Scope

Volume 14 Number 1 March 2024

Bio inspired Synthesis of Silver Nanoparticles from *Terminalia Chebula* Leaves Extract and Evaluation of their Antibacterial Activity

Mandheer Kaur [1*], Deepak Sharma[1], Chaitnyajit Singh[1], Palki Sahib Kaur [1]

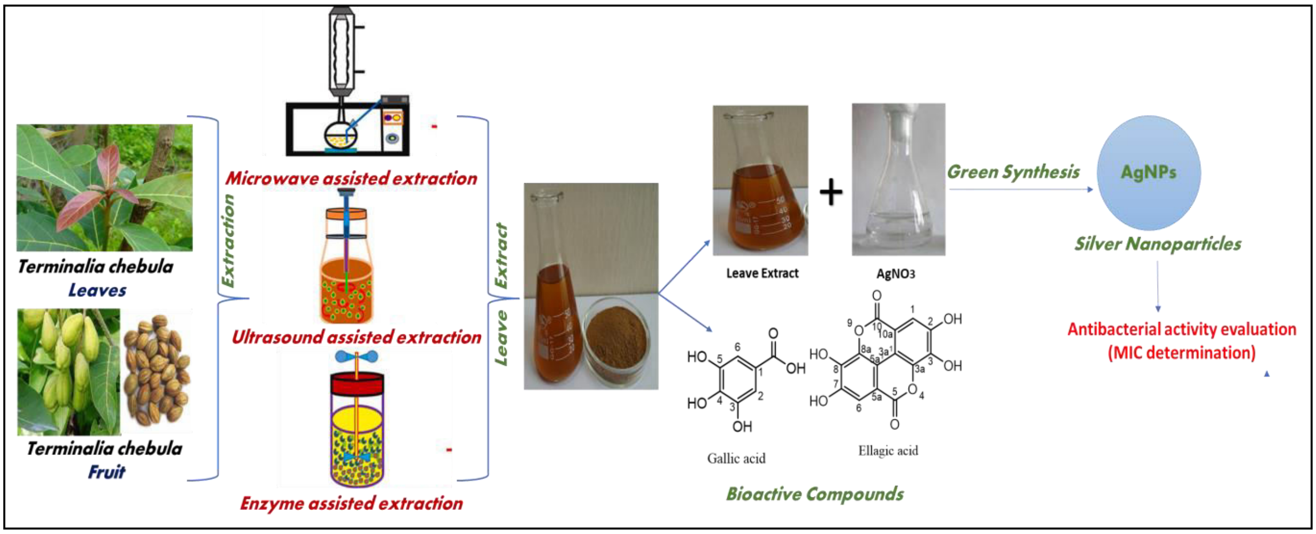
[1]Department of Biotechnology, Chandigarh College of Technology, Landran, Mohali-140307 Punjab, India

Corresponding author: **Dr. Mandheer Kaur**
Orcid ID**:** 0000-0002-6803-099X

Abstract

Metallic nanoparticles have unique properties that make them an effective medication delivery mechanism. Their shape, size, particle distribution, and volume to surface area ratio set them apart from other compounds and elements. Recently, there has been a significant focus on the potential antibacterial and therapeutic properties of metallic nanoparticles. This paper presents a methodology for the production of silver nanoparticles (TC_AgNPs) by a green approach, utilising an aqueous extract derived from the leaves of *Terminalia chebula (T. chebula)*. The extract serves the dual purpose of reducing and stabilising the nanoparticles. The confirmation of TC_AgNPs synthesis was achieved by the observation of a brownish-yellow colour, and this was afterwards corroborated by using UV-Visible spectroscopy. The spectroscopic analysis indicated the presence of a single absorption peak at a wavelength of 420 nm. The synthesised TC_AgNPs were subjected to ATR analysis in order to identify the functional groups present on the surface. Additionally, zeta analysis was performed to quantify the particle size and distribution. The extract of *T. chebula* leaves was subjected to screening for a range of phytochemicals, including flavonoids, alkaloids, terpenoids, amides, and aldehydes. The study of zeta values indicated that the mean size of TC-AgNPs was 58.12 nm, with a zeta potential of -30.2 mV, suggesting a favourable level of stability. The antibacterial investigation demonstrated the efficacy of TC-AgNPs against both Gram-negative Escherichia coli MTCC 452 and Gram-positive Staphylococcus aureus MTCC 737. The determination of the minimum inhibitory concentration (MIC) was conducted in order to evaluate the efficacy of TC-AgNPs. Overall, our findings demonstrate the eco-friendly synthesis of TC-AgNPs with remarkable antibacterial activity, making them a promising candidate for further development as a therapeutic agent.

