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PHYTOSYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES USING *Solanum seaforthianum* Andrews

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ABSTRACT

Phytosynthesis of nanoparticles using higher plants is cost effective, ecofriendly with large scale production potential. The present investigation reports a simple biological method for synthesis of biogenic silver nanoparticles using leaf extract of *Solanum seaforthianum* Andrews which served as a source of reducing and capping agents. The formation of silver nanoparticles was evident by change in colour of reaction mixture to dark brown due to surface Plasmon resonance. The characterization of biogenic silver nanoparticles was done with the help of UV-VIS spectrophotometer, Fourier Transform Infrared (FTIR) Spectroscopy, Atomic Force Microscope (AFM), High Resolution Transmission Electron Microscope (HR-TEM), X–Ray Diffraction (XRD), Energy dispersive X-ray diffraction spectroscopy (EDS). The size of biogenic nanoparticles ranges 5-20 nm. The antimicrobial activity of silver nanoparticles was evaluated using Agar well diffusion method. The results showed that the silver nanoparticles exhibit significant antibacterial activity.

KEY WORDS: ***Solanum seaforthianum Andrews*, Silver nanoparticle, Antimicrobial activity, Phytosynthesis, Biomolecules.**

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INTRODUCTION

Nanotechnology is one of the most promising areas of research in modern medical science. Nanomaterials display exclusive, superior and fundamental properties; due to small size they can be accommodated in various fields of nano science. Nanoparticle research is not because of only application and also for the method of synthesis. In recent years a number of physical, chemical and biological methods were used to synthesis silver nanoparticles with bactericidal effects. The biological methods have advantage over chemical and physical methods, as it is cost effective and environmentally friendly.[1] Therefore, the biological approach for the synthesis of nanoparticles becomes essential.[2] The major biological systems involved in this are bacteria; fungi[3] and plant extract.[4] In recent years, the biosynthesis of nanoparticles using plant extracts has gained more significance. The major advantage of using plant extracts for silver nanoparticle synthesis is that they are easily available, safe, practical, scalable, nontoxic and avoidance of maintaining the microbial culture. [5] In most cases, they provide broad variety of metabolites which can aid in the reduction of silver ions and are quicker than microbes in the synthesis method. Different plants have been successfully used for the synthesis of biogenic metal nanoparticles. [6, 7] Nowadays, research efforts are being strong on integrating nanoparticles with biology. It has been reported that antibiotics often disturb the bacterial flora of digestive tract which may develop multiple drug-resistant isolates, hence novel ways of formulating biocide materials is an upcoming field of attraction. For this reason, there is a need for the use of an agent which does not generate resistance and presents a good bactericidal property. Among several noble metal nanoparticles, silver nanoparticles (Ag NPs) have attracted special attention because of its superior antimicrobial characteristics as compared to bulk silver. [8] The bactericidal effect of silver can be divided into two groups; the reactive component being either silver ions or silver nanoparticles. Silver ions are charged atoms (Ag^+) whereas silver nanoparticles are single crystals of nano-size dimensions. Bactericidal effects of Silver nanoparticles depend on the size and the shape of the particle. [9] Nanoparticles can act as antibacterial and antifungal agents, due to their ability to interact with microorganisms. [10] It has been established that different plant extracts affect the production of nanoparticles in different ways due to presence of specific complex phytochemicals in the extracts. The present study was designed to synthesize, characterize silver nanoparticles and to investigate the antibacterial activity using leaves of medicinal plant *Solanum seaforthianum Andrews* It has been reported that this plant contains modest amounts of various tropane alkaloids such as atropine, scopolamine and hyoscyamine and should be considered mildly toxic and inedible. [11]

MATERIALS AND METHODS

Chemicals and Microorganism

Analytical grade chemicals -Silver nitrate nitrate and sodium hydroxide were used. All glass wares were washed with sterile water and dried in an oven before use. Experimental plant *Solanum seaforthianum Andrews* (Brazilian Nightshade) is a flowering evergreen vine of the Solanum family native to tropical South America. (Fig.1.). Leaves were collected from Dharwad district, Karnataka, India and they were authenticated and kept as a specimen in Dept. Of Botany, Karnataka University. A voucher no. 23082014 I was given to the plant. The standard bacterial strains *Escherichia coli* (MTCC 723), *Pseudomonas aeruginosa* (MTCC 7837), *Staphylococcus aureus* (MTCC 3160) and *Bacillus subtilis* (MTCC 736) were used.

