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Silver Nanoparticles: One Pot Green Synthesis Using *Terminalia arjuna* Extract for Biological Application

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Abstract

Among all the nanoparticles synthesized; silver nanoparticles have attained special place in the area of nano technology because of their antimicrobial and biomedical applications. In general; their syntheses involves the use of hazardous chemicals or costly physical methods. However, the biological processes are making their ways in between and proving their advantages over them. The use of plants and their extracts is one of the most valuable methods which are gaining concerns due to their imperative biological benefits. Plants are not only beautiful but majestic because they are rich sources of various medicinally important substances. They explore the huge diversity which can be utilized towards rapid and single step protocol preparatory method for various nanoparticles keeping intact "the green principles" over the conventional ones and proving their dominance for medicinal importance. Here, in the presented work "*one pot synthesis of silver nanoparticles*" is described. Silver nanoparticles have world-wide desirability due to their exceptional physical and chemical properties in particular to antimicrobial activities plus being non-toxic and environmentally safe. Therefore; a simple, cost effective bio-reduction on the principle of "green synthesis" of silver nanoparticles using the *Terminalia arjuna* plant extract is reported. The beauty of the synthesis is: no involvement of any surfactant, catalyst or template. The aqueous silver ions are reduced to silver nanoparticles when exposed to leaves extract. The bio-reduction and stabilization of so formed silver nanoparticles was monitored by UV-Vis spectrophotometry. The synthesized silver nanoparticles were also characterized by various other techniques viz. FTIR spectroscopy, Dynamic light scattering (DLS), and TEM. FTIR spectroscopy revealed that silver nanoparticles that are functionalized with bio-molecules that are present in the natural aqueous extract are themselves acting as the capping agents and stabilizing the nanoparticles. Biological evaluations of silver nanoparticles were also done against gram positive (*S. aureus*) and gram negative bacteria (*E. coli*) for their future applications in biomedicines especially for the treatment of wounds.

Keywords: *Terminalia arjuna;* Leaf extract; Silver nanoparticle; One pot; Green synthesis

Introduction

Plants have been used from ancient times to attempt cures for diseases and to physical suffering. The progress of medicine has often been guided by the earlier observations and beliefs. The tiny green cells of plants are wonderful laboratories, which produce all the starch and oxygen in the world. Every living fauna is dependent on the flora getting all their food either directly from them or indirectly by eating animals that are fed on plants. The officially documented plants with medicinal potential are 3000 but traditional practitioners use more than 6000. India is called the botanical garden of the world as it is the largest producer of medicinal herbs. In rural India, 70 per cent of the population depends on the traditional type of medicine, the Ayurveda. Many forms of alternative medicines are available for those who do not want conventional medicine or who cannot be helped by conventional medicine.

Nano-science is based on the manipulation of individual atoms and/or molecules to produce materials from them for functioning well below the sub-microscopic level. They involve physical, chemical and biological knowledge at scales ranging between individual atoms and molecules below the nanometer or up to 100 nm and emerging as one of the most active areas of research in modern science. The nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology [1,2]. Silver nanoparticles among various metal nanoparticles have received significant consideration because they are effective antimicrobial agent/s that exhibits low toxicity; and have diverse *in vitro* and *in vivo* applications [3]. Several medicines are available in market based on silver such as silver sulphadiazine, etc. for the treatment of burn and the chronic wound infected with microbes. Silver nano gels/sprays are also worth mention for their use in cosmetic and drug industries for medical purposes. Although there are many routes available for the syntheses of silver nanoparticles including chemical, physical, electrochemical, irradiative, photochemical and biological techniques [4,5]. Drawbacks associated with physico-chemical methods of silver nanoparticles synthesis such as use of toxic chemicals, high temperature, pressure and production of hazardous by-products etc. therefore; it become necessary to search for safer alternative methods of silver nanoparticles syntheses. Bio-inspired synthesis using microorganisms, and plant extracts for silver nanoparticles have been suggested as valuable alternative to chemical methods as it avoids use of toxic chemicals and use of high and temperature.

