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Synthesis and Characterisation of Silver nanoparticles from aqueous extract of Talinum fruiticosum and its antibacterial activity

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Abstract

Synthesis of metal nanoparticles from different biological systems namely bacteria,fungi,algae,yeasts and plants has been reported. Among all biosynthesis methods, plants found to be the most suitable one. The focus of this study was to synthesize silver nanoparticles using aqueous extract of Talinum fruiticosum and study the antibacterial activity of the nanoparticles so produced. The synthesised silver nanoparticles were characterised by UV-Vis, FT-IR, XRD, SEM and TEM techniques. The size of biosynthesised silver nanoparticles between 10nm – 50nm showed significant antibacterial action against both the gram classes of gram negative bacteria, Escherichia coli and gram positive bacteria, Bacillus cereus.

Keywords: Silver nanoparticles, biosynthesis, antibacterial activity

I. Introduction

The field of nanotechnology is one of the most dynamic area of research in modern material science. Nanoparticles extensively used in the fields of medicine, biology, material science, physics and chemistry[1-2]. Silver nanoparticles exhibit unique properties depending upon their size,morphology and distribution.Synthesis of nanoparticles using plant extract [3], micro organisms [4], enzyme [5] have been more advantageous than physical ,chemical and microbial methods [6-10]. It is cost effective, eco friendly and easily scaled up for large scale process. Many research papers reported the synthesis of nanoparticles using various parts of plant extracts such as leaves from Helianthus annuus, Basella alba,Oryza sativa,Saccharum officinarum and Zee mays [11]; pine,ginkgo, magnolia, and platanus leaves[12]; seeds of Jatropha curcas [13]; roots of Trianthema decandra [14]; stems and roots of Ocimum sanctum [15] and Banana peel [16].

In this study we investigated the synthesis of stable silver nanoparticles from aqueous extract of Talinum fruiticosum leaf extract.This plant is an herbaceous perennial plant that is native to Mexico. It is widely grown in tropical regions as a leaf vegetable.It bears small, pink flowers and broad fleshly leaves.

2. Materials and Methods

A.Preparation of plant extract

Fresh and healthy leaves of Talinum fruiticosum were collected and washed with distilled water to remove dust particles and shade dried to remove moisture. (Fig.1) About 10g of Talinum fruiticosum leaves were finally cut into small pieces and transferred into 250ml round bottom flask containing 100ml distilled water. The mixture was refluxed for 1-2 hours until the colour of aqueous solution changed from colourless to yellow. The extract obtained was cooled at room temperature and filtered with Whatmann No.1 filter paper.

Fig.1 Talinum fruiticosum

B. Synthesis of silver nanoparticles

Silver solution (0.1mM) of silver nitrate was prepared and used for the synthesis of silver nanoparticles . 5ml of 10% Talinum fruiticosum extract was treated with 10ml of aqueous solution of 0.1mM silver nitrate and stirred at 65^0C for 15 – 20 minutes. Colour change from pale yellow solution to dark brown precipitate was

observed. The brown precipitate was washed, centrifuged at 50000 rpm for 30 minutes, dried at 60^0C in vacuum oven and stored in a sealed bottle.

C. Characterisation of silver nanoparticles

i) UV - Vis spectral analysis

UV - Vis spectral analysis was done using Shimadzu UV 2450 spectrophotometer.

ii) X-ray diffraction studies

XRD of powdered silver nanoparticle was recorded using a Philip P-W 1710 diffractometer with 0.15405nm Cu K_α radiation. The average particle size was calculated from the width of the XRD peaks using Debye Scherrer's formula

$$D = 0.94\lambda/\beta \cos\theta$$

Where D is the average crystallite domain perpendicular to the reflecting planes, λ is the X-ray wavelength, β is the full width at half maximum (FWHM) & θ is the diffraction angle.

iii) FT-IR

The FT-IR spectrum of silver nanoparticles was obtained using Brucker model within the mid IR region of 400-4000cm^{-1}. The dried experimental sample was mixed with KBr crystals and the spectrum was recorded in transmittance mode.

