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BIOSYNTHESIS OF SILVER NANOPARTICLES FROM *SYZYGIUM CUMINI* (L.) SEED EXTRACT AND EVALUATION OF THEIR *IN VITRO* ANTIOXIDANT ACTIVITIES

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The environmental friendly synthesis of nanoparticles process is a revolutionary step in the field of nanotechnology. In this study, the biosynthesis of silver nanoparticles was carried out using *Syzygium cumini* seed extract as reducing agent. UV–visible spectroscopy was used for quantification of silver nanoparticle synthesis. The synthesized silver nanoparticles were characterized with Scanning electron microscopy (SEM), Energy dispersive X ray analysis (EDX), X-ray Diffraction (XRD) and Fourier transform Infrared Spectroscopy (FTIR). The *in vitro* antioxidant properties of the biosynthesized silver nanoparticles have been evaluated and these nanoparticles were found to have higher antioxidant capacity compared to the seed extract and thus can be used as potential radical scavenger against deleterious damages caused by the free radicals.

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Keywords: Silver nanoparticles, *Syzygium cumini*, free radicals, oxidative stress, *A*ntioxidant activity

1. Introduction

Nanotechnology is unique in that it represents not just one specific area, but a vast variety of disciplines ranging from basic material science to personal care applications [1]. The development of nanoparticles for the delivery of therapeutic agents has introduced new opportunities for the improvement of medical treatment [2]. Nanoparticles are of great scientific interest as they bridge the gap between bulk materials and atomic or molecular structures [3]. They exhibit completely new or improved properties bases on specific characteristics such as size, distribution and morphology [4] than compared to the bulk materials. Metallic nanoparticles are traditionally synthesized by wet chemical techniques, where the chemicals used are quite often toxic and flammable [5]. Nanomaterials are present in some sunscreens, toothpastes, sanitary ware coatings and even food products [6]. Nanoparticles of noble metals, such as gold, silver, and platinum, are widely applied in products that directly come in contact with the human body, so there is a growing need to develop environmentally friendly processes of nanoparticles synthesis that do not use toxic chemicals [7]. Among the various inorganic metal nanoparticles, silver (Ag) nanoparticles have received substantial attention for various reasons. Silver is an effective antimicrobial agent, exhibits low toxicity and has diverse *in vitro* and *in vivo* applications [8]. The most widely used and known applications of silver and silver nanoparticles are in the medical industry [7].

The inorganic nanoparticles are found to be effective in scavenging oxygen based free radicals [9]. A free radical is defined as a molecular species capable of independent existence and which contains one or more unpaired electrons [10]. Reactive oxygen species (ROS) are small, highly reactive, oxygen-containing molecules that are naturally generated in small amounts during

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the body's metabolic reactions and damage complex cellular molecules such as fats, proteins, or DNA [11]. In low/moderate concentrations free radicals are involved in normal physiological functions but excess production of free radicals or decrease in antioxidant level leads to oxidative stress [12] which is apparent in pathology associated with aging and many age-related chronic diseases, including atherosclerosis, diabetes mellitus, rheumatoid arthritis, and neurodegenerative diseases [13]. Antioxidants are the substances which act as free radical scavengers by preventing and repairing damages caused by reactive oxygen species, and therefore can enhance the immune defense and lower the risk of cancer and degenerative diseases [14].

In this present study, silver nanoparticles were synthesized using the seed of *Syzygium cumini* (L.), which is a traditional medicinal tropical plant of Myrtaceae family, originated from India [15], widely known as Jamun, Black plum, Indian blackberry and the seeds of *S. cumini* are considered to have anti-diabetic properties in folklore medicine [15], anti-inflammatory [16], anti-bacterial [17], anti-HIV [18] effects.

2. Materials and methods

2.1 Extraction and fractionation of *S. cumini* seeds:

The seeds of the plant *S. cumini* were thoroughly washed and dried at 37^0C. The dried seeds were further pulverized into fine powder. 25gm of the powdered seed was taken for the extraction purpose in ethanol as the solvent, by using Soxhletor apparatus. This seed extract (Sc) was used for studying the various antioxidant assays.

