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# Synthesis, Characterization and Evaluation of Antimicrobial Efficacy of Silver Nanoparticles using *Paederia foetida* L. leaf extract

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## Abstract

*The present study reports the synthesis of silver nanoparticles using Paederia foetida L. leaf extract were used as reducing agent from silver nitrate solution. The synthesis of silver nanoparticles was analyzed by UV-Visible spectroscopy (UV-Vis), Fourier Transform Infrared spectroscopy (FTIR), X-ray Diffraction (XRD), and Scanning Electron Microscopy coupled with Energy Dispersive X-ray Analysis (SEM-EDX). The synthesized silver nanoparticles were spherical in shape with an average size of 24nm. The antimicrobial activity of the silver nanoparticles shows the diameter of inhibition zones around the disk for Staphylococcus aureus, Klebsella* sp.*, V. cholera, P. aerueginosa and E. coli bacterial suspension are 14mm, 26mm, 12mm, 24mm, and 16mm respectively. Among the four bacterial species, Klebsella* sp. *and P. aerueginosa was showed higher zone of inhibition 26mm and 24mm, respectively. The results were compared with the ciprofloxacin positive control and synergistic effect of silver nanoparticles with ciprofloxacin. In the concluding remarks, the silver nanoparticles synthesized using P. foetida leaf extract would be a better antimicrobial activity against various bacterial species, when applied as individual or combined with commercial antibiotic.*

**Keywords**: Silver nanoparticles, antimicrobial activity, leaf extract, *Paederia foetida* L., Aantibiotic.

## Introduction

In recent past, nanoparticles preparation and its applications in various fields like catalysis, electronics, environmental, pharmaceutical and biotechnology, has been expanded significantly. Metal nanoparticles are usually in sizes ranging from 1-100nm and possess unique physical and chemical properties. Especially, silver nanoparticles have been attributed to their small size, more surface bulk atoms and high surface area permits them to highly interact with microbial membranes. Besides, silver is used in the medical field as best topical bactericides from the ancient time. Many physical or chemical methods that are currently available for silver nanoparticle production include chemical reduction, mechanical smashing, a solid-phase reaction, freeze-drying, spread drying and precipitation. Unfortunately, many of these methods have several disadvantages such as high cost, consumption of high energy; need to maintain high pressure and also high temperatures.

Recently, biological synthesis of silver nanoparticles has received a special attention due to environmental friendly green synthesis and easy to scale-up. Many researchers demonstrated that the green synthesis of silver nanoparticles including bacteria, actinomycetes, fungi and plants. Whereas, the plant materials have been successfully applied for silver nanoparticles synthesis, due to its potential medicinal property, huge availability, possibility of faster rate of synthesis and may also reduce the steps in downstream processing, thereby making the process cost efficient[1,2]. The plant leaf extracts of *Chenopodium album*[3], *Acalypha indica*[4], *Garcinia mangostana*[5], *Myrica esculent*[6], *Capsicum annuum*[7], *Geranium* sp.[8], *Diopyros kaki*[9], *Magnolia kobus*[10], *Coriandrum* sp.[11] have been effectively used for silver nanoparticle synthesis and analyzed its antimicrobial activity against various pathogenic organisms. The plant *P. foetida* (maile pilau), native to eastern Asia, is cultivated in warm regions of the world as an ornamental vine. This plant is a climbing, herbaceous, hairy or smooth slender vine. Perennial twining vine from woody rootstock; stems to 7 m (23ft) or more, climbing, or prostrate and rooting at the nodes. Leaves were arranged in opposite phyllotaxy (rarely in whorls of 3), ovate to oblong-ovate, 6 to 10 cm long, 3.5 to 5 cm wide, with conspicuous stipules[12]. An extensive research has been performed for the medicinal applications of *P. foetida* in antidiarrheal, antiinflammatory, antispasmodic, anthelmintic, antioxidant activity and hepatoprotective activity. In our detailed literature survey, there are no reports available on silver nanoparticles synthesis using medically important *P. foetida.*

In the present study, we have explored the green synthesis of silver nanoparticles using *P. foetida* leaf extract. Synthesized nanoparticles were characterized UV-Visble spectroscopy, XRD, FTIR, and SEM-EDX. Furthermore, the antimicrobial

activity of synthesized silver nanoparticles against *S. aureus, Klebsiella* sp.*, E.coli, Pseudomonas,* and *Vibrio cholera* were explored.

