https://doi.org/10.30799/jnst.217.19050206

J. Nanosci. Tech. - Volume 5 Issue 2 (2019) 669–672

ISSN: 2455-0191

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Green Synthesis, Characterization and Applications of Silver Nanoparticles using *Thunbergia grandiflora* Roxb.

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ARTICLE DETAILS

Article history:
Received 11 March 2019
Accepted 12 April 2019
Available online 06 May 2019

Keywords:
Green Synthesis
Thunbergia grandiflora
Antibacterial Effect
Seed Germination

ABSTRACT

Green synthesis of silver nanoparticles from the leaf extract of *Thunbergia grandiflora* Roxb. was carried out by bioreduction of silver nitrate to silver nanoparticles due to the presence of biological compounds present in the leaf. The reaction mixture showed a colour change from colourless to reddish brown. The characterization of silver nanoparticles was done by using UV-Visible spectroscopy, FTIR, and TEM. For UV-Visible spectrometry the strong surface plasmon resonance centered at 436 nm. The FTIR analysis showed prominent bands of absorbance at 598.35, 1634.05, 2079.30, 2333.3 and 3332.60 cm^{-1}. TEM analysis revealed that most of the particles fall in the size range from 0 – 5 nm with an average size of 2.39 nm and the particles were spherical in shape. The nanoparticles obtained were checked for its antibacterial activity by disc diffusion method. The silver nanoparticles showed positive results with *Klebsiella planticola*, but didn't show any antibacterial effect against *Bacillus subtilis.* The silver nanoparticles also positively influenced on seed germination and growth by escalating the germination percentage and growth rate of seedlings.

1. Introduction

Green synthesis of silver nanoparticles refers to the bio production of silver nanoparticles using plant resources. It is eco-friendly, reliable and cost-effectiveness and does not utilize any harmful chemicals. It is easy to handle and a rapid process when compared to physical, chemical or microbe-mediated synthesis. Biosynthesis of nanoparticles can be achieved through bacteria, extracellular fungus or algae. But plant assisted synthesis of nanoparticles have captured more attention in modern nanoscience and technology due to its flexibility and eco-friendly nature. Plant materials such as leaves, seeds, fruits, latex, and barks are involved in such metal reduction processes [10]. According to earlier reports, the polyol components and water-soluble heterocyclic components are mainly responsible for the reduction of silver ions and stabilization of the nanoparticles. There are also reports on reductase and polysaccharides as factors involved in biosynthesis and stabilization of the nanoparticles [7].

The various applications of nanotechnology in the field of biology are fluorescent biological labels, drug and gene delivery, bio detection of pathogens, detection of proteins, probing of DNA structure, tissue engineering, tumour detection via heating, separation, and detection of biological molecules and materials [10]. The most effectively studied nanoparticles are noble metals such as Ag, Pt, Au, and Pd. Among these, the silver nanoparticles play a significant role in the field of biology and medicine [7]. Because silver act as an effective antimicrobial agent and exhibits low toxicity [5]. Silver ions have strong inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities. Some forms of Silver have been demonstrated to be effective against burns, severe chronic osteomyelitis, urinary tract infections and central venous catheter infections [4].

In recent years, medical science introduces new technologies to halt the spread of infections. Silver is one of the most promising antimicrobial agents introduced by modern medical science. Due to increasing drug resistance and growing concern about over prescribing of antibiotics, there is an increasing interest in the use of antimicrobial silver. The combination of antibiotics and metal nanoparticles could increase the antibiotic efficacy against resistant pathogens. Nanoparticle antibiotic conjugates lower the amount of both agents in the concentration, which reduce harmfulness and increase antimicrobial properties. For this reason, the present research was carried out for biological synthesis of nanoparticles and their use in decreasing the concentration of antibiotics [6].

