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Green Synthesis and Characterization of Silver Nanoparticles using *Tinosopora Cordifolia* Extract and their Antimicrobial activity

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Abstract: In the present research, the synthesis of silver nanoparticles by the green method is done using stem and leaves aqueous extract of Tinospora cordifolia (T.C). The pathway of nanoparticles formation is by means of reduction of silver nitrate by extracts, which act as both reducing and capping agents. The silver nanoparticles characterized by UV-Vis-spectrometer, Fourier transform infra-red spectroscopy, X-ray diffractometer, Scanning electron microscopy, Energy dispersive spectroscopy. The sizes of the synthesized silver nanoparticles are found to be in the range of 27- 58 nm. The energy dispersive spectrum confirmed the presence of silver metal. The silver nanoparticles synthesized in this process have the efficient antimicrobial activity against pathogenic bacteria like Bacillus subtilis, Escherichia coli, Klebsiella pneumonia, proteus mirabilis, Staphylococcus aureus and Serratia marcescens using paper disc diffusion method.

Keywords: Tinospora cordifolia, Nano silver, FTIR, SEM, XRD, EDAX, and Antimicrobial.

INTRODUCTION

Nanotechnology is a rapidly growing science of producing and utilizing Nano-sized particles. Nanoscience research is expected today not because of only application and also by the way of synthesis [1]. The synthesis of noble metal nanoparticles attracts an increasing interest due to their new and different characteristics as compared with those of macroscopic phase, that allow attractive applications in various fields such as optoelectronics, biosensors, bio-nanotechnology, biomedicine etc. [2-8]. Various physical and chemical methods have been formulated for the synthesis of nanoparticles of desired shape and size. However, these methods are not economically feasible and environment friendly. Therefore, green synthesis has been considered as one of the promising methods for synthesis of nanoparticles because of their biocompatibility, non- toxic and eco-friendly nature [9].

In recent years, increasing antibiotic resistance by microbes is imposing a serious threat to the health sector. Nanoparticles have proved to be a likely candidate for an antimicrobial agent since their large surface to volume ratio ensures a broad range of attack on the bacterial surface. One of the most promising nanoparticles which act a highly effective antimicrobial agent is silver [10]. Various investigations on silver nanoparticles have been done to study its antimicrobial activity against *Eschericha coli*, *Staphylococcus aureus* and antifungal activity against *Trichophyton*, *Trichosporon beigelii* and *Candida albican* [11].

T.C is an important medicinal plant, belonging to family Menispermaceae a well-known plant of Indian medicinal system, is gaining more attention for electing a wide spectrum of pharmacological activities [12]. It is known for its general tonic, anti-diabetic, anti-allergic, anti-hyperglycaemic, anti-leprotic, and anti-cancer [13-14]. It improves the phagocytic and bactericidal capacity of polymorphs, protects against gastric mucosal damage and scavenges free radicals [15]. Since this plant has also been reported to possess anti-fibrotic, anti-oxidant, anti-inflammatory, immune modulatory, radio protective and activator of phagocytic and killing activity of macrophages [16].

The aim of the present study was to evaluate the use of *Tinospora cordifolia* extract as a reducing agent for silver nanoparticles formation. The characterization of the synthesized nanoparticles utilizing UV-Visible spectroscopy, Fourier Transform Infrared spectroscopy (FTIR), Scanning Electron Microscopy(SEM), Energy Dispersive X-ray spectroscopy (EDX), and

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X-ray Diffraction (XRD) analysis. Furthermore, the antibacterial activity of synthesized nanoparticles was investigated against different pathogenic bacteria.

EXPERIMENTAL SECTION

Preparation of stems and leaves of *Tinospora Cordifolia* extract

The fresh leaves and stems were treated thoroughly three times with water followed by deionized water and the water is removed. The sample is kept for drying. The fresh leaves and stem were cut into small pieces and were taken a quantity of 25g with the help of electronic balancing scale. Then small pieces were dried in air and a fine powder was made by using pestle and mortar. The powdered material was packed in a separate container until extraction was done. 25g of powdered plant material was weighed and mixed with 100ml sterile autoclaved water and boiled for 15 minutes. Then the material was filtered through Whatmann No.1 filter paper and the extract was prepared. The prepared extract was maintained at 4° C for further investigations.

