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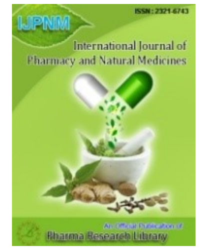


**R ESEAR C HA R T I C L E**

# Green synthesis of silver nanoparticles by using *Tinospora cordifolia* and their evolution of antimicrobial activities and photocatalytic dye degradation studies

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**A B S T R A C T**

In this study, rapid and simple approaches for the green synthesis of silver nanoparticles from *Tinospora cordifolia* aqueous leaf extract (TCLE). The plant extract of TCLE acts as a reducing, stabilizing and capping agent for the synthesis of silver nanoparticles at room temperature. The reaction process was investigated by UV-visible spectrophotometer, FT-IR, HR-SEM. UV-visible spectrophotometer showed absorbance peak in the range of 436–446 nm. To identify the compounds were liable for reduction of silver ions, the functional groups existence in plant extract were FT-IR. The TCLE-Ag NPs showed good antimicrobial activities against both gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*, *Pseudomonas aerouginosa*, *Enterobacter aerogenes*). They demonstrated highest zone of inhibition (34nm) towards gram positive (*Staphylococcus aureus*) microorganism, when compared to other gram negative microorganisms. Moreover, the synthesized silver nanoparticles showed significant photocatytic dye degradation (Methylene blue and Congo red)under UV-light irradiation. Further, the developed TCLE-Ag NPs could be used in the areas of waste water treatment, biomedicine and other biological studies.

**Keywords:** *Tinospora cordifolia*, Green synthesis, Silver nanoparticles, Antimicrobial studies, Photocatalytic dye degradation studies.

**A R TICL E I NF O**

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**C O N T E N T S**

## 1. Introduction

Green synthetic methods of nanoparticles using plant extract [1], microorganism [2], and enzyme [3] have been proposed as possible eco-friendly alternatives to that of chemical and physical methods. The biosynthesis of the silver nanoparticles from natural products such as plant-related parts like leaves, stems, roots, shoots, blooms, barks, seeds, and their metabolites are more attractive and found to be effectively utilized for the proficient biosynthesis [4,5] of nanoparticles.

Phytochemicals such as alkaloids, tannins, saponins, glycosides, phenolic compounds, steroids, proteins, amino acids and secondary metabolites like irridoids, flavonoids, napthoquinone, and volatile constituents that inhibit the development of various microorganisms [2]. Moreover these, phytochemicals that were present in the plant extracts can act as reducing, capping and stabilizing agent in the preparation of nanoparticles.

To synthesize the Ag NPs in solution there needs to have the reducing agents, stabilizing agent or also known as capping agent and the metal precursor. These are some of the most often used reducing agents to assist in the formation of Ag NPs in solution.

The use of plant extracts as reducing agents will effectively reduce the environmental pollution, production cost and produces biocompatible materials. Literature has witnessed the synthesis of NPs according to the green chemistry point of view using eco-friendly reagents such as L-ascorbic acid, L–Lysine, glucose, instead of chemical agents like borohydride, sodium citrate and hydrazine compounds, etc., This method is an excellent alternative for the chemical and physical methods because it is cost effective, can be used for higher yields on a large scale and is eco-friendly.

The biological method including the biosynthesis of metal and metal oxide nanoparticles through using some biological agents such as bacteria, fungi, yeast, plant extracts has gained popularity in the region of nanotechnology [4,5]. Silver nanoparticles have found tremendous applications in catalytic reduction of textile dyes [6], antimicrobial cotton fibers [7,8], antimicrobial property [9,10], antioxidant activities [11], therapeutic applications [12], and photocatalytic dye degradation[13].

*Tinospora cordifolia* (TC) an important medicinal plant with common names which is known by the heart leave moonseed, Guduchi, belonging to family *Menispermaceae* [14], a well-known plant of the Indian medicinal system is obtaining a wider application for selecting a broad spectrum of pharmacological activities.

The present study focused on to prepare the green synthesis of silver nanoparticles by using aqueous plant extract of *Tinospora cordifolia* and their evolution of antimicrobial and photo catalytic dye degradation studies.

