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ENHANCED MECHANISM OF METAL NANOPARTICLES USING *SYZYGIUM CUMINI* AS A POTENTIAL SYNTHESIZER AND ITS INHIBITORY EFFECT

Rajeswari Anburaj [*1] and Vinoth Jothiprakasam [2]

Department of Microbiology [1], MIET Arts and Science College, Affiliated to Bharathidasan University, Tiruchirapalli - 620007, Tamil Nadu, India.
CAS in Marine Biology [2], Annamalai University, Parangipettai - 608502, Tamil Nadu, India.

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Correspondence to Author:
Rajeswari Anburaj

Assistant Professor,
Department of Microbiology,
MIET Arts and Science College,
Affiliated to Bharathidasan University, Tiruchirapalli - 620007,
Tamil Nadu, India.

E-mail: raji.anburaj@gmail.com

ABSTRACT: In recent years metallic nanoparticles, represent one of the most comprehensively studied materials because of their application in biology The synthesis of silver nanoparticles using biological entities has received immense attention in the area of research. Medicinal plants have attracted interest over antibiotics due to a rapid increase in the rate of infections, development of antibiotic resistance in microorganisms and side effects of antibiotics. In the present study biosynthesis of silver nanoparticles was performed using *Syzygium cumini*. Spectrochemical studies indicate the surface plasmon resonance band and the presence of a capping agent responsible for the synthesis of AgNPs. The results revealed that *S. cumini* along with synthesized AgNP found to possess microbicidal effect. 400 µl of synthesized AgNP was found to be resistant against *Bacillus sp.* (23 mm), followed by *S. epidermis* and *A. niger* (22.5 mm). HPLC chromatogram reveals the presence of flavonoids such as quercetin and myricetin responsible for bioassay.

INTRODUCTION: Nanoscale materials have received huge attention because their properties and structure vary considerably from those of atoms and molecules in addition to bulk materials [1]. The synthesis of nanoscale materials with the preferred qualities is one of the most exciting aspects of recent nanoscience and nanotechnology. Nanomaterials synthesized using silver, gold, and zinc is the fascinating area of discovery for researchers, due to a diverse array of their applications in the fields of biomedical engineering, molecular biology, material sciences and medicines [2].

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Silver has unique properties, such as conductivity, catalytic, chemical stability and bactericidal activities. Nanoparticles synthesized using chemical methods are noxious and unsafe for the environment. This increases the growing need to develop an environmentally friendly procedure for the synthesis of silver nanoparticles. Biological methods prove to be particularly cost-effective, nontoxic and provide safer methods for generating AgNPs [3, 4].

Numerous eco-friendly biological sources can be used for biosynthesis of AgNPs which have potent antimicrobial activities [5, 6, 7]. Silver nanoparticles synthesized from plant extract results that silver capped with the functional groups present in the active phytoconstituents of the plant extract acts as antioxidant agents and enhance the biological activity like anticancer effect [8]. *Syzygium cumini* (SC) belonging to Myrtaceae family is dispersed on India and South Asia [9].

The existence of diverse phytochemicals attributes to the medicinal [10], astringent, microbicidal activities of *Syzygium cumini*. The seeds have been reported to be rich in flavonoids and phenols, a well-known antioxidant which accounts for scavenging effects of free radicals and protective effect on antioxidant enzymes [11, 12, 13]. The seeds contain an alkaloid, jambosine, and glycoside jambolin, lowers the blood pressure and this action is attributed to the ellagic acid content [14]. The seeds are well-known to have astringent, antimicrobial and diuretic properties.

Additionally, AgNP has established antimicrobial [13] activities [15]. Silver nanoparticle fabricated using plant extract can be used to improve the property of adhered phytochemicals and secondary metabolites. Failure of antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of numerous medicinal plants for their potential antimicrobial activity [16].

In the present study, we report the biogenic synthesis of AgNPs using seeds of *Syzygium cumini*, to investigate the biomolecules responsible for the synthesis of AgNPs, antimicrobial assessment of silver nanoparticles.

EXPERIMENTAL SECTION:

Phytochemical Analysis: Plant materials were analyzed for the presence of various phytoconstituents like flavonoids, alkaloids, glycosides, steroids, phenols, saponins and tannins according to standard methods [17].

