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ECOFRIENDLY SYNTHESIS OF SILVER NANOPARTICLES FROM ETHANOLIC EXTRACT OF PELARGONIUM GRAVEOLENS L'HER AND THEIR ANTIBACTERIAL PROPERTIES

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Abstract

An efficient and versatile technique was implemented for the synthesis of silver nanoparticles using ethanolic extract of *Pelargonium graveolens*. The biosynthesis of the silver nanoparticles was monitored by colour changing of the solution and then measuring the UV–vis spectroscopy. The crystalline nature of nanoparticles was confirmed from the X-ray diffraction (XRD) pattern.

The morphology and crystalline phase of the nanoparticles were determined using scanning electron microscopy (SEM) with X-ray energy dispersive spectrophotometer (EDAX). The Fourier transform infrared spectroscopy (FTIR) spectra indicated the role of amino acids, amides group I in the synthetic process. The silver nanoparticles thus obtained showed highly potent antibacterial activity toward *Escherichia coli, Shigella flexinaeri* and *Protease mirabillis*. Silver nanoparticles showed a clear well defined inhibition zone compared to control plant extract. The results confirmed that the ethanolic extract of *P.graveolens* is a very good eco-friendly and nontoxic source for the synthesis of nanoparticles as compared to the conventional chemical/physical methods. Therefore, it provides future opportunities in Nano-medicine by tagging nanoparticles with phyto-compounds present in the *P.graveolens*.

Keywords: Bio synthesis, *Pelargonium graveolens*, Silver nanoparticles, Antibacterial activity.

1. Introduction

For the last decades, nanotechnology is a skyrocketing multidisciplinary field of research that interweaves physics, chemistry, bio-Nano science, and materials science (Paulraj Kanmani *et al.*,2013). Recently, biological or green chemistry synthesis of nanoparticles (NPs) received enormous attention over the physical and chemical synthesis, as it is a clean, non-toxic and an ecofriendly approach which includes design and development of syntheses using renewable high energy efficient materials, benign reaction media and non-hazardous as well as non-toxic solvents, utilizing catalysts determining the interactions of nanomaterial's in the environment, their fate and transport as well as the development of real-time monitoring and process control to avoid or minimize accidents (Virkutyte and Varma, 2011, Umesh *et al*., 2013). Therefore, the integration of green chemistry principles into nanotechnology is essential where the development of nanotechnology could benefit from a greener approach that promotes both performance and safety (Mulvihill *et al*., 2011).

Microbes and plants are currently used for nanoparticle synthesis. The use of plants for the fabrication of nanoparticles is a rapid, low cost, eco-friendly and a single step method for biosynthesis process (Huang *et al.*, 2007). The usage of plants can also be suitably scaled up for large-scale synthesis of nanoparticles in a controlled manner according to their size, shape and dispersity. Moreover, the use of plants in process of nanoparticle synthesis is more beneficial than other processes since the nanoparticles are produced extracellular. Among the nanoparticles, silver nanoparticles are attractive especially for antimicrobial sterilization. Silver is the metal of choice as they hold the promise to kill microbes effectively (Asmathunisha Nabikhan *et al*., 2010).

Nano scale materials have emerged as novel antimicrobial agents owing to their high surface area to volume ratio and the unique chemical and physical properties, which increases their contact with microbes and their ability to permeate cells. Also, nanotechnology has amplified the effectiveness of silver particles as antimicrobial agents (Jannathul Firdhouse *et al*., 2012).

Pelargonium graveolens belongs to the family Geraniaceae, and is an evergreen perennial flowering plant generally known for its essential oil, with rose like aroma. The plant is commonly called Rose scented geranium and there are about 300 geranium species widely present. It has several medicinal and aromatic values of commercial importance (Brian *et al*., 2010). Traditionally, geranium was used to staunch bleeding, heal wounds, ulcers and skin disorders as well as treat diarrhea, dysentery and colic (Benazir *et al*., 2013). The extracts of *P. graveolens* are reported to be used as antibacterial and insecticidal agents (Ooshiro *et al*., 2009; Tabanca *et al*., 2013).

