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Green synthesis and characterisation of silver nanoparticles using *Aristolochia bracteata* leaf extract and their antibacterial activity

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ABSTRACT

The silver nanoparticles (Ag NPs) were synthesised using *Aristolochia bracteata* leaf extracts by green synthesis method. The synthesised nanoparticles were characterized by UV-Vis absorption, XRD, FT-IR, TEM techniques. The UV-Visible absorption spectra show that surface plasmon resonance band was appeared at 441-423 nm, which was blue shifted with respect to different leaf extract concentrations. XRD analysis confirms that the prepared AgNPs was in fcc crystal structure and the size of Ag NPs was estimated in the rage of 11 nm. FTIR spectra provide the evidence for the presence of biomolecules responsible for reduction and capping of silver nanoparticles. TEM images revealed that the particles were spherical in shape. The synthesised silver nanoparticles were found to exhibit high antibacterial activity against the bacteria such as *Escherichia coli*, *Bacillus subtilis*.

Keywords: Ag NPs, *Aristolochia bracteata*, Green synthesis, Anti bacterial activity.

1. INTRODUCTION

The field of nanotechnology is an extremely developing field due to its extensive applications in different areas of science and technology. Nanotechnology is an addition of different fields of science which holds comfort in the pharmaceutical industry, medicine, and agriculture [1]. Different types of nanomaterials including copper, zinc, titanium, magnesium, gold, and silver have been useful in biological systems [2]. Amongst all, silver nanoparticles (Ag NPs) have attained promising applications in nanotechnology because of their good optical [3] and electrical [4] applications. The nontoxic environmentally friendly synthesis proposed here produced AgNPs of a suitably small size distribution using the extract of the leaf of the green synthesis in minimal time. The preparation of AgNPs using plant-based extracts is broadly growing in fame recently proposed synthesis use reagents such as several types of leaf extract. AgNPs were both reduced from silver nitrate ($AgNO_3$) and capped by the compounds present in the leaf extract. *A. bracteata* (*Aristolochiaeae*) commonly called as work killer in English and aadutheenadapaalai in Tamil, wildly distributed in Deccan Gujarat, western and southern india, Bihar, sindh, Bundelkhand and Bengal. *A. bracteata* is used in traditional medicine as gastric refreshment and in the treatment of cancer, lung inflammation, dysentery, snake bites and insecticidal properties [5]. In this present work focused and carried out a rapid green synthesis of silver nanoparticles using aqueous leaves extracts of Aristolochia bracteata, their characterisation and has used the synthesised nanoparticles to prove their antimicrobial activity against seven bacterial strains of *Escherichia coli*, *Bacillus subtilis*. In this work is first time synthesis of silver nanoparticles using *Aristolochia bracteata* leaf extract.

2. MATERIAL AND METHODS

2.1. Materials

Silver nitrate ($AgNO_3$) with AR grade was purchased from Sigma Aldrich chemicals. *Aristolochia bracteata* (aadutheenadapaalai) was collected from Alakkudi, Nagappattinam district located in Tamil Nadu, India. Pure culture of gram negative and gram positive bacteria were collected from National Chemical Laboratory, Pune, India. The microbial cultures were

maintained by the Department of Pharmacy, Annamalai University, Annamalai Nagar, Tamil Nadu, India.

2.2. Preparation of leaf extract

Fresh leaves of *Aristolochia bracteata* were collected, then washed thoroughly with distilled water several times to remove the dust and dried under shade. The dried leaves were cut into small pieces and ground to powder. This 5g of *Aristolochia bracteata* leaf powder was boiled in 100ml of distilled water at 80°C for 10 mins and filtered using whatman No: 1 filter paper. The finely prepared extract solution was cooled at 4°C and stored for further synthesis of nanoparticles.

2.3. Synthesis of silver nanoparticles

The prepared leaves extract solution in various concentrations (1ml to 5ml) were added to 10ml of 1mM $AgNO_3$ solutions and then incubated at room temperature. After 10 min the solution was turned from light yellow to dark brown colour indicating that the formation of AgNPs.

2.4. Characterization techniques

The absorption wavelength of prepared samples was recorded using UV-Vis SHIMADU-UV 1800 spectrophotometer. The functional group molecules present silver nanoparticles were analyzed through FTIR RX1-Perkin Elmer in the IR spectral range of 400-4000 cm^{-1}. The crystalline structure of silver nanoparticles was studied using XPERT-PRO using monochromatic Cu kα radiation (λ=1.5406Aº) operated at 40kV and 30mA. The obtained XRD pattern was compared with Joint Committee on Powder Diffraction Standards (JCPDS) card to confirm the crystalline phase of prepared silver nanoparticles. The morphology and size of silver nanoparticles were observed using Transmission Electron Microscope (JEM2100).

