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BIOSYNTHESIS OF SILVER NANOPARTICLES USING LEAVES EXTRACTS OF *ACALYPHA HISPIDA* BURM.F. AND STUDY OF THEIR ANTIBACTERIAL ACTIVITY

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ABSTRACT

The synthesis of metal nanoparticles is an expanding research area due to their potential applications for the development of novel technologies. In this study, we have described a cost effective, green and environment friendly technique for synthesis of silver nanoparticles (AgNPs) and their antibacterial activity. The aqueous Ag^+ ions from 5mM silver nitrate solution were reduced into AgNPs when treated with the leaves extracts of Acalypha hispida. The synthesized AgNPs were characterized by UV–visible spectroscopy, Fourier transform infra-red spectroscopy (FTIR), X-ray diffraction (XRD) and Scanning electron microscopy (SEM) analysis. The phytochemical analysis of the plant Acalypha hispida leaves extracts reveals the presence of flavonoids, saponins, alkaloids, phenols, tannins and cardiac glycosides. The silver nanoparticles were tested for antimicrobial activity and AgNPs have shown good antibacterial activity against Staphylococus aureus, Pseudomonas aeruginosa, Escherichia -coli and Klebsiella aerogenes.

Keywords: Acalypha hispida, Leaves extracts, AgNPs, XRD, SEM, Antibacterial activity.

I. Introduction

Nanotechnology involves the development of experimental processes for the synthesis of nanoparticles of different shapes, sizes and controlled dispersity [1]. Nobel metals such as Ag, Pt, Au and Pd are widely used to prepare metallic nanoparticles [2]. Out of these Nobel metals, silver is the best metal of choice in the field of biological leaves, living organisms and medicine [3]. Silver nanoparticles are widely used because of their unique properties in biosensing, chemical sensing, catalysis, photonics, electronics, and pharmaceuticals [4]. Silver nanoparticles possess good antimicrobial activity [5]. Antimicrobial action of silver nanoparticles allows them to be suitably employed in numerous household products such as food storage containers, textiles, medical devices, and home appliances [6]. Silver is an effective antimicrobial agent which exhibits less toxicity [7]. The most important application of silver and silver nanoparticles is in medical industry such as tropical ointments to prevent infection against burn, cuts and open wounds [8]. A silver nanoparticle plays a very important role in the field medicine. Silver products have long been known to have strong inhibitory and bactericidal effects, as well as a broad spectrum of antimicrobial activities, which has been used for centuries to prevent and treat various diseases, most notably infections [9]. The Silver nanoparticles are reported to possess antifungal, anti-inflammatory, anti angiogenesis, antiviral, and anti platelet activity [10]. The use of environmental friendly materials like plant leaf extract, bacteria and fungi are used for the synthesis of silver nanoparticles as they do not use toxic chemicals in the synthesis protocols, which offers numerous benefits of eco-friendliness and compatibility for the biomedical and pharmaceutical applications [11]. Green synthesis of nanoparticles have advantage over physical and chemical methods as the method are cost effective and environment friendly and no need of using high pressure, temperature, and energy toxic chemicals[12].

Plants provide a best platform for synthesis of nanoparticles as they are free from toxic chemicals and also provide natural capping agents. Moreover, the use of plant extracts also reduces the cost of microorganisms isolation and culture media enhancing the cost competitive feasibility over nanoparticles synthesized by microorganisms [13]. Antibacterial activity of the silver containing materials is used in medicine to reduce infections in burn treatment [14] and arthroplasty [15], as well as prevent bacteria colonization on prostheses [16], catheters [17], vascular grafts, dental materials [18], stainless steel materials [19] and human skin [20].

Acalypha hispida, the chenille plant, is a flowering shrub which belongs to the

family Euphorbiaceae, the subfamily Acalyphinae, and the genus *Acalypha*. *Acalypha* is the fourth largest genus of the Euphorbiaceae family, and contains many plants native to Hawaii and Oceania. This plant is also known as the Philippines Medusa, red hot cat's tail and fox tail in English. *Acalypha hispida* is cultivated as a house plant because of its attractiveness and brilliantly colored, furry flowers. The plant is dioecious, and therefore there are distinct male and female members of the species. The female plant bears pistillate flowers which range in color from purple to bright red, and grow in clusters along catkins. This feature is the primary reason the plant bears the nickname "red-hot cat tail". The pistillates will grow all year long as long as the temperatures are favorable [21-22]. The plant is sometimes grown as a hedge, and the female form is often grown as an ornamental in tropical areas, valued especially for its profusion of showy red inflorescences. The leaves are used to treat thrush. A poultice of the leaves is used in the treatment of leprosy. In Malaysia a decoction of the leaves and flowers is externally applied as an emollient to wounds and ulcers. Internally, it is used as a laxative and diuretic in treating gonorrhea [23-24].

