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# SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF LEAF, SEED AND FRUIT EXTRACTS OF *SYZYGIUM CUMINI* L.

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**Keywords:**

Phytochemicals, *Syzygium cumini* L., Antimicrobial activity, AgNPs

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**ABSTRACT:** *Syzygium cumini* L. belongs to the family Myrtaceae is one of the widely used medicinal plants in the treatment of various diseases particularly diabetes. The present study has been focused on the synthesis of silver nanoparticles of leaf, seed and fruit extracts of *S. cumini* and to observe their antimicrobial activity. The synthesized nanoparticles were characterized by performing UV-Vis spectrophotometer, SEM and TEM. UV visible spectroscopic analysis revealed the Surface Plasmon Resonance (SPR), the final reaction product confirming the reduction of $AgNO_3$ to silver nanoparticles. Spherical, triangular and cubical images are observed in SEM analysis of silver nanoparticles prepared from leaf, seed and fruit extracts of *Syzygium cumini*. TEM analysis also revealed the formation of 5 to 20 sizes of silver nanoparticles with the presence of the layer around the particle. Subsequently tested antimicrobial activity of silver nanoparticles prepared from leaf, seed and fruit extracts of *Syzygium cumini*. Nanoparticles from seed extracts showed more antibacterial activity on *Pseudomonas*, followed by *Bacillus, Klebsiella, Staphylococci* and *E. coli.* Seed extracts also showed more antifungal activity against the fungi *Rhizopus*, *Mucor*, *Aspergillus niger* and *Aspergillus flavus*. Nanoparticles prepared from leaf extracts of *S. cumini* showed less antibacterial activity than the nanoparticles from seed and fruit extracts. This study makes evidence that leaf, seed and fruit extracts of *Syzygium cumini* acts as an excellent capping agent for the formation of silver nanoparticles and showed immense biological activities. Hence, these AgNPs can be used as an antimicrobial agent in treating many medical complications.

**INTRODUCTION:** Nanotechnology is a new and promising tool in the field of phytomedicine and Pharmacognosy. Mainly deals with the development of materials of size ranging from 1 to 100 nm.

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Currently, several researchers are focusing on nano-related applications in various fields [1, 2]. Nano-sized particles ranging below 10 nm are having great significance, because of their chemical and physical behavior [3, 4]. There are different techniques available for the synthesis of silver nanoparticles like ion sputtering, chemical reduction, biological reduction, sol-gel *etc*. [5]

Different applications of nanoparticles include the fields of electronic, chemical and mechanical industries. They can act as drug carriers, sensors and pigments [6, 7].

The techniques involves for obtaining nanoparticles using naturally occurring reagents such as sugars, biodegradable polymers (chitosan, *etc*.), plant extracts, natural products, microorganisms as reductants and capping agents could be measured as an attraction for nanotechnology [8].

Nanoparticles prepared through chemical synthesis may absorb a few toxic chemicals on the surface which can make undesirable effects in their applications. So, that researchers are mainly focusing on biological metal nanoparticles synthesis is a low cost, nontoxic, high yield and environmentally friendly [9, 10]. Though there are different procedures to develop nanoparticles with desired size and shape, biological synthesis becomes very important and alternative for chemical synthesis process [11]. Nanotechnology also affords the delivery of drugs to target site and increasing the efficiency of plant-derived medicines as a novel formulations [12]. In recent years it has been gaining importance for the usage of synthesis of plant-based nanoparticles due to their solvent free nature and less toxicity. As well as their production is faster and which also cost-effective [13].

*Syzygium cumini* L., popularly known as jamolaon and jamun is one of the most generally used medicinal plant [14]. The use of natural products in disease prevention and control as well as in drug discovery and development is increasing these days. *S. cumini* has been widely used in traditional and folk medicine for the treatment of various diseases [15, 16]. To the best of our knowledge and literature study, there are no reports on the comparative analysis of synthesis and antimicrobial activity of silver nanoparticles of leaf, fruit, and seed of *Syzygium cumini* L. and was characterized. The same was also used to assess their effect on different bacteria. Therefore the present study is focused on the preparation of nanoparticles from seed, fruit and leaf extracts to test for their antibacterial activity of *Syzygium cumini* L.

