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# CHARACTERIZATION AND APPLICATIONS OF SILVER NANOPARTICLES SYNTHESIZED FROM *SYZYGIUM CUMINI*

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## ABSTRACT

The present study exhibit a simple environmentally beginning method of synthesis of silver nanoparticles from stem bark *S. cumini*. This method can be further used for industrial production of nanoparticles at room temperature and with a single step. The synthesized nanoparticle size range from 20 – 60 nm was confirmed in SEM analysis. Their functional groups were identified by FTIR spectrum. The nanoparticles thus synthesized shows antimicrobial activity, so they can be used in various fields such as pharmaceutical industry and so on. Silver nanoparticles might be useful for the development of newer and more potent antimicrobial agents. The data represented in our study suggested that nanoparticles of the bark extract possess significant biological activity than crude bark of *S. cumini.*

**KEYWORDS:** *Syzygium cumini*, Silver nanoparticles, Antimicrobial activity.

## INTRODUCTION

Nanoscience has been established recently as a new interdisciplinary science. It can be defined as a whole knowledge on fundamental properties of nano-size objects (Sergeev and Shabatina, 2008). The prefix ‘nano’ indicates one billionth or $10^9$ units. The nature of this unit being determined by the word that follows. It is widely accepted in the context of nanoscience and nanotechnologies, the units should only be those of dimensions, rather than of any other unit of scientific measurement. It is widely agreed that nanoparticles are clusters of atoms in the size range of 1–100 nm. The results of nanoscience are realized in nanotechnology as new materials and functional facilities. Frequently, nanometer-size metallic particles show unique and considerably changed physical, chemical and biological

properties compared to their macro scaled counterparts, due to their high surface-to-volume ratio. Thus, these nanoparticles (NP) have been the subject of substantial research in recent years (Sharma *et al.*, 2009; Iglesias-Silva *et al.*, 2007; Huang and Yang, 2005).

Metallic nanoparticles exhibit size and shape-dependent properties that are of interest for applications ranging from catalysts and sensing to optics, antibacterial activity and data storage (Sudrik *et al.*, 2006; Choi *et al.*, 2007; Yoosaf *et al.*, 2007; Vilchis-Nestor *et al.*, 2008). Metals such as gold (Au), platinum and silver (Ag) are considered as models that were extensively used for the synthesis of nanoparticles (NP). For instance, the antibacterial activity of different metal nanoparticles such as silver colloids is closely related to their size; the smaller the silver nuclei and the higher antibacterial activity. Moreover, the catalytic activity of these nanoparticles is also dependent on their size as well as their structure, shape, size distribution and chemical–physical environment. Thus, control over the size and size distribution is an important task. Generally, specific control of shape, size and size distribution is often achieved by varying the synthesis methods, reducing agents and stabilizers (Zhang *et al.*, 2006).

Application of green technology has reduced the use of has arduous reagents and solvents, improved the material and energy efficiency of chemical processes, and enhanced the design of non-toxic products. Employing these principles toward nanoscience would facilitate the production and processing of inherently safer nanomaterials and nanostructured devices (Dahl *et al.*, 2007). Therefore, there is a growing need to develop eco-friendly processes for nanoparticles synthesis that do not use toxic chemicals. In the present study to synthesis silver nanoparticles, reducing the silver ions present in the solution of silver nitrate by the cell free aqueous extract of *Syzygium cumini* bark and evaluated their antimicrobial activity.

## MATERIALS AND METHODS

### Collection of plant materials

The barks of *Syzygium cumini* were collected from Tholagiripatti, Thanjavur district, Tamil Nadu and India. The barks were rinsed with water thrice followed by distilled water to remove the fine dust materials and then, the bark were dried for 1 week to completely remove the moisture.

**Preparation of alcoholic extract**

The bark of *S.cumini* were first washed well and dust was removed from the barks. The barks were coarsely powdered. The powder was extracted with water and 70% methanol for 24 hours. The extract was stored in refrigerator until used.