Keywords: Green synthesis, Silver nanoparticles, *Terminalia chebula*, Antimicrobial activity



Graphical Abstract: Illustration of synthesis of silver nanoparticles of *Terminalia chebula* and evaluation of antibacterial activity.

Introduction

Since ancient times, people have utilised the tree Terminalia chebula, which belongs to the Combretaceae family, for medicinal reasons. The "king of medicine" is well-known for its extensive usage in Unani, homoeopathic, and Ayurvedic medicine. There is a chance that the tree will grow to be between fifty and eighty feet tall, with a circular canopy and spreading branches. The specimen's leaves are elliptic and oblong in shape, with two conspicuous glands located close to the tip of the petiole. Its bark is a dark brown colour with longitudinal cracks. This plant produces monoecious flowers that range in hue from drab white to yellow and have a terrible smell. Usually, these flowers bloom all of the time. spanning from May to June. On the other hand, the fruits of this plant, which are around 1-2 inches in diameter, are generated between the months of July and December. The fruits of this plant are small, round berries that start off green and gradually turn a deep purple or black when ripe. These berries are highly sought after by birds and other wildlife, who help disperse the seeds. The plant's overall appearance is quite striking, with its tall stature, distinctive leaves, and pungent flowers making it easily recognisable in its natural habitat. Terminalia chebula, a plant species, is often seen in the Sub-Himalayan region, spanning from Ravi towards the east to West Bengal and Assam. It thrives at elevations of up to 1,500 metres in the Himalayas. Terminalia chebula, often known as *T. chebula*, is extensively used in the management of hypercholesterolemia and gastrointestinal ailments such as diarrhoea, constipation, and indigestion. Additionally, it has been used in the treatment of human immunodeficiency virus (HIV) infections. Furthermore, this natural treatment has traditionally been used to treat a variety of diseases, including asthma, sore throat, vomiting, hiccough, diarrhoea, dysentery, bleeding piles, ulcers, gout, heart issues, and bladder disorders. The plant has significant medicinal properties and has been shown to Numerous pharmacological and medicinal effects, such as antioxidant, antimicrobial, antidiabetic, hepatoprotective, anti-inflammatory, antimutagenic, cardioprotective, radioprotective, antiarthritic, anticaries, gastrointestinal motility, and wound healing activity (4-6), have been demonstrated for the plant. Additionally, studies have shown that the plant exhibits potential anticancer properties and may help in the prevention and treatment of certain types of cancer. Furthermore, the plant's extract has also been found to possess neuroprotective effects, making it a promising candidate for the treatment of neurological disorders such as Alzheimer's disease and Parkinson's disease.

Terminalia chebula (T. chebula) is characterized by a high concentration of tannins and phenolic compounds, as well as other components such galloyl glucose, corilagin, terflavin AA, punicalagin, and triterpenemaslinic acid, as well as anthraquinones. Numerous hydrolyzable tannins, such as chebulagic acid, punicalagin, neochebulinic acid, corilagin, chebulanin, ellagic acid, 1,6-dio-galloyl-D-glucose, chebulinic acid, 3,4,6-trio-galloyl-D-glucose, 1,2,3,4,6-penta-O-galloyl-βD-glucose, terchebulin, and casuarinin have all been identified and extracted from the fruits of this plant. Moreover, a variety of compounds have been demonstrated to be present in Terminalia chebula, including laxatives such amino acids, fructose, beta-sitosterol, resin, succinic acid, and ananthraquinone. In addition, it has triterpenoids, flavonol, gallic acids conjugated with coumarin, glycosides, and phenolic compounds. Arjunin, chebulosides I and II, arjunin, 2α-hydroxymicromiric acid, and chebuloside 2α-hydroxyursolic acid are among the many triterpenoid glycosides. Additionally, it is comprised of flavonol, triterpenoids, glycosides, gallic acids that are conjugated with coumarin, and phenolic chemicals. Several triterpenoid glycosides, including arjunin, 2α-hydroxyursolic acid, chebulosides I and II, 2α-hydroxymicromiric acid, and arjunglucoside, have also been isolated from Terminalia chebula. These compounds contribute to the various medicinal properties exhibited by the plant, such as antioxidant, anti-inflammatory, and anticancer activities [(7–16)].