## Material and Methods

All the chemicals and reagents used in the present study were of analytical grade. Silver nitrate was purchased from Sigma–Aldrich Chemicals. The glass wares were washed in dilute nitric acid and thoroughly washed with double distilled water and dried in hot air oven.

**Preparation of plant extract:** The *P. foetida* leaves were collected from Himalayan hills, India, washed thoroughly thrice with distilled water and were shade dried for 10 days. The fine powder was obtained from the dried leaves by using kitchen blender. The leaf powder was sterilized at 121°C for 15 min. Exactly 20 g of *P. foetida* leaf powder was taken and mixed with 200 ml of Milli Q water and kept in boiling water bath for 20 min. The extracts were filtered with Whatman filter paper No 1. The filtered extract was collected in brown bottle and stored in refrigerator at 4°C until further studies.

**Biosynthesis of silver nanoparticles:** For synthesis of silver nanoparticles, 15ml of *P. foetida* aqueous extracts was added to the 250 ml Erlenmeyer flask containing 100 ml of $AgNO_3$ (1mM) and incubated at room temperature for 6 h in a dark condition. To monitor the silver nanoparticle synthesis, the samples were collected and measured at different time intervals (0 min and 6h) in UV–Visible spectroscopy (PerkinElmer Lamda-45). A control reaction mixture was also maintained without plant leaf extract. For the UV-Visible spectroscopic analysis, 0.1mL of the sample was diluted to 2mL with deionized water. The UV–Visible spectra were recorded with difference of 1nm.

**Characterization of silver nanoparticles:** A small aliquot of aqueous plant extract and after silver nanoparticles formation was used to analyze the functional group variation by FTIR analysis. The spectra were recorded using Fourier transform infrared spectrometer (Tensomax Bruker, Tensor-27) in the range of 400-4000 $cm^{-1}$ with samples prepared as KBr discs. Sample mixture was centrifuged at 6000rpm for 10min and the pellet. The particles were then gently washed to remove any organic residue and re-suspended in absolute ethanol. The dried suspension was spread out on the sample mounted on aluminum stab. The thin film was kept in hot air oven to dry. Scanning electron imaging of silver nanoparticles samples was done at a microscope equipped with a filled-emission cathode. The elemental composition of particles was estimated by an energy dispersive X-ray detector fitted with SEM (Hitachi-3400N). Further, the suspension was properly dried in a hot air oven with atm air condition and the powered sample was analysed using powder XRD (Bruker AXS D4 Endeavor) diffractometer operating with Cu Kά radiation source filtered with a graphitic monochromator ($\lambda$ = 1.5406). The samples were crushed to a fine powder and pressed into a sample holder. The XRD scans were recorded from $2\theta = 20^{\circ}$ to $70^{\circ}$ using a step size of $0.02^{\circ}$.

**Antimicrobial studies:** The silver nanoparticles produced using *P. foetida* leaf extract was tested antimicrobial activity by disk diffusion method against pathogenic bacteria *Staphylococcus aureus, Klebsiella, E.coli, Pseudomonas,* and *Vibrio cholera.* The pure bacterial cultures were periodically subcultured were on nutrient agar. The paper disc diffusion method was used to test antimicrobial activity, described by standard procedure[13]. The paper disc was cut downed into small disc (4mm diameter) and sterilized at 150°C for 45 mins in hot air oven. Approximately 20 ml of molten Nutrient agar was poured in sterilized petri plates and plates were left overnight at room temperature. Meanwhile, the sterilized discs were impregnated with the silver nanoparticles solutions and positive control drug. Then the disc was dried in sterile condition. The bacterial species were grown in 100 ml nutrient broth for 24 h. Exactly, 100µl of the diluted cultures (1x $10^6$) of test organisms were spread on nutrient agar plates. The silver nanoparticles impregnated discs were placed on the plates and incubated at 37°C for 24. After incubation, the different levels of zone of inhibition of bacteriae were measured. Ciprofloxacin was used as control antimicrobial drugs. The diameter of the inhibition zones was measured using in mm and the mean values were presented.

