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Green Synthesis of Silver Nanoparticles by using Syzygium cumini and its Characterization, and Evaluation of the Antimicrobial Activity

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Abstract: An extremely worrying and alarming increase in the level of multiple drug resistance is reported in Asia, in which bacterial strains are becoming resistant to many commonly available antibiotics. Eventually, it is becoming extremely difficult to treat debilitating infections. In search of promising solutions to this arising crisis, *Syzygium cumini* silver nanoparticles were synthesized using the green synthesis method. The synthesis of the *Syzygium cumini* silver nanoparticles is confirmed using analytical methods as ultraviolet-visible spectroscopy, X-ray diffractometer, and scanning electron microscopy. Using the ultraviolet visible spectroscopy, an absorption band of around 430 nm was observed. Furthermore, scanning electron microscopy revealed the presence of silver nanoparticles which fell within the range of 1–100 nm, and X-ray diffractometer analysis showed three intense peaks with a maximum intense peak at 24.3 theta. Nanoparticles distribution between 12 nm and 64 nm was observed with an average diameter of 18.115 nm. The synthesized nanoparticles showed antimicrobial activity against pathogenic organisms. The obtained physicochemical properties were correlated with the antibacterial activity of the silver nanoparticles.

***Index Terms* - Green Synthesis, Silver Nanoparticles, *Syzygium cumini*, Antimicrobial Activity**

Introduction

Nanotechnology is a rapidly expanding field with undeniable benefits in the pharmaceutical industry over the last decade. Because nanoparticles vary in shape, size, and physical properties, they are emerging as one of the most important trends in pharmaceutical practice today. The use of nanoparticles is strongly recommended by current research trends, which can be attributed to their Nano-size, which is estimated to be one billionth (109). Nanoparticles are known to have at least one dimension in the 1-100 nm nanoscale range [1]. Silver nanoparticles' unique physicochemical properties have led to a wide range of applications in fields such as biomedicine, catalysis, and energy storage. Furthermore, silver nanoparticles are well known for their broad antimicrobial and anticancer spectrum. Other medical applications such as wound repair, bone healing, dental applications, vaccine adjuvants, and antidiabetic agents would be implicated [2].

Chemical, physical, and biological methods would be used to create silver nanoparticles. The use of biological methods for the synthesis of silver nanoparticles, on the other hand, has a proven economic and environmental value, namely the green synthesis method [3]. Biological molecules derived from plants and microorganisms such as bacteria and fungi have been widely used in nanoparticle synthesis [4]. Biological molecules act as in-situ reducing and capping agents capable of forming well-dispersed nanoparticles, with capping agents imparting properties such as reduced nanoparticle agglomeration and improved antimicrobial activity [5]. Silver nanoparticles are well known for their antimicrobial activity and ability to eradicate various microorganisms at extremely low doses [6]. When combined with the capping properties of the biologic moiety in plants, this intrinsic property of silver provides synergistic and enhanced antimicrobial activity. Furthermore, significant antibacterial spectrums of activity against Gram positive and Gram negative bacteria have been observed [7]. Silver nanoparticles' antibacterial activity is greatly influenced by physicochemical properties such as morphology and size. According to several studies, the smaller the size of silver nanoparticles, the greater the surface area and silver ion release [8]. The antimicrobial activity is attributed to the nanoparticles high surface-to-volume ratio [9]. Better antimicrobial activity was demonstrated when the nanoparticle size was less than 10 nm, implying an important relationship between nanoparticle size and antibacterial activity [10]. *Syzygium cumini*, commonly known as Malabar plum, Java plum, black plum, jamun, jaman, jambul, or jambolan, is an evergreen tropical tree in the flowering plant family *Myrtaceae*, and favored for its fruit, timber, and ornamental value. Many previous studies have found that *Syzygium cumini* has significant antioxidant, antibacterial, and anticancer properties [11]. Fruit of Jamun consists of various kinds of Vitamins, flavonoids, antioxidants, and phenolic compounds which are very beneficial for human health. Recent studies prove that Jambu exhibits various properties like anti-inflammatory, anti-microbial, antioxidant, anti-fungal, anti-bacterial, gastro protective, etc. [12]. It also contains free catechins, which are powerful antioxidants [13]. The purpose of this study was to synthesize, characterize, and determine the antimicrobial activity of *Syzygium cumini* silver nanoparticles formed via green synthesis [14].

1. Methodology

1.1. Preparation of Silver Nitrate Stock Solution. Making a Silver Nitrate Stock Solution Weighing 54 mg of silver nitrate powder (India) and dissolving it in 200 ml of distilled water yielded the stock of silver nitrate ($AgNO_3$) solution. The prepared stock solution was kept at room temperature in a dark place.