In the present study, a novel approach for the synthesis of ethanolic extract of *P.graveolens*-mediated silver nanoparticles by direct reduction of silver nitrate was demonstrated. The synthesized silvernanoparticles were characterized using UV–vis spectroscopy; X-ray diffraction (XRD), scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX), and Fourier transform infrared (FTIR) spectroscopy. In addition, the antibacterial activity of the ethanolic extract of *P.graveolens* -stabilized silver nanoparticles were examined.

2. Materials and methods

Pelargonium graveolens leaves were collected from the herbal garden at Mother Teresa Women's University, Kodaikanal. AgNO3 were purchased from Sigma Aldrich, Mumbai. *Escherichia coli*, *Shigella flexinaeri* and *Protease mirabillis* were purchased from Microbial culture collection, Chandigarh.

Preparation of the plant extract

The ethanolic extract of each plant powder was prepared by soaking 3 g of the material in 80% ethanol at room temperature. After 3 h, the macerate was filtered on Whatmann filter paper no. 1, and the alcoholic extracts dried at 40 °C for 1 week. The dried material was maintained in the dark in a cool, dry place [12].The ethanolic leaf extract of *P.graveolens*(200mg) was weighed and taken in a 250 ml beaker and 100 ml of Millipore water was added to it. The solution was sonicated using ultrasonic bath (PCI Ultrasonic 1.5 L (H)) for 15 mins to disperse the extract in water. The solution was filtered thrice using Whatman filter paper to get a clear solution. From this extract, 20 ml of the sample was used for further analysis.

Synthesis of silver nanoparticles using ethanolic plant extract

1mM silver nitrate solution was taken in a conical flask 20ml of ethanolic extract was added by drop wise to this solution. The solution was kept under dark condition until colour changed to brown colour.

Characterization of silver nanoparticles

To determine the time point of maximum production of silver nanoparticles, the absorption spectra of the samples were taken 340 to 540 nm using a UV–vis spectrophotometer (HITACHI, Model U-2800 spectrophotometer). The deionized water was used as the blank. The mean particle diameter of silver nanoparticles was calculated from the XRD pattern. Powder XRD patterns were recorded using a powder X-ray diffractometer (Model- D8 Advance, made in BRUKER Germany). The morphology of the silver nanoparticles was analyzed using scanning electron microscope (SEM). Elemental composition of silver nanoparticles was analysed using energy dispersive X-ray spectroscopy (EDS). The supernatant from Ag NPs was freeze-dried, and the dried powder was diluted with potassium bromide in the ratio of 1:100. The spectrum was recorded in FTIR in the range of 4000–500 cm^{-1} at a resolution of 4 cm^{-1}.

Antibacterial assay

The efficiency of silver nanoparticles as an antibacterial compound was evaluated against *Escherichia coli*, *Shigella flexinaeri* and *Protease mirabillis.* The disc diffusion method was used to assess the antibacterial activity of the synthesized silver nanoparticles (Geetha *et al.*, 2014). Sterile Whatman filter paper discs (3mm) were containing different concentration of silver nanoparticles (25µl, 50µl and 75µl) and ethanolic extract as a control. Sterile discs were placed on the bacterial strains inoculated Muller-Hinton agar medium and the plates were incubated at 37◦C for 24 hrs in an incubator and observe zone of inhibition.

3. Results and Discussion

The plant leaf *Pelargonium graveolens* L. appears to be a potential source for phytochemicals. It has been reported that the leaves contain various organic compounds such as phenolics, flavonoids, tannins, terpenoids, saponins and glycosides. The alcohol soluble compounds present in the ethanolic extract were found to be responsible for efficient stabilization of nanoparticles and reduction of metal ions.