Figure 1
Experimental plant Solanum seaforthianum Andrews

Preparation of leaf extract

Leaves of *Solanum seaforthianum Andrews* were washed 2-3 times with tap water followed by double distilled water to remove dust and impurities. Leaves were shade dried to remove the residual moisture and about 25gm. were cut into small pieces and boiled in glass beaker containing 250ml of sterile distilled water for 20 minutes. [12] The aqueous extract was separated by filtration with Whatman No. 1 filter paper and stored in refrigerator at 4^0C for further use.

Phytosynthesis of Silver nanoparticles

For reduction of silver ions, 10ml of leaf extract was added to 90ml of 1mM aqueous $AgNO_3$ solution taken in Erleyenmeyer flask (250ml). Simultaneously, the reaction mixture was adjusted to pH 8 by using 1 N.

NaOH. Then the flask containing reaction mixture was incubated at 40-60^{0}C, resulting in the formation of pale yellow to dark brown solution indicating the synthesis of silver nanoparticles. [13]

Detection of silver nanoparticles

A number of different measurement techniques were used for detection of Ag-NPS., including UV-Vis spectroscopy, Fourier Transform Infrared (FTIR), Atomic Force Microscopy (AFM), High Resolution Trasmission Electron Microscopy (HR-TEM), X–Ray Diffraction (XRD) and Energy dispersive spectroscopy (EDS).

Characterization of nanoparticles

The reduction of metal ions was monitored by measuring the UV-Vis spectroscopy of the solution according to the method of Mie (1908), by the sampling of aliquots (3ml) of the aqueous component. The silver nanoparticles were measured in a wavelength ranging from 200-800nm. The UV-Vis spectroscopy measurement of silver nanoparticle was recorded on UV- Vis spectroscopy (Jasco V- 670 UV-Vis NIR spectrophotometer) operated at resolution of 1nm. The solution containing reduced silver ions was centrifuged at 3000 rpm for 40 min to remove the unwanted biomass residue; the resulting suspension was then dispersed in 10ml of double distilled water and centrifuged again at the same condition. Redispersion and centrifugation process was repeated for 2-3 times to obtain silver nanoparticles free from any biomass residue. A sample taken from pellet was dispersed on a slide and dried slide was observed on contact mode of AFM. The pellet thus obtained was redispersed in double distilled water and oven dried at 60^{0}C to obtain the powder. The powder was used for FTIR and HRTEM (TECNAI 20 G2-electron microscope), X-ray diffraction (XRD) analysis and SEM with EDX analysis (Fei Quanta 200 SEM EDAX Genius Xm 4).

Antibacterial activities

The silver nanoparticles synthesized using *Solanum seaforthianum Andrews* leaf extract were tested for antibacterial activity by agar well diffusion method against human pathogenic *Escherichia coli* (MTCC 723), *Pseudomonas aeruginosa* (MTCC 7837), *Staphylococcus aureus* (MTCC3160),*Bacillus subtilis* (MTCC 736). This method depends on the radial diffusion of an antibiotic from the well through semisolid agar layer in Petri plate, which prevents the growth of bacteria in a circular area or the zone around the well. The pure cultures of bacteria were sub-cultured on nutrient broth at 35 °C. The hot sterile medium was poured into the sterile Petri plates to form 2-3 mm thick uniform layer and allowed to solidify. Each strain was swabbed uniformly on the individual plates using sterile cotton swab. Wells of size 6 mm were made on nutrient agar plates using gel puncture. Different concentrations of silver nanoparticles (50, 100, 200, 400, 800 µg/ml) solution were poured on to five wells and in one well 25 µg/ml of gentamycine poured as positive controle on all plates using micropipette. After incubation at 37 °C for 24h, the diameter of zone of inhibition was measured in millimeters and tabulated. [14]