The discipline of materials science has put considerable emphasis on the concept of "green" synthesis, which entails a sustainable and ecologically conscious approach to the production of diverse materials and nanomaterials. This includes the synthesis of metal and metal oxide nanoparticles, hybrid materials, and bioinspired materials[17]. This approach offers an alternative to traditional nanoparticle synthesis methods commonly used in laboratory and industrial settings that have destructive effects. In this study, we investigate the processes and mechanisms involved in green synthesis techniques, focusing on silver (Ag) nanoparticles synthesized using natural extracts. We also conduct stability and toxicity studies on the nanoparticles and implement surface engineering techniques to achieve biocompatibility. Finally, we discuss the potential applications of the synthesized products, including their active phytochemical ingredients and antimicrobial activity.

Materials and Methods

Collection of plant material

After the T. chebula leaves were collected while they were still fresh and healthy, they were shade-dried and ground into a coarse powder using an electric grinder. This powder was then dried and sieved again through a fine plastic sieve to obtain a fine powder, which was stored in an airtight container for later use in the laboratory.

Microorganisms

The strains of bacteria, Staphylococcus aureus (MTCC 737) and Escherichia coli (MTCC 452), were obtained from the Institute of Microbial Technology (IMTECH) located in Chandigarh. These strains were grown in a typical growth medium, and the cultures' slants were produced and kept for later use at 4 °C.

Preparation of *T. chebula* leaf extract

Ten grams of finely powdered T. chebula leaf powder and one hundred milliliters of deionized water were combined to create the T. chebula extract. Following this, the mixture was subjected to magnetic stirring while being heated to 100°C in a temperature-controlled water bath for 15 minutes. The material was centrifuged for 15 minutes at 10,000 revolutions per minute (rpm) after it had been chilled. The resultant extract was then stored at 4°C for later use after being run through cellulose nitrate membrane filter paper with a pore size of 0.2 µ.

Biosynthesis and Characterization of TC_AgNPs

One milliliter of T. chebula leaf extract was combined with nine milliliters of a one-millimeter aqueous solution of silver nitrate (AgNO3). The reaction mixture was adjusted to have a pH of 8 by adding a 0.1M NaOH solution. Two hours later, there was a dramatic color shift in the reactant-containing solution, which went from being brilliant yellow to dark brown. This color change indicates that the silver nanoparticles (AgNPs) were successfully created. Using a UV-Vis spectrophotometer (1900i Shimadzu Spectrophotometer), the resultant TC_AgNPs were examined by obtaining a UV-Vis spectrum ranging from 300 to 800 nm. Additionally, FTIR analysis using a JASCO FT-IR 4100 spectrometer was used to examine TC_AgNPs in order to identify the functional groups. TC_AgNPs were analyzed using SEM (JEOL, USA) to assess their size and shape. Additionally, the Malvern panalytical zeta potential was used to assess the stability of the produced particles. A distinctive peak at about 420 nm was visible in the UV-Vis spectra of TC_AgNPs, signifying the effective synthesis of silver nanoparticles. The surface of TC_AgNPs had functional groups like carboxyl and amine, as shown by the FTIR study. The TC_AgNPs' spherical form and average size of 50 nm were revealed by SEM examination. With a zeta potential of -20 mV, the zeta potential measurement showed that the produced particles were stable.

Antimicrobial analysis and MIC determination

Mueller Hinton Broth (MHB) was used to test the bactericidal activity of TC_AgNPs (1 mg/ml) against E. Coli (ATCC 25922) and S. aureus (ATCC 25923). The CSLI standards were followed in order to calculate the minimum inhibitory concentration (MIC) of TC_AgNPs using the methodology outlined by Sharma and Chaudhary 202119. Furthermore, resazurin dye, an indication of bacterial cell proliferation in the study, was also used to measure the killing time of TC_AgNPs.

