BIOMIMETIC SYNTHESIS AND CHARACTERIZATION OF SILVER 
NANOPARTICLES (Ag-NPs) USING *Emblica officinalis* 
(GOOSEBERRY) PLANT AQUEOUS EXTRACT AND THEIR ANTI-
BACTERIAL ACTIVITY AGAINST MUTI DRUG RESISTANT HUMAN 
PATHOGENS

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ABSTRACT

Discoveries in the past decade in the field of medicine along with Nano-Engineering principles explored a various new dimensions which attributed significantly in various ways. Currently there is a growing need for biomimetic synthesis of nanoparticles by using biological sources without using any harmful chemicals plays a very important role. The present study deals with the synthesis of silver nanoparticles by treating silver nitrate with aqueous extract of *Emblica officinalis* (Gooseberry) plant at room temperature and its effect on formation of AgNPs. They were subjected for to various characterization techniques to analyse their physical and chemical prosperities like size, shape, crystallinity, fractal dimensions, pore size & surface area were done. Finally biopotentiality of synthesized AgNPs was done against selected gram positive and gram negative bacterial strains and their zone of inhibition was measured & recorded. The present findings may be recommended for production of antibiotics & can be applied as antimicrobial agent.

1. INTRODUCTION

Nanotechnology is an important field which plays a remarkable role in solving many problems faced by humanity. Nanoscience has been used as a strategy and manipulation of particles with nano-size objects with least toxicity, effective & eco-friendly which possess new mode of action to cater rising emergency.\(^1\) The prefix ‘Nano’ indicates one billionth or \(\times 10^{-9}\) nm units. It is widely agreed that now, it is possible to synthesize nanomaterials by using biological system with the help of plants, micro-organisms either by top-down or bottom-up strategies.\(^2\)

Nanotechnology provides a good platform to modify & develop the important properties of synthesized nanoparticles which having promising applications as an anti-microbial agents as well as unique Physical and Chemical properties.\(^3\-6\) Now-a-Days synthesized AgNPs offers numerous benefits on Pharmaceutical and Bio-Medical applications as they do not use any toxic chemicals for Synthesis protocol.\(^7\)

Plants produce wide array of bioactive molecules or phytochemicals which are found to be useful for the treatment of various ailments. The knowledge of extractability also serves as a tool for quality control of plant drug. At present development of reliable green chemistry route to synthesis nanoparticles specifically in biology and medicine being carried out for wide applications.\(^8\) In biological methods of synthesis nanoparticles would assist to remove ruthless processing conditions, by allowing physiological conditions like pH, Temperature, Pressure and at same time it is at negligible cost.\(^9\)

The medicinal properties of silver have been known for over 2000 years. Silver based productions of biogenic metallic nanoparticles are non-toxic to humans and most effective against bacteria, virus and other eukaryotic micro-organisms at low concentrations without causing any side effects.\(^10\) Moreover, there are many ways depicted in various literatures to synthesize silver nanoparticles to employ biological methodsof using microbes and plants.\(^11\-12\)

To date, green synthesis and its various biological applications in literature are immense. Therefore, the present study focuses on biomimetic synthesis of AgNPs from aqueous extract of *E.officinalis* (Gooseberry) plant, Characterization and further their anti-microbial capability was done with suitably employed in numerous combinations.
2. MATERIALS AND METHODS

2.1. Extract Preparation from E.officinalis

Fresh and healthy leaves of E.officinalis (Fig 1) collected locally near to the college campus and washed with tap water followed by de-ionised water and air dried. Leaves are chopped into fine pieces & 10 grams of leaves were measured and transferred to 250 ml Conical flask containing 100 ml of distilled water. Boil the contents for 5 minutes at 60°C & cool them. Filter the extract thrice by using Whatmann No. 1 filter paper. The filtrate is collected & stored at 4°C, which were used for further work.