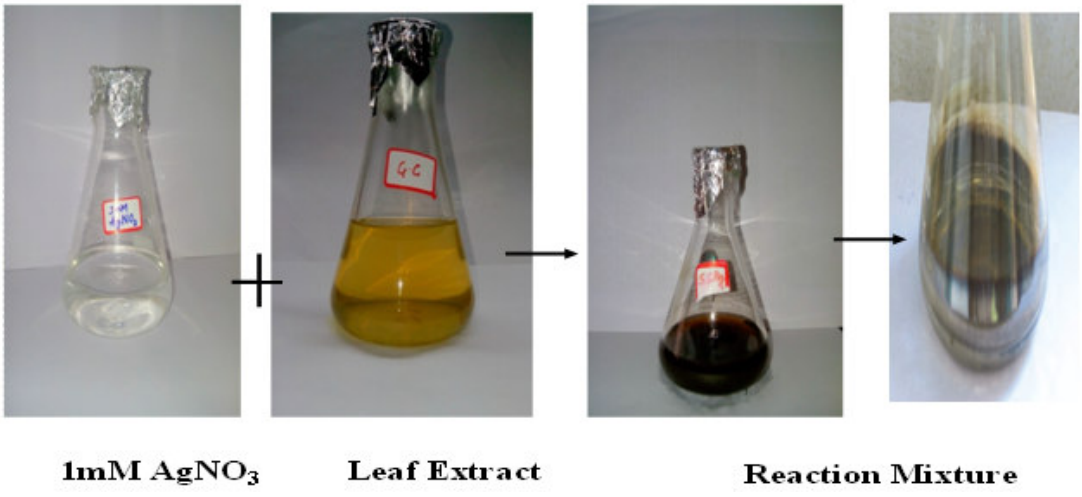
RESULTS

Characterization of silver nanoparticles

The addition of leaf extract to $AgNO_3$ the color of the reaction mixture changes from pale yellow to dark brown within few seconds and after incubation time (24 hours) the walls of the Erlenmeyer flask (which contains reaction mixture) showed mirror like illumination, it clearly indicates the formation of silver nanoparticles in the reaction mixture (Fig. 2).

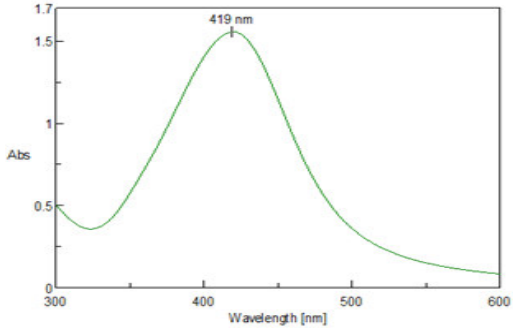
Figure 2

Visual observation of the formation of silver nanoparticle synthesis



The AgNPs formation was confirmed by UV-Vis spectrophotometric analysis, it shows surface plasmon resonance peak at 419nm. (Fig.3).

Figure 3
UV-Vis spectrum of AgNPs in an aqueous solution.



Study of effect of physicochemical parameters on the nanoparticles synthesis

Based on UV–Vis spectroscopy the effect and interaction of various physico-chemical parameters were optimized which would increase the yield of nanoparticle synthesis. Various parameters such as concentration of the leaf extract and $AgNO_3$, pH, temperature and incubation time were optimized for the reduction of Ag+ ions to AgNPs using *Solanum seaforthianum Andrews* extract. The maximum yield of AgNPs is with 1 mM, this concentration was selected for further studies. Among the various parameters, pH is one of the fundamental factors in nanoparticle synthesis. Among 8, 9, 10 pH, the reaction started rapidly at pH 8 of the reaction mixture (as observed by the change in color). The optimal pH for nanoparticle synthesis was preferred to be pH 8.The FTIR study showed sharp absorption peaks located at 3699.75 cm^{-1}, 3413.69 cm^{-1}, 2925.32 cm^{-1}, 2854 cm^{-1}, 1733.44 cm^{-1}, 1620.12 cm^{-1},1383 cm^{-1}, 1036.52 $cm^{-1}$and 824.48 cm^{-1} (Fig. 4).The absorption peaks at 3699 and 3413 cm^{-1} were assigned to strong stretching vibrations of O-H Stretching of phenol & alcoholic bond, the absorption peaks at 2925 cm^{-1} arose from the O-H stretching of the carboxylic acid, The peak around 2854 cm^{-1} region represents the asymmetric stretching of the C-H group, 1733 cm^{-1} peak represents C=O stretching of aldehyde saturated aliphatic compounds, absorption peak at 1620 cm^{-1} represents amide I bond due to carbonyl stretch in proteins, 1383, 1036 cm^{-1} indicates the symmetrical stretching of aliphatic nitro compounds and C-N stretching of alkyl halide and aliphatic amine and 824 cm^{-1} represents C-C, C-H Phenyl ring substitutes.

Figure 4
FT- IR spectrum of silver nanoparticles.

