

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Biosynthesis of Silver Nanoparticles from *E.Tirucalli* and to Check Its Antimicrobial Activity.

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ABSTRACT

Since times plants have been used for various potential and therapeutic uses. In this study, aqueous extract of the stem portions of the plant *Euphorbia Tirucalli* (L.) was made. Further, silver nanoparticles were synthesized and the results recorded from UV-Visible spectrum, Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction (XRD) and Field Emission Scanning Electron Microscopy (FESEM) support the biosynthesis and characterization of silver nanoparticles. Also, antimicrobial activity by agar well diffusion method was tested against *B.subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*. The approach of plant-mediated synthesis appears to be cost efficient, eco-friendly and easy alternative to conventional methods.

Keywords: *Euphorbia Tirucalli*; Nanoparticles; Antimicrobial Activity

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INTRODUCTION

Green synthesis of silver nanoparticles is a kind of bottom up approach. It involves the synthesis, characterization, exploration and application of nanosized (1-100nm) materials for the development of science. The intrinsic properties of metal nanoparticles are mainly determined by size, composition, crystallinity and morphology. Silver nanoparticles have been recently known to be a promising antimicrobial agent that acts on a broad range of target sites both extracellularly as well as intracellularly. It shows very strong bactericidal activity against gram positive as well as gram negative bacteria [1,2]. *Euphorbia tirucalli* L. belongs to genus *Euphorbia*, one of the 8,000 species within family Euphorbiaceae. It is a shrub or a small tree endemic to tropical areas with pencil-like branches. *E. tirucalli* is generally evergreen since its stems and branches remain green all year round and are rarely fed on by herbivores. It bears white poisonous latex, which may possibly account for the low herbivore pressure and medicinal features [3,4]. The present study was aimed to Synthesis of silver nanoparticles from aqueous extract of *E.Tirucalli*, Characterization of nanoparticles and its antimicrobial activity.

MATERIALS AND METHODS

Preparation of plant extract

Fresh plant material was washed thoroughly under running tap water, shade dried and used for extraction. The dried stems were homogenized to a fine powder and stored in airtight bottles. 25 g of stem powder was extracted with 150 ml of water. The extract was dried in a flash evaporator for 30 min and the left over powder was considered 100%.

Biosynthesis of Silver Nanoparticles

The collected plant sample was washed twice with fresh water and distilled water. Cleaned sample was shade dried and was powdered with the help of mortar and pestle. This powder was added to the 100 ml deionized water with constant stirring and exactly 17mg AgNO₃ was added to the obtained solution to get a final concentration of 1 mM and the solution was kept in a water bath at 90°C for 20 minutes in dark condition. Thereafter, the solutions were centrifuged at 5000 × *g* for 20 min to get a clear solution of silver nanoparticles. The change in color from colorless to brown color was taken for visible confirmation of formation of silver nanoparticles. Then, the centrifuged sample was subjected to further characterization.

Characterization Techniques

The characterization of materials is important for understanding their properties and applications. The techniques adopted to characterize the nanoparticles are: X-ray diffraction, UV-Visible Spectroscopy, Fourier transform infrared spectroscopy, Field emission scanning electron microscopy.

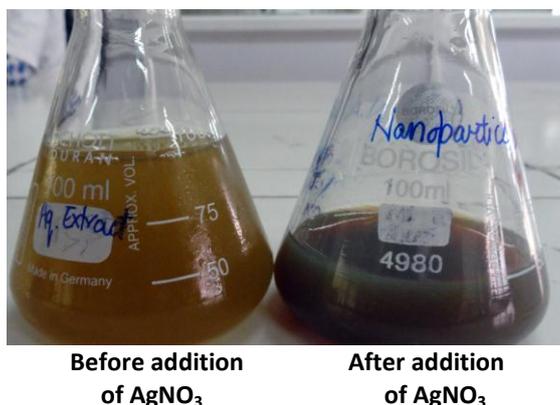
Antimicrobial activity

Antibacterial activity of *Euphorbia Tirucalli* assisted silver nanoparticles was carried out by well diffusion method against Gram Positive and Gram Negative Bacteria. Bacterial cultures used were *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *E.Coli*. These bacterial cultures were freshly cultivated for 24 h in Nutrient broth. Each bacterial culture was spread on the Muller Hinton agar plates. Wells were then made in the agar plate. After the 24 hours of incubation the zone formation was recorded. Experiments were repeated for three times.