**Phytochemical screening**

Phytochemical tests were carried out on the alcoholic extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973, 1984).

**Synthesis of silver nanoparticles (AgNPs)**

Two different conical flasks were taken and add 5 ml of *Syzygium cumini* bark extract separately. 45 ml of 1 mM aqueous $AgNO_3$ solution added to the each conical flask. The flask was then incubated in the dark at 4 hrs (to minimize the photo activation of silver nitrate), at room temperature. A control setup was also maintained without bark extract. The Ag nanoparticle solution thus obtained was purified by repeated centrifugation at 10,000 rpm for 15 min followed by re-dispersion of the pellet in de-ionized water. Then the Ag nanoparticles were freeze dried using SEM analysis (Arunachalam *et al*., 2012).

**UV-Visible analysis**

The extracts were examined under visible UV-Visible spectrum. The sample is dissolved in name of the solvent. The extracts were scanned in the wavelength ranging from 330-920 nm using Systronic Spectrophotometer. These solutions were scanned in turn at intervals of 10 nm and the characteristic peaks were detected. The peak value of the UV-Visible was recorded.

**Fourier Transform Infrared (FTIR) Spectroscopic Analysis**

Spectra were obtained with the aid of an OMNI-sampler attenuated total reflectance (ATR) accessory on a FTIR spectrophotometer (Perkin Elmer Spectrophotometer system, USA) followed by previous methods with some modification (Liu *et al*., 2006). A small amount of liquid of silver nanoparticle was respectively placed directly on sample holder of the infrared spectrometer with constant pressure applied and data of infrared absorbance, collected over the wave number ranged from 4000 $cm^{-1}$ to 400 $cm^{-1}$ and computerized for analyses by using the 21 CFR part 11 software. The reference spectra were acquired from the cleaned blank

crystal prior to the presentation of each sample replicate. The peak values of FTIR were recorded. Each and every analysis was repeated twice and confirmed the spectrum.

**SEM analysis of silver nanoparticles**

Scanning electron microscopic (SEM) analysis was done using VEGA3 LMU machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid. Extra solution was removed using a blotting paper and then the films on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

**Determination of antimicrobial activity**

Antibiogram was done by disc diffusion method (Awoyinka *et al.*, 2007) using bark extracts. Petri plates were prepared by pouring 30 ml NA medium for bacteria. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mints. The surfaces of media were inoculated with bacteria from a broth culture. A sterile cotton swab is dipped into a standardized bacterial test suspension and used to evenly inoculate the entire surface of the Nutrient agar plate. Briefly, inoculums containing *Escherichia coli* and Staphylococcus aureus were spread on nutrient agar plates for bacteria strains. Using sterile forceps, the sterile filter papers (6 mm diameter) containing each 30µl of plant extract, AgNPs and Standard solution as Chloromphenical were laid down on the surface of inoculated agar plate. Each sample was tested in triplicate. The antimicrobial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the extracts were measured using a millimeter scale.

**RESULTS AND DISCUSSION**

Plants synthesize an array of chemical compounds that are not involved in their primary metabolism. These ‘secondary compounds’ instead serve a variety of ecological functions, ultimately to enhance the plants survival during stress. In addition these compounds may be responsible for the beneficial effects of fruits and vegetables on an array of health related measures. Medicinal plants are assumes greater importance in the primary health care of individuals and communities in many developing countries. There has been an increase of demand in international trade because of very effective, cheaply available, supposedly have no side effects and used as alternative to allopathic medicines. Medicinal plants are believed to be much safer and proved elixir in the treatment of various ailments (Liu, 2003).

**Preliminary phytochemical analysis**

The present study was carried out on the bark sample revealed the presence of medicinally active constituents. The phytochemical screening aqueous extract of *Syzygium cumini* showed that the presence of tannin, saponins, steroids, terpenoids, triterpenoids, anthriquinone, polyphenol and glycosides. While phlobatannins, Flavonoids, alkaloids, carbohydrate and protein were absent. Methanol extract of *Syzygium cumini* showed that the presence of saponins, flavonoids, steroids, terepenoids, triterpenoids, carbohydrate, protein, anthriquinone and polyphenol. While tannin, phlobatannins, alkaloids and glycosides were absent (Table 1).