**Preparation of Plant Material and Crude Extract:** The healthy and disease free mature leaves, fruits and the seed of *Syzygium cumini* L. plant material was collected from Osmania University locality, Hyderabad. Plant authentication voucher specimen number UCS-1131. Plant material is washed thoroughly under tap water and rinsed with doubled distilled water to remove dust particles. Later the *S. cumini* leaves, fruits and seeds are dried under shade at room temperature for 24 h under dust-free condition. Dried leaves, fruits and seed were ground with a mortar and pestle to make a powder. 5 grams of leaf, fruit and seed powder of *Syzygium cumini* L. was taken in a sterile conical flask separately, and 100 ml of distilled water was added to each conical flask. Then the mixer was kept on a magnetic stirrer for 24 h at room temperature for further use. 1 Molar solution of $AgNO_3$ was prepared by dissolving 169.87 grams (1 mole of $AgNO_3$) in 1 liter. 0.034 g of silver nitrate is used for the preparation of 200 ml of solution.

**Preparation of Silver Nanoparticles:** Dried powered of 10 grams of *S. cumini* leaves, fruits and seed with double distilled water are kept on a magnetic stirrer for 1 h in conical flasks, at 80 °C until the color of aqueous extract solution changes from watery to pale yellow. A 20 ml of 1 mM aqueous solution of silver nitrate was prepared and taken in Erlenmeyer flask. To the 10 ml of *S. cumini* leaves, fruits and seed 5.0 ml of 1 mM silver nitrate solution was added as dropwise manner at room temperature and stirred for 1 h.

Changing of the colour of the solution indicates the formation of silver nanoparticles also confirms, the color of the solution changed from pale yellow to pink and then to dark brown. The resultant solution is kept in the dark for overnight incubation. Subsequently centrifuged separately and washed with deionized water for two times. Dried samples are kept in a hot air oven for drying. After complete drying, the samples are preserved for further use. This method for synthesis of AgNPs with *S. cumini* leaf, seed and fruit extracts in aqueous solution without any harmful chemicals satisfies pure green, eco-friendly process.

**Characterization of Silver Nanoparticles:** The silver nanoparticles prepared by reducing the silver ion solution with leaves, fruits and seed extracts of *Syzygium cumini* were characterized using UV-visible spectroscopy (Shimadzu Model No. UV – 1800, ENG 240V, soft), Scanning electron microscope (Zeiss, Germany) and ultra-high-resolution transmission electron microscope

(Model No. h-7500, Hitachi) following standard procedures.

**Antimicrobial Activity:** The synthesized AgNPs were screened for their antimicrobial activity determined by disc diffusion method [17, 18]. The potentiality of the AgNPs as antimicrobials was appraised for their antimicrobial activity studies. About five bacterial and four fungal organisms are employed to test antimicrobial activity of normal and silver nanoparticles of fruit, leaf and seed extracts of *Syzygium cumini* L. The bacterial types, *Staphylococcus aureus, Pseudomonas, Bacillus subtilis, Escherichia coli,* and *Klebsiella pneumoniae* strains were used. The fungal types *Mucor, Rhizopus, Aspergillus niger* and *Aspergillus flavus* were tested for antimicrobial activity. The antibacterial effect at various concentrations (10, 25, 50 and 75 µg/ml) was quantitatively assessed as the zone of inhibition. Gentamycin 10 µg/ml was used as a standard for bacterial and Ketoconazole 10 µg/ml as a control for fungi.

## RESULTS AND DISCUSSION:

**UV-Visible Spectroscopy Analysis:** Samples (1 ml) of the suspension were collected and scanned in UV-visible spectra, between wavelengths of nm in a spectrophotometer having a resolution of 1 nm with time intervals of 0, 2, 4, 6, 8, 10, 12, 24 h. There is an increase in the intensity of absorption peaks after regular intervals of time and the color intensity also increased depending on the incubation period. It was observed from **Fig. 1**.

