International Journal of Technical Innovation in Modern Engineering & Science (IJTIMES)
Impact Factor: 5.22 (SJIF-2017), e-ISSN: 2455-2585
Volume 4, Issue 09, September-2018

Household Waste *Carica papaya* Peel Mediated Green Synthesis of Silver Nanoparticles and its Antimicrobial and Antioxidant activity

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Abstract
Nanobiotechnology is the application of nanotechnology in the fields of biological sciences. It holds great potential in different fields of life sciences. Nanotechnology involves the uses of materials with components that have dimensions less than 100 nm. Nanoparticles can be synthesized through various techniques have therapeutic efficacy, targeted drug delivery, anti- microbial, anti-cancerous activity etc. Biosynthesis of nanoparticles using plant waste extracts embrace certain applications of being rapid biocompatible, eco-friendly and non-toxic. The biosynthesis of nanoparticles has been suggested as a cost effective and environmentally friendly alternative to physical and chemical methods. This paper presents anti-microbial activity of silver nanoparticles synthesized by extract of Carica papaya peel. The nanoparticles have been characterized by UV-Vis spectroscopy, Scanning Electron Microscopy (SEM), FTIR, X-ray diffraction (XRD). The antimicrobial impact of AgNPs produced from Carica papaya peel was examined utilizing pathogenic bacterial strains.

Keywords - Green synthesis, Nanoparticles, Carica papaya peel, fruit peel extract (FPE), AgNPs.

Introduction

In biological technique, nanoparticles are prepared using biological agents such as plants, algae, bacteria and fungi as stabilizing and reducing agents and therefore it is termed as green technology for the synthesis of nanoparticles [1]. Nanomaterials can be useful in many fields as catalysis, water treatment, solar energy conversion and medicine [2]. The size and shape of nanoparticles is considerably dictated by chance of reducing agent, solvent and biocompatible agent. To eliminate the organic solvents was impossible due to the solubilizers and stabilizers which was not possible in water. So, the biological methods for the synthesis of nanoparticles has gained more importance due to low cost and eco-friendliness.

Nanomaterials have distinctive properties and potential application in biological, biomedical pharmaceutical and physical applications [3]. Among nanomaterials, AgNPs play an important role in the field of medicine and biology due to their attractive physiochemical properties. AgNPs are reported to possess anti inflammatory, anti-angiogenesis, anti-platelet anti-viral and anti-fungal activity [4]. AgNPs have special chemical and physical property such as SERS and electrical conductivity, chemical activity high thermal, catalytic activity and optical behavior.

Silver nanoparticles have been used as an antibacterial agent for long in Greece and in contrast to antibacterial drugs, bacteria cannot develop resistance against it because silver targets multiple components in the bacterial cell [5]. It is also reported that the mechanism behind its antibacterial activity is to weaken DNA replication and inactivate proteins [6]. Amongst various nanoparticles AgNPs have prominent role in the treatment of disease and targeting of cell.

Use of waste materials in different area of human applications is of great ecological and economical importance both due to national priority and also the need of the time. Use of plant waste materials (e.g. fruit peel) for value added discoveries and for identification of major biological applications is of great socio economical and environmental value, where science has to pay great attention [7-10].

In the present study we have biosynthesized green AgNPs with the help of papaya peel and characterized it with the help of UV-Vis spectroscopy, FTIR, scanning electron microscopy and XRD analysis. We have further investigated antibacterial activity of biosynthesized AgNPs. The effect of AgNPs conjugated with antibiotic (gentamycin) on different bacterial species has also been studied.

Material and Methods

Chemicals-

All of the chemicals and media used in the study were purchased from Hi-media, Laboratories Pvt. Ltd, India.

Source and Maintenance of bacterial culture-

Bacterial strains namely *Bacillus cereus* MCC 2039, *Pseudomonas aeroginosa* MCC 2080, *Esherichia coli* MCC 2246, *Staphylococcus aureus* MCC 2408 and *Enterobacter aerogenes* NCIM 2695, were obtained from the Culture Collection

Center National Centre for Cell Science, Pune. The cultures were maintained by repeated subculturing on nutrient agar slants. 24 hour culture was prepared for each experimental procedure [11].

Preparation aqueous extract of papaya peel-

Carica papaya peels were collected from the local market nearby our university. The fresh and healthy papaya fruit peels were collected rinsed thoroughly first with tap water for 5-10 min followed by distilled water for 10 min to remove all the dust and unwanted visible particles.

