International Journal of Research in Advent Technology, Vol.2, No.3, March 2014

E-ISSN: 2321-9637

PLANT LEAF ASSISTED SYNTHESIS AND APPLICATION EVALUATION OF SILVER NANOPARTICLES

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Abstract- Use of the plant material for the bioreduction of the metal for the nanoparticles synthesis is currently the choice of methods in bionanotechnology. In the present study, silver nanoparticles (AgNPs) were rapidly synthesized usingan aqueous extract of *Ocimum sanctum* (Tulsi) and *Partheniumhysterophorous*(Congress grass). The synthesis process was standardizedbased on the trials. The process of AgNPs formation usingwith Tulsi aqueous extract was carried out with 0.1 mM$AgNO_3$solution and referred as a T1 sample,whereas 2.0 mM$AgNO_3$was used with the Congress grass aqueous extract as a C4 sample.These AgNPs were characterized by means of UV-Vis spectroscopy, scanning electron microscopy(SEM), electron diffraction spectroscopy (EDX) and Dynamic light scattering (DLS).AgNPsformation was confirmed by Surface Plasmon Resonance as determined by UV–Visible spectra analysis in the range of 250 to 750 nm. Maximum absorbance (λ_{max}) peak was reported between 406 to 446 nm in T1 and C4 samplesfollowed by SEM characterization showed a uniform distribution of nanoparticles, with an average size of 68.74 nm and 108.6 nm in T1 and C4 samples respectively. Average size measurement was confirmed by DLS and the presence of silver was confirmed by EDX.Application including mice spleenocytes viability, bactericidal effects, Synergistic effect with Antibiotic and pesticides adsorption suggest the wide scope AgNPs in various fields.

Keywords: silver nanoparticles, Biosynthesis, Application

1. INTRODUCTION

There is an ongoing exploration of bioresources that allows a commercially and environmentally clean synthesis of metal nanoparticles. There has been a huge success by researchers worldwide in finding the effectiveness of various materials of biological origin to bioreduce the metals in ionic form to nano-sized crystalline particles.Biomaterials including bacteria, fungi, algae and plant materials for metal crystallization have mobilizedthe attention of researchers globally [1-4]. So the quest for cleaner methods of nanoparticles synthesis has led to the development of bio-based approaches.Thismethodhas been put forward to be advantageous over other synthetic methods as they are cost effective and do not involve the use of toxic chemicals, high pressure and energy inputs[5].

Plant extracts have a superiorityover the microbial cells for the AgNPsformation, because the microbial mediated synthesis of nanoparticles are not industrially feasible as they require a sterile growth medium and the maintenance of highly aseptic conditionsthroughout the process[6]. So Compared to microbe mediated synthesis, plant assisted synthesis of AgNPsis a relatively less explore field and is recently gaining wide attention with the few contemporary examplesto use of *Capsicum annum* extract[7], leaves of *Azadirachtaindica*[8]and lemongrass plant extract[9], *Citrus limon* aqueous extract[10].

The main aim of this study was to synthesize silver nanoparticles rapidly using plant extracts. Moreover, the Tulsi and Congress grass are not reported earlier for its potential to bioreduce silver ionic metals to nano-sized crystalline form.This is a single-step process without any requirement of toxic chemicals and harsh condition, however elevated temperature was used to facilitate the rapid formation of AgNPs. The rate of formation of AgNPs was related to the incubation temperature of the reaction mixture. High temperature decreases the size of AgNPsand increased the synthesis rate[11], because at high temperature, the reaction rate also increases, causing more silver ions to be used up in the formation of nuclei and thus preventing the

secondary reduction process on the surface of the pre-formed nuclei. The size is reduced initially due to the reductionin aggregation of the growing nanoparticles. Increasing thetemperature beyond a point supports the growth of the crystal aroundthe nucleus[12].

The nanoparticles thus produced using controlled temperature were subjected to further characterization using UV–vis spectrophotometer, SEM, EDX and DLS analysis. Biologically synthesized AgNPshave many applications, as in spectrally selected coatings for solar energy absorption, as intercalation material for electrical batteries, as optical receptors, as catalysts in chemical reactions, as antimicrobials, and in bio-labeling[13]. In the present research we have included multiple application evaluation such asbactericidal, synergistic effect with antibiotic and pesticides adsorption

2. EXPERIMENTAL AND CHARACTERIZATION DETAILS

2.1 Preparation of leaf extract: Leaf extract from the two plants had been prepared with the collection of fresh leaves of *Partheniumhysterophorous* (Congress grass), from coastal areas of Mindhola river, Bardoli, South Gujarat, India as well as *Ocimum sanctum* (Tulsi), collected from commercial plant breeder, Surat,India.Approximately 20 g of healthy green leaves were at first thoroughly washed several times in distilled water, cut into fine pieces and then boiled in 200 ml of distilled water up to 20 min and then filtered with Whatman filter paper No.1 to obtain the clear extract[14].