2.5. Antibacterial activity

Antibacterial activity of the synthesised Ag NPs was studied by the standard disc diffusion method. The overnight grown bacterial suspensions of *Escherichia coli* (ATCC 8739), *Bacillus subtilis* (ATCC 6633), were standardized using Mc farland standard. Whatman filter paper (No: 1) discs of 5 mm diameter were used. The dilutions of biosynthesised Ag NPs varying from 5mg, 10mg and 15mg/ml were prepared with two fold symmetry. 5g of solidified agar was added with 50ml of distilled water and sterilized. This mixture was poured equally in seven petri plates and seven organisms were plated in them. The organisms to be tested were inoculated in four discs (5mm diameter) dipped in different dilutions of AgNPs (5mg, 10mg and 15mg/ml) solutions, and another disc was dipped in 2mg/ml of antibiotic Ofloxcin. Each petri plate was loaded with four discs. The plates containing the bacterial and AgNPs were incubated at 37ºC, and then examined for confirmation appears as a clear area around the disc. The diameter of such zones of inhibition was deliberate using a meter ruler, and the mean value for each organism was recorded and expressed in millimeters.

3. RESULTS AND DISCUSSION

3.1. UV-vis spectroscopy

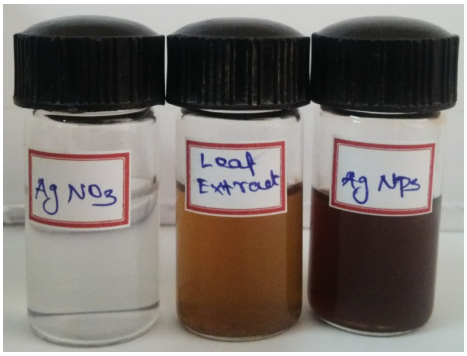

Figure – 1: Photograph of $AgNo_3$, Leaf extract and Synthesised Silver nano particle.

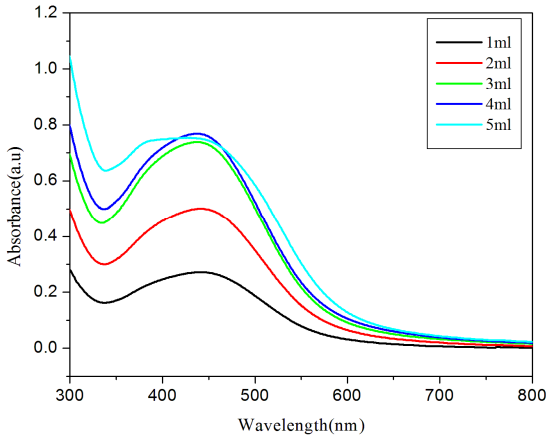

Figure – 2: UV-vis absorption of Ag NPs at different leaf extract concentration.

Figure 1 shows photograph of the aqueous $AgNO_3$ solution without leaf extract. Figure 2 respected the UV-vis spectra of synthesised $AgNO_3$ concentration (1mM) and with different concentration (1ml, 2ml, 3ml, 4ml and 5ml) of the *Aristolochia bracteata* leaf extract. It also revealed that the concentration of the leaf extract plays an important role in the formation of AgNPs. The solution containing the silver nitrate with *Aristolochia bracteata* leaf extract starts to visual change in its colour after 10 mins of starting the reaction [6]. The color changed from light yellow to yellowish brown which is related to the formations of AgNPs. This characteristic color

formation is due to the excitation of the surface plasmon resonance (SPR) band in the metal nano particles [7]. The appearance of the plasmon peak depends on the particle size and shape. It is observed that, there is an appearance of absorbance peaks ranges from 441 nm to 423 nm and steadily increased in intensity with increasing concentration of leaf extract. These appearances of the peaks are shifted to lower side. This blue shift depicts the diameter of the Ag NPs decreases. The SPR band (5ml concentration) is decreased in intensity, when extract quantity increased. This states that a 5ml concentration of the leaf extract is adequate for completion of the reaction, and the decrease of intensity due to the colloids dilution which is caused by increased extract quantity [8]. The 5ml concentration is further analysis.

