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Original Article

PLANT MEDIATED SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES

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ABSTRACT

Objective: The study was aimed to synthesis and characterization of silver nanoparticles from five different herbal plants (*Terminalia chebula, Mimusops elengi, Myristica fragrans, Centella asiatica and Hemidesmus indicus).*

Methods: The qualitative analysis of plant extracts was performed to determine the presence of secondary metabolites. The plant mediated silver nanoparticles were synthesized. The color changed into brown to black color indicating the formation of AgNPs. The characterization of synthesized AgNPs was carried out by different methods such as UV-Vis Spectra, FE-TEM, Particle size analysis, Zeta potential analysis, XRD and FTIR. The antimicrobial activity of synthesized silver nanoparticles also examined against three fungi and bacteria.

Results: The UV wave length of AgNPs is from 300 to 450 nm. The average size of AgNPs 581 d.nm, zeta potential is -13.3 mV. The FTIR results show that AgNPs contains the functional groups. In antimicrobial activity of all AgNPs synthesized by five plants inhibits the growth of bacteria and *Terminalia chebula* showed maximum effect. The XRD pattern clearly confirmed that the synthesized silver nanoparticles are crystalline in nature. TEM results shows that synthesized silver nanoparticles are round in shape.

Conclusion: The green synthesis of nanoparticles shows that cost-effective, environmentally friendly, and safe for human therapeutic use. Color change, UV-Vis spectra, TEM and XRD analysis confirmed the stability of synthesized AgNPs.

Keywords: Nanoparticles, *Terminalia chebula,* Zeta potential, AgNPs, TEM, XRD.

INTRODUCTION

Over the past decade Nanoscience and Nanotechnology is a sprouting interdisciplinary field of research interspersing material science, bionanoscience and technology. Remarkable advances are made in the field of biotechnology and nanotechnology to harness the benefit of life sciences [1] healthcare [2] and industrial biotechnology [3]. Nanomaterials may provide solutions to technological and environmental challenges in the areas of solar energy conversion [4], catalysis [5], medicine [6] and water treatment [7]. This increasing demand must be accompanied by ''green'' synthesis procedures.

Biosynthesis of nanoparticles as an emerging highlight of the intersection of nanotechnology and biotechnology has received increased attention due to growing need to develop environmentally benign technologies in material synthesis. A great deal of effort has been put into the biosynthesis of inorganic material, especially metal nanoparticle using microorganisms and plants. Nanosilver has many important applications. It is used as an antimicrobial agent; it is applied in textiles, home water purification systems, medical devices, cosmetics, electronics, and household appliances. Besides their antimicrobial features, silver nanoparticles exhibit strong optical features making the nanoparticles suitable for biological sensing and imaging. Due to their high conductivity, silver nanoparticles are applied in conductive inks, adhesives and pastes for a range of electronic devices Silver nanoparticles are also used as catalysts in several chemical reactions such as the oxidation of styrene. [8]

Biological methods of nanoparticles synthesis using microorganisms [9], enzymes [10], fungus [11], plants or plant extracts [12] have been suggested as possible ecofriendly alternatives to chemical and physical methods. The chemically synthesized metal nanoparticles are expensive, hazardous to environment and require high energy consumption. Sometimes synthesis of nanoparticles using plants and parts of plants are advantageous over other biological process by eliminating the process of maintaining the microbial culture [12]. Biological approaches using plant extracts for metal nanoparticles synthesis have been suggested as valuable alternative tool towards chemical methods. Since metal nanoparticles are widely applied in biomedical field, sequentially there is an increasing need to produce metal bio-nanoparticles through eco-friendly process which are highly stable for the large scale production (with absence of toxic chemicals). The use of plants for synthesis of nanoparticles is rapid, low cost, eco-friendly, and a single-step method for biosynthesis process [13].

Plant-mediated nanoparticles synthesis is preferred as it is safe for human therapeutic use [1]. Many reports are available on the biogenesis of silver nanoparticles using several plant extracts, particularly neem leaf broth (*Azadirachta indica*), *Pelargonium graveolens*, *geranium* leaves, *Medicago sativa* (Alfalfa), *Aloe vera, Emblica officinalis* (Amla, Indian Gooseberry) and few microorganisms. Similarly different plant constituents such as geraniol possess reducing property and reduce Ag^{+} to silver nanoparticles with a uniform size and shape in the range of 1 to 10 nm with an average size of 6 nm.

