasma-atomic emission spectroscopy (ICP-AES)

Calibration curve was plotted by using standard concentrations of silver in the range of 0.1 to 100 mg/ml. The calibration graph for silver was linear at ppm level concentrations in which the correlation coefficient of the calibration curve of silver was 0.999. Samples on treatment with nitric acid were found to be colourless and warranted that the silver is completely solubilized in solution. The concentration of synthesized AgNPs measured by using ICP-AES was found to be 254±13.6 µg/ml.

4.4 Antibacterial Activity

MIC was determined by visual observation method in which the lowest concentration of AgNPs showing zero growth in tubes when compared to control (growth organism). All the concentrations which were equal to or higher than MIC were inoculated onto the Mueller Hinton (MH) agar plates and incubation for 24 hours. The concentration of AgNPs which showed zero colony forming units (CFU) was recorded as MBC. The MICs was in the range 0.99-7.93 µg/mL for all the cultures while the MBCs was 31.75 µg/mL for MRSA and 7.93 µg/mL for PA with the exception of *S. aureus* where MBC was not detectable even with highest concentration. Effect of AgNPs against PA, SA and MRSA confirms its potent antimicrobial activity against all organisms responsible for infections in burns.

Cytotoxicity evaluation of AgNPs

The cytotoxicity of phytochemical stabilized AgNPs was studied on primary normal L929 mouse fibroblast cell lines under in vitro conditions by using colorimetric MTT assay method. Results of cytotoxicity studies were measured in terms of the intensity of the color formed on solubilization of formazan crystals. Intensity of the color was measured by using ELISA plate reader in terms of absorbance at 492 nm which is directly proportional to the number of live cells. Experiment was performed using varying concentrations of AgNPs (128, 64, 32, 16 and 8 µg/ml) and compared with silver nitrate in varying concentrations and control. The relative cell viability after 24 h of incubation followed by MTT treatment was found to be more than 90% with all concentrations of AgNPs and silver nitrate used in the study, not significantly different from control (figure 6). In the present study, phytochemicals coated AgNPs does not show any

toxicity against normal mouse fibroblast thereby confirming biocompatible nature of synthesized AgNPs. This endorses that phytochemicals present in *Ammania baccifera* not only effectively reduced silver nitrate to AgNPs but also provides non-toxic surface coating for its biomedical applications.

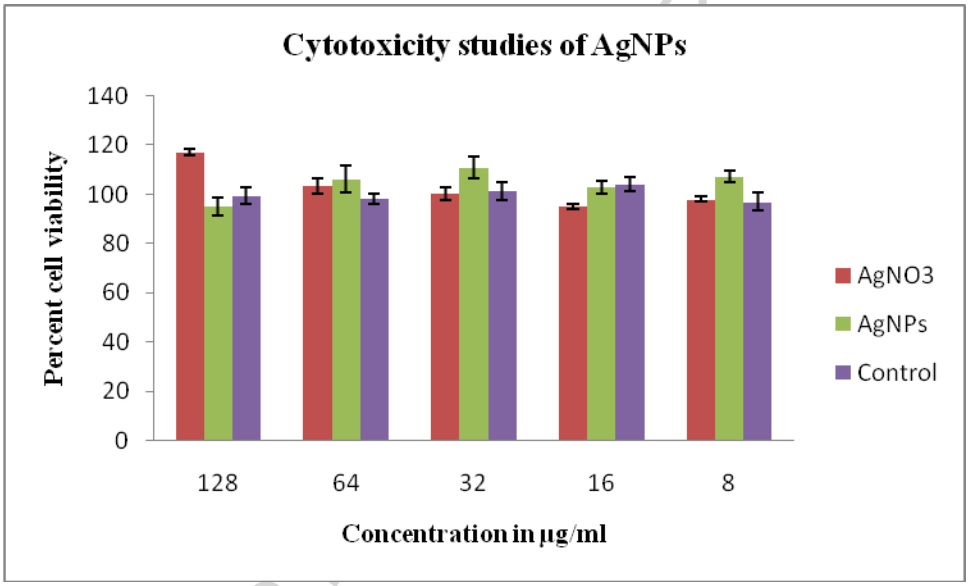


Figure 6: Cytotoxicity studies of synthesised AgNPs and silver nitrate against L929 mouse fibroblast cell lines

