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Research Paper

Green synthesis of silver nanoparticles from fruit extracts of *Terminalia chebula* Retz. and their antibacterial activity

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Abstract

In this investigation, the phytochemical analysis of components present in the fruit extract of *Terminalia chebula* was done with standard protocols. The aqueous and methanolic fruit extracts were prepared and silver nanoparticles were synthesised reacting with silver nitrate solutions. The synthesis of silver nanoparticles was confirmed by change of the color of the solution from yellowish brown to dark brown indicates reduction of silver ions in presence of plant extract. The ultraviolet - visible spectroscopy of the aqueous medium containing silver nanoparticles showed absorption peak at 358nm and 450 nm. The FTIR analysis showed the important groups involved in the extract. The scanning electron microscopy analysis of the silver nanoparticles indicated that the particle size were in the range of 90-120nm. The antibacterial activity of methanolic and aqueous extracts with biological synthesized nanoparticles of *T. chebula* against bacterial species like *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae*, *Bacillus subtilis, Streptococcus* sp. and *Salmonella* sp. was performed. The aqueous extract containing silver nanoparticles found to be more resistant to all the microorganisms tested compared to methanolic and aqueous extracts.

Keywords: *Terminalia chebula,* SEM, Silver nanoparticles, FTIR.

Introduction

The plant resources of our country have made a good contribution to the development of ancient Indian Material Medica. All the wild plants used in one or the other way in the treatment of human diseases in traditional, folklore, Unani or Ayurvedic systems. Nanomaterials are seen as solution to many technological and environmental challenges in the field of medicine. In the context of global efforts to the continuously increasing demand of nanomaterials must be accompanied by green synthesis methods. It has been reported that silver nanoparticles are non toxic to humans and most effective against bacteria, virus and other eukaryotic microorganisms at low concentrations and without any side effects [1]. The bactericidal properties of silver nanoparticles are due to the release of silver ions from the particles, which confers the antimicrobial activity [2]. *Terminalia chebula* is a medicinally important plant that belongs to the family Combretaceae. The whole plant contains a number of medicinally important compounds used in various dysease tretments. *T. chebula* possesses antibacterial, antifungal, antiviral, antidiabetic, antimutagenic, antioxidant, antiulcer and wound healing properties; prevents cardiac damage, used for the treatment of kidney disease. It is one of the constituent of Ayurvedic preparation 'Triphala'. *T. chebula* is distributed in the forests of northern India, Uttar Pradesh, and Bengal and is common in Tamil Nadu, Karnataka and Southern Maharashtra [3]. Phytochemical investigations of *T. chebula* have been reported on the presence of tannins, carbohydrates, glycosides, phenols, alkaloids, terpenoids and flavonoids [4]. Biological synthesis of metal is a traditional method and the use of plant

extract has a new awareness for the control of diseases, pathodenic organisms, beside safe and no phytotoxic effects [5, 6].

Materials and Methods

Collection and preparation of extract from the fruits: Fruits of *Terminalia chebula* Retz. were collected from Shobhavana at Mijar, Moodbidri, Karnataka. The matured fruits from selected plants were collected, cleaned and it was allowed to shade dry for about 30 days. Then it was kept in hot air oven at 60°C for about 48 -72 hours until it was dried completely. The dried fruits grounded to fine powder.

Preparation of aqueous and methanolextract: Each of five gramssample was taken with 50 ml of distilled water to get aqueous extract of the sample and 25ml of methanol to get methanol extract of the sample, this was mixed properly and covered with aluminum foil. Then it was kept in water bath at a temperature of 50-60°C for 7-8 hours.The mixture was allowed to pass through Whatman No. 1 filter paper and the filtrate was transferred to a clean weighed china dish. This was kept in water bath, till all the filtrate evaporated leaving behind a film of plant residue. The residue was dissolved in 10 ml of distilled water to obtain aqueous extract and methanol extract of each plant sample. The extract was storedat -20°C to prevent the loss of bioactive compounds until further use.

Phytochemical screening of the plant extract: Phytochemical tests were carried out on the aqueous and methanolic extracts to identify the constituents like saponins, tannins, alkaloids, terpenoids and resins using standard protocols.

Synthesis of silver nanoparticles: For the synthesis of silver nanoparticles, 350 ml of 1mM aqueous silver nitrate was prepared to which 3g of fruit powder was mixed and centrifuged at 2000rpm for 30 minutes. The supernatant were collected and heated at 95°C for 2 hours. A change in the color of the solution was observed. The extracts were stored at 4°C for further use.

Characterization: The characterizations of synthesized silver nanoparticles were made to understand the characteristic wavelength, to recognize the functional groups bound to the silver and the size of the nanoparticles by using Ultraviolet-visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscope (SEM) respectively.

