GREEN SYNTHESIS AND CHARACTERISATION OF SILVER NANOPARTICLES FROM ANNONA RETICULATA AND ANALYSIS FOR BIOACTIVE COMPOUNDS

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ABSTRACT

Nanoparticle biosynthesis is an essential part of nanotechnology and plays a crucial role in the science of materials. In this research, an aqueous extract of Annona reticulata L was used to synthesise Silver Nanoparticles. UV-Visible spectroscopy, Fourier transform infra-red spectroscopy (FT-IR), X-ray diffraction (XRD), Energy-dispersive x-ray spectroscopy (EDX), particle size analysis of silver nanoparticles, Zeta potential evaluation and scanning electron microscopy (SEM) have characterised the synthesised silver nanoparticles. Standard procedures have been carried out for phytochemical analysis of carbohydrates, proteins and amino acids, alkaloids, flavonoids, phenols, steroids, terpenoids, tannins, saponins and glycosides and for quantitative phytochemical analysis of flavonoids, total phenolic content and tannins. The existence of silver nanoparticles from the aqueous extract of this species was disclosed in the characterization study. From this research, it can be indicated that several phyto-components and the synthesis of nanoparticles are available to the species as an environmentally friendly method. A lower-cost, simple and environmentally friendly alternative to chemical and physical processes is this rapid biosynthesis method.

Keywords: Biosynthesis, Silver nanoparticles, Characterization analysis, phytochemical analysis, *Annona reticulata*

INTRODUCTION

In the pharmaceutical world, nanotechnology attracts considerable interest by opening up new processes ability to overcome bacterial resistance mechanisms(1). A cosmopolitan problem has been hospital-acquired infections with identified susceptibility to all recognised groups of antibiotics. Most adjuvant therapeutics to deal with multi-drug resistant bacteria by doctors and researchers, but wide-spectrum action, drug-drug interactions, comprehensive resistance mechanisms, and incompatible pharmacodynamics and pharmacokinetic profiles has hindered them(2). In a cost-effective approach in related to drug production, design and development methods, Nano-scaled systems in pharmaceutical research pursue various agnostic implementations. Since ancient times, metals have been considered antibacterial before any conventional antibiotics were discovered. In the bacterial cellular process, nanotechnology uses metals as inorganic nanoparticles (NPs) that demonstrate their pleiotropic impact.

Nanotechnology is developing field of science in which particle size vary from 1 – 100 nanometres. It is possible to synthesise nanoparticles via physical, chemical and biological synthesis routes. This study focused on biological synthesis of nanoparticles. Silver has very different and unique properties like antimicrobial activity, protection against cancer cells, and anti-viral activity etc. activity against microorganisms helps to preserve food for longer time. Biological method of silver nanoparticles synthesis is cost efficient and eco-friendly. Even biosynthesis is very simplified way to generate nanoparticles.

In the plant family *Annonaceae*, *Annona reticulata* is a tiny deciduous or semi-evergreen tree and member of the group Annonas. It is best known for its fruit called the custard apple, a small deciduous or semi-evergreen tree with an open, irregular crown increasing 8 to 10 metres tall. The slender leaves are hairless, straight and pointed at the apex

(wrinkled in some varieties), 10 centimetres to 20 centimetres long and 2 centimetres to 7 centimetres broad (3). The plant has historically been used to treat epilepsy, dysentery, heart attacks, and infestations of parasites and worms, constipation, haemorrhage, bacterial infections, dysuria, fever, ulcers and as an insecticide. Bark is a potent astringent and is used as a tonic, while helminthiasis treatment leaves are used (4). Various phytoconstituents have been recognizedfrom various parts of this plant; a broad variety of compounds such as amino acids, proteins, carbohydrates, alkaloids, steroids, tannins, glycosides and phenolics are present in the leaves of *Annona reticulata*.

The current work aims to synthesize silver nanoparticles using an aqueous extract of *Annona reticulata* L. These synthesized silver nanoparticles are being documented and characterized.