Green Synthesis of silver Nanoparticles

For the green synthesis of silver nanoparticles, 10 ml of plant extract was added to the aqueous solution of 1 mM (10^{-3}M) silver nitrate (240 ml) into 250 ml Erlenmeyer flask and kept at room temperature. The solution was stirred for 3 min. A change in colour was observed after mixing plant extract and silver solution.

RESULTS AND DISCUSSION

Visual Observation and UV-Vis Spectroscopy

Fig.1 depicts that silver nanoparticles were synthesized by using a green method through reduction of silver nitrate ($AgNO_3$) solution by plant extract. After the addition of plant aqueous extract of T.C to the $AgNO_3$ solution (1mM/L), a change in colour was observed from yellow to dark brown. The brown colour indicated the green synthesis of silver Nano particles as shown in Fig.1. UV-Vis spectroscopy is an important technique used to confirm the formation of metal nanoparticles in an aqueous solution. As shown in Fig.2 UV-Vis absorption spectrum of the produced silver nanoparticles showed an absorption peak at 437 nm due to excitation of Surface Plasmon Vibration in nanoparticles. Generally, silver nanoparticles having absorbance values which are reported earlier in the visible range of 436-446 nm [17].

SEM and EDX Analysis

The green synthesized silver nanoparticles were characterized by SEM and EDAX analysis. The surface morphology and size of the particles were determined by SEM. It was noted that the particles were predominantly spherical in shape. The particles other than the spherical shaped were also present. The average sizes of nanoparticles are found to be 27 to 58 nm (Fig. 3). The different sizes of particles may be correlated with variable shapes. EDX spectrum reveals strong signal in the silver region and confirms the formation of silver nanoparticles. It is well known that silver nanoparticles show typical optical absorption peak approximately at 3 KeV due to Surface Plasmon Resonance [18]. Fig.4. showed the absorption peak at 3 KeV regions which revealed that nanoparticles were formed exclusively highest proportion of silver with crystalline nature.

XRD Spectrum Analysis

The crystalline nature of silver nanoparticles was confirmed by the analysis of XRD pattern as shown in Fig.5. The diffraction peaks at 2θ values of 38.0°, 44.1°, 64.3°, and 77.3° could be attributed to (111), (200), (220) and (311) planes of pure silver nanoparticles indicating the green synthesis of silver nanoparticles. These peaks were due to the organic compounds which are present the extract and responsible for silver ions reduction and stabilization of resultant nanoparticles [19]. The XRD pattern obtained was consistent with earlier reports [20].

FTIR Analysis

The FTIR spectra were recorded to identify the possible biomolecules responsible for the reduction of the Ag^+ ions and capping of the bio-reduced silver nanoparticles synthesized by the *Tinospora Cordifolia* leaf and stem extract. Active functional groups in the synthesized silver nanoparticles are confirmed in the spectrum as shown in Fig.6. The band at 3696.27 cm^{-1} corresponds to O-H stretching of alcohols and phenolic compounds [21-22]. A similar peak was absorbed in 2922.82 cm^{-1} that could be assigned to C-H stretching vibration of the methyl methylene, and methoxy groups. The peak at 1643.31 cm^{-1} corresponds to -C = O stretching. At 1035 cm^{-1} a peak was observed that could be for plants axis bed to C=C groups. The carbonyl group of the amino acid residues has the stronger ability to bind metal indicating that the proteins could prevent the molecules to be in clusters and stabilize silver nanoparticles in the aqueous medium [23].

