

Rapid Green Synthesis of Silver Nanoparticles (AgNPs) Using (*Prunus persica*) Plants extract: Exploring its Antimicrobial and Catalytic Activities

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Abstract

Fabrication of nanoparticles by using Green synthesis is done because of its wide applications in different field such as biomedical, agriculture and food engineering. In this paper plant extract is used that contain alkaloids, flavonoids, saponins, steroid compounds, acts as reducing and stabilizing agents. The presence of AgNPs in solution was identified by UV-visible spectrophotometer. The size of AgNPs was in the range of 40-98 nm determined by Zetasizer. Spherical Shape and crystalline nature of AgNPs was confirmed by FESEM and XRD. Process Optimization was done by varying leaf extract volume, silver nitrate concentrations, pH, temperature, and time of reaction. The biosynthesized AgNPs exhibited antibacterial and catalytic activity against bacterial strains *E. coli* and *V. cholera*, and heavy metal such as Cadmium (Cd) and Palladium (Pd) in varying extents. Biosynthesized AgNPs were found to have a higher inhibitory action against *E. coli* and removal efficiency of Cd is higher than Pd.

Keywords: *Prunus persica*; Silver nanoparticles (AgNPs); Green synthesis; Zeta potential; Reducing agent

Introduction

Nanobiotechnology is rapidly growing as an interdisciplinary eco-friendly research area and used in broad research section such as biology, chemistry, physics, biomedicine and material engineering [1,2]. It deals with various shapes and size of particles in the range of 1 to 100 nm. From last two decades, top down and bottom-up approaches are used to produce metal nanoparticles with different morphologies, compositions and structures. It is known for its antimicrobial, anti-inflammatory activities other than that it is used in electrical batteries, an optical receptor in solar batteries, bio-labeling and in cancer treatment. Nontoxic and new methods in the field of nano research have been developed that involves microorganism and plants for the synthesis of nano-materials [3]. Though nanoparticle can be fabricated by using different physicochemical methods their synthesis by a biological method such as using microorganisms, single cell plant, plant tissues, fruits or plant extract is a more attractive option as they are nontoxic and therefore environmentally safe especially for their application in food, pharmacy or in medicine. Although several routes are developed for biosynthesis of metal nanoparticles from the salt of the corresponding metal, the most advantageous option is to use plant extract as they are less expensive and very easy, simple, rapid and less energy extensive process as synthesis can be carried out at room temperature within few seconds to few minutes and easy to scale up also. Biogenic synthesis is advantageous not only that it is environmentally friendly but also it is free of contamination and has a well defined size, shape, and morphology than another physicochemical synthesis method. Plant extract acts both as reducing and stabilizing agent in the synthesis of nanoparticles. The activity of the plant extract varies with the varying source as it has different composition and concentration of the particular organic component in the reducing extract. In view of the number of different chemicals involved the bioreduction process is relatively complex.

The pine tree is originated from Northern hemisphere and Sumatra 2°S. In North America, various species occur in the region of latitudes from as far North as 66°N to as far South as 12°S. Few species have introduced to the temperate and subtropical region. Pine extract has a lot of therapeutic effects and used as an alternative medicine for its effect on health. According to Cancer research, UK it is scientifically

proven fact that it can be used as preventive of cancer. The therapeutic properties of pine oil are antimicrobial, antineuralgic, antirheumatic, antiseptic, antiviral, bactericidal, balsamic, cholagogues, deodorant, diuretic, expectorant, hypertensive, insecticidal, restorative, rubefacient, adrenal cortex stimulant as well as a stimulant to the circulation and nervous system.

Recently, a simple and eco-friendly green approach for synthesis of silver nanoparticles by various plant extracts have drawn the attention of academician and researcher because of its advantage over physical and chemical methods [4-12]. Synthesis of nanoparticles by green approach is emerging field because of its various advantages over the other process like nontoxic, ecofriendly and low cost. AgNPs showed promising applications in different engineering fields such as electrical, catalytic properties, water, drug delivery and biomedical engineering because of its high surface to volume ratio [13-16]. Green approach also involved many microorganisms like bacteria, fungi, and yeasts for the synthesis of nanoparticles. Some of the microorganisms have used to produce nanoparticles like magnetite by magnetotactic, siliceous material by radiolarians and diatoms, CdS quantum dots using fungi. The previous study confirms that biogenic silver nanoparticles produced by microorganisms are persistent with excellent (20-50 times higher than chemically synthesized) antimicrobial properties [17-20]. The main disadvantage of microbial nanoparticle synthesis is the aseptically maintenance of microorganisms and the synthesis process is comparatively lengthy. Despite all properties of AgNPs showed many important properties such as antiinflammatory, wounds healing and application in antibiotic, diagnostic, therapeutic etc. AgNPs is non-toxic so it is used for curing different types of diseases causing due to bacterial

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contamination. Silver nanoparticles showed antibacterial property by attaching with highly reactive faces of the bacterial wall and inhibit their metabolism and other major functions [21-24]. Plant extract contains biomolecules such as vitamins, polysaccharides, proteins, amino acids, enzymes, and organic acids can act as both reducing as well as capping agent in the bioprocessing of silver nanoparticles [25]. Various plant extracts had been used for AgNPs synthesis such as *Skimmia laureola*, *Clerodendron serratum*, *Averrhoa carambola* L. [26-28]. Considering the advantages of green synthesis over other methods, synthesis of AgNPs by using pine needles extract was done and also affects the various process parameters on the formation of AgNPs was evaluated. Pine bark extract enhance blood circulation, reduce inflammation, delay aging provide relief from allergy, support immune system improve vascular health and it reported of having antioxidant effect by preventing free radical mechanism. The extract contains pycnogenol, enzogenol, flavangenol, oligonol, oligopin etc. The aglycon, glycoside free, portion of the saponins is termed as saponin acts as reducing agent. Mittal et al. [3] reported that the role of carbonyl group from amino acid and peptide from protein have string affinity to silver and protein works by forming a protective coating over nanoparticles surface by preventing its aggregation and therefore stabilization. Ever green pine tree is found in many parts of the world. In India, pine trees were found in lower Himalaya of Uttrakhand and Himachal Pradesh. In this recent research, green synthesis method at room temperature was developed for the synthesis of AgNPs using *Prunus persica* leaves extract, which is nontoxic, environmentally friendly and low cost. We attempted to evaluate antimicrobial effect of silver nanoparticles (AgNPs) using human pathogen such as *E. coli*, *V. cholera* and also catalytic properties of the green synthesized AgNPs were studied by removing heavy metal from waste water.