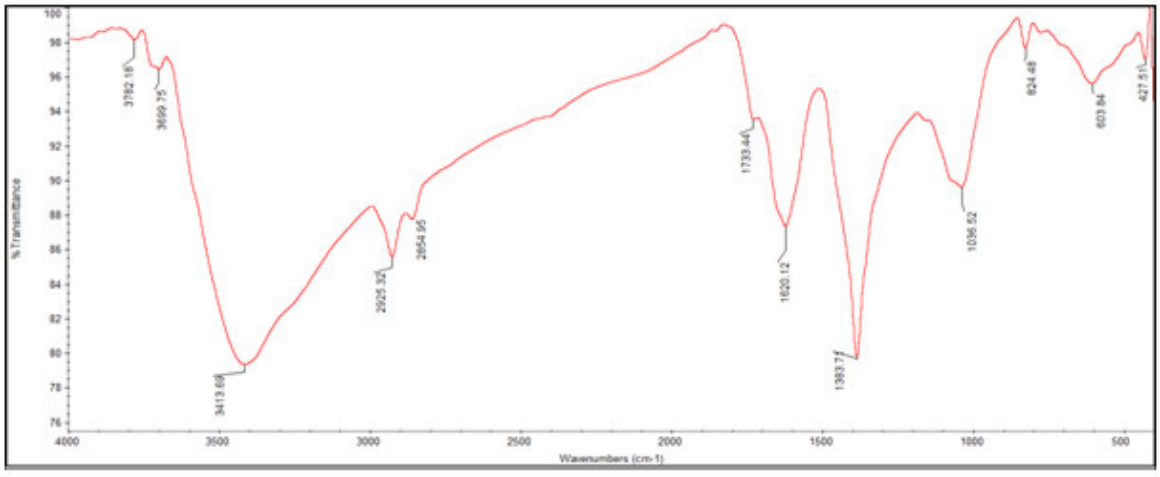
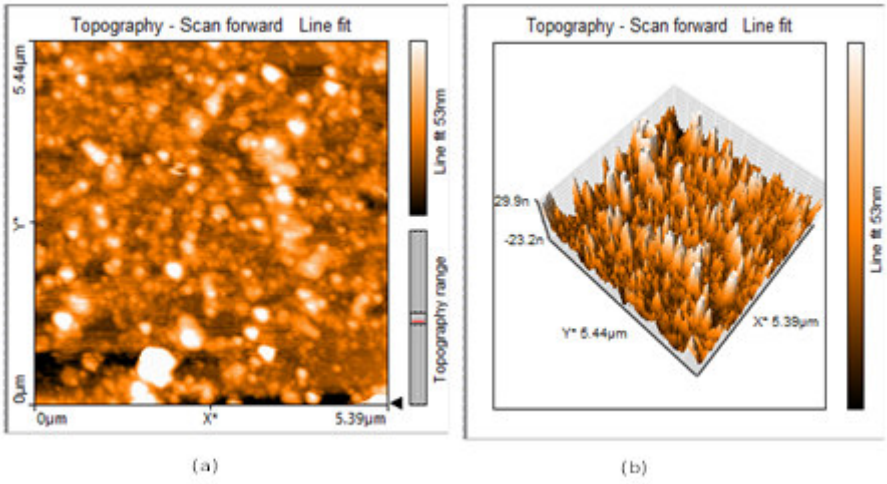


Table 1
FT - IR absorption peaks value and their functional groups

Sl. No.	Absorption peak (cm^{-1})	Functional groups
1	3699.75	O-H Amide N-H Stretch
2	3413.69	O-H Stretch alcohol & phenols N-H
3	2925.32	O-H stretch Corboxylic acid, Alkyl C-H Stretch
4	2854.95	C-H (CH_2 asymmetric stretch)
5	1733.44	C=O aldehyde saturated aliphatic.
6	1620.12	N-H, C=C
7	1383.7	-C-H, C-N alkyl halide and aliphatic amine
8	1036.52	C-N
9	824.48	=C-H Phenyl ring substitutes

