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Green Synthesis of Ag Nanoparticles by *Helicteris Isora.L* Fruit Extract as a Reducing Agent: Antimicrobial Activity

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Abstract: *The present work describes the phytosynthesis of silver nanoparticles using fruit extract of Helicteris isora.L at ambient conditions. The formation of AgNPs was analyzed by visual observation and UV−Visible spectrophotometer. The synthesized silver nanoparticles were characterized by XRD, FTIR, FE-SEM, DLS and HR-TEM. The surface plasmon resonance spectrum of AgNPs was obtained at 435 nm, X-ray diffraction patterns reveal that the particles are crystalline form with face centered cubic structure and the HR-TEM images showed that the synthesized stable AgNPs are approximately 35-40 nm in size with distorted spherical shapes. The results showed that the fruit extract of Helicteris isora.L is sparingly good bioreductant for the synthesis of silver nanoparticles and synthesized nanoparticles active against clinically isolated human pathogens as E.coli, Pseudomonas aeruginosa, Bacillus subtilis.*

Keywords: *Silver nanoparticles, Helicteris isora.L, Human pathogens, Antibacterial activity.*

1. INTRODUCTION

Nanoscience and technology has attracted the attention of scientists all over the world, especially in the areas of medicine, materials, energy, catalysis, and sensors, because of the striking difference in physical, chemical, and optical characteristics compare to bulk materials [1]. Nanostructured materials showed many aspects of characteristics, i.e., optical, catalytic, that greatly depends on the size and shape of nanoparticles as a concern of quantum confinement of electrons. Metal nanoparticles are extensively used in many electrochemical, electro analytical and bio-electrochemical applications owing to their extraordinary electro catalytic activity, antibacterial activity [2, 3]. Silver nanoparticles (AgNPs) have become the focus of intensive research owing to their wide range of applications in areas such as catalysis, optics, antimicrobials, and biomaterial production. Silver nanoparticles exhibit new or improved properties depending upon their size, morphology, and distribution [4]. The strong toxicity of silver against wide range of microorganisms is well known and silver nanoparticles have been recently shown to be a promising antimicrobial material [3, 5-8]. Silver nanoparticles have anti-inflammatory, antiviral, anti-angiogenesis, and anti-platelet activity and cytotoxicity against cancer cells that makes them vital [9-11].

Synthesis of nanoparticles can be done using a several routinely used chemical and physical methods [12-16]. Among these methods, colloidal metal nanoparticles with various morphologies

are synthesized by the reduction of metal ions using reducing agents. In this method, the addition of a stabilizing agent or capping agent is important because of the fact that synthesized nanoparticles are vulnerable to oxidation in the reaction environment. The chemical sythesis and capping agents of nanoparticles are often toxic, costly, and non-ecofriendly. The need for environmental non-toxic synthetic protocols for the synthesis of silver nanoparticles leads to the developing interest in biological approaches that are free from the use of toxic chemicals as by products. Green synthesis of nanoparticles using plant extracts is the almost adopted method of green, eco-friendly production of nanoparticles and also has a special advantage that the plants are widely distributed, easily available, much safer to handle and usually each cause to stable nanoparticles [17-22].

In this present study, we report the synthesis of silver nanoparticles by the reduction of silver ions using Helicteres isora.L fruit extract. Helicteres isora.L (Family: Sterculiaceae) distributed widely in forests throughout India and commonly known as East Indian screw tree, it is a medicinally important sub-deciduous shrub. The plant materials are extensively used in traditional medicinal importance for curing various diseases [23-30]. In the present study, we synthesized silver nanoparticles by green rout method using Helicteres isora.L fruit extract and studied effect of silver nanoparticles against human pathogens.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

Silver Nitrate ($AgNO_3$) was purchased from Sigma Aldrich Corporation (St. Louis. Mo, USA). Methanol HPLC grade was purchased from MERCK Scientific, India and Luria Bertani (LB) commercial medium was purchased from Himedia Scientific, India, throughout the experiment double distilled water was used. *Helicteris isora.L* fruits were collected from Yogi Vemana University, Botanical garden, Kadapa, India.