Materials and Methods

Materials

Silver Nitrate is obtained from NICE Chemicals Pvt. Ltd, Kerala. Antibiotics (rifampicin) and bacteriological agar were purchased from HiMedia; India. Bacterial strains *Escherichia coli* (*E. coli*) and *Vibrio cholera* (*V. cholera*) were procured from Microbial Culture Collection Centre (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. Glassware and Whatman No. 1 filter papers are obtained from local Market, Chandigarh, India. Pine (*Prunus persica*) extract is obtained from botanical garden of Panjab University Campus, Chandigarh, India.

Methods

Preparation of plant extract: Fresh leaves are washed with tap water and then with distilled water, then, leaves were dried at room temperature. Then, leaves were cut into small pieces; 20 g of leaves were added in 50 mL distilled water and then heated at 70°C for 40 min. Biomass of plant is separated through Whatman No.1 filter paper. Plant extract was stored at 10°C temperature for further study.

Synthesis of AgNPs: Silver nanoparticles were synthesized by adding 10 mL of a 0.01 M aqueous solution of silver nitrate into different volume (1.0, 2.0, 3.0, 4.0 and 5.0 mL) of *Prunus persica* needles extract taken into four beakers separately at room temperature. The color of the solution was started changing from yellow to brown within 5 min indicating the formation of nanoparticles and further, no change in color can be observed. The separation of silver nanoparticles from the dispersion was carried out by centrifugation after that AgNPs were washed 4 times with distilled water and acetone to remove water

soluble impurities and then nanoparticles were lyophilized and stored in dry bottles for further study.

Characterization of silver nanoparticles: Characterization of Silver nanoparticles was carried out by using visual observation and various techniques, in visual observation, change in color of the solution was observed by naked eye. X-ray diffractometer, using Cu Ka radiation ($\lambda=0.1546$ nm), with diffraction angle between 10 and 90° was used to find crystal structure of nanoparticles, characterization of the surface chemistry of AgNPs and biomolecules in *Prunus persica* solution were done by using a Fourier transform infrared spectroscopy (FT-IR Shimadzu FTIR spectrophotometer, FTIR 8400). The FTIR spectrum was collected at a spatial resolution of 4 cm⁻¹ in the transmission mode, between 4000-500 cm⁻¹. For identification of AgNPs in solution absorbance value was determined using UV-visible spectrometer in the wavelength range (λ) 200-800 nm. Identification of shape of nanoparticles was done by using Field Emission-Scanning Electron Microscopy (FESEM) in scales of 500 nm and 10 nm, for determination of average particles size of AgNPs Zeta Sizer was used (ZS-90, Malvern Instrument, UK).

Antibacterial activity: Standard agar diffusion method is used to test the antibacterial activity of bio-synthesized AgNPs against two human pathogens such as *E. coli* and *V. cholerae*. Antibacterial activity was performed against all the two bacterial strains as per guidelines by Clinical and Laboratory Standards Institute (CLSI, M02-A12). These were grown overnight and diluted in Mueller-Hinton broth (MHB) to a cell density of 10⁵ Colony Forming Unit (CFU)/mL. 100 μ l of this culture was spread on the Mueller-Hinton Agar (MHA) plate and allowed to dry in a sterile condition. Further AgNPs (200 mg/mL) were added into performed well (6 mm width) on MHA plate. The plate was incubated at 37°C for 24 h antibacterial activity of AgNPs was observed based on the zone of inhibition around the well impregnated with the rifampicin (RIF) and HPLC grade sterile water and biosynthesized silver nanoparticles were used as positive and negative control respectively [29].

Catalytic property of AgNPs: Catalytic property of AgNPs was done in a batch process at optimized process condition for removal of heavy metal such as cadmium and palladium. In experiments, the pH of the batch process was controlled by addition of a solution of HCl and NaOH (1.0 M and 0.1 M). The concentration of heavy metal such as Pd and Cd (1 mg/l) was investigated in 100 mL flasks. A magnetic stirrer is used for stirring at 350 rpm at fixed control temperature. The obtained experimental data at different times were used to calculate the removal percentage of Cd and Pd by using the following relationship (eqn. 1).

$$\% \text{ Cd and Pd removal} = \frac{(C_0 - C_t)}{C_0} * 100 \quad (1)$$

Where C_0 (mg/L) and C_t (mg/L) are the Cd and Pd concentration at initial and after time t (sec) respectively and the equilibrium adsorption capacity of the Cd and Pd were calculated according to following equation:

$$Q_e = (C_0 - C_e) * V / W$$

Where C_0 (mg/L) and C_e (mg/L) are the initial and equilibrium concentrations in solution for Cd and Pd respectively, V the volume of the solution (L) and W is the mass (g) of the adsorbent. Cd and Pd removal percentage on to AgNPs in contact time range (0 to 180 min) at 1 mg/L were investigated to evaluate the required equilibrium time [30].

Results and Discussion

Observation of color change

Color changes of the solution from light yellow to brown were observed and then turned to dark brown when silver salt (AgNO_3) was added to aqueous pine needle extract. Color changes of the solutions are due to some chemical compound such as alkaloids, flavonoids, saponins, steroids, and color present in plant extract acts as a reducing agent that reduced silver ions (Ag^+) to a silver atom (Ag^0). Same color changes were observed by many researchers by using different plant extract [31-33]. The reduction of silver ions to a silver atom in the solution was observed by UV-Visible Spectrophotometer. Detection of silver nanoparticles was observed by its dark brown color. Due to excitation by surface plasmon vibrations, AgNPs are showed brown color in aqueous solution [34,35]. It was noticed that complete color transition took 15 min at room temperature. Thereafter no further color of the solution was changed (Figure 1a and 1b). The presence of silver nanoparticles in the solution was observed by UV-Visible Spectrophotometer presence of peaks at 440 nm. Due to molecular and structural changes, color transitions occur in the test substances leads to corresponding changes in the ability to absorb light in the visible region of the electromagnetic spectrum. As pointed the SPR is caused by the application of the free conduction electrons induced by light.