UV-Visible Spectrum of silver nanoparticles

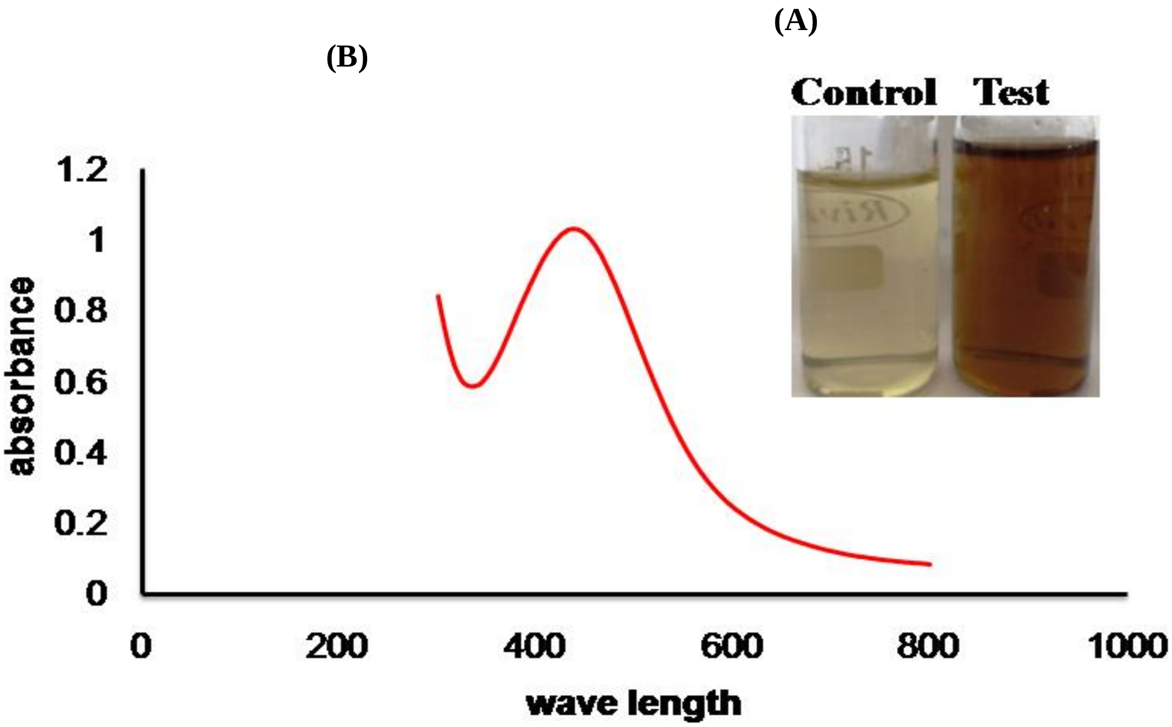


Figure 1: (A) Colour change of synthesized silver nanoparticles shows time interval of incubation **(B)** UV-vis absorption spectrum of silver nanoparticles

Thus, color change of the solution clearly indicated the formation of silver nanoparticles. The color intensity of the cell filtrate with $AgNO_3$ was sustained even after 24 h incubation, which indicated that the particles were well dispersed in the solution, and there was no obvious aggregation (Figure 1A). All these reactions were monitored by ultraviolet-visible spectroscopy of the colloidal silver nanoparticles solutions. The ultraviolet-visible spectra of the cell filtrate with $AgNO_3$ showed a strong broad peak at 436 nm which is surface Plasmon resonances (SPR band), which indicated the presence of silver nanoparticles (Figure 1B). These results were consistent with the reports of Verma *et al* (2010), Gaikwad Sagar (2012). The intensity of the SPR band steadily increased from 6 h to 24 h as a function of time of reaction. It was also observed that the silver nanoparticles formed were quite stable in the supernatant of *P.graveolens*.