2.2 Preparation of stock solutions of Silver nitrate: Aqueous solutions of Silver nitrate ($AgNO_3$) of different concentration including 0.1mM, 0.5mM, 1mM, 2mM, 3mM, 4mM, 5mMwere prepared with double distilled water.

2.3 Synthesis of Silver Nanoparticles: The leaf extract solution was added drop by drop to the solution of silver nitrate with a ratio of 1:50 (Vol: Vol) at the 100^oC temperature. The color changes from colorless to light yellow and finally orange-red in the colloidal solutions occurred indicating the formation of silver nanoparticles.

2.4 UV-Visible spectra analysis: Surface Plasmon Resonance (SPR) spectra were recorded in the range of 250nm to 750nm with quartz cuvette using Shimadzu (model 1800) UV-Vis spectrophotometer.

2.5 Particle size measurement: Particles' size was analyszed by the modern Dynamic Light Scattering (DLS) instrument (Zetasizer Nano system, Malvern Instruments**,** Southborough, MA, USA). Average size determination was done as per the standard methods prescribed by the manufacturer.

2.6 SEM and EDXanalysis: For the analysis of the nanostructures of the samples, thin, dried films of silver nanoparticles were mounted on copper slide with carbon tape followed by observation in SEM at the different magnification power. Air-dried T1 and C4 samples were subjected to EDX spectra using SEM equipped EDX attachment. The micrograph and the corresponding EDX spectrum were recorded by focusing on clusters of particles.

3. APPLICATION EVALUATION

3.1 Cytotoxicity analysis: The cytotoxicity of AgNPs was evaluated with mice spleenocytes using the trypan blue assay.The spleen cell's suspension was mixed with equal volume of AgNPs followed by the addition of an equal volume of 0.4% trypan blue dye and viability count was carried out at specific time interval using haemocytometer under binocular microscope.

3.2 Bactericidal effects: Synthesized AgNPs were tested for antibacterial activity by the agar cup method. Antibacterial activities were tested against clinical pathogenic bacteria including*Esherichia coli*, *Bacillus subtilis*, *Bacillus megatarium*, *Salmonella typhi*, *Salmonella paratyphi*A, *Salmonella paratyphi*B, *Proteus vulgaris*, *Enterobacteraerogens*. All the eight test bacterial cultures were activated on nutrient broth at 37°C in shaking condition for overnight. Next day, all the test organisms from activated culture were spread heavily into respective nutrient agar plates. With the help of the sterile borer, four cups were formed on the respective nutrient agar plate spreaded by test organisms. Then, 50µl of AgNPs samples were added into their three respective cups and 50µl of sterile distilled water was kept as a control in fourth cup. All plates were incubated at low temperature for 30 minutes to facilitate the diffusion followed by incubation at 37°C for overnight. Next day, zone of inhibition was measured and compared with control.

3.3 Synergistic effect of Nanoparticles with Antibiotic: To improve the antibacterial activity of antibiotics, AgNPs was mixed at a specific concentration with streptomycin and tested against *Bacillus megaterium* by the agar cup method.Three cups were formed with the help of the sterile borer on the nutrient agar plate spread by the test organism.Samples were added in each respective cup by following manner. (i) Antibiotic (100µg/ml) (ii) 1:1 Antibiotic (50µg/ml) :AgNPs (T1 sample) and (iii) Antibiotic (50µg/ml).The

plate was incubated at low temperature for 30minutes to facilitate the diffusion followed by incubation at 37°C for overnight. Next day, zone of inhibition was measured[15].

3.4 Adsorption of Pesticides by Nanoparticles: Adsorption experiment was carried out on three different AgNPs mounted copper slide followed by addition of pesticides namely Methyl parathion, Chlorpyrifos, Endosulfan respectively.Washing was done by double distilled water subjected on drier to dry the slide and adsorption of pesticide on AgNPs was observed in the SEM.

