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Plant-mediated Biosynthesis of Silver Nanoparticles and Evaluation of Their Antibacterial Activities

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ABSTRACT

Aqueous seed extract of *Syzygium cumini* (SCE) was used for the synthesis of silver (Ag) nanoparticles. The nanoparticles were characterized with the help of UV–vis spectrophotometer, Particle size analyser (PSA), Transmission electron microscopy (TEM) and Fourier transform infrared spectroscopy (FTIR). UV-Vis spectra of silver nanoparticles shows absorbance peak at 440 nm. The nanoparticles synthesized were relatively spherical in shape with varying sizes. The biosynthesized silver nanoparticles were found to be highly toxic against some human pathogenic bacteria.

Keywords: *Syzygium cumini*, Biosynthesis, Silver nitrate, Seed extract

I. INTRODUCTION

Silver nanoparticles have wide applicability in different areas such as diagnostics [1], electronics [2], catalysis [3], antimicrobials and therapeutics [4, 5]. Silver nanoparticles have been synthesized through various methods such as chemical [6], photochemical [7], radiation [8] and biological methods [9]. The commonly used chemical methods for nanoparticles synthesis make use of toxic substances like hydrazine and high boiling solvents [10]. The biological methods for nanoparticle synthesis are popular these days because they are simple and eco-friendly. These methods use microorganisms [11-13], enzymes [14] and plant extracts [15-19] for nanoparticle synthesis. Silver nanoparticles have been synthesized using extracts of many plants such as *Sorbus aucuparia* [20], *Camellia sinensis* [21], *Aloe vera* [22], *Argemone maxicana* [23], *Azadirachta indica* [24], *Coriandrum sativum* [25], and *Leptadenia pyrotechnica* [26].

Syzygium cumini Linn. (family: Myrtaceae), locally known as Jamun, is a traditional medicinal plant native to India. Besides India, it also occurs in Eastern Africa and South-East Asia [27, 28]. The seeds of *S. cumini* are considered to have anti-inflammatory [29], anti-diabetic [28], antibacterial [30] and antidiarraheal [27] properties. Silver nanoparticles have been synthesized using seeds [31] and leaves [32] of this plant. However the antimicrobial evaluations of silver nanoparticles synthesized using seed extract of *S. Cumini* have not done so far. Here in, we report the synthesis, characterization and antimicrobial activities of silver nanoparticles synthesized using *S. Cumini* seed extract.

II. MATERIAL AND METHODS

A. Collection and identification of plant samples

S. Cumini seeds were purchased from local market and authenticated by Raw Materials Herbarium & Museum, National Institute of Science Communication And Information Resources (NISCAIR), New Delhi.

B. Preparation of extracts

After removing pericarps, the seeds were dried and finely powdered. 5g of seed powder was boiled with 100 ml distilled water and then centrifuged at 5000 rpm for 10 mins. The supernatant was taken for further use.

C. Synthesis of silver nanoparticles

1 mM aqueous solution of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 10 ml of *S. Cumini* seed extract was added into 90 ml of aqueous solution of 1mM silver nitrate in a conical flask wrapped by paper and stirred (750 rpm for

3 hours) at 40˚C. The synthesized nanoparticles were dried by lyophilisation. For comparative analysis, silver nanoparticles were also synthesized chemically using 1 mM silver nitrate and 1% trisodium citrate.

D. UV-Vis Spectra analysis

Silver nanoparticles synthesis was confirmed by analysing the absorption spectra obtained by sampling the aliquots of the suspension using UV-Vis spectrophotometer (Shimadzu, Model: UV-2450) at the wavelength of 300 – 800 nm.

E. PSA Measurements

The particle size distribution of silver nanoparticles was analysed using Zetasizer nano ZS (Malvern instruments, Malvern, UK).

F. FTIR Spectral analysis

For FTIR measurements, the dried samples were grinded with KBr to form pellets. The spectrum was recorded in the range of 4000 - 400 cm^{-1} using FTIR spectrophotometer (Spectrum BX11, Perkin–Elmer) in the diffuse reflectance mode operating at resolution of 4 cm^{-1}.

G. **TEM analysis of silver nanoparticles**

Shape and size of the silver nanoparticles were investigated by TEM images using Morgagni 268D instrument. TEM samples were prepared by placing a drop of the suspension of nanoparticles solution on carbon-coated copper grids and then drying under lamp.

