Research Article

Synthesis, Characterization of Silver Nanoparticles using *Rosa damascena* Hips Extract and Antibacterial, Anti-cancer Activities of the Synthesized Nanoparticles.

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ABSTRACT

Silver nanoparticles were synthesized from *Rosa damascene* hips and its in vitro antimicrobial activity was tried against human pathogenic bacteria in this study. It was accomplished by using plant hips extract as a reducing agent and Ag^+ to AgOas stabilizing agent. Characterizations of the synthesized silver nanoparticles were performed by UV-Vis spectrometer, FT-IR (Fourier Transform Infrared Spectroscopy), SEM-EDAX (Scanning Electron Microscopy-Energy Dispersive Analysis X-ray Spectroscopy) and TEM (Tunneling Electron Microscopy). The UV–visible spectrum clearly revealed the formation of silver nanoparticles on an intense peak due to surface plasmon resonance band at~440 nm and other impurity peaks. The synthesized silver nanoparticle structure and morphology was of the particles was confirmed by SEM. The nanoparticles of produced were of spherical, triangle to in shapes with 10 to 30 nm diameter. The EDAX confirmed the silver nanoparticle and their nanoparticles dispersion at 1.9keV. The silver nanoparticles colloidal solution was tested for their antibacterial activity against five different strains of bacteria *Klebsiella pneumonia (-), Pseudomonas aeroginosa (-), Escherichia coli (-), Bacillus cereus (+) and Staphylococcus aureus (+)*. The different concentration of silver nanoparticles was analyzed on MCF-7 cell. The cell was performed by SRB assay. Syntheses of silver nanoparticles form plant materials is a comparatively rapid and less expensive method and the nanoparticles formed were effective to the antibacterial and anti-cancer therapy in modern medicine.

Keywords: *Rosa damascena,* silver nanoparticles, antibacterial and anti-cancer activities.

INTRODUCTION

Nanotechnology is playing effective role in revolution of biological systems, nano chemistry and medicinal field.[1] Nano biotechnology is well-defined for the nano scale techniques to understand and transform bio system.[2]The noble metals exhibit new physico-chemical properties when brought to nanoparticle size. The size, shape and surface morphologies play an important role in controlling the physical, chemical, optical, and electronic properties of these nanoscopic materials.[3,4] The metal noble nanoparticles Au and Ag have strong surface plasmon resonance fluctuations.[5,6] In biosynthesis of metal nanoparticles from growth and development of clean, nontoxic chemicals, environmentally benign solvents and renewable materials the focus is turned on to green chemistry and bioprocesses now.[7] In recent years metal nanoparticles have been studied extensively for their attractive properties such as electronic, optical, optoelectronics, catalysis, nanostructure fabrication and chemical sensing related to the quantum size effect.[8]

The environmentally friendly conversions of various chemical reagents play an important role in the chemical industries and pharmaceuticals. The importance of the catalytic processes is increasing in recent years.[9, 10] Silver nanoparticles can be synthesized by several physical, chemical and biological methods. Metal nanoparticles are highly effective heterogeneous catalysts; due to their unique properties of electronic and extremely large specific surface areas.[11]The silver nanoparticles have several effective applications as antibacterial, sensors and detectors.[12] Their biomedical applications are attractive physio-chemical properties. The use of metallic silver nitrate and silver nanoparticles are exploited in several fields like medicinal and textile. These are in medicinal field for low toxicity to biological cells, low volatility, high thermal stability, burn treatment, dental materials, coating stainless steel materials, sunscreen lotions, water treatment and textile fabrics.[13,14]

Biosynthetic process is very useful for the development of metal nanoparticles. The plant extracts are useful for the synthesis of nanoparticles by this method without any chemical ingredients. Bio molecules and various plant extracts such as *Azardirachta indica,*[15] *Tamarindus india,*[16] *Pelargonium graveolens,*[17] *Cinnamomum camphora,*[18] *Partheniumleaf,*[19] *Aloe vera,*[20] *Acanthella elongate,*[21] *Coriandrum sativum,*[22] are sources of different types of nanoparticles. Extracts of leaves from *Daucuscarota*, *Solanum lycopersicums*, *Hibiscus cannabinus*, *Murraya koenigii*, *Macrotyl omauniflorum*, *Neem*, *Geranium* and *Anana scomosus* have been found the suitable for the green synthesis of silver nanoparticles,[23-29] Chemical and physical methods has proved successful in produced is well-defined and effective silver nanoparticles. These processes are usually overpriced and very useful of the toxic chemicals. Several biological systems including bacteria, fungi and algae have been used for this purpose,[30,31] It's applications in diverse fields such as drug delivery, biosensors, bio-imaging, antimicrobial activity, food preservation have been reported. The different