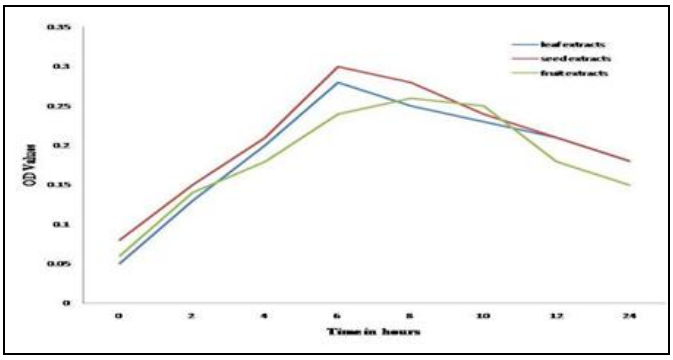



**FIG. 1: UV SPECTROSCOPY ANALYSIS OF NANOPARTICLES OF *SYZYZIUM CUMINI* L. AT TIME INTERVALS**

**SEM Analysis:** The SEM images of the AgNPs are shown in **Fig. 2**. Leaf, seed and fruit extracts of *Syzygium cumini* formed approximately spherical, triangular and cubical AgNPs, respectively. This may be due to the availability of different quantity and nature of capping agents present in the different leaf, seed and fruit extracts. SEM image showed surface morphology of high-density silver nanoparticles which reveals encapsulation of the silver.

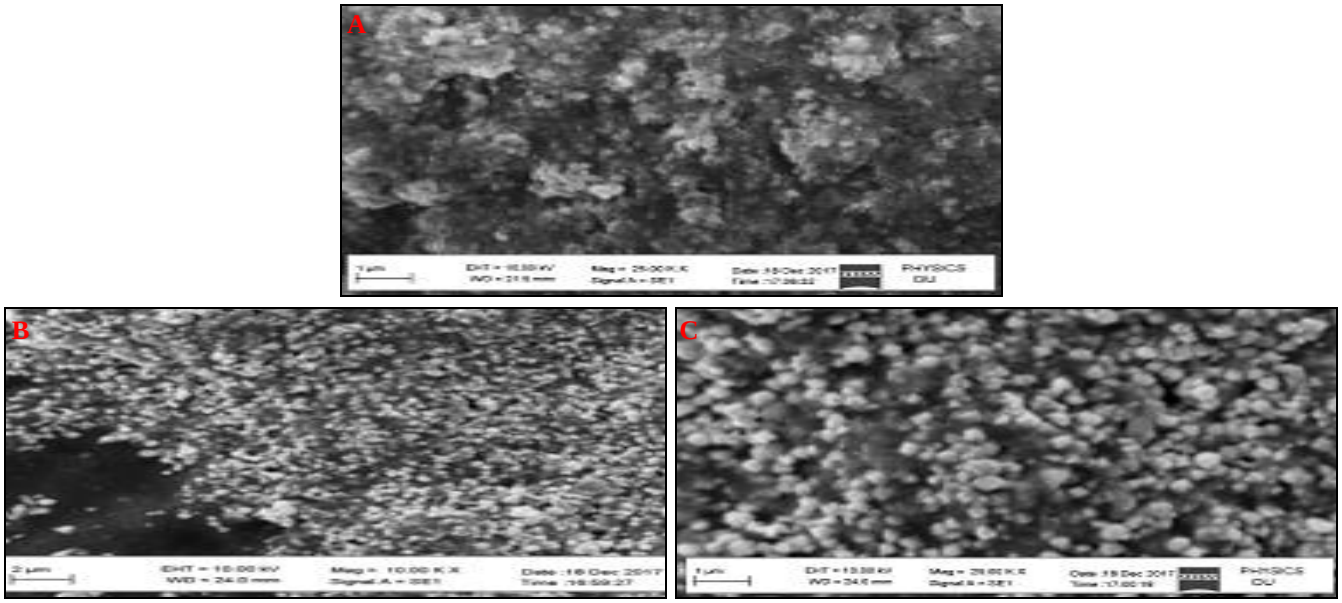



**FIG. 2A, B & C: SEM IMAGES (1 nm & 2 nm) OF AND SEED EXTRACTS OF *S. CUMINI* & 1 mM SILVER NITRATE**

**TEM: Fig. 3** shows the TEM images obtained by the reaction of 5% of each type of leaf, seed and fruit extracts and 1 mM silver nitrate solution separately. TEM analysis showed well-stabilized particles with a mixture of plates (triangles, pentagons, and hexagons) and spheres with sizes of up to 200 nm.