Biosynthesis of Silver Nanoparticles: *S. cumini* seeds were washed with deionized water. 50 g of the seed was powdered and mixed with 150 ml of de-ionized water, heated and filtered to get the extract. The filtrate is used as reducing agent and stabilizer. 7 ml of the extract is mixed with 100 ml of aqueous solution of $AgNO_3$ (5×10^{-4} M), and stirring was continued for 10 min. Reduction takes place gradually at 300 K, color is indicated by the formation of the reddish-brown color of the solution. It is found to be stable for more than 2 months, showing no precipitation or color change.

Purification of Silver Nanoparticles: A measured quantity of finely powdered seed (5 g) was mixed with 100 ml 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 min at 40 °C 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 the reduction of Ag^+ ions and incubated at room temperature in dark condition for 24 h. The solution was then centrifuged at 10,000 rpm for 20 min to separate the silver nanoparticles. These silver nanoparticles were washed three times with deionized water and stored as a lyophilized powder.

Microbicidal Assay: Microbicidal assay of silver nanoparticle was performed by Bauer-Kirby's disc diffusion method [18]. The Muller Hinton Agar (M173 Himedia, India) media was sterilized at 121°C and 15 lbs for 15 min in an autoclave. The culture plates were prepared with a depth of about 4 mm. The cultures were transferred to the center of an agar plate independently and homogeneously seeded the culture on the surface of the plate with a sterile bent-glass rod (purveyor). 10 µl of pure *Syzygium cumini* seed extract along with biosynthesized SNs solution and 1×10^{-3} M $AgNO_3$ were impregnated onto filter paper discs of ~5 mm diameter (which were prepared using Whatman grade no. 1 filter paper) under aseptic conditions, then placed onto cultured plates using sterile forceps. The plates were then incubated for 24 h at 37 °C in an incubation chamber. The antimicrobial activity was evaluated in replicates by quantifying the zone of inhibition (ZoI) for the test organisms *viz. Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella terrigena*, *Mycobacterium mucilaginous*, *Pseudomonas aeuroginosa*, *Aspergillus niger*, *Fusarium oxysporum*, *Penicillium* respectively.

UV Spectroscopic Analysis of Silver Nanoparticles: The reduction of pure Ag^+ ions was monitored by measuring the UV-Vis Spectrum (Shimadzu 1601 model, Japan) of the reaction medium after 24 h incubation by diluting a small aliquot of the sample with distilled water.

FT-IR Analysis: The surface groups of the nanoparticles were qualitatively confirmed by using FT-IR spectroscopy [19], with spectra recorded by a Perkin-Elmer Spectrum 2000 FT-IR

spectrophotometer. FT-IR analyses were performed using the Shimadzu FT-IR model number 8400. Approximately three mg of lyophilized leaves extract under study was mixed with 300 mg of dried KBr, crushed well in mortar and pestle to prepare thin pellet for analysis. The same procedure was performed for synthesized AgNPs using leaves extract. 16 scans per sample were taken in a range of 400-4000 cm^{-1}.

Scanning Electron Microscope: The structure and composition of freeze-dried purified silver particles were analyzed by using a scanning electron microscope. Thin films of the sample were coated on a copper grid by just dropping a very small amount of the sample on the grid, and dried in the UV lamp.

RESULTS AND DISCUSSION: Phytochemical screening of *S. cumini* was done using various solvents such as ethanol, ethyl acetate, methanol, petroleum ether and water **Table 1**. Ethanol indicates the presence of the majority of phytoconstituents. In ethyl acetate and methanol, extract phenols were absent, whereas glycosides and terpenoids were absent in petroleum ether. Steroid, saponin were absent in aqueous extract of *S. cumini*.

TABLE 1: PHYTOCHEMICAL SCREENING OF *S. CUMINI*

Phyto-constituents	*S. cumini*				
	Ethanol	Ethyl acetate	Methanol	Petroleum ether	Water
Alkaloids	+	+	+	+	+
Flavanoids	+	+	+	+	+
Glycosides	+	+	+	-	+
Phenols	+	-	-	+	+
Saponin	+	+	+	+	-
Steroid	+	+	+	+	-
Tannin	+	+	+	+	+
Terpenoid	+	+	+	-	-

Plant Mediated Synthesis of AgNPs: Nano-particles were prepared extracellularly using extracting in boiling water or ethanol [20, 21, 22]. Formation of yellow to brown color indicates the synthesis of AgNPs at 240 nm. The large size of AgNPs was due to the presence of a relatively large amount of polyphenols present in the *S. cumini* seed [23]. Polyphenolic compounds in *Aloe vera* plant extract plays an essential role in the synthesis of silver nanoparticles [24].

