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# SYNTHESIS, CHARACTERIZATION, ANTIBACTERIAL AND CYTOTOXIC STUDIES OF SILVER NANOPARTICLES USING AQUEOUS EXTRACTS OF *CRATAEVA NURVALA* LEAVES - A GREEN NANO-BIOTECHNOLOGICAL APPROACH

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## ABSTRACT

Silver nanoparticle has attracted considerable interest due to its potential applications such as in display technologies, thermoelectric and electronic devices, optoelectronic devices and biomedicine. Metallic nanoparticles are traditionally synthesized by wet chemical techniques, where the chemicals used are quite often toxic and flammable. In the present study, we describe a cost effective and eco-friendly technique for green synthesis of silver nanoparticles from 1mM AgNO3 solution using the extract of *Crataeva nurvala* leaves as reducing as well as capping agent. Nanoparticles were characterized using UV–Vis absorption spectroscopy, FTIR, and SEM. SEM analysis revealed spherical nanoparticles, the nanoparticles were studied for its antimicrobial and cytotoxic effect on MCF-7, a human breast cancer cell line.

**KEYWORDS:** Silver nanoparticle, Green synthesis, UV –Vis absorption spectroscopy, Scanning Electron Microscope (SEM), MTT assay.

## 1. INTRODUCTION

Nanotechnology is a multidisciplinary field, which has wide range of information derived from engineering, biology, physics and chemistry. Therefore, nanotechnology in general refers to building materials, devices and machines at nanoscale dimensions. Nanotechnology

has vast applications in diverse fields like energy storage, agricultural productivity, healthcare, waste water treatment, drug delivery systems, diagnosis, food processing etc.

Silver nanoparticles have applications in many areas, including biomedical, materials science, and catalysis. Its application in medical field is mainly due to its antimicrobial property. It is used in silver based dressings, in drug delivery, in the form of nanogels, nanolotions, etc. [1] Reports suggest that silver nanoparticles can be effectively used against multi-drug-resistant bacteria because of their small size and relatively large surface area making them the new generation of antimicrobials. [2]

In the recent years green synthesis has gained popularity due to its numerous advantages. Green synthesis provides advancement over chemical and physical methods as it is cost effective, environment friendly, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals. [3] Green synthesis involves use of plants, microbes or polysaccharides. Silver nanoparticles have been reported to be synthesized from various herbal plants viz *Piper longum* [4], *Piper nigram* [5] and *Plumeria rubra*. [6]

Here in, we report for the first time synthesis of silver nanoparticles, reducing the silver ions present in the solution of silver nitrate by the aqueous extract of *Crataeva nurvala* leaves. *Crataeva nurvala* is a medicinal tree whose leaves and roots are used for treating wounds, inflammation, loss of appetite, abdominal pain, liver disorders, worm infestation, dysuria, pain in urinary tract, urinary tract infection, fever and general weakness. [7] Nanoparticles biosynthesized from these leaves were found to be highly toxic towards different human pathogenic bacteria and also cancer cells.

## 2. MATERIALS AND METHODS

Silver nitrate powder (Hi-Pure) was purchased from Fine Chem Industries, Chennai. *Crataeva nurvala* leaves were purchased from a local drug store, Chennai, Tamilnadu, India.

**2.1 Preparation of aqueous silver nitrate solution:** 1mM silver nitrate solution was prepared and stored in a dark bottle at room temperature.

**2.2 Preparation of leaf extract:** 10g of *Crataeva nurvala* leaves (Fig1) were washed several times with deionised water. The leaves were cut finely, homogenized with the help of mortar

and pestle, boiled in distilled water for 10 minutes and filtered. The filtrate (Fig2) was allowed to cool down to room temperature and then stored at $4^0$C for further experimentation.

**Fig1: Leaves of *Crataeva nurvala***


**Fig2: Leaf extract**

### 2.3 Optimization and Synthesis of silver nanoparticles

Different volumes of leaf extract (1ml, 3ml, and 5ml) were added to 40ml of 1mM silver nitrate solution. This 3 different mixture solutions were exposed to different conditions like room temperature, sunlight radiation, UV radiation, and several short burst of microwave irradiation in a domestic microwave oven in a cyclic mode (on 15s, off 15s) to prevent overheating as well as aggregation of metals. The mixture solutions were maintained at the aforementioned conditions until a colour change was observed. The colour change was due to the bioreduction of silver ions to silver nanoparticles.