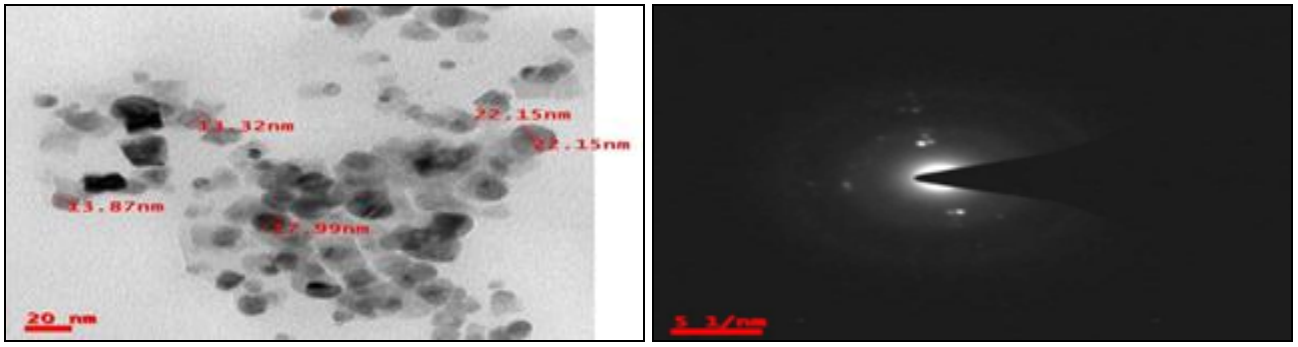



**FIG. 3A: TEM IMAGES OF SYNTHESIZED LEAF *SYZYGIUM CUMINI* SILVER NANOPARTICLES**

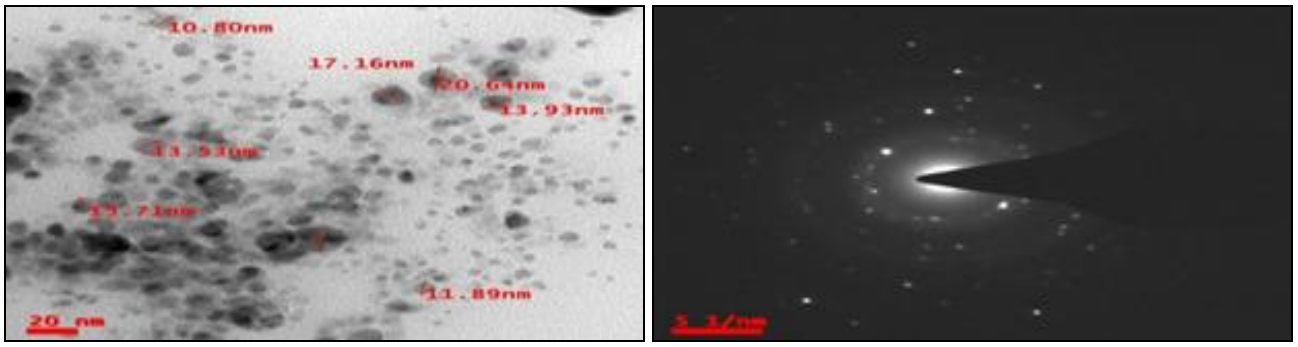



**FIG. 3B: TEM IMAGES OF SYNTHESIZED SEED *SYZYGIUM CUMINI* SILVER NANOPARTICLES**

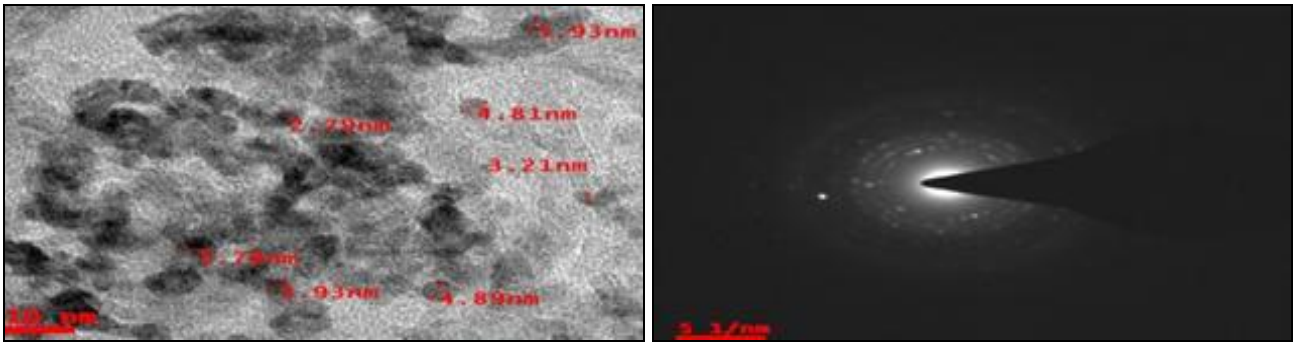


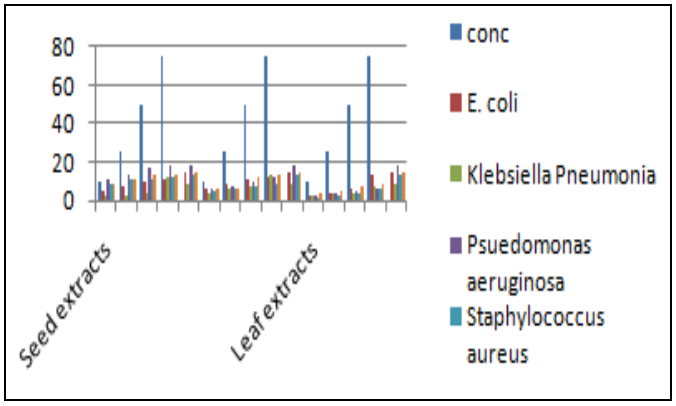

**FIG. 3C: TEM IMAGES OF SYNTHESIZED FRUIT *SYZYGIUM CUMINI* SILVER NANOPARTICLES**

**Antimicrobial Activity:** Antimicrobial activity of *Syzygium cumini* L. fruit, seed, and leaf extracts was assayed and data represented in **Table 1** and **2** and **Graph 1** and **2**. The silver nanoparticles of seed showed antibacterial activity in the order of *Pseudomonas aeruginosa* (17.6 mm), *Bacillus subtilis* (13.8 mm), *Klebsiella pneumonia* (12 mm), and *Staphylococci* sp. (12 mm) *E. coli* (11.5 mm). The silver nanoparticles of seed showed antifungal activity in the order of *Rhizopus* (10 mm), *Mucor* (8 mm), *Aspergillus niger* (7 mm) and *Aspergillus flavus* (6 mm) were tested for antimicrobial activity.