Analytical Characterization: UV-Vis spectroscopy is a significant technique to determine the formation and stability of silver nanoparticles. Colour of silver colloid is recognized using surface plasmon resonance (SPR) bands at 240 nm arising due to the combined oscillation of electrons formed by electromagnetic field [25]. Larger sized nanoparticles tend to show increased scattering that results in broader peaks and shifting of the wavelength to longer wavelengths. This phenomenon is also called red-shifting. Silver nanoparticles are known to have many optical properties. These properties are dependent on the refractive index of the surrounding surface of the nanoparticles. If transferred from a denser medium to a lighter medium, the peak of absorbance of nanoparticles shifts to longer wavelengths. In another case, if nanoparticles are transferred from lighter to a denser medium, the peak of the absorbance shifts to shorter wavelengths, which is also called blue-shift or bathochromic shift. Unstable particles tend to decrease the intensity of absorbance and broadening of the peak due to the formation of polydisperse or various size aggregated nanoparticles.

Optimization Studies for AgNPs Production: Optimization studies were done to enhance the better yield of AgNPs were represented in **Fig. 1-4**. The growth conditions, such as $AgNO_3$ concentration, pH, and temperature directly affecting the productivity were optimized. 2 mM $AgNO_3$ was incubated at different temperatures from 20 to 80°C and monitored for AgNPs synthesis. Maximum synthesis of AgNPs was observed at 70 °C and remains stable for a longer period indicated stabilized synthesis. The high temperature is required for faster synthesis rate and increased kinetic energy [26]. After optimization, synthesis was observed within 6 h. The results indicate that AgNPs were stable at 2 mM concentration at pH 8, incubated at 70 °C for 6 h.

