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Fruit based synthesis of silver nanoparticles-an effect of temperature on the size of particles

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Abstract
The cost effective and eco-friendly technique for synthesis of silver nanoparticles from the extract of seed of *P.granatum*. The reduction process was simple and convient to handle and was monitored by UV-vis spectroscopy. So the green synthesis of silver nanoparticles from 1mM $AgNO_3$ solution was prepared through the extract of *P. granatum* seed which act as a capping and reducing agent. The morphology and uniformity of silver nanoparticles were invastigated by TEM and EDX. The functional group of protein molecule was using identified by FTIR. Increase in temperature leads increase in size of silver nanoparticles.

Keywords: UV-vis spectra analysis, EDX, TEM, FTIR, Silver nanoparticles and Green synthesis.

INTRODUCTION

Nanoparticles are viewed as fundamental building block of nanotechnology(Leela *et al*., 2008).The most important and distinct property based on specific characterstics (Jain *et al*., 2009) such as size distribution and morphology. A number of approaches that is chemical and biological methods. The importance of biological synthesis is being emphasized global at present because chemical methods are capital intensive toxic, non ecofriendly and have low productivity (Kowshik *et al*., 2003).These demerits recommeneded the "greener synthesis" due to the slower kinetics they offer better manipulation and over control growth, stabilization, where it is not necessary to use high pressure, temperature and toxic chemicals (Sinha *et al*., 2009). The use of enviorment benign materials like plant leaf extracts, bacteria, fungi and enzyme for the synthesis of silver nanoparticles offers numerous benefits of ecofriendliness and compatability for the pharmaceuticals and other biomedical applications. Such as drug delivery, cancer treatment (Xu *et al*., 2006). The activity of silver nanoparticles is dependent on size when size increases activity decreases of silver nanaoparticles (Nabikhan *et al*., 2010). Here we observed, synthesis of silver nanoparticles on different temperature and visualize under U.V-vis spectrophotometer. The most important application of silver nanoparticles is in medical industry such as topical ointments to prevent infection and open burn wounds. Here, in this problem we first time synthesis of silver nanoparticles from seed of *P. granatum* reducing the silver ions present in the solution of silver nitrate by the aqueous extracts of seed *P. granatum*. Further synthesized silver nanoparticles were highly toxic against pathogens.

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MATERIALS AND METHODS

Preparation of extract

The fresh *P. granatum* seed weighed 10 g added into 100 ml of distilled water and allow it to boil the filter it with the Whatmann filter paper, the extract used for further analysis.

Biosynthesis of Silver nanoparticles

1Mm fresh silver nitrate solution prepared (Brown bottle), 10 ml *P. granatum* seed extract added into 90 ml of silver nitrate and incubate it at different- different Temperature 4^0C , 27 ^{0}C, and 50^0C for 5 hrs.

UV-vis spectra analysis

The bioreduction of Ag^+ in aqueous solution at different temperature interval was monitored by UV-vis spectroscopy .1ml of sample taken in eppendroffs centrifuge it at 10,000 rpm for 10 min , discard the supernatant washed the pellet two times at 7000 rpm for 2 min and then dilute the pellet with 1ml of D.W used for further analysis.

Transmission electron microscopy (TEM)

For Transmission electron microscopy was prepared placing a drop of sample placed on carbon coated grid and allowing the water to evaporate TEM observations were performed on JOEL-2100 F (AIRF-JNU) operated at a voltage of an accelerating of 2100 Kv and EDX analysis were performed on Burker (PAN Analytical Epsilon -5) in AIRF department JNU.

Fourier Transform Infrared spectroscopy (FTIR)

FTIR technique is used to remove free biomass residue or compound that is not the capping ligand of the synthesized silver nanoparticles. The silver nanoparticles were synthesized after 5 hrs of incubation with the seed of *P. granatum* aqueous extract, 100 ml of residual solution after reaction, centrifuged at 10,000 rpm for 10 min at 18 ˚C and the pellets were redispersed in sterile distilled water to get rid of any uncoordinated biological molecules. The centrifugation and redispersion in sterile distilled water were repeated for three times to ensure better separations of free entities from silver nanoparticles. The purified pellets were then dried with lyophilization and powder particles were subjected to FTIR spectroscopy measurement (Paragon 500, Perkin Elmer-RX1 spectrophotometer) in the diffuse reflectance mode at a resolution of 4 cm^{-1} in KBr pellets.