**TABLE 1: ANTIBACTERIAL ACTIVITY OF LEAVES, FRUITS AND SEED SILVER NANOPARTICLES OF *SYZYGIUM CUMINI***

| Extract | Conc. (µl) | Zone of inhibition in mm | | | | |
|---|---|---|---|---|---|---|
| | | ***E. coli*** | ***Klebsiella Pneumonia*** | ***Psuedomonasaer uginosa*** | ***Staphylococcus aureus*** | ***Bacillus subtilis*** |
| Seed | 10 | 5 | 2 | 10.6 | 8.6 | 9 |
| extracts | 25 | 6.9 | 3 | 13.9 | 10.2 | 11 |
| | 50 | 10 | 3.8 | 16.6 | 11.2 | 13 |
| | 75 | 11.5 | 12 | 17.6 | 12 | 13.8 |
| | Control | 15 | 8 | 18.5 | 13 | 14 |
| Fruit | 10 | 6 | 4 | 6.1 | 4.8 | 5.9 |
| extracts | 25 | 8 | 6 | 7.5 | 5.3 | 6.6 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 50 | 11.5 | 7.6 | 9.2 | 6.8 | 12.1 |
| | 75 | 12.5 | 13.5 | 12 | 8 | 13.1 |
| | Control | 15 | 8 | 18.5 | 13 | 14 |
| Leaf | 10 | 3 | 2 | 3 | 1 | 4.1 |
| extracts | 25 | 4.1 | 4 | 3.4 | 2.3 | 5.3 |
| | 50 | 6 | 4.2 | 4.7 | 3 | 7.1 |
| | 75 | 13 | 6.9 | 6.1 | 6 | 8.2 |
| | Control | 15 | 8 | 18.5 | 13 | 14 |