2.2 Biosynthesis of Silver Nanoparticles from *S. cumini* Seed (ScSNPs):

A measured quantity of finely powdered seed (5gm) was mixed with 100mL of deionized water and then boiled the mixture for 5 min before finally decanting it. This suspension was then centrifuged at 5,000 rpm for 15 minutes at 4^0C using fresh deionized water. The extract volume was adjusted to an appropriate volume by adding deionized water, and filtered through Whatman filter paper No.1. 10 mL of seed extract was added to 90 mL of 1 mM aqueous $AgNO_3$ solution for reduction of Ag^+ ions and incubated at room temperature in dark condition for 24 hours. The solution was then centrifuged at 10,000 rpm for 20min to separate the silver nanoparticles. These silver nanoparticles were washed three times with deionized water and stored as lyophilized powder.

2.3 Characterization of ScSNPs:

2.3.1 UV Spectroscopic analysis of Silver nanoparticles: The reduction of pure Ag^+ ions was monitored by measuring the UV- Vis Spectrum (Hitachi U-2910 Spectrophotometer, Japan) of the reaction medium after 24 hours incubation by diluting a small aliquot of the sample with distilled water.

2.3.2 SEM and EDX Analysis: Purified ScSNPs in suspension were characterized for their size using Scanning Electron Microscope (JEOL-JSM 6390, Japan). EDX (Energy Dispersive X-ray) analysis of purified ScSNPs was carried out using the same instrument for confirming the elemental composition of the sample.

2.3.3 XRD Analysis: The characterization of purified ScSNPs was carried out by X-ray diffractometer (XRD-600, Shimadzu, Japan) for the crystallographic structural analysis. The nanocrystallite domain size was calculated from the width of the XRD peaks using Scherrer formula, assuming they are free from non-uniform strains. $D=0.94\lambda / \beta\cos\theta$ …. (Eq i), where D is the average crystallite domain size perpendicular to the reflecting planes, λ is the wavelength of X-ray, β is the full width at half maximum (FWHM) and θ is the diffraction angle.

2.3.4 FTIR Analysis: To identify biomolecules attached to *S. cumini* seed and ScSNPs that is, before and after the synthesis of silver nanoparticles, Fourier Transform Infrared (FTIR) spectra of aqueous extract of seed and purified ScSNPs powder were recorded on FTIR spectroscopy (FTIR Shimadzu 8400S, Japan) carried out at PSG Arts and Science, Coimbatore.

2.4 *In vitro* Antioxidant Assays:

2.4.1 Determination of Total Phenolic Content: The total phenolic content was determined by the Folin-Ciocalteau method [19, 20]. The total phenolic content was expressed in terms of Gallic acid equivalent (mg/g of dry mass).

2.4.2 DPPH Free Radical Scavenging Assay: The DPPH free radical scavenging assay was carried out by the method of Tagashira *et al*., [21] and Chang *et al*., [22]. The percentage of inhibition or scavenging of free radicals was determined by the formula % Inhibition= [(Control OD – Sample OD)/ Control OD] * 100, where control was prepared as above without extract.

2.4.3 Reducing Power Assay: The reducing power assay was carried out by the method of Koleva *et al*., [23] and Makari *et al*., [24]. Ascorbic acid was used as standard and phosphate buffer used as blank solution. Increased absorbance of the reaction mixture indicates stronger reducing power.

2.4.4 Total Antioxidant Capacity: The total antioxidant capacity was assayed following the method of Preito *et al*., [25]. Ascorbic acid was used as the standard and the total antioxidant capacity was expressed as equivalents of ascorbic acid.

2.4.5 Statistical Analysis: All the grouped data were statistically evaluated by using Student't test with SPSS/16 software. *P* values of less than 0.05 were considered to indicate statistical significance. Values are presented as the mean ± S.D. of each three replicates in each experiment.

3. Results

Fig. 1 shows the change of the color of the *S. cumini* seed extract from light brown to yellowish brown after the addition of 1mM of $AgNO_3$.