XRD pattern of synthesized silver nanoparticles

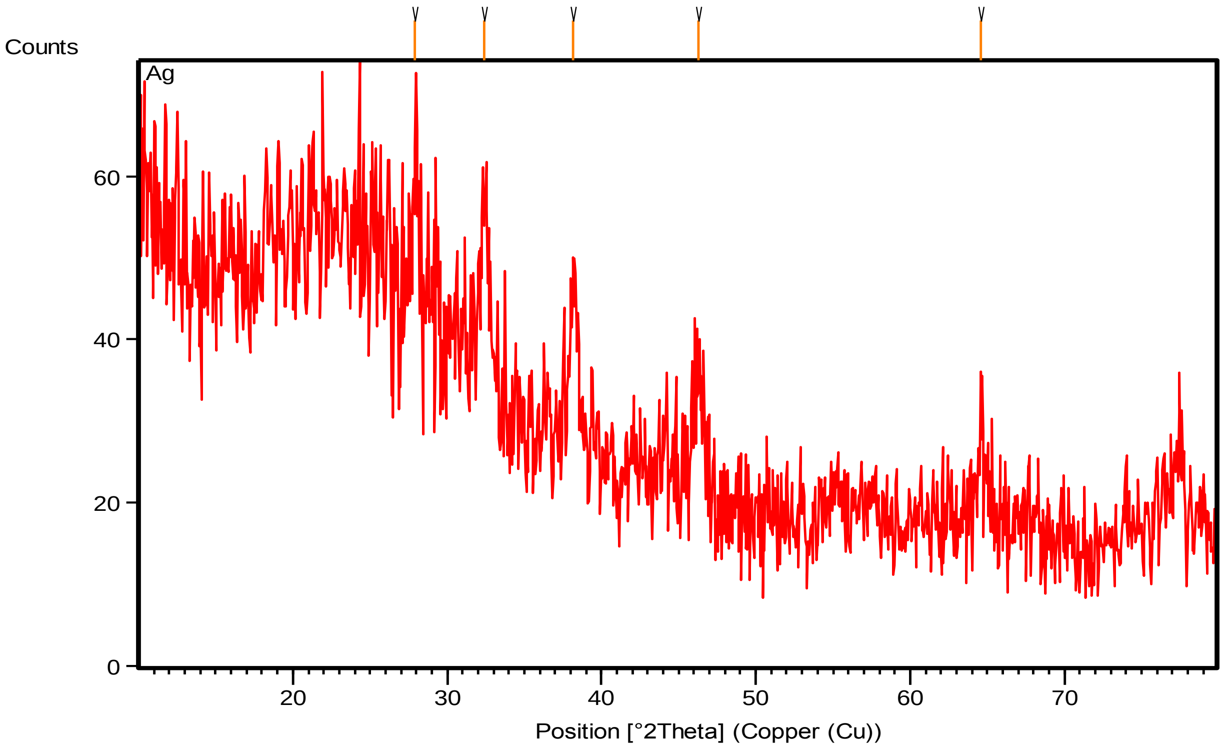


Figure 2: XRD pattern of synthesized silver nanoparticles

The crystalline nature of silver nanoparticles was confirmed from the analysis of X-ray diffraction (XRD) pattern of synthesized silver nanoparticles. Figure 2 shows numbers of Bragg reflections corresponding to (1 11), (2 0 0) and (2 2 0) planes of face centred cubic (fcc) structure of silver (JCPDS, file no. 89- 3722). The appearance of this sharp peak could have resulted from crystallization of silver nanoparticles which was due to reducing and stabilizing agent present in the ethanolic leaf extract of *P.graveolens.* The mean size of nanoparticles was calculated using Debye–Scherrer's equation ($D = K\lambda / \beta \cos \theta$, where D is the size of the particles, K is the shape dependent Scherrer's constant, λ is the X-Ray wavelength, β is the Full Width at Half Maximum (FWHM) and θ is the diffraction angle) by determining the width of the peaks and it was found to be 70-80 nm. The XRD pattern thus clearly shows that the silver nanoparticles are crystalline in nature.