Silver has long been recognized as having inhibitory effect on microbes present in medical and industrial process [14]. The most important application of silver and silver nanoparticles is in medical industry such as topical ointments to prevent infection against burn and open wounds.

Further these biologically synthesized nanoparticles were found highly toxic against different multidrug resistant human pathogens. In the present study to synthesize and characterization of phyto-silver nanoparticles from five medicinal plants *Terminalia chebula, Mimusops elengi, Myristica fragrans, Centella asiatica and Hemidesmus indicus*.

MATERIALS AND METHODS

Collection of Plants

The *Terminalia chebula* fruits were collected from the villages around Kumbakonam and authenticated. Selection of plant were based on their availability and medicinal important. The collected plants materials were washed with sterile double distilled water, finely cut and air dried for a week under shade. The dried plant materials were finely powdered and stored in airtight containers for analysis.

Preparation of aqueous plant extracts

The powdered plant materials were used for extract preparation. About 10 gram of powder was mixed with 100 ml of double distilled water and boiled in water bath for 20 mins for the formation of plant extract. The obtained plant extract was filtered through whatman no 1 filter paper then centrifuged at 6000 rpm for 20 mins. The centrifuged samples were transferred into autoclaved vials and stored at 4^0C for further analysis.

Phytochemical analysis of plant extracts

The qualitative analysis of plant extract was performed to determine the presence of secondary metabolites [15].

Preparation of silver nitrate solution

The silver nitrate was collected from sigma-Aldrich. The Molecular Weight of $AgNO_3$ is 169.87g/mol. For preparation of 1mM $AgNO_3$ solution 16.987 mg of $AgNO_3$ was added to 100 ml of double distilled water and mixed thoroughly. The solution was stored in an amber colored bottle.

Synthesis of silver nanoparticles

To synthesis silver nanoparticles, 90 ml of 1mM $AgNO_3$ solution was taken in a sterile conical flask and 10 ml of aqueous plant extract was added to it. The solution was mixed well and kept in a rotator shaker for overnight. As a result, a brown to black color solution was formed, indicating the formation of silver nanoparticles. It showed that aqueous silver ions could be reduced by aqueous extract of plants part to generate extremely stable silver nanoparticles in water [16].

Characterization of silver nanoparticles:

UV-VISIBLE Spectrometry

Preliminary characterization of the AgNPs was carried out using UV–vis spectroscopy. The bioreduction of pure Ag+ ions was monitored by a periodic sampling of the aliquots (0.5 ml) of the suspension, then diluting the samples with 5 ml deionized water and subsequently measuring UV–Vis spectra of the resulting diluents[17]. UV–Vis spectroscopic analyses of the silver nanoparticles produced were carried out as a function of bioreduction at room temperature on UV–Vis spectrometer (Perkin-Elmer lambda 25) in the range of wave length from 300 to 700 nm. The presence and reduction of silver ions was highlighted by a peak of absorption in the 350 to 500 nm range [18]. The results show the presence and reduction of silver ions in the tested samples.

Particle size analysis

Particles size analyzing experiment were carried by laser diffractometry (laser particle analyzer) using extremely compact optical bench, the CILAS integrates 2 sequenced laser sources pointed at 0^0 and 45^0 measurements were taken in the range between 0.04 into 500 µm. Through the software, the particle distribution curve represented.

Zeta potentiometer

Size and zeta potential of the silver nanoparticles were determined by Malvern Zetasizer ZEN 3600 (United Kingdom). This instrument allows the measurement of particle sized distribution in the range 2 nm–3 nm [19].

TEM analysis of AgNPs

The morphological analysis of the nanoparticles was done with transmission electron microcopy (TEM).The size and shape of the silver nanoparticles was determined by TEM. A drop of aqueous silver nanoparticle sample was loaded on carbon-coated copper TEM grid. It was allowing water to evaporate and dry completely for an hour at room temperature.The TEM micrograph images were recorded on a JEOL 1200 EX instrument on carbon coated copper grids with an accelerating voltage of 100 to 200 kV. The clear microscopic views were observed and documented in different ranges of magnifications. The presence of silver elements was confirmed through Energy Depressive spectroscopy [20].

Lyophilization of sample

The plant extract was lyophilized for XRD and FTIR analysis.The sample was centrifuged at 10,000 rpm for 20 minutes and the action was carried out twice. The samples were kept in the freezer at 4^oC for further analysis.