2.2. Preparation of *Helicteris Isora.L* Fruit Extract

Helicteris isora.L fruits were collected from Yogi Vemana University, Kadapa, India. The surface of the fruits was cleaned with double distilled water those fruits were dried up to 10 days at room temperature under shade, we used two layered plastic mesh to avoid dust and moisture. 5 grams of dried *Helicteris isora.L* fruits was pulverized with mortar and pestle into fine powder, boiled with 100 ml of deionized water at 80^0 C for 30 minutes under magnetic stirrer with constant stirring. The extract was filtered through Whatman No.1 filter paper. The filtrate was collected and then refrigerated for further use.

2.3. Bio Synthesis of Silver Nanoparticles Using *Helicteris Isora.L* Fruit Extract

Silver nanoparticles are synthesized by adding 10 ml of extract into 100 ml of 1 mM $AgNO_3$ solution at ambient conditions for about 150 min with constant stirring. The formed silver nanoparticles are confirmed by visually (light yellow color to reddish brown). The surface plasmon resonance also confirms formation of silver nanoparticles. Formed silver nanoparticles are separated by using centrifugation at 8000 rpm, 10 min it is repeated at about five times. Each time we washed with double distilled water and finally these particles are again washed with methanol solution and dried for overnight in vacuum oven, separated nanoparticles are stored for further characterization purpose.

2.4. Characterization of Silver Nanoparticles

UV-Visible spectra were recorded as function of reaction time by Shimadzu-UV 1800 spectrophotometer operated at resolution of 1nm. Fourier transform infrared spectroscopy (FTIR) spectrum of the sample was recorded by Perkin-Elmer, Two Model FT- IR spectrometer. The FTIR ranged from 4000 to 400 cm^{-1} at resolution of 4 cm^{-1} by making a KBr pellet with AgNPs. The structure and morphology of the samples were characterized by powder X-ray diffractometry (XRD) Bruker D8 using CuKα1 (1.5406 A^0) and Kα2 (1.54439 A^0) radiations, morphologies of as-obtained products were studied by a Field emission scanning electron microscope (FE-SEM) imaging using a (SUPRA 55) - CARL ZEISS instrument operating at 5 to 20 kV. Structural analyses were conducted by a High resolution Transmission electron microscope (HR-TEM) with

energy dispersive X-ray spectroscopy (EDX) measurements were done on JEOL 3010 instrument operating at 200 kV and particle size analyzed by Malvern Nano S90.

2.5. Antibacterial Activity

Antibacterial activity of biosynthesized silver nanoparticles was evaluated by using standard disc diffusion method [36], *E. coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* was followed for testing positive (Kanamycin) control comparative with AgNPs containing solution. The bacterial suspension of 24-40 hours grown strains was swabbed on Luria Bertani (LB) /agar (10g of LB and 6 g of agar dissolved in 100 ml of double distilled water) media plates using sterile cotton swab. Double sterilized paper disc was placed on Luria Bertani agar media plates. The discs were soaked with kanamycin (30µl/disc) and solution containing silver nanoparticles (10, 20, 30µg) of each rather separately. Then the discs were air dried in sterile condition. Plates containing media as well as culture were divided into three equal parts and previously prepared discs were placed on each part of the plate. Then, the maximum zone of inhibition was observed and measured for analysis against each kind of test microorganism.

3. RESULTS AND DISCUSSIONS

3.1. UV-Visible Analysis of Silver Nanoparticles

UV–visible spectra were recorded for the fruit extract of *Helicteres isora.L* was introduced with 1mM $AgNO_3$ solution at various time intervals as 9, 30, 60, 90, 120, 150 min. UV-visible spectra showed the presence of a surface plasmon resonance band at 435 nm in Fig.1 [31, 32]. It indicates the formation of silver nanoparticles. The possible synthesis mechanism can involve reduction of silver ions to convert toxic Ag^+ to stable Ag^0 and subsequent stabilization of particles using capping agents were present in fruit extract.