### 2.4 Recovery of silver nanoparticles

After bioreduction, the solution containing silver nanoparticles was centrifuged at 10,000 rpm for 20 minutes. The pellet was dried in hot air oven and the powdered form of silver nanoparticles was stored for experimental analysis. The powdered nanoparticles were weighed to obtain the yield per 100ml (Table 1).

### 2.5 Characterization of the biosynthesized AgNPs

The colour change is due the optical property of nanoparticles called surface plasmon resonance. Sample solution was scanned using UV-Vis Double Beam Spectrophotometer Systronics 220 between the wavelength 300 and 800nm to obtain the maximum absorbance or the surface plasmon resonance peak value. This value is used to determine the presence of AgNPs in the solution. SEM study was done to know the size and shape of the nanoparticles biosynthesized. SEM analysis was done using FEI Quanta 200 SEM machine at IITM, Chennai. A thin film of the sample was obtained by loading a small amount of the dried

sample on a circular metallic sample holder and the sample was covered with a thin layer of gold by sputter coating. Then the film was allowed to dry and the images of nanoparticles were taken. The dried silver nanoparticles were subjected to FTIR analysis by Potassium Bromide pellet (FTIR grade) method in 1: 100 ratios and spectrum was recorded in Perkin Elmer Spectrum1 FTIR Spectrophotometer. Peaks in FTIR spectra gives the possible organic and inorganic molecules that responsible for reducing silver ions to silver nanoparticles.

**2.6 Antibacterial assay**

The biosynthesized AgNPs were tested for antibacterial activity by agar well diffusion method against human pathogenic bacteria *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli and Klebsiella pneumonia*. Each bacterial culture was grown in nutrient broth for 24 h. Each overnight grown culture (100µl) was spread onto a sterilized plate containing nutrient agar by spread plate method. Wells were made on the nutrient agar plates using a micropipette. 20µl of the sample of nanoparticle solution was poured onto wells on all plates. 20µl distilled water added in the other well of each plate as a control (C). All the plates were incubated at $37^0$ for 24 hours. Zone of inhibition was measured on the next day (Table 2).

**2.7 Invitro Assay of Cytotoxic Activity (MTT assay)**

The assay was carried out at at Biozone, Chennai, India. The cell line used was MCF-7, a breast cancer cell line.

The MTT assay [8] is based on the ability of live but not dead cells to reduce a yellow tetrazolium dye to a purple formazan product. Cells were maintained in DMEM medium, supplemented with 10% Fetal Bovine Serum, at $37^oC$ in humidified atmosphere with 5% $CO_2$. The cells were plated in 96 well flat bottom tissue culture plates at a density of approximately 1.2 X $10^4$ cells/well and allowed to attach overnight at $37^oC$. The medium was then discarded and cells were incubated with different concentrations of the extract for 24 hours. After the incubation, medium was discarded and 100µl fresh medium was added with 10µl of MTT (5mg/ml). After 4 hours, the medium was discarded and 100µl of DMSO was added to dissolve the formazan crystals. Then, the absorbance was read at 570nm in a microtitre plate reader. The assay was performed for all 10 fractions obtained from column chromatography

Cell survival was calculated by the following formula:

Viability % = (Test OD/ Control OD) X 100

Cytotoxicity % = 100 – Viability%

## 3. RESULTS AND DISCUSSIONS

### 3.1 Observation of colour change

It is well known that silver nanoparticles exhibit dark brown or yellowish brown color in aqueous solution due to the surface plasmon resonance phenomenon. [3] As the *Crataeva nurvala* leaf extract was mixed in the silver nitrate solution, the colour started to change from yellow to dark brown and then to yellowish brown (Fig 3) due to the reduction of silver ions to silver nanoparticles. The yellowish brown colour remained unchanged for several days. Among various concentrations and methods used, sunlight irradiation method was very effective and 3ml of leaf extract had shown maximum yield of nanoparticles (Table 1).