The plant extracts have come up nano factory for synthesizing metal nanoparticles of gold and silver. Its use for the synthesis of nanoparticles is potentially advantageous over microorganisms due to the ease of scale up, less biohazard, eco-friendly and elaborate process of maintaining cell cultures [6]. It is considered to be the best platform for synthesis of nanoparticles being free from toxic chemicals as well as providing natural capping agents for stabilization of silver nanoparticles. Moreover, use of plant extracts has drawn special attention because

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it reduces the cost of micro-organisms isolation and culture media enhancing the cost competitive feasibility over nanoparticles synthesis by microorganisms. A lot of literature is available on green synthesis of silver nanoparticle till date. Gold and silver nanostructures were produced using *C. sinensis* extracts, as a reducing and stabilizing agent, in aqueous solution at ambient conditions [7]. *Desmodium* plant biomass was used to synthesized stable and spherically shaped nanoparticles of average size of 10 nm [8]. Triangular or spherical shaped silver nanoparticles size ranging from 55-80 nm and gold nanoparticles, were fabricated using the novel sundried extract of *Cinnamomum canphora* leaf [9]. Silver nanoparticles were successfully synthesized using the latex of *Jatropha curcas* [10]. A green straight forward method of synthesizing silver nanoparticles in an aqueous medium was designed using *Emblica officinalis* fruit extract as stabilizer and reducer. And the synthesized nanoparticles were found to be in spherical shape showing inhibition and significant antibacterial activities against both gram-positive and gram-negative bacterial strains [11]. Ethanolic leaf extract of *Premna serratifolia* was used to fabricate silver nanoparticles and their anticancer activities were also investigated. The sizes of silver nanoparticles were 22.97nm synthesized by using these ethanolic leaf extract [12]. Chitosan, a natural biopolymer was also used to synthesize silver nanocomposites for various biological activities [13]. A clean and green process was used to synthesize 30nm sized silver nanoparticle using leaf extract of *Petroselinum crispum* at room temperature [14].

In the present study, we established that an aqueous extract of *Terminalia arjuna* leaves were used in reduction of Ag(I) and in the formation of stable silver nanoparticles and tested the effect of antimicrobial activities. *Terminalia arjuna* is a large 20 to 30 m tall evergreen tree with spreading crown and drooping branches belongs to Combretaceae family distributed in India, Sri Lanka and Burma and its leaf extract contains leucoanthocyanidins and hydrolysable tannins [15]. The method used is simple, one pot, clean and required only non-hazardous reactants like plant extract, water and silver nitrate, and is advantageous in large scale production of silver nanoparticles.

Experimental

Silver nitrate was purchased from Merck Chemicals. All glassware are sterilized with nitric acid and further with distilled water and dried in oven before use. *Terminalia arjuna* leaves were collected from university campus in the month of February.

Preparation of leaf extract

The fresh leaves were washed several times with running tap water and after that with distilled water. Around 20 g of leaves were weighed and boiled for 1h in 100 mL double distilled water at 60°C and then the extracts were filtered through whatman filter paper. Then the filtered extract was stored in refrigerator at 4°C for further use in synthesis of silver nanoparticles.

One pot green synthesis of silver nanoparticles

100 mL (10^{-3}M) aqueous solution of silver nitrate was prepared in Erlenmeyer flask. Then 1.0, 2.0, 3.0, 4.0 and 5.0 mL of leaf extract were added separately to 10mL aqueous silver nitrate solution kept in separate beakers at room temperature (their notation shown in Table 1). The solution was kept in dark chamber until solution colour changes to yellow to dark yellow. After, 15 min, the solution turns yellow to yellow-red or dark brown indicating the formation of silver nanoparticles. The bioreduction of silver ions was monitored by periodic sampling by the UV spectrophotometer.

Sample	Plant extract (mL)	$AgNO_3$ Solution (mL)
A	10	-
1A	1	10
2A	2	10
3A	3	10
4A	4	10
5A	5	10

Table 1: Notation of silver nanoparticles synthesized using *Terminalia arjuna* extract.

Characterization of silver nanoparticles

A colour change from pale yellow to dark brown upon incubation due to surface plasma resonance (SPR) vibration was observed indicating the formation of nanoparticles. The periodic scans of the optical absorbance between 200 nm and 800 nm with a Shimadzu UV-Visible spectrophotometer (UV-1800, Japan) were performed to investigate the reduction of silver ions by leaf extract. Deionised water was used as reference for background correction of experiments. The size distribution or average sizes of the synthesized silver nanoparticles were determined by dynamic light scattering (Spectroscatter 201). The particle size and surface morphology was confirmed using Transmission electron microscopy (TEM), operated at an accelerated voltage of 120 kV. A Fourier Transform Infrared Spectrometer (Bruker Tensor 37 spectrometer) is used to obtain the infrared spectra of absorption and emission of the formed silver nanoparticles. FTIR spectra were recorded from wave number 600-4000 cm^{-1}.