1.2. Preparation of Syzygium cumini Solution. *Syzygium cumini* Solution Preparation were emptied and visually inspected for impurities. On a sensitive balance, 1.5 g of jambu powder was weighed, and 250 ml of distilled water was added. It was then heated for 20 minutes in an 80°C water bath. After that, the solution was filtered. After obtaining the filtrate, it was allowed to cool at room temperature.

1.3. Synthesis of Silver Nanoparticles (AgNPs). 80 mL of extract solution was withdrawn. It was then transferred to a flask that was magnetically stirred. The stirring was kept to a bare minimum. Silver nitrate ($AgNO_3$) stock solution was added in 5 ml increments. The solution was stirred after each addition to optimize silver reduction, and the resulting electronic absorption was measured using ultraviolet visible spectroscopy

1.4. Characterization of Synthesized Silver Nanoparticles

1.4.1. Ultraviolet-Visible Spectroscopy (UV-Vis Spectroscopy). A UV spectrophotometer (Shimadzu, Japan) was used to measure the formation of *Syzygium cumini* silver nanoparticles at wavelengths ranging from 300 nm to 800 nm. 5 ml of $AgNO_3$ was added sequentially, and the silver ion reduction caused by the jambu infusion was monitored [15].

1.4.2. Scanning Electron Microscopy (SEM). The prepared silver nanoparticles (AgNP) were centrifuged at 3000 rpm, and the filtrate was dispersed in distilled water before being centrifuged again. The filtrate was spread over plastic Petri dishes and dried in the oven for 15 minutes. The dried brown deposit of silver nanoparticles was scraped off and subjected to scanning electron microscopy analysis.

1.4.3. X-Ray Diffractometry (XRD). The prepared silver nanoparticles were centrifuged using Eppendorf tubes, and the obtained supernatant layers were collected using a micropipette. The *Syzygium cumini* silver nanoparticles were characterized in powdered form using X-ray diffractometry.

1.5. Antimicrobial Activity of Synthesized Silver Nanoparticles. The synthesized silver nanoparticles were tested for their antimicrobial activity against two bacterial strains: *Staphylococcus aureus* and *Escherichia coli*.

2. Results

2.1. Visual Appearance and UV-Visible Spectroscopy. As shown in Figure 1, the green synthesis of *Syzygium cumini* nanoparticles was distinguished by a brownish-orange color. UV-visible spectroscopy was used to confirm the formation and growth of the nanoparticles. It exhibited characteristic absorbance at around 440 nm. As shown in Figure 2, this absorption band is known as the surface Plasmon resonance absorption band [16].