iv) SEM & TEM

The surface morphology of silver nanoparticle was obtained by SEM & TEM.

v) Antibacterial activity

Antibacterial activity of silver nanoparticle against gram negative Escherichia coli and gram positive bacteria Bacillus cereus were observed using a agar well diffusion & disk diffusion method respectively. Mueller Hinston agar were seeded with 4 hours old fresh culture of clinical isolates and referral strains.Various fractions of silver nanoparticles loaded discs were dispersed on the solidified Mueller Hinston agar with test organisms. Oxtetracycline and solvent loaded discs were used as positive and negative control respectively and the discs were incubated at 37^0C for 24 hours.

3. Results & Discussion

UV-Vis

Visual color change from pale yellow to brown when Talinum fruiticosum leaf extract added dropwise to silvernitrate was the primary evidence for the formation of silver nanoparticles.(Fig.2) The silver nanoparticles formed by the reduction of Ag^{+} into Ag^{0}. The reduction reaction is facilitated by the extract of Talinum fruiticosum. The appearance of brown color in aqueous solution is due to excitation of surface plasmon vibrations. It is observed that the surface plasmon resonance of silver nanoparticles is between 200 - 450nm [17].As the concentration of the Talinum fruiticosum leaf extract increases,the absorption peaks gets more sharpness and blue shift was noted which indicates a reduction in the size of silver nanoparticles.(Fig.3) This result was in correlation with the report of green synthesis of silver nanoparticles by Aegle marmelos leaf extract [18].

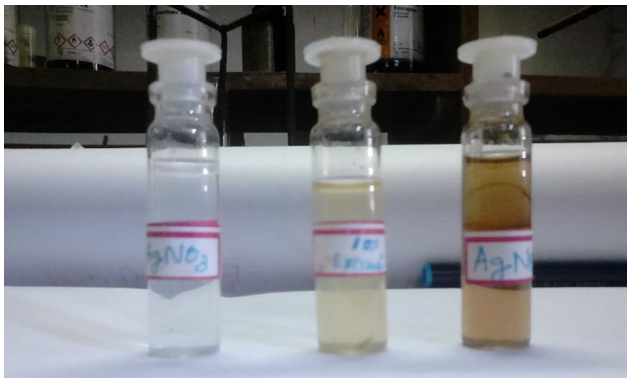

Fig.2 Color change of leaf extract from yellow (without silver nitrate) to brown (with silver nitrate)

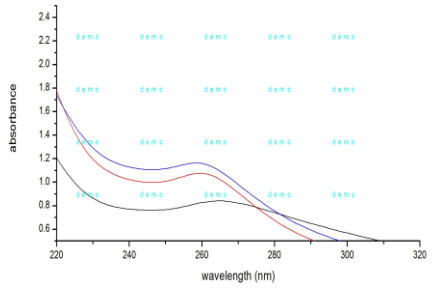

Fig.3 UV-Vis absorption spectrum of leaf extract of Talinum fruiticosum

FT-IR

FT-IR spectrum was recorded to identify the possible phytochemical in Talinum fruiticosum leaf extract responsible for capping lead to efficient stabilisation of silver nanoparticles. (Fig.4) The absorption at 3377, 2926 cm^{-1} correspond to the O-H stretching vibrations of phenols. Peaks correspond to the wave numbers 1602, 1384, 1117, 1087 cm^{-1} show the presence of O-H stretching of alcohol/ phenol. The absorption peak between 917cm^{-1} & 617 cm^{-1} is responsible for C-H out of plane bending of aromatic hydrocarbons.(Fig.4) These results confirms the presence of poly phenolic groups responsible of capping & stabilization of silver nanoparticles.