In the present work, we investigated the simple, effective, low cost biological (green) synthesis of stable silver nanoparticles by the bioreduction method using aqueous leaves extracts of *Acalypha hispida* and evaluated their antibacterial activity against drug resistant bacterial strains.

Fig. 1 Acalypha *hispida*.

II. Experimental

A. Materials

Acalypha hispida leaves were collected from the campus garden of Shridevi Institute of Engineering and Technology, Sira Road, Tumakuru, Karnataka, India. The lyophilised bacterial strains *Staphylococus aureus, Klebsiella aerogenes, Pseudomonas aeruginosa and E-coli* were procured from Department of Microbiology, Shridevi Institute of Medical Sciences and Research Hospital, Tumakuru, Karnataka, India. The nutrient media was supplied by Hi-Media Laboratories. $AgNO_3$ was procured from Merck, Mumbai, India.

B. Methods

1) Preparation of leaves extracts:

For the synthesis of silver nanaoparticles, the leaves of *Acalypha hispida* were washed thoroughly with tap water to remove the dust and dirt particles and then washed with double distilled water. 20 g of chopped leaves were added to 100 ml double distilled water and stirred at 60°c for 30 min on heating mantle. After boiling, the mixture was cooled for 20 min and filtered through Whatman filter paper No.1. The collected leaves extracts (bright green color) was used for reducing and as capping agents in AgNPs synthesis.

(A) Leaves extracts	**(B)** 5mM $AgNO_3$solution	**(C)** Leaves extracts + $AgNO_3$ after 24 hour

Fig.2 Formation of AgNPs.

2) Synthesis of Silver Nanoparticles using leaves extracts:

5 ml of *Acalypha hispida* leaves extracts was added to the 45 ml of 5mM $AgNO_3$ solution at ambient temperature and the mixture was stirred continuously for 15 min using magnetic stirrer. Slow reduction was observed and kept for 24 hours for complete reduction. After 24 hours bright green color of the mixture was turned into dark brown color which indicates the formation of AgNPs (Fig. 2) The AgNPs obtained from the mixture was purified by repeated centrifugation at 8,000 rpm for 15 min using Remi cooling centrifuge C-24. The AgNPs obtained were dried and stored for further analysis.

3) Analysis of Silver nanoparticles

a) Phytochemical analysis.

The leaves extracts of *Acalypha hispida* were assessed for the qualitative determination of chemical constituents i.e. phenols, alkaloids, saponins, flavonoids, tannins, terpenoids and cardiac glycosides by applying standard procedures [25-29].

b) UV-Vis Spectra analysis:

The reduction of pure silver ions was observed by measuring the UV-Vis spectrum of the reaction at different time intervals taking 1ml of the sample, compared with 1ml of distilled water used as blank.The sample was analysed by UV-Vis spectrophotometry (model Shimadzu UV) for its maximum absorbance v/s wavelength to confirm the formation of AgNPs.

c) Fourier Transform Infra-Red spectroscopy (FT-IR) analysis:

The sample was mixed with KCl procured from Merck. Thin sample disc was prepared by pressing with the disc preparing machine and placed in Fourier Transform Infra Red [FTIR] for the analysis of the nanoparticles. The FTIR measurement sample was recorded in the range of 400-4000cm^{-1} using Nicolet Avatar model. It gives information on the rotations and vibrations modes were identified and purposed to determined the distinct functional groups present.

d) X-Ray diffraction analysis:

The reduced AgNPs powder was coated on a glass substrate and the X-ray diffraction measurement were carried out by using a powder X-ray (PAN analytical BV model) instrument operating at a voltage of 40kV and current of 30mA. The output was recorded in the form of a graph with 2θ on x-axis and then intensity on y-axis. The crystallite average size of particle was calculated by using the Debye-Scherrer formula.

$D=k\lambda/\beta\cos\theta$,

Where λ is wavelength, D is particle diameter size, β is the full width half maximum, k is a constant (value 0.9) and θ is Braggs diffraction angle.

e) Scanning Electron Microscopy (SEM) of silver nanoparticles:

After the preparation of the nanoparticles, the particle size and its morphological distribution were assessed with Scanning Electron Microscopy (SEM).

f) Antimicrobial activity of silver nanoparticles:

The antibacterial activity of AgNPs produced by *Acalypha hispida* leaves extracts were evaluated by the disc diffusion method. *Staphylococus aureus, Klebsiella aerogenes, Pseudomonas aeruginosa and Escherichia coli* bacterial strains were developed in nutrient broth (NB) media for 24 h at 37°c and 1 ml of each broth culture was spread over the nutrient agar media. 5 mm sterilized filter paper discs were dipped in synthesized Silver nanoparticles suspension (10µg/ml), double distilled water was used as negative control, Taxim (1µg/ml) was used as standard and leaves extract was placed over the agar plates and incubated for 24 h at ambient temperature.