Effect of concentration of leaf extract on AgNPs sizes and concentration

Silver ions above a particular limit can be toxic to protein (present in plant extract) and they can cause precipitation. To stop precipitation ionic forms are transformed to nano-particle by plant extract. To see the effect on the size and AgNPs concentration in the reaction mixture by varying the concentration of the leaf extract was studied by using UV- spectrophotometer and Zetasizer. Size and shape of AgNPs can be controlled by changing the concentration of extract from 1 to 5 mL in 2 mM AgNO_3 of 20 mL solution, when we increased the leaves extract concentration, the absorbance of the sample increased. It indicates that the increasing concentration of the silver nitrate particle size reduced till the optimum concentration beyond that particle size increase. Optimum particles size was observed at 2 mM AgNO_3 (Figures 2a and 2b). Similar results were reported in previous research [36]. At higher concentration, it might increase the possibility of particles agglomeration.

Effect of concentration of AgNO_3 on size and concentration of AgNPs

A higher ratio of reducing agent to substrate accelerates the reduction of Ag^+ to Ag^0 immediately followed by capping by the capping



Figure 1: (a) *Prunus persica* aqueous extracts (b) Extract with AgNO_3 .

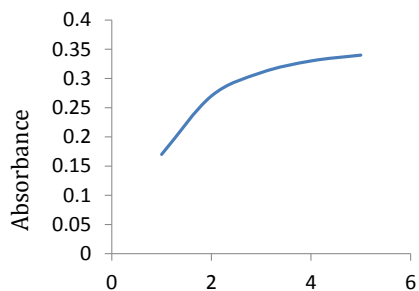


Figure 2a: Effect of concentration of leaf extract on AgNPs concentration in solution.

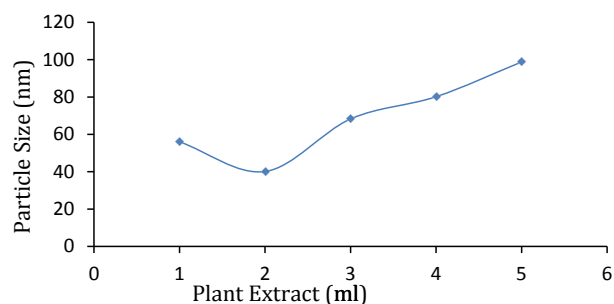


Figure 2b: Effect of varying concentration of leaf extract on AgNPs sizes.

agent preventing from aggregation. Changes in absorption peak of the reaction mixture were observed at varying salt concentrations from 1 mM to 5 mM. Dark brown colors and maximum peak intensity were observed at salt concentrations 2 mM. Thereafter absorption peak intensity was decreased from 2 mM to 3 mM and then remained constant from 3 mM to 5 mM because, Ag^+ concentration was increased in the solution by adding more AgNO_3 , the maximum peak intensity was obtained at 2 mM of AgNO_3 as shown in Figures 2c and 2d similar variation in peaks intensity due to changing AgNO_3 concentration in reaction mixture was observed with many plant extract (Ibrahim, 2015; Metz, 2015). A variation in silver nitrate concentration was also known to influence nanoparticles size. AgNPs size was observed to decrease from 1 mM to 2 mM and then increase from 2 mM AgNO_3 to 5 mM because of increase in the concentration of AgNO_3 , agglomeration may occur [37,38].

Effect of pH on AgNPs size and concentration

Solution pH played an important role in the formation of nanoparticles. At low pH (2-5), the formation of AgNPs in the solution is very slow and large size nanoparticles are obtained that was shown by color change during the reaction. pH of the solution affects the shape and size of the particles and also have the ability to change their capping as well as stabilizing abilities. When pH of the solution was increased from acidic to basic (pH 5-13), absorption peak intensity increased as pH increased from 5 to 13. Concentration of AgNPs in solution also increased due to increase in reduction rate of Ag^+ in the solution and color change was observed very fast when AgNO_3 mixed with pine needles leaf extract and achieved maximum concentration of AgNPs and highly uniform size of nanoparticles were obtained with an average size of 40 ± 2 nm at pH 10 as shown in Figures 2e and 2f. Similar results were reported in many papers [39,40]. At pH 13, AgNPs are very unstable in solution and agglomeration of silver

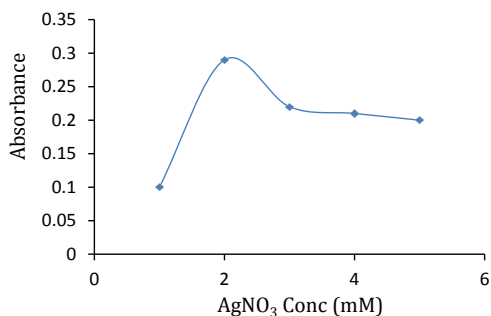


Figure 2c: Effect of concentration of AgNO₃ on AgNPs concentration.

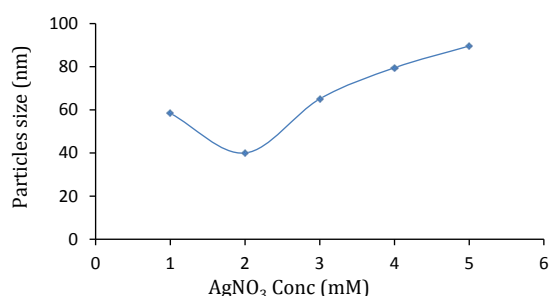


Figure 2d: Effect of concentration on AgNPs size.

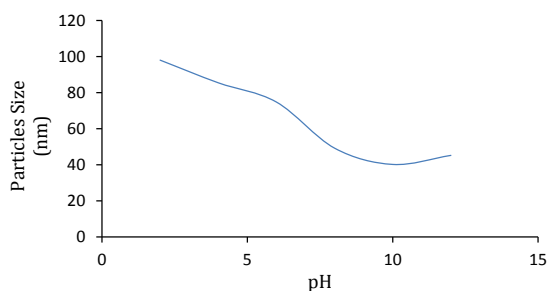


Figure 2e: Effect of pH on AgNPs size.