4. RESULT AND DISCUSSION

4.1 Spectral and particle size analysis

Color changes of the reaction medium is the visual confirmation for the formation of silver nanoparticles (Figure 1), more precise characterization was done using Surface Plasmon Resonance (SPR) spectral analysis.The color change of the reaction is due to the excitation of surface plasmon vibrations in the nanoparticles[4, 16]. The solution was extremely stable, with no evidence of aggregation or flocculation of the particles even several months after synthesis indicating superior findingcompares to the earlier report[17].

Fig. 1.Color changes of the reaction medium

Absorption of thesynthesized AgNPsusing*Ocimum sanctum* and *Partheniumhysterophorous*samples were measured in UV-Vis spectrophotometer at 250 to 750 nm and λ_{max} was reported in the range of 406 to 446 nm that clearly indicate the formation of AgNPs(Figure 2). This spectral analysis result is supported by the reports of Geranium leaf assisted biosynthesis of silver nanoparticles[18].Plant extract contains the various reductants which helps to reduce the Ag+ to Ag^{o}. Plant extract contains various organic acids, aromatic compounds and secondary metabolites which are acting as a bioreductants for the synthesis of silver nanoparticles.

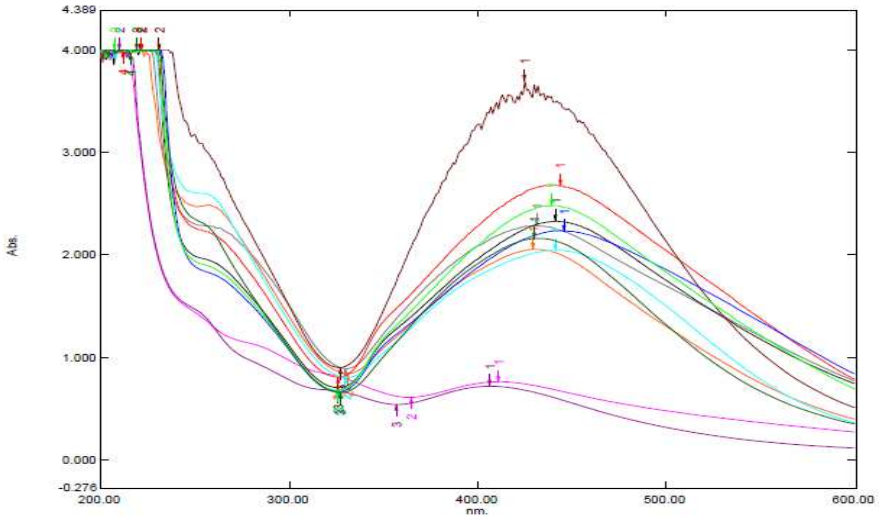


Fig. 2. Combined spectra of all the samples of plant mediated synthesized AgNPs

Synthesized AgNPs by *Ocimum sanctum* were showed λ_{max} at 416 to 446 nm, with smallest average size nearly 68.74 nm in the T1 sample containing 0.1 mM$AgNO_3$concentration, while highest size was 146.8 nm reported in T6 sample containing 4.0 mM$AgNO_3$Concentration (Table 1 and Figure 3). Whereas, AgNPs synthesized from *Partheniumhysterophorous* had shown λ_{max} at 406 to 441 nm, with smallest average size nearly 108.6 nm in the C4 sample holding 2.0 mM$AgNO_3$Concentration and highest size was 164.9 nm reported in C7 sample holding 5.0 mM$AgNO_3$concentration (Table 2 and Figure 4).

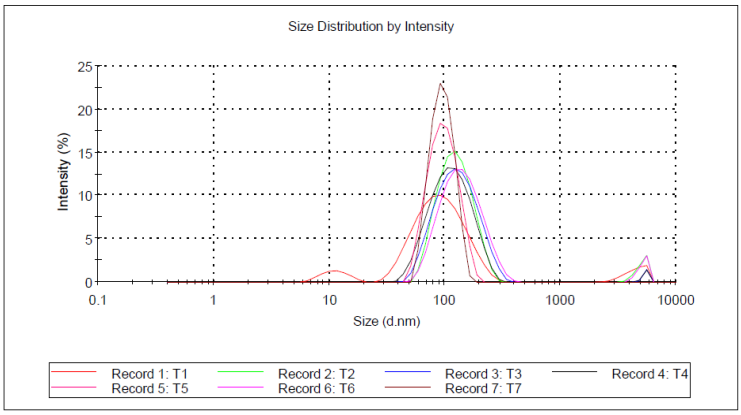


Fig. 3. Combined report of size distribution of AgNPs from *Ocimum sanctum* (Tulsi)