Results and Discussion

TC_AgNPs were created using an aqueous extract of T. chebula leaf powder extract (10%) and silver nitrate (1 mM) at pH 8 (Fig. 1a-c). The silver nitrate and plant extract's light yellow color changed to a dark brown hue as a result of plant molecules reducing Ag+ ions into Ag0 (Fig. 1d). To create TC_AgNPs, Ag+ ions were reduced using an aqueous extract. The T. chebula leaf powder extract contains a number of significant biological components, including alkyl halides, aromatic compounds, and phenolics (20–23). This extract could help TC_AgNPs by acting as a capping and stabilizing agent. By examining the UV-Vis spectra, it was possible to confirm the synthesis of TC_AgNPs because the maximum absorption was the detection of nanoparticles at 410 nm in wavelength. Previous studies have reported the synthesis of silver nanoparticles using a variety of plant extracts. The fact that the peak absorbance of silver nanoparticles is about 400 nm has also been highlighted by these experiments. [20–21.] As a result, the present TC_AgNPs synthesis agrees with the published reports.

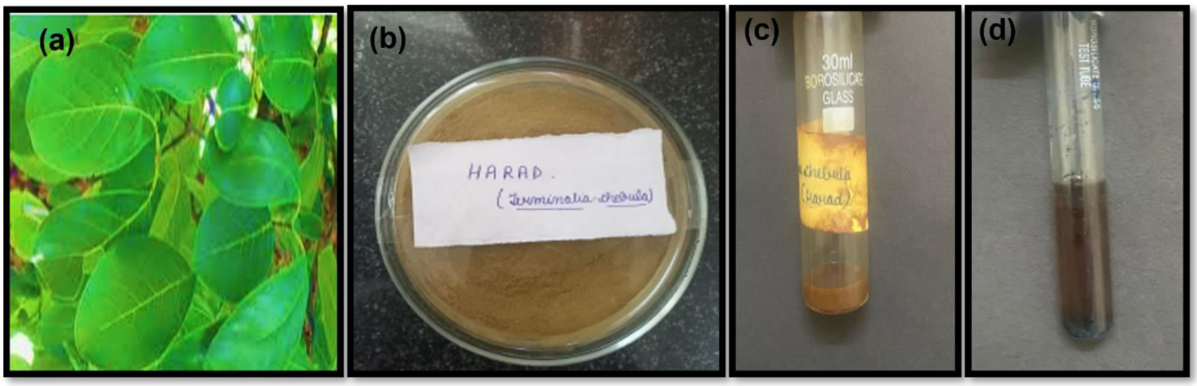


Fig. 1: Images of (a) *T. chebula* leaf (b) *T. chebula* leaf powder (c) *T. chebula* leaf extract (d) Synthesized TC_AgNPs

Characterization of TC_AgNPs

The greatest absorption spectra of TC_AgNPs were found at 410 nm by UV-vis spectroscopy analysis (Fig. 2). It has been discovered that the aqueous extract of the leaves and roots of Berberis vulgaris produces silver nanoparticles with an absorbance peak at around 450 nm. Similarly, Lysiloma acapulcensis-synthesised silver nanoparticles showed absorbance maxima at 400 nm21. Similarly, absorption maxima have been reported for silver nanoparticles produced using Tectona grandis seed extract at around 440 nm22.