types of living organisms are well-known to explain the nanostructured composites such as cyanobacteria, bacteria and fungi.[32-34] In this present study, an attempt is made to synthesis silver nanoparticles using the aqueous extract of *Rosa damascene* hips and its characterization using UV–Visible, FT-IR, SEM, EDAX and TEM. The antimicrobial activity was studied by using agar well diffusion method.[35] The evaluated its anticancer activity against human breast cancer with help of MCF-7 cell. The process variables such as concentration of extract, mixing ratio of reactants, silver salt concentration and interaction time were analyzed.[36, 37]

Materials and Methods

Rosa damascene hips were collected from Department of Horticulture, SHUATS, campus, Allahabad, India. The *Rosa damascene* hips were thoroughly washed. All chemicals used in this present study were of all analytical grade. $AgNO_3$ purchased from (SDFC Limited, Mumbai, India) and deionized water was obtained from (LAB CHEM, Scientific agencies and general supplier, Allahabad, India). The different bacterial strains were collected from culture bank; and experimental work also done in department of microbiology, SHUATS, Allahabad, India). For this experimental works purpose used Agar powder, Peptone, Beaf extract purchased from (HIMEDIA Laboratories Pvt. Ltd, Mumbai, India) and sodium chloride (MERCK Limited, Mumbai, India).

Preparation of hips extract

Rosa damascene hips were collected and dried in shade at room temperature and made into fine powder with the help of laboratory grinder. 4gm of powder was taken in 100ml of deionized water and boiled for 5 to 10 minutes. Finally 70 to 80 ml fresh aqueous extract of *Rosa damascene* hips were filtered with the help of Whatman filter paper No.1 and centrifuged at 10,000 rpm for 30 minutes for removing heavy metal impurities.

Synthesis of silver nanoparticles

Rosa damascene hips extract was treated with 10^{-3}mM $AgNO_3$ solution. 90ml of $AgNO_3$ solution was added to 10ml of aqueous solution and was mixed properly with help of rotary shaker. It was boiled for 10 minutes and covered with aluminum foil. The extract was kept in dark place to cool and stored in incubator for further studies. The solution turned from yellowish to brown color within 30 to 45 minutes. The appearance of brown color indicates the presence of silver nanoparticles in the presence of figure 1(B).[38] This solution was tested by UV-Visible spectroscopy for evaluating the nanoparticles and the same solution was used for further characterization and applications.

Characterization of silver nanoparticles

Synthesized silver nanoparticles were confirmed by UV-Visible spectrophotometer (SL-164, Elico).

Figure 1: (A).Image of *Rosa damascena* hips, (B) Changing color intensity from yellow to brown of synthesized silver nanoparticles from extract of *Rosa damascena* hips

Strong peak appeared at wavelength 440 nm. This solution was tested by Fourier Transform Infrared Spectroscopy (FT-IR), (Varian-1000, USA). Silver nanoparticles morphology, sizes and shape were analyzed using Scanning Electron Microscope and EDAX analysis was also done to confirm the elemental composition of the sample (SEM-EDAX, JSM-6510, JEOL/EO, VOLT 15, MAG 20000, SPOT_SIZE 30, USIF, AMU) and confirmed the silver nanoparticle and their nanoparticles dispersion at 1.9keV. The sample was coated on copper grid and the silver nanoparticles were studied using Transmission Electron Microscopy (TEM, JEOL JEM-2100F operated at high voltage of 200.0kV).

Antibacterial Assay

The synthesized silver nanoparticles were tested for antibacterial activity on pathogenic bacteria by agar well diffusion method. Petri plates were washed with distilled water and dried at room temperature. Freshly prepared agar media was poured into petri plates and swabbed. These plates were kept in Laminar air flow chamber for solidification. Four wells made in the petri plates with 6 mm diameter a sterile cork borer that was already seeded with check organisms. Every micro-organism was swabbed uniformly on nutrient agar plates using sterile pin. In first well extract, in second silver nanoparticle, in third one standard sample of Ciprofloxacin and in fourth well silver nitrate was poured and observed for the inhibition zone after 24h at room temperature. After 24h incubation was evaluated for antimicrobial activity and it was measured in diameter (in millimeters). The sample incubation zone was evaluated with the standard controls. The research was checked in triplicates.

