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EVALUATION OF ANTIBACTERIAL ASSAY AND CHARACTERIZATION OF SILVER NANOPARTICLES PRODUCED BY GREEN SYNTHESIS METHOD USING HYLOCEREUS UNDATUS FRUIT EXTRACT

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Abstract
The present work mainly deals with the study pertaining to the synthesis, characterization and evaluation of antibacterial properties of silver nanoparticles (AgNPs) synthesized from the pulp extracts of Hylocereus undatus fruit (Pitahaya). The AgNPs were synthesized by using a rapid, single step green synthesis method. The synthesized AgNPs were characterized by various instrumental techniques such as ultraviolet-visible spectroscopy (UV-Vis), X-ray diffraction (XRD) and scanning electron microscope (SEM). The synthesized AgNPs were found to be spherical in shape with average diameter of 31 nm. The AgNPs have shown good antibacterial activity against Klebsiella aerogenes, E-coli, Staphylococus aureus andPseudomonas aerogenes.

Keywords**: **Hylocereus undatus, Pulp Extracts, SEM, XRD, Antibacterial Activity.

I. Introduction

Nanotechnology is mainly deals with the production of nanoparticles and their uses in various fields of chemistry, physics, medicine, materials science, and engineering. It is rapidly growing by synthesizing nanoparticles and nano products. The nanoparticles differ significantly from other matter as they possess novel and size-related physico-chemical properties. Nanoscience is an interdisciplinary subject which [1] depends on the fundamental properties of nano size matter [2, 3]. Nanoparticles possess amazing optical, electronic, magnetic, and catalytic properties than the mass material owing to their high surface area to volume ratio [4, 5].The nanoparticles can be prepared by chemical methods by using chemical reducing agents such as hydrazine, sodium citrate and sodium borohydride [6] and bio reduction using extracts of yeast, fungi, bacteria and plant [7]. Recent studies have shown that green biologically based methods using microorganisms and plants to synthesize nanoparticles are safe, inexpensive, and an environment-friendly in nature. Green synthesis of nanoparticles uses water as solvent which replaces toxic organic solvents. Silver nanoparticles (AgNPs) have unique optical, electrical, and thermal properties and therefore these are used in various products that range from photo voltaic to biological and chemical sensors. AgNPs are widely used in the medicinal, pharmaceutical, agricultural industry and in water purification. Silver nanoparticles have received special attention because of their physico – chemical, catalytic, bactericidal and biological properties which found many applications in nano biotechnological research [8, 9]. AgNPs are used as antimicrobial agents in wound dressings [10–12], as topical creams to prevent wound infections [13], and as anticancer agents [14]. A number of plant extract mediated synthesis of AgNPs have been reported in the literature. For instance the use of Red Apple (*Malus domestica*)[15], M*yristica fragrans* (nutmeg) [16], *Avocado* [17], *Abelmoschus esculentus* [18], *Limonia acidissima* [19], and fruit extract of Andean blackberry [20].

In the present study the ***Hylocereus undatus*** fruit is selected for the synthesis of AgNPs. *Hylocereus undatus* (white-fleshed pitahaya) is a species of *Cactaceae* and is the most cultivated species in the genus. It is used both as an ornamental vine and as a fruit crop - the pitahaya or dragon fruit (Fig.1).

Fig.1: *Hylocereus undatus* fruit

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II. Experimental

Collection and Preparation of Pulp Extracts

Fresh ***Hylocereus undatus*** fruits were collected from Tumakuru city market and then washed thoroughly 2-3 times with tap water and once with sterile water. 20 g of fresh pulp was finely crushed and added to 100ml of distilled water and stirred at 60°C for 30 minutes. After boiling, the mixture was cooled and filtered with Whatman filter paper number 1. The filtrate was collected and used for further investigations.

Synthesis of Silver Nanoparticles (AgNPs) using Pulp Extracts

5mM of aqueous solution of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of AgNPs. 10mL of pulp extracts was added to 90mL of 5mM $AgNO_3$ solution for bio-reduction process at room temperature in dark condition and allowed for 24 h.

a) Pulp extracts	b) 5mM $AgNO_3$solution	c) Pulp extracts + $AgNO_3$ after 24 h.

Fig.2: Synthesis of silver nanoparticles

A reduction of Ag^+ ions was clearly observed when pulp extracts was added with $AgNO_3$ solution. The color changes from off-white to dark brown color indicates the formation of silver nanoparticles. The produced silver nanoparticles were purified by centrifugation using Remi Cooling Micro Centrifuge (CM-12 PLUS) at 10,000 rpm for 25 min. Supernatant was disposed and the obtained pellet was washed 2-3 times with double distilled water to fling off un reacted $AgNO_3$ and pulp extracts. The refined pellet collected was air dried and used for further characterization.