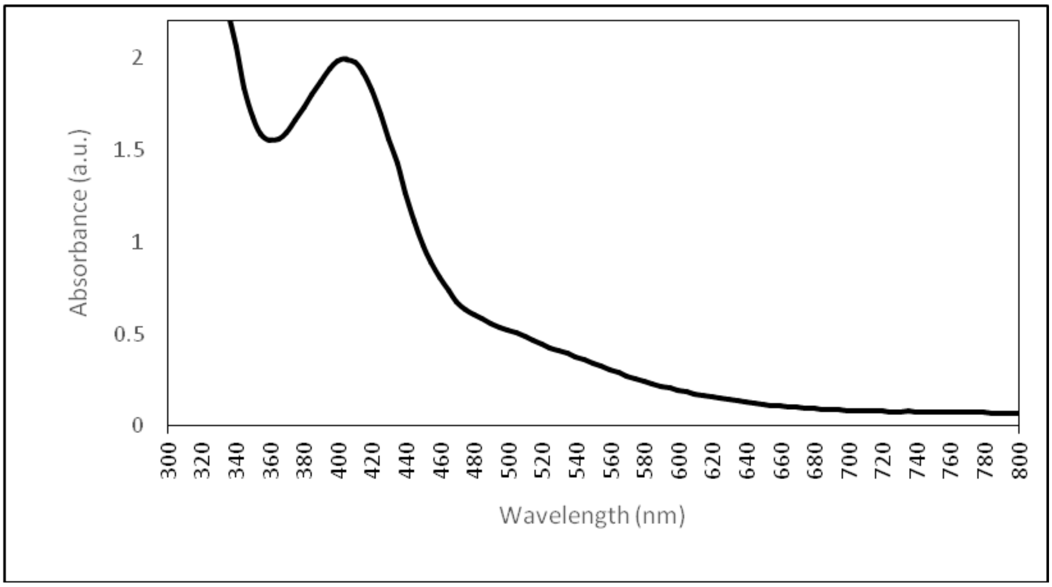


Fig. 2: UV-Vis spectrum of synthesized TC_AgNPs

The FTIR spectra of the TC_AgNPs were recorded to identify functional groups present on the nanoparticles. The transmission peaks at 798, 897, and 1637 cm1 were believed to be of the N-H, C-C, C-OH, C-H (ring), C-CO, and C-O functional groups, respectively, of the plant extract (Fig. 3a). Moreover, the OH of plant metabolites and the aqueous solution were responsible for the peak at 3323 cm1 (Fig. 3a). Extant publications [21, 23] provided strong support for the FTIR analysis results. The shape of TC_AgNPs was found to be spherical, and their average size was around 48±2 nm, according to SEM examination (Fig. 3b). It was also discovered that the silver nanoparticles produced using Berberis vulgaris leaf extract had a spherical shape and were around 50 nm in size[20]. The form and size of the silver nanoparticles produced by Melia azedarach leaf extract were found to be spherical, with a nanoscale of 15–30 nm[21].

