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Research Article

GREEN SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLE USING LEAF EXTRACT OF *Capparis zeylanica*

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ABSTRACT

The green synthesis of Silver nanoparticles (AgNPs) using the aqueous extract of *Capparis zeylanica* plant leaves act as a reducing and capping agent has been reported in the present work. The formation of silver nanoparticles was observed by the change of colour from colourless to dark brown by the addition the leaf extract. Nanoparticles were characterized with the help of UV-Vis absorption spectroscopy analysis, Fourier Transform Infrared (FTIR) analysis, X-ray diffraction analysis (XRD), Scanning Electron Microscopy (SEM) & EDX analysis, and Transmission Electron Microscopy (TEM) analysis. The prepared AgNPs were monodispersed, spherical in shape with the particle size in a range of 50-90 nm.

Keywords: Capparis zeylanica, bioreduction, Ag NPs, antimicrobial activity.

INTRODUCTION

In modern research, nanotechnology plays a vital role, it involves the engineering and the manipulation of particles at the nano scale ranging from approximately 1-100 nm [1]. Nanotechnology is gaining importance in various fields such as health care, food and feed, cosmetics, environmental health, biomedical science, chemical industries, drug and gene delivery, energy science, electronics, mechanics, and space industries [2]. It also have extensively been achieved for the treatments of cancer [3], diabetes [4], allergy [5], infection [6] and inflammation [7]. Green chemistry is a development, design, implementation of chemical products and processes to reduce the use and generation of substances that are hazardous to human health and environment [8]. There are many ways to synthesize nanoparticles such as solid reaction, co-precipitation, chemical reaction, and sol gel method etc.,. In recent years green synthesis of NPs has several advantages over chemical synthesis, such as simplicity, cost effectiveness. Moreover it is compatible for biomedical and food applications, and this technique eliminates the use of energy, high pressure, temperature, and toxic chemicals [9,10].

The growing need of environmental friendly nanoparticles has attracted many researchers to use green synthesis methods of various metal nanoparticles [11] due to their interesting and remarkable properties with a variety of applications over their bulk material [12]. Considering the photochemical reduction, chemical reduction methods, electrochemical reduction, heat evaporation etc., the biological method is more advantageous [13]. In this biological method, the plant extract has been used as reducing agent and capping agent for the synthesis of nanoparticles [14] due to their reducing properties [15]. Some properties such as size, distribution, and morphology of the particles are clearly obtained from the nanoparticles [16].

The various nanoparticles like gold, silver, copper, iron, palladium, zinc, quantum dots(CdS, ZnS), among these, Silver nanoparticles places a major role because it has several important properties such as optical, chemical, electronic, photo electro chemical, catalytic, magnetic, antibacterial, and biological labelling, antimicrobial, catalytic. Silver nanoparticle acts as antimicrobial agent which finds applications in medical field such as AgNPs coated blood collecting vessels, coated capsules, band aids etc [14-17]. The silver is non-toxic to animal cells and highly toxic to bacteria, and other microorganisms (*E-coli, Pseudomonas aeruginosa, Staphylococcus aureus*). Due to these phenomena it is considered to be safe and effective bactericidal metal [18-20].

The synthesis of silver nanoparticles has been synthesized using green methods which are non-toxic, less usage of chemicals and Environmental friendly and low cost. Plants used for green synthesis of AgNPs like, *Acalypha indica* leaf, *Coriandrum sativum, Sorbus aucuparia* leaf, *Gliricidia sepium, Rose* leaf, *Cinnamomum camphora, Aloe vera and Neem, Camellia sinensis, Magnolia kobus and Diopyros kaki* leaf, *Geranium* leaf etc.,. All the parts of the plant like leaf, stem, flower, seed and skin of the fruits were used earlier for the synthesis of AgNPs. Plants have been used for the synthesis of nanoparticles were coated by the plant extract which has medical benefits and can be used as drug and cosmetic applications [21]. In this report, *Capparis zeylanica* Linn belonging to the family of capparidaceae commonly known as Indian caper, is a climbing scandant shrub found throughout India and has been used as a 'Rasayana' drug in the traditional medicine [22]. The plant mainly shows the presence of fatty acids, flavonoids, alkaloids which are already reported. *Capparis zeylanica* Linn is reported to possess antioxidant, antimicrobial, anti inflammatory and immune stimulant activity [23].