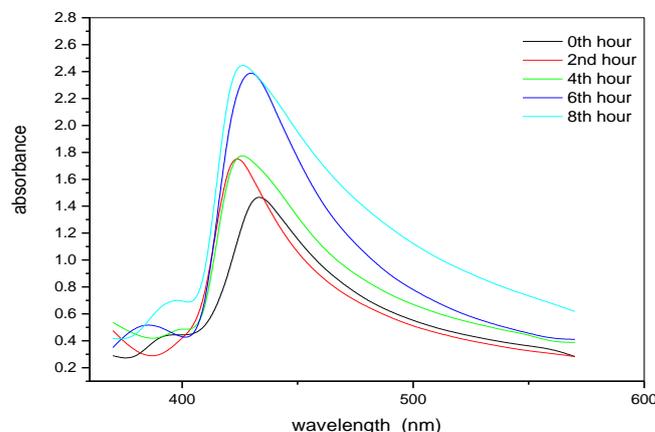
RESULTS AND DISCUSSION

Biosynthesis of Silver Nanoparticles

To 10 ml of prepared aqueous extract of *E.Tirucalli*, 40ml of 1mM silver nitrate solution was added. The solution formed was heated up to 60 °C for 20 min and the change in colour to dark brown was visualized. The colour change is due to reduction of silver metal ions into silver nanoparticles. The intensity of the colour change increased in direct proportion to the incubation period of nanoparticle synthesis. It may be due to the excitation of surface plasmon resonance (SPR) and also may be because of the presence of various phytochemicals present in the *Euphorbia Tirucalli*.