## Results and Discussion

Silver is known as very good antimicrobial effect against various pathogenic organisms and it was used from ancient times. Further, the silver also used as water purification and air filtration system to eliminate the pathogenic microorganisms. In the recent times, researchers paid more attention on the green synthesis and applications of silver nanoparticles using various plant extracts. In the present study, an attempt was made to green synthesis of silver nanoparticles using *P. foetida* leaf extract.

**Characterization of silver nanoparticles: UV-Visible spectroscopic analysis:** The silver nanoparticles synthesis was confirmed by monitoring the changing of the colorless solution changed into brownish yellow color which indicates the formation of silver nanoparticles. UV-Vis spectroscopy is an important technique to ascertain the formation and stability of metal nanoparticles in aqueous solution. It is well known that silver nanoparticles exhibit different colors and size, and these colors arise due to excitation of surface plasmon resonance (SPR) in the silver nanoparticles[14]. The reduction of aqueous silver ions into silver nanoparticles was monitored from the aqueous silver nitrate - aqueous *P. foetida* extract reaction medium as a function of time of reaction was shown in figure 1. The silver surface plasmon resonance band occurs at ca. 420nm and steadily increases in intensity as function of time. This band is indicative of the presence of spherical nanoparticles in reaction mixture. The maximum and stable silver nanoparticles were achieved in 6h of reaction. The same trend was observed by a research team[15]. However, in another report, the reduction

of silver ions to silver nanoparticles using *Camellia sinensis* extract was occurring within 4 h.

**FTIR Analysis:** FTIR analysis was used to characterize the nature of capping ligands that stabilizes the silver nanoparticles formed by bioreduction process. The spectra were obtained in the wavelength range between 400 and 4000cm$^{-1}$ and the FTIR spectra of before and after plant extract addition into silver ions reaction products are given in figures 2 (a and b). The peaks appeared at 3420cm$^{-1}$, 3431cm$^{-1}$ (strong O-H bonding) indicate the presence of O-H stretching of carboxyl groups and N-H stretching of secondary amides. Further, these peaks also indicate the presence of bonded hydroxyl groups. The peaks observed at 2920 cm$^{-1}$, 2851cm$^{-1}$, 2660cm-$^{1}$ and 2318 cm$^{-1}$ represent the C-H stretching bonds. The peaks observed at 1625 cm$^{-1}$ and 1460 cm$^{-1}$ represent the bonds with C-N stretching, N-H deformation, COO- anions and C=C aromatic conjugates, and 1400-1200 cm$^{-1}$ represent the C-H stretching vibrations, N-H bending, -$CH_3$ wagging and C-OH stretching vibrations, whereas the sharp peaks appeared at 1074 cm$^{-1}$ and 746 cm$^{-1}$ represent the C-O stretching and aromatic -CH deformation respectively. As shown in figure 4a, indicates the presents of many functional groups involved in the conversion of silver ions to silver nanoparticles. The disappearance of these bands, or intensity decrease, such as the band at 1460cm$^{-1}$and 562cm$^{-1}$, can be attributed to reduction of silver ions coupled with oxidation of phenolic components[16]. An intense and sharp peak was observed in figure 4b, at 562 cm$^{-1}$ indicates the presents of silver ions.