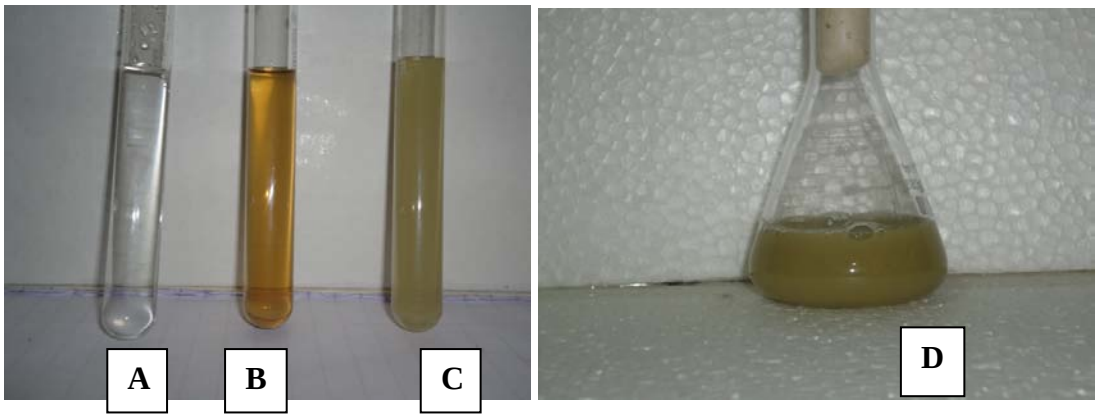

Fig. 1 Change in color of seed extract solution after addition of $AgNO_3$ (A) 1mM Silver nitrate solution, (B) S. cumini seed extract, (C) ScSNPs after 24 hours and (D) ScSNPs after 48 hours

Fig. 2 depicts the UV-Visible absorption spectrum of the reaction solution taken after incubation period of 24 hours showing the maximum absorbance at ~450 nm.

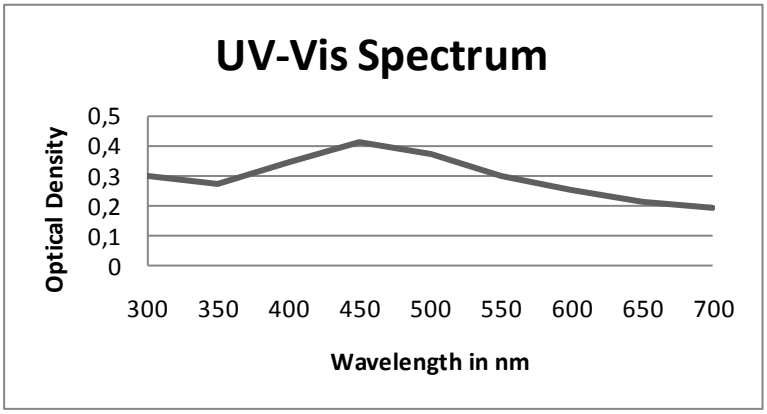

Fig. 2 UV-Vis Spectrum of ScSNPs after 24 hours

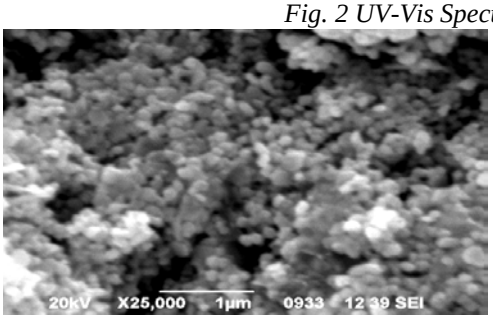

Fig. 3a SEM picture of ScSNPs

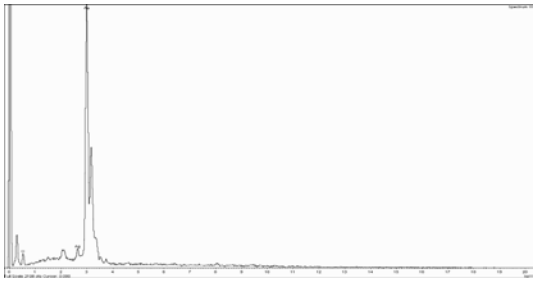

Fig. 3b EDX analysis of ScSNPs

From the Scanning Electron Microscopy (SEM) analysis, the size of ScSNPs was averagely found to be ~93 nm. The Energy Dispersive X-ray (EDX) analysis of the ScSNPs confirmed the elemental composition of nanoparticles as silver. The biosynthesis of silver nanoparticles from the *S. cumini* seed was further demonstrated by the characteristic peaks observed in the X-Ray Diffraction (XRD) image. The XRD pattern showed three distinct diffraction peaks at 38.2◦, 32.4◦ and 44.4◦, corresponding to diffraction from the 111, 101 and 200 planes of the cubic face centered silver (JCPDS no. 04-0783). The 100% intensity was found at 2θ value with 38.2◦. The average nanocrystallite size calculated from Scherrer formula was found to be 3.5 nm.