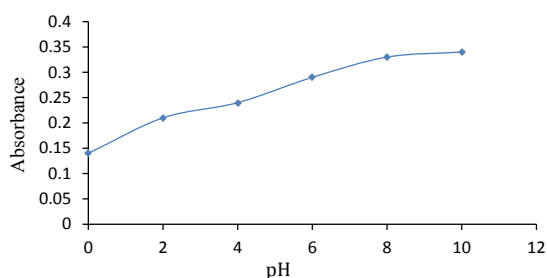


Figure 2f: Effect of PH on AgNPs concentration.

nanoparticles was observed, however, higher pH facilitates reduction of Ag⁺, subsequently, a large number of nanoparticles were formed with the smaller size.

Effect of Temperature on AgNPs size and concentration

The temperature was also played a crucial role for the size and shape of AgNPs. The effect of temperature was observed by varying

the temperature from 20°C to 100°C at an interval of 20°C. When the temperature of reaction mixture was increased, an increase in the AgNPs concentration was observed which is due to increase in reduction rate of Ag⁺ ions and color of the solution turned light brown to dark brown within 5 min and also decrease the average particle size (Verma et al., 2015). Optimum temperature was obtained for our experiment at 40°C (Figures 2g and 2h). Whereas with an increase in temperature above 40°C showed very broad absorbance peak which showed an increasing particle size [41].

Effect of contact time on AgNPs concentration

UV-vis spectrums were used to monitor the effect of contact time on AgNPs concentration in the reaction mixture at different intervals of time with a change of color of the solution. The intensity of absorption peaks is increased gradually and then became constant because the concentration of AgNPs was initially increased with increase in contact time which is due to the effect of surface plasmon resonance of AgNPs. Nanoparticles size was measured and it varied from 20 nm to 130 nm (Figure 2i). It was observed by Zetasizer, after 15 min of reaction time,

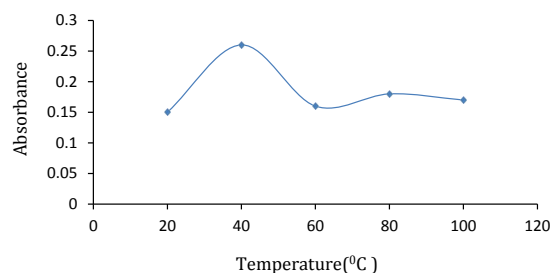


Figure 2g: Effect of Temperature on AgNPs concentration.

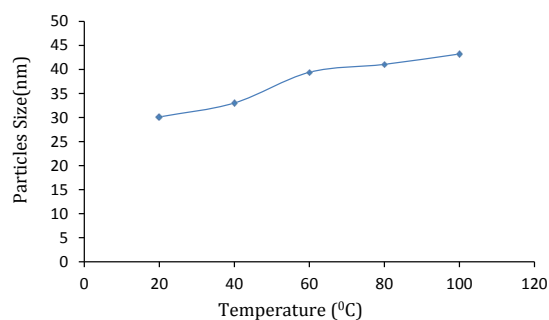


Figure 2h: Effect of Temperature on AgNPs size.

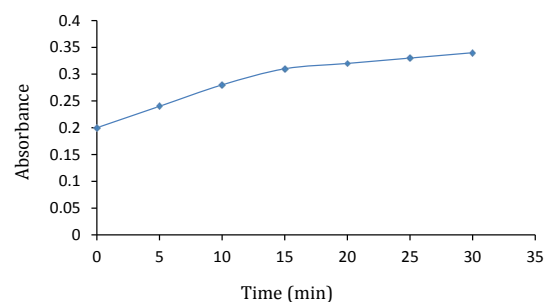


Figure 2i: Effect of Time on AgNPs concentration.

there was no significant change in AgNPs concentration. Small reaction times were recommended for higher production rate and small size of AgNPs [42].

XRD analysis

XRD analysis of dried green synthesized AgNPs by *Prunus persica* extract was done to find the crystalline nature and structure of silver nanoparticles. Pattern showed numbers of diffraction peaks at 2 theta such as 38.20, 44.23, 64.33, 77.40, 27.741, 32.221, 46.261, and 81.67 (Figure 3) The spectrum showed five distinct separate peaks at $2\theta=38.20, 44.23, 64.33$ and 77.40 that could be indexed to (111) (200), (220), (311) and (222) reflection planes of face centered cubic structure of silver respectively. XRD spectrum clearly shows that the silver nanoparticles formed by a green approach using *Prunus persica* extract through reduction process are crystalline in nature. Other unknown peaks of XRD pattern showed the presence of an organic compound in the extract was also discussed earlier [43]. Average size from XRD data of greensynthesized AgNPs was calculated by Debye Scherrer's equation:

$$D = K\lambda / \beta \cos\theta$$

Where 'D' is AgNPs crystal size, β is the full-width half maximum (FWHM) of diffraction peaks and θ is Bragg diffraction angle and λ

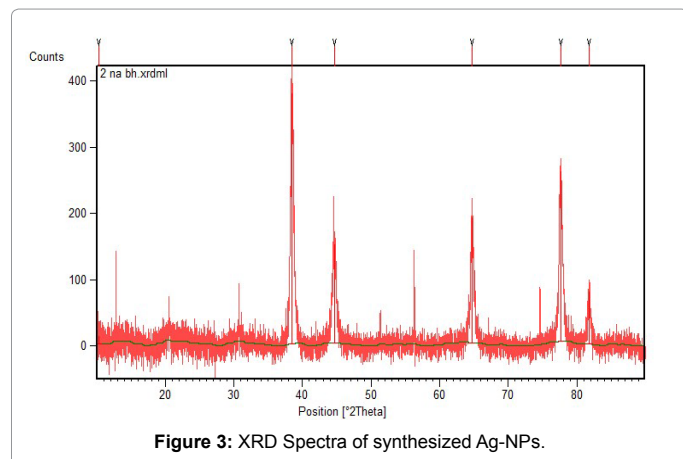


Figure 3: XRD Spectra of synthesized Ag-NPs.

is the wavelength of CuK α and found to be 40 nm. Biosynthesized AgNPs was showing high catalytic and antibacterial properties because of small size [44].

UV-Visible spectral analysis

Most important properties of silver nanoparticles are their optical properties which change with altering the size, the shape of the particle. The surface plasmon resonance of nanoparticles is responsible for the unique and beneficial optical properties of nanoparticles which depend on the size, shape of the nanoparticles, their distance from each other and the refractive index changes. UV-Visible spectrum was carried out to monitored the reduction process when *Prunus persica* extract was added into silver nitrate (1 mM) solution resulted in colour change of the solution from yellowish to brown due to excitation of surface plasmon vibrations of the silver nanoparticles, also indicating the formation of silver nanoparticles in the solution and got a peak centered near about 440 nm. It was also noticed that the reduction process of silver ions into silver atom was very rapid. Generally, most of the papers were reported the wavelength range of AgNPs absorption peak is in the range of 440-460 nm [45]. In our work, solution showed absorbance peak near at 440 nm as shown in Figure 4 which was confirmed from the reported specified range of Ag nanoparticles.