Thunbergia grandiflora Roxb. is an evergreen vine. It is used for the treatment of blood dysentery, cataract, conjunctivitis, diabetes, gout, hydrocele, hysteria, malaria, marasmus, ophthalmia, postpartum, pre-eclampsia, rheumatism, spermatorrhoea, stomach complaints, elephantiasis and urinary bladder stone. They are used as traditional medicine for cardiac diseases and blood purification. Leaves and stem of *T. grandiflora* are used as a poultice in stomach complaints and to treat eye diseases in the Chittagong hill tracts and it has anthelmintic activity. The methanol extract of *T. grandiflora* flowers showed the presence of alkaloids and phenols. The novel iridoid, glycosides, isounedoside and grandifloric acid were isolated from *T. grandiflora* [9].

In the present investigation, the synthesis and characterization of silver nanoparticles from *T. grandiflora* through a green method has been identified along with some applications of silver nanoparticles such as antibacterial property and effect on seed germination and growth were also studied.

2. Experimental Methods

2.1 Plant Materials and Chemicals

Thunbergia grandiflora leaves were collected from the Botanical garden of St. Berchmans College, Changanassery, Kottayam, Kerala. AR grade silver nitrate was used for the biosynthesis.

2.2 Preparation of T. grandiflora Leaf Extract and $AgNO_3$ Solution

Fresh leaves of *T. grandiflora* (25 g) were diced into fine pieces and transferred to sterile 250 mL conical flask. Type 1 Water 200 mL was added to the flask and heated at 100 °C for 30 min in the water bath to facilitate the formation of aqueous extract. The extract was filtered using Whatman No. 1 filter paper and the filtrate was stored at 4 °C for further use [2]. Silver nitrate 0.017 g was added to 100 mL of double distilled water and dissolved thoroughly. The solution obtained was transferred to an amber colour bottle to prevent auto-oxidation of silver.

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https://doi.org/10.30799/jnst.217.19050206

Cite this Article as: Anumol Mathew, Salvy Thomas, Green synthesis, characterization and applications of silver nanoparticles using *Thunbergia grandiflora* Roxb., J. Nanosci. Tech. 5(2) (2019) 669–672.

2.3 Synthesis and Determination of Silver Nanoparticles

The aqueous leaf extract of *T. grandiflora* and $AgNO_3$ solution were mixed in the ratio of 1:4 and heated on a water bath at 90 °C until change in colour was observed. The colour change from colourless to reddish-brown indicated the formation of silver nanoparticles [2]. A positive control of diluted leaf extract with 5 mL type 1 water and a negative control of aqueous silver nitrate alone were also maintained under the same condition.

2.4 Characterization

2.4.1 UV–Visible Spectrometric Analysis

UV–Visible spectral analysis of silver nanoparticles was analyzed by Specord - 200 Plus (Analytikjena®) spectrophotometer. The reduction of silver was measured periodically at 200–800 nm at a resolution of 1 nm and the peaks were recorded. Absorbance at 420 - 450 nm was used as an indication of the formation of silver nanoparticles. Diluted leaf extract was used as a blank. A spectrum of silver nanoparticles was plotted with wavelength on X-axis and absorbance on Y - axis. The UV-Visible spectral scan was done at different intervals such as 0 min, 15 min, 30 min, 45 min and 60 min and a peak was noted [2].

2.4.2 Fourier Transforms Infrared (FTIR) Analysis

FTIR analysis was done using Perkin- Elmer Spectrum 400. The diffuse reflectance mode in the range of 450-4000 cm^{-1} at a resolution of 4 cm^{-1} of the Ag-NPs from leaf extract of *T. grandiflora* [8].

2.4.3 TEM Analysis

Transmission electron microscopy (JOEL JEM – 2100) was used to visualize the morphology of the silver nanoparticles. The instrument was operated at an accelerating voltage of 200 kV with high-resolution of 0.23 nm and magnification is from 8K X to 500K X. TEM's grid size is 3 mm diameter which was prepared placing a 4 drop of the silver nanoparticles solutions on carbon-coated copper grids and drying under mercury lamp and then analyzed [2]. The size and dimensions of the silver nanoparticles were determined using the software ImageJ.

