

Evaluation of Bactericidal and Fungicidal Properties of Silver Nanoparticles Fabricated Using *Jasminum sambac* (L.)

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Abstract: Plant mediated silver nanoparticle synthesis has been reported as a good alternative for physical and chemical methods. In the present study, plant mediated synthesis of silver nanoparticles (AgNPs) was carried out using the leaf extract of *J. sambac* and its antimicrobial, potential was evaluated. Bio reduction of silver ions occurred at a faster rate (i.e. 15 - 20 minutes) which was confirmed by the visible colour change from pale yellow to dark brown in the reaction mixture. The UV-Vis spectra showed maximum absorbance at 420 nm. SEM result revealed that the surface morphology of the synthesized silver nanoparticles was spherical in shape with the diameter range of 14 to 17 nm. The presence of peak of Ag in EDAX spectra further confirmed the presence of elemental silver in the suspension. The FTIR spectrum of synthesized silver nanoparticles showed ten different functional groups. The synthesized silver nanoparticles exhibited antimicrobial effect against eight bacterial and four fungal pathogens. From the present study, it is concluded that the plant mediated synthesis of silver nanoparticles using leaf extract of *J. sambac* is quick with potent antimicrobial activity.

Key words: Phytofabrication • Silver Nanoparticles • *Jasminium sambac* • Antimicrobial Activity

INTRODUCTION

Bio nanotechnology has emerged as an integration of biotechnology and nanotechnology to develop biosynthetic and environmental-friendly technology for the synthesis of nanomaterials [1]. Bio nanotechnology is also considered as a new frontier in medical field. Shrivastava *et al.* [2] and Kumar *et al.* [3] suggested that bio nanotechnology may fulfil the needs of medical sciences in various ways such as imaging, sensing, targeted drug and gene delivery and artificial implants.

Nanoparticles are being considered as fundamental building blocks of nanotechnology and it is a cluster of atoms in the size range of 1 to 100 nm. The smaller particle size has unique, chemical and physical properties and is

very useful in biomedical sciences [4]. These particles are of great scientific interest as they bridge the gap between bulk materials and atomic or molecular structures. The nanoparticles have more fascinating properties than bulk materials. Silver nanoparticles have several industrial applications and are being used in many home appliances due to their strong bactericidal activity [5]. When compared with other metals, silver exhibits stronger activity against microorganisms and lower toxicity to mammalian cells [5]. Hence the researchers are concentrating to develop new pharmaceutical products using silver nanoparticles.

Nowadays, AgNPs are synthesized by various approaches such as chemical, physical and biological routes. Among these methods, biological route is a novel

method because of its variability, availability, easy procedure, cost effective, eco-friendly and amenable for large scale production [6]. In the biological synthesis of silver nanoparticles, microorganisms like fungi, yeasts (eukaryotes) or bacteria, actinomycetes (prokaryotes), plant extracts or enzymes and templates like DNA, membranes, viruses and diatoms are used [7]. Among the biological routes, plant and plant materials mediated silver nanoparticle synthesis is more advantageous than microbes and animal products. This is due to the presence of broad variability of bio-molecules in plants that act as capping and reducing agents which in turn increase the rate of reduction and stabilization of silver nanoparticles [8].

Now more evidences are available to prove the efficiency of silver nanoparticle synthesis by green synthesis method. The procedure is simple and quick i.e. the plant extract is mixed with silver nitrate solution and allowed for reduction at room temperature. The reduction reaction is completed within few minutes that results in the synthesis of AgNPs [9]. The reducing agents involved in the synthesis include various water soluble metabolites such as alkaloids, phenolic compounds, terpenoids, flavones, quinines, organic acids, polysaccharides, proteins and co-enzymes which are available in the plant extract [10]. Nature is an important source of products that are currently being used in medical practice [11]. Medicinal plants play a significant role as therapeutics that aid health system all over the world. A major factor impeding the development of the medicinal plant is lack of information about utilization of them. Nanoparticle synthesis using medicinal plant would give many applications in medical field.