Antimicrobial Efficacy

The Silver ions, as well as silver nanoparticles, were known to have strong antimicrobial activities [24-27]. The antimicrobial activity of silver nanoparticles synthesized from leaves and stem of *Tinospora cordifolia* aqueous extract was investigated against various pathogenic organisms such as *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia, Proteus mirabilis, Staphylococcus aureus* and *Serratia marcescens* using Whatmann No.1 filter paper disc diffusion technique of 10 µg/disc concentrations. Gentamycin was used as a standard for comparison. The average antibacterial activity of silver nanoparticles against microbial strains ranged from 17 to 26 mm (Table 1). The maximum activity of silver nanoparticles is found for *Staphylococcus aureus* with 26 mm zone of inhibition. The results are good agreement with earlier reports [28]. Fig. 7a-f show the zones of inhibition of silver nanoparticles against *Bacillus subtilis* (a) *Escherichia coli* (b), *Klebsiella pneumonia* (c), *Proteus mirabilis*(d), *Staphylococcus aureus*(e) and *Serratia marcescens* (f). Researchers have proposed different mechanism accounting for the antibacterial effect of silver nanoparticles, however, the mechanism showing the action of silver nanoparticles is still unclear. Also, it should be noted that the antimicrobial activity of Nano silver depends on particle size [29], preparation method etc. The smaller

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size of the silver nanoparticles with the spherical shape can have good antimicrobial efficacy [30-32]. Finally, the results of this study indicated that the Nano- sized silver produced by *Tinospora cordifolia* showed excellent antimicrobial activity.

CONCLUSION

The silver nanoparticles were green synthesized using stem and leave extract of *Tinospora Cordifolia*. The synthesized nanoparticles were spherical, 27-58 nm in size, crystal in nature and showed absorption spectrum at 437 nm characterized by using different techniques. This green synthesis approach appears to be a non- toxic, cost effective, simple and eco-friendly alternatively to the conventional methods and would be suitable for developing a biological process for large scale production. Green synthesized silver nanoparticles are found to have enhanced antimicrobial activity against different pathogenic bacteria. Due to the enhanced antimicrobial activity of silver nanoparticles, it is effectively used in the field of medicine as well as in food and cosmetic industries.

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Table 1 Assay of Antimicrobial Activity

S.No	Bacteria	Zone of inhibition (mm in diameter)		
		Control	Standard	Sample
1	*Bacillus subtilis*	-	18	21
2	*Escherichia coli*	-	20	22
3	*Klebsiella pneumonia*	-	18	20
4	*proteus mirabilis*	-	14	17
5	*Staphylococcus aureus*	-	16	20
6	*Serratia marcescens*	-	22	26

*** Gentamicin (10 mcg)**

Fig.1. Change in colour from yellow to dark brown indicating the green synthesis of Silver nanoparticles of Tinospora *Cordifolia.*

a→ Aqueous Silver nitrate solution, b→ Aqueous Silver nitrate solution + Plant extract after 6 Hours c → Aqueous Silver nitrate solution + Plant extract after 12 Hours. d → Aqueous Silver nitrate solution + Plant extract after 24 Hours.

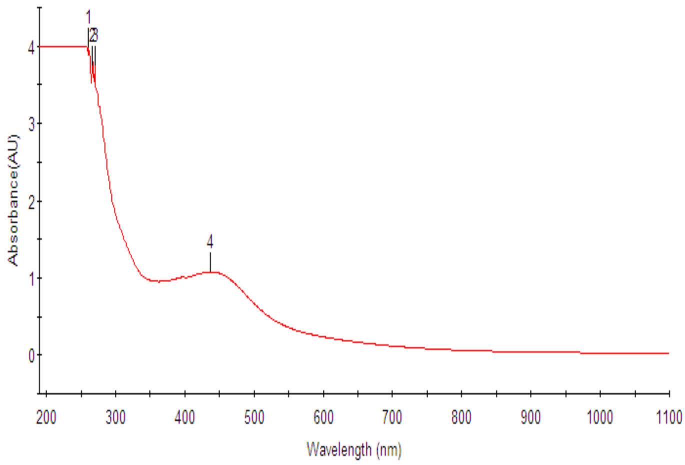

Fig.2.Uv-Vis absorption spectra of silver nanoparticles of Tinospora *Cordifolia*