**Table. 1: Phytochemical screening of bark of *Syzygium cumini.***

| S.No | Phytochemical analysis | Aqueous | 70% Methanol |
|---|---|---|---|
| 1 | Tannin | + | - |
| 2 | Phlobatannins | - | - |
| 3 | Saponin | + | + |
| 4 | Flavonoids | - | + |
| 5 | Steroids | ++ | + |
| 6 | Terpenoids | + | + |
| 7 | Tritrpenoids | + | + |
| 8 | Alkaloids | - | - |
| 9 | Carbohydrate | - | + |
| 10 | Protein | - | + |
| 11 | Anthroquinone | ++ | + |
| 12 | Polyphenol | + | + |
| 13 | Glycoside | + | - |

(+) Presence (-) Absence.

**Synthesis of silver nanoparticles**

The green synthesis of silver nanoparticles through plant extracts were carried out. Silver nitrate is used as reducing agent as silver has distinctive properties such as good conductivity, catalytic and chemical stability. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other inorganic materials (nanomaterials). The aqueous silver ions when exposed to herbal extracts were reduced in solution, there by leading to the formation of silver hydrosol. The time duration of change in colour varies from plant to plant. The phytochemicals present in the grain extract were considered responsible for the reduction of silver ions. It is well known that silver nanoparticles exhibit yellowish - brown colour (Fig 1) in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles (Thirumurgan *et al.*, 2010). The appearances of yellowish-brown colour

in the reaction vessels suggest the formation of silver nanoparticles (SNPs) (Shankar *et al.*, 2004).


**Fig.1: Aqueous solution of 1mM $AgNO_3$ (B) Silver nanoparticles.**

**Ultraviolet/visible (UV/VIS) spectroscopy**

It is generally recognized that UV–Vis spectroscopy could be used to examine size and shape-controlled nanoparticles in aqueous suspensions. Figure 2 shows the UV-Vis spectra recorded from the reaction medium after 5 hours. The UV–vis spectra of the reaction mixture of silver nitrate solution with *Syzygium cumini* leaf extract at the peaks observed at 420nm indicate the presence of silver nanoparticles which is synthesized by *Syzygium cumini* extract, the peak was raised due to the effect of surface plasmon resonance of electrons in the reaction mixture and the broadening of peak indicated that the particles are polydispersed. Appearance of this peak assigned to a surface plasmon, is well-documented for various metal nanoparticles with size ranging from 2 nm to 100 nm (Henglein, 1993).

