Silver nanoparticles: Synthesized by leaves extract of Avocado and their antibacterial activity

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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 by the leaves extracts of Avocado*(Persia americana)*. The silver nanoparticles were synthesized by using a rapid, single step and completely green synthetic method. The synthesized silver nanoparticles were characterized by using various instrumental techniques such as ultraviolet-visible spectroscopy (UV-Vis), Fourier transformed infrared spectroscopy (FTIR), X-ray diffraction (XRD) and scanning electron microscope (SEM). The synthesized silver nanoparticles were found to be spherical in shape with average diameter of 35.6 nm.The synthesised silver nanoparticles (AgNPs) have shown good antibacterial activity against *E-coli, Klebsiella aerogenes, Staphylococus aureus* and *Pseudomonas aerogenes.

***Key words*— Avocado, Leaves extracts, silver nanoparticles (AgNPs), antibacterial activity.**

I. INTRODUCTION

Nanotechnology is the one of fastest growing areas of manufacturing nanoparticles in recent days. Many researchers have witnessed biosynthesis of nanoparticles and their applications in catalysis, chemical sensing, bio-sensing, photonic, electronics, area of medicine and drug delivery [1-5]. There is increasing optimism of nanotechnology has applied to medicine, will bring significant advances in the diagnosis and treatment of diseases. One of the fields in which nanotechnology finds extensive applications for nanomedicine, an emerging new field which is an outcome of a fusion of nanotechnology and medicine. Nanotechnology can improve our understanding of the living cells and molecular level interactions. A number of nanoparticles based therapeutics has been approved clinically for vaccines, infections and renal diseases. One of the applications of silver nanoparticles in drug discovery, drug delivery and new drug therapies has declared war on dreadful diseases and they use on body natural transport pathway and natural mechanism of uptake of drug by the diseased cell. Nanoparticles can be synthesized by using various methods including physical, chemical, and biological methods. Although chemical method of synthesis requires a short period of time for synthesis of nanoparticles in large quantity, environmental nontoxic synthetic for nanoparticles synthesis leads to developing interest in biological approaches, which are free from the use of toxic chemicals as a by products. Many biological approaches for intracellular and extracellular nanoparticles synthesis have been reported till date using microorganisms including plants, fungi and bacteria. Plants have been providing a better platform for synthesis of nanoparticles, they are free from toxic chemicals and providing a natural capping agents. A number of plant extract mediated synthesis of AgNPs have been reported in the literature. For instance the use of Red Apple (*Malus domestica*)[6], M*yristica fragrans* (nutmeg)[7], *Portulaca oleracea*[8], *Piper betle* leaves[9], *Adansonia digitata* leaf extract[10], *Adansonia digitata* leaf extract[11], *Aeglemarmelos [12], Murraya koenigii[13], Cardiospermum halicacabum* L. leaf extract[14].

The Avocado *(Persia americana)* is a tree that is native to south central Mexico [15], classified as a member of the flowering plant family Lauraceae. *Avocado* additionally refers to the tree's fruit, which is botanically a large berry containing a single seed. In India, *Avocado* is not a trade fruit crop. This plant was introduced from Sri Lanka in the twentieth century. A very small scale and in a scattered in different directions, it is grown in Karnataka, Maharashtra, Tamil Nadu, Kerala in the south-India and in eastward Himalayan state of Sikkim.

Varieties: All the three horticulture races make suitable to tropical and sub-tropical conditions those are Mexican, Guatemalan and West Indian have been tried in Indian country. The selective breeds of West Indian race are grown in Karnataka, Maharashtra and Tamil Nadu.

Fig. 1: *Avocado* leaves

The plant is utilized as a part of conventional medication for the treatment of different diseases, for example, monorrhagia, hypertension, stomach hurt, bronchitis, looseness of the bowels, and diabetes. Peptone, b-galactoside, glycosylated abscisic corrosive, alkaloids, cellulose, polygalacto urease, polyuronoids, cytochrome P-450, and unpredictable oils are accounted for to be available in Avocado plant [16].

In the present study the Avocado plant is selected as it has got many medicinal values as said above and the plant extracts were used for the synthesis of silver nanoparticles along with their characterization and evaluation of antibacterial activity.

II. EXPERIMENTAL

Collection and preparation of leaves extract:

Fresh leaves from the plant *Avocado* free from diseases were collected from Tumakuru city and then washed thoroughly 2-3 times with tap water and once with sterile water. 20 g of fresh leaves was finely chopped 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 for further investigation.