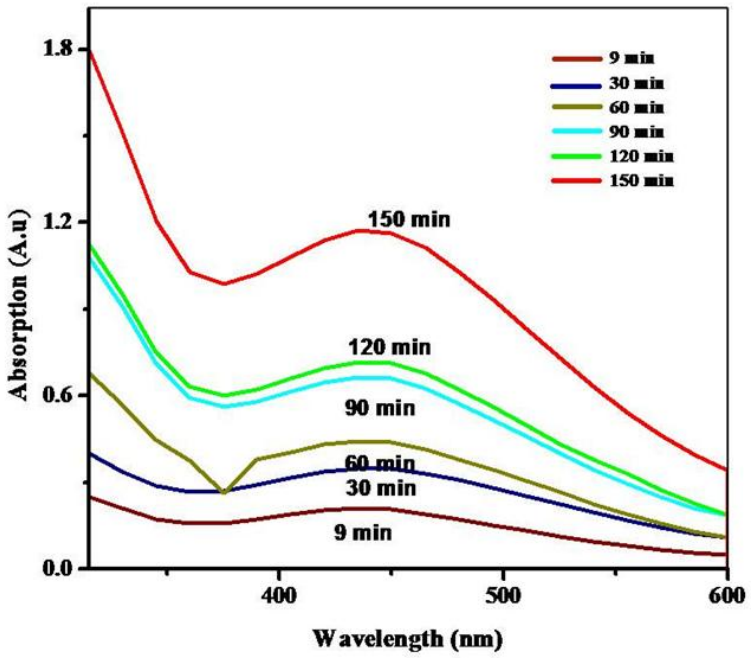

Fig1. *UV-Vis spectra of the Ag nanoparticles prepared with 1mM aqueous $AgNO_3$ solution with Helicteris isora.L fruit extract with different time intervals.*

3.2. FT-IR Spectrum of Silver Nanoparticles

The FT-IR spectroscopy is used to identify the possible biomolecules are responsible for the reduction of Ag^+ ions and capping of the reduced silver nanoparticles from the fruit extract of *Helicteris isora.L.* The IR spectrum of silver nanoparticles showed the distinct peak in the range of 3435.81, 2925.23, 1626.02, 1384.29, 1035.16 and 553.31 cm^{-1}in Fig. 2. Mainly we assigned the broad peak present at 3435.81, 2925.23 cm^{-1} corresponded to N-H, and –OH stretching vibration respectively. The absorption band at 1626.02 cm^{-1} can be attributable to $–NH_2$. The sharp band appearing at 1384.29 cm^{-1} indicates C-N, the absorption peak at 1035.16 cm^{-1} indicates C-O

stretching vibrations. The absorption peak at 553.31 cm^{-1} indicates C-X (Chloroalkanes or Bromoalkanes) stretching vibrations. This indicates that silver nanoparticles synthesized using *Helicteris isora.L* fruit extract are capped proteins having functional groups.