The current investigation focuses on the aqueous leaves extract of *Capparis zeylanica* used to synthesize AgNPs using different experimental conditions and thereby enhancing the importance of plant sources and implementing green chemistry for the future research.

MATERIALS AND METHODS

Materials

Silver nitrate was purchased from Sigma Aldrich chemicals India. Double distilled water was used throughout the process. Whatman no.1 filter papers were used for filtration. Glasswares used for the complete reactions were washed well and rinsed with double distilled water and dried in hot air oven before use.

Preparation of *Capparis zeylanica* leaf extract

The fresh *Capparis zeylanica* leaves were collected from surrounding areas of Mannargudi, India. The leaves were washed several times using normal and distilled water to remove impurities present on the leave. The cleared leaves were dried under sunlight with closed pack to remove moisture completely. By using mechanical grinder the dried leaves were powdered. The 5g of plant powder was taken along with 100 ml of distilled water in a round bottom flask and then allowed to boil at 60°C for 30 min under reflux condition, then it was cooled down to room temperature. Thus the prepared solution was initially filtered through normal filter paper thereby powdered leafy

materials will be filtered out, the filtrate was again filtered through Whatman No.1 filter paper to get clear solution. The filtrate was then stored at 4°C which is to be used for further characterization and future works [24].

Synthesis of AgNPs

25 ml stock solution of leaf extract was slowly added into 100 ml of 1mM solution of silver nitrate under continuous stirring for 20 min. After the complete addition of leaf extract, the colourless solution changed into pale yellow colour, after 20 min colour changed from pale yellow to reddish brown which indicates formation of silver nanoparticles. Then the solution was centrifuged at 10,000 rpm for 15 min [25], consequently dispersed in double distilled water to remove any heavy-handed biological materials [26].

Characterization

The UV-Visible spectral measurements were used to confirm the formation of silver nanoparticles by using JascoV-550 spectrophotometer instrument in the range between 400-900 nm [27]. FTIR experiment were carried out to determine the biomolecules present in the leaf extract responsible for the reduction of Ag ions with 400 - 4000 $cm^{-1}$of spectral range. The sample was centrifuged at 9000 rpm for 15 min, then dried and ground with KBr to form a pellet and analyzed on Jusco 5300 model. The X-ray diffraction analysis was used to check the crystalline structure of the nanoparticles. Rigaku X-ray diffractometer (Miniflex, UK) instrument used to measure the XRD pattern of synthesized AgNPs operating at a voltage 40 kV with the time interval of 2 sec at room temperature 27 ºC. The SEM and TEM analysis was carried out to determine the morphology and the mean particle size of nanoparticles. The sample were prepared on a carbon coated grid by just dipping a very small amount of the prepared AgNPs on the grid, by using blotting paper the extra solution was isolated, then the sample were allowed to dry for SEM analysis using Supra Zeiss with resolution of 1nm at 30 kV with 20 mm Oxford EDS detector, EDX analysis was done, to reveals the presence of elemental composition in the reaction mixture. The TEM analysis was done using HITACHI H-7650 at an operating voltage of 80 kV.

Antimicrobial activity

The bacterial sensitivity of pathogens were tested using Well diffusion method [28].The antimicrobial study of AgNPs was estimated against pathogenic bacteria such as *E-coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*. Nutrient agar was used to cultivate the bacteria. Medium was sterilized by using autoclave then poured into petriplates. After solidification of medium the holes were punched on the solidified medium by using stainless steel cylinder. The prepared Silver nanoparticles were added into the holes with different concentrations like 25 µl, 50µl and 100 µl. Plates were incubated for 24 hr at 37°C. After incubation period zone of inhibition was measured around the well [25].

