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Green Synthesis of Silver Nanoparticles Using Aqueous Solution of *Syzygium cumini Flowering* Extract and its Antimicrobial Activity

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ABSTRACT

A simple, green method is described for the synthesis of silver nanoparticles (AgNPs) from the extract of Syzygium cumini flowering as capping and reducing agent. The AgNPs were characterized by a ultraviolet-visible spectrometer, infrared spectroscopy, X-ray diffraction analysis and scanning electron microscopy. Further, these NP exhibited potent antibacterial and moderate antifungal activity toward tested strains than the standard drugs.

Key words: *Silver nanoparticles, X-ray diffraction, Scanning electron microscopy.*

1. INTRODUCTION

Nowadays nanotechnology is gaining profoundness owing to its increase of vital role in most dynamic areas of research in modern materials science. Recently is has broadened the scope of elevating research in various scientific disciplines due to their unique properties compared with bulk counterparts. Metal nanoparticles (NP) have been of great interest due to their distinctive features such as catalytic, optical, magnetic, and electrical properties [1-3]. A number of methods including physical and chemical methods [4-7], electrochemical reduction [8,9], photochemical reduction [10,11] and heat evaporation [12,13] have been used for the synthesis of silver.

In particular, silver NP (AgNPs) have expunged a considerable attention in present tremendous research due to their exquisite fascinating spectrum of physical properties such as catalytic, optical, electrical and antimicrobial applications [14,15], and their significant potential selective activity in a very wide range of applications. Simple, cost effective, easily scaled up for large-scale synthesis, without using toxic and redundant chemicals in the solid, gaseous and liquid form [16]. Of these, a biological method is named green synthesis of AgNPs and does not release harmful byproducts in the environment [17]. Colloidal silver is of particular interest because of distinctive properties, such as good conductivity, chemical stability, catalytic and antibacterial activities.

In the present study, we report the biogenic synthesis of AgNPs using an aqueous flowering extract of *Syzygium cumini* to investigate the biomolecules responsible for the synthesis of AgNPs. *S. cumini* trees start flowering from March to April. The flowers of are fragrant and small, about 5 mm in diameter. The fruit has a combination of sweet, mildly sour and astringent flavor and tends to color the tongue purple. The pulp of the fruit, extracts from the bark and seeds is of great benefit when it comes to lowering of blood glucose level. Taking dried extract of the seeds orally greatly reduces the blood sugar and glucosuria. *S. cumini* has been known to have antibacterial and antifungal activities. In addition, the green synthesized AgNPs reveals excellent antibacterial and antifungal effect against clinical isolates of bacterial pathogens, Gram-positive and Gram-negative.

2. EXPERIMENTAL

2.1. Characterization

The ultraviolet-visible (UV-Vis) absorption spectra of the transparent colloid solution were performed on UV-Vis spectrometer (Shimedzu 2400 UV-Vis double beam model) at a resolution of 1 nm in 200-800 nm wavelength range. The phase purities of as synthesized compounds were checked by X-ray diffraction (XRD) technique. The XRD measurements were recorded on a Seifert 3003 TT X-ray diffractometer with Cu Kα radiation with a wavelength of 1.52 Å. The quantitative elemental analysis of the NP were carried out an Oxford instruments Inca Penta FET × 3 energy dispersive spectrum. The Fourier transforms infrared (FTIR) spectra of AgNPs and *S. cumini* flowering extract was carried out with a Thermo Nicolet FTIR-200 Thermo Electron Corporation.

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2.2. Preparation of Extract from *S. cumini* Flowering

S. cumini flowering are thoroughly rinsed with double distilled water to remove the fine dust particles and later, the *S. cumini* flowering is dried under shade at room temperature for 24 h under dust free condition. Dried *S. cumini* flowering grinded with a mortar and pestle to make a powder. An amount of 10 g of *S. cumini* flowering powder is mixed into 100 ml double distilled water and refluxed for 1 h, at 80°C until the color of aqueous extract solution changes from watery to pale yellow. The resultant composition is cooled to room temperature and filtered with a Whatman No. 1 filter paper and the final extract is stored at −4°C for further use.

2.3. Green Synthesis of AgNPs

A 30 ml of 1 mM aqueous solution of silver nitrate was taken in Erlenmeyer flask and 5.0 ml and 10 ml of *S. cumini* flowering extract was added to it separately at room temperature and stirred for 1 h. Then the reaction flask was kept at room temperature for overnight. Finally, the color of solution changed from pale yellow to dark brown. The AgNPs present in the solution were confirmed by the dark brown color. As the present method for synthesis of AgNPs with *S. cumini* flowering extract in aqueous solution was made without any additional hazardous chemicals, this pathway satisfies pure green eco-friendly process.