## 2. Materials and Methods

### 2.1. Materials:

The fresh and healthy leaves of *Tinospora cordifolia* (TC ) were collected from waste land, near to Gachibowli, Hyderabad, T.S-500032. $AgNO_3$, Methylene blue, Congored were purchased from Merck India.


**Fig: 1.**Confirmation of Silver nanoparticles synthesized from leaf extract of *Tinospora cordifolia.* **A)** *Tinospora cordifolia* (TC) plant leaves **B) a**. *Tinospora cordifolia* leaf extract (TCLE), **b.** *Tinospora cordifolia*-Ag nanoparticles (TLCE-Ag Nps), **c.** Distilled water.

**2.2. Preparation of *Tinospora cordifolia* leaf extract (TCLE)**: The extraction of the fresh leaves was thoroughly washed with running water, further with double distilled water. 5gms of leaves of *Tinospora cordifolia* (TC) was grinded using mortar pestle boiled for 10 min in 100 mL ultra-pure water and filtered through Whatmann No. 1 filter paper. The filtered *Tinospora cordifolia* leaf extract (TCLE) was used for the synthesis of silver nanoparticles.

### 2.3. Preparation of *Tinospora cordifolia* leaf extract-Ag nanoparticles (TCLE-Ag):

The new leaf of *Tinospora cordifolia* was set up by taking 10 g of thoroughly washed and finely cut leaves in a 300 mL Erlenmeyer flagon alongside 100 mL of cleaned twofold refined water and afterward heating up the blend for 5 min before at long last tapping it. The concentrate was separated through Whatman no 1 paper and put away at -15 °C and could be utilized inside 1 week. The filtrate was treated with watery 1 mM $AgNO_3$ arrangement in an Erlenmeyer jar and brooded at room temperature. Accordingly, a darker yellow arrangement was shaped, showing the development of silver nanoparticles. It demonstrated that fluid silver particles could be lessened by watery concentrate of plant parts to produce to great degree stable silver nanoparticles in water.

### 2.4. Characterization techniques

The UV-visible spectroscopy analysis was carried out on a UV-Visible absorption spectrophotometer with a resolution between 200 to 600 nm possessing a scanning speed of 300nm/min. Elemental analysis of the synthesized *Tinospora cordifolia* silver nanoparticles (TCLE-Ag NPs) was studied by Fourier Transform Infra-Red spectroscopy (FT-IR). Scanning Electron Microscope (TESCAN - Vega TC software) was performed for the phase identification of Ag NPs from a size range of (30 ± 15nm) and poly-dispersed.

### 2.5. Biological activity studies

#### 2.5.1 Antibacterial activity:

The antibacterial activity was monitored by agar gel method [15]. The stabilized *Tinospora cordifolia* silver nanoparticles (TCLE-Ag) were tested with some bacterial strains with *S. aureus, E. coli*, *P. aeruginosa,* and *E. aerogenes*. The obtained results indicated the zone of inhibition (mm) of Ag NPs on depending on bacterial strains at 100$\mu$L concentrations.

**2.5.2 Anti-fungal activity:**

Antifungal activities of *Tinospora cordifolia* silver nanoparticles (TCLE-Ag NPs) and TCLE against fungi *Aspergillu sniger* were investigated by the agar disk diffusion method [15]. The determination of zone of inhibition, fungal strains were taken as a standard antibiotic for comparison of the results. *Streptomycin* and distilled water were used as positive and negative control respectively. All the extracts were screened for antifungal activities against *A.niger.*

**2.5.3. Antimicrobial activity:**

The biosynthesized *Tinospora cordifolia* leaf extract using sliver nanoparticles (TCLE-Ag NPs) has been decorated over cotton fabric through in-situ chemical reaction. Zone of inhibition on culture media observed. The formation of TCLE-Ag NPs and their stability was observed by zone of inhibition on the culture. The biological approach is a cost effective method as compared with the chemical synthesis.