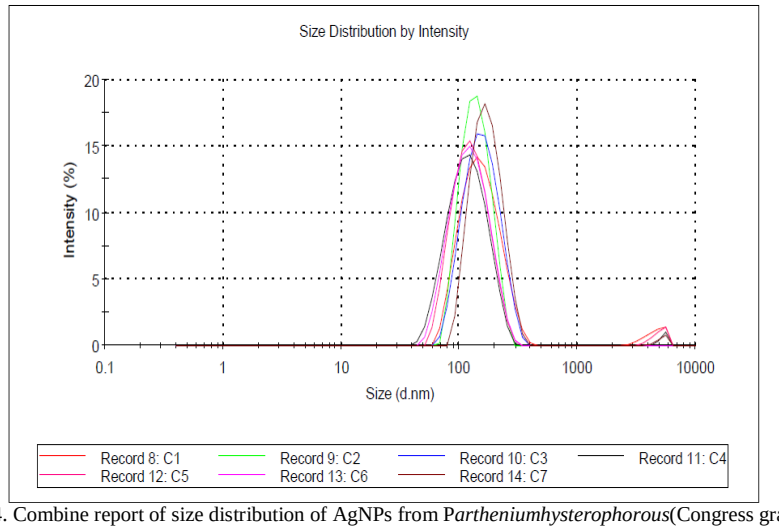


Fig. 4. Combine report of size distribution of AgNPs from P*artheniumhysterophorous*(Congress grass)

Table 1. AgNPs synthesized from *Ocimum sanctum* (Tulsi) using different concentration of $AgNO_3$

Sample	$AgNO_3$Conc. (mM)	λ max (nm)	Absorbance	Z-Average (d.nm)
T1	0.1	416	1.739	68.74
T2	0.5	440	2.138	123.3
T3	1.0	444	1.573	134.1
T4	2.0	441	2.328	109.0
T5	3.0	444	2.678	135.1
T6	4.0	446	2.234	146.8
T7	5.0	439	2.484	122.8

Table 2. AgNPs synthesized from *Partheniumhysterophorous* (Congress grass) using different concentration of $AgNO_3$.

Sample	$AgNO_3$Conc. (mM)	λ max (nm)	Absorbance	Z-Average (d.nm)
C1	0.1	411	0.760	146.3
C2	0.5	429	2.054	130.8
C3	1.0	441	2.044	141.4
C4	2.0	430	2.159	108.6
C5	3.0	406	0.716	116.1
C6	4.0	425	3.697	114.9
C7	5.0	431	2.285	164.9

This average size measurement was done by DLS instrument. The experiments suggest that the low concentration of $AgNO_3$facilitates the formation of nanoparticles having less than 100 nm size. This may be due to $AgNO_3$ forms a coat on growing particles, thereby preventing their aggregation and thus, yielding particles of nanoscale size.However The reason for the decrease in particle size is also depends on various factors including concentration of reductants in extract, pH, temperature of reaction system etc.

4.2 Scanning Electron Microscopy and EDXanalysis

Size and surface of the AgNPswere also analyzed with the SEM, data from the microscopy revealed the heterogeneous AgNPsin both the sample T1 and C4. SEM determinations of T1 and C4 sample showed the formation of nanoparticles, which were confirmed to be of silver by EDX spectra. As shown in Figure5 and 6 well-dispersed nanoparticles could be seen both samples. EDX analysis also showed a peak in the silver region, confirming the formation of silver nanoparticles (Figure5 and 6).