UV-VIS spectra analysis: The reduction of pure Ag^+ ions was monitored by measuring the UV-VIS spectrum of the reaction medium at 5 hours after diluting a small aliquot of the sample indistilled water using UV-VIS spectrophotometer.

Fourier Transform Infrared Spectroscopy (FTIR): It was used to recognize the functional groups bound to the silver surface and involved in the formation of silver nanoparticles. The lyophilized powder sample was used and examined by Infra red (IR) spectrum at the spectral range at 1000-4000 cm^{-1}.

SEM analysis of silver nanoparticles: Scanning Electron Microscopic analysis was done using SEM machine. Thin films of the sample was prepared on a carbon coated copper grid by dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid was allowed to dry by putting it under a mercury lamp for 5 minutes.

Antimicrobial screening of the crude extracts:

Antibacterial activity test by Agar well diffusion method: To examine the bactericidal effect of aqueous, methanolic and silver nanoparticle, the well method was employed on the bacterial culture used.The extracts of the plant at different concentrations viz 50µl, 100µl and 150µl were dispensed into the wells. A negative control of water is kept into the one well and Penicillin G was taken as a positive control in another well. Extract of aqueous, methanolic and silver nanoparticle were allowed to diffuse for 30mins at room temperature. The plates were incubated at 37°C for 24 hours and the zones of inhibition were measured.

Results

Both the methanolic and aqueous fruit extract showed positive result for terpenoids, resins and saponins whereas it showed negative result for tannins and alkaloids (Table1). The *T. chebula* fruits extract

reacted with silver ions and the reaction mixture colour changes from yellowish brown to dark brown (Fig. 1).

Table 1: Phytochemical analysis for methanol and aqueous extract of *T. chebula*

Chemical constituents	Aqueous extract	Methanol extract
Tannins	-	-
Alkaloids	-	-
Terpenoids	+	+
Resins	+	+
Saponins	+	+

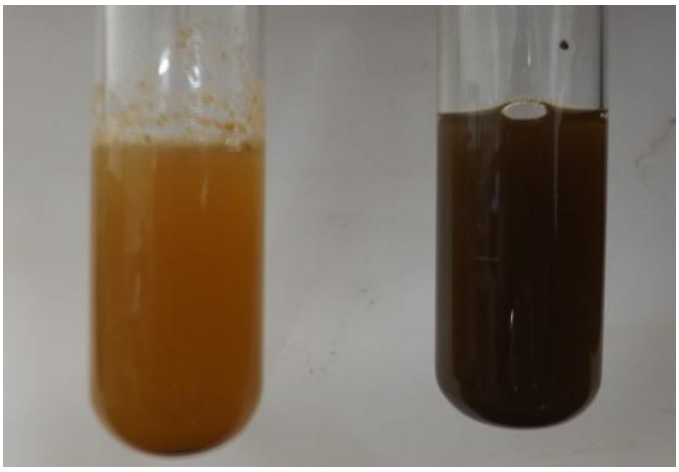

Figure 1: Synthesis of silver nanoparticles confirmed by change in color

Figure 2 indicates the two absorption peaks of synthesised silver nanoparticle at 358 nm and 450 nm in the fruit extracts.

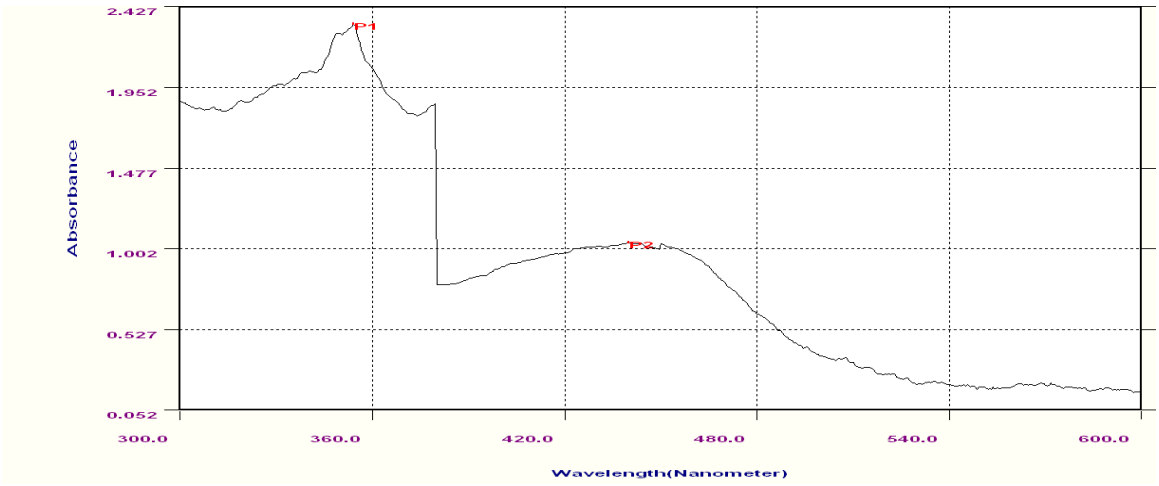

Figure 2: Absorption spectra of silver nanoparticles of *T. chebula* fruit extracts