3.2. Structural study

The XRD pattern shows the synthesised Ag NPs using *Aristolochia bracteata* leaf extracts. The XRD patterns are used to confirm the crystalline nature of the silver nanoparticle exhibited in figure 3. The spectra of XRD is clearly indicate the synthesized silver nanoparticles at 5 ml using the mentioned extracts are crystalline nature and the high intensity of (111) plane is revealed. At higher concentration of plant extract, showed diffraction 2θ peaks at 38.09°, 44.17°, 64.49° and 77.06° which could be indexed to (111), (200), (220) and (311) planes of silver confirmed by JCPDS card No: 89-3722. The average crystal size of the silver nanoparticles was determined by full with half maximum (FWHM) data along with the Debye scherrer's equation [9]

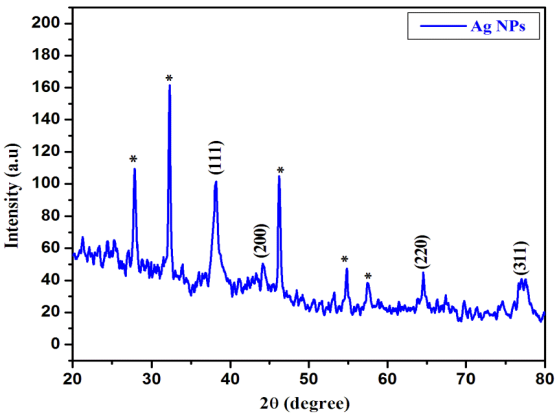

Figure – 3: X-ray diffraction of green synthesised Ag NPs.

$$D = k\lambda/\beta \cos\theta$$

In which D is particles diameter size, k is the scherrer coefficient (0.9), λ is wavelength of X-ray source (1.5406nm), β is the full width at half maximum (FWHM) and θ is the diffraction angle. The synthesis of AgNPs average crystal size was calculated. An additional few sharp unidentified crystalline peaks at 27.79°, 32.19° and 54.74° were also occurred. The early reported unidentified crystalline peaks (27.89◦, 32.30◦, 54.79◦) are also obvious in many works in which the XRD pattern includes the relevant 2θ range and these peaks are due to the organic compounds which are present the extract [10].

3.3. Functional group analysis

FTIR measurements were carried out to identify the possible reducing and stabilizing biomolecules in the bioreduced AgNPs synthesised by *Aristolochia bracteata* leaf extract. The FTIR spectrum in *Aristolochia bracteata* leaf extract Fig.4 shows peaks at 611, 1079, 1416, 1612 and 3381 cm^{-1} corresponds to C=O out of plane bending vibration, C-O-C stretching vibration in ethers, O-H bending in carboxylic acids, carbonyl group of carboxylic acid in the extract [11], O-H stretching vibration in alcohols and phenols. The weak band at 888 cm^{-1} is C-H out of plane in bending vibration. The broad peaks at 2361 cm^{-1} corresponding to N-H stretching of any ammonium ions which are characteristics of aromatic phenols [12].

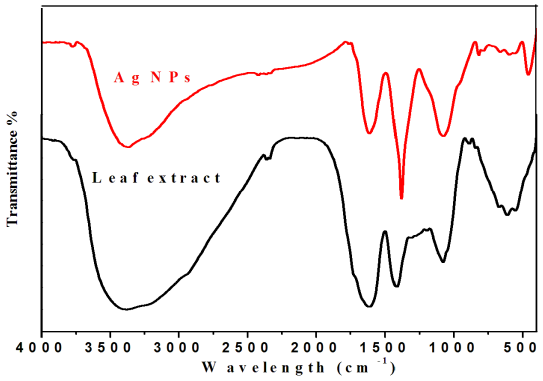

Figure – 4: FTIR spectra of leaf extract and AgNPs.

The FTIR spectrum of silver nanoparticle Figure 4 showed different bands at 666,821, 1080, 1384, 1616, 2425 and 3371 cm^{-1}. The band at 666 cm^{-1} is O-H bending of phenols [13].The weak band at 821 cm-1 is CH out of plane deformation. The strong band at 1080cm^{-1} corresponds to C–N stretching vibration of the amine [14]. The very strong band shifted at 1,384 developed for C–C and C–N stretching, respectively, and was commonly found in the proteins. The strong band obtained at 1,616 cm^{-1} corresponds to carbonyl groups and secondary amines [15]. The weak band at 2425 cm^{-1} is O-H stretching vibration in carboxylic acids. The intense broad band at 3371 cm^{-1} is the characteristic of the hydroxyl functional group in alcohol and phenol compounds.

Table – 1: The anti bacterial activity in *Escherichia coli, Bacillus subtilis* bacteria

Bacterium name	Zone of inhibition of Ag NPs (mm)			
	Control (Ofloxcin) 2mg/ml	5mg/ml	10mg/ml	15mg/ml
Escherichia coli	25	14	16	19
Bacillus subtilis	27	11	12	14

3.4. Transmission electron microscopy (TEM)

TEM images were used to observe the morphology and size of as synthesised Ag NPs by using *Aristolochia bracteata* leaf extract. Figure 5 (a) shows TEM images. TEM micrographs revealed that particles are spherical shape and uniformly distributed (mono dispersed) without significant agglomeration. The TEM image was employed so that the synthesised nanoparticles were in the size of 12 nm.