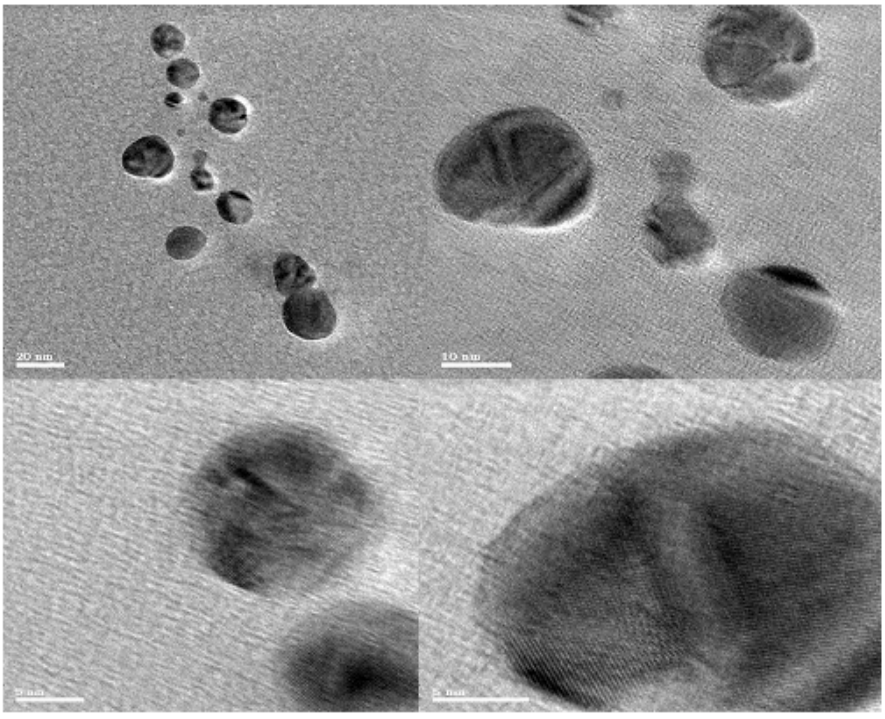
Figure 5
AFM images of silver nanoparticles synthesized from Solanum seaforthianum Andrews



(a) Topography and (b) 3D image

The biogenic silver nanoparticles were characterized by AFM. The topographic image of silver nanoparticles was shown in Fig. 5(a) where the formation of spherical silver nanoparticles and their agglomeration was clearly observed. Fig. 5(b) represents three dimensional views of synthesized silver nanoparticles. The size of the silver nanoparticles ranges from 5 -20 nm. AFM images were taken with silicon cantilevers with force constant and the particle size was measured using line profile.The silver nanoparticles were further characterized by HR-TEM micrograph, these Silver nanoparticles showed spherical shape with the size range from 5 to 20 nm. (Fig. 6). Further, it also shows that the biomolecules of leaf extract bound the nanoparticles as capping agents to hinder further oxidation of nanoparticles. HRTEM analysis confirmed that all the particles (AgNPs) exist in the nanoscale range and possess spherical shape.

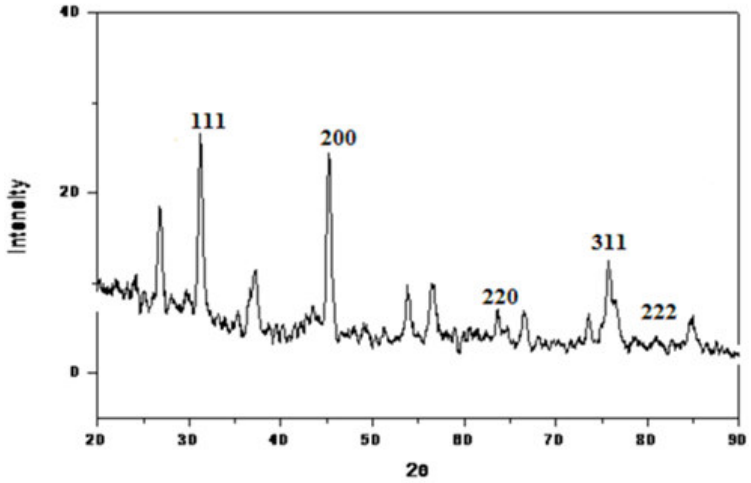
Figure 6
HR-TEM image of silver nanoparticle synthesized from Solanum seaforthianum Andrews



The X-ray Diffraction patterns of silver nanoparticle were recorded according to the description of Wang (2000).The X-ray diffraction pattern of the biosynthesized silver nanostructure produced by the leaf extract was further demonstrated and confirmed by the characteristic peaks observed in the XRD image (Fig. 7). The XRD pattern showed three intensive diffraction peaks at a 2θ value of 38.18º from the (111) lattice plane of face centered cubic (fcc) silver unequivocally indicates that the particles are made of pure silver. Three additional broad bands are observed at 32.50º (2θ), 44.32º (2θ), 64.50º (2θ), and 77.05º (2θ) they Correspond to the (111), (200), (220) and (311) planes of silver respectively (Fig. 7). Other spurious diffractions are due to crystallographic impurities. Table 2 explains the X-ray diffraction peak list of silver nanoparticles. In