Assessment of antimicrobial activities

Disc diffusion method was used to assess antimicrobial activities of synthesized silver nanoparticles against *S. aureus* and *E. coli*. Microbes were sub culture and incubated at 37°C for 24 h. Fresh cultures were taken and spread on Mackonkey agar (HiMedia) plates to cultivate bacteria. Sterile paper discs of 5 mm diameter were saturated with double distilled water (as control), plant extract and silver nanoparticles solution were placed in each agar plate and incubated again at 37°C for 24 hrs. Based on inhibition zones around the discs, the antimicrobial activities were measured saturated with plant extract and synthesized silver nanoparticle.

Results and Discussions

Spectroscopy plays a very important role in the identification of different compounds and their qualitative and quantitative determinations. Nothing can complement these characterizations now a day. The syntheses and the biological evaluation of silver nanoparticles are also dependent relatively on these phenomenal techniques. Therefore, a lot of spectrochemical evaluations were carried out along with the biological evaluations. Silver nanoparticles are proving to be an alternative for the development of new antibacterial agents with their unique physical and chemical properties [16] and is still charming scientists to explore new dimensions of their further usefulness. Silver nanoparticles have been gaining renovation in catalyst, light emitter, silver nanoparticle impregnated textile fabrics, wound dressings, cosmetics and coatings for medical devices, etc.

Visual observations

The synthesis of nanoparticles was initiated once the plant extract of *Terminalia arjuna* was introduced into 10^{-3}M aqueous $AgNO_3$ solution. Silver ions were reduced to silver nanoparticles and showed the UV-visible spectra of synthesized silver nanoparticles with varying concentrations of leaf extract. Silver nanoparticles were synthesized

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at different concentrations of leaf extract (1, 2, 3, 4 and 5 mL). A preliminary visual observation showed that the initial colour of the reaction mixture after the addition of leaves extract to the aqueous silver nitrate solution was nearly colourless. Interestingly, the colour of the reaction mixture changed from pale-yellow then light brown to dark brown exponentially with reaction-time as aggregation proceeds (Figure 1b) due to excitation of surface plasmon vibration [17] confirming the formation of silver nanoparticles (also confirmed by UV and DLS). Appearance of different colours at different time intervals indicated that the morphology (shape, size and the size distribution) of silver nanoparticles alters with the reaction time.

UV-Visible spectral studies

The formation and stability of silver nanoparticles in aqueous solution was confirmed by using UV-Vis spectrophotometer analysis. It is generally recognized that UV-Vis spectroscopy could be used to examine size and shape-controlled nanoparticles in aqueous suspensions. The UV-Vis spectra of silver nanoparticle after 1 h, 2 h, 3 h, 4 h, 5 h, 18 h and 24 h of the reaction were documented, indicating the formation of silver nanoparticles due to excitation of surface plasmon vibrations in silver nanoparticles [18]. Silver nanoparticles are analyze by UV spectra of Surface Plasmon Resonance(SPR) band observed at 448-440 nm [19]. If we increase the leaf extract concentration upto 4 mL, there is a decrease in the absorbance up to 440 nm (blue shift) as presented in Figure 1a. However, it can't be denied the presence of some of $AgNO_3$ in the colloidal solution attributed to the absorbance band at 370 nm. The slight variation in the values of absorbance signifies the changes due to variation in the particle size. Increasing the concentration of extract increases the intensity of absorbance. A dramatic increase in SPR band observed at 455 nm with low intensity indicates that there is little formation of nanoparticle with 5 mL plant extract concentration. The variation in values of absorbance confirms to the changes in the particle size.