2.5 Studies on the Application of Silver Nanoparticles

2.5.1 Assessment of Antibacterial Activity using Disc Diffusion Method

The antibacterial assays were done on human pathogenic strains like, *Klebsiella planticola* and *Bacillus subtilis* by the standard disc diffusion method. Briefly, Muller Hinter broth/agar medium was used to cultivate bacterial strains. Fresh overnight inoculums (100 μL) of each culture were spread on to Muller Hinter agar plates. Sterile Whatman No. 1 paper discs of 6 mm diameter containing 30 μL of *T. grandiflora* leaf extract, 30 μL of TGL- silver nanoparticles of different concentrations (25%, 50% and 75%) 30 μL of silver nitrate solution and gentamycin were placed in each plate in a serial order. After incubation overnight, the zone of inhibition was measured (diameter in mm). The bactericidal activity is evaluated by the size of the clear zone and greater the zone of inhibition greater the bactericidal activity [14].

2.5.2 Effect on Seed Germination and Plant Growth

Four different concentrations (25%, 50%, 75% and 100% (v/v)) of AgNP dispersions were prepared in distilled water. The germination test was carried out in sterile petridishes of 12 cm diameter by placing a Whatman No. 3 filter paper on them. Fifty seeds of each receptor crop, Moong Bean (*Vigna radiata)* and Chickpea (*Cicer arietinum),* were placed in the respective petri dishes. The seeds were surface sterilized with 0.1% $HgCl_2$ solution and rinsed three times with distilled water. The solution of each concentration was added to each petri dish of respective treatment daily in such an amount just enough to wet the seeds. The petri dishes were then placed in the dark. Seeds with root tip 1 mm and higher were considered as germinated. Percentage of germination and length of root and shoot (in mm) were recorded in each 24 hours up to 72 hours and the percentage of germination were calculated [3].

3. Results and Discussion

3.1 Synthesis of Silver Nanoparticles

The reaction mixture containing the leaf extract of *Thunbergia grandiflora* and silver nitrate solution after incubation of 15 minutes showed colour change from colourless to reddish brown due to the reduction of silver ion, indicated the formation of silver nanoparticles. No colour change was observed in the positive and negative control even after longer periods of incubation (Fig. 1). The production of silver nanoparticles was initiated with 2–10 minutes and vigoursly increase up to 1 hour, and it became stabilized.

The colour change is due to many factors such as coherent electron motion, the frequency of the electromagnetic field [9]. The presence of water soluble bioactive compounds such as ascorbic acid and other phenol compounds in the plants [12]. The polyol, water soluble heterocyclic components, reductase,polysaccharides, plant source, organic compounds in the leaf extract, concentration of silver nitrate, temperature and the pigments in the leaf [8], flavanoids in the leaf extract such as apigenin, quercetin, myricetin, isorhamnetin and kampferol and its oxidation and reduction. These flavanoids serve as the reducing and stabilizing agents in the synthesis process[15]. Mallikarjuna et al. reported that silver nanoparticles surrounded by a thin layer of protiens and metabolites, such as terpenoids, having functional groups of amines, alcohols, ketones, aldehydes and carboxylic acids.

According to the investigation of Mbachu and Moronkola, *T. grandiflora* leaf oil is dominated by the presence of alcohols, carboxylic acids, aldehydes and ketone. Ibrahim et al., reported that 70% aqueous methanolic leaf extract of *T. grandiflora* shows the presence of quercetin and kaempferol. Flavanoids such as apigenen were reported in *T. grandiflora* [14]. The presence of terpenoids, flavanoids, phenolics and alkaloids may be the reason for the formation of silver nanoparticles in *T. grandiflora.*