Anti-cancer

The synthesis of silver nanoparticles was tested for anti-cancer activity against on MCF-7 cancer cell, the different concentrations with SRB assay. The results obtained are represented in table 1. The workers were documented the potential of this medicinal plant for future development of therapeutic drugs against breast cancer[39-40].

Table 1: The activity of silver nanoparticles in different concentration incubation at 37 °C for 1h.

Experiment	25%	50%	75%	100%
Experiment 1	87.9	80.5	75.2	75.5
Experiment 2	87.6	82.8	74.2	75.3
Experiment 3	81.8	76.6	74.1	75.2
Average Values	85.8	80.0	74.5	75.3

RESULTS AND DISCUSSION

UV–visible spectra analysis

The synthesis of nanoparticles was initiated once the hips extract deionized water of *Rosa damascena* was introduced into 1mM aqueous $AgNO_3$ solution. Silver nitrate solution was added to the aqueous extracts of *Rosa damascena and* silver nanoparticles were synthesized. The sample suspension showed silver nanoparticles by UV–Visible spectral analysis. After 24h of AgNPs formation, brown precipitate was observed. However, in all the extract concentrations, Ag NPs precipitated. The observed brown color solution showed silver nanoparticles ranges between 300 and 600 nm and it shows in fig.2. The absorption spectrum was observed at 440nm wavelength. It might be due to the excitation of surface plasmon resonance effect and reduction of $AgNO_3$. This sample was checked again after 24h and same peak appeared at same wavelength. The color change of solution from light yellowish to brown color is due to the reduced silver metal ions (Ag^+) into silver ions (AgO). The color change occurred of the presence of active molecules in the extract due to the excitation of surface plasmon resonance formed silver nanoparticles [Fig. 2 (A)].

FTIR analysis

Fourier Transform Infrared Spectroscopy (FT-IR) was done using a ranging from 4000 to 450cm^{-1}, 5ml of sample using small amount with KBr reagent. During FT-IR analysis strong peaks of *Rosa damascena* hips at 3734.79, 3648.53, 1698.04, 1541.14, 660.48, 608.96, 565.70, 542.31 samples were observed [Fig. 2 (B)].

Transmission Electron Microscope (TEM)

The synthesized silver nanoparticles colloidal solution (6ml) was centrifuged twice at 10,000 rpm for 30 min and the precipitation was part collected under the test tube. The resulting pellets were used for TEM analysis. For this analysis few drops of suspension sample were placed on a carbon coated copper grid. And it was kept into hot air oven for drying the sample at 60 to 80 °C for 4 h. Transmission Electron Microscope analysis was performed for the sample suspension.

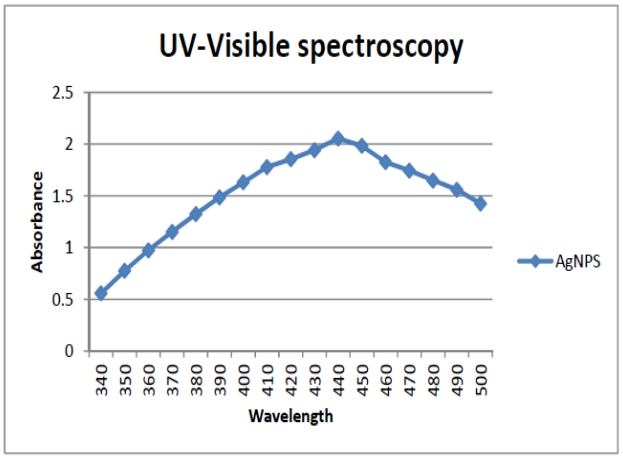


Figure 2: Characterization of synthesized silver nanoparticles using *Rosa damascena* hips extract **(A)** UV spectra