III. Characterization

UV-Vis Spectroscopy

An aliquot of collected pellet containing silver nanoparticles was subjected to UV–Vis spectroscopy (Shimadzu model-UV3600) at the resolution of 1 nm in range of 340 to 900 nm. Equal amounts of the suspension (0.5mL) were taken and analyzed at room temperature. The progress of the reaction between metal ion solution and fruit pulp extract was recorded by UV-Visible spectra.

X-Ray Diffraction Analysis

The sizes of particle and nature of AgNPs were resolved by XRD employing a Rigaku diffractometer at a voltage of 40 keV and a current of 30 mA with Cu-Kα radiation with a wavelength of 1.5418 Å. A thin film of the dried silver nanoparticles was coated on an XRD grid and carried out for X-ray diffraction studies. The obtained data which is helpful for analysis having peak corresponding to different planes of crystal was compared with the data in JCPDS card. The average size of ccrystalline AgNPs can be calculating by using the Debye-Scherrer equation,

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

where, k value 0.9, λ is wavelength, θ is Braggs diffraction angle, β is full width at half maximum of peak and D is average particle size.

Scanning Electron Microscopy (SEM) analysis

A drop of aqueous solution containing purified AgNPs obtained after repetitive centrifugation was placed on the carbon coated copper grids and dried under infrared lamp for characterization of their morphology using FEI Quanta 200 Scanning electron microscope at accelerating voltage of 20 Kev.

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Antibacterial Activity

The silver nanoparticles were mixed with de-ionized water and were tested for their antibacterial activity by the agar disc diffusion method. Four bacterial strains, *Pseudomonas aeruginosa*, *Klebsiella aerogenes*, *Staphylococus aureus* and *E-coli* were used for these analyses which were collected from The Department of Microbiology, Shridevi Institute of Medical Sciences and Research Hospital of Tumakuru, Karnataka, India. These bacteria were grown in NB media for 24 hours prior to the experiment and seeded on agar plates by the pour plate technique. The plates were incubated at 37^0C overnight. Next day the inhibition zones around the wells were measured.

IV. Results

UV-Vis-spectroscopy Analysis

The aqueous solution of synthesized silver nanoparticles was observed by recording the absorption spectra at a wavelength range of 340–900 nm. The maximum absorbance of AgNPs synthesized using pulp extracts of *Hylocereus undatus* fruit was found at 438 nm (Fig. 3).

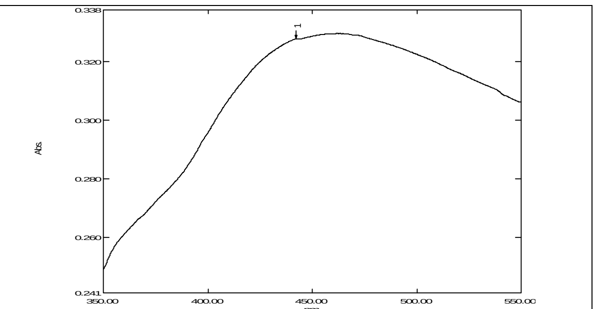

Fig.3: UV-vis spectrum of AgNPs

X-ray Diffraction Studies

From the X-ray diffraction studies confirm that the biosynthesis of silver nanoparticles (AgNPs) from pulp extracts of *Hylocereus undatus* have cubic crystalline structure of Ag nanoparticles. The XRD pattern shows distinct peaks of diffraction at 2θ. Lattice planes of face centered cubic crystal at 2θ values were indexed with respective degrees, 38° (111), 44° (200), 64° (220) and 77° (311) and compared with the data of JCPDS card No. 04-783. The average particle sizes were calculated according to Debye-Scherrer equation. According to the equation, average Ag nanoparticle size was found to be 31.62 nm range at different θ values (Fig. 4).

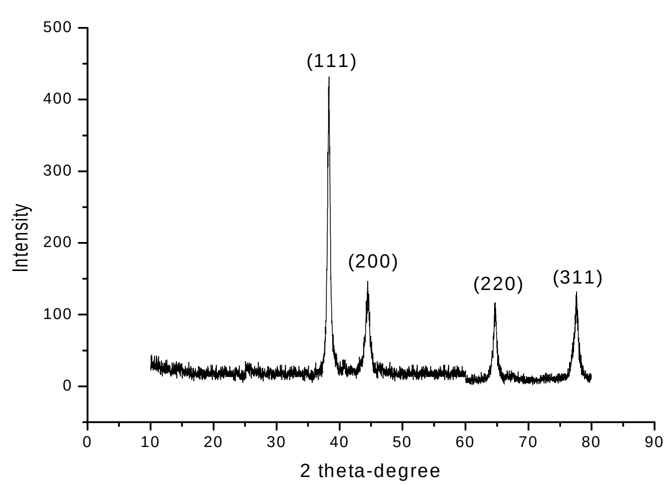

Fig.4: XRD pattern of AgNPs

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Scanning Electron Microscopy Analysis