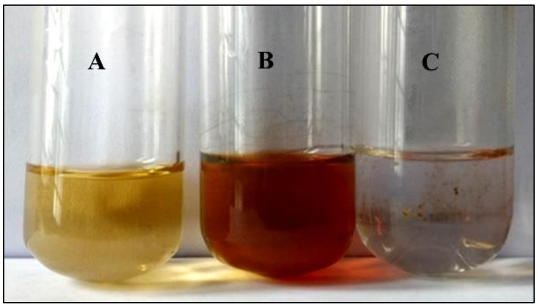

Fig. 1 Formation of silver nanoparticles (A – Positive control, B- Reaction mixture, C – Negative control)

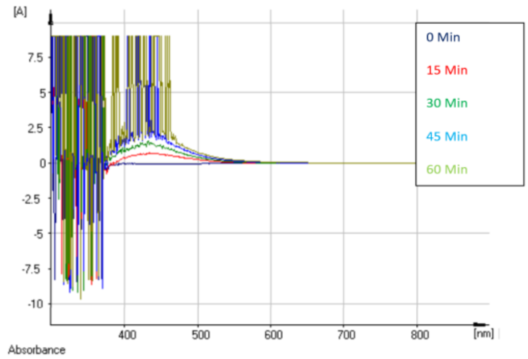

Fig. 2 UV-Vis spectrum of *T. grandiflora* silver nanoparticles at various time intervals

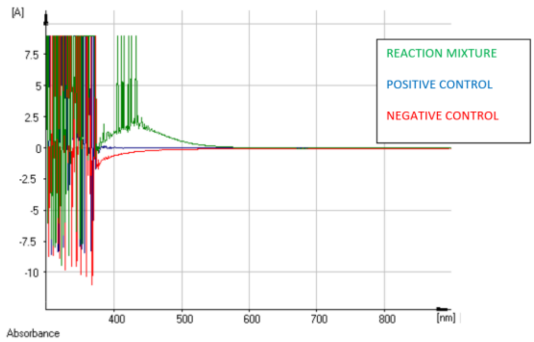

Fig. 3 UV-Vis spectrum of positive control, negative control and reaction mixture

3.2 Characterization

3.2.1 UV–Visible Spectral Analysis

The formation of nanoparticles was primarily characterized by UV-Visible spectroscopy analysis. The formation of dark brown colour clearly indicates the formation of silver nanoparticles and it is conformed by the surface plasmon resonance an the free oscillation of free electrons in the

https://doi.org/10.30799/jnst.217.19050206

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silver nanoparticles [15]. The characteristic silver surface plasmon resonance bands were detected around 400-450 nm [16]. The UV-Visible spectra recorded from *T. grandiflora* leaf extract reaction mixture at different time intervals were plotted in Fig. 2. The strong surface plasmon resonance centered at 436 nm.The production of silver nanoparticles was initiated from 2–10 min onwards and the spectra clearly showedan increase in intensity of silver solution with time, indicating the formation of more silver nanoparticles in the solution. The solution was stable after 1 hour, with no evidence of aggregation of particles. The positive and negative control does not showed any colour change indicating the absence of silver nanoparticles in the positive and negative control (Fig. 3).

Silver nanoparticles from *Ocimum sanctum* shows the surface plasmon resonance bands at 436 nm, the broadening of peak which indicated the formation of poly dispersed large nanoparticles due to slow production rates [9]. *Argemone mexicana* leaf extract showed absorbance peak at 440 nm, broadening of peak indicated that the particles were polydispersed [13]. In the present investigation, UV-Visible spectrum showed the same pattern of absorbance peak as the early reports. Hence, the silver nanoparticles formed may be polydispersed nanoparticles.

3.2.2 FT–IR Analysis

In the AgNP solutions, prominent bands of absorbance were observed at 598. 35, 1634.05, 2079.30, 2333.33 and 3332. 60 cm^{-1}. The observed peaks denote –C – I-, -C = C-, N-H, -C-H-, alkine, amine, alkyne, alcohol, aromatic compounds respectively (Fig. 4). These bands denote stretching vibrational bands responsible for compounds like flavonoids and terpenoids and so may be held responsible for efficient capping and stabilization of silver nanoparticles obtained [3].