2.4. Antimicrobial Activity

The synthesized AgNPs were screened for their antimicrobial activity determined by well plate method [18]. The potentiality of the AgNPs as antimicrobials was appraised for their antimicrobial studies against various Gram-positive such as *Staphylococcus aureus* and *Bacillus subtilis* and Gram-negative such as *Escherichia coli* and *Klebsiella pneumoniae* strains of human pathogens. The result obtained as zone of inhibition (mm) was presented in Table 1. The antibacterial effect of AgNPs at various concentrations (5, 10, 25, and 50 µg/ml) was quantitatively assessed on the basis of zone of inhibition. Ciprofloxacin was used as standard antibacterial agent whereas the fluconazole was used as an antifungal agent and both are prepared as described in the related references. Dimethyl sulfoxide was also taken in a control experiment which showed no effect in the experiment.

3. RESULTS AND DISCUSSION

3.1. UV-Vis Analysis of AgNPs

Figure 1 shows the formation of AgNPs was indicated by the change in color of the reaction mixture from pale yellow to dark brown and this color change was due to surface plasma resonance and reduction of silver ions by *S. cumini* flowering extract. In this study, the UV-Vis spectrum of AgNPs was recorded at 24 h which showed a single broad peak with the λ max at 435 nm corresponding to the surface plasmon resonance of AgNPs.

3.2. FTIR Characterization

The synthesis solutions of AgNPs in each case contained many molecules and some of these become adsorbed on the surface of AgNPs. FTIR analysis was conducted to further demonstrate the successful

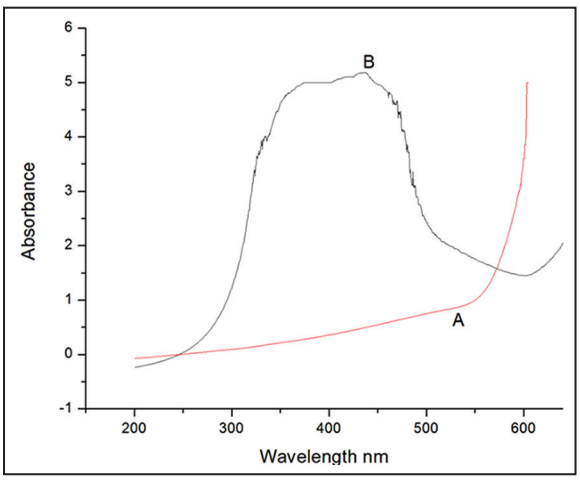

Figure 1: Ultraviolet-visible absorption spectra of the flowering extract (a) and synthesized silver nanoparticles (b).

Table 1: Antimicrobial activity.

Nanoparticles	Zone of inhibition (mm)											
	Antibacterial activity								Antifungal activity			
	Gram-positive bacteria				Gram-negative bacteria				*A. niger*		*C. albicans*	
	S. aureus		*B. subtilis*		*E. coli*		*K. pneumonia*					
Concentrated (µg/ml)	5	10	5	10	5	10	5	10	5	10	5	10
AgNPs	4.8	8.6	4.9	8.2	6.0	8.9	6.1	9.3	2.0	3.4	2.1	3.6
Ciproflaxcin	5.2	8.4	5.4	8.3	5.1	8.2	5.2	8.2	–	–	–	–
Fluconazole	–	–	–	–	–	–	–	–	2.4	4.0	2.6	4.1
Blank	–	–	–	–	–	–	–	–	–	–	–	–

–: Indicates no effect, AgNPs=Silver nanoparticles, *A. niger=Aspergillus niger, S. aureus=Staphylococcus aureus, B. subtilis=Bacillus subtilis, E. coli=Escherichia coli, K. pneumonia=Klebsiella pneumoniae*

conjugation of some such molecules associated with AgNPs. The FTIR spectra of *S. cumini* flowering extract AgNPs are shown in Figure 2. FTIR spectra were carried out to identify the potential biomolecules *S. cumini* flowering responsible for the reduction and capping of the bioreduced AgNPs. Some pronounced absorbance bands were observed at around 3321 cm^{-1} (N–H stretching), 2126 cm^{-1} (aliphatic alkynes) and 1637 cm^{-1} (carbonyl groups), suggest the presence of proteins on the surface of Ag particles. The absorption peaks were located at 3321 cm^{-1} the peaks corresponding to presence of fatty acids, carbonyl groups, flavanones, and amide band of proteins.

3.3. XRD Analysis

The green synthesized AgNPs are highly crystalline with diffraction peaks could be obviously assigned to the face-centered cubic (FCC) phase of metallic silver. Figure 3 shows five main characteristic diffraction peaks for Ag were observed at 2θ values of 38.32°, 44.56°, 64.45°, and 76.12° are indexed to the (111), (200), (220), and (311) reflections of the FCC structure of metallic silver. The average grain size of the AgNPs formed in the bio reduction process was determined using Scherr's formula:

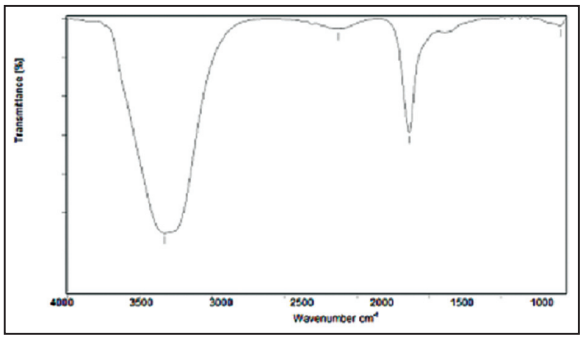

Figure 2: Fourier transforms infrared spectrum of synthesized silver nanoparticles.