FESEM analysis

Morphology study of bio synthesized silver nanoparticles was carried out with the help of field emission-scanning electron microscopy (FESEM) of AgNPs by using the scales of 500 and 10 nm. Images of the surface morphology of the bio synthesized nanoparticles were clearly indicated that bio synthesized AgNPs was roughly spherical in shape and uniformly distributed and agglomeration was found in AgNPs as shown in Figure 5. It can also be observed that few nanoparticles have slightly deviated from their shape from spherical, may be due to the presence of different groups of natural chemical which helps in reducing and stabilizing the nanoparticles during their initial stage and the average size of AgNPs was obtained of 35 nm [46].

FTIR spectral analysis

FT-IR spectra taken to detect the major functional groups in plant extract and their possible role in reduction process during the synthesis

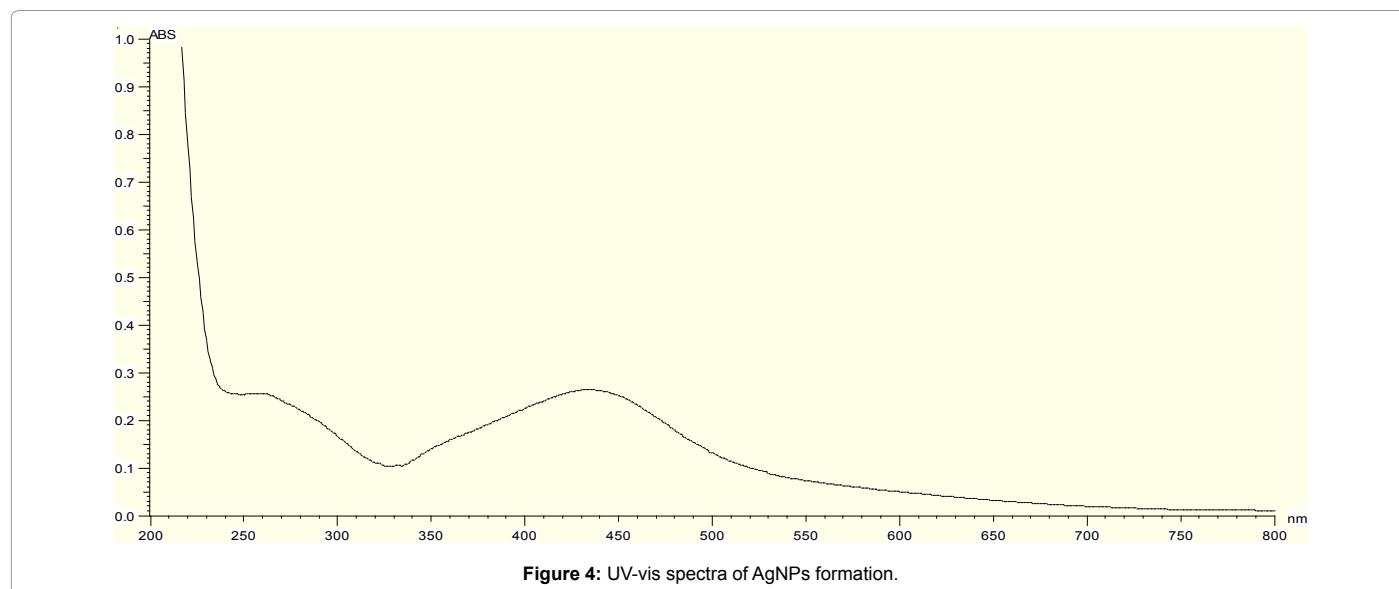


Figure 4: UV-vis spectra of AgNPs formation.

of AgNPs and stabilization of silver nanoparticles and spectrum peaks were compared with standard values to identify the main biomolecules present in the plant extract. The spectra showed absorption peaks at 3313 cm^{-1} , 1635 cm^{-1} , 1070 cm^{-1} and 1032 cm^{-1} indicating the presence of capping and stabilizing agents respectively. The role of these main biomolecules in stability/capping of AgNPs was found in many papers (Niraimathi et al., 2013; Krishnaraj et al., 2010). The peak 3318 cm^{-1} indicated phenolic or amide N-H, O-H stretching peak was observed at 1635 cm^{-1} and last important peak at 1032 cm^{-1} characteristics to glycoside or other (C-O-C) groups present in the plant extract are observed in Figure 6 (Kumar et al., 2014; Prabhu and Poulouse, 2012; Benakashani et al.). Major functional groups such as C-O, N-H and C-N groups in different chemical classes such as flavonoids, triterpenoids, polyphenols, proteins, and pigments present in the plant extract might be responsible for bioreduction of Ag^+ to AgNPs (Venu, 2011). The result of FTIR confirmed the presence of protein in the nanoparticles sample which will bind with protein either by free amine group or by cysteine residues in the protein. The peak at the region of 1638 nm is a characteristic of amide bond that is responsible for the stretching of carbonyl group coupled to the amide bond as the peptide has the ability to bind with metal and thus most of the cases protein coats the metal nanoparticles.

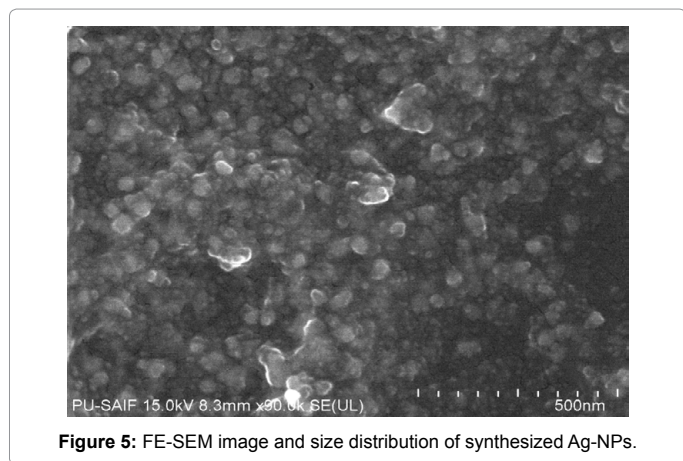


Figure 5: FE-SEM image and size distribution of synthesized Ag-NPs.