Synthesis of Silver Nanoparticles using leaves extracts:

5mM of aqueous solution of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 5mL of leaf extract of *Avocado* was added to 45mL of 5mM $AgNO_3$ solution for bio-reduction process at room temperature in dark condition and allowed incubation for 24 hours.

a) Plant extract	b) 5mM $AgNO_3$ solution	c) Plant leaves extract+$AgNO_3$

Fig.2: Synthesis of silver nanoparticles.

A reduction of Ag NPs was clearly observed when *Avacado leaves* extract was added with $AgNO_3$ solution immediately. The colour change from yellowish to dark brown indicates the formation of silver nanoparticles. The produced silver nanoparticles were purified by centrifugation in Remi CM-12 PLUS Cooling Micro Centrifuge at 10,000 rpm for 20 min. Supernatant was disposed and the obtained pellate was washed thoroughly with double distilled water to fling off unreacted $AgNO_3$ and leaves extract. The refined pellate collected was air dried and used for further necessary characterization.

III. CHARACTERIZATION

UV-Vis spectroscopy: An aliquot of collected pellate 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 + leaf extract was recorded by UV-Visible spectra of silver nanoparticles in aqueous solution with different wave length in nanometers.

FT-IR analysis:

FT-IR analyses were performed using Shimadzu FT- IR model number 8400 to identify probable biomolecules present in the leaves extracts of *Avacado* which may be responsible for the reduction of metal ions and even for the nanoparticle stabilization. Approximately 3 mg of lyophilized leaves extract under study was mixed with 300 mg of dried KBr, crushed well in mortar and

pestle to prepare thin pallet for analysis, 16 scans per sample were taken in range of 400-4000 $cm^{-1.}$

X-Ray diffraction analysis: The sizes of particle and nature of silver nanoparticles 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 crystalline silver nanoparticles was calculated from the width of the XRD peaks and the average size of the nanoparticles can be calculating by using the Debye-Scherrer equation,

Eq. 1: $D=0.9\lambda/\beta\cos\theta$.

where, 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) of silver nanoparticles:

A drop of aqueous solution containing purified silver nanoparticles 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. Energy-dispersive X-ray (EDX) analysis was carried out by the same instrument and employed to confirm the presence of silver in the particles as well as to detect the other elementary compositions of the particles.

Antimicrobial activity of silver nanoparticles:

The silver nanoparticles were mixed with deionized water and were tested for their antibacterial activity by the agar 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 nutrient broth (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 and DISCUSSION

The extracellular silver nanoparticle synthesis occurred during the exposure of leaf extract of *Avacado* to 5mM aqueous silver nitrate solution. The complete reduction of silver ions was observed immediately. The change in dark brownish color of the reaction mixture was observed during the incubation period because the formation of silver nanoparticles. The impression of this dark brownish color (Fig. 2.c) distinctly affirms the formation of silver nanoparticles succeeding addition of the leaf extract.

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 (Fig. 3). It was noticed that solution of silver nitrate turned brown on addition of leaves extract. It indicated the formation of AgNPs. In the UV-Vis spectrum; a single, strong and broad Surface plasmon resonance (SPR) peak was observed at 432nm that confirmed the synthesis of AgNPs. Reaction parameters viz. concentration of silver salt, aqueous extract and reaction time were optimized for maximum yield of silver nanoparticles. Figure 3 shows the UV-vis spectra of the nanoparticles in 24 hr reaction time.

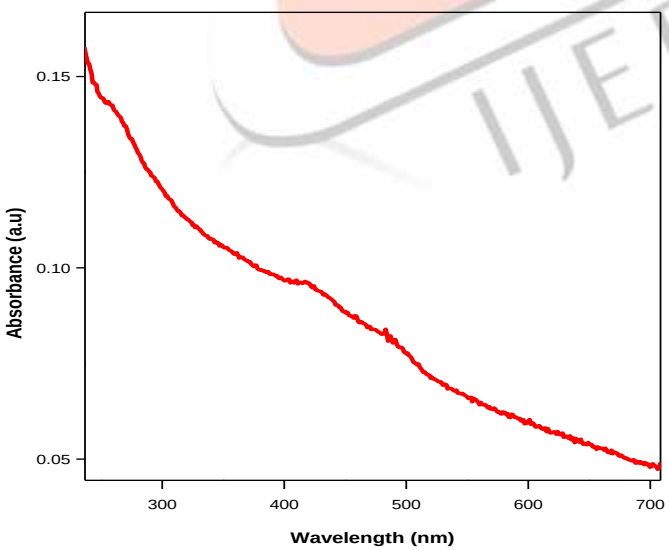

Fig.3: UV-vis spectrum of Ag NPs synthesized by Avocado leaves extract.