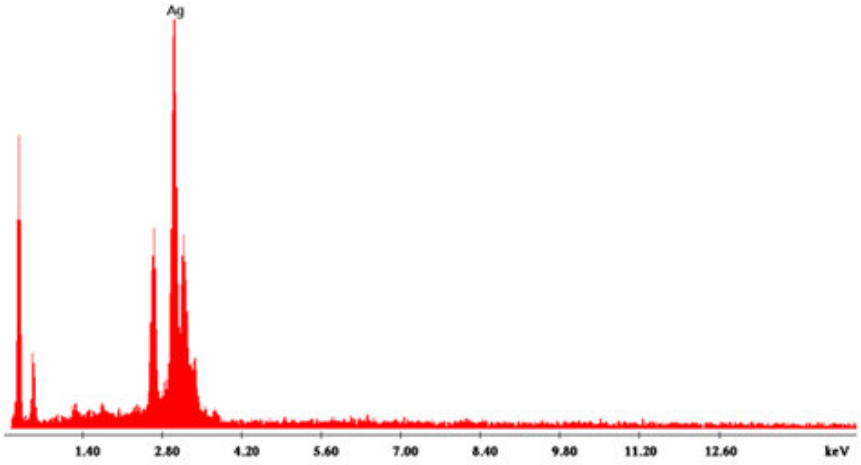
the spectrum obtained the Bragg peak position and their intensities were compared with the standard JCPDS files 89-3722. The software gave the information about the face centered cubic (fcc) structure of silver nanoparticles. The average size of the nanoparticles is 20nm. It can be estimated using the Debye–Scherrer equation.

Figure 7
X-ray Diffraction Spectrum of Silver Nanoparticles from Solanum seaforthianum Andrews



The SEM image showed the high density silver nanoparticles synthesized by the *S.seaforthianum* leaf extract and EDX analysis was conducted to confirm the elemental composition of the sample.The EDS images (Fig. 8.) confirmed the presence of significant amounts of elemental silver in the nanoparticles.

Figure 8
EDS spectra of silver nanoparticles synthesized by leaf extract of S. seaforthianum



Antibacterial activity of silver nanoparticles

Synthesized silver nanoparticles exhibited antibacterial activity against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* (Fig. 9). The antibacterial effect of silver nanoparticles at different concentrations (50-800 µg/ml) was quantitatively assessed on the basis of the zone of inhibition (Table 2). Silver nanoparticles obtain very strong inhibitory action against *Staphylococcus aureus, Bacillus subtilis, and Escherichia coli* than Pseudomonas *aeruginosa* (Table 2). Ag-Nps exhibited strong antibacterial activity against human pathogens even at the lowest concentrations (50µg/ml) used, except against Pseudomonas aeruginosa for which the growth was inhibited only at 800µg /ml (Fig. 9). The AgNPs exhibited a maximum zone of inhibition against E. coli. When these results were compared with a positive control Gentamycine antibiotic (Table 2), it was found that the Ag-NPs were more effective against Staphylococcus aureus, Bacillus subtilis, and Escherichia coli than the test antibiotic (Gentamycine) at higher concentrations. The MIC values of the Ag-NPs evaluated against the human pathogens are shown in Table 2.

Figure 9
The zone of inhibition at different concentrations of Ag-Nps against (a)Staphylococcus aureus (b) Bacillus subtilis (c) E. coli and (d) P. aeruginosa