Fig. 4 XRD pattern of ScSNPs

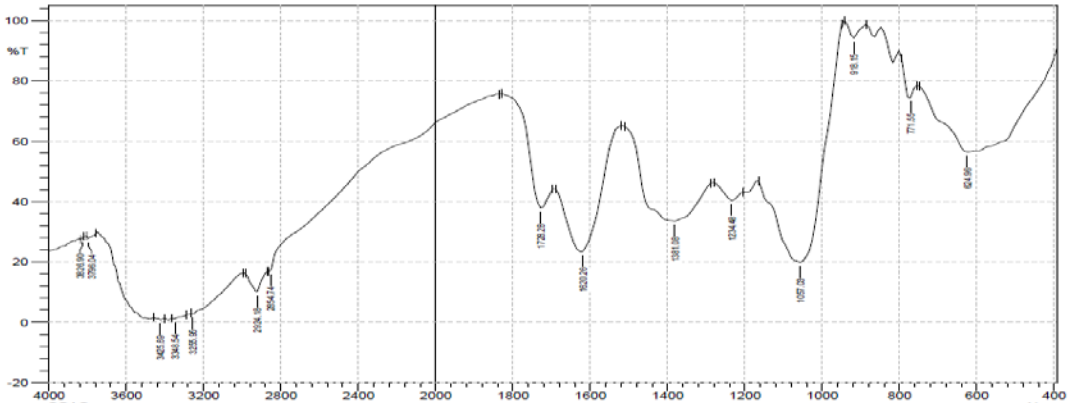

Fig. 5a FTIR spectra of S. cumini seed aqueous extract.

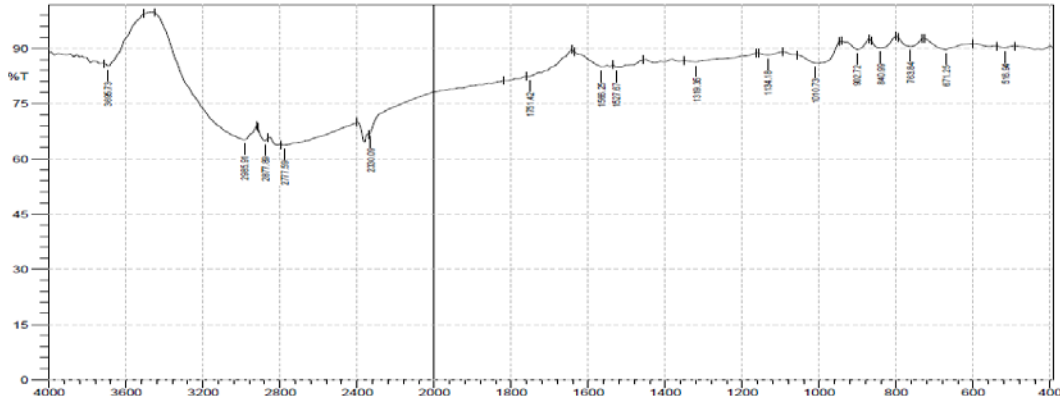

Fig. 5b FTIR spectra of ScSNPs

The Fourier Transform Infrared (FTIR) spectra were analyzed for the characterization of the seed aqueous extract and the resulting silver nanoparticles (ScSNPs). The FTIR spectra (Fig. 5a) showed the presence of various polyphenols in the seed of *S. cumini*. The presence of high amount of polyphenols in the *S. cumini* seed was also evaluated from the estimation of total phenolic content of the seed extract by the Folin-Ciocalteau method, which was found to be 200.83±3.81 mg per gm of ethanolic extract of the *S. cumini* seed expressed as mg of gallic acid equivalents.