It is presumed that the source of plant extract may influence the characteristics of metallic nanoparticles [9, 12]. *Jasminum* species is one of the important medicinal plants. Leaves of *Jasminum sambac* L. are rich in antioxidant compounds and help to treat fever, ulcers and gallstones. They are also useful in gonorrhoea, ophthalmopathy and skin diseases (bacteria and fungi). They heal wounds and prevent inflammation [13]. They also possess antidiabetic, antitumor, antimicrobial, antioxidant, anticancer, anti-stress properties and anesthetic stimulating effect [13, 14]. These medicinal properties necessitate the use of this plant for silver nanoparticle synthesis. The present study was aimed at the detection of bioactive compounds and to synthesize silver nanoparticles using leaf of *Jasminum sambac* L. and to evaluate the competence of the synthesized silver nanoparticles as microbicide agent.

MATERIALS AND METHODS

Chemicals: Pure and analytical grade chemicals were used for synthesis of silver nanoparticles. The media for growth of microbial cells and all the other chemicals used in this study were purchased from Himedia laboratories Pvt. Ltd. Mumbai, India. The bacterial and fungal cultures were obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India.

Silver Nanoparticle Synthesis: Jasmine (*Jasminum sambac* L.) leaves were collected from Anaiyur, Sivakasi, Virudhunagar district, Tamil Nadu, India. For the synthesis of silver nanoparticles, 10 ml of leaf extract was added to 90 ml of 1 mM AgNO₃ solution. This solution mixture was kept in dark condition at room temperature for bio reduction process and the colour change was observed. After the colour change, the silver nanoparticle solution thus obtained was purified by repeated centrifugation at 7000 rpm for 20 minutes. Then the pellet was resuspended with deionized water. This process was repeated three times to obtain silver nanoparticles devoid of free entities.

Characterization of Silver Nanoparticles: Initial characterization of silver nanoparticles was carried out by UV-Visible spectroscopy. Change in color was visually observed in the silver nitrate solution incubated with leaf extract of *Jasminum sambac*. Absorbance of synthesized nanoparticles was measured between 200 and 800 nm in a UV-Visible Spectrophotometer (LabKit, Hongkong) at 1 nm resolution. Scanning Electron Microscopic (SEM) analysis was done in a Hitachi S-4500 SEM machine. A thin film of the silver nanoparticle was made by dipping a glass plate in the silver nanoparticle solution and X-ray diffraction studies were carried out. EDAX was tested in a Hitachi S-340 N SEM attached with Thermo EDAX attachments. FTIR was carried out on a SHIMADZU model equipment in the d reflectance mode operating at a resolution of 4 cm⁻¹.

Antimicrobial Activity Assay: Bactericidal and fungicidal assay was carried out using standard agar well diffusion method [15, 16] against both Gram positive (*Bacillus subtilis* MTCC121, *Bacillus cereus* MTCC430, *Bacillus coagulans* MTCC492, *Staphylococcus aureus* MTCC3160) and Gram negative bacteria (*Escherichia coli* MTCC40, *Klebsiella pneumoniae* MTCC109, *Proteus vulgaris* MTCC426 and *Pseudomonas aeruginosa* MTCC424) and virulent strains of fungal pathogens such

as *Alternaria alternata* MTCC149, *Curvularia lunata* MTCC283, *Cladosporium herbarum* MTCC351 and *Penicillium chrysogenum* MTCC161.

Identification of Bioactive Compounds in the Leaf Extract by GC-MS: For GC-MS analysis, Dichloromethane (DCM) was used as solvent. 2 ml of leaf extract was mixed with 1 ml of DCM in the ratio of 2:1. Then the DCM layer was filtered through silica gel G (50-60 μ mesh size). The filtrate was collected in a glass vial and sealed with air-tight screw cap. The vials were stored at -20°C until analysis. The sample was fractionated and chemical compounds were identified by Gas Chromatography-linked Mass Spectrometry (GC-MS; QP- 5050, Shimadzu, Japan). Identification of unknown compounds was made by probability-based matching using the computer library built within the NIST 12 system.