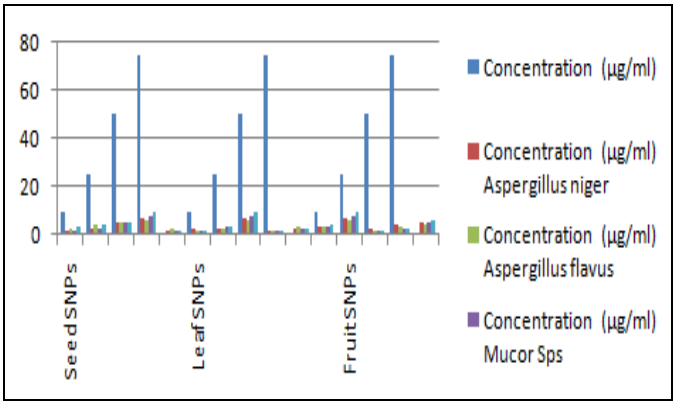



**GRAPH 1: ANTIBACTERIAL ACTIVITY OF LEAVES, FRUITS AND SEED SILVER NANOPARTICLES OF *SYZYGIUM CUMINI***

**FIG. 4: ANTIBACTERIAL ACTIVITY OF LEAVES, FRUITS AND SEED SILVER NANOPARTICLES OF *SYZYGIUM CUMINI***

**TABLE 2: ANTIFUNGAL ACTIVITY OF LEAVES, FRUITS AND SEED SILVER NANOPARTICLES OF *SYZYGIUM CUMINI* L.**

| Extracts | Concentration (µg/ml) | Zone of inhibition in mm | | | |
|---|---|---|---|---|---|
| | | *Aspergillus niger* | *Aspergillus flavus* | *Mucor sps* | *Rhizopus sps* |
| Seed SNPs | 10 | 1.8 | 3 | 2 | 3.1 |
| | 25 | 3 | 4 | 2.2 | 4 |
| | 50 | 5 | 5 | 5 | 5.2 |
| | 75 | 7 | 6 | 8 | 10 |
| | Control | 1.5 | 2.8 | 1.5 | 1.6 |
| Leaf SNPs | 10 | 2.2 | 2 | 1.8 | 2.1 |
| | 25 | 3 | 2.8 | 3.8 | 3.6 |
| | 50 | 7 | 6 | 8 | 10 |
| | 75 | 1.8 | 2 | 2 | 1.8 |
| | Control | 2.2 | 3.6 | 3 | 2.8 |
| Fruit SNPs | 10 | 3.6 | 3.4 | 3.9 | 4 |
| | 25 | 7 | 6 | 8 | 10 |
| | 50 | 2.5 | 2 | 2 | 2 |
| | 75 | 4.2 | 3.5 | 2.8 | 2.5 |
| | Control | 5 | 4.1 | 5.2 | 5.9 |




**GRAPH 2: ANTIFUNGAL ACTIVITY OF LEAVES, FRUITS AND SEED SILVER NANOPARTICLES OF *SYZYGIUM CUMINI***

**CONCLUSION:** Identification of therapeutically and industrially important compounds like alkaloids, phenolic compounds, saponins, flavonoids, tannins, steroids and terpenoids having various activities is possible through phytochemical screening [19]. Many researchers worked on different parts of *S. cumini* and proved the presence of alkaloids, phenolic compounds, saponins, flavonoids, tannins, steroids and terpenoids [20]. Plants provide a better platform for nanoparticle synthesis since they are free from toxic chemicals in addition to provide natural capping agents. Nanoparticles synthesis from plant extract with silver is safe to be used as an antimicrobial agent and in medical research. The conformation of Silver nanoparticles conformed by UV-Visible, FTIR, DLS and TEM studies. In future, selection of medicinal plants which synthesize nanoparticles may create a new platform for recognition of natural medicines in nanoscience for biomedical applications and antibacterial and antiviral properties of nanoparticles depends upon on the morphology and size [21, 22]. Silver nanoparticles (AgNPs) were successfully obtained from bioreduction of silver nitrate solutions using crude seed, fruit, and leaf of the *Syzygium cumini* L.

Among all the extracts tested, nanoparticles from seed extracts showed more antibacterial activity in the order *Pseudomonas*, followed by *Bacillus, Klebsiella, Staphylococci,* and *E.coli.* Seed extracts also showed more antifungal activity against the fungi *Rhizopus*, *Mucor*, *Aspergillus niger* and *Aspergillus flavus*. Comparatively nanoparticles from leaf extracts of *S. cumini* showed less antibacterial activity than the nanoparticles prepared from seed and fruit extracts.

The present study revealed synergetic antimicrobial and antifungal activities of seed, fruit, and leaf synthesized silver nanoparticles of the *Syzygium cumini* L. with the presence of phytochemicals that can be effectively used as medicine.

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**CONFLICT OF INTEREST:** The authors declared no conflict of interest.

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