Antimicrobial Test
Antibacterial test (Food poisoning method)

Food poisoning method- Luria Bertani (LB) agar medium was used to cultivate bacteria. Silver nanoparticles 300 µl were added to LA (Luria agar) media at the time of pouring, after solidification the medium and *E. coli* culture were spread and the media devoid of silver nanoparticles was used as control.

Antifungal test

Antifungal assay was done on *Aspergillus flavus*. Potato dextrose agar (PDA) medium was prepared and autoclaved at 121^0C and 15 Psi. The fungal cultures were point inoculated at the center incubated at the 28^0C for 5 days. The medium without silver nanoparticles inoculated with fungal culture served as control. The percentage of inhibition was calculated by following formula:

$$\% \text{ Inhibition} = \frac{C - E}{E} \text{ X } 10$$

Where C is the diameter of fungal mycelium on control plate synthesized from leaves, epicarp and seeds of *P. granatum* plate and E is the diameter of fungal mycelium on the experimental plate.

RESULTS AND DISCUSSION
Localised surface Plasmon resonance characteristics

The colour changes was noted by virtual observation in extract of pomegranate (*Punica granatum*) fruit seed incubated with aqueous solution of $AgNO_3$ at different different temperature. It started to change colour from watery to yellowish brown due to the reduction of silver ions, this exhibit the formation of silver nanoparticles (Fig.1). Synthesized silver nanoparticles from seed extract from *P. granatum* were analyzed under UV-Vis spectrum.The optical properties of silver nanoparticles are related to excitation of Plasmon resonance or interband transmission particularly on the size effect. The UV-vis spectroscopy method can be used to track the size evolution of silver nanoparticles based on localized surface Plasmon resonance band exhibitely at different wavelength. (Fig.2) shows the UV-vis spectra obtained from solution at different reaction temp. The spectra show peaks at 405 nm (50^0C), 420 nm (27^0C) and 451 nm (4 ^{0}C). These peaks are characteristics Plasmon band for silver nanoparticles. The increase in reaction temp, UV-vis spectra show sharp narrow peak at lower wavelength regions (405 nm at 50^0C) which indicate disaggregation of nanoparticles occurs, when peaks observed at higher wavelength region (420 nm at 27^0C) and (451 nm at 4^0C) broad peak observed.

Fig 1. (A)

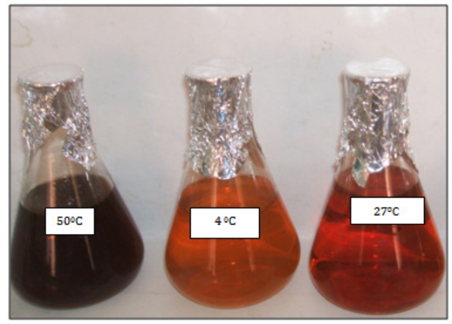

Fig 2. (A) Fruit seed extract without silver nitrate and (B) Synthesis of silver nanoparticles from the seed extract incubated with silver nitrate solution.

Transmission electron microscope

The TEM image of synthesized silver nanoparticles reveals the size of silver nanoparticles and shape. The typical TEM showing the size and morphology is given in Fig. 3 (A), (B) and (C).The morphology of monodisperse silver nanoparticles spherical which is observed in micrograph. The TEM micrograph suggest that the size of particles is 5- 20nm. In TEM micrograph Fig. 3(A) show silver nanoparticles synthesized at 27 ^{0} C show spherical in shape and size 5 nm. In lower temperature of 4 ^{0}C Fig. 3 (B) shows the formation of silver nanoparticles was the size of 10 nm. When we increase the reaction temperature 50 ^{0}C at results increase the particles size rapidly that is 20 nm Fig. 3 (C). As reaction rate is increased the reactants consume faster, hence reactant depletion takes place leading to the formation of nanoparticles and narrow size distribution at higher temperature (Lee *et al*., 2004)