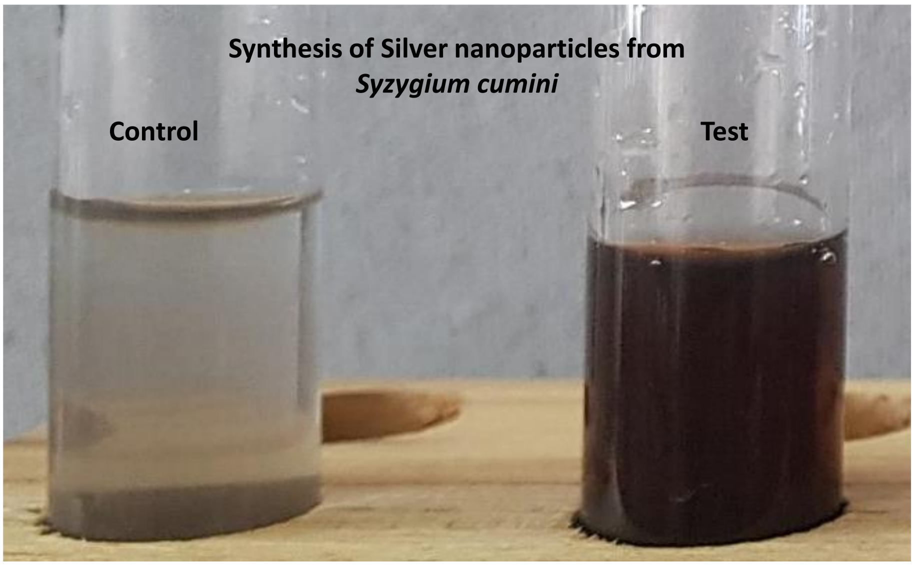

Figure: 1: Visual characterization of AgNPs from *Syzygium cumini*

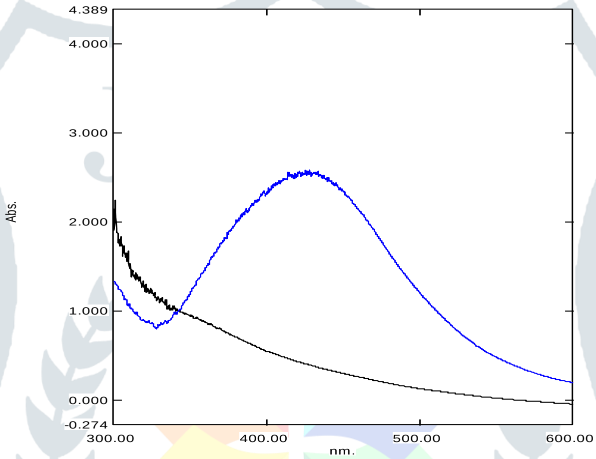

Figure: 2 SPR by UV-Vis spectroscopy of *Syzygium cumini*

2.2. *SEM Analysis of Syzygium cumini Silver Nanoparticles. Syzygium cumini* Silver Nanoparticles SEM Analysis the morphological features of the synthesized silver nanoparticles were examined using scanning electron microscopy (SEM). SEM micrographs of the synthesized silver nanoparticles are shown in Figure 3. The size was found to be within the actual size range of nanoparticles, which is 1-100 nm, when a voltage of 15 kV was applied.

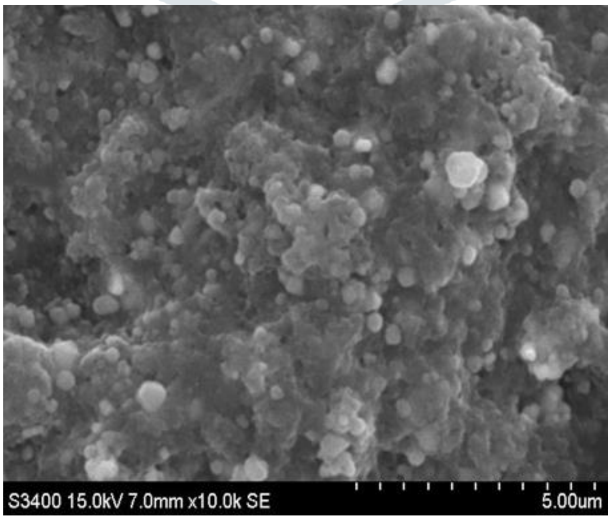

Figure: 3 SEM of AgNPs of *Syzygium cumini*

2.3. *XRD Analysis of Syzygium cumini Silver Nanoparticles.*The size distribution and crystal structure were determined using X-ray diffractometry XRD analysis. The XRD analysis yielded the following results, as shown in Figure 4. The other peaks detected by

XRD were thought to be for the other components present in the silver nanoparticle on derived from the plant extract. Diffraction peaks were observed in the XRD analysis of synthesized silver nanoparticles from leaf extract at 32.50, 38.30, 44.40, 64.60, and 76.80 and 32.40, 38.30, 44.50, 64.50, and 76.70, respectively. The obtained XRD spectrum confirmed that the synthesized silver nanoparticles were in nanocrystal form and crystalline in nature when compared to the standard. The fact that the peaks can be assigned to the planes (122), (111), (200), (220), and (311), respectively, indicates that the silver nanoparticles are face centered, cubic, and crystalline in nature.

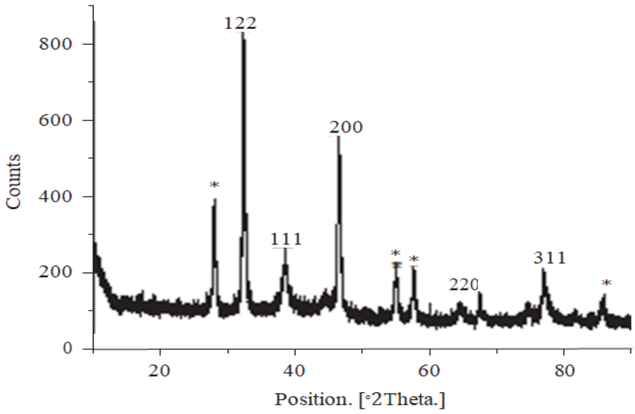

Figure: 4 XRD of AgNPs from *Syzygium cumini*

2.4. *Antimicrobial Activity of AgNP*. The antimicrobial activity of the AgNP was demonstrated using two bacterial strains, namely, *Escherichia coli* and Staphylococcus aureus. Specifically, the agar well diffusion method has been utilized for the determination of antimicrobial activity. After an incubation period of 24 hours, a zone of inhibition of 21 mm and 23 mm were obtained against *Staphylococcus aureus* and *Escherichia coli* respectively, using the positive control antibiotic (Streptomycin), a zone of inhibition of 14 mm and 12 mm were observed with S. aureus species and E. coli. As shown in Figure 5.