The absorption peaks were observed at 3415.63 cm^{-1}, 2928.87 cm^{-1}, 1647.38 cm^{-1}, 1559.30 cm^{-1}, 1442.00 cm^{-1}, 1376.63 cm^{-1} and 1315.32 cm^{-1} can be assigned as absorption bands of –NH group of amines, -OH group of phenols, C-H aromatic stretch of groups, -NHCO group of amides and C-Cl functional groups (Fig. 3). The size of the synthesized silver nanoparticles in the present study was in the range of 90-120 nm (Figure 4).

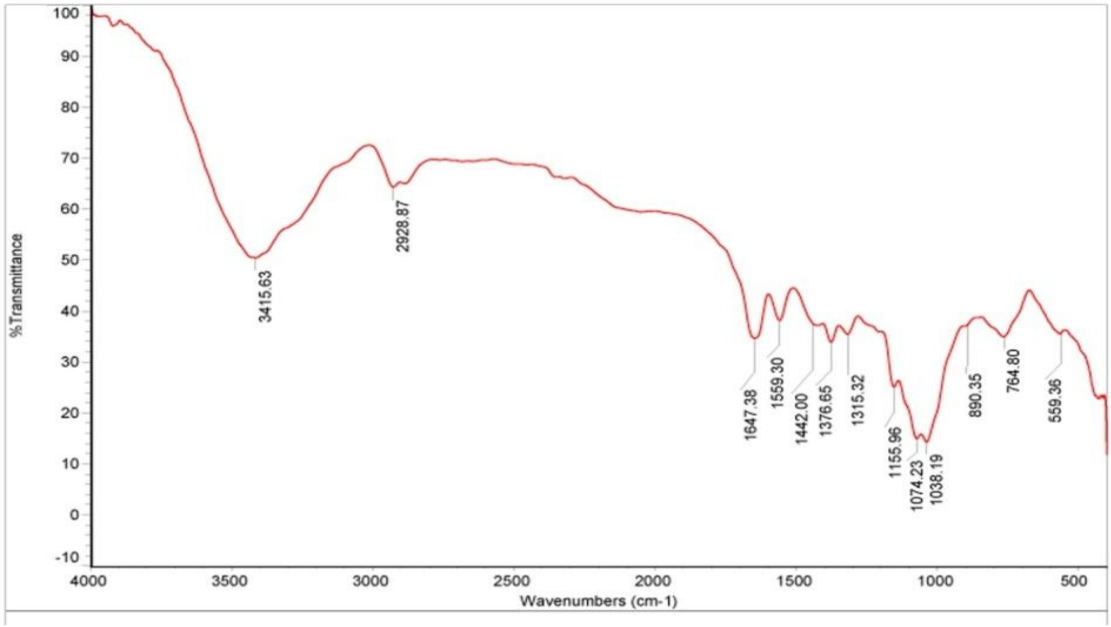

Figure 3: Absorption peaks for various functional groups

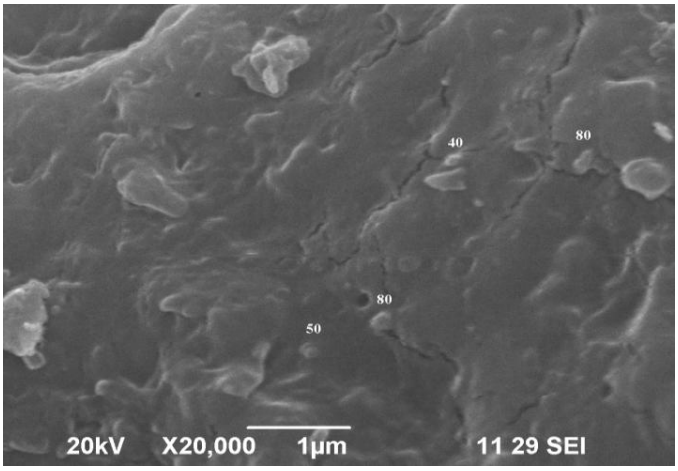

Figure 4: Synthesized silver nanoparticles

Table 2: Antibacterial activity of aqueous extract of the *T. chebula* for different concentrations

Organisms	Zone of inhibition (mm) for different concentration of aqueous extract				
	50µl	100µl	150µl	Positive control	Negative control
Pseudomonas aeruginosa	17	19	21	13	-
Bacillus subtilis	14	20	21	18	-
Staphylococcus aureus	19	23	24	26	-
Klebsiella pneumoniae	17	19	23	20	-
Streptococcus sp.	15	17	19	21	-
Salmonella sp.	15	19	23	-	-
Escherichia coli	13	18	19	-	-