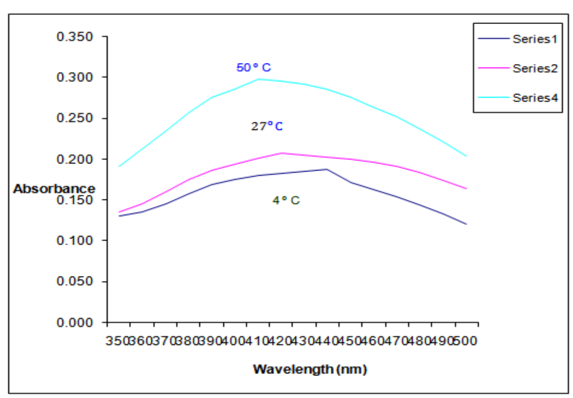

Fig. (3) UV-Vis absorption spectra of silver nanoparticles synthesized from seed extract at of *P. granatum* at different Temperature of silver nitrate solution.

Energy Dispersive X-ray Crystallography:

EDX is analytical technique used for elemental analysis or chemical characterization of the nanoparticles. It is the interaction between electromagnetic radiation and X- ray emitted by the response to being hit with charged particles. To stimulate the emission of characteristic X-rays from a specimen a high energy beam of charged particles such as electrons is focused into the sample being studied. As the energy of the X-rays is the characteristic of the difference in energy between the two shells and of atomic structure of the element from which they were emitted, this allows the elemental composition to be measured. The EDX analysis of silver nanoparticles synthesized from extract of seed of *P. granatum* is shown in (Fig. 4). This revealed that Cu grid normal weight and unnamed weight will be same and same as that for silver, error percentage is 0.9.so all the weight of metal element found in sample will be 100%.

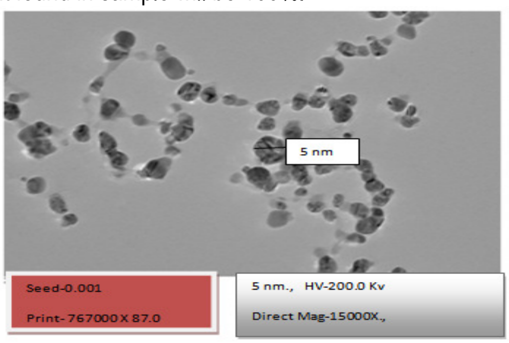

Fig 4 (A).

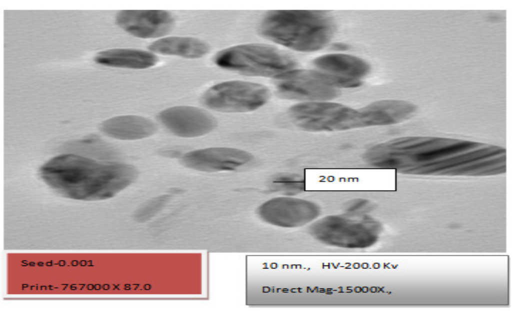

Fig 4 (B).

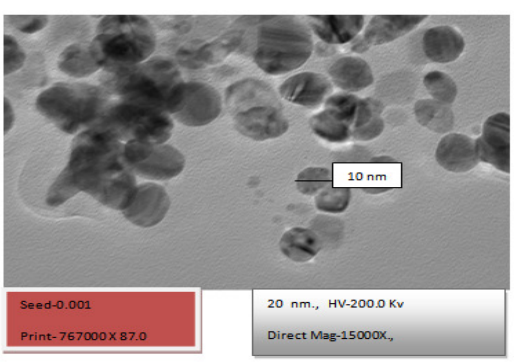

Fig 4 (C).

Fig. 4 (A) TEM micrograph of silver nanoparticles synthesized from seed extract of *P. granatum* at 27^{0}C, (B) TEM micrograph of silver nanoparticles synthesized from seed extract of *P. granatum* at 50 ^{0}C and (C) TEM micrograph of silver nanoparticles synthesized from seed extract of *P. granatum* at 4 ^{0}C.

Fourier Transform Infra red spectroscopy (FTIR)

FTIR is used for determination of find out the functional group associated with the synthesized silver nanoparticles (Fig.5). Silver nanoparticles synthesized from seed absorption peak located at 1519 Cm^{-1}, 1242 Cm^{-1} and 3441Cm^{-1} in the region of 1200 to 3800 Cm^{-1} These associated with stretch vibration of N-H and O-H respectively and have medium intensity.