RESULTS AND DISCUSSION

UV-Vis spectroscopy analysis

The reduction of silver ions to silver nanoparticles was confirmed by UV-Visible spectroscopy analysis and shown in figure 1. It is well known that the silver nanoparticles shows yellowish brown colour in water. These colours occur due to the observable fact of surface Plasmon excitations in the metal nanoparticles [29]. When the plant extract was added into the $AgNO_3$ solution the pale yellow colour solution was obtained. After 20 minutes, the colour changes from pale yellow to dark reddish brown. The absorption spectra of silver nanoparticles formed in the reaction mixture was obtained by the UV-Vis analysis at the range between 300-800 nm, the AgNPs has sharp absorbance with highest peak at 438 nm and progressively decreased while nanometre increased [30].

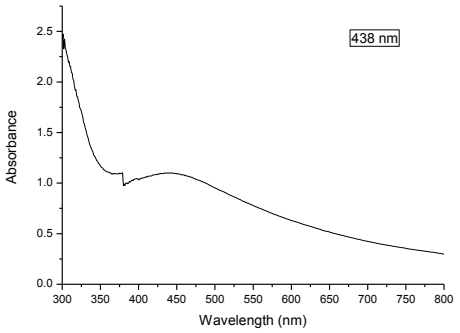
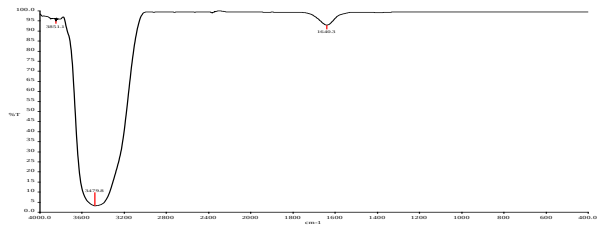


Fig.1: UV-vis spectra of silver nanoparticles

FTIR analysis

FTIR measurement was done to identify the reducing, capping and stabilizing capacity of *Capparis zeylanica* leaf extract. The FTIR analysis was done for both plant extract and AgNPs. In Figure 2(a), aqueous plant extract shown the peaks at 1640, 3479, 3851 cm^{-1}, the peak at 3479 cm-1 shows the bonds due to O-H stretching of phenolic compound [31] and 3851 cm^{-1} corresponds to O-H stretching of hydroxyl groups [32]. Peak at 1640 cm^{-1} was a strong absorption peak which indicates the characteristics IR absorption of polysaccharides shows the bonds due to C=O stretching, amines [33**]**. Whereas the silver nanoparticles present in the solution shows the peaks at around 1636, 3460, 3852, 3840, and 3901 cm^{-1} shown in a Figure 2(b). The peak at 1636 cm^{-1} corresponds to C=O stretching, (amides). Peak at 3460 cm^{-1} corresponds to O-H stretching of phenolic compound. Whereas the other peaks obtained in silver nanoparticle sample are 3852, 3840, 3901 cm^{-1} due to O=H stretching of hydroxyl groups [34,35]. The band 1636 cm^{-1} represents the stretching vibration of C=O due to the amide 1 bond of proteins. Particularly the plant leaves contained the chemical constituents of phenolic compounds, Alkaloids, flavonoids, and fatty acids, such as thioglycoside, β-carotene, glycocapparin, α-amyrin are acting as a capping and reducing agents [16]. The reduction of silver ions to silver nanoparticles was confirmed from the peak value of plant extract (1640 cm^{-1} to 1636 cm^{-1}).