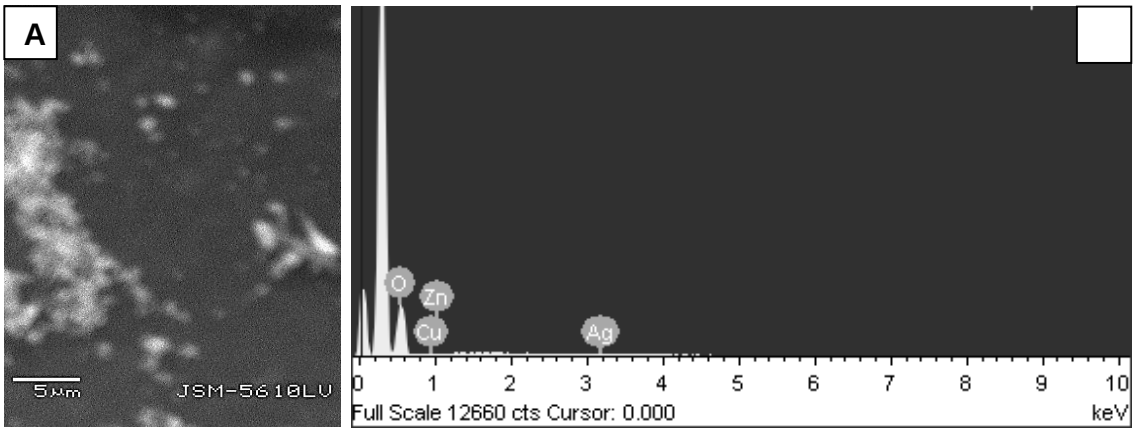


Fig. 5.SEM and EDX of the AgNPs of T1 sample.

From left to right: (a) SEM micrograph of the T1 sample (scale bar at 10µm), and (b) EDX spectrum indicating signals from silver could be observed in the graph near 3keV.

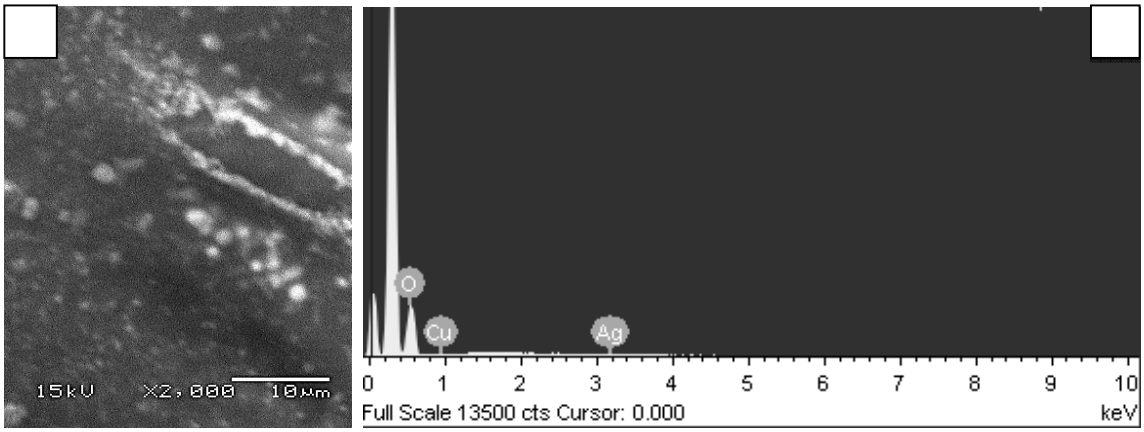


Fig. 6.SEM and EDX of theAgNPs of C4 sample.
From left to right: (a) SEM micrograph of the C4 sample (scale bar at 5µm), and (b) EDX spectrum indicating signals from silver could be observed in the graph near 3keV.

4.3 Cytotoxicity analysis

Viability of mice spleenocytes against AgNPs synthesized from T1sample (Figure 7) was found considerable even after couples of hours. Dark blue colored non-viable cells after 20 hours incubation were nearly 50%. Similar result was also found in AgNPs from C1sample (Figure 8). Viability assay suggeststhe less toxic effects of AgNPson spleenocyteswas observed hence It is effective in targeted drug delivery system.AgNPs destabilize the cell membrane and producing the depletion of vital molecules inside the cell leads to the death of cells. [19]