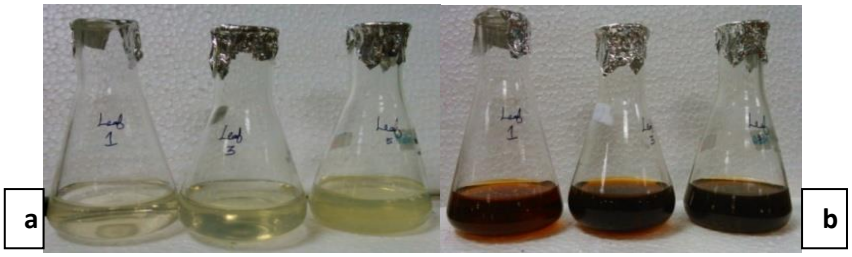


**Fig. 3 a. Sample before exposure to sunlight b. Sample after exposure to sunlight**

**Table 1: Yield of AgNPs obtained at varying conditions and concentrations**

| Condition | Volume of leaf extract (ml) | Time taken for colour change | Yield (mg/100ml) |
|---|---|---|---|
| Sunlight | 1 | 5 min | 26 |
| Sunlight | 3 | 3 min | 37 |
| Sunlight | 5 | 3 min | 30 |
| Room temperature | 1 | 3 hours | 10 |
| Room temperature | 3 | 1 hour | 22 |
| Room temperature | 5 | 1 hour | 20 |
| Microwave | 1 | 60 sec | 10 |
| Microwave | 3 | 60 sec | 11 |
| Microwave | 5 | 45 sec | 10 |

### 3.2 UV-Vis absorption spectroscopy

Figure 4 shows the UV-Vis spectra recorded from the reaction medium after 2 hours. Absorption spectra of silver nanoparticles formed in the reaction media has absorbance peak

at 444 nm. Absorption bands in the range 425-475 correspond to spherical AgNPs of fine nature [9] thus proving the synthesised particles are silver nanoparticles.

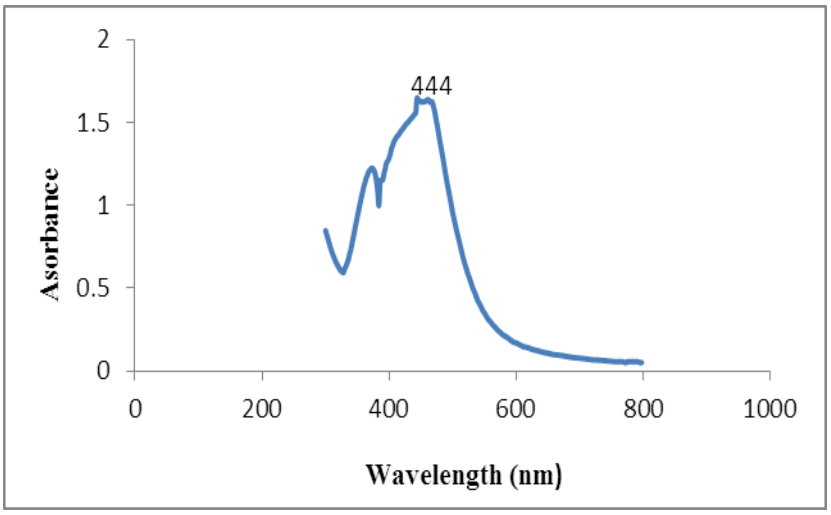


**Fig 4: UV spectra of silver nanoparticles synthesized**

### 3.3 Scanning Electron Microscopy

Morphology of the synthesized nanoparticles can be studied from SEM analysis. Fig 5 shows that the particle size ranges between 32 and 38 nm. It also reveals the spherical structure of the biosynthesized AgNPs.