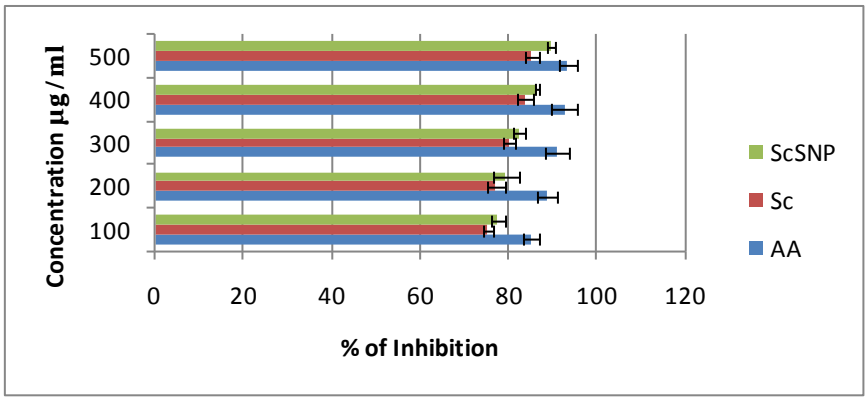

Fig. 6 DPPH Free Radical Scavenging Assay of SC, ScSNP and AA (Values are mean ± SD of three determinations)

The DPPH free radical scavenging assay showed potent inhibitory capacity of both Sc and ScSNPs when compared with ascorbic acid (AA). The percentage of inhibition of free radicals increased with increase in concentration of substrates.

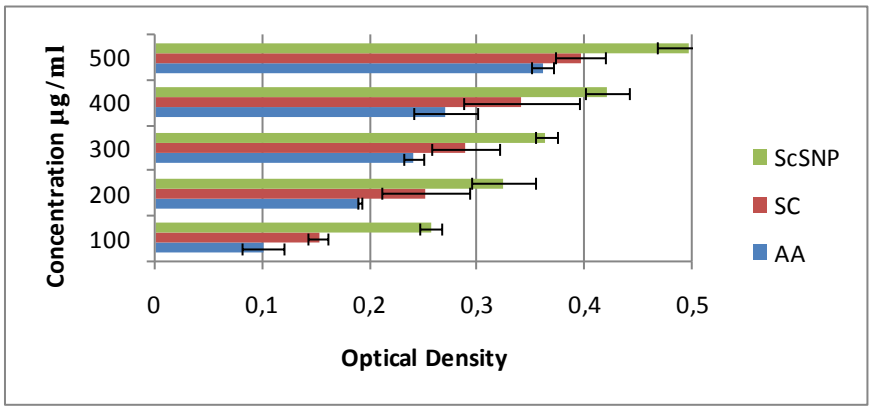

Fig. 7 Reducing Power Assay of SC, ScSNP and AA (Values are mean ± SD of three determinations).

Fig. 7 shows the reductive capabilities of Sc and ScSNPs along with ascorbic acid (AA). The reducing power of Sc and ScSNPs were found to be very potent and increased with increasing concentration of substrates.

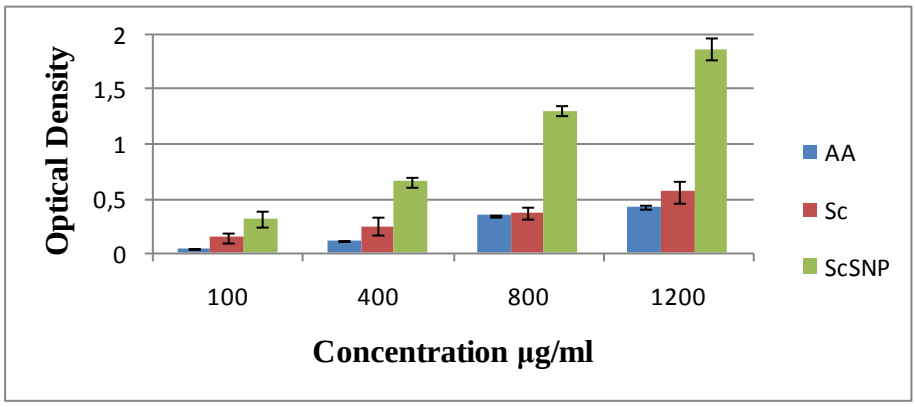

Fig. 8 Total Antioxidant Capacity of SC, ScSNP and AA (Values are mean ± SD of three determinations).