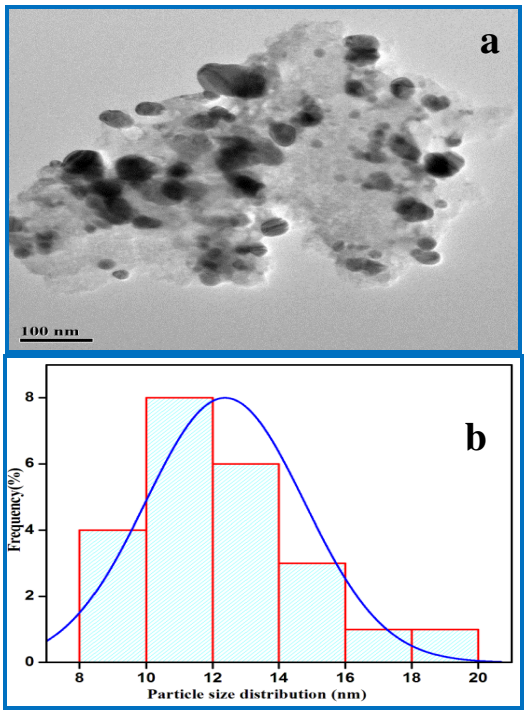

Figure – 5: (a) TEM images of synthesised Ag NPs and (b) particle size distribution.

3.5. Antibacterial activity

Silver nanoparticles have strong bactericidal activity against that is *Escherichia coli*, *Bacillus subtilis* at (5, 10, 15 mg/ml) different concentrations using the disc diffusion method. Therefore the present study was performed to study the growth inhibition effect of biosynthesized Ag NPs for drug conflict micro organisms. Figure 6 clearly indicates that the concentrations of silver nanoparticles tested (5, 10, 15 mg/ml), inhibited the growth of the tested gram negative and gram positive bacterial strains of *Escherichia coli*, *Bacillus subtilis*. The difference observed in the diameter of zone inhibition may be due to the difference in the susceptibility of different bacteria to the prepared silver nanoparticles (Table 1). The differential sensitivity of gram positive and gram negative bacteria towards AgNPs possibly depends upon their cell structure, physiology, metabolism and their interaction with changed Ag NPs [16].

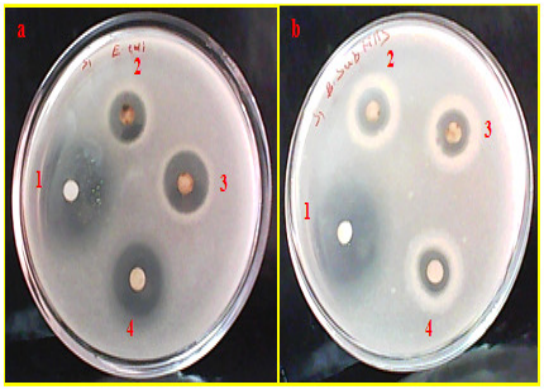

Figure – 6: The anti bacterial activity of E.coli (a) and *B.Subtilis* (b) organisms at different volume of Ag NPs (1(control), 2 (5 mg/ml), 3 (10 mg/ml), 4 (15 mg/ml)).

As form the table.1, the inhibition zone of gram negative bacteria *Escherichia coli* are 19 mm (15mg). Whereas inhibition the inhibition zone of gram positive bacteria *Bacillus subtilis* are 14 mm (15mg) respectively. The maximum inhibition zone was observed for the gram negative a bacterium which demonstrates that the bacterial effect of silver nanoparticles on these bacteria was depends on the anionic of silver ions realest from the silver NPs [17]. There is no much difference in the inhibition zone of the gram negative bacteria which enplanes that the smaller NPs reduces more cell permeability by crafting in cellular lose and their by leading to cell death [18].

4. CONCLUSION

In this study, we have successfully investigated and ecofriendly and low cost synthesis procedure for the Ag NPs particles using *Aristolochia bracteata* leaf extract is the reducing agent for silver ions. The crystalline nature of silver nanoparticles was confirmed by XRD and SAED results. It was observed that almost spherical nanoparticle and the lattice plane being confirmed by TEM and XRD studies. The

antibacterial activity showed potential efficiency exhibited the green synthesised Ag NPs against tested micro organisms.

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