H. **Antibacterial assays**

The antibacterial assays were performed on pathogenic gram-positive (*Staphylococcus aureus*) and gram-negative (*Klebsiella pneumoniae, Enterobacter aerogenes, Escherichia coli* and *Pseudomonas aeruginosa*) bacteria by using agar well diffusion method. Nutrient agar media was prepared and autoclaved at 121°C for 15 minutes. Media was solidified in petri plates and an inoculum (turbidity adjusted to approximately 10^8 CFU/ml of bacterium, compared with 0.5 Mc Farland standards) of each bacterial strain was spread on it. Wells were bored into the solidified media using a sterile 7 mm diameter cork borer. Biosynthesized and Chemically synthesized silver nanoparticles samples, aqueous seed extract samples and standard antibiotic were added in respective wells. Amikacin was used as standard antibiotic. The plates were incubated at 37°C for 24 hours. Three replicate trials were conducted against each bacteria and the mean values are presented.

III. RESULTS AND DISCUSSION

It is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles [15]. As the *S. Cumini* seed extract was mixed in the aqueous solution of the silver ion complex, it started to change the color from watery to yellowish brown due to reduction of silver ions (Fig.1); which indicated silver nanoparticles formation.

UV-Vis spectra of aqueous component of silver nanoparticles is shown in Fig. 2. Absorption spectra of silver nanoparticles shows peak at around 440 nm, broadening of peak indicates that the particles are polydispersed. Fig.3. shows the particle size of the silver nanoparticles as determined by PSA in aqueous solution. The PSA observation shows that the average diameter of silver nanoparticles formed is 70.76 nm.

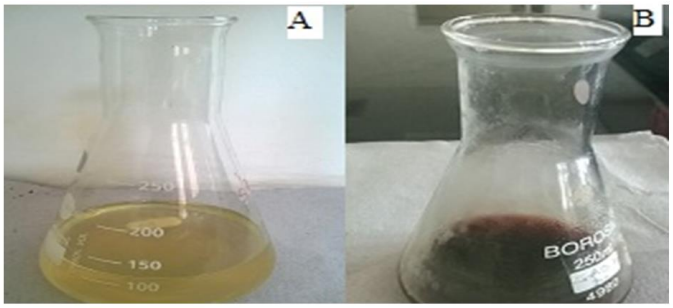

Figure 1. S. Cumini seed extract solution before (A) and after (B) addition of 1mM AgNO3 solution

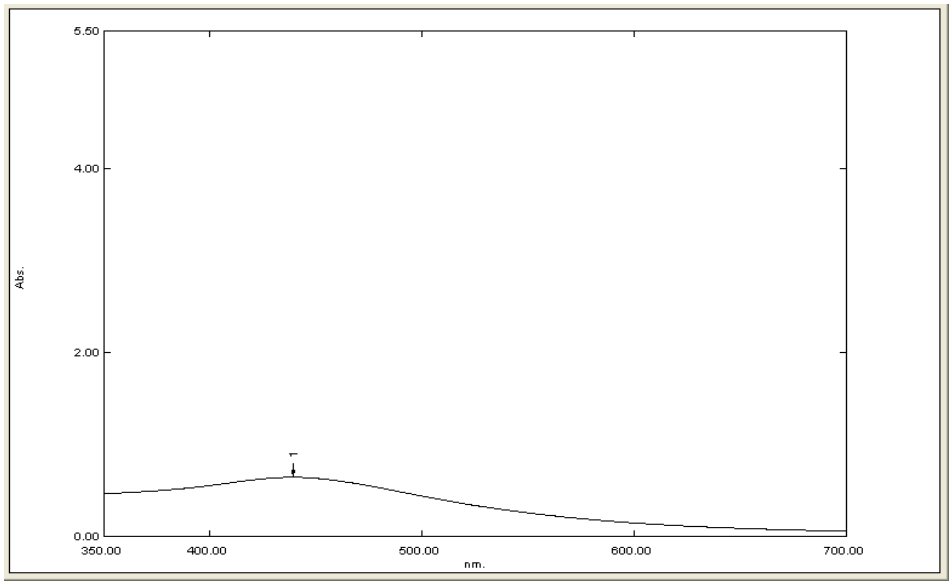

Figure 2. UV-Vis absorption spectrum of silver nanoparticles synthesized by treating 1mM aqueous $AgNO_3$ solution with *S. Cumini* seed extract