Figure 2 shows UV-Vis spectra recorded at different time intervals from aqueous solution of silver nitrate with *Terminalia arjuna* extract. The sample 1A displays an optical absorption band peak at about 447 nm, typical of absorption for metallic Ag nanoparticles due to the SPR. The sample 2A, 3A, 4A and 5A typically show absorption band of synthesized silver nanoparticles at 446.5, 440, 440 and 455 nm [20] respectively. Effect of the reaction time on silver nanoparticles syntheses was also evaluated with UV-Visible spectra and it is noted that with an increase in time the peak becomes sharper and intense. The increase in intensity could be due to increasing number of nanoparticles formed as a result of reduction of silver ions presented in the aqueous solution [21]. The spectra of silver nitrate solution and plant extract did not show any SPR band. The colloidal solution of silver nanoparticles was kept as such in closed sample tubes and absorbances was noted for two

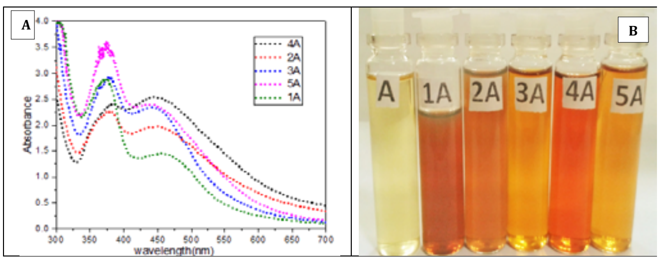

Figure 1: (a) UV-Vis spectra and (b) visual observation of synthesized nanoparticles.

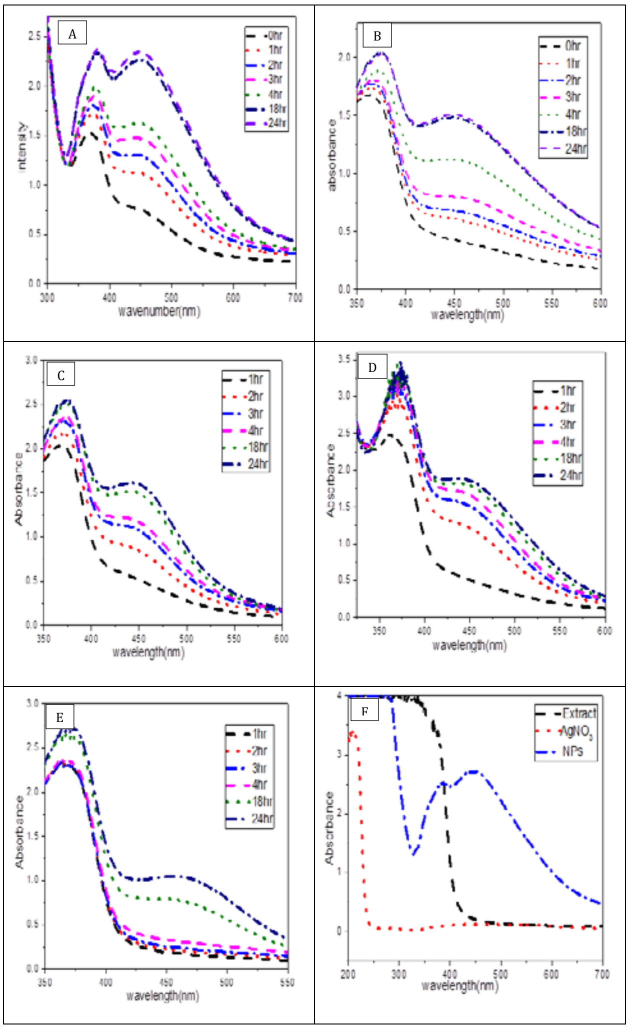

Figure 2: UV-Visible spectra of silver nanoparticles at different time interval using different concentration of plant extract (a) 1A, (b) 2A, (c) 3A, (d) 4A, (e) 5A and (f) extract, $AgNO_3$ and Nanoparticles.

months at regular 10 days interval and were found to be stable after 60 days showing the absorbance band (data not shown).

Particle size and distribution

Figure 3 shows the dynamic light scattering (DLS) pattern of the suspension of Ag nanoparticles synthesized using *Terminalia arjuna* aqueous leaf extract. The size distribution histogram of DLS indicates that the size of these silver nanoparticles varies between 3 and 50 nm and shows an asymmetric distribution. High intensity distribution at lower range of particle size indicates that the most of the synthesized particles are in lower range of particle size. This was further confirmed by TEM images of silver depicted in Figure 4. The shape, size and morphology of the synthesized silver nanoparticles were elucidated with the help of transmission electron microscopy further confirming the formation of silver nanoparticles. The size was in the range of 8-16 nm; and the shape of the nanoparticles was spherical and irregular in shape with moderate variation in size, confirming the results obtained by DLS. The average particle size was determined by DLS method, and it was found to be 5.20 nm as revealed in the size distribution graph.