Fruit peel were cut into small pieces and weighed. 20 gm of papaya peel was taken with 50 ml double distilled water in soxhlet apparatus. Then it is boiled for 30 min in soxhlet and left at room temperature. The extract was filtered through whatmann filter paper No. 1 and used as such for silver nanoparticles synthesis. The extract was freshly prepared for the biosynthesis of AgNPs [12].

Preparation of silver nanoparticles-

The aqueous solution of 1 mM conc. silver nitrate was prepared to synthesize silver nanoparticles from *Carica papaya* fruit peel extract. Different conc. of fruit peel extract (FPE) was taken and added slowly to aqueous solution of 1 mM $AgNO_3$ while stirring. In the study we have taken 1 ml, 1.5 ml, 2 ml, 2.5 ml, 3 ml, 3.5 ml and 4 ml of FPE respectively and then they are added slowly to stirring aqueous solution of 1mM $AgNO_3$ reduction into Ag^+ ions.

The colour change of the reaction mixture from colourless to faint yellow to yellowish brown and finally to reddish brown was observed after overnight incubation at room temperature [13].

Characterization of silver nanoparticles-

UV-Vis - The bio-reduction of Ag^+ ion in aqueous solution was observed with the help of Multimode microplate reader (Thermo Fischer Scientific Varioskan Flash), in a wavelength range of 300-800 nm at resolution of 1 nm.

FTIR- To evaluate chemical bonds in surface atoms and functional atoms on the surface of the nanoparticals all of which will have characteristic vibrational frequencies in IR region, FTIR analysis was carried out for the reduction of Ag^+ ions with the spectral range of 400-4000 cm^{-1}. The sample was centrifuged at 1000 rpm for 20 min, dried using hot air oven and ground with KBr to form a pellet. Then, the pellet was analyzed using JASCO 860 model FTIR instrument.

XRD - Crystalline nature of the AgNPs was analysed by XRD at 2θ range from 20 to 80^o using X-ray diffractometer (Rigaku, Smart Lab, Japan) equipped with Cu/Kα radiation source using Ni as filter at a setting of 30 KV/30 mA. Sample was prepared by subjecting to freeze drying under high vaccum.

SEM - The morphology and elemental composition of the green synthesized silver nanoparticles was identified by Scanning Electron Microscopy (SEM, JEOL). For this freeze dried samples of silver nanocolloids were taken.

Antibacterial activity-

Silver nanoparticles synthesized from *Carica papaya* peel extract were tested for antimicrobial activity by standard well diffusion method against human pathogenic micro-organisms Bacillus *cereus* MCC 2039, *Pseudomonas aeroginosa* MCC 2080, *Esherichia coli* MCC 2246 and *Enterobacter aerogenes* NCIM 5139. For experiment, fresh bacterial inoculums were prepared in nutrient broth so as to achieve the turbidity as per 0.5 Macfarland standard which is equivalent to 1.5×10^8 cfu/ml. Experiment was carried out on Muller Hinton agar. Bacterial lawn of pure cultures was prepared and three wells were punched, with the help of sterile cork borer of 4 mm, on each plate dispensed with 10 µl of freshly prepared silver nanoparticles, 10 µg/ml of antibiotic (gentamicin) and 10 µl conjugate of AgNPs and antibiotic mixed in the ratio of 1:1. The latter was prepared to screen the silver nanocolloids for its synergistic effect. The experiment was setup in triplicate to avoid any errors. The antibacterial activity was analyzed by measuring zone on inhibition [14].

Antioxidant assay

DPPH (1, 1 Diphenyl-2-picryl Hydroxyl) scavenging assay:-

DPPH Scavenging activity was calculated by the considerably modified spectrophotometric technique of Brand Williams [15]. DPPH is a Stable free radical with red colour. It reacts with an antioxidant compound that can donate hydrogen and get reduced which is indicated by development of yellow colour. The intensity of the yellow colour developed depends on amount and nature of radical scavenger present in the sample. This will allows visual monitoring of the reaction and the number of initial radicals can be counted from the change in the optical absorption at 520 nm. For this, different concentrations of peel extract and biosynthesized silver nanoparticles were added separately, in equal volume to 0.1 mM methanolic DPPH solution. The reaction mixture was incubated for 30 min at room temperature under shaking condition and absorption was recorded at 520 nm.