Morphology studies of synthesized silver nanoparticles

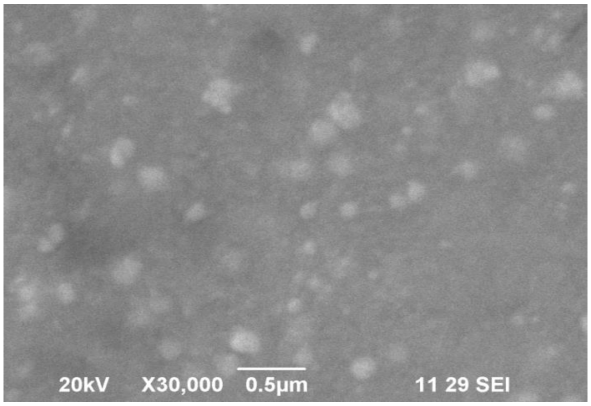


Figure 3: SEM micrograph of silver nanoparticles with the Magnifications of 30,000X

According to the scanning electron micrograph, uniformly distributed silver nanoparticles on the surface of the cells are observed. However, it does not indicate that all the nanoparticles are bound to the surface of the cells, because those dispersing in the solution may also deposit onto the surface of the cells. Figure 3 shows aggregation of spherical and square shaped silver nanoparticles was observed below 80nm with the magnification of 30,000X.

In the analysis by energy dispersive spectroscopy (EDS) of the AgNPs the presence of elemental metal signal was confirmed (Fig.4) (Selvaraj Mohana Roopan *et al.*, 2013). Metallic silver nanocrystals are generally show typical optical absorption peak approximately at 3 keV due to SPR (Harekrishna Bar *et al*., 2009).