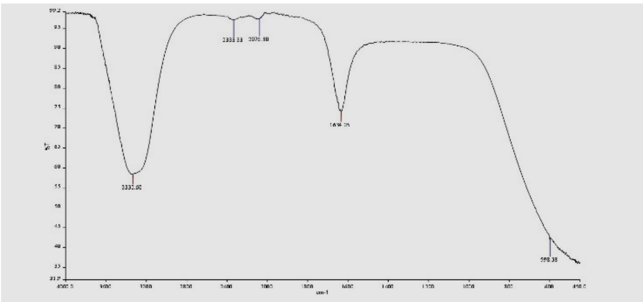

Fig. 4 Graphical representation of FT-IR Analysis of silver nanoparticles from *T. grandiflora*leaf extract

3.2.3 TEM Analysis

The TEM micrograph (Fig. 5) showed individual silver nanoparticles as well as aggregates. The TEM provided further insights into the morphology and size details of silver nanoparticles. The size distributions were obtained by measuring the diameter of about 250 nanoparticles in the different TEM images and are depicted in Fig. 6. Most of the nano particles were spherical in shape and falled in the size range of 0 nm – 5 nm with an average size of 2.39 nm (Fig. 7).

Based on the earlier reports it has been found that the TEM micrograph of *Ocimum* and *Carica* showed the size ranges from 3 – 20 nm with an average size of 9.5 nm and 5-40 nm respectively. The silver nanoparticles obtained in the present study with *T. grandiflora* is so small in size compared with the previous reports.

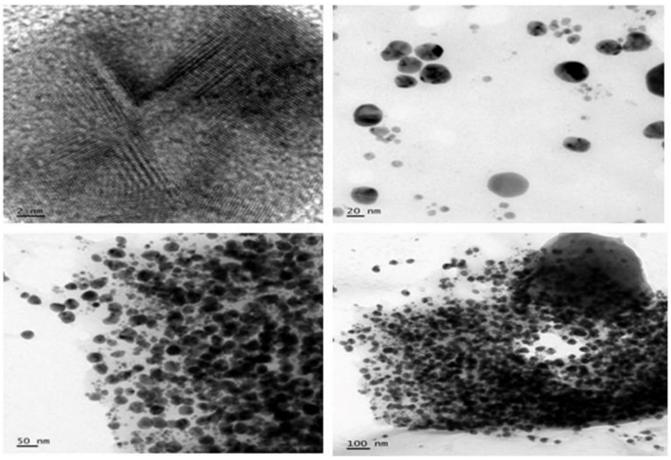

Fig. 5 TEM micrograph at the resolution of 2 nm, 20 nm, 50 nm and 100 nm

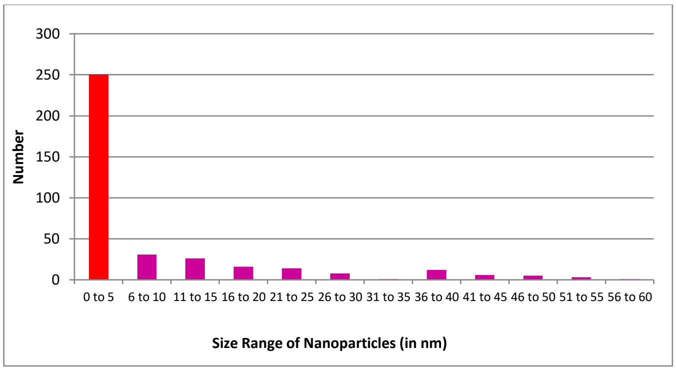

Fig. 6 Nanoparticle size distribution

3.3 Applications of Silver Nanoparticles

3.3.1 Antibacterial Effects

It has been observed that the green synthesized silver nanoparticles were more highly toxic against *K. planticola* than *B. subtilis*as investigated by disc diffusion method. The maximum zone of inhibition was observed at 25%, with 30 μL concentrations in the case of *K. planticola*. The zone of inhibition was decreased with increasing concentration of silver nanoparticles. In the present study, *B. subtilis* does not show any zone of inhibition. The inhibition zone of *Klebsiella planticola* was 11.36±0.36 mm by silver nanoparticles, it was somewhat similar to the inhibition zone obtained with gentamycin (positive control). So silver nanoparticles showed a high antibacterial effect against gram-negative *Klebsiella planticola* than gram-positive bacteria. The result has been summarized in Table 1 and Fig. 7.