MATERIALS AND METHODS

Collection and preparation of plant materials

The *Annona reticulata* leaves were controlledfrom Coimbatore District, Tamil Nadu. Fresh and young leaves were picked and washed with water. Leaves were air-dried, powdered in a mixer blender.

Dried and powdered leaves were filled in thimble. 40 ml solvent was taken in the flask. Temperature was maintained at the boiling point of respective solvent. Soxhlet exhaustion was continued till the solvent become colorless in tube. Extracts were collected and dried at 40ºC in hot air oven. Dried extracts were collected and preserved in a refrigerator for further tests.

Silver Nanoparticle Synthesis

The aqueous solution of 1 mM silver nitrate ($AgNO_3$) was formulated and used for production of nanoparticles. Out of 1 mL A. Reticulate leaf solution was mixed to 100 mL of 1 mM silver nitrate aqueous solution for decrease in Ag+ ions and held for incubation at room temperature for 20 minutes.

Characterization techniques

UV-Vis absorption spectra were analysed using the spectrophotometer Shimadzu UV-1601. Crystalline-metallic silver nanoparticles were evaluated with an X-ray diffractometer (Shimadzu XRD-6000) equipped with a Cu Kaf radiation source using Ni as a philtre at a 30 kV/30 mA setting. Under the same experimental conditions, all XRD data was obtained. Electron microscopy field emission scanning (FESEM) study of silver nanoparticles analysis was performed. To evaluate the hydrodynamic diameter of both variations of AgNPs, a VASCO DLS device (Cordouan innovations, Pessac, France) was used. The hydrodynamic diameter decided to show the distribution of the particle size of the differences of colloidal AgNPs and organizations determine of the dispersion of particles. The NanoQ software package has made it easier to control hardware and analyse data.

By centrifuging the solution of ag nanoparticles for 20 min at 10,000 rpm, the powder sample of AgNPs was formulated. The solid residue was washed with water in order to eliminate certain uncommitted biological substituents on the nanoparticle surface, which are not able to take responsibility for bio functionalization and reducing. The resulting coating is then fully closed, and the powdered sample was used for FTIR analysis on a Nicolet iS5 FTIR. Dispersions of both differences of nanoparticles (NP1 and NP2) were sonicated for 20 min and diluted in phosphate buffer saline (PBS) at a final concentration of 75 μg / mL pH =

7.4. The zeta potential of the variants NP1 or NP2 and E of either pure nanoparticle. Using a Zetasizer Nano ZS instrument, coli cells were assessed.

RESULTS AND DISCUSSION

PHYTOCHEMICAL SCREENING OF *Annona reticulata* LEAF EXTRACT

S.No.	Phytochemicals	Ethanol	Methanol	Aqueous	Ethyl alcohol	Acetone
1.	Carbohydrates	++	+	+++	-	-
2.	Proteins and amino acids	-	-	++	+	-
3	Alkaloids	++	+++	+++	+++	+++
4	Flavonoids	++	++	++	++	+++
5	Phenols	+	++	+++	+	++
6	Steroids	+++	+++	+	+++	+++
7	Terpenoids	+	+	++	+	+
8	Tannins	+++	+++	+	+	+++
9	Saponins	++	+++	+	++	++
10	Cardiac glycosides	+++	++	+++	++	++
11	Gums and Mucilages	-	-	+	-	-

12	**Thiol's**	++	++	+++	+++	+

+ → present in small concentration; ++ → present in moderately high concentration; +++ → present in very high concentration; - → absent.

Table 1 - Qualitative phytochemical analysis of different solvent extracts of *Annona reticulata*

The development of primary and secondary metabolites from the leaf extract was discovered by the phytochemical examination of different solvent extracts of Annona reticulata. The aqueous Annona reticulata extract was selected for further studies. The aqueous extract showed the presence of very high concentrations of carbohydrates, proteins and amino acids, alkaloids, flavonoids, phenols, hormones, terpenoids, tannins and saponins. There are many biological and therapeutic properties of these secondary metabolites (5.