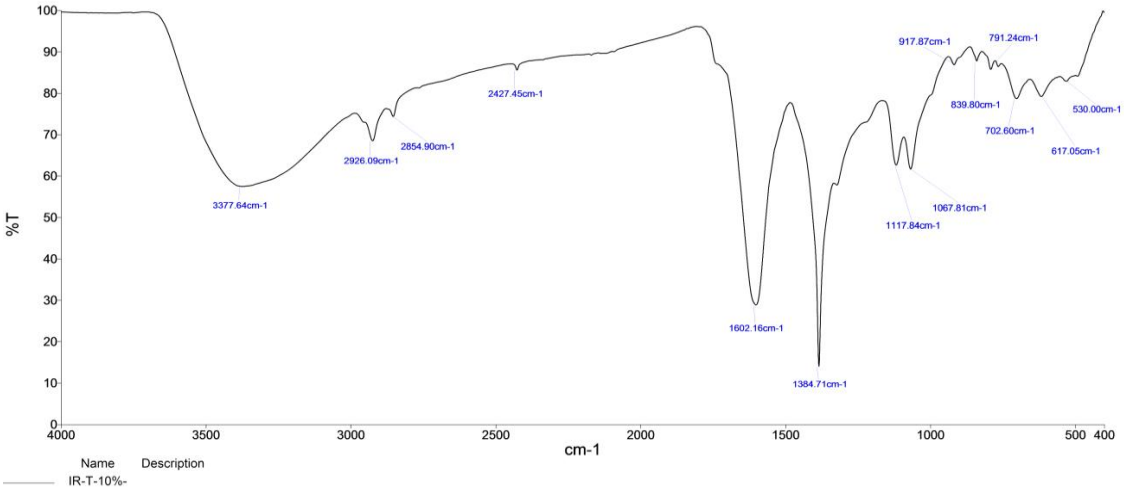

Fig.4 FTIR spectra of silver nanoparticles synthesized from Talinum fruiticosum

XRD

XRD pattern showed three intense diffraction peaks at 38.16^0, 44.24^0 and 64.32^0 corresponding to 111,200 and 220 reflection planes respectively confirmed the face centered cubic structure (JCPDS) No.84-0713.Thus, XRD result confirmed the formation of silver nanoparticles by the reduction of Ag^+ ion to Ag^0 by Talinum fruiticosum.The mean size of silver nanoparticle was calculated using Debye- Scherrer's equation by determining the full width at half maximum and found to be approximately 50nm. (Fig. 5)

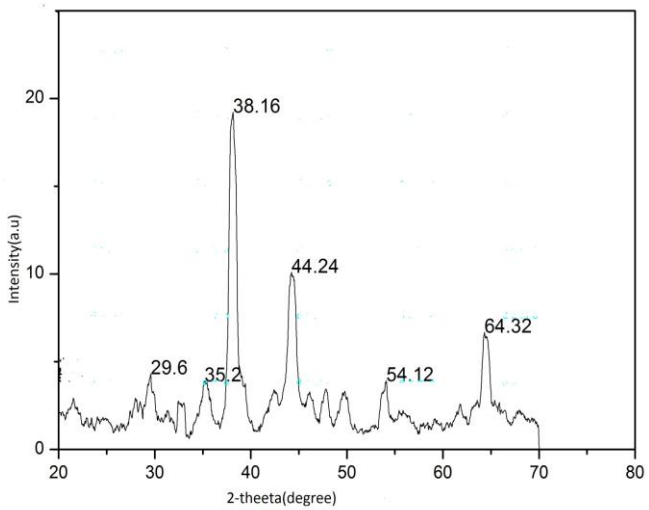

Fig.5 XRD spectra of silver nanoparticles synthesized from Talinum fruiticosum

SEM & TEM

SEM image show a uniform dispersed silver nanoparticles and TEM showed the nanoparticle formed are with diameter range 10-50nm. (Fig. 6 and Fig. 7)