$$\%\ \text{scavenging activity} = \frac{\text{Absorbance of control- Absorbance of sample}}{\text{Absorbance control}} \times 100$$

Results and discussion -

Synthesis of silver nanoparticles

When the papaya peel extract was added to 1 mM solution of $AgNO_3$ a change in colour from colourless to dark brown was observed in 30 min which indicates the reduction of silver ions and the formation of nanoparticles occurred rapidly within an hour of reaction (Fig.1) this result is concurrence with [16] in which they studied on *Euphorbia hirta* leaves extract. The colour intensified with increase in time. In all the seven different concentrations of extract used for reduction, change in color was observed which was seen maximum in 3.5 ml. The sample prepared was taken for further characterization. The colour change indicated that the elemental silver have been converted to nanometric range silver ions in reaction medium. Metal nanoparticles such as silver have free electrons, which give rise to SPR absorption band somewhat similar result was seen in *Mangosteen* leaf extract as cited by Veerasamy R et. al. [17].

UV-Visible analysis

Out of all the seven different concentrations of extract used for reduction 3.5 ml showed the best result giving strong surface Plasmon resonance band of green silver nanoparticles at 416 nm after 12 hrs of synthesis. Control silver nanoparticles solution (without papaya peel extract) neither developed the brown colours nor did they displayed the characteristic peak, the resultant peak are very similar to the peak as referred in *Acalpha indica* leaf extract as cited by Krishnaraj C et. al. [18].

The study revealed the effect of varying the concentration of *Moringa* leaf extract on the size of silver nanoparticles. The results obtained are concurrent with the *Carica papaya* peel extract synthesized AgNPs [19]. As the concentration of leaf extract is increased, more number of biomolecules are available in the leaf extract which helped in the metal reductive process.

FTIR spectroscopy

It has been shown that phytochemical analysis of *Carica papaya* peel extract reveals whether the aqueous extract contains carbohydrates, glycosides and flavonoids [20]. The presence of carbohydrates, glycosides and flavonoids in *Carica papaya* peel extract may play an important role in Ag reduction reaction. FTIR spectroscopy was used to characterize and identify the chemical composition of the AgNPs surface. As can be seen in Fig. 3 the presence of bands at about 3778.5 cm^{-1}, 3730.3 cm^{-1}, 3406.2 cm^{-1}, 2915.1 $cm^{-1,}$ 1634.5 cm^{-1}, 1251.2 cm^{-1},1152.4 cm^{-1}, 1070.2 cm^{-1}, 836.3 cm^{-1} and 671.8 cm^{-1}. The absorption band at 3778.5 cm^{-1}, 3730.3 cm^{-1}, 3406.2 cm^{-1} and 2915.1 cm^{-1} which is characteristic of the primary and secondary amines respectively. The absorption band at 1152.4 and 1251.2 corresponds to carbonyl group present in the extract. The sharp band at 1070.2 indicated C-O group of ester and the band at 1634.5 is due to aromatic CH stretching vibrations. The absorption bands that appear in the IR spectrum of the aqueous extract could also be seen in the IR Spectra of phytocapped AgNPs [21].

Scanning Electron Microscopy (SEM) -

SEM micrographs revealed the size and structural patterns of silver nanocolloids. Silver nanoparticles were somewhat spherical and irregular shaped including some particles aggregate. One of the smallest aggregate molecule appeared to be 2.968 µm in size. Others were too large aggregate as the SEM micrographs were taken after 25 days after preparation of the nanocolloids as shown in Fig. 4

To know exact size of the Ag nanoparticles, they were sonicated (Model No.UP100H) at amplitude of 20 KHz for 1 hour and scanned again. The particle size observed was of 15 nm and spherical in shape.

XRD

The XRD graph of the dried silver nanoparticles is shown in fig. 6. The XRD peaks at 2Ө degree of 32.27°, 38.25°, 46.60° and 64.78° could be attributed to the (111), (200), (311) and (220) crystalline plane of the face centered cubic crystalline structure of metallic silver (JCPDS file no 04-0783). The results clearly revealed that the AgNPs formed by the reduction of Ag^+ ion by *Carica papaya* peel extract are crystalline in nature.