QUANTITATIVE ESTIMATION OF CHEMICAL CONSTITUENTS OF AQUEOUS EXTRACT OF *Annona reticulata*

S.No.	PHYTOCHEMICALS	QUANTITY(mg/g of plant extract)
1.	Alkaloids	54.50±0.89
2.	Flavonoids	70.78±0.53
3.	Total phenols	93.76±.60
4.	Tannins	50.13±0.14
5.	Steroids	80.09±1.31

Values are expressed in Mean ± SEM (n=3)

Table 2 - Quantitative estimation of secondary metabolites of aqueous extract of *Annona reticulata*

The quantitative estimation of aqueous extract of *Annona reticulata* exhibited the appearance of alkaloids, flavonoids, total phenols, tannins and steroids. The considerable

quantity of secondary metabolites includes as alkaloids (54.50± 0.89 mg/g of Caffeine), flavonoids (70.8±0.53 mg/g of Quercetin), total phenols (93.8± 0.60 mg/g of Gallic acid), tannins (50.1±0.14 mg/g of Gallic acid) and steroids (80.09±1.31 mg/g of Cholesterol) were present.

Visual analysis

Synthesis of the Silver nanoparticle by the biological process of elimination. As reduction agents that generate AgNP, silver nitrates are a source of Ag+ and NaBH4. Chemical processes form the Silver nanoparticle by sequentially adding reactants, namely the AgNO3 substance, drop by drop into the plant extract. In reverse direction, the addition of reactants creates the formulated Silver nanoparticle to instantly settle (6). The colour of the plant extract was initially green when silver nitrate was introduced to the plant extract, changing the colour to brown (Fig 1). Further processing, such as colloidal way to solve centrifugation, drying at 400 c to obtain dry silver particles and reducing the size of silver nanoparticles even by particle size. The colour of the produced AgNP colloid depends on the concentration of the AgNO3 solution added and indicates the typical UV-vis spectra of colloidal silver nanoparticles with varying concentrations of AgNO3 processed.

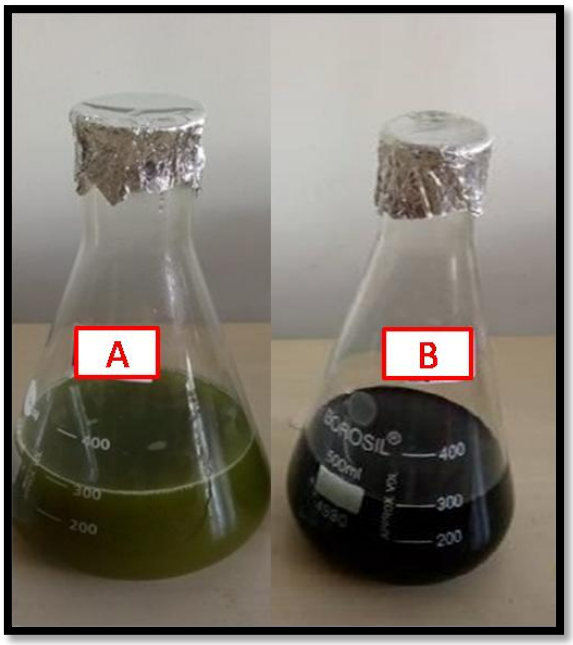

Figure 1 - Plant Extract, B- Synthesized Sliver Nanoparticles

UV-vis spectroscopy

One of the common analysis methods to evaluate particle structure and its properties is UV-visible spectroscopy. A poor absorption limit of surface plasmon peaks was found at 406 nm at low AgNO3 concentration, with the amplitude of the maximum plasmon peak increased with growing AgNO3 concentration and silver nanoparticles were produced (7). 2015, according to Suresh K. Ghotekar. The absorption spectrum of silver nanoparticles produced in the response media has been shown to have an absorption peak at 482 nm.