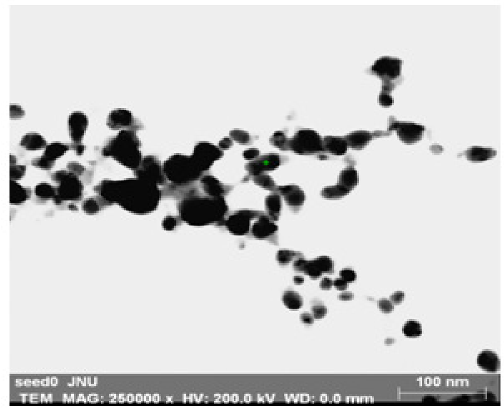

Fig 5 (A).

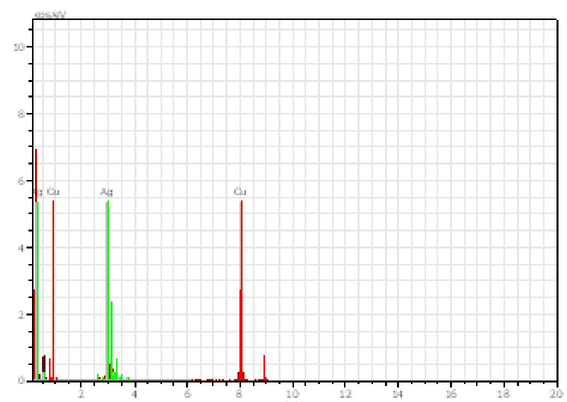

Fig 5 (B).

Spectrum: seed 0

Element	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [wt.%]
Copper	K-series	31.07	31.07	43.34	1.0
Silver	L-series	68.93	68.93	56.66	0.9
	Total:	100.00	100.00	100.00	

Fig 5 (A). TEM Magnification Image of silver nanoparticles synthesized from seed of *P. granatum*. (B) EDX pattern of silver nanoparticles.

Antimicrobial test

Antibacterial Test (Food poisoning Method): The growth of *E. coli* and *P. aerugiosa* in Luria Bertani agar medium contain silver nanoparticles synthesized from seed of *P. granatum* had not shown bacterial growth (Media devoid of silver nanoparticles were used as control (Fig. 6).

Antifungal test: The plate containing the (silver nanoparticles of seed) showed small fungal zone as compared to the control plate of *A. flavus* and 36% respectively showed in (Fig.7).

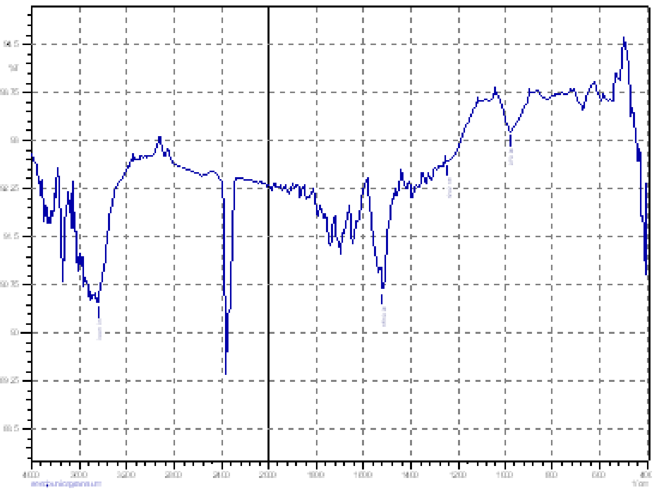

Fig (6). FTIR absorption spectra of the nanoparticles synthesized from seed extract of *P. granatum.*

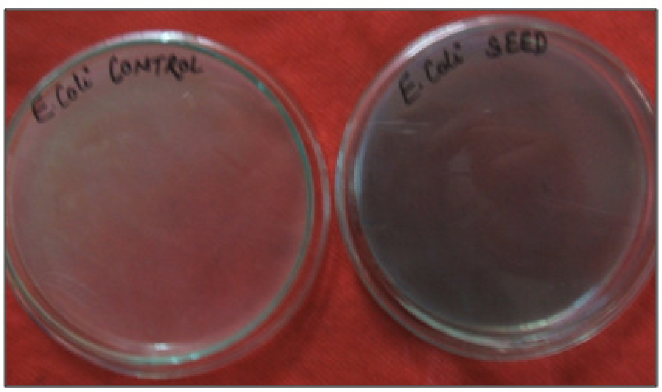

Fig (7). Antibacterial activity of silver nanoparticles synthesized by seed of *P. granatum* against *E. coli* by food poisoning method.