To estimate the average particle size of AgNPs, the Debye-Scherrer equation was $D = K\lambda \backslash \beta \cos$ where D is the crystalline size of nanoparticles, K is Scherrer constant with a value ranging from 0.9 to 1λ is the wavelength of the X-rays source (0.1541) used in XRD, β is width at the half maximum of the diffraction peak and Ө is the Bragg's angle [22].

Antimicrobial activity

In vitro, antibacterial activity of silver nanoparticles biosynthesized from papaya peel was performed against some selected bacterial pathogens by well diffusion method. Antibacterial activity was compared among different bacterial strains along with standard gentamycin as zone of inhibition (mm). Maximum zone of inhibition was seen in *E. aerogenes* (18mm) and the least activity was observed in *S. aureus* (7mm).

While performing the antibacterial assay with conjugate, we have seen synergistic effect on each bacterial species. This shows the AgNPs have strengthened the activity of antibiotic both in the case of susceptible and resistant bacteria irrespective of their mode of action. The most remarkable and interesting point is in the case of *P. aeruginosa* where no antibiotic activity is seen but still synergistic effect was observed. We are not able to find exact reason but may be AgNPs must have provided the route to the antibiotic to the target point in the bacteria. It is known that AgNPs change membrane permeability, collapse the plasma membrane potential and leads to the formation of pits, which may be the reason for synergistic effect in anti-microbial activity of conjugate [23].

Antioxidant assay

DPPH (1, 1-Diphenyl-2-Picryl Hydroxyl) radical scavenging assay

Silver has been very known for strong toxicity in different chemical forms to a wide range of bug explicitly. In recent times, silver nanoparticles have shown a great potential as antioxidant. The antioxidant activity of papaya peel extract and silver nanoparticles was assessed by DPPH scavenging assay. DPPH was a stable compound and accepts hydrogen or electrons from silver nanoparticles. The results obtains from the DPPH assay showed valuable free radical inhibition by both silver nanoparticles and papaya peel extract. The average percentage inhibition of synthesized silver nanoparticles was 64% as compared to that of peel extract (43%) at different concentrations used in this study and the activity increased with increasing concentrations of silver nanoparticles.

Conclusion

The present study showed silver nanoparticles with mean diameter of 15 nm were synthesized using waste fruit peel extract of carica papaya as a reducing agent. But we got the agglomerated particles as we have done characterization via SEM after 15 days of sample preparation. The Detailed characterization of the nanoparticles carried out using UV- VIS spectroscopy, SEM and XRD. The UV-VIS spectrum shows the characterized Plasmon absorption peak for silver nanoparticles at 416 nm. SEM micrograph images shows that the nanoparticles synthesized were nearly spherical and and average size of 15 nm. Furthermore, antibacterial activity, as a function of nanoparticles amount was carried out against the five most pathogenic gram positive and gram negative bacteria. From the results we can conclude that silver nanoparticles showed a potent antibacterial potential displaying synergistic effect also.

Acknowledgements

Authors greatly acknowledge USIC of BBAU Lucknow and AIIMS, New Delhi for providing their services in Charaterization of silver nanocollids.

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Fig. 1 Showing Silver nanocolloids made by different concentrations of papaya peel extract

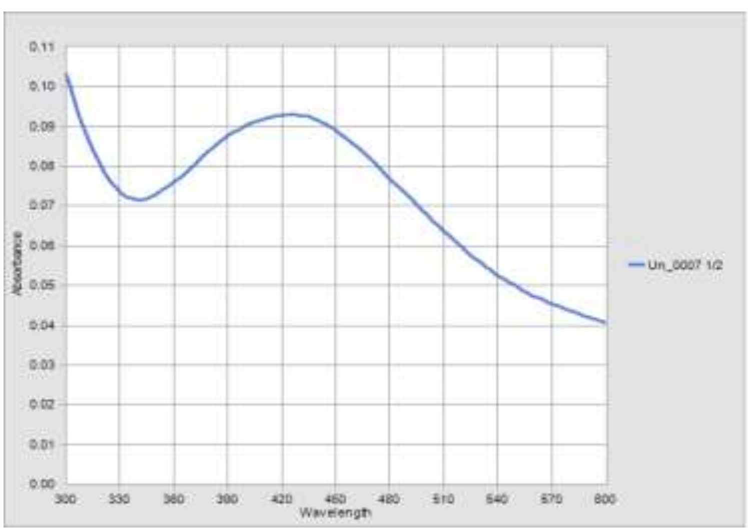


Fig. 2. Showing UV –visible absorption spectra of silver nanoparticles synthesized from *Carica papaya* peel extract