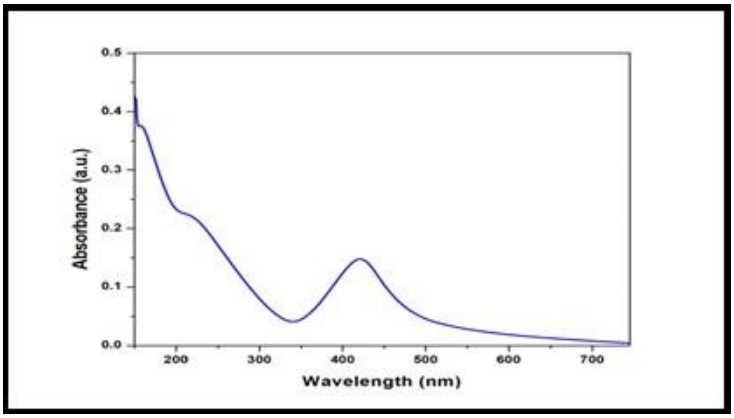

Figure 2 - UV – Visible spectra of silver nanoparticles of aqueous extract of *Annona reticulata*

A UV-VIS spectrophotometer was used to classify the blend silver nanostructures. This figure showed the absorption spectrum of silver nanoparticles synthesized by *Annona reticulata* at the range of 420 nm (Fig 2).

FT-IR ANALYSIS OF SILVER NANOPARTICLES

To classify the potential biomolecules in A, the FTIR calculation was performed. Responsible for capping reticulata leaf extract leading to successful stabilisation of AgNPs. These are extracted from water soluble substances that are found in leaves, such as flavonoids, alkaloids, and polyphenols. Biological element is known to interfere with chemical reagents through these molecules and mediate their degradation into nanoparticles.

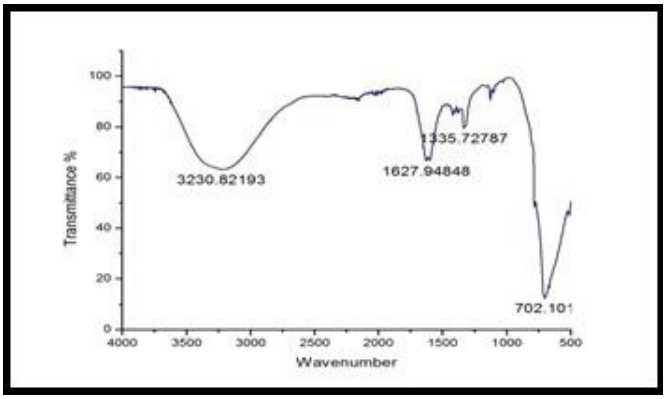

Figure 3 - FT-IR patterns of synthesized silver nanoparticles in aqueous extract of *Annona reticulata*

S.No	Wave number (cm^{-1})	Group	Possible compounds present
1	3230	-OH, -NH, -C≡H	Alcohol, phenol, aldehydes, amides, amines and alkynes
2	1627	-C=O, C=C, -NH_2, C=N	Amides, amines, amino acids, unsaturated aliphatics, aromatics and unsaturated heterocycles
3	1335	-S=O, -NO_2, -CH_2, -CH_3	Sulfate, nitro compound, alkanes and alkenes
4	702	-C-O, -C-N, Si-O, P-O	Primary amine, organosilicons and phosphorous

Table 3 - Possible functional groups and compounds present in silver nanoparticles of *Annona reticulata*

Stretching vibration of 3186cm-1 (alcohols and phenols) of OH bonds and two peaks of 1595cm-1 and 1496cm-1 lead to C = C stretching vibration of plant metabolites from aromatic rings. Alcohol-extending C-O leads to peaks at 1065cm-1, all of which are due to protein structure and metabolite groups covering the AgNPs (Fig.3). After bio-reduction, the 1601-1595cm-1 absorption edge is modified, the presence of green synthesised AgNPs is revealed and bio-molecules are coated (8). This promotes the difficult roles played in the

isolation of water-based variants in the bio-reduction of precursors and the development of AgNPs.

SCANNING ELECTRON MICROSCOPY (SEM) ANALYSIS

Further insight into the morphology and the size of the synthesised AgNPs was given by the SEM picture. AgNPs were accumulated on a copper grid covered with carbon (Fig.4). Due to its spherical nature, AgNPs have been found to be highly dispersed. Small AgNPs are seen in the present analysis, attached to the surface of very large biomolecules (9).