RESULT AND DISCUSSION

Numerous physical, chemical and biological methods have been adopted for silver nanoparticle synthesis. Among these methods, biological synthesis is gaining importance at a faster rate. The present study also provides the evidence that *J. sambac* leaf extract is a successful agent for the synthesis of silver nanoparticles.

The leaf extract of *J. sambac* has the potential to convert silver nitrate to silver nanoparticles by the reduction of silver ions. The colour change in the reaction mixture was considered as a preliminary confirmation for the synthesis of silver nanoparticles. UV-Vis absorption spectrum of reaction mixture was obtained at specific wavelength. In the present study, the colour change was observed in the reaction mixture within a short period of 15-20 minutes which indicated the reduction of silver nitrate (Fig. 1). It is reported that the colour change in the solution may be due to the excitation of surface plasmon vibrations in the nanoparticles [17] and may also be attributed to the presence of large amount of phytochemicals and wide range of metabolites present in the plant extract [18]. The differences in time taken for colour change may be due to the concentration, availability and variability of biomolecules present in the leaf extract of *J. sambac* used for nanoparticle synthesis. In the present study we have identified 20 chemicals in the leaf extract which may participate in the bio reduction of silver nitrate. It is evident from literatures that there is no correlation between duration of bio reduction of silver nitrate and its activity.

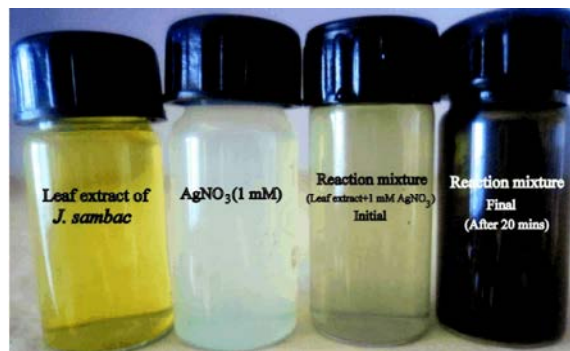


Fig. 1: Occurrence of colour change in reaction mixture

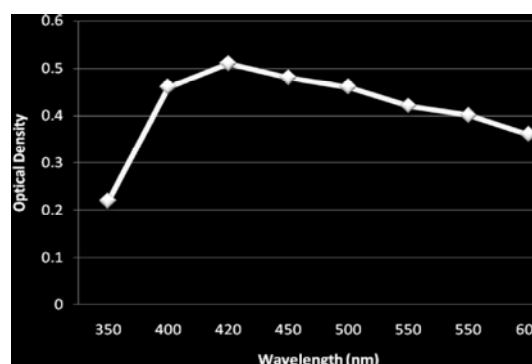


Fig. 2: UV- visible absorption spectrum of silver nanoparticles synthesized using *J. sambac* leaf extract

The presence of nanoparticle was further confirmed by UV-Vis spectroscopy. It is very useful to identify the formation of metal nanoparticle in reaction mixture [19]. In the present study, nanoparticles showed sharp absorbance maximum at 420 nm and gradually decreased with increase in wavelength. This can be attributed to the surface plasmon resonance of synthesized silver nanoparticles (Fig. 2). Broadening of peak indicates the formation of polydispersed nanoparticles in the reaction mixture.

The morphology and size of the biosynthesized silver nanoparticles using aqueous leaf extract of *J. sambac* were determined by SEM (Fig. 3). The SEM image showed that the synthesized silver nanoparticles were clustered. The particles were more or less spherical in shape and their size range was 14-17 nm.