FTIR (Fourier Transforms Infrared Spectroscopy)

FTIR was used to identify the possible functional groups responsible for the reduction of the Ag ions and capping of the bioreduced silver nanoparticles synthesized. In order to determine the functional groups and their possible involvement in the synthesis of silver nanoparticles, FTIR analysis was carried out [21]. The silver nanoparticle solution was centrifuged at 15,000 rpm for 15 mins and the pellet was collected. The pellet washed twice and lyophilized. The dry powder was used for FTIR analysis. The powder was grinded in Kbr pellets and analyzed on a Thermo Ncolet model 6700 spectrum instrument in the diffuse reflectance mode operation at a resolution of 4cm $^{-1.}$ In order to obtain good signal/noise ratio. 512scans ware recorded. The peaks obtained were plotted as % transmittance in Y axis and wave number (cm^{-1}) in x axis [16].

XRD (X-ray diffraction)

The characterization of the purified IhAgNO3 was conducted with an XRD 6000 X-ray diffractometry (shimadzu, Japan) operated at voltage of 40 kV and current of 30 mA with cu k radiation in θ 20 configurations. The crystallite domain size was calculated from the width of the XRD picks by assuming that they were free from non uniform strains and using the sharer formula

$$D = \frac{0.94}{\beta \cos \theta}$$

Where the D is the average crystalline domain size perpendicular to the reflecting planes, λ is the X-ray wave length, β is the full width at of maximum (FWHM) and θ is the diffraction angle.

To eliminate the additional instrumental broadening, the FWHM was curetted using FWHM from a large grained Si sample.

β corrected = $(FWHM^2 \text{ sample} - FWHM^2 n)^{1/2}$

This modified formula is valid only when the crystallite size is smaller than 100 nm **[22]**.

RESULTS AND DISCUSSION

Phytochemical analysis

The phytochemical constituents of the aqueous extract of five different plants given in Table1. The qualitative analysis of plant extract was performed to determine the presence of secondary metabolites [15]. The carbohydrates, flavonoids, alkaloids, steroids, glycosides, Saponins, terpenoids, amino acids, proteins, tannins and phenols are tested in the plants.

In *Terminalia chebula* carbohydrates, proteins, amino acids, tannins, Saponins, and steroids are present. Flavonoids, alkaloids and phenolics are absent. *Mismusops elengi* contains all compounds except phenolics. In *Myristica fragrans* carbohydrates, proteins, amino acids, tannins, steroids, Flavonoids, alkaloids are present and Saponins, phenolics are absent. In *Centella asiatica chebula* carbohydrates, proteins, amino acids, tannins, Saponins, steroids, alkaloids are present and Flavonoids, phenolics are absent.

In *Hemidesmus indicus* carbohydrates, proteins, amino acids, tannins, Saponins, Flavonoids, alkaloids are present and steroids, phenolics are absent. [22] demonstrated that *Myristica fragrans* contains phytochemical constituents such as proteins, amino acids, tannins, steroids, Flavonoids, alkaloids and potassium salts. SuryaPrakash, **[23]** reported that *Terminalia chebula* contains phytochemical constituents such as anthraquinone, flavonoids, chebulic acid, chebulinic acid, tannic acid, ellagic acid, 2,4-chebulyi–β-D-gluco pyranose, gallic acid. Rajan *et al.,* [24] reported that *Hemidesmus indicus* contains steroids, terpenoids, flavonoids, phenolic compounds, tannins, lignin, carbohydrates and proteins.

Table 1: Phytochemical screening of extracts of experimental plants

Phyto-constituents	*Terminalia chebula*	*Mimusops elengi*	*Myristica fragrans*	*Centella asiatica*	*Hemidesmus indicus*
Carbohydrates	++	++	++	++	++
Proteins	++	++	++	++	++
Amino acids	++	++	++	++	++
Tannins	++	++	++	++	++
Saponins	++	++	--	++	++
Flavonoids	--	++	++	++	++
Alkaloids	--	++	++	--	++
Steroids	++	++	++	++	--
Phenolics	--	--	--	--	--

Synthesis of silver nanoparticles

The plate 1 and 2 shows the photographs 1mM $AgNO_3$ with double distilled water (control) and the 1mM $AgNO_3$ with plant extract (test).