Fig. 8 shows the Total antioxidant capacity of ascorbic acid (AA), Sc and ScSNPs. The Total antioxidant capacity was calculated based on the formation of the phosphomolybdenum complex where, ScSNPs showed much higher total antioxidant capacity compared to AA and Sc.

4. Discussion

From this study, it has been found that the seed of *Syzygium cumini*, a traditional medicinal plant has the potential to reduce silver nitrate ions to silver nanoparticles. The light brown color of aqueous extract of *S. cumini* seed was changed to yellowish brown after 24 hours of incubation due to the excitation of surface plasmon vibrations in silver nanoparticles [26]. The intensity of the color increased with the incubation time. Nanoparticle size can also be determined by the change in the color of the reaction solution. The smaller the size of nanoparticles greater is the color shift towards red [27]. The synthesis of silver nanoparticles was also confirmed from the UV spectra of ScSNPs where the maximum absorbance was found ~450nm after 24 hours of incubation.

The size of the ScSNPs was determined by the Scanning Electron Microscopy (SEM) and elemental composition of the nanoparticles was further confirmed by the Energy Dispersive X-ray

(EDX) analysis. The large size of ScSNPs was due to the presence of relatively large amount of polyphenols present in the *S. cumini* seed [28]. The presence of high polyphenolic compounds in the seed has been reported to play an important role in the synthesis of silver nanoparticles in Aloe vera plant extract [29]. The morphology and the nanocrystallite size were determined from characteristic peaks obtained from the XRD image.

The FTIR spectra, before the bioreduction of Ag+ ions (Fig. 5a) showed the peak at 1057 cm^{-1} ,which corresponds to C-N stretching vibration of aliphatic amines or to alcohols or phenols, representing the presence of polyphenols [7]. The absorbance peak at 3425 cm^{-1} indicates polyphenolic OH group [30]. The absorbance peak around 3000 cm^{-1} indicates aromatic C-H stretching [31]. The peak at 2547-2973 cm^{-1} indicates C-H stretching and peak at 3250-3450cm^{-1} corresponds to primary aliphatic amines and the aromatic C-H out of plane deformation bands occur below 700 cm^{-1} [32]. The absorbance peak at 1000-1200 cm^{-1} indicates C-O single bonds and the peak at 1620 cm^{-1} indicates the presence of carbonyl groups (C=O) from the polyphenols such as catechin gallate (CG), epicatechin gallate (ECG), epi-gallocatechin (EGC), epigallocatechin gallate (EGCG), gallocatechin gallate (GCG), theaflavin [30,33,34]. The total disappearance of this band after bioreduction of Ag+ ions (Fig. 5b) may be due to the major involvement of polyphenols in the biosynthesis process of silver nanoparticles.

In this study, antioxidant activity of the seed extract (Sc) and biosynthesized silver nanoparticles (ScSNPs) were investigated where, both Sc and ScSNPs were found to be effective antioxidants in compared to standard ascorbic acid. A freshly prepared DPPH solution exhibited a deep purple color with a maximum absorption at 517 nm. This purple color disappears when an antioxidant is present in the medium. Thus, antioxidants molecules can quench DPPH free radicals and convert them to a colorless product [35]. The DPPH free radical scavenging assay showed that ScSNPs have higher free radical scavenging activity in compared to seed extract alone. The reducing ability of a compound depends on the presence of reductants [36] which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom [37]. Presence of reducers causes the conversion of the Fe3+/ferricyanide complex used in this method to the ferrous form [38]. The biosynthesized silver nanoparticles (ScSNPs) are found to have very potent reducing ability. The total antioxidant capacity of the Sc and ScSNPs was based on the phosphomolebdnum method where the reduction of Mo(VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo(V) complex [39]. The ScSNPs were found to have very high total antioxidant capacity as compared to *S. cumini* seed extract.

5. Conclusion

This study suggests the seed of *Syzygium cumini* has the potential to produce silver nanoparticles and the biosynthesized silver nanoparticles using *Syzygium cumini* seed have potent antioxidant activity. Since free radicals are important contributors to various degenerative diseases, thus the observed antioxidant properties of the seed extract of *Syzygium cumini* and biosynthesized silver nanoparticles might be useful for the development of newer and more potent antioxidants. The biosynthesized silver nanoparticles can be used as potential free radical scavengers and can be used against the various damages caused by free radicals.

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