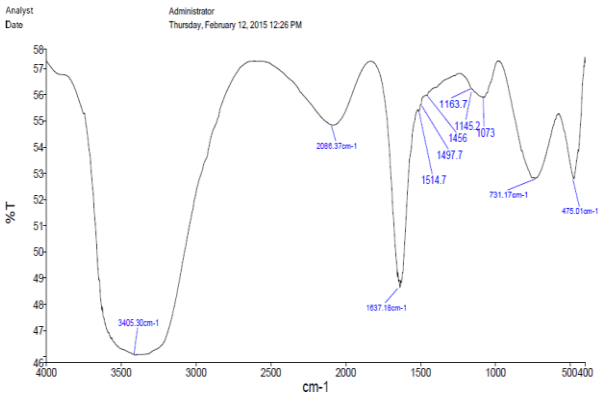


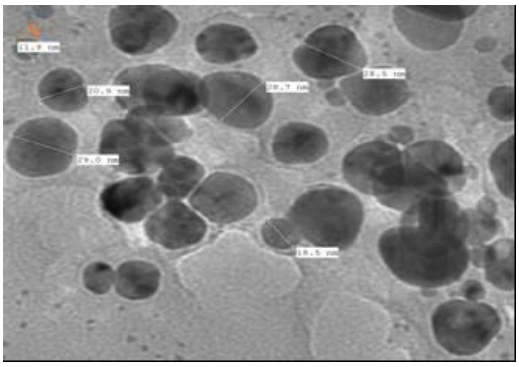
Figure 2: Characterization of synthesized silver nanoparticles using *Rosa damascena* hips extract **(B)** FTIR Spectra

The TEM of the silver nanoparticles clearly exhibited the morphology of the particles being spherical and oval in shape. The synthesized silver nanoparticles size ranges from 18 to 30nm. The uniform shapes acquired by the silver nanoparticle explain that there was no secondary nucleation in the synthetic process [Fig. 3 (A)].

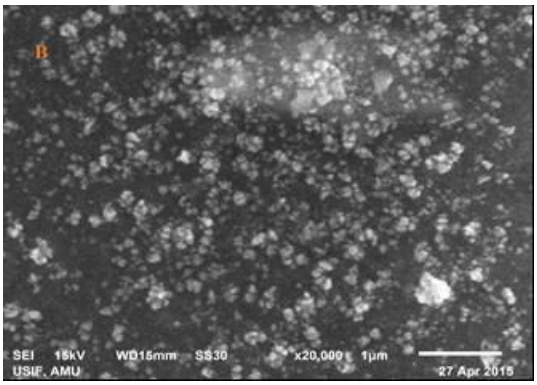
SEM

Scanning Electron Microscopic (SEM) analysis was performed using the VOLT 15, MAG 20000, JEOL/EO JSM-6510 and Japan. The silver nanoparticles observed were predominantly spherical, square and uniform in size. A small percentage of resulting nanoparticles were spherical and in the size of 30 nm. The thin sample film was prepared on a copper grid carbon coated. When the dropping a small amount of sample on the copper grid and dry. After drying the sample copper grid and excess of solution was removed with the help of blotting paper. The film on the SEM grid was then allowed to dry by putting the grids under a mercury lamp for 5 min and they showed representative SEM images were recorded at different magnifications from drop-coated films of the silver nanoparticles synthesized by treating silver nitrate solution with *Rosa damascena* hips. The SEM analysis of Ag nanoparticles from *Rosa damascena* showed different

shapes and sizes of nanoparticles increased by high concentrations of *Rosa damascena* hips extract [Fig. 3 (B)].



(A)



(B)

Figure 3: Characterization of synthesized silver nanoparticles using *Rosa damascena* hips extract **(A)** High resolution transmission electron microscopic image **(B)** Scanning electron microscopic

EDAX

The particles were isolated by centrifuging 20 ml of suspension in deionized water containing Ag nanoparticles for 30 min at 10,000 rpm two times. The suspension solution was sent for scanning electron microscopy with EDAX instrument. The extract reduced silver nanoparticles were dried and drop coated on carbon film. The EDAX spectrum showed silver nanoparticles synthesized following in figure 6. Silver nanoparticles generally showed the typical absorption peak approximately at 1.9keV in [fig 4]. The presence of the elemental silver nanoparticles of *Rosa damascena* hips was observed in the graph obtained from EDAX analysis. This result indicates the reduction of silver ions to elemental silver. The synthesized nanoparticles were stable for 6 months at room temperature. Due to the surface plasmon resonance of the metallic silver nano crystals shows typical optical absorption peak approximately at 1.9keV peak.