Antimicrobial activity of AgNPs

Silver nanoparticles have been using in many industries such as the health, pharmaceuticals, water treatment, paint, food storage because of its antibacterial properties [47]. In the present study, the antibacterial activity of bio synthesized AgNPs was tested against two different human pathogens (Figure 7). It is apparent that the AgNPs showed inhibition zone against two tested organisms (Table 1). Concentration of AgNPs and rifampicin (positive control) were 200 mg/mL and 2 mg/mL respectively. The HPLC grade sterile water was used as negative control. The power of AgNPs against human pathogen was depended up the size and dose. The synthesized AgNPs were found to have a higher inhibitory action against *P. aeruginosa* compared to other bacteria. The larger sizes of nanoparticles have less activity than smaller size nanoparticles due to small surface area. One of the possible modes of action of the plant mediated AgNPs might be to attach to the cell surface and disrupt the cell membrane and interact [48]. After penetration, AgNPs released silver ion. These ions interacted with DNA, proteins, and sulfur containing cell constituents, therefore, the organisms were prevented [49].

Catalytic property

Short equilibrium time indicates the high surface area of bio synthesized AgNPs and its suitability for fast and quantitative removal of Cd and Pd. It was found that the adsorption rate is rapid at the initial stages (Figure 8) because of the high vacant site to adsorb heavy metal and the system reaches equilibrium after 120 min and 150 min respectively. After equilibrium time, removal process became slow because of pore diffusion or saturation of adsorbent with heavy metals [5-7]. The degree of removal was very high for heavy metal by using AgNPs, more than 80% of Cd and Pd removal was achieved in 120 and 150 min respectively and after equilibrium time, the removal rate became slow [9].

Conclusion

There are a number of reports which describe methods making use of plant extract for the synthesis of nanoparticles as nonconventional economic and non-toxic procedures as compared to traditional chemical synthesis process. Green technique for fabrication of AgNPs

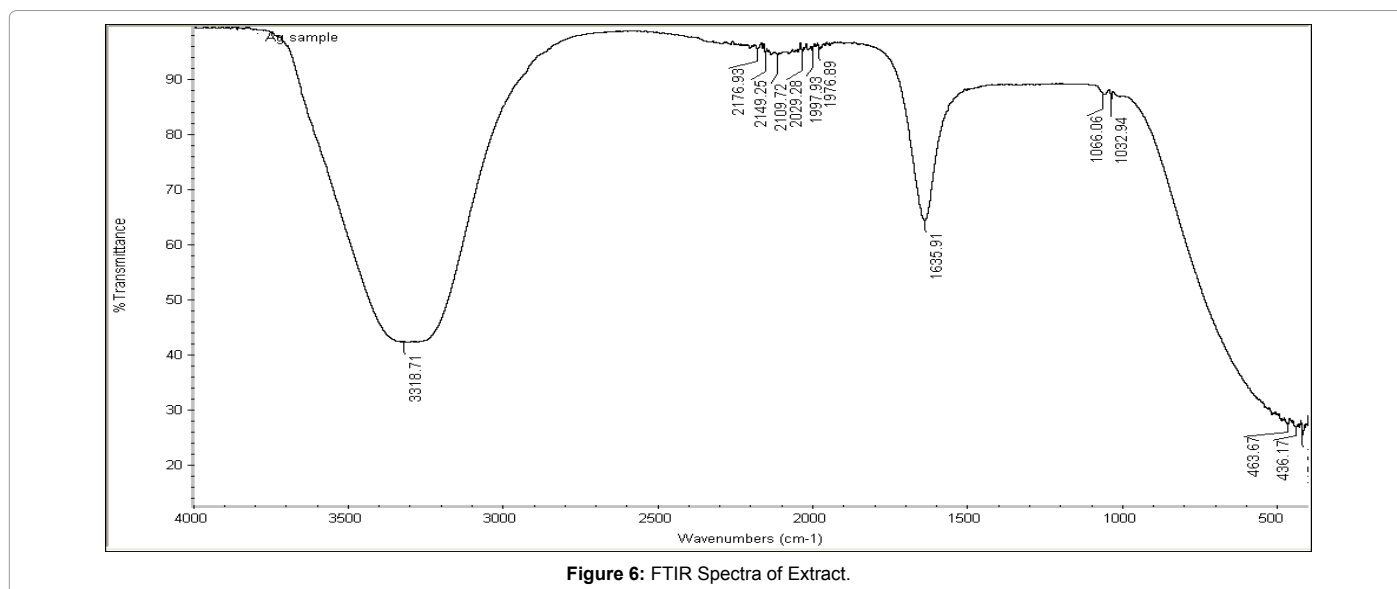


Figure 6: FTIR Spectra of Extract.



Figure 7: Panel-I *Escherichia coli* (*E. coli*), Panel-II *Vibrio cholera* (*V. cholera*).

Strains	Zone of inhibition	
	<i>E. coli</i>	<i>V. cholerae</i>
AgNPs	12	6
Rifampicin (Positive control)	4	24

Table 1: Zone of inhibition of AgNPs and rifampicin against bacterial strains.

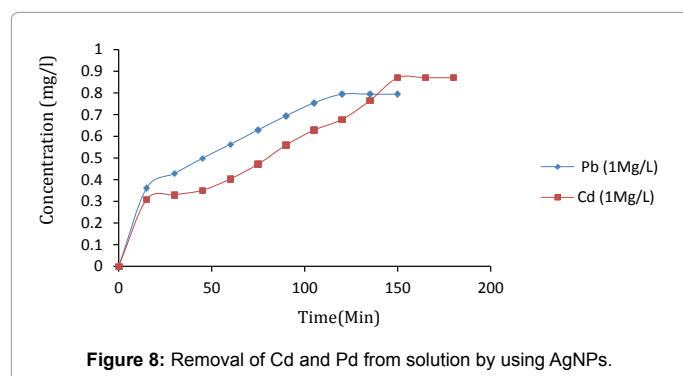


Figure 8: Removal of Cd and Pd from solution by using AgNPs.