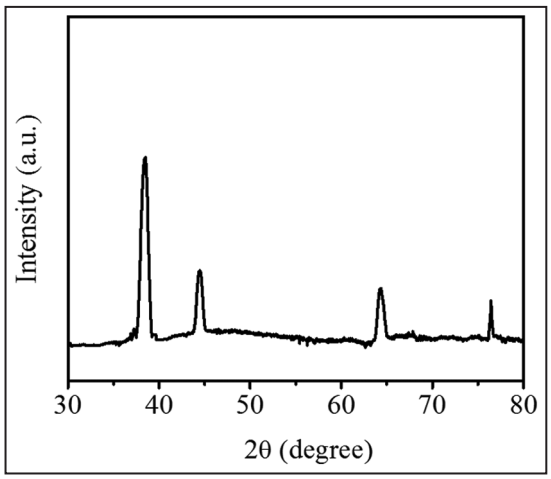

Figure 3: X-ray diffraction pattern of silver nanoparticles synthesized using *Syzygium cumini* flowering extract.

$$D = 0.89\ \lambda/\beta\ \text{Cos}\theta$$

Where, D is the average particle size, λ is the wavelength of the X-ray, β is the full width at half maximum intensity of the diffraction peak and θ is diffraction angle of the (111) plane of cubic AgNPs and the calculated he bio reduction of silver ion to elemental silver.

3.4. Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDX) Analysis

The morphology and size of the synthesized AgNPs were determined by SEM images and they are shown in Figure 4. The particles formed were spherical in shape. The nanospherical formed where shown to have high surface area. Formed NP was in the range of 5-20 µm in size with 10 µm average size. The particles were monodisperse, with only a few particles of different size the result of EDX analysis was shown in Figure 5. This confirmed the significant presence of elemental silver. The above results indicate the spherical shape and elemental silver formed by a facile manner.

3.5. Antimicrobial Activity

From the data, the NP synthesis by green method was found harmful against four bacterial species such as Gram-positive; *S. aureus* and *B. subtilis* and Gram-

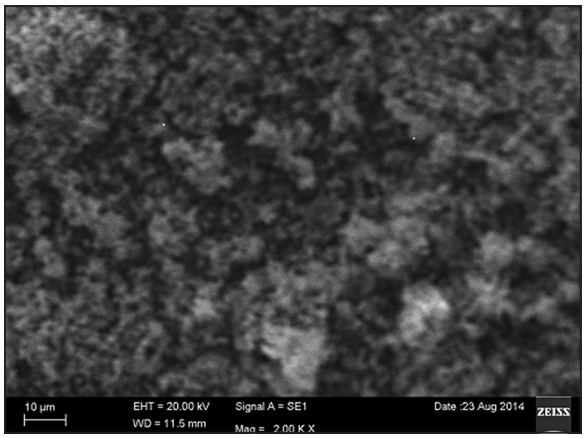

Figure 4: Scanning electron microscope image of synthesized silver nanoparticles.

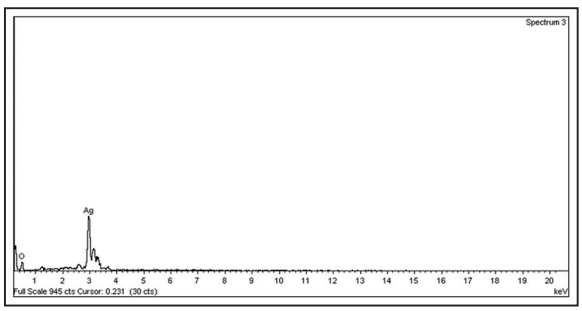

Figure 5: Energy dispersive X-ray spectroscopy (energy dispersive spectrum) image of synthesized silver nanoparticles.

negative; *E. coli* and *K. pneumoniae.* And it revealed higher antibacterial activity against *K. pneumoniae* whereas intermediated activity was shown against.

S. aureus, B. subtilis and *E. coli.* In addition, that the synthesized AgNPs reveal moderate antifungal activity. These results also suggest that green synthesized AgNPs were very much useful in various biomedical and biotechnological applications.

4. CONCLUSION

We have established that were synthesized form extract of *S. cumini* flowering in a quick method by using environmentally and eco-friendly green synthesis. These NP are found to be highly crystalline as evidenced by the peaks in the XRD pattern corresponding to Bragg reflections from the (111), (200) and (220) planes of the FCC structure. The average size of the particle is found to be 10 μm from SEM image analysis.

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***Bibliographical Sketch**

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