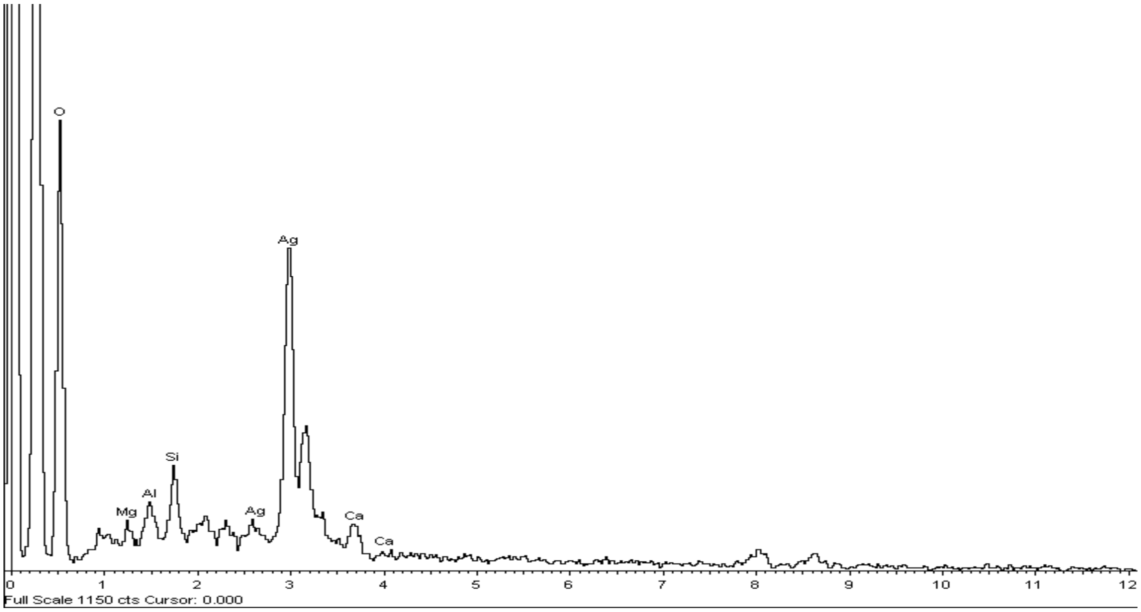


Figure 4: EDX spectrum of silver nanoparticles

FTIR studies of synthesized silver nanoparticles

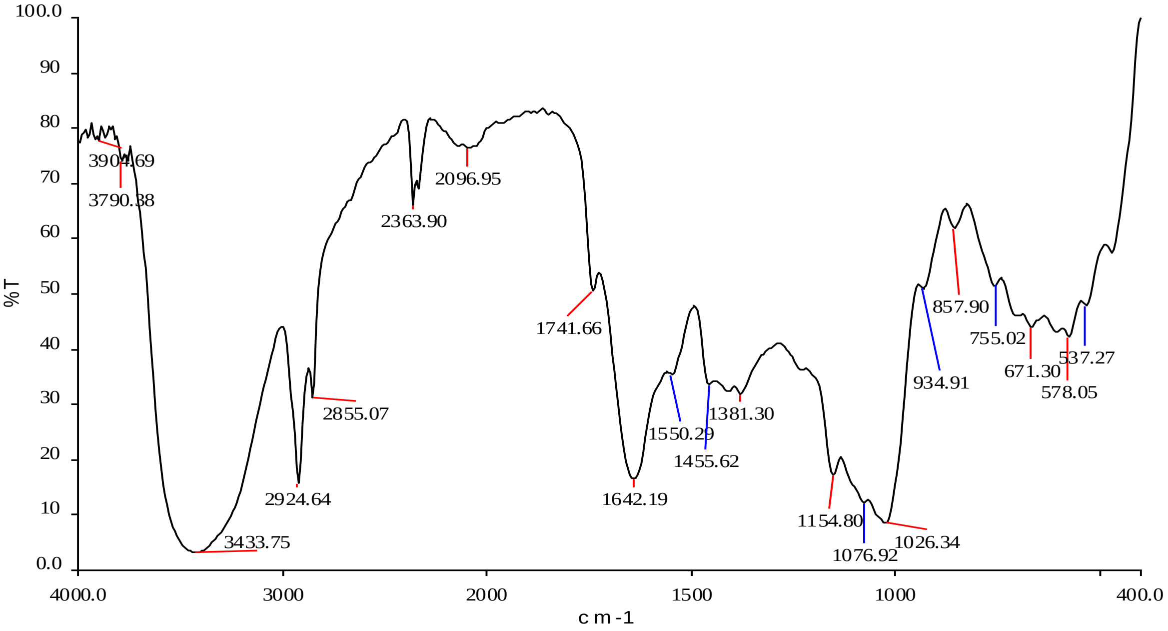
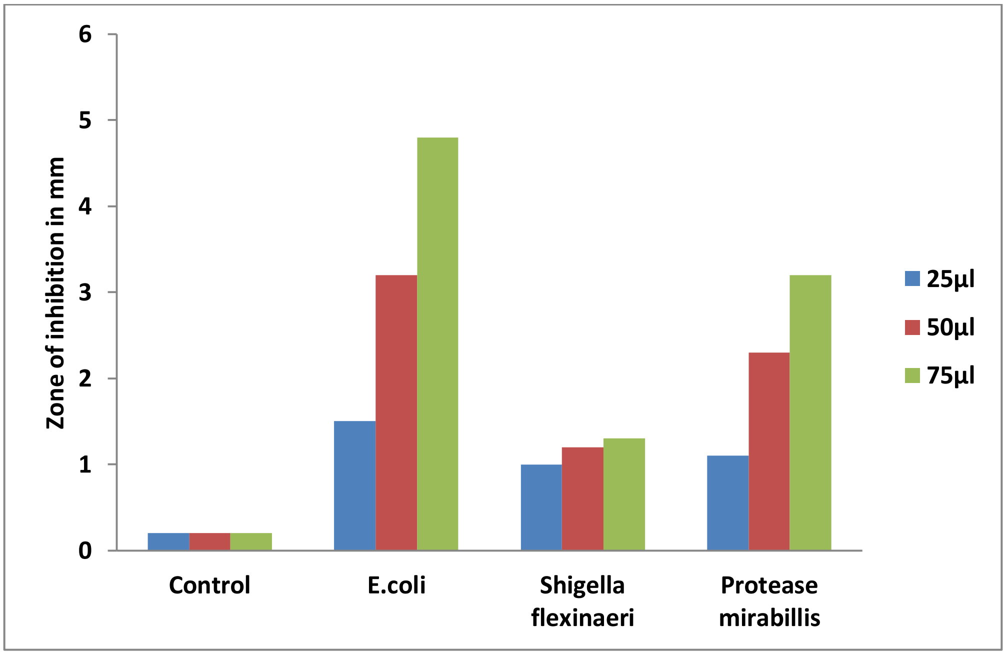