The results of XRD pattern analysis revealed three intense peaks in the whole spectrum of 2 θ values ranging from 10 to 80 for the silver nanoparticles (Fig. 4). The synthesized silver nanoparticles were in the form of nanocrystals as evidenced by the peaks at 2 θ values of 29.73, 38.27 and 43.61. The XRD pattern showed strongest peaks in the whole spectrum of 2 θ value ranging from

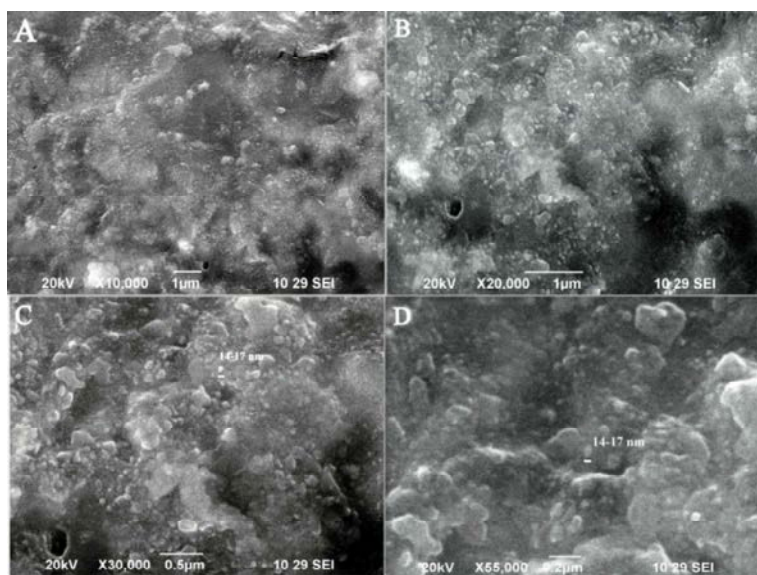


Fig. 3: SEM images of synthesized silver nanoparticles at different magnifications: (A) 20 kV X 10,000; (B) 20 kV X 20,000; (C) 20 kV X 30,000 and (D) 20 kV of X 55,000

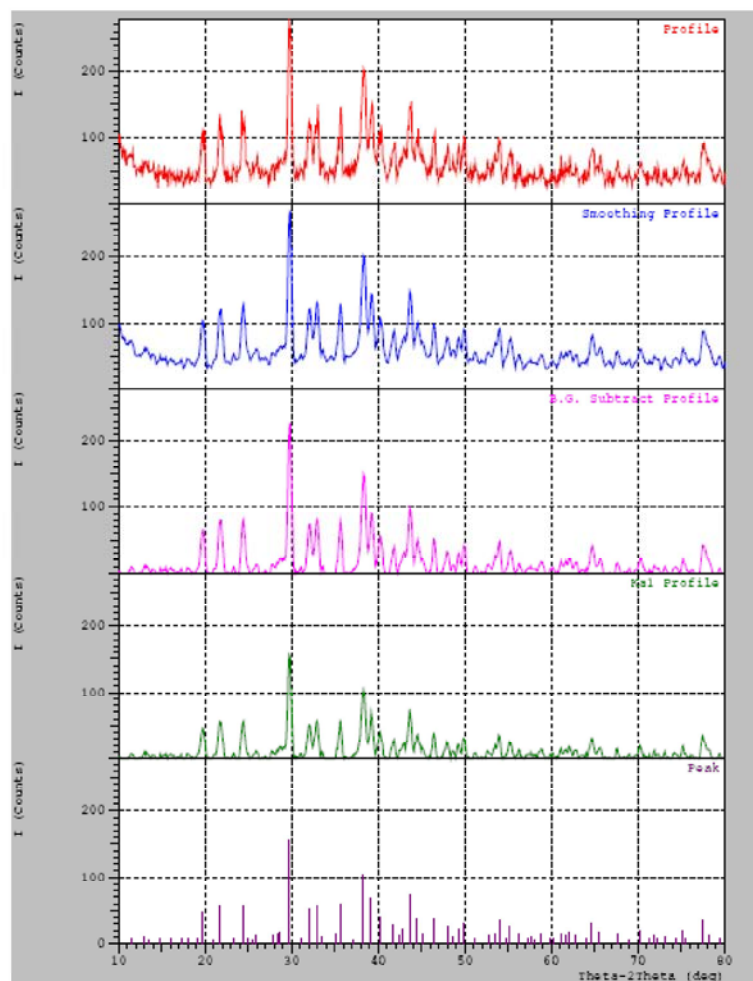


Fig. 4: XRD patterns of synthesized silver nanoparticles using *J. sambac* leaf extract