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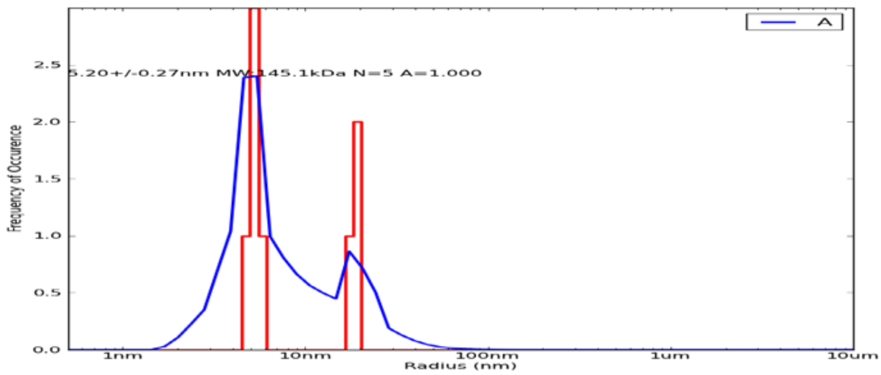

Figure 3: Showing Particle size distribution of synthesized silver nanoparticles.

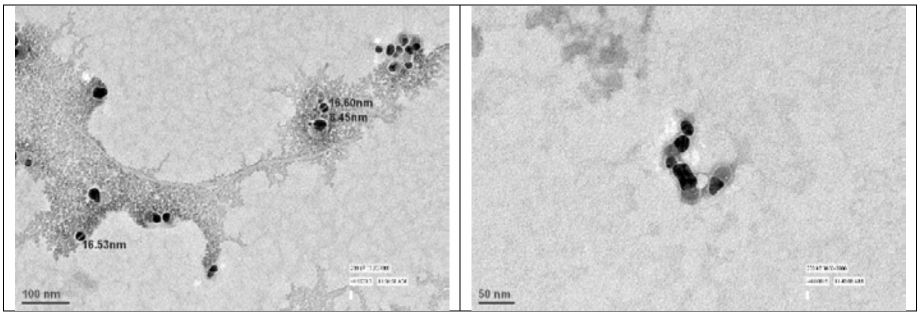

Figure 4: TEM micrographs of the silver nanoparticles synthesized by using aqueous plant extract.

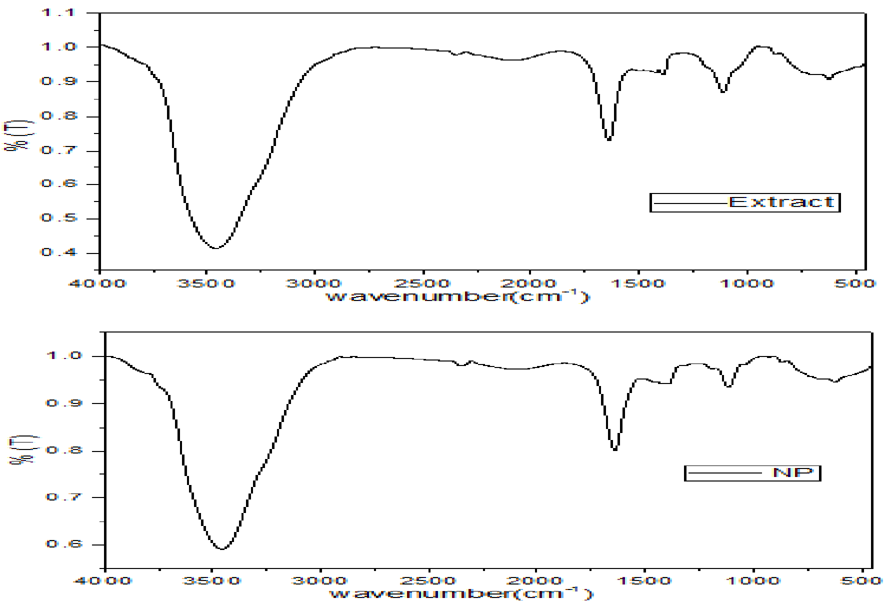

Figure 5: FTIR spectra of plant extract and synthesized silver nanoparticles.