Fourier transform-infrared spectroscopy analysis

FTIR measurements were carried out to identify the possible biomolecules responsible for the capping and efficient stabilization of the silver nanoparticles synthesized by the plant extract. Figures 4(a) and 4(b) show the IR spectra of AgNPs synthesized using *Avocado* and *Avocado* leaf extract, respectively. Absorbance bands of *Avocado* AgNPs were observed at 3575 cm-1 Amide N-H

stretch, 3224 Alcohol O-H, 2907 Alkyl C-H, 2221 Nitrile CN, 2105 Alkyne C≡C, 1826 Carbonyl C=O, 1543 Nitro N-O, 1423 Aromatic C=C, 1004 Alkyl halide C-F, 758 Alkyl halide C-Cl, 574 Alkyl halide C-Br, 501 Alkyl halide C-I. Absorbance bands of *Avocado* leaf extract observed at 3283 cm-1 Alcohol O-H stretch, 2162 Alkyne C≡C, 1630 Alkenyl C=C, 571 Alkyl halide C-Br, 501 Alkyl halide C-I.

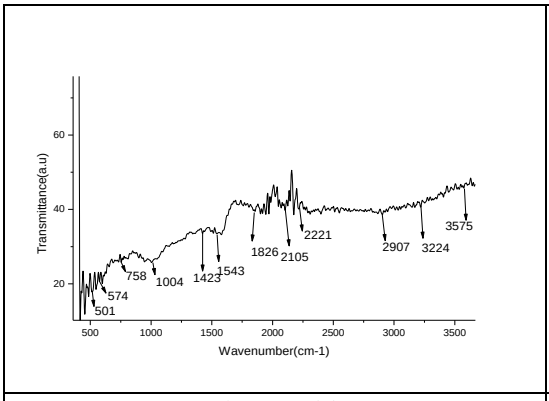

Figure 4(a)

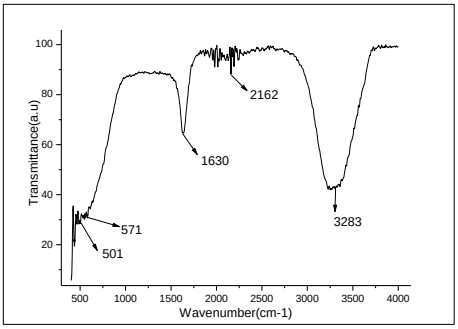

Figure 4(b)

Fig. 4(a) - IR spectra of silver nanoparticles synthesized using *Avacado* leaves extract.
4(b) - IR spectra of Avocado leaves extract.

X-ray diffraction studies:

The silver oxide molecules formed are necessarily subjected to XRD analysis for the measurement of size of these particles. Figures (5) shows the XRD pattern obtained for the silver nanoparticles synthesized using the leaf extract of *Avocado*. The intense peak of nanoparticles 38, 44, 64 and 77° in *Avocado* appeared which are indexed as crystalline silver. The sharpening of the peaks clearly indicates that the particles are the spherical nanoparticles. The average size of the silver nanoparticles is estimated by using Debye Scherrer's formula. The average of silver nanoparticles synthesized by *Avocado* leaves extract is 35nm.

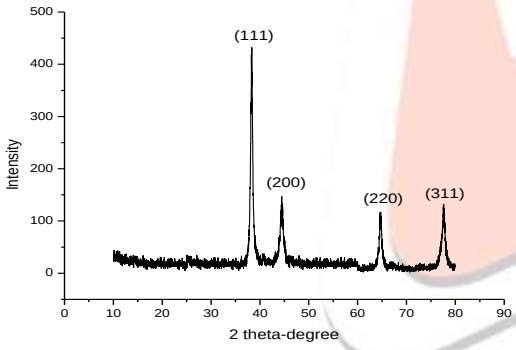

Fig.5: XRD pattern of synthesized silver nanoparticles synthesized using the leaf extract of *Avocado*.