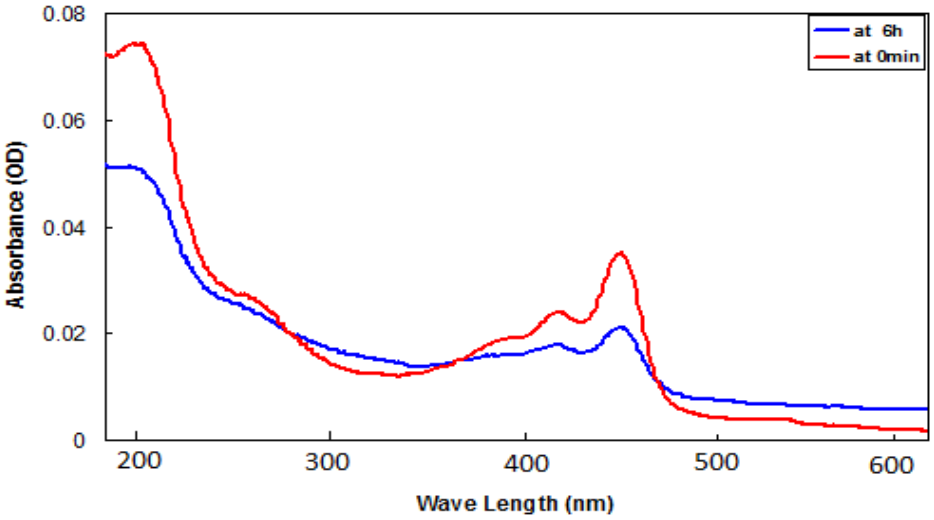


**Figure-1**
**UV–Vis spectra of silver nanoparticles synthesis using aqueous *P. foetida* leaf extract**

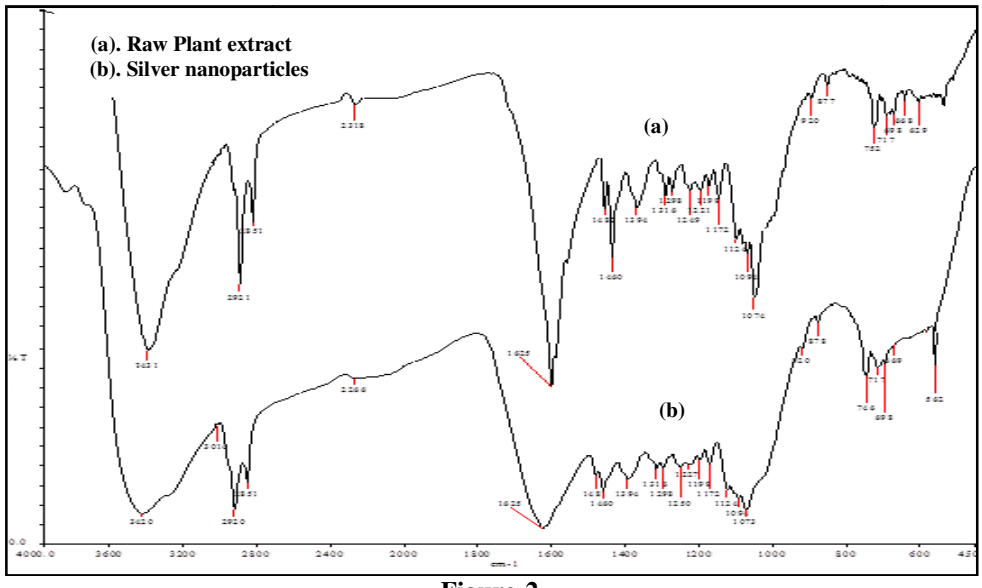


**Figure-2**
**FTIR analysis of raw plant extract (a) and silver nanoparticles (b) synthesized using *P. foetida* leaf extract**

**X-ray diffraction analysis:** X-ray diffraction is a very important method to characterize the structure of crystalline material and used for the lattice parameters analysis of single crystals, or the phase, texture or even stress analysis of samples. X-ray diffractogram of the silver nanoparticles showed three distinct diffraction peaks at 26.459°, 32.555°, 46.705°, 55.632° and 73.628° and these 2ɵ (degree) values were indexed in the angle values of (110), (111), (211) crystalline planes of cubic Ag was observed in figure 3. This analysis revealed that nanoparticles are in orthorhombic crystals. The high peaks in the analysis indicate the active silver composition with the indexing. Each crystallographic facet contains energetically distinct sites based on atom density facets such as (111) that are known to be highly reactive because this is the fact that contains more atom density.