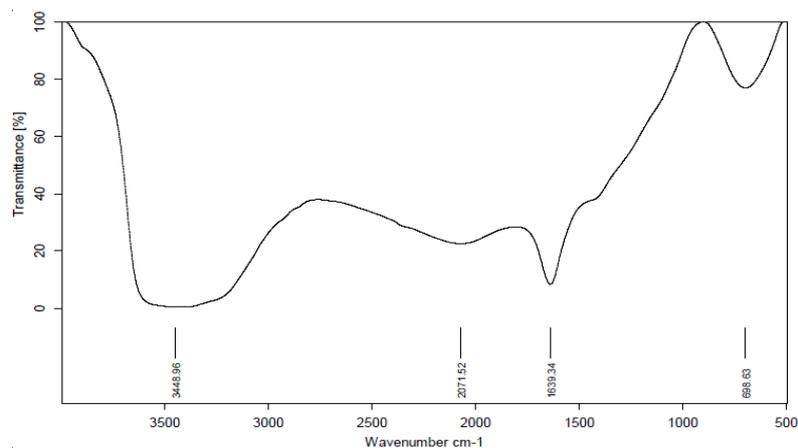
UV Spectrum Analysis



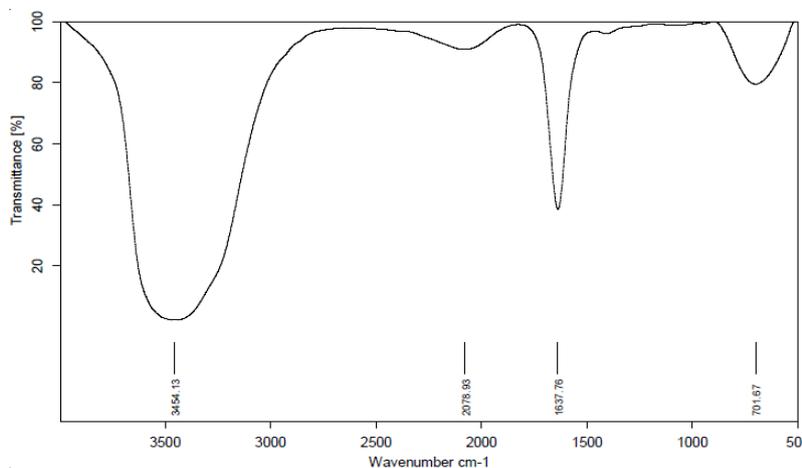
UV Spectrum of Silver Nanoparticles formed at different intervals of time.

UV-vis spectra was recorded for the plant extract of *E.Tirucalli*. It was introduced with 1 mM AgNO₃ solution and absorbance was documented at various time intervals. After 24 hrs. of incubation in the dark room condition yellow colour reaction mixture was turned into dark brown. It may due to the excitation of surface plasmon resonance for the synthesised silver nanoparticles. The silver nitrate plant extract reaction mixture exhibits the strong peak at 435 nm. Silver nanoparticles exhibit unique and tunable optical properties on account of their surface plasmon resonance; dependent on shape and size distribution of the nanoparticles. The plant extract of *E.Tirucalli* without adding the silver nitrate was used as a control showed the peak at 280–300 nm. It may due to the reducing protein molecules present in the extract of *E.Tirucalli*.

FTIR Spectrum Analysis



FTIR Spectrum of *E.Tirucalli* extract.



FTIR Spectrum of Silver nanoparticles mediated by *E.Tirucalli* extract

The major factor liable for the biological reduction of silver ions (Ag⁺) into silver nanoparticles (Ag) present in the plant extract of *E.Tirucalli* were identified using FTIR spectroscopy. The biologically synthesized nanoparticles and the powdered plant extract were mixed with the Potassium bromide to make a pellet. The IR spectrum of plant extract alone showed the distinct peak in the range of 3448.96, 2071.52, 1639.34, 698.63.

The spectrum of IR peak at 3448.96 indicated strong bonding of primary amines. The vibrational mode at 2071.52 corresponds to c=c variables in the plant protein. The peak

obtained at 1639.34 shows similar conjugation effects to carbonyl group while a medium bonding of haloalkanes was observed at 698.63.

The FTIR spectrum of silver nanoparticles showed the distinct peak in the range of 3454.13, 2078.93, 1637.76, 701.67. The comparison of FTIR spectrum between the plant extract and silver nanoparticles were observed with only minor changes. Due to the silver nanoparticles the O H stretching vibration shifted from 3488.76 to 3419.18.

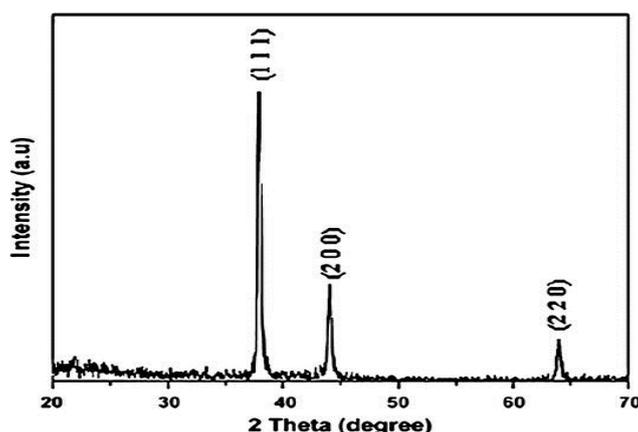
Analysis of XRD Pattern

The X-ray diffraction studies were performed to confirm the crystalline structure of synthesized silver nanoparticles. XRD spectrum of plant extract reduced silver nanoparticles showed three distinct diffraction peak at 38.1, 44.3, 64.4. The lattice plane value was observed which may indexed at 1 1 1, 2 0 0, and 2 2 0 of the cubic silver. The crystallite domain size was calculated from the width of the XRD peaks, assuming that they are free from nonuniform strains, using the Scherrer's formula.