Table 3: Antibacterial activity of the methanol extract of *T. chebula* plant for different concentrations

Organisms	Zone of inhibition (mm) for different concentration of methanol extract				
	50µl	100µl	150µl	Positive control	Negative control
Pseudomonas aeruginosa	14	19	20	12	-
Bacillus subtilis	17	20	23	17	-
Staphylococcus aureus	15	18	20	26	-
Klebsiella pneumoniae	17	22	24	19	-
Sterptococcus sp.	14	18	20	20	-
Salmonella sp.	15	20	23	-	-
Escherichia coli	17	20	22	-	-

The antibacterial activity of methanolic and aqueous extract of the plant *Terminalia chebula* exhibited varied levels of sensitivity. *Staphylococcus* sp. showed high sensitivity for the aqueous extract compared to other bacterial stains, whereas *Streptococcus* sp. was less sensitive (Table 2). *Klebsiella* sp. was highly sensitive for the methanolic extract and *Streptococcus* sp. and *Pseudomonas* sp. showed less sensitivity compared to that of other organisms for the methanolic extract (Table 3). *Staphylococcus* sp. and *Bacillus* sp. were highly sensitive for the extract of silver nanoparticle. On the other hand *E. coli* showed less sensitivity (Table 4).

Table 4: Antibacterial activity of silver nanoparticles synthesis from *T. chebula*

Organisms	Zone of inhibition (mm) for different concentration of silver nanoparticles				
	50µl	100µl	150µl	Positive control	Negative control
Pseudomonas aeruginosa	15	16	17	25	-
Bacillus subtilis	16	18	20	9	-
Staphylococcus aureus	24	26	27	21	-
Klebsiella pneumoniae	14	16	17	8	-
Streptococcus sp.	16	17	18	9	-
Salmonella sp.	14	17	19	-	-
Escherichia coli	9	15	16	-	-

Discussion

The phytochemical analysis *T. chebula* showed the presence of terpenoids, resins and saponins where as it showed absence of tannins and alkaloids which is omparable to the works of Kathirvel and Sujatha[7] who investigated on bioactive compounds of *T. chebula* fruits and concluded that, it contains classes of compounds like phenol, flavonol, flavonoid, ascorbic acid, and proteins, carbohydrates.

In the present study formations of silver nanoparticles by reduction of silver nitrate during exposure to *Terminalia chebula* fruit extract was easily monitored from the change in color of the reaction mixture from yellowish brown to dark brown after 2 hours. Similar reports made by earlier workers also [1, 8, 9].

Maribel et al.[10] reported that the absorption peak of silver nanoparticles by chemical reduction method was 412nm whereas Lakshmi et al., reported a peak of 358 nm in *Mesua* fruit extract[11]. But in the present study we observed two absorbance peaks one at 358 nm and other one at 450 nm. The present work reveals that the FTIR analysis supported the reducing property of silver nanoparticles synthesized by *Terminalia chebula* fruit extract in which different functional groups were observed. Saifuddin et al. [12] observed the FTIR measurements to identify the possible bimolecular responsible for capping and efficient stabilization of the metal nanoparticles synthesized in leaf broth. Charusheela et al.[13] reported spherical shaped nanoparticles of 10-25nm size and Hemanth et al. [14] obtained the nanoparticles size 52-104nm. In this work we observed the particles with the diameter range of 90-120 nm. This work supports earlier report on *Mesua* flower nanaparticles where the particles are of hexagonal shape wirh 90- 120nm size [11].

In the study of silver nanoparticles synthesized using *T. chebula* fruit extract exerted a significant antibacterial action on concentration compared with positive control (Penicillin G). *T. chebula* fruit extract showed activity against *Psedomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus* sp.*, Salmonella* sp. and *E. coli* with zone of inhibition less than 17mm in diameter.In this study finding antibacterial activity of methanolic and aqueous extract from floral parts of *T. chebula* has resulted that *Staphylococcus aureus* showed the highest zone of inhibiton of 24mm in 150µl in aqueous extract and *Klebsiella pneumoniae* has shown the highest inhibitory zone of 24mm at 150 µl in methanolic extract.

In this study, with silver nanoparticles, *Staphylococcus aureus* showed the highest zone of inhibition at 150 µl. By increasing the dose of the extract more inhibitory zone will be found with silver nitrate extract. Savithramma et al.[1] reported the antibacterial activity can also be compared with the other silver nanoparticles synthesized leaf extracts of *Svensonia* which also showed activity against *E.coli, B. subtilis, S. aureus and K. pneumoniae*. Similar results were recorded in earlier studies too[9, 11].

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