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Synthesis of silver nanoparticle using *Tinospora cordifolia* and its characterization and beneficial effects on wastewater treatment

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ABSTRACT
Nanotechnology is one of the recent upcoming areas of research in the field of modern material science that has gained more interest among researchers. Recent advances in themanipulation of nanomaterials have facilitated the application of nanotechnology in water and wastewater treatment. In the present study, the green synthesis of silver nanoparticles using aqueous extracts extracted from Tinospora cordifolia was performed and further the synthesized nanoparticles were characterized using different characterization techniques like UV-vis., FE- SEM (Field emission - Scanning Electron Microscope) with energy-dispersive X-ray (EDX or EDAX), and FT-IR (Fourier Transform Infrared Spectroscopy) techniques. The catalytic competence of the synthesized nanoparticles was tested using the antibacterial and antioxidant activities employing the agar well diffusion method. In addition, the biocidal activity of silver nanoparticles was exploited for water disinfection and has been an evident study for thesmall scale employment of green synthesized silver nanoparticles in wastewater remediation.
Keywords: Tinospora cordifolia, Silver nanoparticles, Wastewater, Heavy metal, Nanotechnology

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INTRODUCTION

Nanotechnology is one of the rapidly growing technologies that have been developed by producing nanoproducts and nanoparticles (NPs) that can have novel and size-related physicochemical properties differing significantly from larger matter [1]. The innovative properties of NPs have been implemented in a wide range of potential applications in various fields such as medicine, cosmetics, renewable energies, environmental remediation and biomedical devices [2-4]. Though there are many nanoparticles produced to date, silver nanoparticles (Ag-NPs or nanosilver) have engrossed increasing interest due to their unique physical, chemical and biological properties [5].
Ag-NPs have distinctive physical-chemical properties, including a high electrical and thermal conductivity, surface-enhanced Raman scattering, chemical stability, catalytic activity and nonlinear optical behaviour [6]. These properties make them of potential value in inks, microelectronics, and medical imaging [7]. Besides, Ag-NPs exhibit broad spectrum bactericidal and fungicidal activity [8] that has made them extremely popular in a diverse range of consumer products, including plastics, soaps, pastes, food and textiles, increasing their market value [9-11].
Water is one of the most important substances on Earth and is essential to all livingthings. About 70% of the Earth is covered with water, but only 0.6% is suitable for human consumption. Safe drinking water is an important health and social issue in many developing countries [12]. The WHO survey showed that at least 1 billion people worldwide do not have access to drink safe water. Contamination of drinking water and the subsequent outbreak of waterborne diseases are the leading cause of death in many developing nations [13]. The silver-based NPs are very ideal for use in water disinfection. The silver-based NPs can be incorporated into core materials and polymeric membranes to disinfect the water contaminated with bacteria and viruses. The application of silver-based NPs is of utmost importance to prevent outbreaks of waterborne diseases related to poor treatment of drinking water.
Tinospora cordifolia (Thunb.) Miers is an important medicinal plant that belongs to the family Menispermaceae [14] that is obtaining more attention for employing a wide spectrum of pharmacological activities. The botanical description of the plant shows that it is a large, deciduous broadly spreading climbing shrub with several elongated twining branches. Leaves of the plant are simple, alternate,

exstipulate, long petioles that are measured up to 15 cm long, roundish, pulvinate, alternatively seen at the base and the apex with the basal region seen longer and twisted partially [15]. Flowers of the plant are unisexual, small on separate plants that appear when it is leafless, greenish- yellow on axillary and terminal racemes. The male flowers are clustered and the female flowers are usually solitary [16].
The fruits of the plant aggregate to be of 1-3cm, ovoid smooth drupelets on a thick stalk with subterminal style scars, scarlet or orange coloured. It is well known for its anti-cancer, anti- leprotic, and general tonic, anti-hyperglycemic, anti-allergic and anti-diabetic properties [17, 18]. It improves the phagocytic and bactericidal capacity of polymorphs by protecting them against gastric mucosal damage and scavenges free radicals [19]. The fruits of the plant develop during the winter season and appear fresh, smooth.

MATERIALS AND METHODS

PLANT COLLECTION

The *T. cordifolia* fruits were collected fresh from the foothills of the Yercaud regions of Salem district. The collected plant fruit said above was shade dried and powdered to produce 107.8g of the plant extract as a fine powder.