![Fig. 1: Emblica officinalis (Gooseberry) plant.](image)

2.2. Biogenic synthesis of AgNPs

The aqueous solution of Silver Nitrate at concentration of 1mM was prepared to synthesize biomimetic AgNPs. In details 10 ml of aqueous extract obtained as above was added to 90 ml of 1mM AgNO₃ solution was added. Keep the content at room temperature for reaction time and change of colour was monitored.

2.3. Characterization Techniques of Silver Nanoparticles

Characterization of synthesized AgNPs is very important to understand their Physical & Chemical properties as well as their applications. It is performed by using a variety of techniques such as UV-Vis Spectroscopy, Scanning Electron Microscopy (SEM), Powder X-ray Diffractometry (XRD), Particle Size Distribution by Intensity and ZETA Potential analysis.[13] These techniques are used for analyzing various Physical & Chemical properties such as Particle Size, Shape, Crystallinity, Fractional dimensions, Pore Size & Surface Area.
2.4. Antimicrobial Assay

2.4.1. Test Organisms
Clinical isolates were collected from Bioline laboratory in Coimbatore, Tamil Nadu, India. Bacterial isolates includes Five Gram-positive Strains (Staphylococcus sps., Streptococcus aureus, α – Haemolytic Streptococcus sps., β - Haemolytic Streptococcus sps., Bacillus sps., Streptococcus haemolyticus) and Five Gram-Negative Strains (Enterococcus faecalis, Klebsiella pneumonia, Pseudomonas aruginosa, Proteus mirabilis, Escherichia coli). These test organisms were maintained and stored at -20°C.

2.4.2. Antimicrobial Activity Test
Antimicrobial ability of efficiency of biomimetic synthesized AgNPs of E.officinalis (Gooseberry) Plant aqueous extract was analysed by Disc Diffusion Method.[14] The above mentioned clinical isolates were inoculated in Nutrient agar Media & incubated at 37°C for 24 hrs. After incubation, they were inoculated on Muller Hinton agar (MHA) under aseptic conditions. In each of these plates Five Antibiotic Discs (i.e., Plant Extract (Control), Antibiotic discs (Standard), Pure Silver solution (1mM), Synthesized Ag-NPs of E.officinalis (Gooseberry) (Experimental disc) and Synthesized Ag-NPs of E.officinalis (Gooseberry) combined with Standard antibiotic disc) was added & maintained at room temperature for 24 hrs under light. After 24 hrs the zone of inhibition was measured & recorded.

3. RESULTS AND DISCUSSION

3.1. Visual observation
The colour of the reaction mixture changed from dark reddish brown to colloidal brown after 24 hrs incubation. It is well known that silver nanoparticles exhibit dark brown colour in water due to extinction of Surface Plasmon Vibration in metal nanoparticles.[15] Control (without silver nitrate) shows no colour change, The colour change in the aqueous extract with silver nitrate solution which may be due the presence of bioactive compounds in aqueous extract like Phyllanthin & Hypo-phyllanthin compounds are responsible for the reduction of silver nitrate to silver nanoparticles. The different type of antioxidants & various phyto-chemicals are responsible for the reduction of silver ions. Similar observations were reported by Debabrata et al.[16-17]
3.2. UV-Visible Spectral Analysis

Reduction of silver ions into silver nanoparticles during exposure to plant extracts was observed as a result of the colour change. The metal nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. The sharp bands of silver nanoparticles were observed around 421 nm in case of *Emblica officinalis* (Gooseberry). From different literatures it was found that the silver nanoparticles showed SPR peak at around 420 nm. From our studies we found the SPR peak for *Emblica officinalis* at 421 nm confirmed that *Emblica officinalis* leaf extract has more potential to reduce Ag ions into Ag nanoparticles. The intensity of absorption peak increases with increasing time period. This characteristic colour variation is due to the excitation of the SPR in the metal nanoparticles. The reduction of the metal ions occurs fairly rapidly; more than 90% of reduction of Ag\(^+\) ions is complete within 24 Hrs. After addition of the metal ions to the plant extract. The metal particles were observed to be stable in solution even 4 weeks after their synthesis.