The size of the silver nanoparticles was calculated by Debye-Scherrer's equation using FWHMs obtained from the diffraction peaks.

$$D = \frac{K\lambda}{\beta_s \cos\theta}$$

Where, D - average crystallite size in direction perpendicular to the diffracted crystal planes, K - shape-dependent Debye-Scherrer's constant correlating to the true shape of crystallite (typical values used vary but in this case 0.94 is used to correspond spherical crystallites with cubic symmetry); $\lambda$ - radiation wavelength (1.5406 A); $\beta_s$= peak width caused by structural broadening due to crystallite size, thus the effect of instrumental broadening is already subtracted from this value, and the resulted value is given in radians. From the Debye-Scherrer equation, the average size of silver nanoparticle synthesized was found as 24nm.

**SEM-EDX analysis:** The Scanning Electron Microscopy-Energy Dispersive X-ray analysis (SEM-EDX) was done to determine the size and elemental composition of the silver nanoparticles synthesized. As shown in figure 3, the SEM analysis confirmed the in the size range of 22 - 40nm, a clear indication of the formation of silver nanoparticles. The EDX pattern (silver counts in three places with high peaks) of the silver nanoparticles synthesized using *P. foetida* leaf extract.

**Antimicrobial activity of silver nanoparticles:** Recently, silver nanoparticles were found to be a best biocide, for example in wound dressings and as an antimicrobial coating on consumer products, little is known about its mode of toxicity[17]. A wide research reports were available for using silver nanoparticles as a antimicrobial agent against *S. aureus* and *E. coli*[18], Gram positive strains (*S. aureus, S.epidermidis* and Gram negative strains (*E. coli, S. typhi, Pseudomonas aueroginosa, Klebsiella pneumonia, Proteus vulgaris*)[19], *B. substilis* and *S. aureus* and *E. coli*[20] and *Staphylococcus aureus, E.coli, Corynebacterium diphtheria and Micrococcus sp.,*[21]. In this study, the antimicrobial activity of silver nanoparticles and ciprofloxacin was tested by disk diffusion method against pathogenic bacteria *S. Aureus*, *Klebsella* sp., *V. cholera, P. aerueginosa* and *E. coli.* As shown in table 1, the diameter of inhibition zones around the disk containing silver nanoparticles in *S. Aureus*, *Klebsella* sp., *V. cholera, P. aerueginosa* and *E. coli* bacterial suspension are 14mm, 26mm, 12mm, 24mm, and 16mm respectively. Among the four bacterial species, *Klebsella* sp. and *P. aerueginosa* was showed higher zone of inhibition 26mm and 24mm, respectively figures 5 (a & b). The variation in the diameter of zone of inhibition observed for different bacterial species, due to bacterial cell wall composition. When the positive control (ciprofloxacin) tested the antimicrobial activity against *S. Aureus*, *Klebsella* sp., *V. cholera, P. aerueginosa* and *E. coli,* the zone of inhibition (diameter) was observed 09mm, 20mm, 21mm, 19mm and 11mm, respectively. However, the combined effect of silver nanoparticles and ciprofloxacin antibiotic shows, higher antibacterial activity than individual.