GREEN SYNTHESIS OF SILVER NANOPARTICLES

The plant *T. cordifolia,* was chosen selectively and analyzed for its useful morphologies and the leaves were collected and were used for the silver nanoparticle synthesis, respectively, which was prepared using the traditional biological or the green synthesis protocol [20]. 99ml of the 1% silver nitrate ($AgNO_3$) solution was added with the 1ml of respective pre-dissolved and filtered plant extracts. The observations of colour change from a transparent, colourless solution to deep a brownish yellow colour formation for the confirmation of the plant-silver nanoparticle synthesis completion were also made and were confirmed using the spectra analysis at 400 - 570nm in the UV-spectrophotometer system using the samples collected at various time intervalsduring the synthesis procedure.

CHARACTERIZATION OF NANOPARTICLES

The synthesized *T. cordifolia* nanoparticles (TcAgNPs) of the collected parts of the plant prepared were checked for the presence of various functional groups using the Fourier Transform-Infrared Radiation (FT-IR) characterization tool and the size, morphology and the silver nanoparticle confirmations were made by the Field Emission-Scanning Electron Microscope (FE-SEM), Energy Dispersive Analysis of X-rays (EDAX) characterization tools.

WASTEWATER TREATMENT ANALYSIS

PREPARATION OF TCAGNP

The TcAgNP Ag-NP suspension of 200 mg Ag/L was prepared by dispersing Ag-NPs in filtered (0.22 µm) wastewater and vortexing for 30 s at the maximum speed.
Organic matter is mainly composed of compounds such as proteins, carbohydrates, and fats, biodegradable organics that are measured in terms of biochemical oxygen demand (BOD) and chemical oxygen demand (COD). If there is a presence of any untreated discharge to the environment, its biological stabilization can cause the depletion of natural oxygen sources andthe quality of freshwater in available sources leading to freshwater contamination [21]. The collected tap water and wastewater was taken in each separate 500 ml in a round-bottom flask.
The pre-prepared green synthesized $AgNO_3$ leaf extract was added into the 500 ml beakers containing the tap water and wastewater with a concentration rate of 5 ml and the tap water and the wastewater respectively with the concentrations approximately set to around 10 ml in the round bottom flask. A set of three experiments was conducted for calculating the approximate results. The flasks were initially stirred with the nanoparticle pellets and mixed for around 15 minutes and then heated up to 45 minutes using a water bath at 70° C. After heating, thesamples were drawn at regular intervals of every 5 minutes and were checked for pH, TDS, total hardness, BOD, COD as per APHA (American Public Health Association) standards.
BOD or biodegradable soluble Chemical Oxygen Demand (bsCOD):
The bsCOD of the effluent was calculated the following formula,

$$S = \frac{K_S\left(1 + k_d\,(SRT)\right)}{(SRT)\left(Yk - k_d\right) - 1}$$

where, S = effluent soluble substrate concentration (bsCOD), SRT = Sedimentation Retention Time, Ks = half-velocity constant, kd = endogenous decay coefficient, Y = biomass yield, k = maximum specific soluble substrate utilization rate, fd = fraction of biomass that remains as celldebris.
Similarly, the TDS is calculated by the following formula:

mg Dissolved Solids/L = (A-B) X 1000mL Sample
Whereas the Total hardness of the samples were calculated using the formula, Water hardness (mg/L) ＝Ca (mg/L) × 2.497 ＋Mg (mg/L) × 4.118

RESULTS AND DISCUSSION

UV-VISIBLE SPECTRA

The synthesized TcAgNPs retrieved at various time intervals such as 5min, 10 minutes,
15 minutes, 20 minutes and 25 minutes from light yellowish-brown to deep dark brown colouration show the results are given in Table 1 and the spectra confirm the presence of AgNPs in the synthesized TcAgNPs mixture. The observed colour change, with the corresponding single peak absorbance spectra was due to characteristic vibrations as a result of changes in the electronic energy levels of metal nanoparticles [22]. The reactions were relatively fast, showing that the phytochemicals in *T. cordifolia* such as alkaloids, glycosides and terpenoids were adequate for the AgNPs formation [23]. The biomolecules acted as both the reducing andcapping agents for the newly formed $AgNO_3$ [24].