After the addition of 1mM $AgNO_3$ to the aqueous plant extract the color starts to change from brown to black. Plate 2 and 3 shows the control the aqueous plant extract without 1mM $AgNO_3$ and the color change of the plant extract due to synthesis of silver nanoparticles. Reduction of silver ion into silver particles during exposure to the plant extracts could be followed by color change. Silver nanoparticles exhibit dark brown to black color in aqueous solution due to the surface plasmon resonance phenomenon.

Plate 1: The aqueous extract of experimental plants without silver nitrate

1 = *Myristica fragrans,* **2** = *Centella asiatica,* **3** = *Hemidesmus indicus,* **4** = *Mimusops elengi,* **5**= *Terminalia chebula*

Plate 2: Synthesized AgNPs of five different experimental plants

The *Centella asiatica* took over night for color change. The color changed from light yellow to dark black. The *Mimusops elengi* plant extract color changed from turbid white to black. The color change of *Hemidesmus indicus* was from light brown to dark brown. The color change of *Hemidesmus indicus* was from light brown to dark black. The color change of *Terminalia chebula* was from yellow to light brown [24]

Prabu *et al.,* [25] reported the *Chenopodium murale* leaf extract produced dark brown color after addition of silver nitrate. Ponarulselvam [24] have demonstrated that leaf extract of Catharanthus roseus Linn formed light brown color with silver nitrate. Fouzia Banu., 2012 have reported the *Cleome Viscosa* with silver nitrate produced dark black color.

Biosynthesis of nanoparticles by plant extracts is currently under exploitation. The development of biologically inspired experimental processes for the synthesis of nanoparticle is evolved into an important branch of nanotechnology. The present study emphasizes the use of plants medicinal for the synthesis of silver nanoparticles with potent antimicrobial effect.

Characterization of silver nanoparticles

UV-Vis spectrophotometry

The formation of silver nanoparticles was confirmed by color changes followed by UV–Visible spectrophotometer analysis. The UV–Visible spectrophotometer has proved to be a very useful technique for the analysis of some metal nanoparticles and is a significant technique to authenticate the formation and stability of AgNPs in aqueous solution.

It is renowned that AgNPs exhibit dark brown colors, depending on the intensity and the size of nanoparticles. The colors arise due to the excitation of surface plasmon resonance (SPR) of the AgNPs.[17]. It is generally recognized that UV- vis soectroscopy could be used to examine size and shape controlled nanoparticles in aqueous suspension [26]. It is one of the most widely used techniques for structural characterization of silver nanoparticles [18]. The UV–vis spectra were recorded for aqueous leaf extract of plants. The absorption peaks were from 300 to 400 nm.

The maximum range of silver nanoparticles in UV-Vis spectrometer is 300 to 500 nm. In this study the results of UV-Vis spectrometer were from 300 to 400 nm.It confirms that the synthesized particles are silver.

The absorption of *Hemidesmus indicus* and *Mimusops elengi* was 277 and 528 nm respectively. It indicates that no AgNPs synthesis was not carried out. The absorption of *Centella asiatica*, *Terminalia chebula* and *Myristica Fragran*s were 328, 398, 373 nm respectively and indicating that synthesis of AgNPs was carried out. The maximum absorption of *T.chebula* (398 nm) shows the maximum synthesis of AgNPs.

In the UV-Vis spectrum, the broadening of peak indicated that the particles are poly dispersed. The reduction of silver ions and the formation of stable nanoparticles occurred rapidly within 24 h of reaction making it one of the fastest bioreducing methods to produce silver nanoparticles [27]. The surface plasmon band in the silver nanoparticles solution remains close to 400 nm throughout the reaction period indicating that the particles are dispersed in the aqueous solution, with no evidence for aggregation. It was observed that the nanoparticles solution was stable for more than six months with little signs of aggregation. [28, 29].

Ponarulselvam, [24]have demonstrated that Synthesis of silver nanoparticles using leaves of *Catharanthus roseus Linn. G.* In that they got UV-Vis spectra results from 390 to 400 nm under different time intervals. have reported the UV-Vis spectra results of

Biosynthesis of Silver Nanoparticles from *Aloe vera* Plant Extract is from 410 nm. Dubey et al., [30] have investigated the Green synthesis, antimicrobial and cytotoxic effects of silver nanoparticles using *Eucalyptus hybrida* leaves extract. In that the nanoparticles UV-Vis was 413 nm. Among the five plants mediated AgNPs *T.chebula* mediated AgNPs was selected for further characterization.