III. Results and discussion

a) Phytochemical analysis

The results of phytochemical analysis of *Acalypha hispida* leaves extracts has shown the presence of flavonoids, saponins, alkaloids, phenols, tannins and cardiac glycosides (table.1).

Table.1 Phytochemical analysis of leaves extracts of *Acalypha hispida*.

Sl.No.	Phytochemicals	Leaves extract
1	Flavonoids	+++
2	Alkaloids	+++
3	Phenols	++
4	Tannins	+++
5	Cardiac glycosides	++
6	Saponins	++
7	Terpenoid	—

+: Confirms, —: Absent.

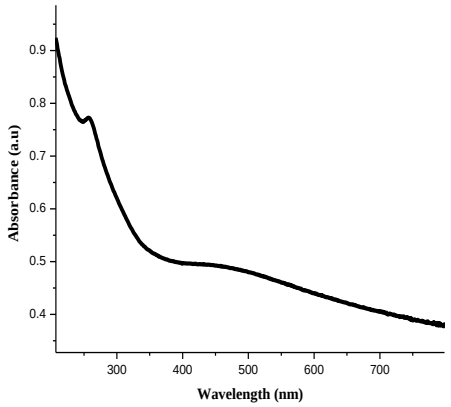


Fig. 3 UV-vis spectrum of AgNPs synthesized by *Acalypha hispida* leaves extracts.

b) UV-Vis-spectroscopy analysis:

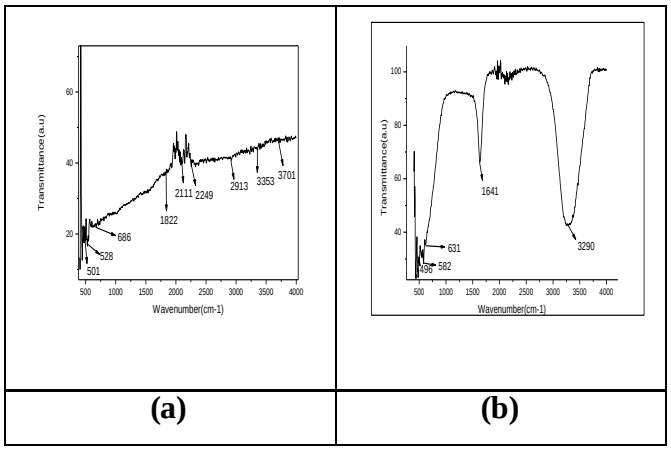
Reduction of silver ions present in the aqueous solution of silver Nitrate by the bioreductants present in the *Acalypha hispida* plant leaf extracts have been studied by the UV-Vis spectroscopy. UV-Vis spectrograph of the colloidal solution of silver nanoparticles was recorded as a function of time by using a quartz cuvette with water as reference. Maximum absorbance peak was observed at 456 nm indicating the formation of silver nanoparticles (Fig. 3).

c) FT-IR analysis.

FT-IR spectrum was performed to identify and to determine the different functional groups present in the AgNPs (Fig. 4. (a)). The IR bands were observed at 3701, 3353, 2913, 2249, 2111, 1822, 686, 528 and 500cm^{-1} (fig.4.(a)). The strong bands which appeared at 3701cm^{-1} Amide N-H stretch and 3353cm^{-1} Alcohol O-H, the bands at 2913cm^{-1} Alkyl C-H, 2249cm^{-1} Nitrile CN, 2111cm^{-1} Alkyne C≡C, 1822cm^{-1} Carbonyl C=O, 686cm^{-1} Alkyl halide C-Cl, 528 cm^{-1}Alkyl halide C-Br and the low band at 500cm^{-1} corresponds to Alkyl halide C-I.

In *Acalypha hispida* leaves extracts, FT-IR spectrum was performed to identify and to determine the different functional groups present (Fig. 4. (b)). The strong band were observed at 3290cm^{-1} Alcohol O-H stretch, the bands at1641 cm^{-1} Alkenyl C=C, 631 Alkyl halide C-Cl,582cm^{-1}

Alkyl halide C-Br and low band at 496cm^{-1} Alkyl halide C-I.



(a) (b)

Fig.4 (a) - IR spectra of silver nanoparticles synthesized using *Acalypha hispida* leaves extracts.

Fig.4 (b) FT-IR spectrumum of *Acalypha hispida* leaves extracts.

d) X-ray diffraction.