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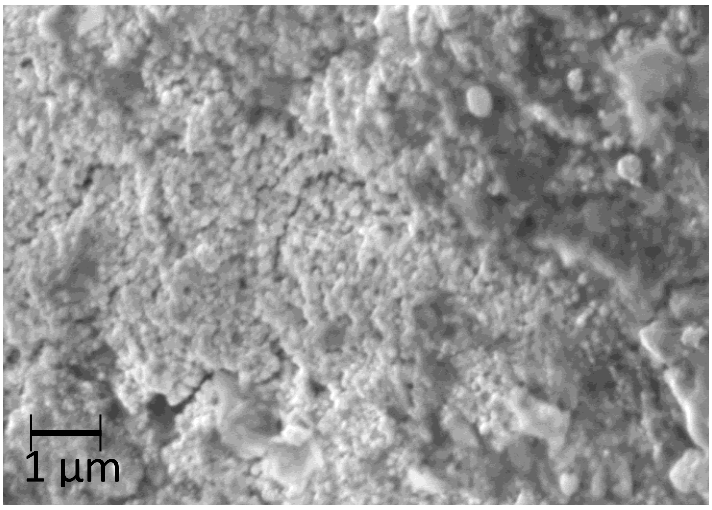

Fig.3. SEM images of silver nanoparticles of Tinospora *Cordifolia.*

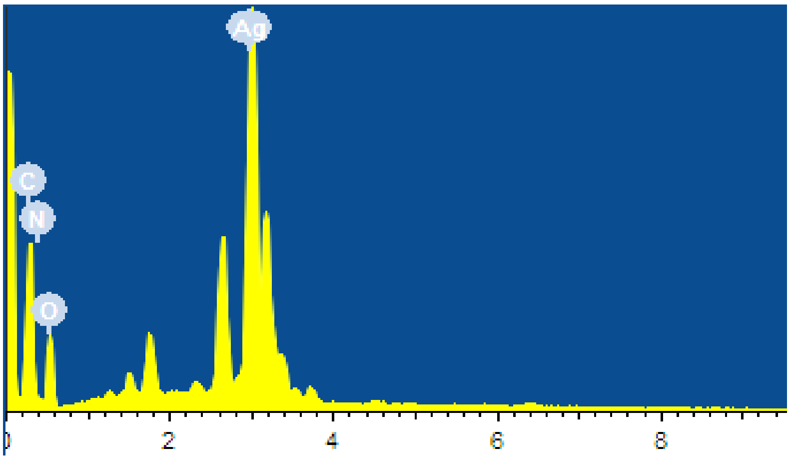

Fig.4. EDX images of silver nanoparticles of Tinospora *Cordifolia.*

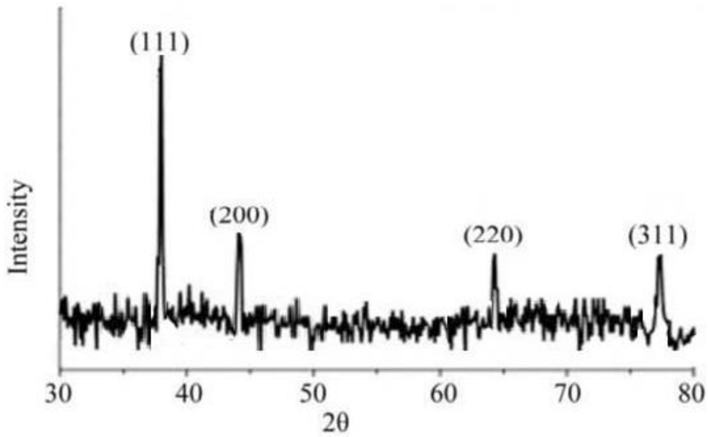

Fig.5. XRD pattern of green synthesised silver nanoparticles of Tinospora *Cordifolia*