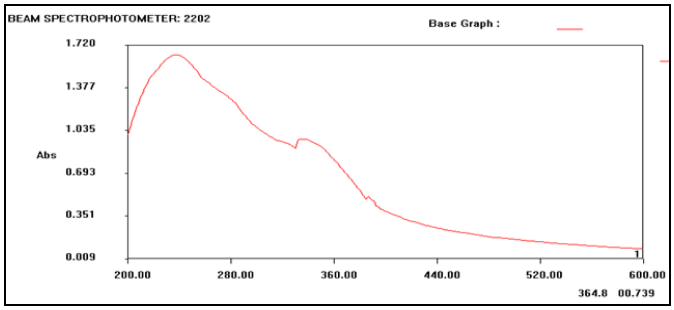

FIG. 1: AgNPs SYNTHESISED AT 6 h

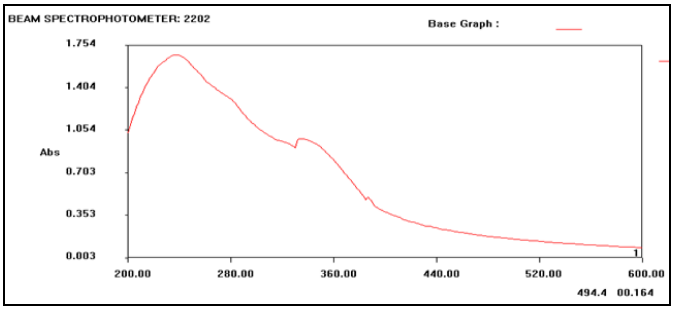

FIG. 2: AgNPs SYNTHESIZED AT 70 °C

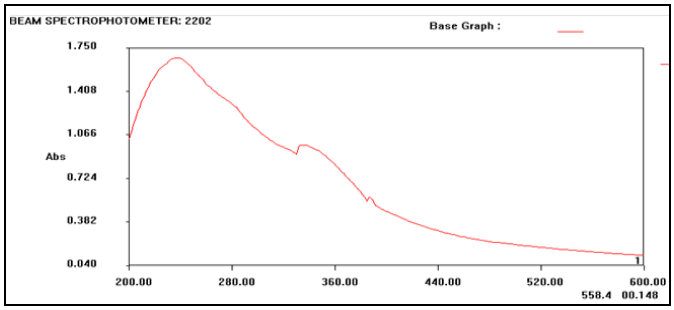

FIG. 3: AgNPs SYNTHESIZED AT 2mM

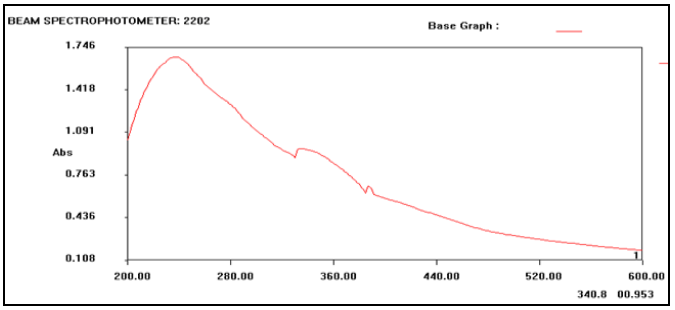

FIG. 4: AgNPs SYNTHESIZED AT pH 8

FT-IR analysis of synthesized AgNPs using *S. cumini* was represented in **Fig. 5**. The wave number at 2978.65 cm^{-1}, 2931.73 cm^{-1} corresponds to O-H stretching of carboxylic acid group, followed by 1726.28 cm^{-1} belongs to aromatic C=O stretching of esters, 1358.59 belongs to aromatic C-N stretching of amines.

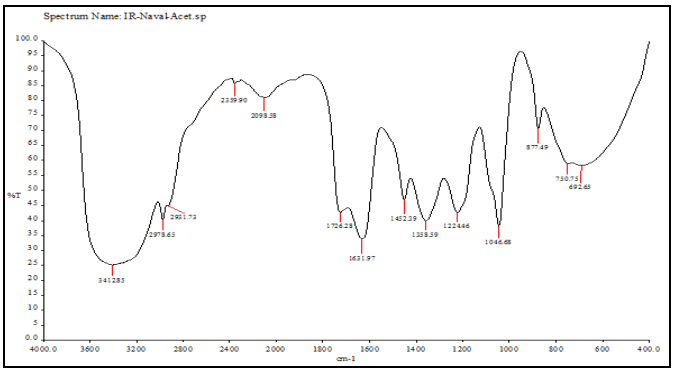

FIG. 5: FT-IR ANALYSIS OF AgNPs SYNTHESIZED USING *S. CUMINI*

Li *et al*., (2007) used an aqueous extract of *Capsicum annuum* L. for the synthesis of AgNPs which revealed that the proteins having amine groups play an important regulatory role (reduction) during the formation of AgNPs in the solutions [27]. The peak at 1631.97 cm^{-1} indicates the presence of primary NH=2 bending of amides, whereas 1452 cm^{-1} of C=C stretching belongs to the aromatic group. The functional groups at 1224.46 cm^{-1}, 1046.68 cm^{-1} indicates the presence of C-O stretching of alcohols and phenol.

FT-IR spectra indicate the presence of phenolic hydroxyl groups in the structure of flavonoids, which essentially substantiates the presence of friedelin, lupeol, and *β*-sitosterol groups, acting as reducing agents *Vitex negundo* L. extract [1]. The peak at 750.75 cm^{-1} indicates the NH_2 wagging of amines, followed by 692.65 cm^{-1} which corresponds to =C-H bending of the alkyne group. Compounds present in plant *Aristolochia bracteata* leaf extract were responsible for the capping of the AgNPs and reduction of the silver ions [28].

SEM image of the AgNPs synthesized using *S. cumini* are shown in **Fig. 6**. Colloids consist mainly of large nanoparticles having nearly spherical shape particles of size 20-30 nm. It is clear from the images that the particles in a colloid are well dispersed with a more uniform size of 20 nm.