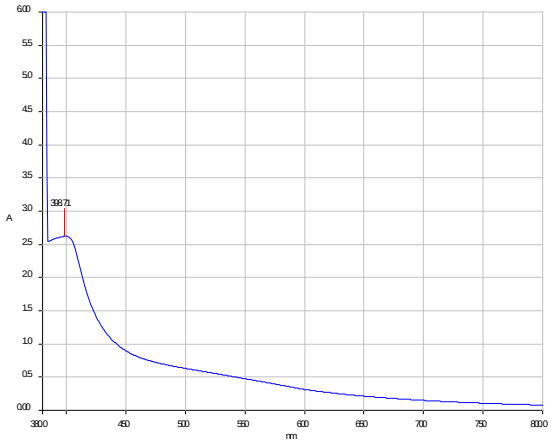

Fig. 1: UV-VIS absorption spectra of Ag NPs synthesized by *Terminalia chebula.*

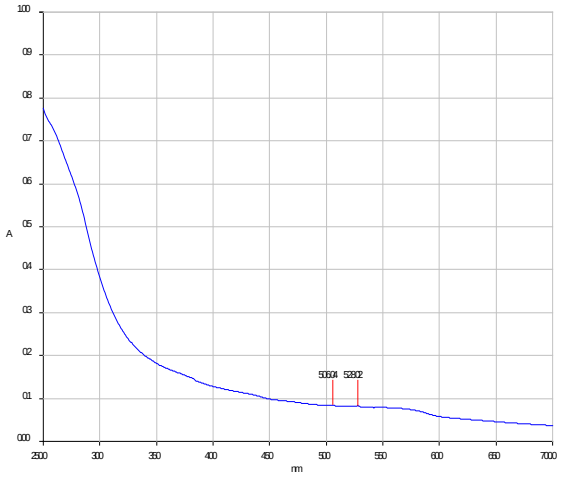

Fig. 2: UV-VIS absorption spectra of Ag NPs synthesized by *Mimusops elengi*

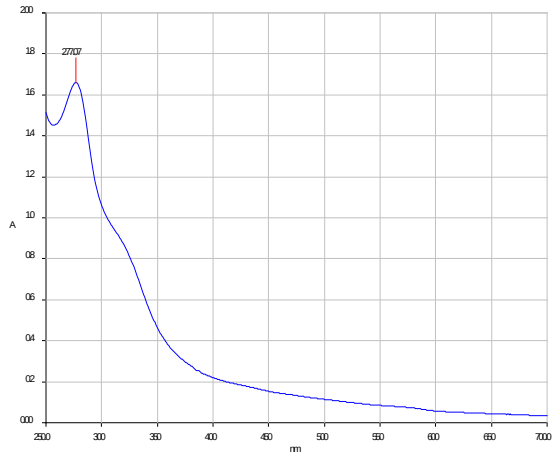

Fig. 3: UV-VIS spectra absorption of Ag NPs synthesized by *Hemidesmus indicus*

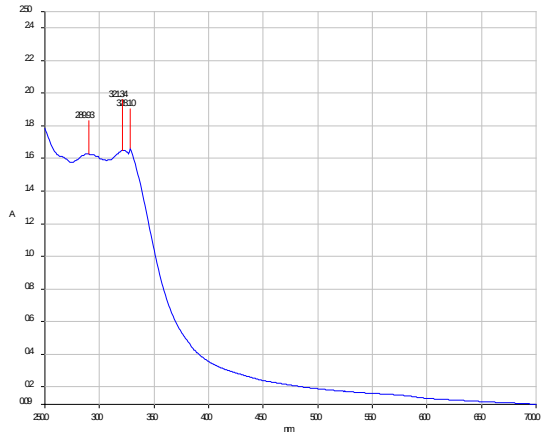

Fig. 4: UV-VIS absorption spectra of Ag NPs synthesized by *Centella asiatica*

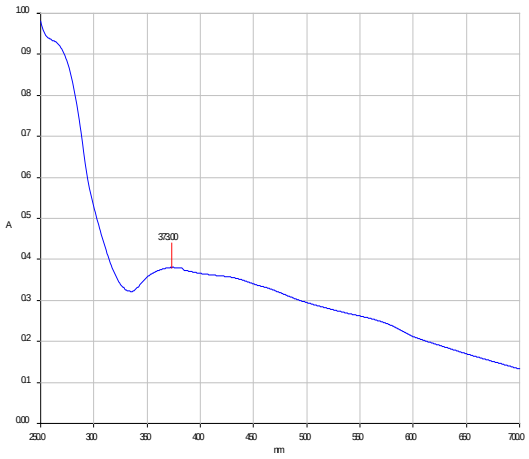

Fig. 5: UV-VIS spectra absorption of Ag NPs synthesized by *Myristica fragrans*

TEM analysis

The morphology and size of the synthesized nanoparticles were also determined by TEM images. *T. chebula* mediated AgNPs images shown in plate 3. Images reveal that the AgNPs are predominantly spherical in shape and are not in physical contact with each other. Lower magnification image reveals the nanoparticles are embedded in a dense matrix which may be the organic stabilizing components of T. *chebula* extract. The presence of organic content associated with AgNPs can be further confirmed by observing the sharp Braggs reflection in XRD spectrum. The size of synthesized AgNPs are approximately 25 nm. The results of TEM results coincides with Daizy Philip, [19], Jebakumar Immanuel Edison and Sethuraman,[16]. Daizy Philip., [19] have demonstrated the Green synthesis of gold and silver nanoparticles using *Hibiscus rosasinensis.* He reported that the nanoparticles are triangular, hexagonal, dodecahedral and spherical in shape and the size was 14 nm.