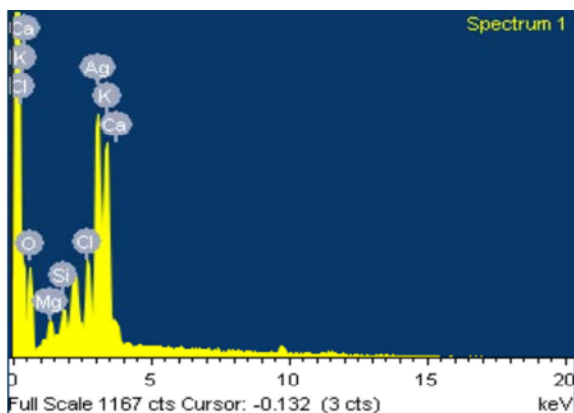


Fig. 5: EDAX spectrum of synthesized silver nanoparticles using *J. sambac* leaf extract

20 - 35. The XRD is a valuable tool to calculate the size of crystalline silver nanoparticle size. In the present study, the particle sizes were ranging between 14 and 17 nm.

EDAX provides the supporting confirmation for the formation of silver nanoparticles. In the present study, EDAX spectrum showed the signal for silver which confirmed the presence of silver nanoparticles (Fig. 5). The signal was observed at 3 KeV, which is typically for silver nanoparticles due to the surface plasmon resonance. The other spectral signals such as Ca, K, Cl, O, Mg and Si were also noticed in the EDAX spectrum. The signals other than silver signal in EDAX spectrum may arise from the organic content of leaf extract that are already bound with the surface of silver nanoparticles.

FTIR provides the information about functional groups present in the leaf extract which is responsible for the transformation of AgNO_3 from simple inorganic to elemental silver. It may be attributed to the action of different phytochemicals present in the plant extract which would act as reducing, stabilizing and capping agent [20]. In the present study, FTIR spectrum indicates that the leaf extract of *J. sambac* assisted the production of silver nanoparticles. It is shown by the functional groups on the silver nanoparticles. The FTIR spectrum of produced silver nanoparticles showed many absorption bands (Fig. 6) at 3377.12 cm^{-1} , 3317.34 cm^{-1} , 3174.61 cm^{-1} , 2923.88 cm^{-1} , 2306.71 cm^{-1} , 1622.02 cm^{-1} , 1400.22 cm^{-1} , 1309.58 cm^{-1} , 1114.78 cm^{-1} and 995.20 cm^{-1} which were assigned to the O-H stretching of alcohol, C=H stretching of alkynes, N-H stretching of amides, C-H stretching of alkanes, C=N stretching of nitriles, N-H bend of amines, N=O bend of nitro groups, O=C-O-C stretching of esters, O-H stretching of alcohol and =C-H stretching of alkenes (Fig. 6; Table 1). These results reveal that the silver

Table 1: Functional groups in synthesized silver nanoparticles revealed by FTIR

S.No.	Absorption (cm^{-1})	Class of compounds	Bond
1.	3377.12	Alcohol	O-H stretch
2.	3317.34	Alkynes	C=H stretch
3.	3174.61	Amides	N-H stretch
4.	2923.88	Alkanes	C-H stretch
5.	2306.71	Nitriles	C=N stretch
6.	1622.02	Amines	N-H bend
7.	1400.22	Nitro Groups	N=O bend
8.	1309.58	Esters	O=C-O-C stretch
9.	1114.78	Alcohol	O-H stretch
10.	0995.20	Alkenes	=C-H stretch

nanoparticle synthesized using *J. sambac* leaves extract was found to have all these functional groups bound on it.