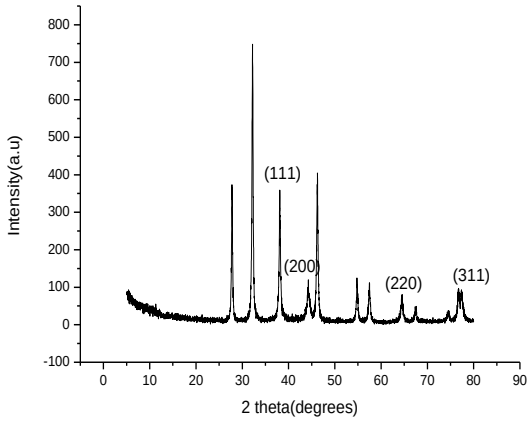


Fig. 5 XRD patterns recorded for the AgNPs synthesized from *Acalypha hispida* leaves extracts.

An X-ray diffraction (XRD) pattern was recorded for the synthesized AgNPs (Fig. 5), shows a number of Bragg reflections corresponding to (111), (200), (220) and (311) sets of lattice planes are observed. Which may be indexed based on the structure of silver. The diffraction peaks at 2θ= 38.04°, 44.21°, 64.39° and 77.33° were indexed with the planes (111), (200), (220) and (311) for the fcc lattice of obtained silver as per the Joint Committee on Powder Diffraction Standards (JCPDS) card no. 89-3722 was matched with database. The average size (D) of synthesized Silver nanoparticles was found to be 34.31 nm as calculated by using Debye-Scherer formula. The XRD pattern thus clearly shows that the silver nanoparticles are crystalline in nature.

e) Scanning Electron Microscopy (SEM) of silver nanoparticles:

SEM analysis shows the uniformly distributed silver nanoparticles on the surface of the cells. However, it does not indicate that all the nanoparticles are bound to the surface of the cells, because those dispersing in the solution may also deposit onto the surface of the cells. The SEM image (Fig. 6) has shown separate AgNPs as well as particle agglomeration. This indicates, the particle size is irregular and shape of the particles was spherical in morphology with an average size of 34.5 nm ranging from 29 to 42 nm.

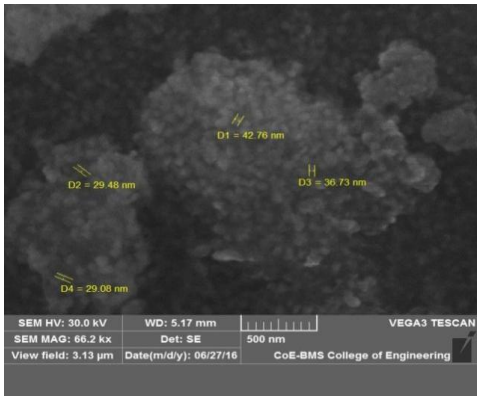
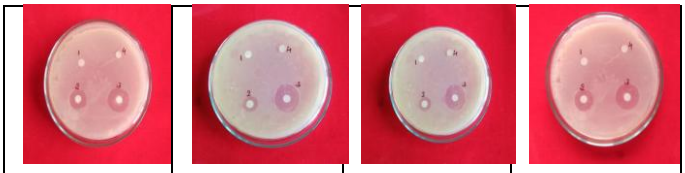


Fig. 6 SEM images of synthesized AgNPs from *Acalypha hispida* leaves extracts.

f) Antibacterial Assay

The synthesized AgNPs have shown a significant antibacterial activity against *Pseudomonas aeruginosa*, *Klebsiella aerogenes*, *Escherichia coli*, and *Staphylococus aureus* (Fig. 7; Table 2).



Staphylococus aureus	*Pseudomonas aeruginosa*	*Klebsiella aerogenes*	*Escherichia coli*

Fig. 7 Antibacterial activity of AgNPs synthesized byleaves extracts of *Acalypha hispida*

Table.2 Antibacterial Zone of Inhibition.

Zone of Inhibition (in mm)					
Sl. No	Strains	(1) Control	(2) Standard	(3) AgNPs	(4) Leaves Extract
1	*Escherichia coli*	–	19	26	–
2	*Pseudomonas aeruginosa*	–	12	27	–
3	*Klebsiellaaerogenes*	–	11	23	–
4	*Staphylococus aureus*	–	16	24	–

Control - double distilled water, AgNPs - Silver Nanoparticles, Standard -Taxim, Leaves Extract - *Acalypha hispida* leaves extracts.

IV. Conclusion

It is concluded that the leaves extracts of *Acalypha hispida* is capable of producing silver nanoparticles and are stable in solution. SEM analysis shows the particle size was irregular and shape of the particles was spherical in morphology with an average size of 34.5 nm ranging from 29 to 42 nm. The XRD pattern clearly shows that the silver nanoparticles are crystalline in nature and the average size of Silver nanoparticles was found to be 34.31 nm. Green synthesis is an alternative to chemical synthesis protocols as the method is low cost and eco-friendly. It was confirmed that the silver nanoparticles are capable of rendering antimicrobial

efficacy and proved to be active against the *Escherichia coli*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa* and *Staphylococus aureus*.

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