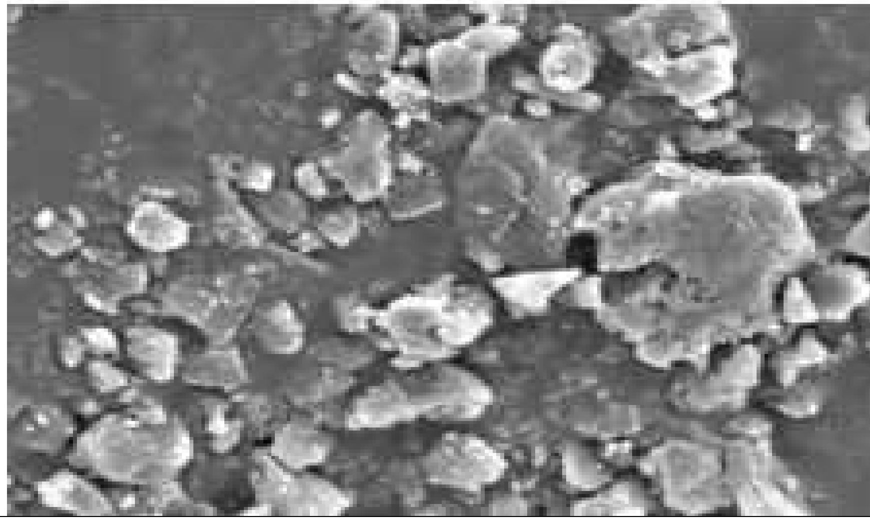

Fig. 5: SEM image of AgNPs

The scanning electron microscopy (SEM) image (Fig.5) further ascertains that the silver nanoparticles are pre-dominantly spherical in morphology with their sizes ranging from 19 to 52 nm with average diameter of 31.3 nm.

Antibacterial Assay

The antibacterial assay was performed against bacterial pathogens like nosocomial pathogens such as *Pseudomonas aeruginosa, Klebsiella aerogenes, Staphylococus aureus* and *E-coli* by standard disc diffusion method. 24 h broth culture was aseptically spread by sterilized cotton swab over solidified Mueller Hinton agar plates. Paper discs are placed at equal distance and discs diameter (5 mm). Each disc was dipped with synthesized silver nanoparticles suspension (30µg/ml). Taxim and distilled water were maintained as positive and negative control respectively. 20% pulp extracts is also used for antibacterial study. The plates were kept for incubation at 37°C for 24 h. The sensitivities of the test organisms to the different samples were indicated by clear zone around discs (Fig. 6: Table. 1).

E-coli	Klebsiella aerogenes	Staphylococus aureus	Pseudomonas aeruginosa

Fig.6: Antibacterial activity of Ag NPs.

Table 1: Antibacterial Zone of Inhibition

Zone of Iinhibition (in mm)					
S. No	**Bacterial Species**	**Negative Control (Distilled water)**	**Positive Control (Taxim)**	**AgNPs**	**Pulp Extracts**
1	E-coli	0	8	12	0
2	Klebsiellaaerogenes	0	7	10	0
3	Staphylococus aureus	0	8	13	0
4	Pseudomonasaerogenes	0	6	9	0

V. Discussion

The formation of AgNPs using pulp extracts of *Hylocereus undatus* was viewed by the colour change from off-white to dark brown color. UV-Visible spectrum the maximum absorbance peak for AgNPs was observed at 438 nm. FT-IR analysis confirmed that the bio-reduction of Ag^+ ions to AgNPs is due to the reduction by pulp extracts. The XRD studies reveal that Ag nanoparticles are poly dispersed and the average size of silver nanoparticles was found to be about 31.62 nm. From the SEM micrograph, it was observed that the spherical in morphology with their sizes ranging from 19 to 52 nm with average diameter of 31.3 nm. Silver nanoparticles obtained from the *Hylocereus undatus* have have got a very good zone of inhibition on *Staphylococus aureus, Pseudomonas aeruginosa, Klebsiella aerogenes* and *E-coli*. Further studies have scope to carry out the effect of AgNPs in wound healing and also in other medical applications.

VI. Conclusion

The present work shows that, medicinally significant aqueous pulp extracts of *Hylocereus undatus* was found to have as a reducing properties for the green synthesis of Ag nanoparticles. The synthesized Ag nanoparticles have been characterized by UV-vis, SEM and XRD to determine the sizes and shapes of the AgNPs. The developed method is one of the good method to produce Ag nanoparticles in absence of toxic reducing chemicals. Our outcomes suggest that the biologically synthesized AgNPs are most prominent against human pathogens.

VII. Conflict of Interest

Conflict of interest declared none.

VIII. Acknowledgment

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