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

where D is the average crystallite domain size perpendicular to the reflecting planes, λ is the X-ray wavelength, β is the full width at half maximum (FWHM), and θ is the diffraction angle. This formula is valid only when the crystallite size is smaller than 100 nm.

The lattice constant calculated from the diffraction spectrum was $a = 4.0857 \text{ \AA}$ and the resultant data was matched with the database Joint Committee on Powder Diffraction Standards (JCPDS) file no. 01-087-0717. The resultant XRD spectrum clearly suggests that the silver nanoparticles synthesized from the extract of *E.tirucalli* L. was crystalline.

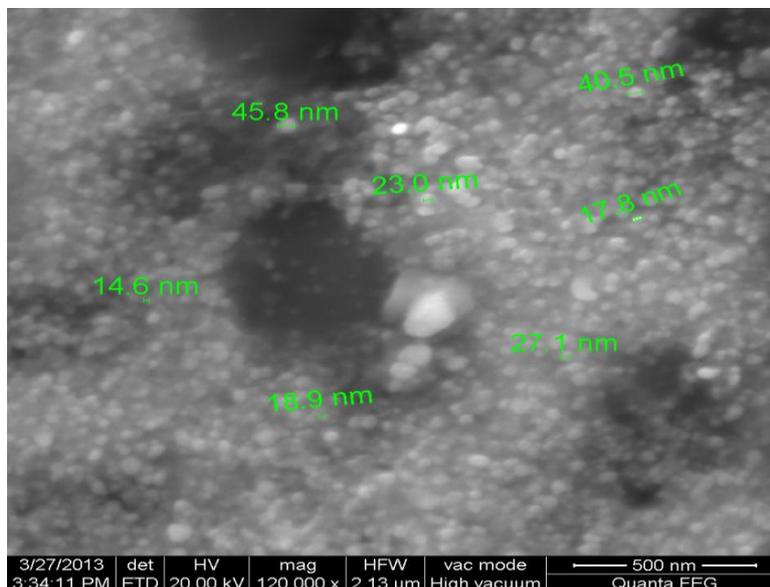


XRD Pattern of Silver nanoparticles mediated by *E.Tirucalli* extract

Analysis of Field Emission Scanning Electron Microscopy

The biologically synthesized silver nanoparticles were high in density, spherical shaped, well distributed without aggregation in solution. The aliquot of silver nanoparticle solution was placed into a drop coated copper grid and the sample was allowed to dry. The SEM images were recorded at different magnification to find the individual particles. The

synthesized silver nanoparticles were observed in spherical shape and average size of the particles was 24 nm. The variation in the particle sizes such as 14, 23, 27, 40 and 45 nm difference in size is possibly due to the fact that the nanoparticles are being formed at different times.



SEM Image of Silver nanoparticles mediated by *E. Tirucalli* extract

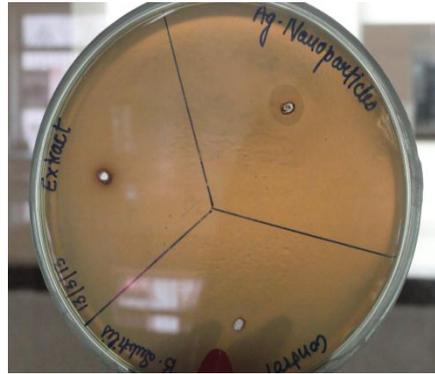
Antibacterial Assay

The biologically synthesized silver nanoparticles showed excellent antimicrobial activity against clinically isolated human pathogens such as Gram positive bacteria *Staphylococcus aureus*, *Bacillus subtilis* and Gram negative bacteria *Pseudomonas aeruginosa* and *E. coli* [5-7].