(a)



(b)

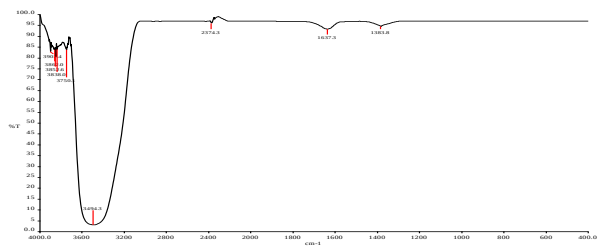


Fig.2: FTIR analysis of plant extract (a), Silver nanoparticles (b)

XRD analysis

The XRD spectrum analysis indicated two different diffraction peaks at 38.08°, 43.47° (figure 3). All diffraction peaks correspond to the characteristics of cubic face centred AgNPs. These diffraction lines are obtained at 2θ angle, it have been indexed as (111), (200). The Debye-Scherrer equation is used to determine the average grain particle size of the nanoparticles.

$D = K \lambda / \beta \cos\theta$

Where, D is the crystalline size of NPs, λ is the wavelength of the X-ray source (1.54 nm) used in XRD, β is the full width at half maximum of the diffraction peak, (FWHM) K is the Scherrer constant with a value from 0.9 to 1, and θ is the Bragg angle. According to Debye Scherrer equation the average particle size was found to be 18 nm [21].

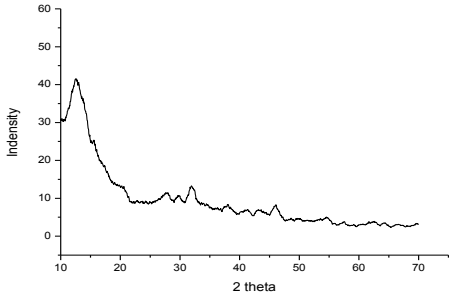
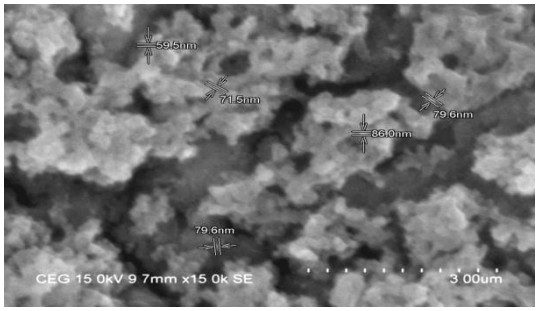


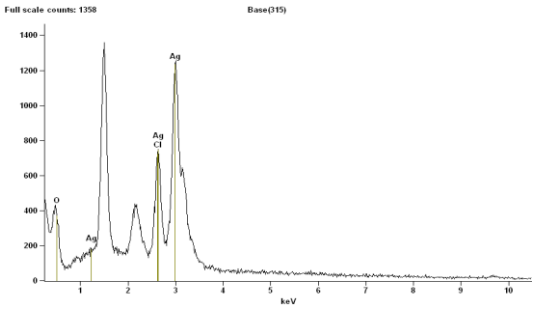
Fig.3: XRD analysis of AgNps

SEM analysis

Scanning Electron Microscopy (SEM) analysis provided the morphology and size details of the nanoparticles. The figure 4(a) shows high density AgNPs synthesized by the plant extract of *Capparis zeylanica* more confirmed the presence of AgNPs. The interactions such as hydrogen bond and electrostatic interactions between the bio-organic capping molecules bond are the reason for synthesis of silver nanoparticles using plant extract [8].It was shown that spherical and relatively uniform shape of the silver nanoparticles in the range 50-90 nm. The direct contacts even within the aggregates of nanoparticles were not found, indicating the stabilization of the nanoparticles by a capping agent [14, 36]. The qualitative and quantitative position of elements that may be concerned in the formation of silver nanoparticles was analyzed by EDX analysis. The elemental profile of silver has been confirmed from the sample using plant leaf extract shown in Figure 4(b). It confirms the formation of silver nanoparticles. Due to the Surface Plasmon Resonance the silver nanoparticles shows the absorption peaks of higher counts. [37].