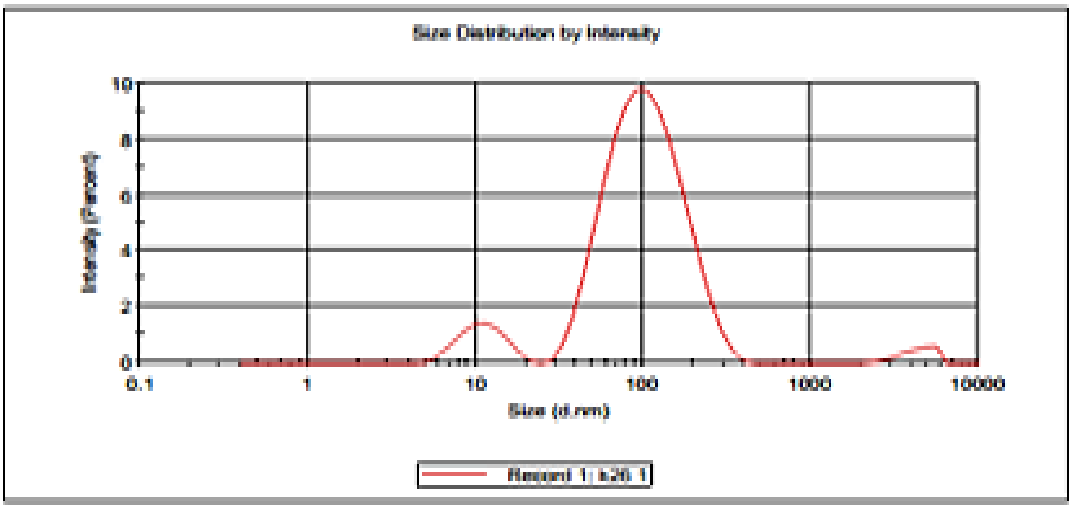

Figure 3. Particle size analyzer results of biosynthesized silver nanoparticles

FTIR analysis was used to identify the biomolecules for efficient stabilization and capping of the silver nanoparticles synthesized by *S. Cumini* seed extract (Fig. 4). The band at 3399.54 cm^{-1} corresponds to O-H stretching H-bonded phenols and alcohols. The peak at 2930.46 cm^{-1} and 1734.44 cm^{-1} corresponds to O-H stretch carboxylic acids and C=O stretch carbonyl groups respectively. The peak at 1631.26 cm^{-1} corresponds to N-H bend primary amines while the band at 1384.52 cm^{-1} corresponds to C-N stretching of aromatic amine group. The 1233.79 cm^{-1} and 1030.60 cm^{-1} bands arises most probably from the C–O group of polyols such as hydroxyflavones and catechins. The peak at 832 cm^{-1} belongs to the characteristic absorptions of polysaccharides. From these results it can be concluded that the synthesized nanoparticles are surrounded by plant metabolites having functional groups of carboxylic acids and alcohols. It is suggested that these biomolecules could possibly perform dual functions of silver nanoparticles formations as well as their stabilization in the aqueous medium [25]. TEM images of silver nanoparticles synthesized using *S. Cumini* seed extract are shown in Fig. 5. It is observed that relatively spherical nanoparticles are formed with varying sizes (8-75 nm). Particle size distribution histogram determined from TEM is shown in Fig. 6. The silver nanoparticles formed are polydisperse and some of them are agglomerated. It is also observed that nanoparticles are surrounded by a faint thin layer of other materials, which we suppose are capping biomolecules from *S. Cumini* seed extract.

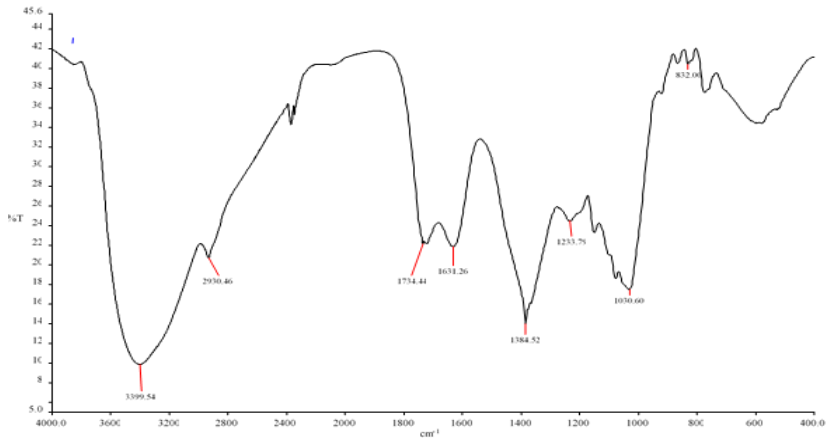

Figure 3. FTIR spectra of freeze dried silver nanoparticles synthesized using *S. Cumini* seed extract