Table 1 Effect of silver nanoparticles on different bacteria

Bacteria	Zone of inhibition (mm) - Disc diffusion method					
	Treated (30 μL Silver nanoparticles)			Control (30 μL)		
	25%	50%	75%	$AgNo_3$	Extract	Gentamycin
Klebsiella planticola	11.36±0.3	7.7±0.2	7.63±0.3	0	8.1±0.2	12.33±0.8
Bacillus subtillis	0	0	0	0	7.8±0.1	14.13±0.8

Value indicated as mean ± SD of 03 samples

There are several mechanisms to kill microorganisms by silver nanoparticles. They closely associated with the cell wall of bacteria forming pits finally it affects the permeability and cause cell death. Silver nanoparticles were small in size, so it easily enters into the bacterial cell and affects the intracellular processes such as DNA, RNA, and protein synthesis. The smaller particles affect the larger surface area of the bacteria thus it has more bactericidal activity than the larger sized nanoparticles [15].

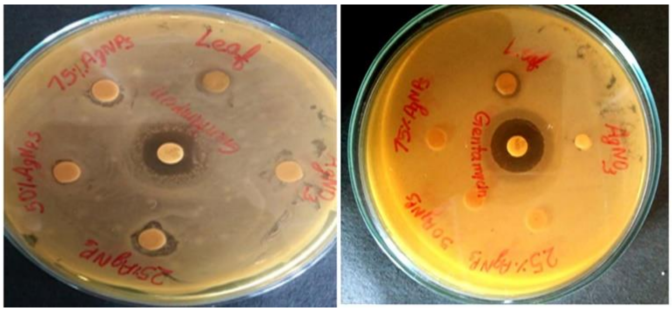

Fig. 7 The antibacterial effect of silver nanoparticles against *Klebsiella planticola* and *Bacillus subtillis*

Antibacterial effect of silver nanoparticles was dose dependent and more pronounced against gram-negative bacteria than gram-positive bacteria [12]. In the present study, similar findings were obtained with gram-negative bacteria *Klebsiella planticola* and no inhibition was noticed with gram-positive bacteria *Bacillus subtillis*.

3.3.2 Effect Silver Nanoparticles on Seed Germination and Growth

Germination studies with *Vigna radiata* showed a high germination rate when treated with 50% and 75% of Silver nanoparticles. High rate of shoot and root growth was observed at concentrations 25% and 50%, and no significant effect was noticed on seeds treated with 100% Silver nanoparticles (Fig. 8).

https://doi.org/10.30799/jnst.217.19050206

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In the case of *Cicer arietinum*, high seed germination percentage and root growth was observed at seeds treated with 25% silver nanoparticles. Low seed germination was observed when seeds treated with 50%, 75% and 100% silver nanoparticles enhanced shoot length was noticed at concentrations of 50%, 75% and 100% of silver nanoparticles. It has been noticed that silver nanoparticles have no any effect on shoot growth (Fig. 9). Exposure to specific concentrations of silver nanoparticles could enhance plant growth compared with non-exposed plants. Whereas higher and lower concentrations of silver nanoparticles could have an effect on the plant growth positively or negatively [1]. In the present study, 25% of silver nanoparticles gave maximum growth, whereas at 100% of silver nanoparticles, no effect was observed.