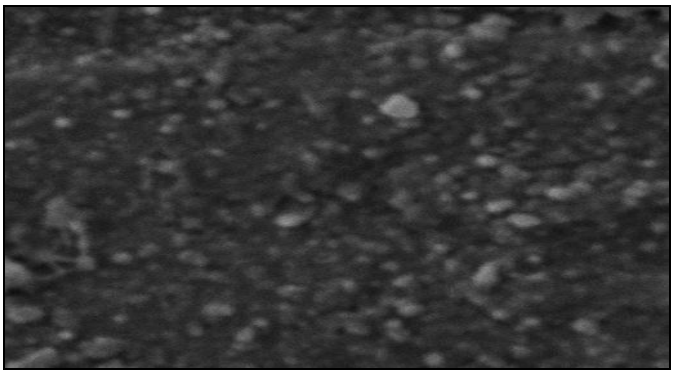

FIG. 6: SEM ANALYSIS OF AgNP SYNTHESISED USING *S. CUMINI*

HPLC chromatogram of *S. cumini* is represented in **Fig. 7**. The peak indicates the presence of flavonoids. *S. cumini* contains quercetin and myricetin [29]. Flavonoids are well-known antioxidant constituents in plants [30]. The biological activity of *S. cumini* may be related to its flavonoid constituents. Catechin, a monomeric flavanol, is reported to have hydroxyl [31], peroxyl [32], superoxide [33] and DPPH [34] radical scavenging activities. It is used as a supplement for animal feeds both to improve animal health and to protect animal products, an antimicrobial agent in foodstuffs and a health functional ingredient in various foods and dietary supplements [35].

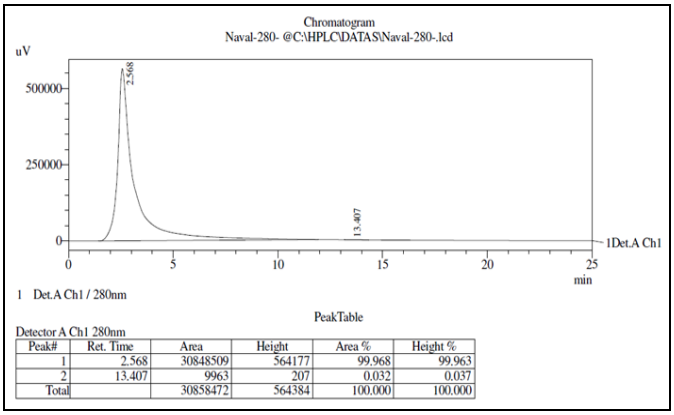

FIG. 7: HPLC CHROMATOGRAM OF *S.CUMINI*

Antimicrobial Activity of *S. cumini:* Flavonoids play a vital role in defense against microorganism and activity has been reported [36]. Compounds such as quercetin, isorhamnetin and kaempferol extracted from plants have antimicrobial activity [37]. **Table 2** indicates the antimicrobial screening of *S. cumini* against several bacteria and fungi. The maximum inhibitory effect was recorded in methanol extract against *B. cereus* and *A. niger* (22 mm), followed by acetone against *B. cereus* (21.5 mm) and *S. aureus* (21 mm). Antibacterial activity of hydro-methanol extract (70%) of fresh immature fruits of *S. cumini* was carried against gram positive and gram negative organisms [38]. *E. coli* was found to be sensitive against methanol and acetone possessing an inhibition of 20.5 mm. Chloroform extract acquired an inhibition of 19.5 mm and 18.7 mm against *S. aureus* and *E. coli*. Moderate zone of inhibition was observed in K. pneumonia against ethyl acetate (16.5 mm) and chloroform (16 mm). The inhibitory effect was found to be minimum in *M. mucilaginous* in chloroform (14.2 mm) and petroleum ether (13 mm). Tannins and other phenolic constituents, gallic, ellagic acid and polyphenol derivatives in the leaves are responsible for the bactericidal activity [39, 40].

TABLE 2: ANTIMICROBIAL ASSESSMENT OF *S. CUMINI*

		Syzygium cumini			
S. no.	**Microorganism**	**Acetone**	**Chloroform**	**Methanol**	**Ethyl acetate**
1	*Bacillus cereus*	21.5 ± 0	19.4 ± 0.2	22.3 ± 0.2	20.2 ± 0.2
2	*Klebsiella pneumonia*	17.3 ± 0.2	16 ± 0	18 ± 0	16.5 ± 0
3	*Pseudomonas aeuroginosa*	18 ± 0	17.2 ± 0.2	19.1 ± 0.2	17.5 ± 0.5
4	*Staphylococcus aureus*	21 ± 0	18.3 ± 0.2	21.3 ± 0.2	19.5 ± 0
5	*Escherichia coli*	20.5 ± 0	18.5 ± 0	20.5 ± 0	18.7 ± 0.2
6	*Mycobacterium mucliaginosus*	15 ± 0	14.2 ± 0.2	15.1 ± 0.2	14.5 ± 0
7	*Klebsiella terrigena*	16.5 ± 0.5	15 ± 0	17.3 ± 0.3	15.6 ± 0.7
8	*Fusarium oxysporum*	18.5 ± 0.5	16.5 ± 0	19.6 ± 0.1	17.3 ± 0.2
9	*Penicillium*	19.5 ± 0.1	17.6 ± 0.2	20.5 ± 0	18 ± 0
10	*Aspergillus niger*	20.5 ± 0	18.7 ± 0.2	22 ± 0	19.1 ± 0.2