was utilized using pine (*Prunus persica*) plant needle extract. This technology for synthesis of AgNPs seems to be low cost, not toxic. Some natural bio-compounds found in leaves extract that is confirmed by FTIR peaks that are responsible for reducing and stabilizing agent. Nanoparticles obtained with green approach were smaller in size than other chemical and physical methods but uniform in size monodispersed in nature and was synthesized very rapidly. This method does not require tedious downstream processing and it may be scaled up to develop a viable technology for the silver nanoparticles synthesis. The spherical shape of bio synthesized AgNPs was well dispersed in solution with an average size of 40 nm as evidenced by FESEM. The bio-synthesized AgNPs was proved as an excellent antimicrobial agent against *E. coli* and *V. cholerae*. Biosynthesized AgNPs were found to have a higher inhibitory action against *E. coli* compared to *V. cholera*. The present work showed potential for removal of heavy metals such as Cd and Pd from waste water by exploring catalytic property of bio synthesized AgNPs in a batch process. AgNPs showed higher catalytic activity against Cd heavy metal as compared to Pd. Development of the continuous process for removal of heavy metals and to explore biomedical properties for the preparation of biosensors using AgNPs and its application in medicine by using herbal medium is the thrust area for future work.

Conflict of Interests

None of the authors have any financial interest in any of the products, plant extract or devices mentioned in this paper. The authors declare that they have no competing interests.

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References

- Daniel M, Astruc DG (2004) Nanoparticles: Assembly, Supramolecular Chemistry, Quantum-Size-Related Properties, and Applications toward Biology, Catalysis, and Nanotechnology Gold Nanoparticles: Assembly, Supramolecular Chemistry, Quantum-Size-Related Properties. Chem Rev 104: 293-346.
- Arunachalam KD, Annamalai SK, Hari S (2013) One-step green synthesis and characterization of leaf extract-mediated biocompatible silver and gold nanoparticles from Memecylon umbellatum. Int J Nanomedicine 8: 1307-1315.
- Mittal AK, Chisti Y, Banerjee UC (2013) Synthesis of metallic nanoparticles using plant extracts. Biotechnol Adv 31: 346-56.
- Patil RS, Kokate MR, Kolekar SS (2012) Bioinspired synthesis of highly stabilized silver nanoparticles using Ocimum tenuiflorum leaf extract and their antibacterial activity. Spectrochim Acta - Part A Mol Biomol Spectrosc 91: 234-238.
- Devi TA, Ananthi N, Amaladhas TP (2015) Photobiological synthesis of noble metal nanoparticles using Hydrocotyle asiatica and application as catalyst for the photodegradation of cationic dyes. J Nanostructure Chem 6: 75-92.
- Alishah H, Seyedi SP, Ebrahimipour SY, Esmaeili-Mahani S (2016) A Green Approach for the Synthesis of Silver Nanoparticles Using Root Extract of Chelidonium majus: Characterization and Antibacterial Evaluation. J Clust Sci 27: 421-29.
- Bansal V, Bharde A, Ramanathan R, Bhargava SK (2012) Inorganic materials using "unusual" microorganisms. Adv Colloid Interface Sci 179: 150-168.
- Benakashani F, Allafchian AR, Jalali SAH (2016) Biosynthesis of silver nanoparticles using Capparis spinosa L. leaf extract and their antibacterial activity, Karbala. Int J of Modern Sci 2: 251-58.
- Bindhu MR, Umadevi M (2015) Antibacterial and catalytic activities of green synthesized silver nanoparticles. Spectrochim Acta - Part A: Mol Biomol Spectrosc 135: 373-378.
- Zúñiga O, García Jiménez F, Meléndez GR (2005) Comparative study of carotenoid composition in three mexican varieties of Capsicum annum. L Food Chem 90: 109-114.
- Dandu KRVR, Kim M (2015) Removal of Pb(II) using silver nanoparticles deposited graphene oxide: equilibrium and kinetic studies. Monatshefte Fur Chemie 146: 1445-1453.
- Elias E, Charles O, Aleruchi C, Hassan LA, Christiana OM, et al. (2014) Evaluation of antibacterial activities of silver nanoparticles green-synthesized using pineapple leaf (Ananas comosus). Micron 57: 1-5.
- Gao X, Yourick JJ, Topping VD, Black T, Olejnik N, et al. (2015) Toxicogenomic study in rat thymus of F1 generation offspring following maternal exposure to silver ion. Toxicology Reports 2: 341-350.
- Ghaedi M, Heidarpour S, Nasiri Kokhdan S, Sahraie R, Daneshfar A, et al. (2012) Comparison of silver and palladium nanoparticles loaded on activated carbon for efficient removal of Methylene blue: Kinetic and isotherm study of removal process. Powder Technol 228: 18-25.
- Hutchison JE (2008) Greener nanoscience: A proactive approach to advancing applications and reducing implications of nanotechnology. ACS Nano 2: 395-402.
- Ibrahim HMM (2015) Green synthesis and characterization of silver nanoparticles using banana peel extract and their antimicrobial activity against representative microorganisms. J Radiat Res Appl Sci 8: 1-11.
- Jamil M, Murtaza G, Mehmood A, Bhatti TM (2015) Green synthesis of silver nanoparticles using leaves extract of Skimmia laureola: Characterization and antibacterial activity. Mater. Lett 153: 10-13.
- Joy Prabu H, Johnson I (2015) Plant-mediated biosynthesis and characterization of silver nanoparticles by leaf extracts of Tragia involucrata, Cymbopogon citronella, Solanum verbascifolium and Tylophora ovata, Karbala. Int J Mod Sci 1: 237-246.
- Kaviya S, Santhanalakshmi J, Viswanathan B, Muthumary J, Srinivasan K (2011) Biosynthesis of silver nanoparticles using citrus sinensis peel extract and its antibacterial activity. Spectrochim Acta - Part A Mol Biomol Spectrosc 75: 594-598.

20. Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P, Kalaichelvan PT, et al. (2009) Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens. *Colloids and Surfaces A: Physicochem Eng Aspects* 76: 50-56.
21. Kumar B, Smita K, Cumbal L, Debut A (2014) Synthesis of silver nanoparticles using *Sacha inchi* (*Plukenetia volubilis* L.) leaf extracts. *Saudi J Biol Sci* 21: 605-609.
22. Kumar V, Yadav SK (2009) Plant-mediated synthesis of silver and gold nanoparticles and their applications. *J of Chem Technol and Biotechnol* 84: 151-157.
23. Manjari P, Kumar S, Kumar G, Parida K (2015) Biomimetic synthesis, characterization and mechanism of formation of stable silver nanoparticles using *Averrhoa carambola* L. leaf extract. *Mater Lett* 160: 566-571.
24. Marambio-Jones C, Hoek E (2010) A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. *J Nanoparticle Res* 12: 1531-151.
25. Maurya AK, Ben J, Zhao Z, Lee RTH, Niah W, et al. (2013) Positive and Negative Regulation of Gli Activity by Kif7 in the Zebrafish Embryo. *PLoS Genet* 9.
26. Metz KM, Sanders SE, Pender JP, Dix MR, Hinds DT, et al. (2015) Green Synthesis of Metal Nanoparticles via Natural Extracts: The Biogenic Nanoparticle Corona and Its Effects on Reactivity. *ACS Sustain Chem Eng* 3: 1610-1621.
27. Morones JR, Elechiguerra JL (2005) The bactericidal effect of silver nanoparticles. *Nanotechnol* 16: 2346-2353.
28. Mulvaney P (1996) Surface Plasmon Spectroscopy of Nanosized Metal Particles. *Langmuir* 12: 788-800.
29. Narayanan KB, Sakthivel N. (2010) Biological synthesis of metal nanoparticles by microbes. *Adv Colloid Interface Sci* 156: 1-13.
30. Nayak D, Pradhan S, Ashe S, Rauta PR, Nayak B (2015) Biologically synthesised silver nanoparticles from three diverse family of plant extracts and their anticancer activity against epidermoid A431 carcinoma. *J. Colloid Interface Sci* 457: 329-338.
31. Niraimathi KL, Sudha V, Lavanya R, Brindha P (2013) Biointerfaces Biosynthesis of silver nanoparticles using *Alternanthera sessilis* (Linn.) extract and their antimicrobial, antioxidant activities. *Colloids and Surfaces B: Biointerfaces* 102: 288-291.
32. Oluwaniyi OO, Adegoke HI, Adesuji ET, Alabi AB, Bodede SO, et al. (2015) Biosynthesis of silver nanoparticles using aqueous leaf extract of *Thevetia peruviana* Juss and its antimicrobial activities. *Appl Nanosci* 6: 903-912.
33. Panigrahi T (2013) Synthesis and characterization of silver nanoparticles using leaf extract of *Azadirachta indica*. Dep Life Sci Natl Inst Technol Rourkela (Doctoral dissertation).
34. Pantidos N, Horsfall LE (2014) Biological Synthesis of Metallic Nanoparticles by Bacteria, Fungi and Plants. *J of Nanomed & Nanotechnol* 5: 1.
35. Noginov MA, Zhu G, Bahoura M, Adegoke J, Small C, et al. (2007) The effect of gain and absorption on surface plasmons in metal nanoparticles. *Appl Phys B Lasers Opt* 86: 455-460.
36. Philip D (2010) Honey mediated green synthesis of silver nanoparticles. *Spectrochim Acta - Part A Mol Biomol Spectrosc* 75: 1078-1081.
37. Prabhu S, Poulouse EK (2012) Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. *Int Nano Lett* 2: 32.
38. Priyadarshini R, Parthiban S, Srinithya B, Kumar VV, Philip S, et al. (2015) Biogenic silver nanoparticles synthesis using the extract of the medicinal plant *Clerodendron serratum* and its in-vitro antiproliferative activity. *Mater Lett* 160: 400-403.
39. Rai A, Singh A, Ahmad A, Sastry M (2006) Role of halide ions and temperature on the morphology of biologically synthesized gold nanotriangles. *Langmuir* 22: 736-741.
40. Roopan SM, Madhumitha G, Rahuman AA, Kamaraj C, Bharathi A, et al. (2013) Low-cost and eco-friendly phyto-synthesis of silver nanoparticles using *Cocos nucifera* coir extract and its larvicidal activity. *Ind Crops Prod* 43: 631.
41. Sana SS, Badineni VR, Arla SK, Naidu Boya VK (2015) Eco-friendly synthesis of silver nanoparticles using leaf extract of *Grewia flaviscences* and study of their antimicrobial activity. *Mater Lett* 145: 347-350.
42. Saravanakumar A, Ganesh M, Jayaprakash J, Tae H (2015) Biosynthesis of silver nanoparticles using *Cassia tora* leaf extract and its antioxidant and antibacterial activities. *J Ind Eng Chem* 28: 277-281.
43. Singh RRN (2015) To Study the Effect of the Concentration of Carbon on Ultraviolet and Visible Light Photo Catalytic Activity and Characterization of Carbon Doped TiO₂. *J Nanomed Nanotechnol* 6: 1-7.
44. Suresh G, Hariharan P, Kokila D, Prabhu D (2014) Green synthesis of silver nanoparticles using *Delphinium denudatum* root extract exhibits antibacterial and mosquito larvicidal activities. *Spectrochimica Acta Part A : Molecular and Biomolecular Spectroscopy* 127: 61-66.
45. Swamy MK, Sudipta KM, Jayanta K, Balasubramanya S (2015) The green synthesis, characterization, and evaluation of the biological activities of silver nanoparticles synthesized from *Leptadenia reticulata* leaf extract. *Appl Nanosci* 5: 73-81.
46. Uygur B, Craig G, Mason MD, Ng AK (2009) Cytotoxicity and genotoxicity of silver nanomaterials. *Tech Proc NSTI Nanotechnol Conf Expo NSTI-Nanotech* 2: 383-386.
47. Venu R, Ramulu TS, Anandakumar S, Rani VS, Kim CG (2011) Bio-directed synthesis of platinum nanoparticles using aqueous honey solutions and their catalytic applications. *Colloids and Surfaces A: Physicochem Eng Aspects* 384: 733-738.
48. Verma A, Mehata MS (2015) ScienceDirect Controllable synthesis of silver nanoparticles using Neem leaves and their antimicrobial activity. *J Radiat Res Appl Sci* 9: 1-7.
49. Yilmaz M, Turkdemir H, Kilic MA, Bayram E, Cicek A, et al. (2011) Biosynthesis of silver nanoparticles using leaves of *Stevia rebaudiana*, *Mater Chem Phys* 130: 1195-1202.