Table 1 Synthesis of TcAgNPs retrieved at various time intervals and their OD values

Time (minutes)	Optical density value at 420nm	Colour change
5	0.247	Pale yellowish-brown
10	0.531	↓
15	0.743	
20	2.118	
25	2.972	Deep yellowish-brown

FT-IR SPECTRAL ANALYSIS

The pellets retrieved after the centrifugation at 5000rpm for 30 minutes of green synthesized TcAgNPs were dried and analyzed for the FT-IR spectra and Figure 1 depicts a clear picture of the results. It is confirmed that to identify the bio-molecules for reduction of the metal nanoparticles in the TcAgNPs show the band at 3367 cm^{-1} of O-H stretch and H- bonds, 2926 cm^{-1} of alcohols and phenols with shifts from 3460 cm^{-1} and 3168 cm^{-1} seen for the crude plant extract in line with Premasudha *et al.* studies [25]. These results suggest that the molecules perform the dual functions of formation and stabilization of silver nanoparticles.

The previous study of Peter Amaldhas *et al.* [26] has studied the biogenic synthesis of AgNPs from the leaf extract of *Cassia angustifolia.* The findings of the results proved that the extract which contains the largest number of biomolecules, especially sennosides. These sennosides are responsible for the conversion of silver to AgNPs. In the present study, the extract which contains various bioactive compounds among these some compounds may be responsible for the production of AgNPs.

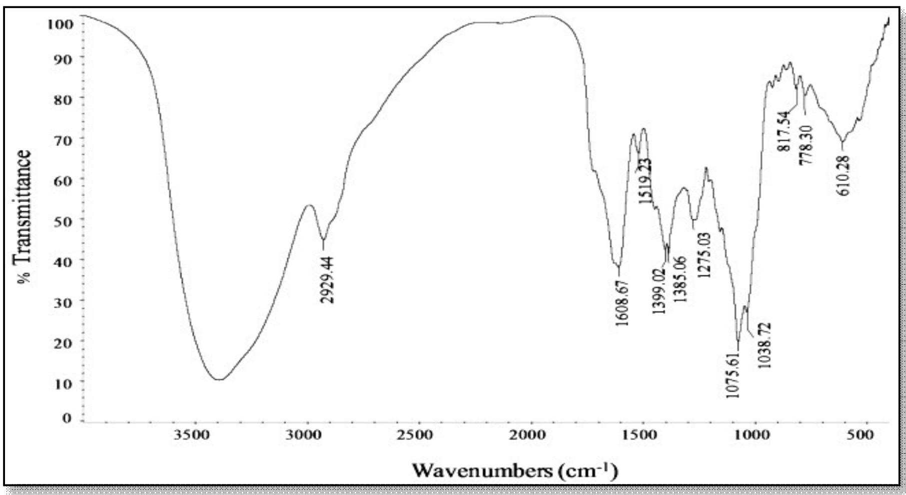

Figure 1. Showing the various peak values seen for the *Tinospora cordifolia* NPs in the FT-IR analysis spectrum

FE-SEM AND EDAX ANALYSIS

The TcAgNPs were checked for their structural confirmations to be 108 nm using the FE-SEM analysis and are shown in Figure 2 and the EDAX of the sample was also characterized and is given in Figure 3 respectively. Recently the studies are given by Dakeshwar *et al.* [27] on the green synthesis of silver nanoparticles from *F. vulgare* plant extract also show similar results of sphere-shaped nanoparticles in SEM analysis [27].

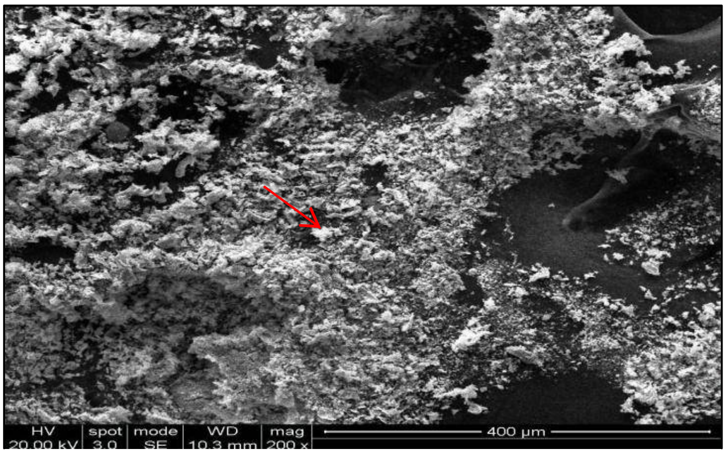

Figure 2. Showing the three dimensional surface characterization of the *Tinosporac ordifolia* NPs under the Scanning electron Microscope (SEM) specified with spherical shaped NPs characterized