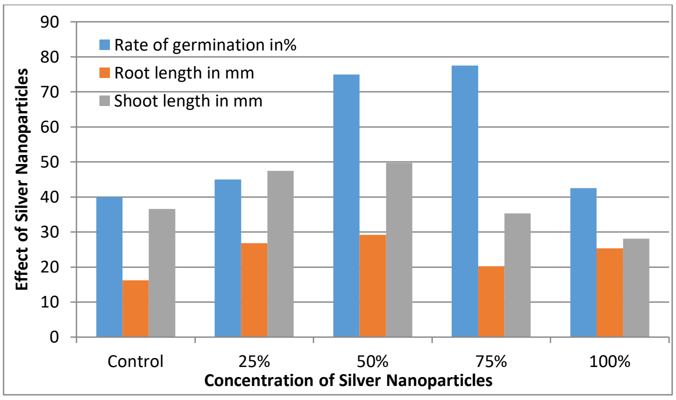

Fig. 8 Effect of silver nanoparticles on *Vigna radiata* seed germination and seedling growth

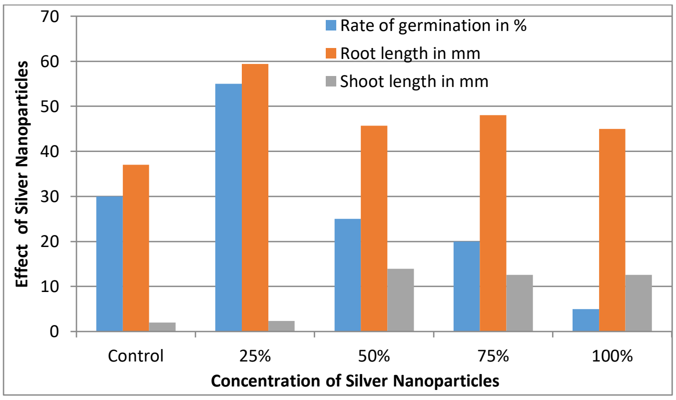

Fig. 9 Effect of silver nanoparticles on *Cicer arietinum* seed germination and seedling growth

4. Conclusion

Green synthesis of silver nanoparticles from *Thunbergia grandiflora* leaf extract is eco-friendly, without the use of harmful chemicals. Presence of silver nanoparticles was determined by a colour change from colourless to reddish-brown due to the reduction of silver ions. The compounds such as flavanoid, terpenoid, alcohols, aldehydes, water-soluble compounds etc., in the leaf interact with the silver nitrate and cause its reduction. As a result, the silver nanoparticles were formed. Silver nanoparticles were characterized by UV-Visible Spectroscopy, FTIR analysis, and TEM. The UV-Visible spectra showed the surface plasmon resonance bands at 436 nm. The production of silver nanoparticles was initiated after 15 min and it increased up to 1 hour, and it became stabilized. The FTIR analysis showed prominent bands of absorbance at 598.35 cm^{-2}, 1634.05 cm^{-2}, 2079.3 cm^{-2}, 3332.60 cm^{-2}. The observed peaks denote –C–I-, -C=C-, N-H, -C–H-, alkene, alkyne, alcohol, aromatic compounds respectively. The TEM analysis represented that most of the nanoparticles fall in size range from 0 – 5 nm with an average size of 2.39 nm. The silver nanoparticles showed more antibacterial effect against *Klebsiella planticola* than *Bacillus subtillis.* The antibacterial effect showed by the silver nanoparticles was as similar to the antibiotic gentamycin. The effect of silver nanoparticles on seed germination was dependent on many factors such as the concentration of silver nanoparticles, nature of the species etc. and similar results were obtained with *Vigna radiata* and *Cicer arietinum* against various concentrations of silver nanoparticles. Hence, it has been concluded that *T. grandiflora* is not only an evergreen vine but also have much potential with a rich source of phytochemicals and silver nanoparticles can be synthesized by a green synthesized method without the use of harmful chemical compounds.

Acknowledgement

We are greatly thankful the principal St. Berchmans College Changanacherry, Kottayam, Kerala, India, for providing the lab facilities. Also, we acknowledge the School of Chemical Sciences and the Centre for Nanoscience and Nanotechnology, Mahatma Gandhi University, Kottayam, Kerala for their help and support.

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