**2.6. Photocatalytic dye degradation activity**

The photocatalytic activities of TCLE-Ag NPs were scaled against two dyes *viz.,*Methylene blue and Congo red in aqueous medium were used to investigate the photo catalyticactivity of TCLE-Ag NPs and compared with TCLE.In this experiment, 10 mg of Methylene blue and Congo red dyes to 1000 mL of distilled water. About 10 mg of TCLE-Ag NPs are dispersed to 100 mL of two dyes solution. A control was also maintained without UV light of silver nanoparticles (TLCE). Before exposing to irradiation, the reaction suspension was well mixed by being magnetically stirred for 30 min to clearly make the equilibrium of the working solution. Afterwards, the dispersion was put under the UV light and monitored from different time intervals. At specific time intervals, aliquots of 2-3 mL suspension were filtered and used to evaluate the photocatalytic degradation of dye. Aliquots with a suspension of 2-3mL were filtered at specific time intervals and used for evaluating photocatalytic dye degradation. Percentage of dye degradation was estimated by using the formula

$$\%\text{ of dye degradation}=\frac{\text{Initial concentration of dye solution}}{\text{After degradation of dye solution}}$$

## 3. Results and Discussion

**3.1 UV-Visible spectral analysis:**

Silver nanoparticles were synthesized at different concentrations of leaf extract such as 1–5 mL using 1 mm of silver nitrate were analyzed by UV spectra of Plasmon resonance band observed at 436–446 nm similar to those reported in literature [16].

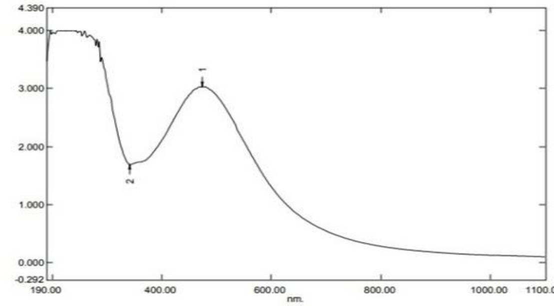

**Fig: 2.**UV-Visible spectra of TCLE-Ag NPs.

**3.2 FT-IR spectral analysis**

The reducing and capping agency of TCLE-Ag NPs existence of several functional groups was confirmed by FT-IR analysis. A broad band between 3600-3200 $cm^{-1}$ is due to the O-H stretching vibration of group $NH_2$ and OH the overlapping of the stretching vibration of attributed for water and *Tinospora cordifolia.* The prominent peaks shows at 2982 $cm^{-1}$due to C-H stretch, 1641 and 1631$cm^{-1}$due to C=O sharp and 1045 $cm^{-1}$due to H-C-H deformation extract molecules. The observed peaks are mainly attributed to flavonoids and terpenoids excessively present in *Tinospora cordifolia* leafextract.

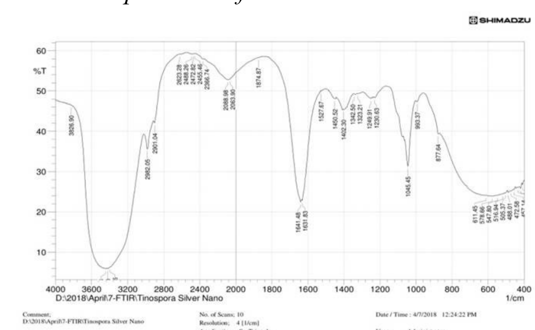

**Fig: 3.** FT-IR spectra ofTCLE-Ag NPs.

**3.3 High-resolution of scanning electron microscopy analysis:** The biosynthesized TCLE-Ag nanoparticles obtained by using HR-SEM technique explore that aggregated particles. However, the depth of focus is only 0.5 μm at ×1000 and the diffraction effects increase with small particles, which causes blurring at the edges in the determination of particles. The image, explains the silver nanoparticles size of *Tinospora cordifolia.*