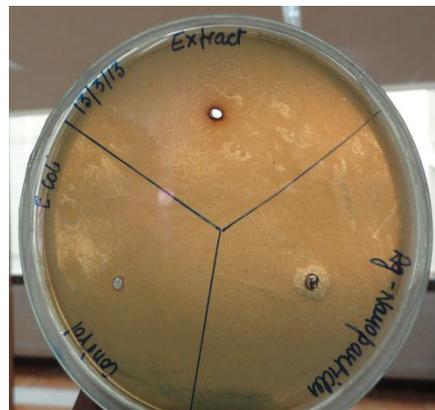
The Gram negative bacterium *E. coli* showed maximum zone of inhibition which may be due to the cell wall of Gram positive bacteria composed of a thick peptidoglycan layer, which is consisting of linear polysaccharide chains cross linked by short peptides thus forming a more rigid structure leading to difficult penetration of the silver nanoparticle compared to the gram negative bacteria where the cell wall possesses a thinner peptidoglycan layer [8].

The high bactericidal activity is certainly due to the silver cations released from Ag nanoparticles that act as reservoirs for the Ag⁺ bactericidal agent. Big changes in the membrane structure of bacteria as a result of the interaction with silver cations lead to the increased membrane permeability of the bacteria. Silver nanoparticles are believed to become attached to the negatively charged bacterial cell wall and rupture it, which causes accumulation of envelope protein precursors, which results in dissipation of the proton motive force which leads to denaturation of protein and finally cell death [9]. On the other hand, it is elucidated that silver nanoparticles exhibited destabilization of the outer membrane and rupture of the plasma membrane, thereby causing depletion of intracellular ATP. Silver has a greater affinity to react with sulfur- or phosphorus-containing biomolecules in the cell.

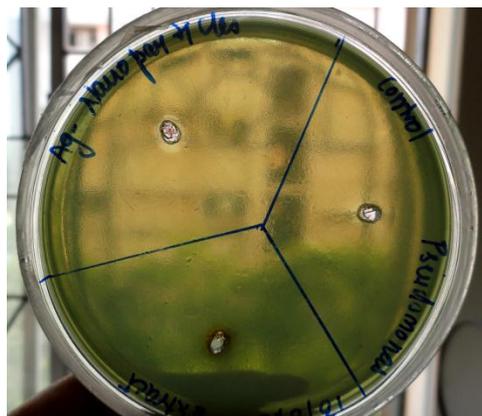
Thus, sulfur-containing proteins in the membrane and phosphorus containing elements like DNA are likely to be the preferential sites for silver nanoparticles binding.



Zone of inhibition of *E. Tirucalli* mediated silver nanoparticles against *Bacillus Subtilis*.



Zone of inhibition of *E. Tirucalli* mediated silver nanoparticles against *E.Coli*.



Zone of inhibition of *E. Tirucalli* mediated silver nanoparticles against *Pseudomonas Aeruginosa*.



Zone of inhibition of *E. Tirucalli* mediated silver nanoparticles against *Staphylococcus Aureus*.

CONCLUSION

Highly monodisperse silver nanoparticles were synthesized using leaf extract of a well-known medicinal plant, *E.Tirucalli*. The synthesis was found to be efficient in terms of reaction time as well as stability of the synthesized silver nanoparticles. This nanoparticulate solution exhibited excellent stability for six months from the date of synthesis. The process is truly scalable owing to lesser specificity of reaction parameters. Investigation on the antibacterial activity of the nanoparticles against *E. coli*, *S. aureus*, *P.Aeruginosa* and *B.Subtilis* reveals high potential of *E.Tirucalli* extract stabilized AgNPs to be used as antimicrobial agent in medical field as well as food and cosmetic industries.

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