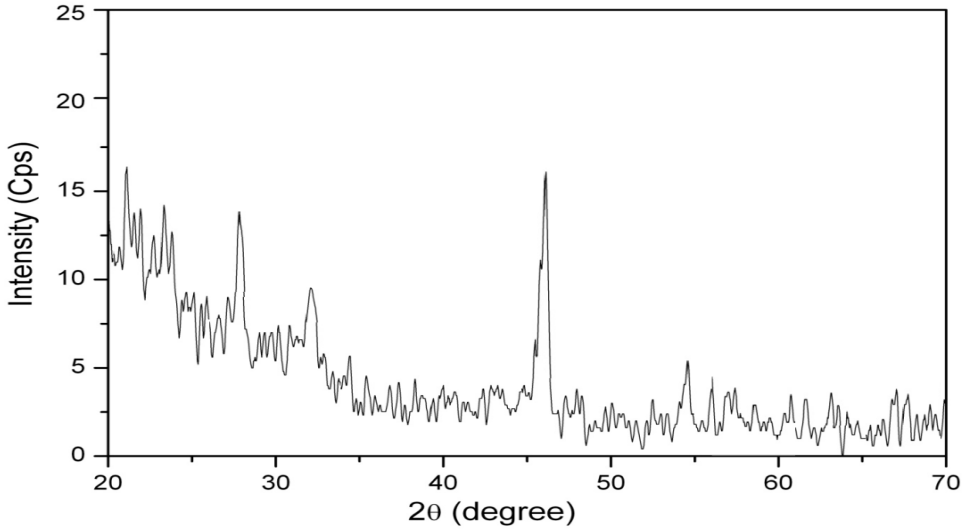


**Figure-3**

**XRD pattern of silver nanoparticles synthesized using *P. foetida* leaf extract**

**Table-1**

**Zone of inhibition of silver nanoparticles synthesized using *P. foetida* leaf extract**

| S.No. | Organisms | NPs(mm) | Antibiotic(mm) | NPs + Antibiotic(mm) |
|---|---|---|---|---|
| 1. | *Staphylococcus aureus* | 14 | 09 | 22 |
| 2. | *Klebsella* sp. | 26 | 20 | 27 |
| 3. | *Vibrio cholera* | 12 | 21 | 24 |
| 4. | *P. aeruginosa* | 24 | 19 | 26 |
| 5. | *E.coli* | 16 | 11 | 18 |