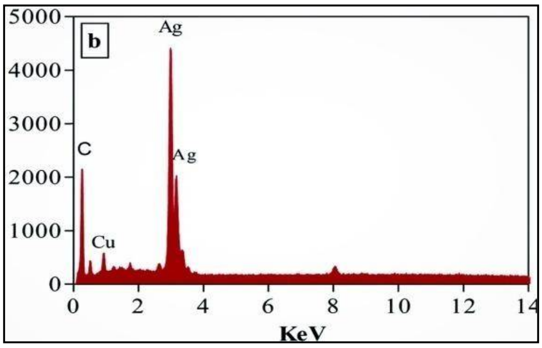

Figure 3. Showing the key compounds present in the *Tinosporac ordifolia* NPs analyzed under theEDAX characterization system

ADSORPTION ANALYSIS FOR TREATED WASTEWATER PRODUCTS

A recent study showed that the chem-Ag-NPs were successfully formed macroporous methacrylic acid copolymer beads that can be used for the disinfection of water [28]. This showed that the chem-Ag-NPs formed on these copolymer beads by the chemical reduction method were stable underwater washing and their stability was due to the interaction of the chem-Ag-NPs with the -COO− carboxylic functional group on the copolymer beads. Polymeric microspheres containing chem-Ag-NPs displayed highly effective disinfection against two gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and two gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*). The chem-Ag-NPs bound copolymer beads were used and performed efficiently in bringing down the bacterial count to zero for all the tested strains. The bacterial adsorption/adhesion tested revealed that copolymer beads containing chem-Ag-NPs do not haveany adsorption/adhesion of bacterial cell.

Similarly, a new class of polyethersulfone (PES) hybrid ultrafiltration membranes bending with modified halloysite nanotubes (HNTs) loaded with the chem-Ag-NPs for water purification was reported [29]. The results of antibacterial activity tests showed that the hybrid membrane had a good antibacterial property, and the antibacterial rates against ***Escherichia coli*** and *Staphylococcus aureus* were about 99.9 and 99.8%, respectively. Noticeably, this novel hybrid ultrafiltration membrane was observed to exhibit both organic antifouling and antibacterial properties by the addition of the chem-Ag-NPs. The pH, TDS, total hardness, BOD, COD were calculated for the effluents of Tap water and wastewater before the TcAgNPs treatment and after the treatment for three consecutive days.

Table 2 shows the complete data of adsorption analysis for all the above parameters for both the tap water and the wastewater effluents taken.

Table 2 Adsorption analysis of water samples

	Tap Water			Wastewater			Standard Limits		
	Day 1	**Day 2**	**Day 3**	**Day 1**	**Day 2**	**Day 3**	**Day 1**	**Day 2**	**Day 3**
pH	7.9	7.75	7.63	6.8	6.92	7.4	7.4	7.43	7.52
(Total Dissolved Solids) TDS mg/l	3000	3030	3070	3500	3479	3562	3000	2535	2321
(Biological Oxygen Demand) BOD mg/l	370	367	390	450	428	416	59	37.5	35.7
(Chemical Oxygen Demand) COD mg/l	483	499	500	500	497	481	150	142	137
Total Hardness	618	599	567	720	690	650	310	290	270

CONCLUSION

The chronic effluent exposure of the wastewater contaminants shows a wide range of hazardous effects on the environment. The present study depicts that the TcAgNPs show a well-built antibacterial and effluent degradation capacity compared to that of the standard carriedout by the control $AgNO_3$ solution. These results confirm earlier findings that suspended cells are more susceptible to Ag+ inhibition than intact biofilms and this moreover proves that the plant extract apart from the fact of showing an evident antibacterial effect from the past studies also has a positive effect on wastewater effluent treatment from the current study and its data interpretations.

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