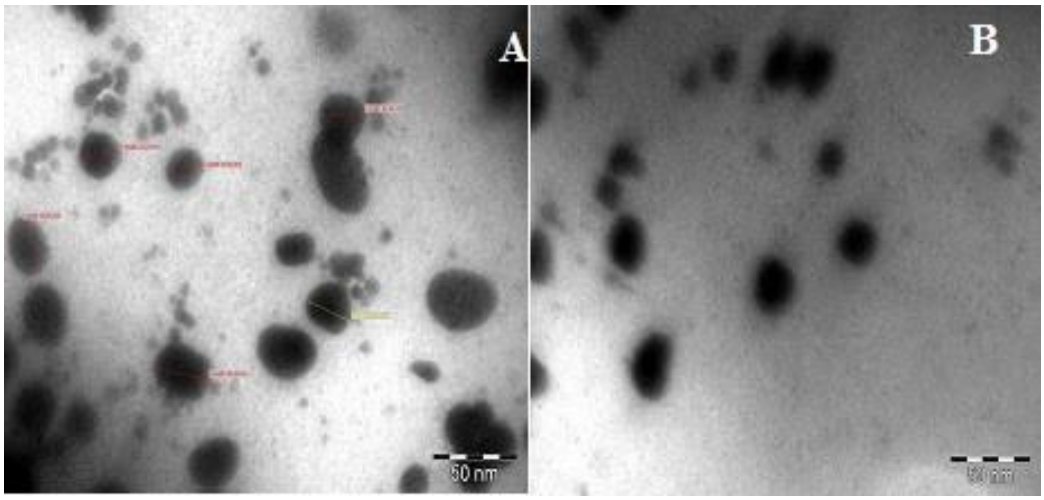

Figure 3. TEM images of biosynthesized silver nanoparticles

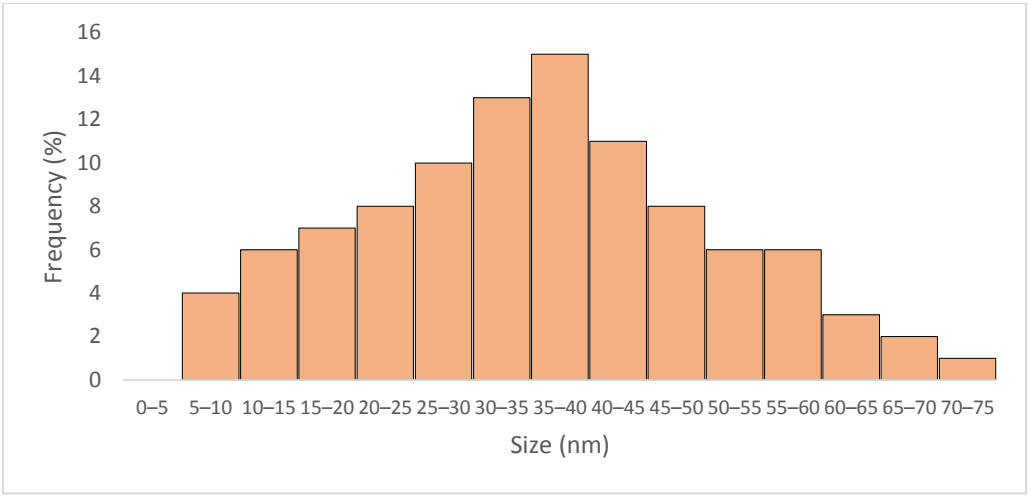

Figure 3. Particle size histogram of silver nanoparticles synthesized using *S.Cumini* seed extract

The antibacterial assays results (Fig. 7) show that silver nanoparticles synthesized using *S. Cumini* seed extract have potential antibacterial properties against human pathogenic bacteria *E. coli*, *S. aureus*, *E. aerogenes*, *K. pneumoniae* and *P. aeruginosa*. Zone of inhibition of SCE, SCE mediated silver nanoparticles, chemically synthesized silver nanoparticles and standard antibiotic drug are shown in Table 1. The activity index (A.I.) and percent inhibition (P.I.) were calculated at a concentration of 50 mg/ml of aqueous solutions of both types of nanoparticles as well as SCE using the following formula:

$$A.I. = \frac{\text{Mean zone of inhibition of sample}}{\text{Zone of inhibition obtained for standard antibiotic}}$$