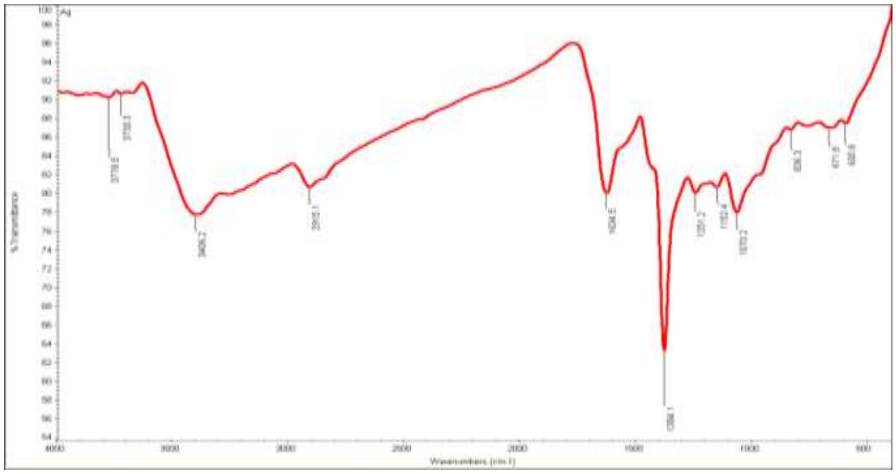


Fig. 3. Showing FTIR of Silver nanoparticles Synthesized from Carica papaya

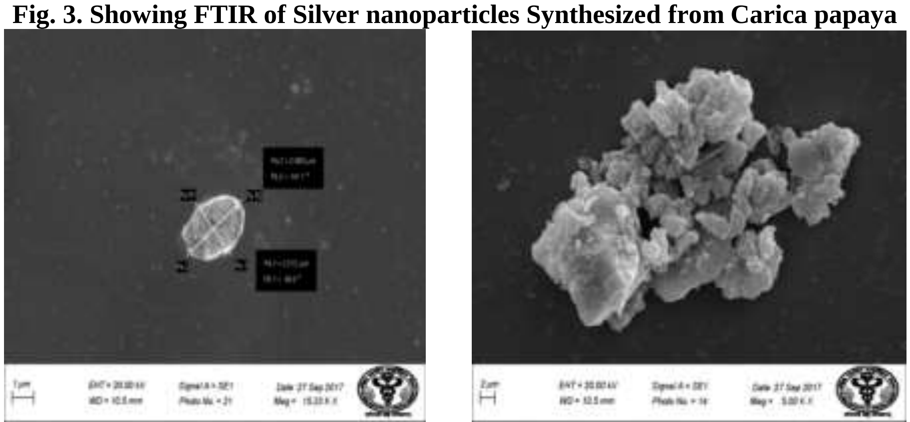


Fig. 4 Showing SEM micrographs of the silver nanocolloids

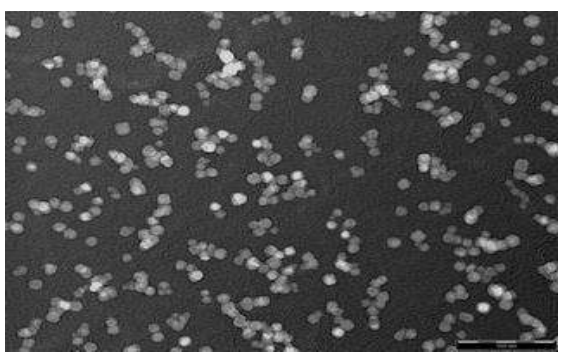


Fig.5 Showing SEM micrograph after sonication

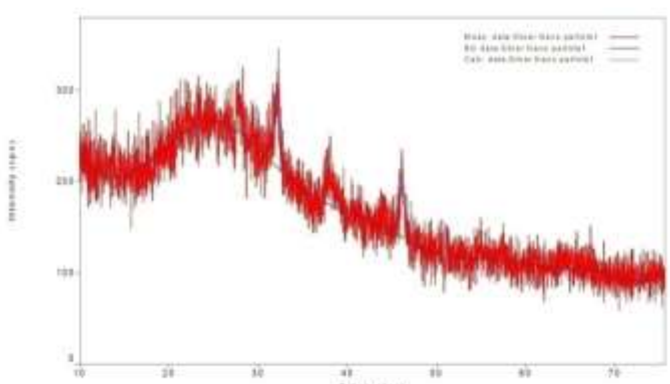
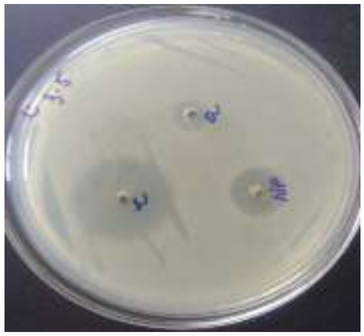
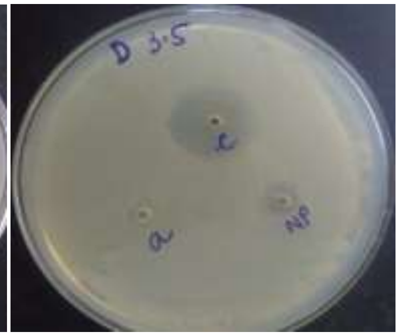


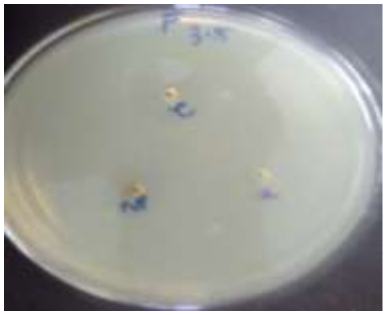