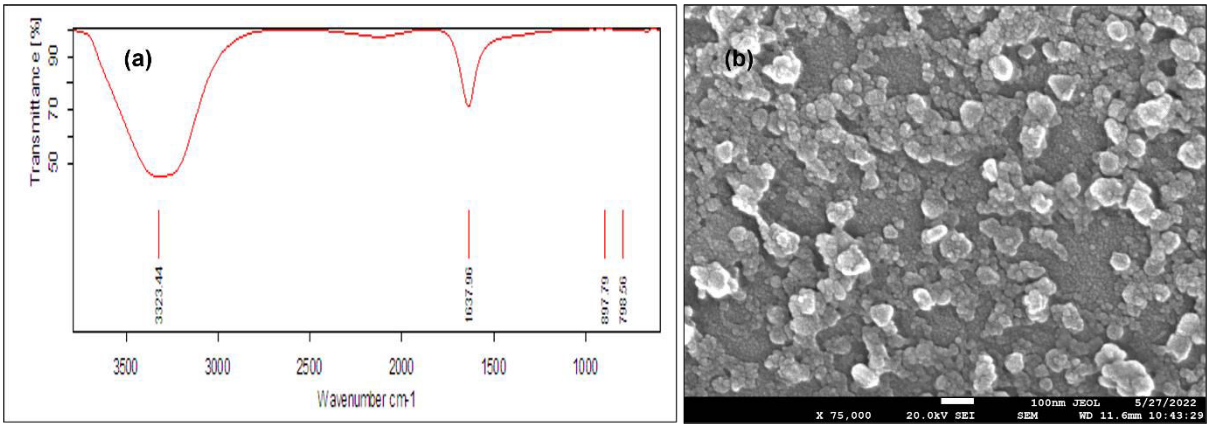


Fig. 3:TC-AgNPs(a) ATR analysis (b) SEM analysis

Zeta potential measurement is a crucial metric that affects the electrical potential of nanoparticles by characterizing their surface charge. Both the sign and the amplitude of the zeta potential are largely determined by the type of particles and the medium in which they are suspended. Silver nanoparticles that have a zeta potential over 33 mV or below -33 mV are generally considered to be very stable nanoparticles. The zeta potential of TC-AgNPs was measured as part of the current inquiry, and the result was a value of -30.2 mV. This finding implies that, under the given experimental settings, the particles show stability. The results obtained suggest that the TC-AgNPs have a negatively charged surface, indicating a propensity for interparticle repulsion. This repulsive process is essential for keeping TC-AgNPs from aggregating and, as a result, increases their stability in a suspension medium. The results of this investigation are consistent with previous studies that have demonstrated a relationship between the stability of silver nanoparticles and their zeta potential[(20–25).] According to the previously stated studies, nanoparticles with higher zeta potentials are more stable and less likely to clump together in the suspension medium. Therefore, our work's reported zeta potential of -30.2 mV for TC-AgNPs offers empirical support for the particles' stability within the given experimental settings.

Antimicrobial activity and MIC

Using the disc diffusion method at a working concentration of AgNO3 and T. chebula leaf extract, the antimicrobial impact of TC-AgNPs generated by aqueous extracts of T. chebula leaf extract was measured and compared with manufactured TC-AgNPs. Table 1 mentions the same outcomes. Antimicrobial analysis was conducted using kanamycin discs (30 µg/disc) as the reference. The findings demonstrated that TC-AgNPs had a synergistic antibacterial effect against S. aureus (MTCC 737) and E. coli (MTCC 452). TC-AgNPs demonstrated a minimum inhibitory concentration of 80 µg/ml against E. coli (MTCC 452) and 85 µg/ml against S. aureus (MTCC 737), respectively.

Table 1: Antimicrobial activity analysis by disc diffusion assay.

Microorganism	*T. chebula* leaf extract (working conc.)	$AgNO_3$ (working conc.)	TC_AgNPs (100µl of 1mg/ml)	Kanamycin (30µg/disc)
E. coli (MTCC 452)	8.5±1 mm	12.0±1 mm	21.0±1 mm	23.5±1 mm
S. aureus (MTCC 737)	9.0±1 mm	12.2±1 mm	19.5±1 mm	23.0±1 mm

A comparison study was carried out to evaluate the antibacterial effectiveness of commercially obtained nanoparticles against six harmful bacterial strains versus silver nanoparticles synthesized using saffron extract. The findings showed that neither the saffron extract nor the commercially available nanoparticles showed any appreciable antibacterial activity. On the other hand, the biologically synthesized nanoparticles demonstrated the capacity to successfully hinder the development of every bacterium that was investigated[26]. The antibacterial activity of silver nanoparticles produced in an environmentally friendly manner with Pedalium murex leaf extract was also demonstrated against a range of bacteria, including S. aureus, E. coli, Klebsiella pneumoniae, Mariniluteicoccus flavus, Pseudomonas aeruginosa, Bacillus subtilis, and Bacillus pumilus [(20–27).] A different study assessed the susceptibility of L. acapulcensis extract-derived silver nanoparticles to four pathogenic bacteria, and the results showed that E. coli ≥ S. aureus ≥ P. aeruginosa >C. albicans were the most sensitive to the nanoparticles[21].

Conclusion

The study created environmentally friendly silver nanoparticles by utilizing a water-based solution of T. chebula leaves and then evaluated the antibacterial activity of the particles. All things considered, the results show that using T. chebula leaf extract to produce silver nanoparticles is a safe, economical, and non-hazardous method that has no negative effects on the environment[28]. The manufactured nanoparticles showed significant antibacterial properties and could be a good substitute for traditional medicines in the treatment of bacterial illnesses. Nonetheless, further cytotoxicity and in vivo studies are needed to support the results that have been found thus far.

Acknowledgement

The Chandigarh Group of Colleges has been a great help and source of support for the authors.

Conflicts of Interest

The authors say they have no conflicting agendas.

Funding Source

There was no funding provided to the author or authors for their research.

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