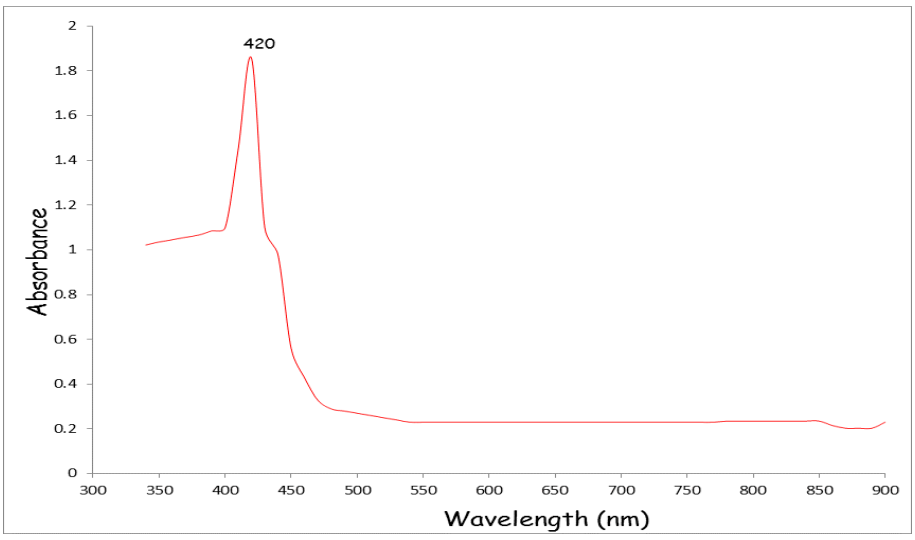


**Fig. 2: UV-Visible spectrum of AgNPs synthesized from *Syzygium cumini* extract.**

**FTIR Spectroscopic analysis of silver nanoparticle**

FTIR is an important tool which enables us to understand the involvement of functional groups in the interactions between metal particles and biomolecules. In the present work, FTIR spectra are used in the identification of biomolecules responsible for capping and stabilizing the silver nanoparticles. FTIR spectrum of *Syzygium cumini* Fig 3, extract shows bands at 668, 1313, 1407, 1531, 1645, 2064, 2767, 3398 and 3464. The FTIR spectra of the *Syzygium cumini* is given in the Fig 3 which show the presence of silver nanoparticles, peak at 3464cm$^{-1}$ which are assigned as – OH stretching in alcohols and phenolic compounds. The band appeared at about 1645 cm$^{-1}$can be assigned for aromatic rings. The strong broad band appearing at 3398 cm$^{1}$can be associated to the stretching vibrations of alcoholic and phenolic O–H. At 1313cm$^{-1}$a peak is observed that could be for plant described to multiplet C=O group.

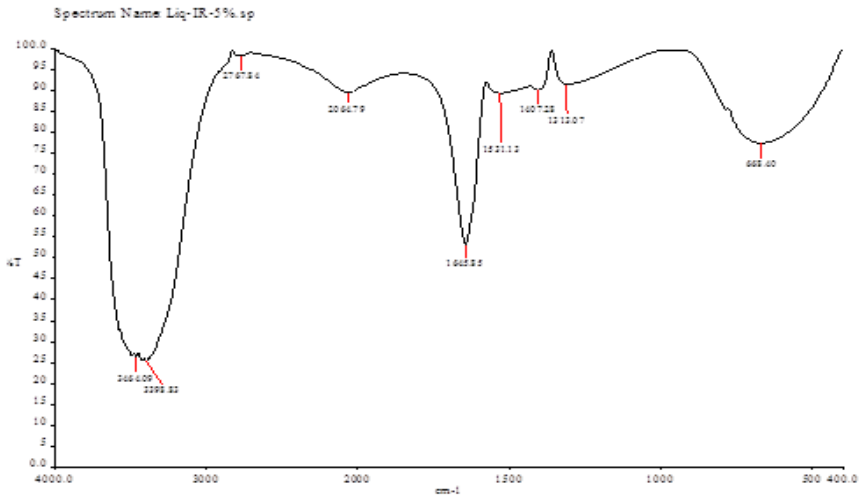


**Fig.3: FTIR Spectroscopic analysis of silver nanoparticle**

**SEM analysis**

SEM analysis was carried out to understand the topology and the size of the Ag-NPs, which showed the synthesis of higher density polydispersed spherical Ag-NPs of various sizes. The SEM image showing the high density silver nanoparticles synthesized by the bark of *S. Cumini* was further confirmed the development of silver nanostructures. Most of the nanoparticles aggregated and only a few of them were scattered, as observed under SEM. The SEM analysis showed the particle size between 20-60nm as well the cubic, face-centred cubic structure of the nanoparticles. In the present investigation the peak was decreased due to destabilization of nanoparticles (Fig 4).