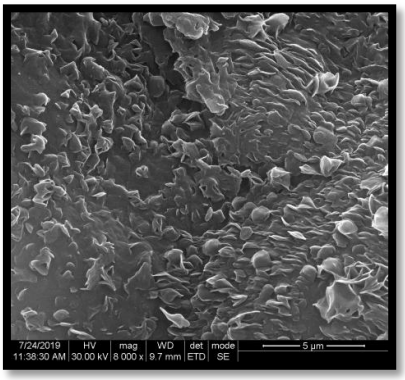

Figure 4 - Scanning electron micrographs of silver nanoparticles synthesized by aqueous extract of *Annona reticulata*

ENERGY DISPERSIVE X-RAY SPECTROSCOPY (EDX)

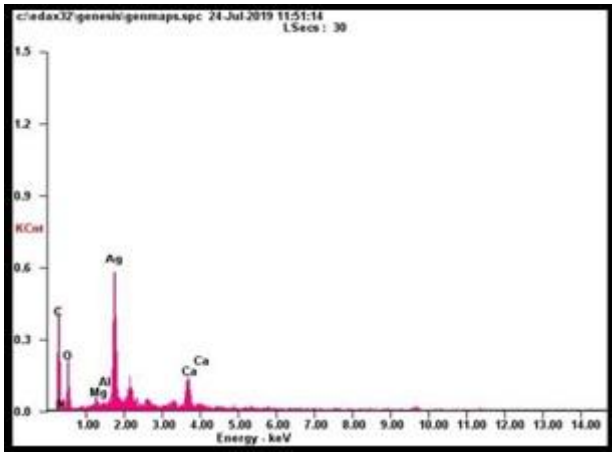

Figure 5 - EDX spectrum of elemental composition of silver nanoparticles from aqueous extract of *Annona reticulata*

The energy dispersive X-ray (EDX) study of silver nanoparticles synthesised with Annona reticulata leaves showed that silver had a high signal peak. At different time periods, differences in particle sizes were due to the formation of AgNPs. A typical absorption peak at 3 keV due to surface plasmon resonance is seen in the EDX spectrum of AgNPs (10). From the carbon coated Cu grid was another signal observed at 8 keV. The formation of AgNPs was clearly proven by the elementary verification by EDAX (Fig 5).

PARTICLE SIZE DISTRIBUTION HISTOGRAM OF SILVER NANOPARTICLES FROM AQUEOUS EXTRACT OF *Annona reticulata*

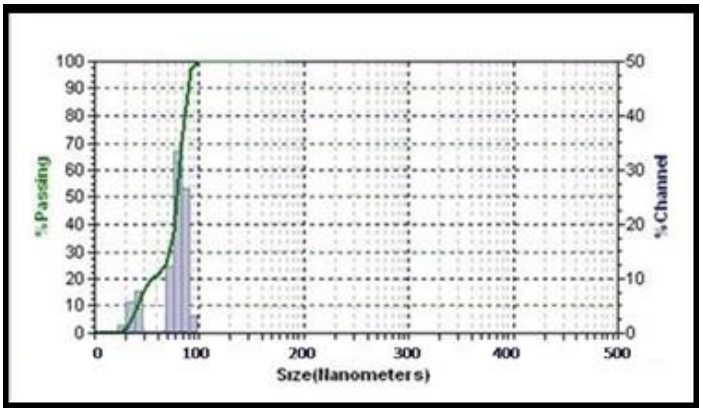

Figure 6 - Particle size distribution histogram of silver nanoparticles from aqueous extract of*Annona reticulata*

The average size for sliver nanoparticles synthesized by *Annona reticulata* wasfound to be 50 nm and size varies from 10 nm to 100 nm.

The particle amounts varied from 10 to 60 nm for the green synthesised AgNPs, with a maximum particle size of 40 nm. Zeta's potential for recorded stability of green synthesised AgNPs and leaf metabolite reducing of AgNPs. It is evident from (Fig. 6 and 7) that AgNPs displayed a low distribution of particle size with a z-average of 40 nm and a low polydispersity index of 0.31 (11). The high value -31.2 showed that the capping compounds found on the surface of the AgNPs consisted predominantly of negative charge categories and were also important for the medium balance of the AgNPs.