FTIR

FTIR measurements were carried out to identify the potential biomolecules in *T. chebula* responsible for reduction and capping of the bioreduced silver nanoparticles. The major phytoconstituents present in the myroblan fruitare hydrolysable tannins, gallic acid, chebulic acid, chebulic ellagitannins and gallate esters. The presence of three bands at about 3887.98 cm^{-1}, 3732.44 cm^{-1}, 2921.50 cm^{-1}, 2319.62 cm^{-1}, 1702.26 cm^{-1}, 1605.50 cm^{-1}, 1173.85 cm^{-1}, 1045.96 cm^{-1}, 757.11 $cm^{-1.}$ (Figure 6). The absorption band at 3887.9 cm−1 which is characteristic of the OH stretching of phenolic group. The absorption bands at 1173.8 and 1605 cm−1 correspond to carbonyl

group present in the extract. The sharp band at 1045.9 cm−1 indicated C O group of ester and the band at 17021.2 cm−1 is due to aromatic CH stretching vibrations. The absorption bands that appear in the IR spectrum of the aqueous extract could also be seen in the IR spectra of phytocapped AgNPs. This shows that the phytoconstituents (mostly tannins) protect the AgNPs from aggregation **[19]**. Daizy Philip, [19] have reported the FTIR pattern of AgNPs synthesized by Hibiscus rosa sinensis. It shows that the plant contains functional groups such as carboxylic acid, amide group.

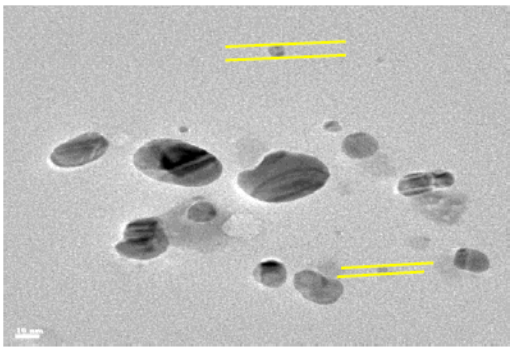

Plate 3: TEM images of synthesized AgNPs by fruit extract of *T.chebula*.

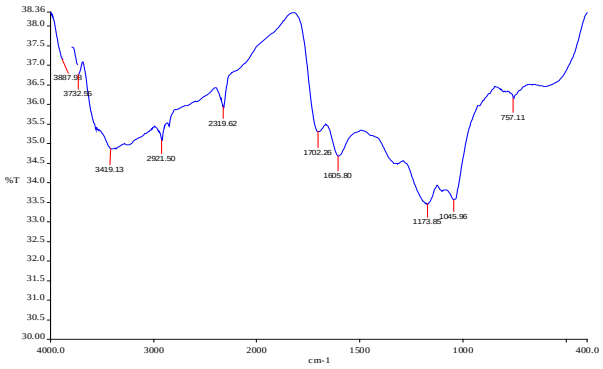

Fig. 7: FTIR results of synthesized AgNPs using fruit extract *Terminalia chebula.*