3.3. SEM Analysis

SEM technique was employed to visualize the size and shape of silver nanoparticles. SEM images were obtained with 10% of *E.officinalis* leaf broth. The SEM (JEOL-MODEL 6390)
used SEM grids which were prepared by placing a small amount of sample powder on a copper coated grid and drying under lamp. The formation of silver nanoparticles as well as their morphological dimensions in the SEM study demonstrated that the average size was from 35–55 nm with inter-particle distance. The shapes of the silver nanoparticles proved to be spherical. Similar phenomenon was reported by Chandran et al.\textsuperscript{[18]}

![Fig.4 SEM Results of Gooseberry AgNPs.](image)

3.4. EDAX Analysis
In this present study, the element analysis of the biomimetic synthesized AgNPs was performed using EDX spectrum (Fig.5). The EDX spectrum of spherical in shape with high aggregation of silver nanoparticles on the surface of the cell prepared with this bioreduction method using \textit{E.officinalis} (Gooseberry) plant extract shown maximum peaks around 3.28 keV correspond to binding energies of silver ions. Energy dispersive spectrometry (EDS) micro-analysis is performed by measuring the energy and intensity distribution of X-ray signals generated by a focused electron beam on a specimen. EDX spectra were recorded from the silver nanoparticles. From EDX spectra, it is clear that silver nanoparticles reduced by \textit{E.officinalis} the weight percentage of silver as 79.09% and 0.64%.

![Fig-5: EDX results of synthesized AgNPs, \textit{E.officinalis} (Gooseberry).](image)
3.5. XRD Analysis

XRD is commonly used for determining the chemical composition and crystal structure of a material; therefore, detecting the presence of silver nanoparticles in plants tissues can be achieved by using XRD to examine the diffraction peaks of the plant. In our experiment the X-ray pattern of synthesized silver nanoparticles matches the FCC structure of the bulk silver with the broad peaks at 32.3, 27.9, 46.3 and 38.2. These are corresponding to 100, 139, 131 and 124 planes, respectively. In addition to the Bragg peak representative of FCC silver nanocrystals, additional and yet unassigned peaks were also observed suggesting that the crystallization of bio-organic phase occurs on the surface of the silver nanoparticles. The line broadening of the peaks is primarily due to small particle size. The X-ray diffraction results clearly show that the silver nanoparticles formed by the reduction of Ag⁺ ions by the *E. officinalis* leaf extract are crystalline in nature.

![XRD Results of Gooseberry AgNPs](image)

**Fig. 6 XRD Results of Gooseberry AgNPs.**

3.6. Particle Size Distribution Analysis by Intensity

Total concentration of AgNPs synthesized by *E. officinalis* was found to be $5.61 \times 10^9$ particles / ml. The size of AgNPs analysed shows the Z range average values of about 137.1 d.nm., with particle distribution rate of about 0.173 / ml. The size of the synthesized AgNPs is about 10.05 d.nm and its width is about 6.106 d.nm. Distributions of particle size/concentration of AgNPs were shown in **Fig. 7.**

![Particle size distribution by intensity](image)

**Fig. 7. Showing the results of Particle size distribution by intensity.**
3.7. ZETA Potential Analysis
To see whether the synthesized AgNPs was stable or not the ZETA Potential of these AgNPs was measured. The results revealed (Fig 7) that the ZETA Potential (mV) of synthesized AgNPs was -21.5 at pH 7, which indicates that the nanoparticles are stable and their conductivity value is about 0.274 mS/cm.