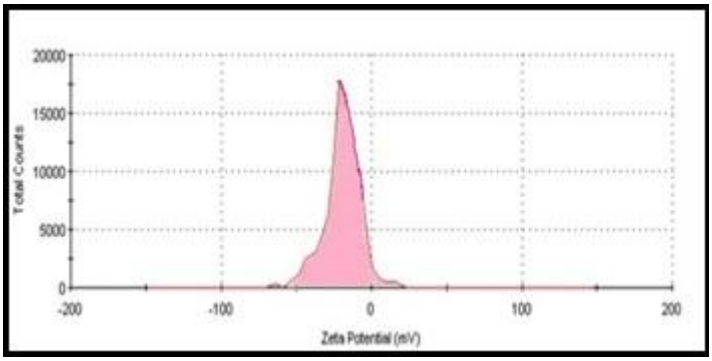

Figure 7 - Zeta potential analysis for synthesized silver nanoparticles

Zeta potential measurement of synthesized AgNPs potential value was found to be -25 mV (negative).

X-RAY DIFFRACTION ANALYSIS

The crystalline nature of AgNPs has been verified by XRD research methods, as seen in the Fig.8. At 38.28^{o}, 44.33^{o}, 64.33^{o} and 77.53^{o}, the XRD results exhibited four different diffraction peaks; the corresponding lattice plane measure was measured to (111), (200), (220) and (311) face-centered cubic (fcc) silver planes with a lattice parameter that was in strong correlation with the fcc geometry comparison. Using the Debye Scherrer equation (12), the mean size of AgNPs was determined by calculating the width of the reflection of Bragg. For AgNPs synthesised at room temperature, the size of the AgNPs was thus determined to be around 10-80 nm . The collected XRD trends are consistent with previous studies. Unselected peaks (*) have been observed because of bio-organic phase crystallisation occurring on the surface of the AgNPs. The smaller scale of AgNPs has indirectly been shown by the great bottom of the mountain. The Debye Scherrer equation was used in predicting the crystallite size from the line expansion spectrum of the reflection: D = 0.94 k / β cos. If D is the average size of the crystallite, k is the wavelength of the X-ray, b is the complete width at half the limit and y is the angle of diffraction (13).

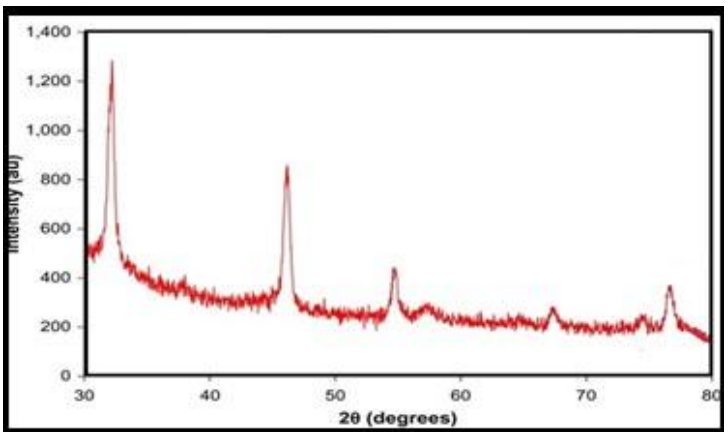

Figure 8 - XRD pattern of silver nanoparticles from aqueous extract of *Annona reticulata*

The XRD pattern of silver nanoparticles obtains using *Annonareticulata* showed unassigned peaks at (111), (200), (142), (220) and (311).

CONCLUSION

The present study demonstrated that the leaf extracts of *Annona reticulata* are excellent source of bioactive phytocompounds. Nanoparticles synthesized from the aqueous extract showed high intensity bands observed in the plasmon peak and presence of possible functional groups and compounds in silver nanoparticles of *Annona reticulata.* This study can form the basis of further extended biomedical applications in cancer therapy.

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