Silver nanoparticles are being extensively synthesized using many different biological sources including fungi, bacteria and plants (Shivaji *et al.*, 2011; Shaligram *et al.*, 2009). Among them the plant mediated nanoparticles synthesis is getting more popular because of the high reactivity of plant extract and easy availability of plant materials. This method of nanoparticles synthesis involves no toxic chemicals and termed as green chemistry procedure. In this present study, bark of *S. Cumini* extract was used for the synthesis of silver nanoparticles. The aqueous $AgNO_3$ solution turned to brown colour formed after 4 hours with the addition of bark extract indicating the formation of AgNPs in the reaction solution probably as a result of the excitation of Surface Plasmon Resonance (SPR) bands (Mulvaney, 1996). The control tubes ($AgNO_3$) showed no change in colour when incubated in a similar condition.

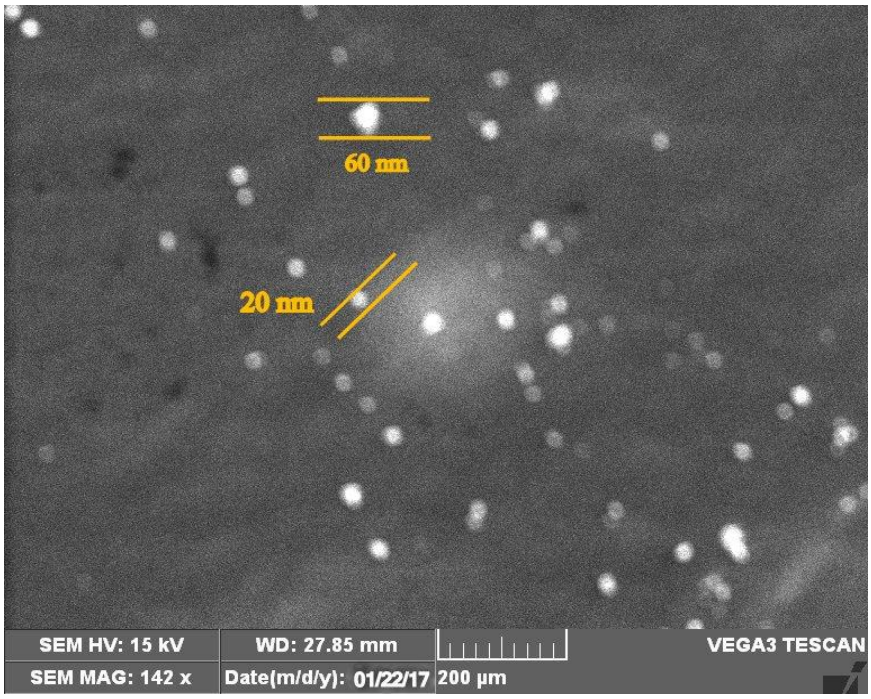


**Fig.4: High resolution scanning electron microscopic (SEM) image of silver nanoparticles (AgNPs). Polydispersed AgNPs ranged between 20–60 nm.**

**Antimicrobial activity**

Silver nanoparticles the stembark of *Syzygium cumini* has maximum inhibitory activity against *E.coli*, than *Staphylococcus aureus* Table 2.

**Table. 2: Antimicrobial activity the bark of S. *cumini* AgNPs**

| Zone of inhibition in diameter (mm) | | | | |
|---|---|---|---|---|
| **Microorganism** | **AgNPs** | **$AgNO_3$** | **Extract** | **Std.** |
| *Escherichia coli* | 12.20±0.85 | 10.50±0.73 | 10.70±0.74 | 15.50±1.08 |
| *Staphylococcus aureus* (mm) | 2.82±0.16 | 6.97±0.40 | 0.95±0.08 | 7.26±0.52 |

Values were expressed as Mean ± SD.