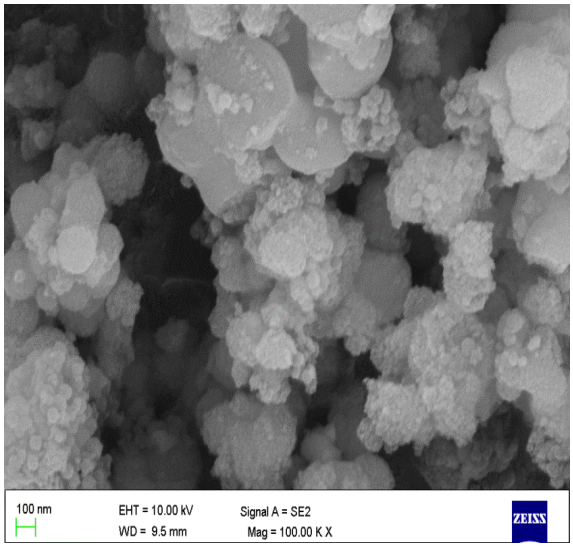

Fig.6 SEM image of silver nanoparticles synthesized from Talinum fruiticosum

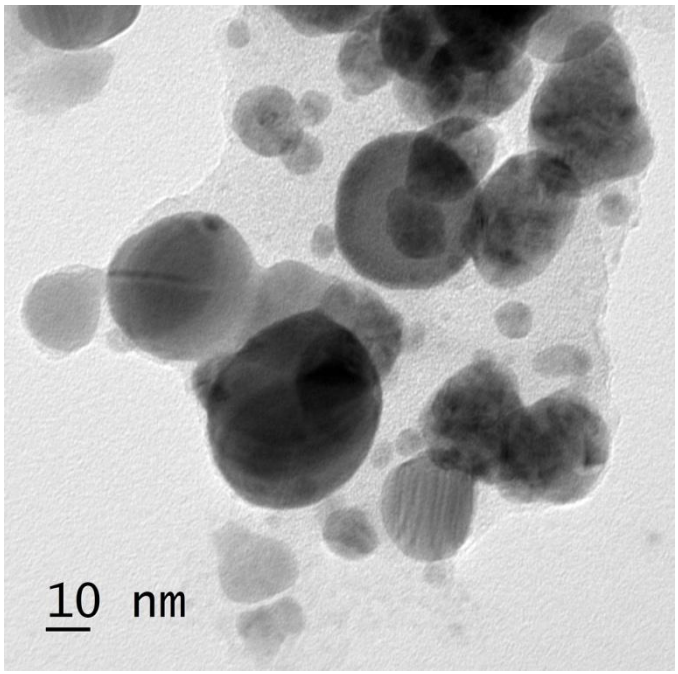

Fig.7 TEM image of silver nanoparticles synthesized from Talinum fruiticosum

Antibacterial Activity

Nanoparticle synthesis by green route are found to be highly effective against multi-drug resistant human pathogenic bacteria. Antibacterial activity of silver nanoparticles against Escherichia coli and Bacillus cereus were observed and compared. As the concentration of silver nanoparticles increases the zone of inhibition also increases. (Fig. 8 and Fig. 9) The maximum activity was found to be 35mm and 30mm for 400mg gram of both negative and 350mg of gram positive respectively.The antibacterial activity has been calculated by measuring the zone of inhibition and listed in Table 1.

Table 1. Zone of inhibition observed for Escherichia coli and Bacillus cereus

AgNPs (mg)	Zone of Inhibition (mm)	
	Gram Negative Bacteria Escherichia coli	Gram Positive Bacteria Bacillus cereus
50	-	23
100	22	25
150	-	20
200	22	25
250	-	27
300	30	29
350	-	30
400	35	-

Fig. 8 Represent the Zone of inhibition of AgNPs for gram negative bacteria Escherichia coli

Fig. 9 Represent the Zone of inhibition of AgNPs for gram positive bacteria Bacillus cereus

The exact mechanism of antibacterial activity is partially understood. Silver nanoparticles generate free radicals that damage the membrane,the effect is dose dependent. As the activity of antibacteria is depend on the dosage of silver nanoparticles, the present study may follow this mechanism, but it still remains to clarify the exact mechanism of silver nanoparticles against micro organism.

Conclusion

Talinum fruiticosum is acting as an excellent capping and stabilizing agent for the rapid and ecofriendly synthesis of silver nanoparticles. XRD revealed that the biosynthesised silver nanoparticles is crystalline and of 10-50nm size.It acts as an antibacterial agent against gram negative and gram positive bacteria. The presence study showed a lucid, rapid and eco friendly route to synthesize silver nanoparticles.

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