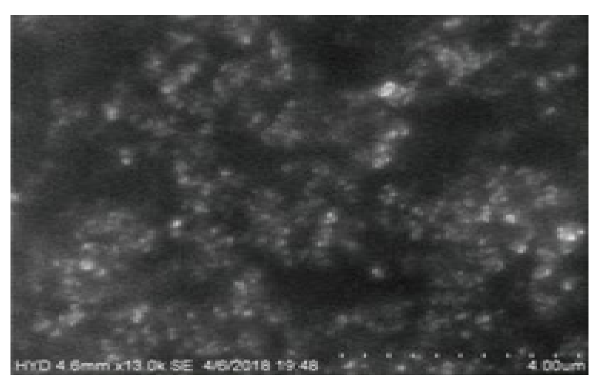

**Fig: 4.**HR-SEM image of TCLE-Ag NPs.

### 3.4. Biological activity studies

#### 3.4.1. Antibacterial activity:

The stabilized TCLE-Ag nanoparticles were screened for their antibacterial activity with Gram-positive (*S. aureus*) and Gram-negative bacterial strains (*P. aeruginosa, E. aero genes)* and by obtaining agar diffusion method and the results are outlined in the Table 1.

The zones of growth inhibition around the disk were measured after 18 to 24 hours of in incubation at 37°C for bacterial cultures.

The obtained results of TCLE-Ag NPs showed higher inhibition *S. aureus* with a zone of inhibition 34 nm and *E.coli*with zone of inhibition 30 nm with standard *streptomycin*, 23 nm on *S. aureus* and 26 nm on *E. coli*and $AgNO_3$20 nm on*S. aureus* and 15 nm on *E.coli* respectively. It was clearly that the synthesized TCLE-Ag NPs showed good antibacterial activity against comparing with TCLE, distilled water (DW), $AgNO_3$ and standard with bacterial strains.

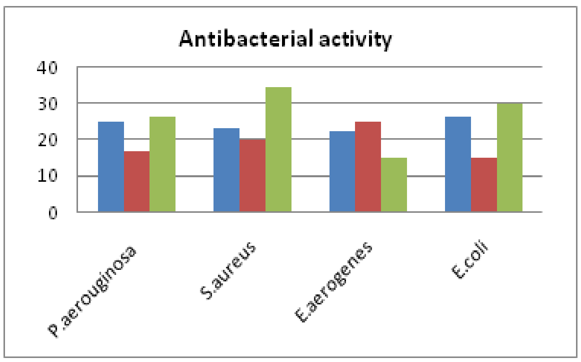


**Fig: 5.**Graph represents the percentage of antibacterial activity of TCLE-Ag NPs (green), $AgNO_3$ (red), *Streptomycin* (blue).

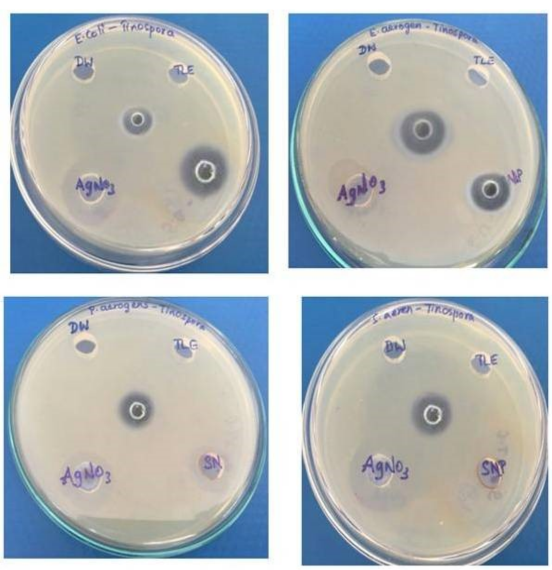

**Fig: 6.** Representative plates showing antibacterial activity of TCLE-Ag NPs (SNP) against different bacterial strains.

#### 3.4.2. Anti-fungal activity:

Antifungal activities of TCLE - Ag NPs against fungi *Aspergillus niger* was investigated by the agar disk diffusion method [15]. The determination of zone of inhibition, fungal strains was taken as a standard antibiotic for comparison of the results. All the extracts were screened for antifungal activities against *Aspergillus niger,* The 100 µg/mL concentrations of TCLE, DW, $AgNO_3$, TCLE-Ag NPs and *Streptomycin* was employed as standard were prepared in double-distilled water.