AgNPs = Silver Nanoparticles; $AgNO_3$ = Silver nitrate

Bacterial standard - Chloramphenicol

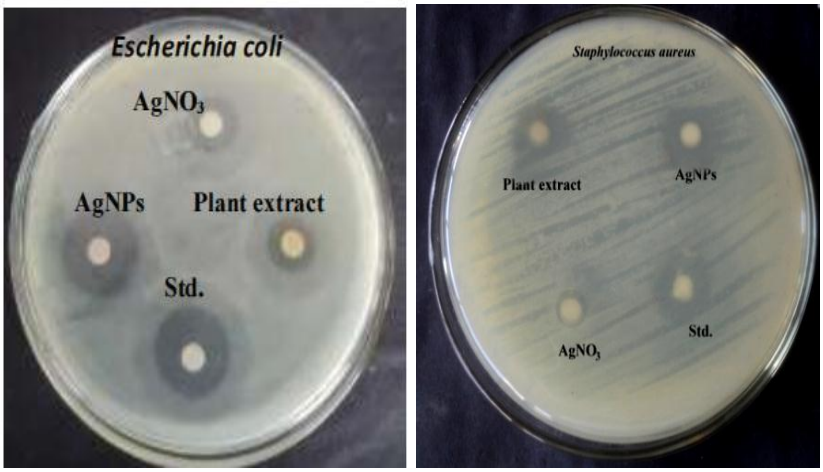


**Fig.5: Shows the antibacterial activity the bark of *S. cumini* AgNPs**

Toxicity studies on pathogen opens a door for nanotechnology applications in medicine. Biological synthesis of metal NPs is a traditional method and the use of plant extract has a new awareness for the control of disease, besides being safe and no phytotoxic effects (Torresdey *et al*., 2003). The biologically synthesized silver nanoparticles using medicinal plants were found to be highly toxic against different pathogenic bacteria of selected species. The SNPs of shows highest antibacterial activity than *S.cumini* was observed against *E. coli* and *Staphylococcus aureus* The inhibitory activities in culture media of the Ag nanoparticles reported in table 2 comparable with standard antimicrobiotic viz. chloromphenical.

In this study to evaluate the antimicrobial effect of Ag nanoparticles against *E. coli* and *Staphylococcus aureus*. When silver nanoparticles were tested they effectively inhibited bacterial growth. The results show that Ag nanoparticles having antimicrobial activity against *E. coli* and *Staphylococcus aureus* that was similar to that found by Sondi and Salopek-Sondi (2004).

These results suggest that the antimicrobial effects of Ag nanoparticles may be associated with characteristics of certain bacterial species. The growth of microorganisms was inhibited by the green synthesized SNPs showed variation in the inhibition of growth of microorganisms may be due to the presence of peptidoglycan, which is a complex structure and after contains teichoic acids or lipoteichoic acids which have a strong negative charge. This charge may contribute to the sequestration of free silver ions. Thus gram positive bacteria may allow less silver to reach the cytoplasmic membrane than the gram negative bacteria (Ahmad *et al.*, 2011). We think that the lower efficacy of the Silver nanoparticles against *E. coli* and *Staphylococcus aureus* may derive from the difference as a point of membrane structure. To confirm this hypothesis, further comparative study between various gram-negative and gram-positive bacterial species is needed. The peptidoglycan layer is a specific membrane feature of bacterial species and not mammalian cells. Therefore, if the antibacterial effect of Ag nanoparticles is associated with the peptidoglycan layer, it will be easier and more specific to use Ag nanoparticles as an antibacterial agent. The AgNPs synthesized from plant species are toxic to multi drug resistant microorganisms. It shows that they have great potential in biomedical applications.

The present study exhibit a simple environmentally beginning method of synthesis of silver nanoparticles from stem bark *S. cumini*. This method can be further used for industrial production of nanoparticles at room temperature and with a single step. The synthesized nanoparticle size range from 20 – 60 nm was confirmed in SEM analysis. Their functional groups were identified by FTIR spectrum. The nanoparticles thus synthesized shows antimicrobial activity, so they can be used in various fields such as pharmaceutical industry and so on. Silver nanoparticles might be useful for the development of newer and more potent antimicrobial agents. The data represented in our study suggested that nanoparticles of the bark extract possess significant biological activity than crude bark of *S. cumini.*

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