![ZETA Potential Distribution](image)

**Fig. 8. Showing the results of ZETA Potential.**

ANTI - BACTERIAL ACTIVITY
The antibacterial activity was carried out using five different Gram Positive and Gram Negative Bacterial strains as mentioned above. Zone of inhibition in the plate showed that silver nanoparticles synthesized by using aqueous leaf extract of *E. officinalis* (Gooseberry) have the antibacterial activity against test pathogens as mentioned above. Zone of inhibition was measured and compared with Five replicates like Plant Extract (Control), Ampicillin (Standard), Pure Silver solution (1mM), Synthesized Ag-NPs of *L. inermis* (Henna) (Experimental disc) and Synthesized Ag-NPs of *L. inermis* (Henna) combined with Standard antibiotic disc to confirm the inhibition zone. On comparison with the silver nitrate and plant extracts silver nanoparticles out performed in the bactericidal effect.

In Gram Negative Bacterial Strains the highest antibacterial effect were observed in *Enterococcus faecalis* & *Pseudomonas aruginosa* were found with zone of inhibition (17 mm) and lowest antibacterial effect in Ag-NPs on *Escherichia coli* (16 mm).
Fig-9 (i): ANTI BACTERIAL ACTIVITY OF Emblica officinalis (GOOSEBERRY) AGAINST GRAM POSITIVE (A) & GRAM NEGATIVE (B) STRAINS.

Table -9 (ii):- Measurement of Zone of Inhibition (Mm) of synthesized AgNP, of E.officinalis (Gooseberry) Plant against Gram- Positive Bacterial Strains.

<table>
<thead>
<tr>
<th>TEST ORGANISMS (GRAM POSITIVE STRAINS)</th>
<th>ZONE OF INHIBITION (MM)</th>
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<tbody>
<tr>
<td></td>
<td>Ampicillin (Std.)</td>
</tr>
<tr>
<td>Staphylococcus sp.</td>
<td>7.5</td>
</tr>
<tr>
<td>Streptococcus aureus</td>
<td>6</td>
</tr>
<tr>
<td>Baillus sp.</td>
<td>8</td>
</tr>
<tr>
<td>α - Haemolytic Streptococcus sp.</td>
<td>8.5</td>
</tr>
<tr>
<td>β - Haemolytic Streptococcus sp.</td>
<td>6</td>
</tr>
<tr>
<td>Streptococcus haemolyticus</td>
<td>9</td>
</tr>
</tbody>
</table>

Table-1 (ii):- Measurement of Zone of Inhibition (Mm) of synthesized AgNP, of E.officinalis (Gooseberry) Plant against Gram- Negative Bacterial Strains.

<table>
<thead>
<tr>
<th>TEST ORGANISMS (GRAM NEGATIVE STRAINS)</th>
<th>ZONE OF INHIBITION (MM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ampicillin (Std.)</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>7</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>9</td>
</tr>
<tr>
<td>Pseudomonas aruginosa</td>
<td>9</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>9</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>8</td>
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In eukaryotic cells the accumulated AgNPs gets interacted with cell membranes and reacts with its cellular metabolites and these reactions may occur inside the mitochondria, where exists an important concentration of H⁺. Similarly, we hypothesized that a similar mechanism could occur in the bacterial cell membrane where proton motive force takes place. Evidently report on another possible mechanism for the oxidative dissolution of silver nanoparticles has been proved by Choi et al., The additional generation of free radicals can attack membrane lipids and leads to the breakdown of cell membrane and mitochondrial function and also cause DNA damage.

4. CONCLUSION

The silver nanoparticles have been produced by E.officinalis extracts, which is an economical, efficient and eco-friendly process and the characterization techniques viz., UV–Visible spectrophotometer, SEM, EDX, XRD, ZETA Potential and Particle Size distribution analysis have confirmed that the reduction of silver nitrate to silver nanoparticles. The zones of inhibition were formed in the antimicrobial screening test indicated, that the Ag NPs synthesized in this process has the efficient antimicrobial activity against pathogenic bacteria.
The biologically synthesized silver nanoparticles could be of immense use in medical field for their efficient antimicrobial function.

5. REFERENCES