The zones of growth inhibition around the disks were measured after 48 to 96 hours for fungus at room temperature (28°C). The results of the extracts are reported in Table 2.

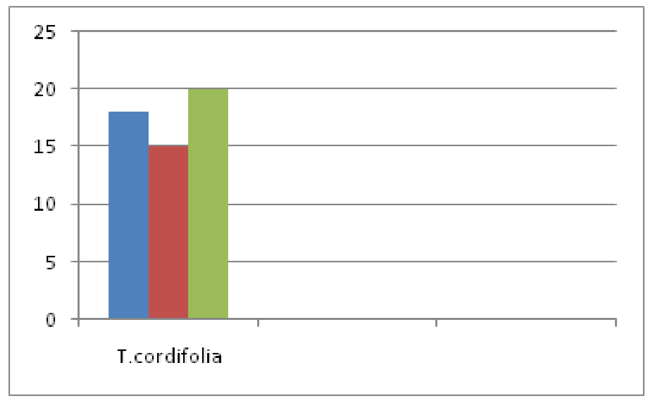


**Fig: 7.**Graph showing antifungal activity of TCLE-Ag NPs (green), $AgNO_3$ (red), *Streptomycin* (blue).

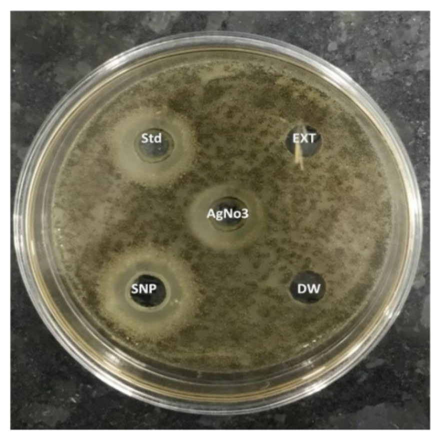


**Fig: 8.** Representative plate showing antifungal activity of TCLE-Ag NPs (SNP).

The synthesized TCLE-Ag NPs showed highest antifungal activity when compared with standard *Streptomycin* and $AgNO_3$. So the TCLE-Ag NPs exhibited potential activity on *A. niger* strain compared with standard.

#### 3.4.3. Antimicrobial efficiency of cotton fabrics:

The synthesized TCLE-Ag NPs are coated on cotton fabric material exhibit effective antimicrobial effect against microorganisms. The biological approach is a cost-effective method as compared with chemical synthesis. Further it is proved that the sliver nanoparticles coated cotton finally the

5g TCLE produces highest antimicrobial effect and release properties as compared with 3g and 4g leaf extracts. Due to the highest control release properties of this coating utilized for wound healing dressing.

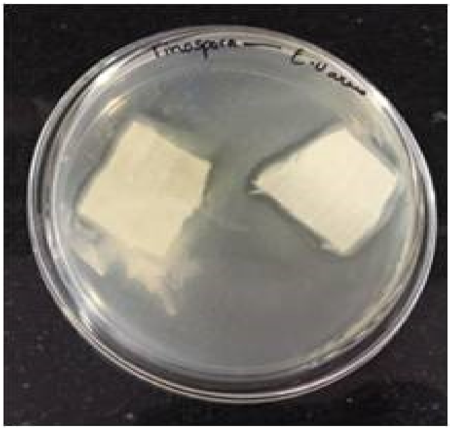

**Fig: 9.** Antimicrobial activity of silver nanoparticles synthesized from aqueous TCLE on cotton fabric material.

### 3.5. Photocatalytic dye degradation studies:

In UV light irradiation with Methylene blue had shown maximum degradation with 53.72 % at 60 min and minimum degradation 23.72 % at 0 min. Upon UV light irradiation with Congo red dye solution exhibited maximum photo degradation activity with 28.49% at 60 min and the minimum percentage degraded with 16.53% at 0 min was observed. Therefore, in the presence of UV light, TCLE-Ag NPs with Methylene blue dye showed maximum percentage degradation due to prominent absorption property.