Escherichia coli ***Staphylococcus aerues***

Figure: 5 Antimicrobial activity

3. Discussion

Syzygium cumini silver nanoparticles were synthesized using the green synthesis method in this study. When compared to physical or chemical synthesis methods, this method was found to have significant advantages in terms of simplicity, efficacy, lower toxicity, and cost-effectiveness [17].This manuscript also described plant phytochemicals' ability to detoxify heavy metals, reducing the undesirable toxic effects of silver nanoparticles and making them a preferable choice [4]. The use of the green synthesis method is primarily due to the phytochemicals' reducing and capping properties. Multiple studies have found that the polyphenols and flavonoids found in main biomolecules responsible for the reduction of nanoparticles [18]. Furthermore, it is widely accepted that the bio compounds responsible for silver ion reduction have a significant impact on the size and size distribution of the synthesized silver nanoparticles. The formation of smaller-sized silver nanoparticles has been linked to phytochemicals with stronger reducing properties [19]. However, little is known about the effect of phytochemicals on the size and morphology of silver nanoparticles, and even less is known about the effects of size and morphology on antibacterial activity. The reducing and capping properties of inspired the creation of *Syzygium cumini* silver nanoparticles. The appearance of a dark brown color further confirmed this process. Following the addition of the metal salt, an ultraviolet-visible analysis revealed a characteristic absorption band of 412 nm. Several previous studies reported similar results, obtaining a similar characteristic absorption wavelength [20]. The surface refers to the rapid oscillations of electrons on the nanoparticle surface. Plasmon resonance has been identified as the primary cause of this characteristic wavelength. The size and shape of the silver nanoparticles play a role in this interaction [21].

The morphology and stability of *Syzygium cumini* silver nanoparticles were investigated using scanning electron microscopy. In this study, characteristic spherical clusters with a diameter of 26.9 nm were obtained. *Syzygium cumini* silver nanoparticles with sizes ranging from 10 to 20 nm were synthesized and demonstrated antimicrobial activity with similar morphological features [22].

The nanoscale crystallographic structure was investigated using X-ray diffractometry [23]. Three distinct peaks were observed, and a consistent distribution between 12 and 64 nm was obtained. The average size was used to calculate the crystallite size of 18 nm. In addition, an orthorhombic structure has been discovered. It is worth noting that the antimicrobial activity of the synthesized silver nanoparticles has been established using the disc diffusion method. Interestingly, combining the capping properties of jamu with the inherent antibacterial properties of the silver nanoparticle would result in increased antibacterial activity. The individual physicochemical properties of the silver nanoparticles, on the other hand, may have an effect on the resulting biological activity.

The obtained physicochemical properties limited the expected antimicrobial activity of the plant-mediated silver nanoparticles. In our study, we obtained particle sizes larger than 20 nm. Smaller nanoparticles have higher antibacterial activity because they have more stability and surface area, resulting in better interactions and intracellular penetration of bacterial cell walls [24]. Another study found that smaller particle size particles had greater antibacterial activity against *Escherichia coli*. [25]. This phenomenon could be explained by the high concentration of silver nanoparticles used in our study, given that both studies obtained a similar range of particle size and shape. Thus, differences in concentrations and synthesis methods may have resulted in a different level of interaction of AgNPs with the cell wall of microorganisms, resulting in variations in the spectrum of antimicrobial activity SEM analysis of the morphology of the *Syzygium cumini* silver nanoparticles revealed the presence of roughly spherical silver nanoparticles. Some research has found a link between the shape of the nanoparticles and their antibacterial activity. The antibacterial activity of silver nanoparticles is undeniable. Silver nanoparticles of various shapes were synthesized, and each shape demonstrated distinct antimicrobial activity against Escherichia coli. Because of their larger specific area, nanocubes and nanospheres had higher antibacterial activity than nanowires, which had low antibacterial activity [26]. Controlling the shape and size of silver nanoparticles allows for antimicrobial activity control [27]. Spherical, triangular plate, and disk-shaped nanoparticles were created, and their antibacterial activity against Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa was tested. According to the findings, silver spheres had the highest antimicrobial activity followed by silver disks and then silver triangles [24].

5. Conclusion

The green synthesis method was used to create *Syzygium cumini* silver nanoparticles. The nanoparticles were tested using ultraviolet-visible spectroscopy, scanning electron microscopy, and X-ray diffractometry. Antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* was demonstrated. It is clear that nanoparticles smaller than 10 nm have a larger surface area and higher activity. It should be noted that the physicochemical properties of silver nanoparticles have a significant positive impact on their intrinsic antimicrobial activity. The study adds to our understanding of the effects of nanoparticle size and shape on the claimed antimicrobial activity of *Syzygium cumini* silver nanoparticles; however, further research into the possible effects of size and morphology is strongly encouraged in the future. Furthermore, the effects of phytochemicals on physicochemical properties should be investigated in order to improve the antimicrobial properties of silver nanoparticles.

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