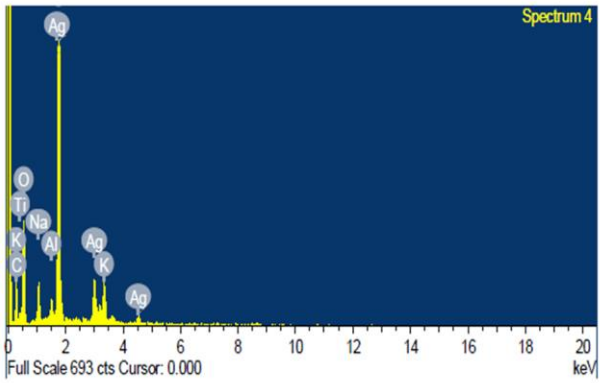


Figure 4: EDAX image of synthesized silver nanoparticles using extract of *Rosa damascena* hips.

Antimicrobial activity

The nanoparticles synthesized by green route were found harmful against 5 bacterial species with concentration of silver nanoparticles. It revealed high antibacterial activity against *Enterococcus faecium (+)* showed highest activity, than *Pseudomonas aeroginosa* (-) and *Escherichia coli* (-). *Bacillus cereus* (+), and *Salmonella aureus* (+) bacteria showed very less activity compare with antibiotic, silver nitrate and extract. The antibacterial activity of phyto-synthesized silver nanoparticles was investigated against these micro-organisms. The silver nanoparticles also exhibited antibacterial activity against all gram positive and gram-negative bacteria and formed the zone of inhibition of *Rosa damascena* silver nanoparticles showed more antimicrobial activity on *Klebsiella pneumonia (-), Pseudomonas aeroginosa (-), Escherichia coli (-), Bacillus cereus (+) and Staphylococcus aureus (+)* other bacterial also in,[39] [Table.2 and Fig.5].

Table 2: The antibacterial activity of metallic silver nanoparticles synthesized by using hips extracts of *Rosa damascena* plant

Name of the microorganisms	Antibiotic (mm)	$AgNO_3$(mm)	Nanoparticles (mm)	Extract (mm)
Enterococcus faecium (+)	14.5±0.408	14.2±0.110	13.333±0.235	0
Pseudomonas aeroginosa (-)	14.833±0.235	14.833±0.235	10.833±0.288	0
Escherichia coli (-)	13.333±0.288	12.333±0.288	12.333±0.235	0
Bacillus cereus (+)	111.23±0.251	11.23±0.251	10.13±0.230	0
Salmonella aureus (+)	10.10±0.793	10.10±0.793	9.555±0.322	0

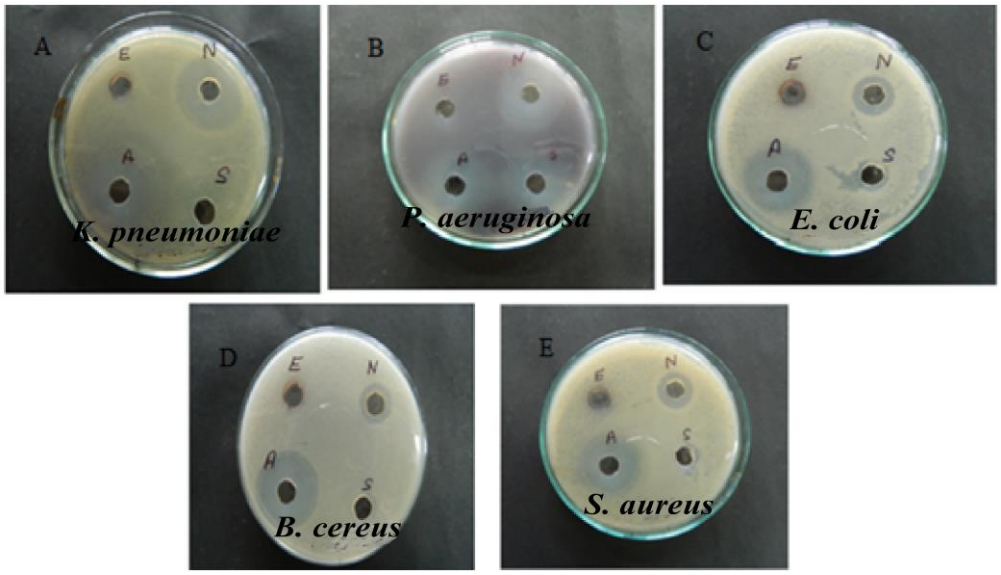


Figure 5: (A) *Enterococcus faecium,* (B) *Pseudomonas aeroginosa,* (C) *Escherichia coli,* (D) *Bacillus cereus and* (E). *Salmonella aureus*