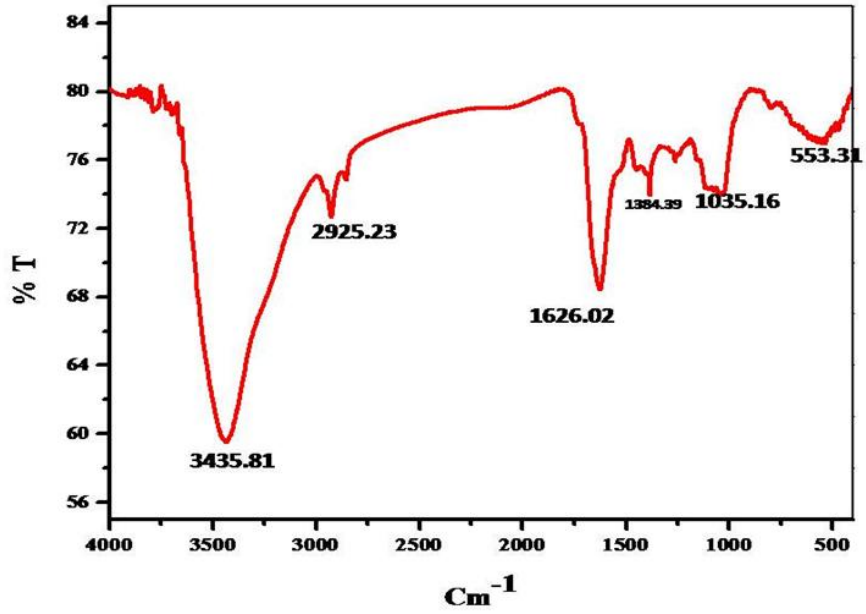

Fig2. *FT-IR spectra of reduced silver nanoparticles.*

3.3. Powder X-Ray Diffraction Analysis of Silver Nanoparticles

The X-ray diffraction studies were done to confirm the crystalline structure of synthesized silver nanoparticles. The XRD pattern showed numbers of Bragg reflections that can be indexed by the face-centered cubic structure of silver in Fig.3. The diffraction peaks at 37.9, 43.9, 64.2, 77.3 and 81.44 corresponding to the (111), (200), (220), (311) and (222) planes (JCPDS file No.03-0921). Some extra peaks were noted that corresponded to plant extract [33].

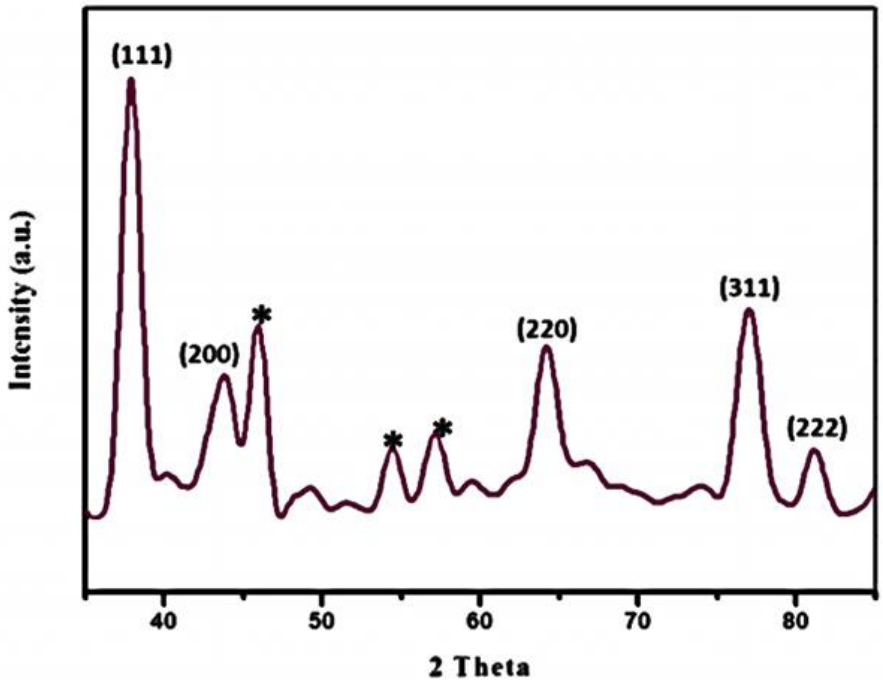

Fig3. *XRD pattern of Synthesized AgNPs by Helecteris isora.L fruit extract.*

3.4. Field Emission Scanning Electron Microscope Analysis of AgNPs

Field emission scanning electron spectroscopy (FE-SEM) shows the size and shape of biosynthesized AgNPs in Fig. 4. Fig. 4 represents spherical shape of AgNPs with particles size

range from 35-40 nm and some of the AgNPs showed large size attributable to the aggregation of poly dispersed AgNPs. Above results suggested that the silver nanoparticles are synthesized attributabe to the action of fruit extract, *Helicteris isora.L*, acts as good bioreductant for biosynthesis. It is also confirms by particle analyzer in Fig 4.