P.I. = Activity index x 100

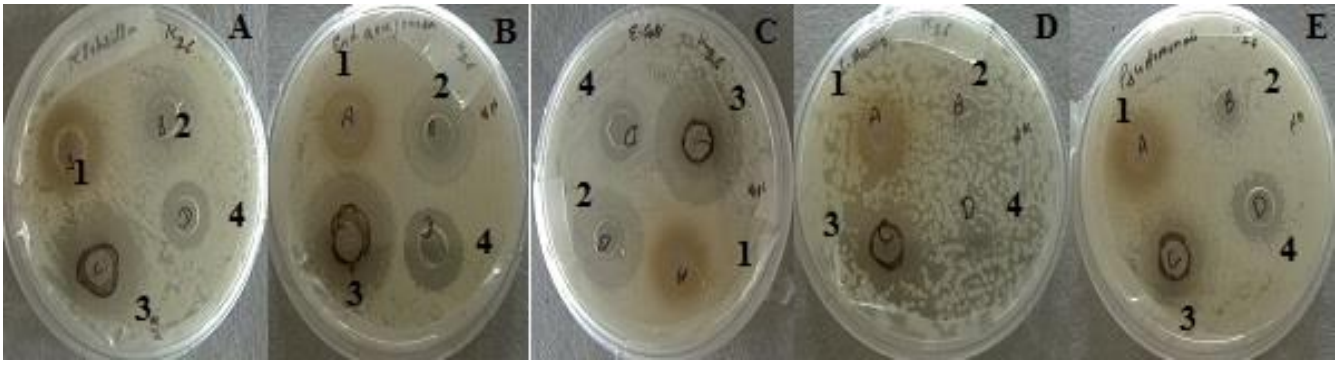

Figure 7. Antibacterial activity of silver nanoparticles against (A) *Klebsiella pneumoniae*, (B) *Enterobacter aerogenes*, (C) *Escherichia coli*, (D) *Staphylococcus aureus* and (E) *Pseudomonas aeruginosa*.

1 - SCE, 2 - Chemically synthesized silver nanoparticles, 3 - SCE mediated silver nanoparticles, 4 - Standard antibiotic Amikacin (85 µg/ml)

TABLE 1

Antibacterial activity of aqueous SCE extract and silver nanoparticles synthesized chemically and using *S. Cumini* seed extract at concentration of 50 mg/l

Bacterial Strain	Zone of inhibition of standard drug (mm)	SCE			Chemicaly synthesized AgNPs			SCE mediated AgNPs		
		Zone of inhibition (mm)	Activity Index	% Inhibition	Zone of inhibition (mm)	Activity Index	% Inhibition	Zone of inhibition (mm)	Activity Index	% Inhibition
S. aureus	17.00	9.66	0.57	57	14.00	0.82	82	21.00	1.24	124
E.coli	20.00	9.33	0.49	49	16.66	0.88	88	19.17	0.96	96
E. aerogenes	18.66	8.66	0.46	46	15.66	0.84	84	19.00	1.01	101
P. aeruginosa	19.33	8.33	0.43	43	16.33	0.84	84	18.33	0.95	95
K. pneumoniae	18.83	8.33	0.45	45	15.66	0.85	85	18.50	0.98	103

Well diameter – 7 mm, Standard drug amikacin concentration – 85 µg/ml

SCE - *S. Cumini* extract, AgNPs - Silver nanoparticles

IV. CONCLUSION

Silver nanoparticles with spherical shapes and varying sizes (8-75 nm) were synthesized using aqueous seed extract of *Syzygium cumini*. Silver nanoparticles were characterized by UV-Visible, FTIR, PSA and TEM measurements. Green synthesis of silver nanoparticles using resources like *Syzygium cumini* is a better alternative to chemical synthesis, since this method of synthesis is pollutant free and eco-friendly. The results suggested that *Syzygium cumini* seed extract plays an important role in the reduction of silver and stabilization of synthesized silver nanoparticles. Study also found that these biosynthesized nanoparticles show potent antibacterial activity on gram-negative as well as gram-positive bacteria and should be explored further for antimicrobial applications.

V. ACKNOWLEDGMENT

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