Anti-cancer

The in vitro anticancer effect of *Rosa damascene* hips silver nanoparticles was tested on MCF-7 cell lines. A dose dependent decrease in the viability results of MCF-7 cells was observed on treatment with silver nanoparticles, as shown in [Figure 6 and 7]. The half maximum inhibitory concentration (IC50) was observed at 75% µg/mL. At this concentration, the silver nanoparticles inhibited the growth of MCF-7 cells by 50%, table 3. The silver nanoparticles were found to exhibit anticancer activity against human breast cancer. However, several possible mechanisms for anticancer activity of silver nanoparticles can be proposed. In addition, the presence of pharmacologically active anticancer compound which includes flavonoids such as quercet in, etc., in the hips of *Rosa damascena* could also be one of the reasons for the silver nanoparticles to exhibit anticancer activity. Further, studies are needed to unravel the exact mechanism behind the anticancer efficacy of *Rosa damascena* silver nanoparticles[41-42].

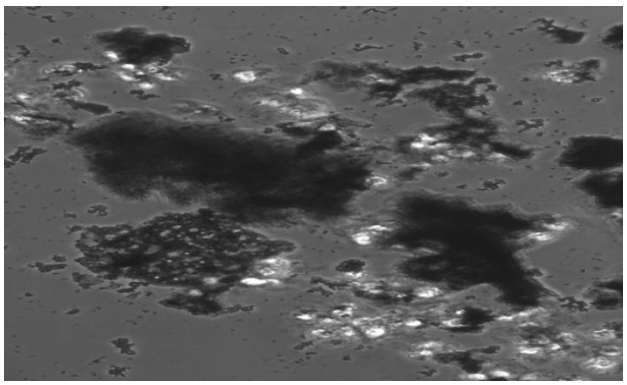


Figure 6: In vitro anticancer effect of *Rosa damascena* silver nanoparticles on MCF-7 cell proliferation SRB assay. Fifty percents inhibition of cells was observed at 75%µg/mL silver nanoparticles concentration.

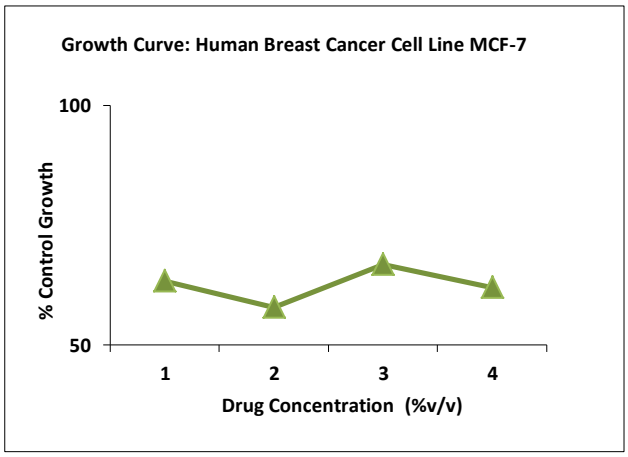


Figure 7: In this Graph, Cell proliferation assay using MTT for analyzing dose dependent effect of silver nanoparticles on MCF-7 cells for 24 h.

CONCLUSIONS

The silver nanoparticles of average size have been synthesized using dried hips of *Rosa damascena* plant. The characterizations from UV–Vis, SEM, EDAX and TEM analysis were documented of the stability of biosynthesized silver nanoparticles. The FTIR spectra indicate that the functional groups (CHO, CO, COOH, and OH) may be responsible for the reduction and stabilizing of silver nanoparticles. The structural analysis was confirmed by the SEM and TEM. The biosynthesized silver nanoparticles were characterized; they are crystalline, spherical and uniform and mono dispersed nanoparticles with average particle size 11 to 30nm. To conclude, we have demonstrated that silver nanoparticles were rapidly synthesized using aqueous extracts of *Rosa damascena* within the first minute of the reaction at room temperature. The chemical and physical methods were useful for developing a biological process for very high production. The silver nanoparticles showed excellent

antimicrobial activity against these five different micro-organisms. The biosynthesis approach appears a cost-effective, nontoxic and eco-friendly alternative to the conventional microbiological. In effluent treatment process for reducing the microbial load, these silver nanoparticles were used. Various process parameters involved in the green synthesis were analyzed for the better yield of silver nanoparticles were found to be biocompatible. The silver nanoparticles exhibited inhibitory effect against MCF-7 cell line.

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