XRD

XRD is commonly used for determining the chemical composition and crystal structure of a material; therefore, detecting the presence of silver nanoparticles in plants tissues can be achieved by using XRD to examine the diffraction peaks of the plant. The crystalline nature of Ag nanoparticles was further confirmed from X-ray diffraction (XRD) analysis shows the XRD pattern of the dried nanoparticles obtained from colloid samples. Four peaks were observed at 32.77^0, 23.59^0, 20.44^0 in the 2θ range $10\text{-}60^0$ (Figure 6). These Braggs reflections clearly indicated that presence of (3.2), (2.35) and (2.04) sets of lattice planes and further on the basis that they can be indexed as face-centered-cubic (FCC) structure of silver. The observed peak broadening and noise were probably macromolecules present in the plant extract which may be responsible for the reduction of silver ions. Hence XRD pattern thus clearly illustrated that the silver nanoparticles formed in this present synthesis are crystalline in nature. In addition to the Bragg peaks representative of fcc silver nanocrystals, additional as yet unassigned peaks are also observed suggesting that the crystallization of bio-organic phase occurs on the surface of the silver nanoparticles**.** The line broadening of the peaks is primarily due to small particle size.

The X-ray diffraction results clearly show that the silver nanoparticles formed by the reduction of Ag^+ ions by the *T.chebula* fruit extract are crystalline in nature. [1]. Neveen Abdel-Raouf et al., [20] demonstrated the pattern of AgNPs synthesized by Galaxaura elongate is coincides with results of plant mediated synthesis of AgNPs.

Zeta potential analysis

The biosynthesized silver nanostructure was further demonstrated and confirmed by the characteristic peaks observed in Zeta sizer image, which indicate that the average of the diameter was in the range is 581 d.nm and corresponding average zeta potential value is −13.3 mV suggesting higher stability of AgNPs. The large negative potential value could be due to the capping of polyphenolic constituents present in the extract. The figure 8-10 shows the results of Zeta potential results.

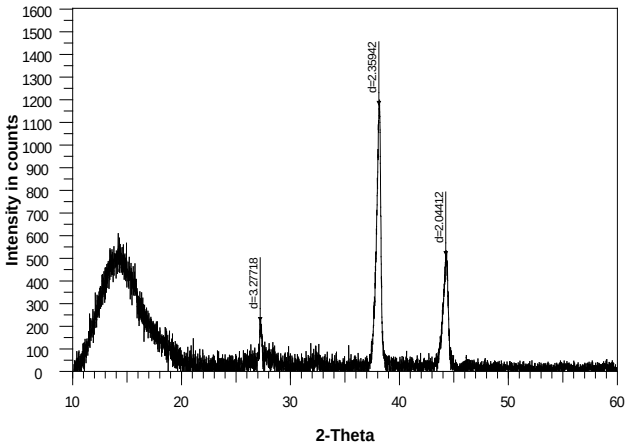

Fig. 8: XRD pattern of silver nanoparticles synthesized by *Terminalia chebula.*

Angle 2-Theta °	d value	Intensity Angstrom	Intensity % Count %
27.189	3.27718	221	18.7
38.110	2.35942	1180	100.0
44.276	2.04412	513	43.5

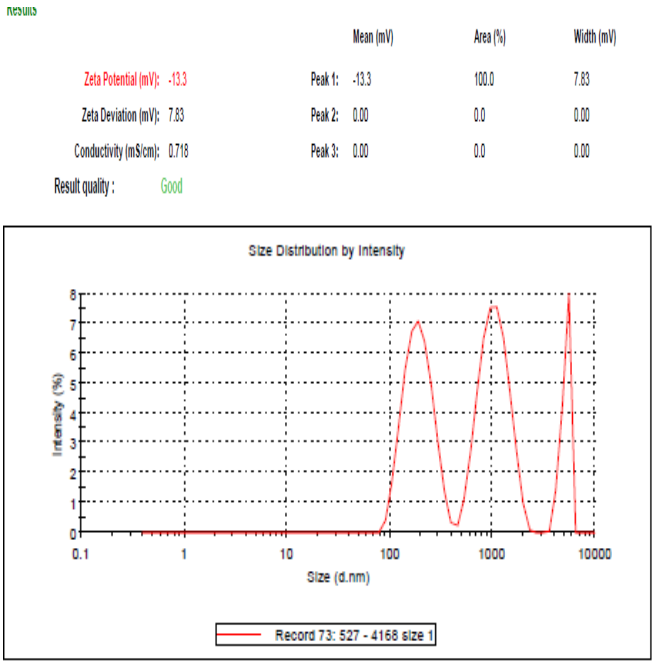

Fig. 9: Zeta sizer analysis of synthesized AgNPs using fruit extract of *T.chebula*