It can be suggested that the silver nanoparticles obtained in the present study may be surrounded by proteins having the functional groups such as Alcohols, Alkynes, Amides, Alkanes, Nitriles, Amines, Nitro Groups, Esters and Alkenes. Tripathi *et al.* [21] reported that functional residues have stronger capability to bind with silver nanoparticles to prevent agglomeration and also provide longer stability. It is also stated that the biomolecules present in the plant extract may play dual role i.e. silver nanoparticle synthesis as well as stabilization of the synthesized particles.

As another phase of the present study, antimicrobial effect of the synthesized AgNPs was evaluated against eight clinically important both Gram positive and negative bacterial pathogens like *Bacillus subtilis*, *Bacillus cereus*, *Bacillus coagulans*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and four fungal pathogens viz. *Alternaria alternata*, *Curvularia lunata*, *Cladosporium herbarum* and *Penicillium chrysogenum*. As evident from Table 2 and Table 3, the silver nanoparticles showed promising antimicrobial activity against all the tested pathogens. The activity was found to be higher in silver nanoparticles than the leaf extract and silver nitrate solution alone. The $60\text{ }\mu\text{l}$ concentration of synthesized silver nanoparticles exhibited highest bactericidal as well as fungicidal activity. The present study provides the evidence that silver nanoparticles are effective against both Gram positive and Gram negative bacteria and also inhibit the growth of fungal pathogens. Guzman *et al.* [22] reported that silver nanoparticles exhibited very good activity against both Gram positive and negative bacteria and the activity increased with the reduction in the size of the nanoparticle. In the present study, the particle size was found to be 14-17 nm. Thus, it may be suggested that

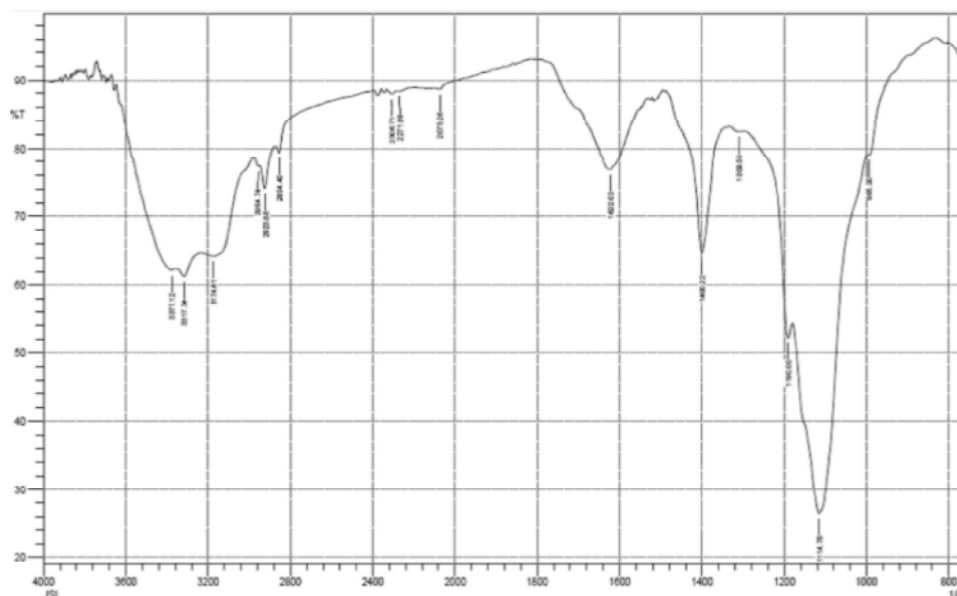
Fig. 6: FTIR spectrum of silver nanoparticles synthesized using *J. sambac* leaf extract

Table 2: Bactericidal activities of different concentrations of leaf extract, silver nitrate and silver nanoparticles against eight bacterial strains