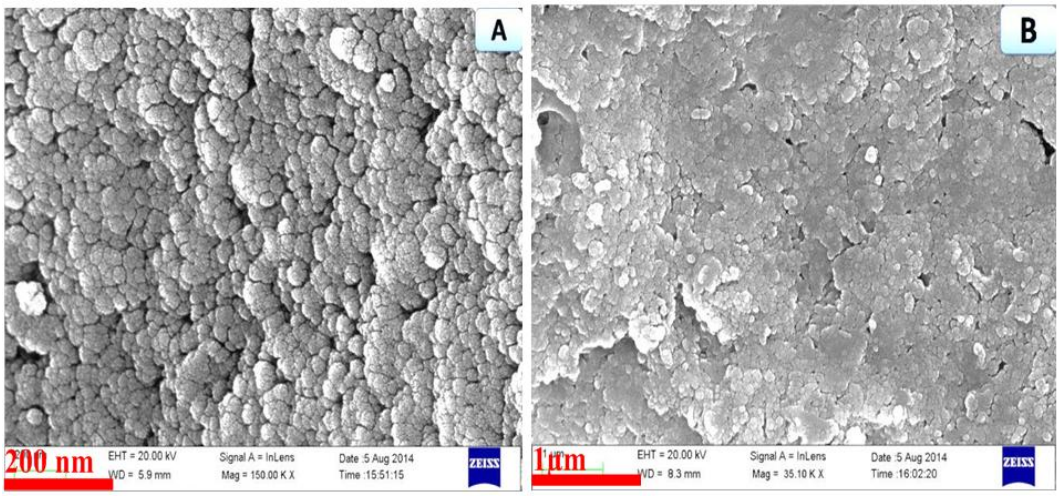

Fig4. *Ag nanoparticles at high magnification (B) Ag nanoparticles at low magnification.*

3.5. Dynamic Light Scattering (DLS) Analysis of AgNPs

In order to determine the particle size distribution of AgNPs in solution, the qualitative DLS size distribution image of AgNPs is shown in Fig.5. The size of synthesized AgNPs range from 35 to 40 nm and average diameter of the silver nanoparticles were found as 38 nm.