4.6 Formulation and evaluation of AgNPs

Gel was formulated using 0.5, 1.0, 1.5 and 2 % concentrations of carbopol and marked as F1, F2, F3 and F4 respectively. Formulations were evaluated for pH and were found to be in acceptable range of 5.9 ± 0.2 which is similar with the natural pH of skin. Under normal circumstances, an acidic milieu is found on the skin surface which varies depending on anatomical location and age of the person. This acidic milieu is very important which makes skin resistant to harsh chemicals and pathogenic bacteria. In wounds, the internal body pH 7.4 is exposed to the external skin surfaces leading to generation of alkaline pH, which become favorable for bacterial colonization. It is well known that most the pathogenic bacteria growth is inhibited in acidic milieu and promoted in alkaline milieu. It is also known that bacterial colonization increase the pH value to generate alkaline milieu for their growth. Although there are no proofs for claims that bacterial

growth hinders wound healing, it is well reported that *Staphylococcus aureus* presence decrease wound healing efficiency. Hence, clinical application of any wound or burns wound infections showed is targeted to decrease the pH of infected area which would show synergistic effect with antibacterial activity. It is very interesting to know that even body's defense mechanism follow the same strategy by invading neutrophils at wound infected area leading to formation of pus which generates acidic environment for decreasing bacterial colonization [33].

Viscosity of all four formulations was found to be 38 ± 5.03, 79 ± 4.5, 126 ± 8.7, 164 ± 11.6 pa.S respectively. Viscosity of gel increased with increase in concentration of carbopol. Carbopol® 934 polymer is a cross-linked polyacrylate polymer *having ionized carboxyl groups of* which undergoes conformational changes in the polymer chain followed by swelling of polymer matrix and drug release. It offers excellent stability at high viscosity and produces thick formulations for opaque gels [34].

4.7 Antibacterial activity of AgNPs gel

Comparative antimicrobial testing of marketed formulation and AgNPs gel showed that the zone of inhibition of AgNPs gel was equivalent with 0.2 % silver nitrate marketed gel having equal zone of inhibition (17 mm) (figure 7). These observations suggest that reduced particle size (nanosize) of AgNPs plays important role for usage of silver as an antibacterial agent. These results further confirms that AgNPs gel formulation with 95 % lesser silver concentration is equally effective when compared to marketed formulation and can be used as better alternative to commercially available silver formulation. Antibacterial results were supported by the existing literature which suggests that silver in nanocrystalline forms not only reduces wound infection but also promotes wound healing. It further helps in reducing the frequency of dressing changes which would subsequently reduce associated pain and costing [35]. A meta-analysis of randomized control trials on application of silver compounds in wound effects suggests that patients receiving nano-silver had significantly lower incidence of infections than those treated with existing silver products in market. The study concluded that silver in nanoscale possessed significantly higher antimicrobial efficiency that other silver formulations [36].

Bactericidal activity of AgNPs could be due to the generation of the positively charged Ag^+ ions, which act on bacterial cell wall, inhibiting membrane permeability and inactivating necessary enzymes by interaction with the thiol groups of proteins leading to cell death. On

exposure of AgNPs (Ag°) with water or other oxidising agent, it gets oxidized to silver ions (Ag^{+}) which are very toxic to bacterial cell and best explains the molecular mechanism of AgNPs. Hence, small particle size of AgNPs leads to more surface area which gets exposed to water leading to generation of high amount of silver ions (Ag^{+}) which subsequently deactivate proteins necessary for bacterial survival [13].

Figure 7: Antibacterial activity of AgNPs gel formulation (F2) compared with marketed formulation against *P.aeruginosa*, *S.aureus* and methicillin resistant *S. aureus* where 1: AgNPs colloidal dispersion (0.03%) 2: AgNPs gel (0.03%) and 3: Marketed gel containing 0.2% silver nitrate (Silverex™ ionic)

5. CONLUSION

AgNPs were successfully synthesized by using extract of *Ammania baccifera* and evaluated for their antibacterial activity. Plant extract of *Ammania baccifera* served as a green source for reducing silver nitrate to AgNPs. AgNPs gel (0.025% w/w) when compared with marketed 0.2% w/w silver nitrate gel showed equal zone of inhibition against all pathogenic bacteria responsible for infections in burns. Approximately 95% lesser dose of silver (formulated gel) was found to be equally effective when compared to marketed formulation. Hence, the formulated AgNPs gel could serve as an effective and better alternative tropical antimicrobial in antibiotic resistant genotypes for treatment of infections in burns by promoting cellular growth and relieving pain.

ACKNOWLEDGEMENTS

Authors wish to acknowledge Dr. Prabhakar Kore Basic Science Research Center, KLE University, Belgaum and Regional Medical Research Center, ICMR, Belgaum for providing required facilities. Authors are also thankful to SAIF, Panjab University, Chandigarh for TEM analysis and SAIF, Indian Institute of Technology, Bombay for ICP-AES analysis.

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Highlights

Silver nanoparticles (AgNPs) synthesized based on 12 principles of green chemistry.

Ammania baccifera used as green source for synthesis of AgNPs.

Stabilized and biocompatible AgNPs formulated into gel form.

Remarkable antibacterial activity of AgNPs gel against bacteria's responsible for infections in burns.