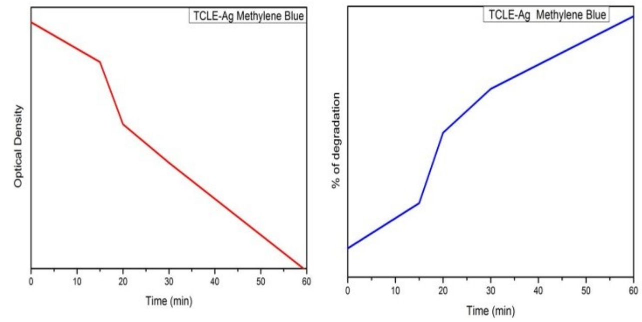


**Fig: 10**. Photocatalytic dye degradation kinetics of Methylene blue with TCLE-Ag NPs.

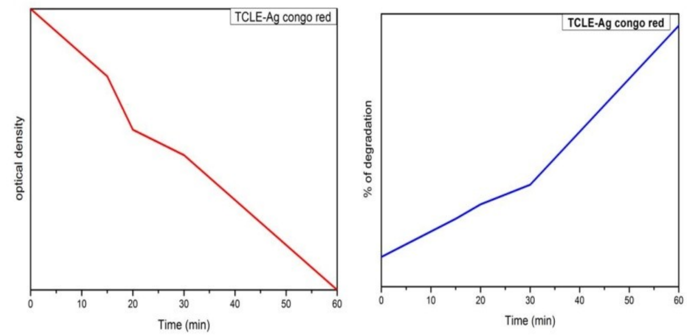


**Fig: 11.** Photocatalytic dye degradation kinetics of Congo red with TCLE-Ag NPs.

**Table: 1.**Theantibacterial activity and zone of inhibition of TCLE-Ag NPs (SNT), silver nitrate ($AgNO_3$), *Streptomycin* standard, distilled water, aqueous *Tinospora cordifolia* leaf extracts (TCLE) against organisms.

| **Organisms** | TCLE (EXT) | Distilled water (DW) | *Streptomycin* (standard) | $AgNO_3$ | TCLE-Ag nanoparticles (SNP) |
|---|---|---|---|---|---|
| Zone of inhibition in mm | | | | | |
| *P. aerouginosa* | 0 | 0 | 25 | 17 | 26 |
| *S. aureus* | 0 | 0 | 23 | 20 | 34 |
| *E.aerogenes* | 0 | 0 | 22 | 25 | 15 |
| *E. coli* | 0 | 0 | 26 | 15 | 30 |

**Table: 2.**The antifungal activity and zone of inhibition of TCLE-Ag nanoparticles (SNP), silver nitrate ($AgNO_3$), standard, distilled water, aqueous TCLE against organism.

| Organisms | TCLE | Distilled water (DW) | *Streptomycin*(standard) | $AgNO_3$ | TCLE -Ag nanoparticles (SNP) |
|---|---|---|---|---|---|
| Zone of inhibition in mm | | | | | |
| *Aspergillus niger* (100$\mu$g/mL) | 0 | 0 | 18 | 15 | 20 |

## 4. Conclusion

In this protocol, *Tinospora cordifolia* aqueous leaf extract (TCLE) was used successfully to synthesize silver nanoparticles. This method is simple, rapid, and cost-effective and carried out at room temperature, without any waste generation. Green synthesized TCLE-AgNPs further displayed promising antimicrobial and photocatalytic activities. The TCLE-Ag NPs shows superior to the antimicrobial activities against Gram positive microorganism (*S.aureus*) than Gram negative microorganisms. Furthermore, the photocatalytic study concludes that the TCLE-Ag NPs have adaptability to degrade Methylene blue and Congo red dyes under UV light .Consequently these can be highly effective in water purification in industries. Based on these results, it can be concluded that the green synthesized TCLE-Ag NPs can be served as a phenomenal functions for degraded dyes in industrial waste water pollutants and the kind of TCLE-Ag NPs may have potential applications in biomedical field.