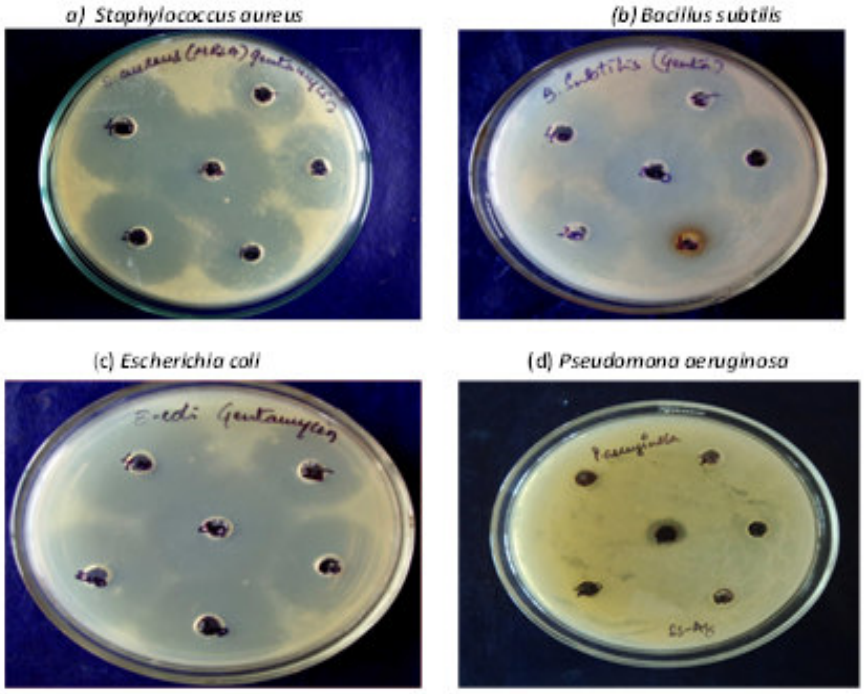


Table 2
Antibacterial activites of Silver nanoparticles synthesized from Solanum seaforthianum Andrews

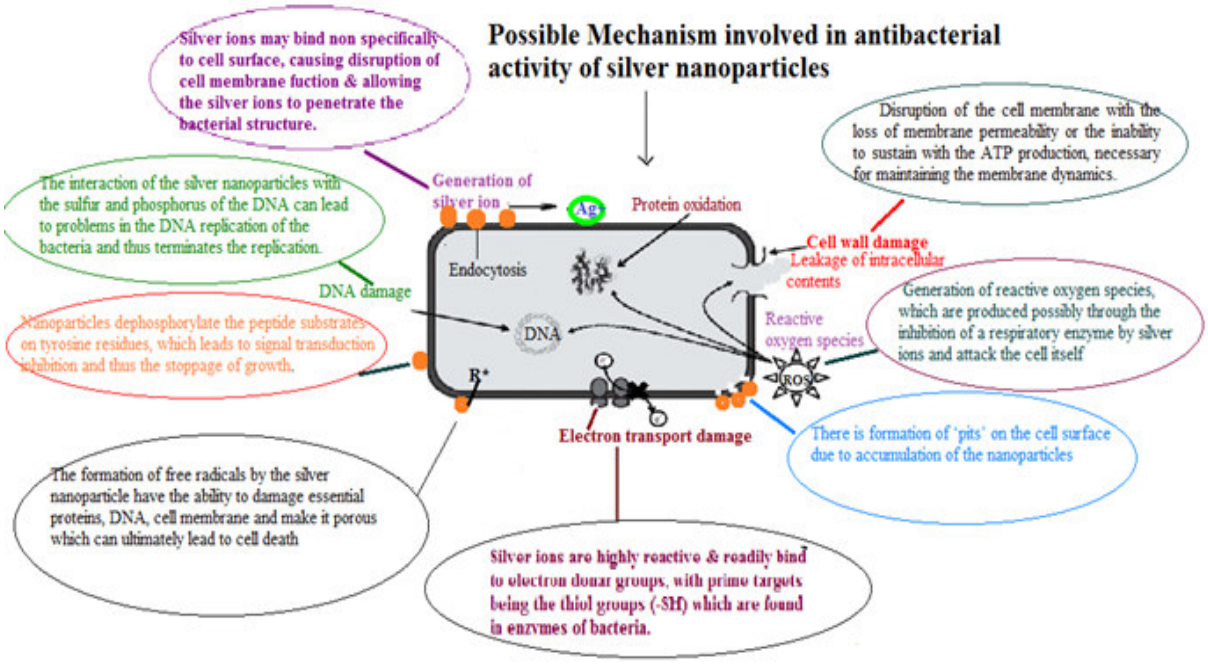
Organism	Gentamycine (+ ve control)	Zone of inhibition (mm) by Silver nanoparticles(µg/ml)					
	25 µg/ ml	50 µg/ml	100 µg/ml	200 µg/ml	400 µg/ml	800 µg/ml	MIC µg/ml
S. aureus	14	16	19	23	25	30	50
B. subtilis	09	08	13	17	20	23	50
E. coli	19	18	21	24	26	29	50
P. aeruginosa	0	0	0	0	0	06	800