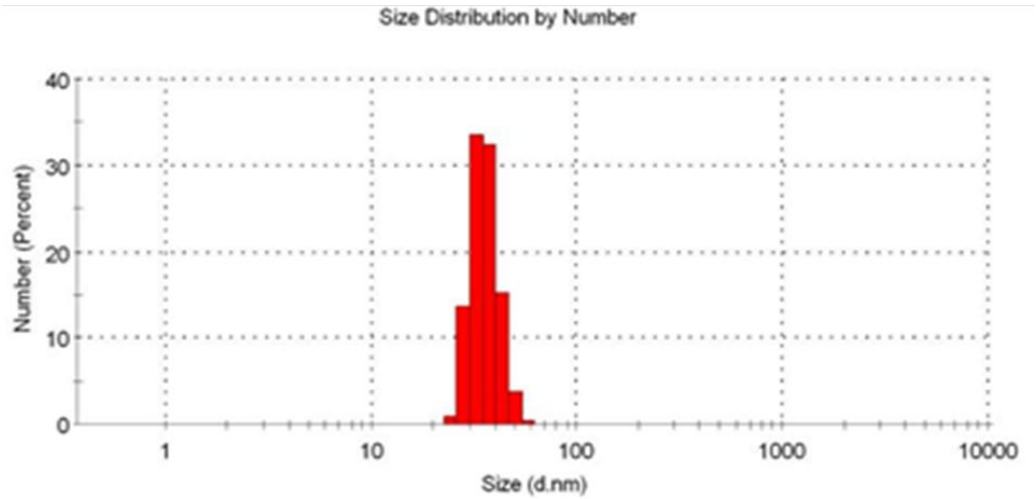

Fig5. *Particle size distribution analysis.*

3.6. High Resolution Transmission Electron Microscope Analysis of AgNPs

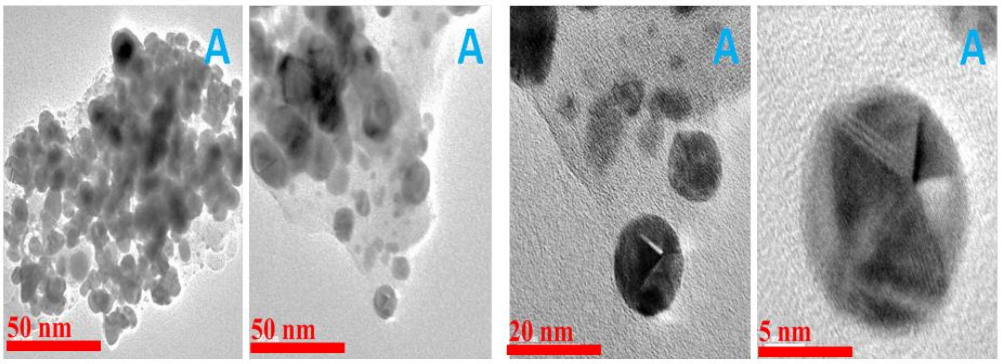

Fig6. *(A) HR-TEM images of silver nanoparticles at different resolutions*

The morphology of biosynthesized AgNPs was showed by HR-TEM images in Fig. 6. Most of the AgNPs shape spherical with size 35-40 nm and also shows little bit agglomeration in the Fig. 6. The HR-TEM images showed what appears to be a layer of organic material surrounding the synthesized AgNPs are used as capping agent [34, 35]. Moreover, the size of the AgNPs was around 38 nm with spherical shapes. The EDX spectra recorded from the silver nanoparticles are shown in Fig.7 and the elements are framed in Table.1. The EDX spectra shows a strong silver signal along with carbon peak may originate from bio molecules bind to the surface of the silver nanoparticles, copper peak is originate from copper grid, chlorine is appeared in less amount it is came because of water.

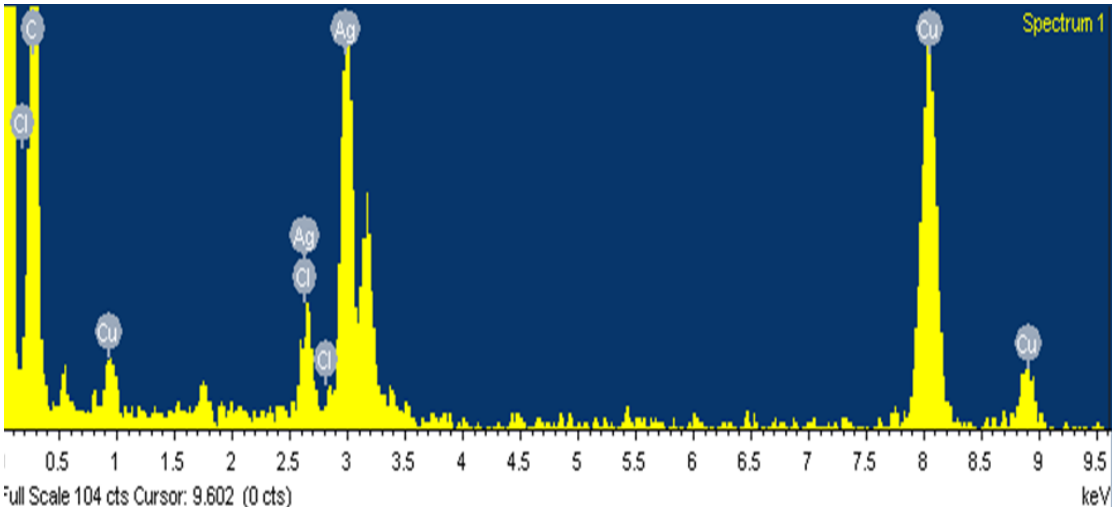

Fig7. *EDX data of Silver nanoparticles.*

Table1. *Elemental composition of AgNPs from HR-TEM with EDX*

Element	Weight (%)	Atomic (%)
1. Ag	46.23	15.92
2. C	20.36	62.96
3. Cu	29.98	17.53
4. Cl	3.43	3.59