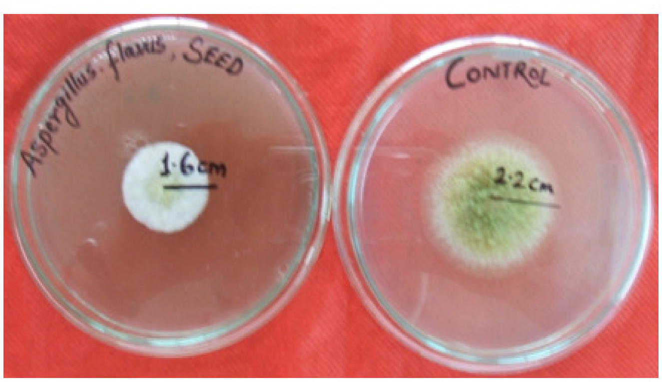

Fig (8). Antifungal activity of silver nanoparticles synthesized by seed of *P. granatum* against *A. flavus.*

The synthesized nanoparticles by "*Green route*" are found highly toxic against resistant human pathogens. The antimicrobial effect of synthesized silver nanoparticles obey a dual mechanism of antibacterial activity several studies proposed that AgNps of the cell membrane and disrupting permeability may attach to the surface and respiration function of the cell.

CONCLUSION

In conclusion we demonstrated simple, stable and efficient biological method for synthesis of silver nanopartciles using fruit *P. granatum*. The synthesis is carried out at various temperature conditions and at lower reaction temperature the size of the nanoparticles were increased and we decrease the temperature size of nanoparticles also increased than the normal room temperature. An important potential benefit of this research is the development of ecofriendly protocol for biosynthesis of silver nanoparticles range at 5 nm by controlling the reaction temperature. We use standard technique, TEM, EDX and FTIR for characterization the shape and size of silver nanoparticles. Applications of such ecofriendly nanoparticles in wounds healing, Cancer therapy, antimicrobial agents and cosmetics. Toxicity study of silver nanoparticles on human pathogens opens a new door for a new range of antimicrobial agents. Silver nanoparticles have emerged as potentially sources of bactericidal agents. The biological synthesis of nanoparticles have paved for better methodological and approaches in medical field.

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REFERENCES

[1] Gil-Jae Lee, Seung-Il Shin, Young-Chai Kim, Seong-Geun Oh, Mater, 2004. Chem. Phys.84 , 197–204

[2] Jain D, Kumar D, Kachhwaha S K and Kothari S L. 2009. Synthesis of Plant mediated silver nanoparticles using *Papaya* fruit extract and evaluation of their antimicrobial properties. *Nanomaterials and Biostructures*; 4: 557-563.

[3] Leela A and Vivekandan M; 2008. Tapping the unexploited plant resource for the synthesis of silver nanoparticles. *African Journal of Biotechnology*; 7: 3162-3165.

[4] Kowshik M, Ashtaputre S, Kharrazi S, Vogel W, Urban J, Kulkarni SK and Paknikar KM ,2002. Extraceelular synthesis of silver nanoparticles by silver-tolrent yeat strain MKY3. *Nanotechnology*; 14: 95- 100.

[5] Nabikhan A, Kandasamy K, Raj A and Alikunhi N; 2010. Synthesis of antimicrobial silver nanoparticles by callus and leaf extract of saltmarsh palnt *Sesuvium Portulacastrum* L. *Colloids and surface B: Biointerfaces*; 79: 488- 493.

[6] Sinha S, Pan I, Chandra P and Sukanta K Sen.2009. Nanoparticles fabrication using ambient biological resource. *Applied Science*; 9: 1113-1130.

[7] Xi Su, Xin Z, Fang T, Wu Jingyani Y and Ji Wu. 2011. Magnetic Nanoparticles I Brain diseases diagnosis and targeting drug delivery. *Current Science* ; 7 : 37-46.