DISCUSSION

Nanoparticles are generally characterized by their size, shape and surface area. Homogeneity of these properties is important in many applications. [15] The reduction of metal ions was primarily monitored by visual inspection of the reaction mixture. [16] The change in color has been attributed to excitation of surface Plasmon resonance of the metal nanoparticles.[17] UV-Vis absorption spectroscopy is one of the main tools to analyze the formation of metal nanopartcles in aqueous solution. [18] The exact position of absorbance depends on a number of factors such as the dielectric constant of the medium, size of the particle.[19] Baishya *et. al*., (2012), reported the strong SPR band at 418 in case of *Bryophyllum pinnatum* (Lam) [20]. Further Baishya *et. al*., (2012), Kiruba *et al*., (2012) reported the characteristic SPR of colloidal nanoparticles (ranges between 390 – 420) due to the Mie scattering in case of *Dodonaea viscose* leaf extract. [20] Silver nanoparticles were synthesized rapidly by using plant system. [21, 22, 23, 24] Physicochemical parameters like pH of the reaction mixture plays very important role in the nanoparticle synthesis. Kiruba *et al*., 2012, reported the formation of AgNPs with adjustment of pH 8-10. [25] Acidic condition suppresses the formation of AgNPs (pH 2 and 4); whereas the slight basic condition enhances the formation of the nanoparticles (pH 6-8). Large sized nanoparticles were formed at lower pH which is indicated by the color change and aggregation in the solution, but small and highly dispersed nanoparticles were formed at pH 8-10, at pH 11 agglomerations of nanoparticles was observed, which is in agreement with the early reports.The FTIR analysis was carried out to identify the possible interfacial groups between the capping agents and silver nanoparticles. Different functional groups indicate that the silver nanoparticles synthesized from the extract are surrounded by some proteins and metabolites such as terpenoids that have amine, alcohol, ketone, aldehyde and carboxylic acid functional groups. [26] This result suggests that the biological molecules could probably perform a function involving the formation and stabilization of Ag NPs through free amine groups in the proteins. [27] AFM analyses reveals the size of nanoparticles, this could be attributed to the fact that the compounds present in the leaf extract were responsible for the particle morphology and were kinetically controlled. [28] AFM and HR-TEM analysis shows the particles shape and size. The optical absorption band of the EDX peak in the range of 3–4 keV is typical for the absorption of metallic silver nanoparticles.[29] Silver nanoparticles are good antibacterial agents; Dipankar and Murugan have reported dose- dependent inhibition by Ag NPs synthesized from *Iresine herbstii* leaf aqueous extract. It was noticed that the zone of inhibition increased with

increased concentration of Ag NPs. This might be due to the denaturation of bacterial cell wall, blocking bacterial respiration, destabilization of outer membrane, and depletion of intracellular ATP. [30, 31, 32] The high bactericidal activity due to the silver cat ions released from Ag nanoparticles that act as reservoirs for the Ag+ bactericidal agent. Change in the bacterial membrane structure of bacteria as a result of the interaction with silver cations leads to the increased membrane permeability. [33, 34] Lin *et al*. explained that in general, silver ions from silver nanoparticles are believed to become attached to the negatively charged bacterial cell wall and rupture it, which leads to denaturation of protein and finally cell death. [35] The attachment of either silver ions or nanoparticles to the cell wall causes accumulation of an envelope protein precursor, which results in dissipation of the proton motive force. On the other hand, silver nanoparticles exhibited destabilization of the outer membrane and rupture of the plasma membrane, thereby causing depletion of intracellular ATP. [36] Silver has a greater affinity to react with sulphur or phosphorus-containing biomolecules of the cell. [37] Thus, sulphur-containing proteins in the membrane or inside the cells and phosphorus-containing elements like DNA are likely to be the preferential sites for silver nanoparticle binding. [38] In the present investigation inhibition of bacterial growth which could be due to distraction of the cell membrane structures with the loss of membrane permeability or the failure to sustain with the ATP production, required for maintaining the membrane dynamics.

Figure 11
Mechanism involved in antibacterial activity of silver nanoparticles.



CONCLUSION

In this study method for biosynthesis of stable and spherical shaped nanoparticles using aqueous leaf extract of *Solanum seaforthianum Andrews* has been described. Role of phytochemicals such as flavonoids, tannins, saponins and triterpenoids may be significant in reduction and stabilization of the silver nanoparticles. The synthesized silver nanoparticles were showed significant antimicrobial activity.A lower MIC value exhibited by the silver nanoparticles against *S. aureus B. subtilis, and E. Coli* is of great significance in the health care delivery system.

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