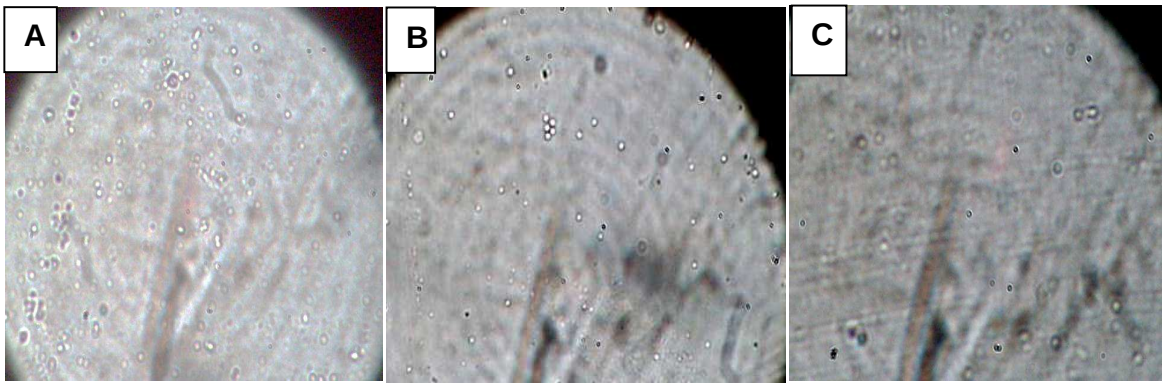
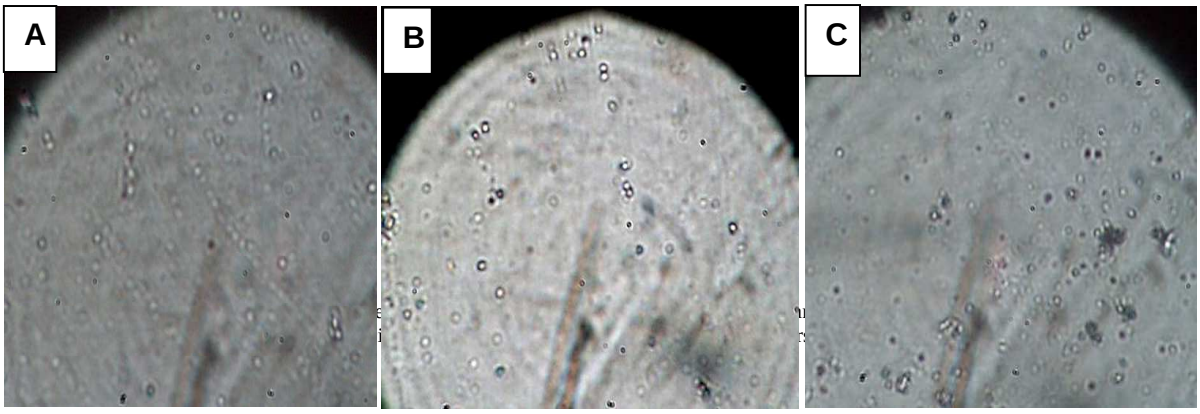


Figure 7. Viability of mice spleenocytes with AgNPs synthesized from T1 sample (Tulsi)
(From left to right: (a) after 1 minutes, (b) 3 hours and (c) 20 hours interaction)



4.4 Antibacterial activity

The bactericidal effect of theAgNPswas tested against eight pathogenic bacterial strains. Zone of growth inhibitions were observed in each agar plate after 24 hours incubationand measured in mm (Figure 9& 10). Inhibition zone against *Bacillus subtilis* and *Escherichia coli* was found remarkable however rest of all the strains were also inhibited notably by AgNPs of Tulsi and congress grass. Our findings were correlated with earlier reports[14].The exact mechanism for the growth inhibition by silver nanoparticles has not yet been fully clarified, but many possible mechanisms have been proposed including rupture of plasma membrane and cell wall components' denaturation[19-20]. In general, Ag ions from nanoparticles are believed to become attached to the negatively charged bacterial cell wall and rupture it, which leads to denaturation of protein and finally celldeath[21].