*values are mean of ± S.D, n=3

TABLE: 3 ANTIMICROBIAL ASSESSMENT OF SYNTHESIZED SILVER NANOPARTICLE USING *S. CUMINI*

Microorganism	**Plant samples used in the study (Zone of inhibition in mm)**			
	Syzygium cumini			
	100	**200**	**300**	**400**
Bacillus sp.	18.7 ± 0.2	20.5 ± 0	22.3 ± 0.2	23 ± 0
Escherichia coli	17 ± 0	19.1 ± 0.1	20 ± 0	21.2 ± 0.2
Mycobacterium mucliaginosus	15.2 ± 0.2	16.2 ± 0.2	18.7 ± 0.2	18.5 ± 0
Klebsiella terrigena	15.7 ± 0	19.7 ± 0.2	19 ± 0	19.7 ± 0.2
Pseudomonas aeruoginosa	16.5 ± 0	19 ± 0	20.2 ± 0.2	21 ± 0
Klebsiella pneumoniae	16.1 ± 0.1	18.7 ± 0.2	19.5 ± 0	20.3 ± 0.2
Staphylococcus epidermis	18.5 ± 0	20.2 ± 0.2	21.1 ± 0.2	22.5 ± 0
Fusarium oxysporum	14 ± 0	16.3 ± 0.2	18.2 ± 0	20.7 ± 0.2
Penicillium	15.3 ± 0.2	17 ± 0	19.3 ± 0.2	21.2 ± 0.2
Aspergillus niger	16 ± 0	18.3 ± 0.2	20 ± 0	22.5 ± 0

*****values are mean of ± S.D, n= 3

Antifungal activity was found to be maximum in methanol extract against *A. niger* (22 mm) and *Penicillium* (20.5 mm). Acetone extract remained resistant towards *A. niger* (20.5 mm), *Penicillium* (19.5 mm) and *F. oxysporum* (18.5 mm). Moderate inhibition was observed in *F. oxysporum* against ethyl acetate (17.3 mm) and chloroform (16.5 mm). Antifungal activity was found to be minimum in petroleum ether extract against *Penicillium* (15.3 mm) and *F. oxysporum* (13.2 mm).

Antimicrobial Efficacy of AgNPs Synthesized Using *S. cumini*: The results of antimicrobial activity of synthesized silver nanoparticles assayed *in-vitro* by the agar well diffusion method was represented in **Table 3**. 400 µl of synthesized AgNP produced maximum inhibition against *Bacillus sp.* (23 mm), followed by *S. epidermis* and *A. niger* (22.5 mm). This may be due to perforation and lysis of AgNPs to the bacterial cell wall followed by generation of free radicals [41] and degradation of DNA [42]. 300 µl produced inhibition against *Bacillus sp.* (22.3 mm), followed by *S. epidermis* (21.1 mm). *P. aeruoginosa*, *A. niger*, and *E. coli* produced an inhibition in the range of 20 mm. Zone of inhibition of *E. coli* and *S. aureus* against $AgNO_3$ and increasing concentration of AgNPs has been reported [43].

200 µl acquired inhibition against *Bacillus sp.* (20.5 mm), *S. epidermis* (20.2 mm) and *E. coli* (19.1 mm). This can be due to a reduction of $AgNO_3$ into which resulted in an increased surface area that leads to better surface contact with bacteria and hence better bactericidal [41]. Moderate zone of inhibition was observed in *Penicillium* (17 mm), followed by *M. Mucilaginous* (16.2 mm). Minimum inhibitory activity was observed in *M. mucilaginous* (15.2 mm), followed by *F. oxysporum* (14 mm). These zones were developed against both gram-positive (*Bacillus subtilis*) and gram-negative (*Escherichia coli*); it was interestingly noted that *Escherichia coli* with thin cell wall are sensitive to cell wall damage compared to *Bacillus subtilis* [44].

CONCLUSION: In this study optimization of silver nanoparticles (AgNP) was done and the best reaction conditions were selected. Flavonoids synthesized from *S. cumini* along with AgNP are found to possess maximum microbicidal effect.

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CONFLICT OF INTEREST: The authors declare that there is no conflict of interest.

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