Fig. 6 Showing XRD pattern of Silver nanoparticles biosynthesized by Papaya peel



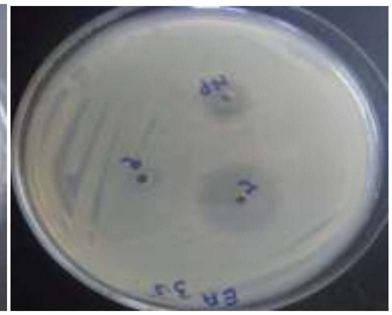
A. Plate of *Bacillus cereus*



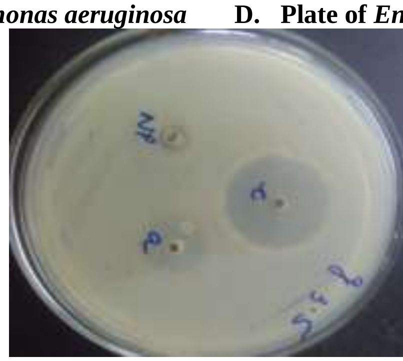
B. Plate of *Escherichia coli*



C. Plate of *Pseudomonas aeruginosa*



D. Plate of *Enterobacter aerogenes*



E. Plate of *Staphylococcous aureus*

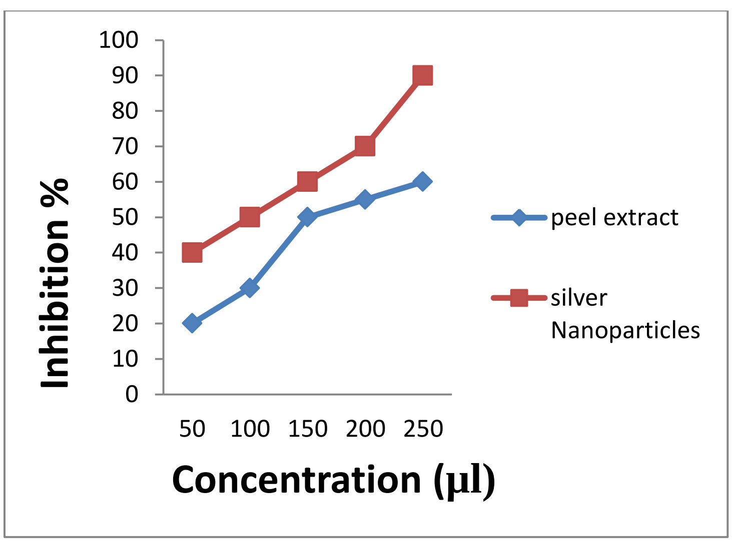


Fig 7.DPPH free radical scavenging assay for peel extract and Silver nanoparticles