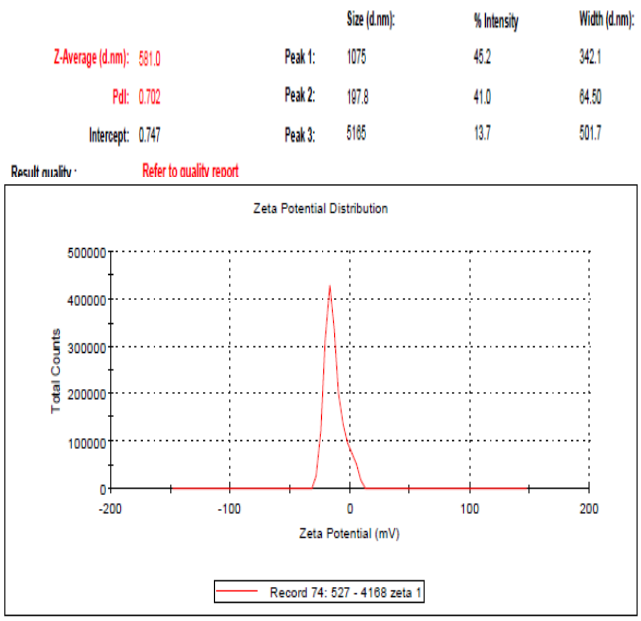

Fig. 10: Zeta potential illustration of silver nanoparticles produced by *T.chebula*.

Over the past decade Nanoscience and Nanotechnology is a sprouting interdisciplinary field of research interspersing material science, bionanoscience and technology. Remarkable advances are made in the field of biotechnology and nanotechnology to harness the benefit of life sciences, healthcare and industrial biotechnology. Nanomaterials may provide solutions to technological and environmental challenges in the areas of solar energy conversion, catalysis, and medicine and water treatment. This increasing demand must be accompanied by ‘‘green’’ synthesis procedures. Nanosilver has many important applications. It is used as an antimicrobial agent. It is applied in textiles, home water purification systems, medical devices, cosmetics, electronics and household appliances. The chemically synthesized metal nanoparticles are expensive, hazardous to environment and require high energy consumption. Biological approaches using plant extracts for metal nanoparticles synthesis have been suggested as valuable alternative tool towards chemical methods. The five different herbal plants were selected for this study (*Terminalia chebula, Mimusops elengi, Myristica fragrans, Centella asiatica and Hemidesmus indicus).*The plant samples were collected from the villages around the kumbakonam and authenticated. The aqueous plant extract was prepared. The qualitative analysis of plant extracts was performed to determine the presence of secondary metabolites. The plant mediated silver nanoparticles were synthesized. The color changed into brown to black color indicating the formation of AgNPs. The characterization of synthesized AgNPs was carried out by different methods such as UV-Vis Spectra, FE-TEM, Particle size analysis, Zeta potential analysis, XRD and FTIR. The antimicrobial activity of synthesized silver nanoparticles also examined against three fungi and bacteria. The UV wave length of AgNPs is from 300 to 450 nm. The average size of AgNPs 581 d.nm, zeta potential is -13.3 mV. The FTIR results show that AgNPs contains the functional groups. In antimicrobial activity of all AgNPs synthesized by five plants inhibits the growth of bacteria and *Terminalia chebula* showed maximum effect. The XRD pattern clearly confirmed that the synthesized silver nanoparticles are crystalline in nature. TEM results shows that synthesized silver nanoparticles are round in shape. The green synthesis of nanoparticles shows that cost-effective, environmentally friendly, and safe for human therapeutic use. Color change, UV-Vis spectra, TEM and XRD analysis confirmed the stability of synthesized AgNPs.

CONFLICT OF INTERESTS

Declared None

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