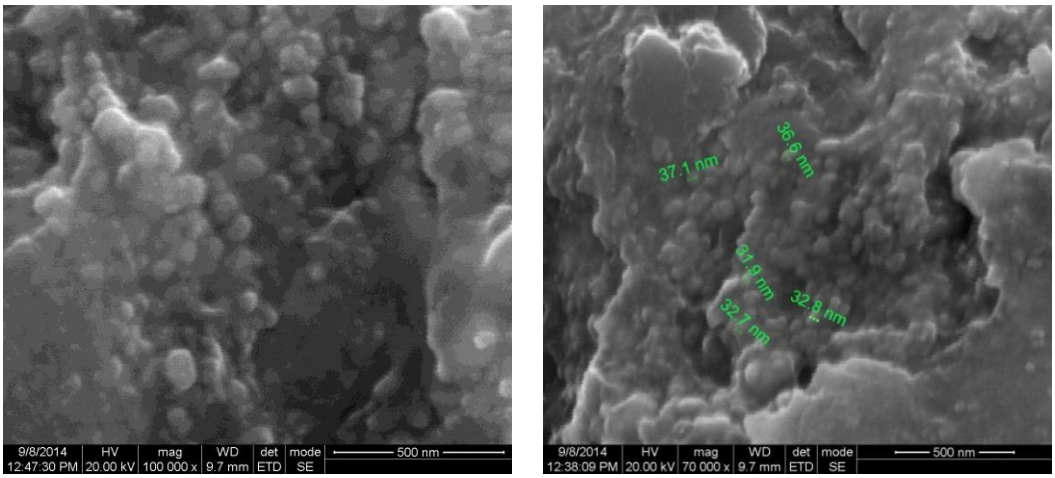


**Fig 5: SEM image of biosynthesized AgNPs**

### 3.4 Fourier Transform Infra red Spectroscopy analysis (FTIR)

FTIR absorption spectra of AgNPs produced by the *Crataeva* extract are shown in the figure 6, bands are observed at 3430, 2920, 2360, 1640, 1380, 1100 and 598 cm-$^{1}$. These bands were compared with the FTIR spectra of $AgNO_3$ and extract alone. The bands at 3430 and 2920 cm-$^{1}$ are broadened in the extract alone, but the narrow band in the AgNPs showed that

reduction of silver ions in NPs. The band at 1640 cm-$^{1}$ showed the N-H bonding vibrate of amides indicated the involvement of amides from the plant extract in the reduction of AgNPs. The shift of the band in plant extract at 1422 to 1380 cm-$^{1}$ after the bio reduction of AgNPs indicated the C=C stretching mode in the aromatic compounds which confirmed the aromatic compounds like quercetin, kaempferol in the leaf extract are responsible for the reduction of AgNPs.

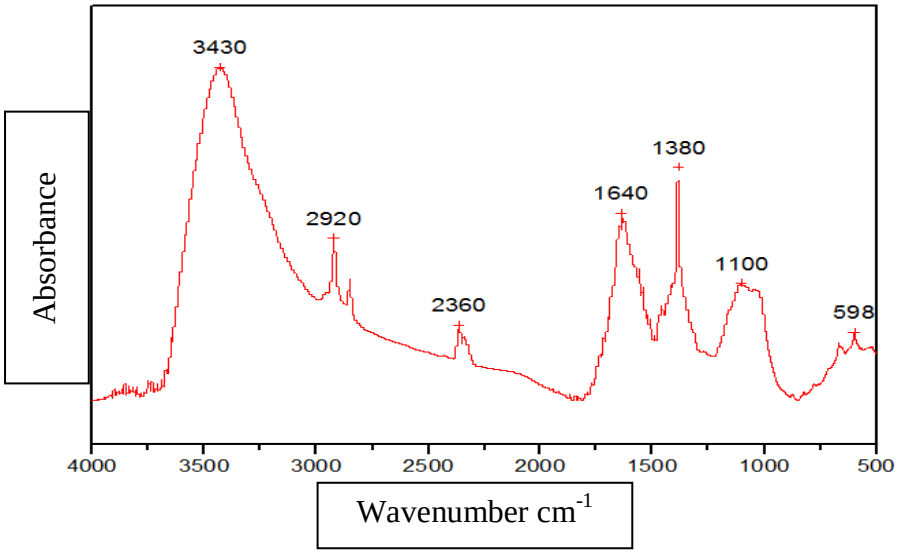


**Fig 6: FTIR spectrum of the AgNps synthesiszed**

**3.5 Antibacterial assay:** The bactericidal effect of AgNPs causes the zone of clearance as shown in figure 7. Zone of clearance formed against *Staphylococcus aureus* is smaller in comparison to the other bacteria (Table 2). This is because Gram positive bacteria possess a thick peptidoglycan layer in its cell wall which makes it difficult for the nanoparticles to penetrate whereas the peptidoglycan layer of Gram negative bacteria is thin allowing easy penetration of the nanoparticles and causing destruction to bacterial cells. [10] In the case of *Escherichia coli* maximum inhibition was observed.

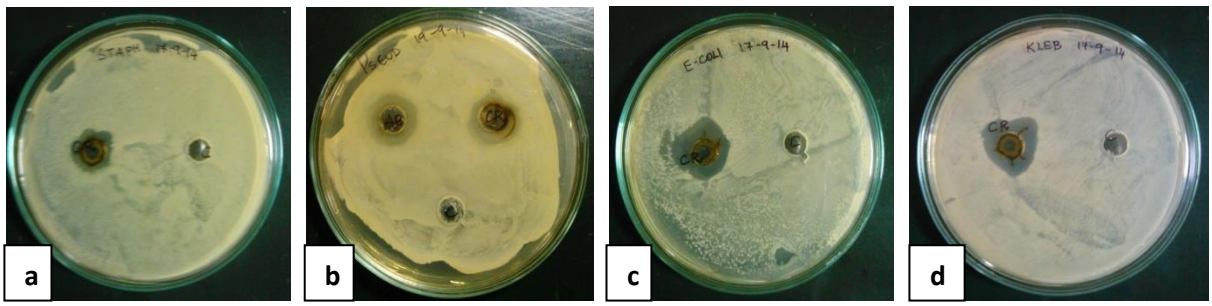


**Fig 7: Zone of inhibition observed against (a). Staphylococcus aureus (b). Pseudomonas aeruginosa (c). Escherichia coli and (d) Klebsiella pneumonia**