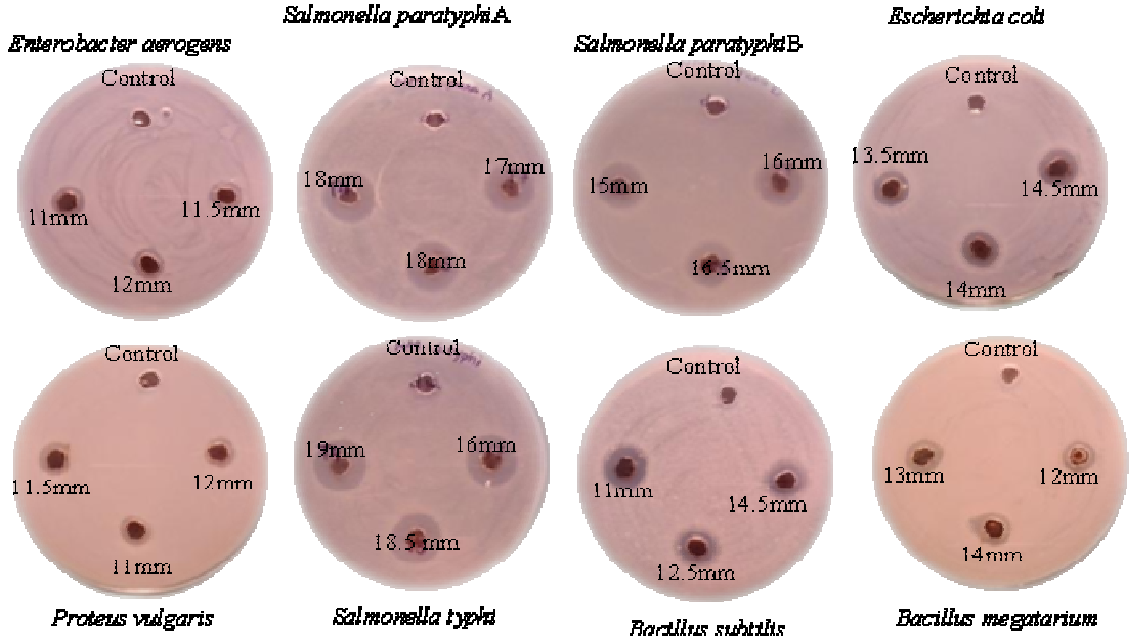


Fig. 9. Antibacterial activity of AgNPsT1 sample from Tulsi

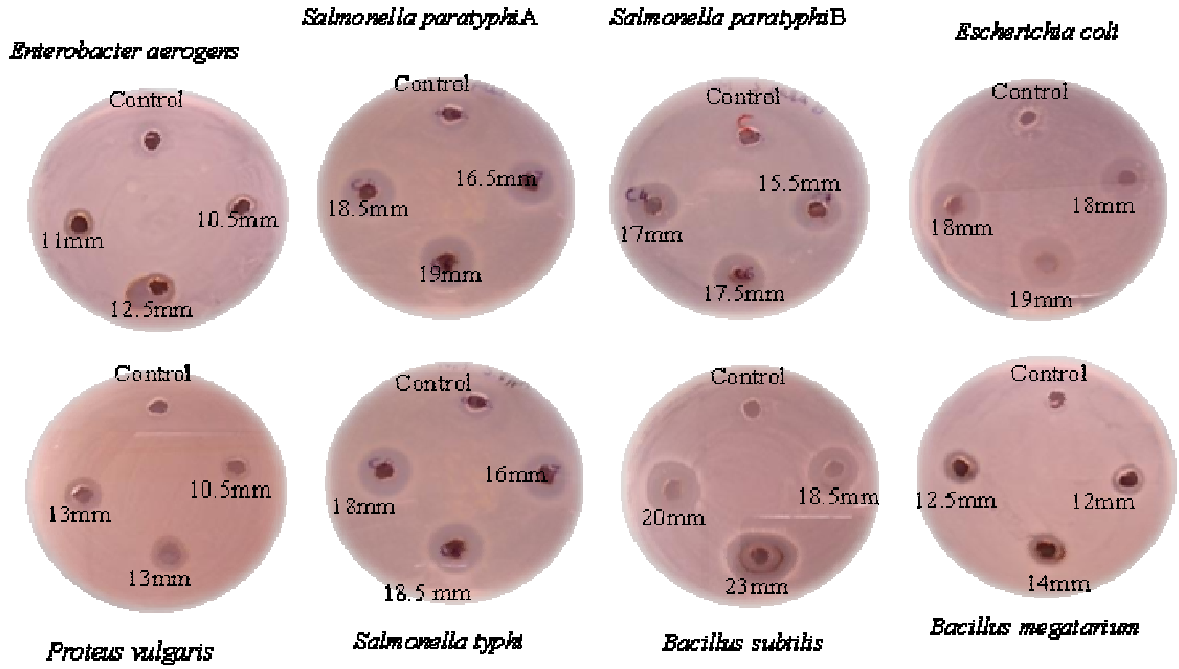


Fig. 10. Antibacterial activity of AgNPsof C4 sample from Congress grass

4.5 Synergistic effect of Nanoparticles with antibiotic

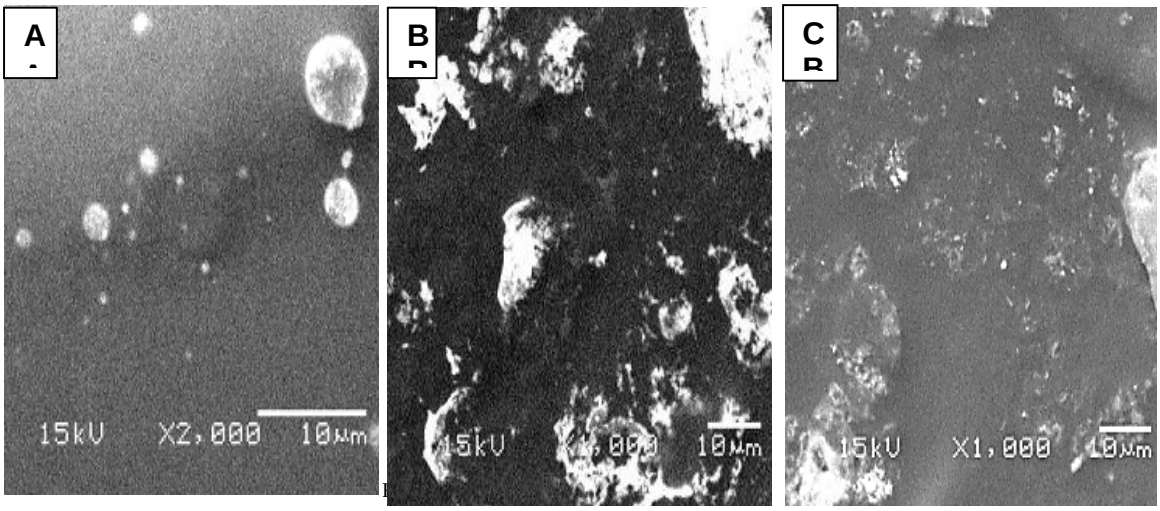
Growth inhibition of *Bacillus megatarium* on the nutrient agar plate was clearly indicating the zone of inhibition by Streptomycin antibiotics and AgNPs. The synergistic effect of silver nanoparticles with aminoglycosides antibiotics such as streptomycin was also observed significant (Table 3). Synergistic approach is effectively used to treat the multi drug resistant bacterial strains.Synergistic antibacterial effects of beta lactam antibiotic combined with silver nanoparticles was already evaluated using *E. coli*[22]. The antibacterial activity of AgNPs was influenced by ATP-associated metabolism rather than by the permeability of the outer membrane. Additionally, AgNPs are also generated hydroxyl radicals, a highly reactive oxygen species induced by bactericidal agents[23].

Table 3.Synergistic effect of Nanoparticles with Antibiotics

Antibiotic used	Zone of inhibition against *Bacillus megatarium* (mm)		
	Antibiotic (100µg/ml)	Antibiotic +AgNPs (50µg/ml)	Antibiotic (50µg/ml)
Streptomycin	32	29	27

4.6 Adsorption of Pesticides by Nanoparticles