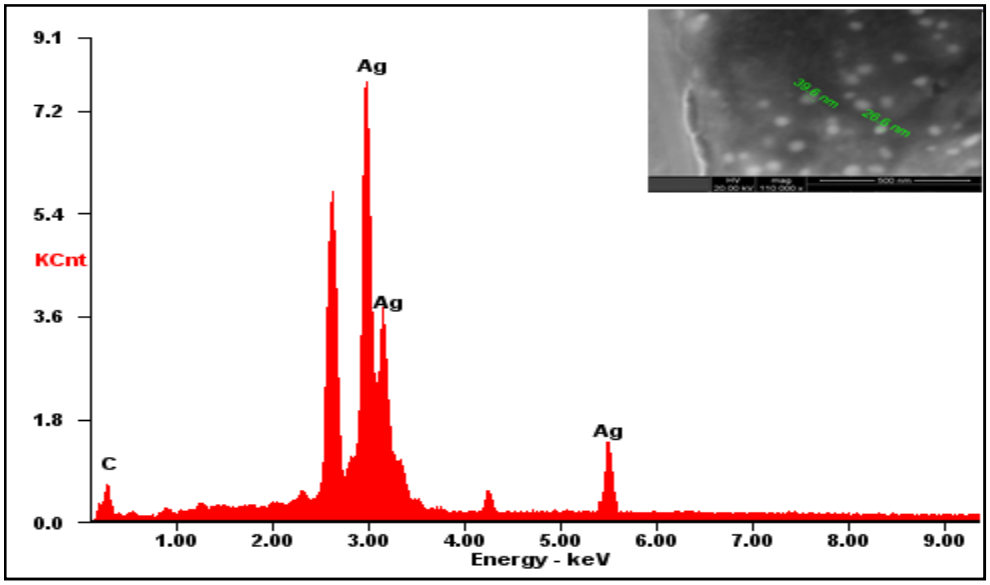


**Figure-4**

**SEM-EDX analysis of silver nanoparticles synthesized using *P. foetida* leaf extract**

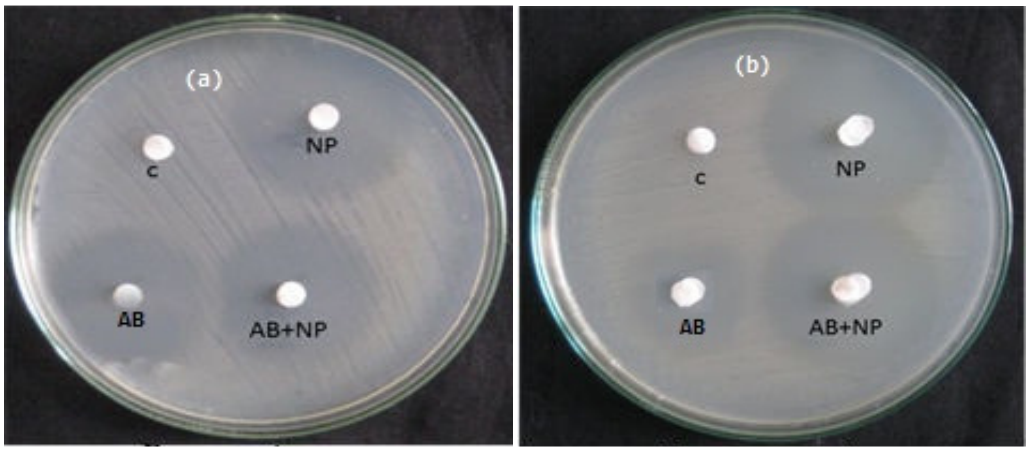


**Figure-5**

**Zone of inhibition silver nanoparticles synthesized using *P. foetida* leaf extract (a). *Klebsella* sp. and (b). *P. aeruginosa***

The mechanism involved in the inhibition of bacterial growth by silver nanoparticles is unclear. However, the general mechanism of antibacterial activity of silver nanoparticles was proposed by many researchers, but the detailed mechanism remains to be understood. The bacterial growth was inhibited by silver ions, which accumulated into the vacuole and cell walls as granules[22]. The silver nanoparticles attached the surface of the cell membrane disturbing the permeability and respiration functions followed by dysfunction of metabolic pathways including, silver ions can interact with nucleic acids they preferentially interact

with the bases in the DNA rather than with the phosphate groups. Thereby, inhibiting the cell division and also damaged the cell envelope and cellular contents of the bacteria[23]. In another study, the mechanism of silver nanoparticles against bacterial cells due to the sizes of the bacterial cells increased, and the cytoplasmic membrane, cytoplasmic contents, and outer cell layers exhibited structural abnormalities[24]. It is also possible that silver nanoparticles not only interact with the surface of membrane, but can also penetrate inside the bacteria. When the silver nanoparticles enters into the bacteria that, generating hydrogen peroxide radicals followed by inactivated metabolic enzymes, which leads bacterial cell death. When the nanomolar concentrations of silver nanoparticles also have killed *E. coli* cells within minutes possibly due to immediate dissipation of the proton motive force[25]. However, at lower concentration of silver ions result in massive $H^+$ leakage through bacterial membranes and makes proton motive fore. This is possibly due to $H^+$ leak might be happening from either any silver ions modified membrane protein or phospholipids bilayer and causes deenergization of the membrane and consequently cell death.

## Conclusion

The present study demonstrated the biological synthesis of silver nanoparticles using *P. foetida* leaf extract was performed. The average size of silver nanoparticle synthesized was found as 24nm with spherical shaped structures. The antimicrobial screening test indicated, that the zones of inhibition was observed in all the five bacterial species, among these *Klebsella* sp. and *P. aeruginosa* was showed higher zone of inhibition. The synergistic effect of synthesized silver nanoparticles and ciprofloxacin was showed higher inhibition effect in all the tested bacterial species. The antimicrobial activity of the nanoparticles showed that the silver nanoparticles synthesized using *P. foetida* plant derived reducing agent, have a great potential antimicrobial against many pathogenic microorganisms. In addition, this green synthesis process would be a better alternative to the existing methods.

## Acknowledgements

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