Scanning Electron Microscopy analysis:

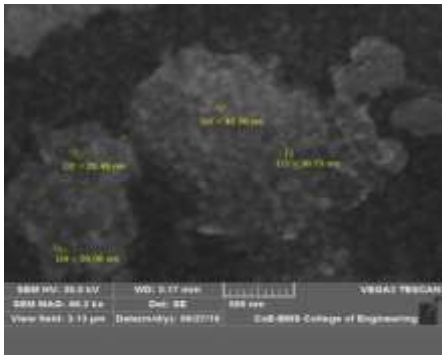

Fig.6: SEM image of Ag NPs using leaves extract of *Avocado*.

The scanning electron microscopy (SEM) image (Fig 6) further ascertains that the silver nanoparticles are pre-dominantly spherical in morphology with their sizes ranging from 29 to 42 nm with average diameter of 35.6 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 well diffusion method. 24 h broth culture was

aseptically spreaded by sterilized cotton swab over solidified Mueller Hinton agar plates. Wells of equal distance and equal diameter (4 mm) were made by sterilized gel borer. Each well was filled with 50 µl of synthesized silver nanoparticles suspension (30µg/ml). Taxim and distilled water were maintained as positive and negative control respectively and leaf extract alone). 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 wells. Triplicates were maintained and for each replicates the diameter were measured in eight different directions and the average values were noted.

Investigation of the antibacterial properties demonstrated that antibiotics with silver nanoparticles synthesized by leaves extract of *Avocado* displayed a noteworthy zone of inhibition when contrasted with standard antibiotic Taxim alone (Tab. 1, Figure: 7), and the effects of the synthesized silver nanoparticles were analyzed, based on the zone of inhibition around the microbial colonies.

E-coli	*Klebsiella aerogenes*	*Staphylococus aureus*	*Pseudomonas aeruginosa*

Fig.7: Antibacterial activity of Ag NPs synthesized by leaves extract of *Avocado*.

Table 1: Antibacterial activity of Ag NPs synthesized by leaves extract of *Avocado*.

Zone of Iinhibition (in mm)					
S.No.	**Bacterial Species**	**Negative Control (Distilled water)**	**Positive Control (Taxim)**	**Synthesized AgNPs**	**Plant leaves Extract**
1	*E-coli*	0	3mm	9mm	0
2	*Klebsiellaaerogenes*	0	5mm	11mm	0
3	*Staphylococus aureus*	0	2mm	8mm	0
4	*Pseudomonasaerogenes*	0	5mm	12mm	0

V. DISCUSSION

The formation of silver nanoparticles using plant leaves extract of *Avocado* was viewed by the colour change from yellowish to dark brown. [17]. UV-Visible spectrum the maximum absorbance peak for *Avocado*was observed at 432 nm. [18]. FT-IR analysis confirmed that the bio-reduction of Ag^+ ions to silver nanoparticles is due to the reduction by plant extract[19]. The XRD studies reveal that silver nanoparticles are poly dispersed and of size from 29 to 42 nm. The average size of silver nanoparticles is synthesized by Avacado was found to be about 35.6 nm[20]. From the SEM micrograph, it was observed that the spherical nanoparticles were in the size ranging from 10 to 50 nm with a variety of morphology and spherical shaped. Silver nanoparticles obtained from the *Avocado*have have good zone of inhibition on *Pseudomonas aeruginosa*, *Klebsiella aerogenes*, *Staphylococus aureus* and *E-coli* [21]. Reports shows that antibacterial assay was done using Papaya fruit extract mediated silver nanoparticles on human pathogen, showing high toxicity against multidrug resistance bacteria[22]. Further studies can be carried out the effect of synthesized nanoparticles in wound healing and also in the field of medicine.

VI. CONCLUSION

The present study shows that aqueous leaves extract of *Avocado* can be used efficiently for the synthesis of silver nanoparticles at room temperature. The synthesized silver nanoparticles were found to be stable at room temperature. Formation of silver nanoparticles was confirmed by ultraviolet-visible (UV-vis), SEM and XRD. The sharpening of the peaks from powder diffraction study clearly indicates that the particles are the spherical in nature. The anti- bacterial activity is well demonstrated by disc diffusion method and antibacterial activity of Ag NPs to shows significant effect against the gram-positive and gram-negative bacteria. The green-synthesized method is convenient, eco-friendly and can be applied in various applications and the use of *Avocado* has added advantages that the plant has many medicinal properties.

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