4(a)



4(b)

Fig.4: SEM micrograph of AgNPs (a), EDX analysis of AgNPs (b)

TEM analysis

TEM analysis is used to visualize the size and shape of the AgNPs synthesized using plant extract [38]. The TEM image of the biosynthesized AgNPs are shown in Figure 5. The obtained AgNPs were almost spherical in shape with particle size in nano range. The particle size of synthesized silver nanoparticles is highly subjective by the concentration of leaf extract. The TEM analysis confirmed that the particle size is decreased when the concentration of leaf extract is increased in the reaction mixture [39].

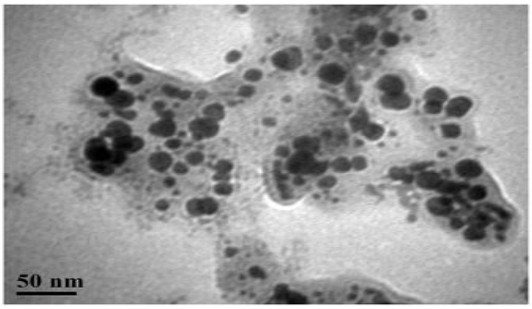


Fig.5: TEM analysis of AgNPs

Antimicrobial study on AgNPs

The antimicrobial activity of synthesized AgNPs by using aqueous $AgNO_3$ and plant extract, against both gram positive and gram negative pathogenic bacteria were shown in table 1. The well diffusion method was used to analyze the antimicrobial study of pathogens such as *E-coli, Pseudomonas aeruginosa* (gram negative) and *Staphylococcus aureus* (gram negative). The Fig. 6 shows that the zone of inhibition (ZOI) was increased when increasing the concentration of Silver nanoparticles. However the zone of inhibition was observed higher in gram negative bacteria than gram positive is mainly due to the differences in their membrane structures [33]. From the table 1, maximum zone of inhibition was observed in *E-coli* bacteria for 100 µl as 9mm. Minimum ZOI was observed from *Pseudomonas aeruginosa* and *Staphylococcus aureus* when compared to other pathogens [25,40].

Table 1: Zone of inhibition of AgNPs (mm)

Species	25 µl	50 µl	100 µl
E-coli	±3	±4	±9
Pseudomonas aeruginosa	±2	±3	±4
Staphylococcus aureus	±4	±3	±7

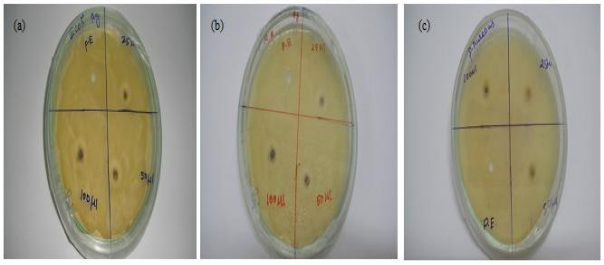


Fig.6: Antimicrobial activity of AgNPs *E-coli* (a), *Pseudomonas aeruginosa* (b), *Staphylococcus aureus (c)*

CONCLUSION

We have established an eco-friendly, rapid biological approach for the synthesis of AgNPs by using C*apparis zeylanica* plant leaves, which provides easy, cost effective, proficient way for the synthesis of AgNPs. The biological synthesis involves the utilization of plant leaves. The plant leaves used for the synthesis of nanoparricles has no other use and considered as a waste by product. The synthesizing of AgNPs using green source can be used in various biological applications. In this study, the development of AgNPs was observed by appearance of the solution and UV-vis spectroscopy. The XRD pattern was used to confirm that the synthesized AgNPs are found to be highly stable with the crystalline nature. The FTIR analysis was used to identify the functional group present in the leaf extract which are responsible for the reductions AgNPs. Both SEM and TEM studies confirmed that the synthesized AgNPs were spherical in shape with the range between 50-90 nm.

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