Adsorption of pesticides- Methyl parathion, Chlorpyrifos and Endosulfan onto the nanoparticles were observed in SEM analysis. Aggregation of AgNPS with the negative charge surface of pesticides molecules leading to the adsorption of all the three pesticide. Result suggested impending application of AgNPS in the removal of pesticide from the contaminated sites (Figure 11).



(A) Methyl parathion adsorption by AgNPs of T1 sample, (B) Endosulfan adsorption by AgNPs of T1 sample, (C) Chlorpyrifos adsorption by AgNPs of T1 sample. In all the figure aggregation formation is observed which is the confirmation of interaction of the AgNPs with pesticides. 99.9 % pure grade of each pesticide were used.

5. CONCLUSION

We propose an environment-friendlyone step rapid green synthesis method for the preparation of silver nanoparticles. This method is a novel and can be used for the bulk production of AgNPs at industrial scale. The concentration of $AgNO_3$ solution and mixing ratio with plant extract was optimized to produce nearly spherical shaped AgNPs. Further characterization was done by SEM, EDX and DLS.The smaller-size of AgNPs has many positive attributes including chemical stability, catalytic and antibacterial activity, pesticide adsorption, which would make them suitable for many practical applications. Synergistic effects with antibiotic is key investigation and suggest the good potential of AgNPs as a combination therapeutic agent for the treatment of infectious diseases caused by bacteria.

ACKNOWLEDGEMENT

The authors gratefully acknowledge Department of Biosciences, Veer Narmad South Gujarat University for providing the laboratory facilities for cell viability test.

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