## 5. References


[1] Khushboo Singh, ManjuPanghal, Sangeeta Kadyan, Jaya Parkash Yadav. Evaluation of Antimicrobial Activity of Synthesized Silver Nanoparticles using Phyllanthusamarus and Tinospora cordifolia Medicinal Plants, Journal of Nanomedicine & Nanotechnology.5(6); 1000250-1000255, 2014.

[2] Konishi Y, Ohno K, Saitoh N, Nomura T, Nagamine S, Hishida H, Takahashi Y, Uruga TBioreductive deposition of platinum nanoparticles on the bacterium Shewanella algae. Journal of Biotechnology. 128; 648–653,2007.

[3] Willner I, Baron R, Willner B Growing metal nanoparticles by enzymes. Advanced Materials.18; 1109–1120,2006.

[4] Husen A, Siddiqi KS. Photosynthesis of nanoparticles:concept, controversy andapplication. Nano scale research letters. 9(1);229, 2014.

[5] Husen A, Siddiqi KS. Plants and microbes assisted selenium nanoparticles: characterization and application. Journal of Nano biotechnology. 12(1);28,2014.

[6] Sharma P, Pant S, Rai S, Yadav RB, Dave V. Green synthesis of silver nanoparticle capped with Allium cepa and their catalytic reduction of textile dyes: an ecofriendly approach. Journal of Polymers and the Environment. 26 (5);1795-803, 2018.

[7] Ibrahim HM, Hassan MS. Characterization and antimicrobial properties of cotton fabric loaded with green synthesized silver nanoparticles. Carbohydrate polymers. 20(151);841-850,2016.

[8] Ullah N, Yasin S, Abro Z, Liu L, Wei Q. Mechanically robust and antimicrobial cotton fibers loaded with silver nanoparticles: synthesized via Chinese holly plant leaves. International Journal of Textile Science. 3(1);1-5,2015.

[9] Logeswari P, Silambarasan S, Abraham J. Synthesis of silver nanoparticles using plants extract and analysis of their antimicrobial property. Journal of Saudi ChemicalSociety. 19;311–317, 2015.

[10] Lokina S, Stephen A, Kaviyarasan V, Arulvasu C, Narayanan V. Cytotoxicity and antimicrobial activities of green synthesized silver nanoparticles. European Journal of Medicinal Chemistry. 76;256-263, 2014.

[11] Niraimathi KL, Sudha V, Lavanya R, Brindha P. Biosynthesis of silver nanoparticles using Alternantherasessilis (Linn.) extract and their antimicrobial, antioxidant activities. Colloids and Surfaces B: Biointerfaces.102;288-291, 2013.

[12] Wei L, Lu J, Xu H, Patel A, Chen Z-S, Chen G. Silver nanoparticles: synthesis, properties, and therapeutic applications. Drug discovery today. 20(5);595-601, 2013.

[13] Chandan Tamuly, Moushumi Hazarika, Manobjyoti Bordoloi, Pradip Kr. Bhattacharyya, Rahul Kar, Biosynthesis of Ag nanoparticles using pedicellamide and its photocatalytic activity: An eco-friendly approach. Spectrochimica Acta Part A: Molecular and Bimolecular Spectroscopy, 132, (11); 687-691, 2014.

[14] S. M. Ahmad, S. B. Hoot, P. H. Qazi & V. Verma.Phylogenetic patterns and genetic diversity of Indian Tinospora species based on chloroplast sequence data and cytochrome P450 polymorphisms.Plant Systematics and Evolution, 281; 87-96, 2009.

[15] N S Alzoreky, K Nakahara. Antibacterial activity of extracts from some edible plants commonly consumed in Asia. International Journal of Food Microbiology, 80(3); 223-30, 2003.

[16] A. Y. Obaid, Shaeel Ahmed Al-thabaiti, E.H. El. Mossalamy, Laila M, Al-Harbi, Zaheer Khan, Extracellular bio-synthesis of silver nanoparticles,Arabian Journal of Chemistry, 10 (2); 226-231, 2015.