**Table 2: Measurement of zone of inhibition formed against human pathogenic bacteria**

| S.No | Microorganisms | Zone of inhibition (in mm) |
|---|---|---|
| 1 | Escherichia coli | 6 |
| 2 | Klebsiella pneumonia | 5 |
| 3 | Staphylococcus aureus | 2 |
| 4 | Pseudomonas aeruginosa | 4 |

### 3.6 MTT Assay

Silver nanoparticles synthesized using *C.nurvala* leaves were tested for its cytotoxic effect using MTT (3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide) on MCF-7, a breast cancer cell line (Fig 7a). Biosynthesized AgNps of varying concentrations in cell lines were tested after 24 hrs of incubation at 37°c in 5% CO2. Morphological changes (Fig 7b). in the cell line as well as a significant cytotoxic effect of IC50 at 265.579µg of AgNps (Table 3) were observed. Aromatic compounds like quercetin, kaempferol contribute anticancer property to the leaves. Since FTIR result of biosynthesized AgNPs shows the presence of aromatic compounds, it can be assumed that anticancer property of the AgNP is also may be due to the same aromatic compounds viz quercitin or kaempferol. [7]

**Table 4: Cytotoxicity effect of *A.exclesa* mediated AgNPs on MCF-7 cell line**

| Concentration (µg/ml) | A at 570nm | A at 570nm | Average | SD | % of viability | % of toxicity | IC50 value |
|---|---|---|---|---|---|---|---|
| 250µg | 1.063 | 1.062 | 1.0625 | 0.000707 | 59.994 | 40.00 | 695.657 |
| 500µg | 0.986 | 0.985 | 0.9855 | 0.000707 | 55.64 | 44.35 | |
| 750µg | 0.881 | 0.88 | 0.8805 | 0.000707 | 49.71 | 60.28 | |

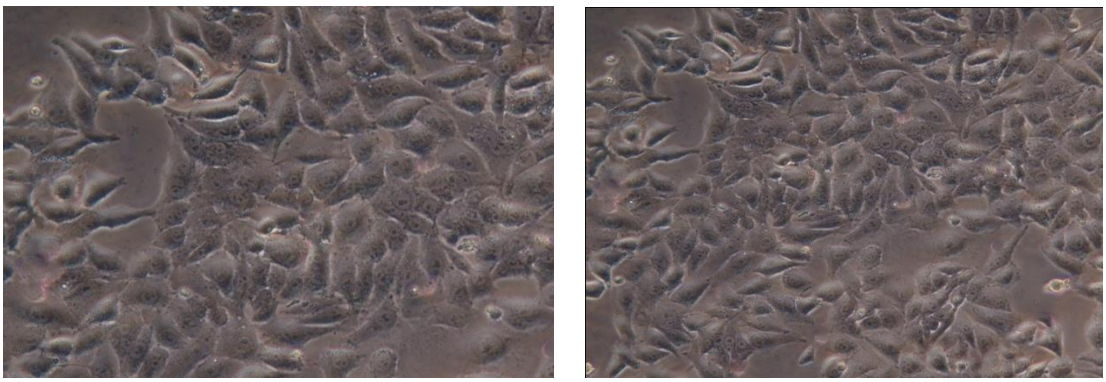

**Fig 7 a). MCF-7 Cell Line- Control b). Cell line after treated with AgNps.**

## 4. CONCLUSION

In conclusion, silver nanoparticles synthesized using the leaves of medicinal tree *Crataeva nurvala* is simple, rapid, economical and ecofriendly. Plant mediated synthesis has advantages such as faster rate of synthesis, highly stable and biocompatible nanoparticles. Biocompatability of the nanoparticles is due to the surface capping with natural polymers like plant metabolites present in the leaf extract. Biosynthesized AgNP were characterized using SEM, UV-VIS and FTIR spectroscopic techniques. SEM analysis revealed fairly well dimensioned nanoparticles. The AgNPs showed excellent antibacterial and anticancer effect, which could be due to the same compounds that confer antibacterial and anticancer effect to the leaves such as quercetin and kaempferol. Antimicrobial and cytotoxic property of the biosynthesized nanoparticles has made them a potential candidate for medical applications.

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