Figure 5: FTIR spectrum of silver nanoparticles

FTIR measurements were carried out to identify the possible biomolecules responsible for reduction, capping and efficient stabilization of the silver nanoparticles (Mervat *et al.*, 2012). The FTIR spectra indicate various functional groups present at different positions. The appearance of peaks in the amide I and amide II regions characteristic of proteins/enzymes that have been found to be responsible for the reduction of metal ions when using the plant extract for the synthesis of silver nanoparticles. The FTIR spectrum obtained for raspberry leaf extract (Fig.6) displays a number of absorption peaks, reflecting its complex nature due to biomolecules. The IR spectrum of silver nanoparticles manifests prominent absorption bands at 3904 cm^{-1}, 3790 cm^{-1}, 3433cm^{-1}, 2924cm^{-1}, 2855 cm^{-1}, 2363 cm^{-1}, 2096 cm^{-1}, 1741 cm^{-1}, 1642 cm^{-1}, 1550 cm^{-1}, 1455cm^{-1}, 1381 cm^{-1}, 1154cm^{-}1,1076 cm^{-1}, 1026cm^{-1}, 934cm^{-1}, 837cm^{-1}, 755cm^{-1} ,671cm^{-1}, 578cm^{-1} and 537 cm−1. The strong band at 3433 cm^{-1} may result from the N-H stretching vibration band of amino groups and indicative bond of –OH hydroxyl group. The well known band at 2924 cm^{-1}, 2855cm^{-1}, 2363cm^{-1}and 2090 cm^{-1} can be assigned as absorption bands of –C=H, -O-H, -S-H, and -N=C=N, stretching vibration respectively. Band at1741 cm^{-1} corresponds to amide I due to carbonyl stretch in proteins. 1550 cm^{-1}, 1455cm^{-1}, 1381 cm^{-1} were aroused for C-N strechinh vibrations of aromatic. Therefore, biosynthesized silver nanoparticles, which have the stronger ability to bind silver metal, so that the proteins could most possibly form a coat covering on the Ag NPs (i.e. capping of Ag NPs) and stabilize the nanoparticles in the medium.

Antibacterial Screening

Synthesized nanoparticles against the following species of gram negative bacteria produced the zone of inhibition (cm) by well diffusion method which was shown in (fig 6). In this study ethanolic extract of *P.graveolens* was used as control. Antibacterial efficacy of 25µl, 50µl and 75µl concentration of silver nanoparticles were effective against the growth of bacterial strains were tested. The highest antibacterial activity was observed against *Escherichia coli* (4.8 cm) at the concentration of 75 µl followed by *Protease mirabilis* (3.2cm); and the least was noticed against *Shigella flexinaeri* (1.3 cm) at the same concentration. The variations in the bactericidal activity of Ag NPs were also due to the different sizes and shapes of Ag NPs produced by various biological systems (Bonde *et al*., 2012).



4. Conclusion

We have demonstrated an eco-friendly, rapid green chemistry approach for the synthesis of silver nanoparticles by using ethanolic extract of *P.graveolens*, which provides a simple, cost effective and efficient way for the synthesis of AgNPs. Since, the biosynthesis involves the utilization of jackfruit seeds which otherwise has no other use and considered as a waste by product. The present study has opened up an innovative way for synthesizing antimicrobial AgNPs using natural products which can be used in various biomedical applications.

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