3.7. Estimation of Anti-bacterial activity

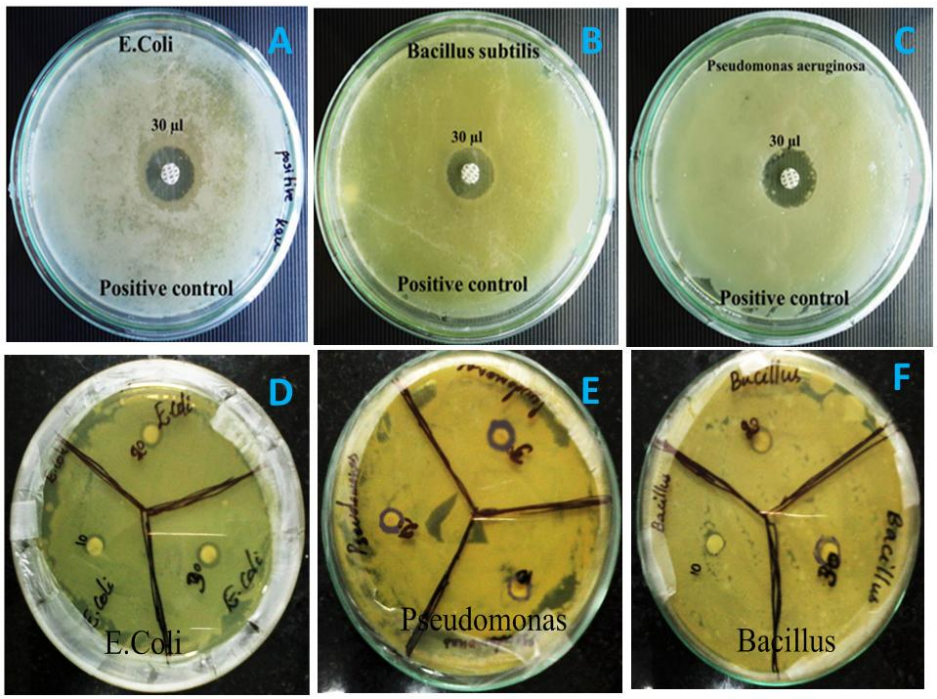

Fig8. *Images of antibacterial activities of discs of different concentration of AgNPs and antibiotics (A,B,C) Positive control(D) E.coli (E) Pseudomonas aeruginosa (F) Bacillus subtilis.*

The antibacterial activity of the commercial drug (kanamycin) and AgNPs was examined against *E.coli, Pseudomonas aeruginosa* and *Bacillus subtilis* bacteria by disc diffusion test. Luria Bertani (LB) /agar were used to cultivate bacteria. Positive control (Kanamycin) is maintained inhibition in separate agar plate; Fig.8 shows the inhibition zones of different bacterial cultures and represents the inhibition zones are obtained maximum antibacterial activity of AgNPs in Table 2. The zone of bacterial inhibition by AgNPs prepared from *Helicteris isora.L* fruit extract show maximum inhibition for *E. coli*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*, which can be concluded from the fact that these particles had the smallest diameter, which in turn exhibited equal antimicrobial property. It is concluded that the synthesized silver nanoparticle shows good antimicrobial property at low concentrations compare with commercial drug Kanamycin.

Table2. *Influence of bio synthesized AgNPs by using Helicteris isora.L fruit extract against human pathogens.*

Ag NPs Concentration	Pathogenic bacteria (Mean of three replicates)	Zone of diameter in mm
30 µl	Positive control (kanamycin)	±20.5
(10, 20, 30 µl)	Ecoli	± 16
(10, 20, 30 µl)	Pseudomonas aeruginosa	±15.5
(10, 20, 30 µl)	Bacillus subtilis	±16

4. CONCLUSIONS

In this study, we described eco-friendly and convenient green method for the synthesis of silver nanoparticles using *Helicteris isora.L* fruit extract in ambient conditions and characterization of synthesized AgNPs was carried out by UV-Visible spectroscopy, XRD, FT-IR, FE-SEM, and HR-TEM. These AgNPs showed characteristic surface plasmon resonance at 435 nm. The XRD pattern showed Bragg reflections that may be indexed by the face-centered cubic structure of AgNPs. The HR-TEM images showed that the synthesized stable AgNPs are approximately 35−40 nm in size with spherical and poly dispersed shapes. The synthesized AgNPs have excellent antibacterial activity against resistant human pathogens. We concluded that the synthesis method we used is almost straight, nontoxic and eco-friendly method compare to chemical and physical methods. The Green synthesized nanoparticles are useful in various biomedical and catalysis applications.

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