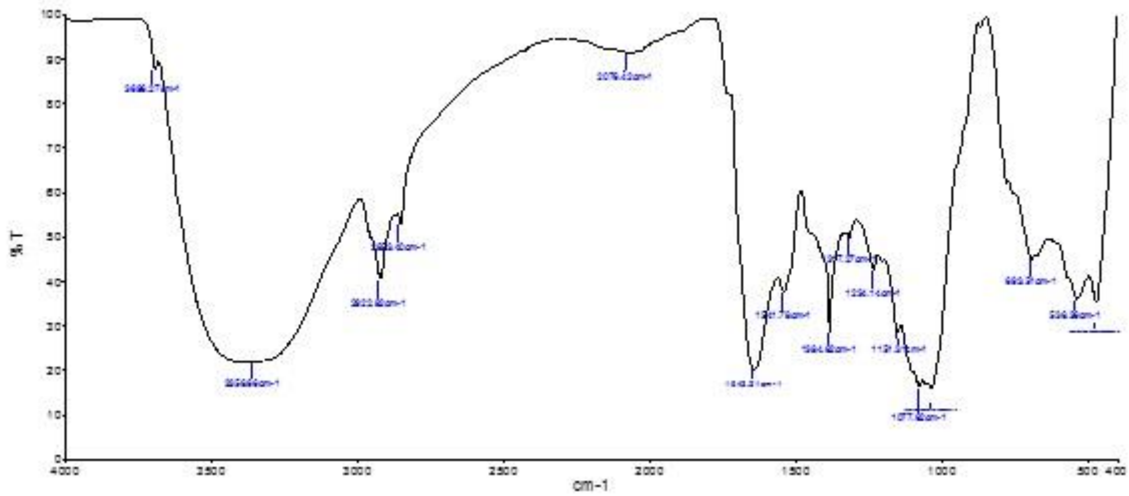

Fig.6.FTIR Spectra Representing the Functional Groups Associated with the reduction and Stabilization of Silver Nanoparticles of Tinospora *cordifolia*

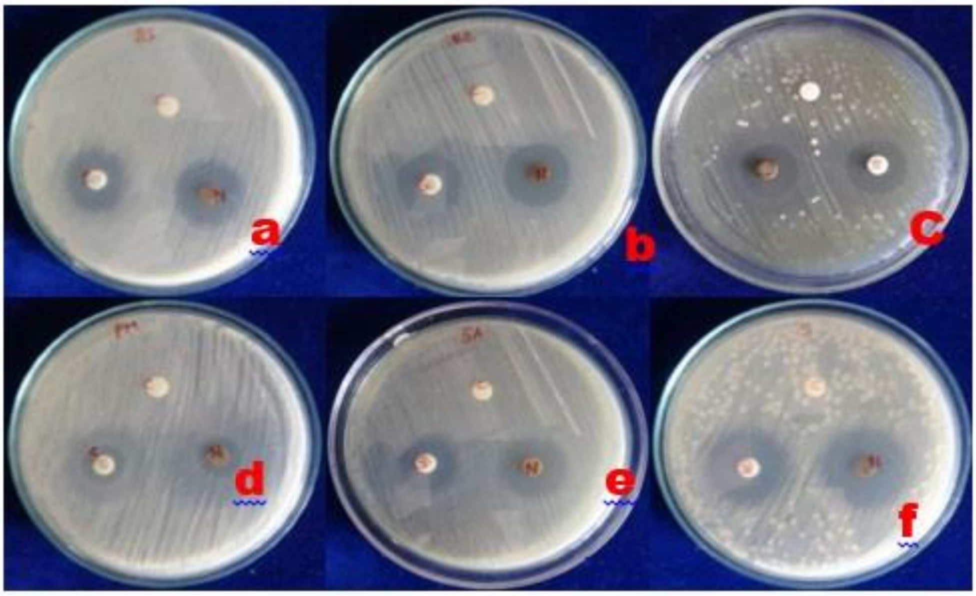

Fig.7. Antimicrobial activities of silver nanoparticles against a) *Bacillus subtilis,* b) *Escherichia coli,* c) *Klebsiella pneumonia,* d) *proteus mirabilis,* e) *Staphylococcus aureus* and f) *Serratia marcescens*.