Structural studies

FTIR has become an important tool in understanding the involvement of functional groups in relation between metal particles and biomolecules which is used to search the chemical composition of the surface of the silver nanoparticles and identify the biomolecules for capping and efficient stabilization of the metal nanoparticles [21]. There were many functional groups present which may have been responsible for the bio-reduction of Ag^+ ions.

The band intensities in different regions of the spectrum for plant extract and silver nanoparticles were analyzed and are shown in Figure

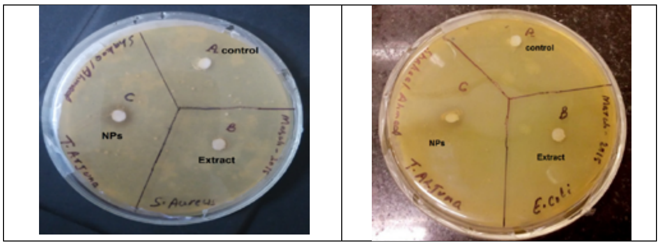

Figure 6: Antimicrobial activities of silver nanoparticles synthesized against *S. aureus* and *E. coli*.

Components	Zone of inhibition(mm)	
	E. coli	***S. aureus***
Control	NZ	NZ
Plant extract	7	6
Silver nanoparticle	14	13

Table 2: Zone of inhibition (mm) obtained by disc diffusion method.

5. FTIR spectrum shows different major peak positions at 3464, 2922, 2886, 2073, 1639, 1419, 1405, 1384, 1109, 868, 861 and 628 cm^{-1}. The similarities between the spectra with some marginal shifts in peak position, clearly indicate the presence of the residual plant extract in the sample as a capping agent to the silver nanoparticles. The peak located at 1639 cm^{-1} could be assigned to C=O stretching or amide bending [22]. The broad and intense peak at 3464 cm^{-1} corresponds to OH stretching vibrations of phenol/carboxylic group present in extract. A peak observed at 2922 and 2886 cm^{-1} is due to C-H stretching of alkanes [23]. The peak at 1384 cm^{-1} assigned to nitro N-O bending [24] and a peak at 1109 cm^{-1} to C-O-C stretching aromatic ring. It showed peak in the range of 628 cm^{-1} relating to the alkyl halides band especially the C-Cl bond [25]. Therefore, it may be inferred that these biomolecules are responsible for capping and efficient stabilization of synthesized nanoparticles.

Antimicrobial activities

The antimicrobial activity of silver nanoparticles was carried out against both gram positive and gram negative bacteria (Figure 6). The synthesized silver nanoparticles exhibited good antibacterial activity against both Gram-negative and Gram-positive bacteria. Based on the zone of inhibition produced, synthesized silver nanoparticles exhibited good antibacterial activity against *E. coli* and *S. aureus*. The aqueous extract of *T. arjuna* also showed good antimicrobial activities against both *E. coli and S. aureus*. The zone of inhibition of synthesized silver nanoparticles was found to be more than that of plant extract (Table 2).

Conclusions

One pot green synthesis of silver nanoparticle using *Terminalia arjuna* leaves extract has been reported. Silver nanoparticle has been successfully synthesized by this simple, fast, cost effective, environment friendly, efficient method supported by the physicochemical characterization viz. TEM and DLS. FTIR and UV-Vis analysis confirmed the reduction of Ag(I) ions to Ag(0) which is supposed through the plant extract as capping agents i.e. the phytochemical constituents which are acting as the reducing agents. The synthesized silver nanoparticles showed efficient antimicrobial activities. The silver nanoparticles synthesized by this one pot green synthesis method will be proving of potential use in medical applications.

Research over last three decades has increased concern about the treatment of various types of wounds causes and their remedies. The major concerns which are threatening and spend worldwide are different types of microbial infections into them and are the major factor of amputations/surgical removals of the different organs and sometimes death in all developed and well developing countries. Therefore, this synthesis of nanosilver in combination with biomaterials will generate considerable interest in the field of medical science especially the domain of wound dressings. These materials may include chitin, chitosan, collagen, hydrogels, hydrocolloids, alginates and polyurethanes individually or in combination with each other. Hence, the reported work may prove its endeavours in the designing of biocompatible and biodegradable wound dressing inheriting the principle of "Green Chemistry" which of course will be reducing exposure of post use disposal or medical waste generation.

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