		Bacterial strain (Zone of inhibition in mm)							
Sample	Concentration (µl)	Gram positive				Gram negative			
		<i>B. subtilis</i>	<i>B. cereus</i>	<i>B. coagulans</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. vulgaris</i>	<i>P. aeruginosa</i>
Leaf extract	15	10.33±0.40	16.00±1.2	09.00±0.70	08.66±0.40	07.66±0.40	07.33±0.40	09.33±0.81	11.66±1.52
	30	12.33±1.08	20.33±1.47	11.66±1.08	10.66±0.40	09.66±0.40	10.33±0.40	12.66±0.40	14.00±0.70
	45	15.33±1.47	24.00±0.12	15.33±0.40	13.00±0.70	12.33±0.40	12.66±1.08	18.00±0.70	18.00±0.70
	60	17.33±1.47	25.00±0.70	18.00±0.70	14.66±0.40	15.00±0.70	15.66±1.08	22.00±0.70	22.00±0.70
Silver nitrate	15	08.33±1.52	08.00±1.22	12.66±1.08	09.00±0.70	08.66±1.08	07.00±1.22	08.33±1.63	10.00±0.70
	30	11.33±1.52	09.66±1.08	14.00±1.87	11.66±1.08	10.33±1.47	08.00±1.22	09.33±1.63	12.00±0.70
	45	13.00±1.41	12.00±0.00	16.33±1.47	15.00±0.70	11.66±1.63	09.00±1.22	12.00±1.22	14.00±0.70
	60	13.00±1.41	14.00±0.70	18.00±1.22	18.66±0.40	13.33±2.041	10.33±1.63	13.33±1.08	16.00±0.70
Synthesized silver nanoparticles	15	11.66±1.52	17.00±0.70	13.66±0.81	14.66±0.40	11.66±0.40	11.00±0.70	17.33±1.47	15.00±0.70
	30	15.66±1.52	19.66±0.40	16.33±1.08	17.00±0.70	14.00±0.70	12.66±1.08	19.66±1.08	16.00±0.70
	45	17.66±1.52	23.00±0.70	18.33±1.08	19.33±0.40	16.00±0.70	14.00±0.70	22.66±0.40	21.66±1.52
	60	21.00±0.70	27.33±1.63	21.00±0.70	22.66±0.40	18.00±0.70	15.66±0.40	25.33±0.81	24.33±0.40

Values are expressed in Mean ± SE.

Table 3: Fungicidal activities of different concentrations of leaf extract, silver nitrate and silver nanoparticles against four fungal strains

		Fungal species (Zone of inhibition in mm)			
Sample	Concentration (µl)	<i>A. alternate</i>	<i>C. lunata</i>	<i>C. herbarum</i>	<i>P. chrysogenum</i>
Leaf extract	15	07.33±1.08	11.00±0.70	08.33±0.40	10.00±1.41
	30	09.00±1.41	13.00±0.70	10.33±0.40	13.00±0.70
	45	11.00±1.41	15.33±0.40	12.00±0.70	15.33±1.08
	60	12.66±1.08	18.33±0.40	14.33±0.40	21.00±1.08
Silver nitrate	15	07.00±1.22	07.66±1.08	07.00±0.70	08.66±1.47
	30	09.00±1.22	10.00±1.22	08.33±0.81	09.66±1.47
	45	10.66±1.63	12.00±1.22	09.66±1.08	11.00±1.87
	60	12.66±2.27	13.66±1.08	11.00±1.41	12.66±1.47
Synthesized silver nanoparticles	15	13.00±0.70	12.66±1.08	12.00±1.41	15.33±0.40
	30	15.00±0.70	16.33±0.40	15.00±0.70	17.33±0.40
	45	17.00±0.70	19.00±0.70	17.33±1.08	21.66±1.47
	60	19.00±0.70	21.33±1.08	19.33±1.08	27.00±1.22

Values are expressed in Mean ± SE.

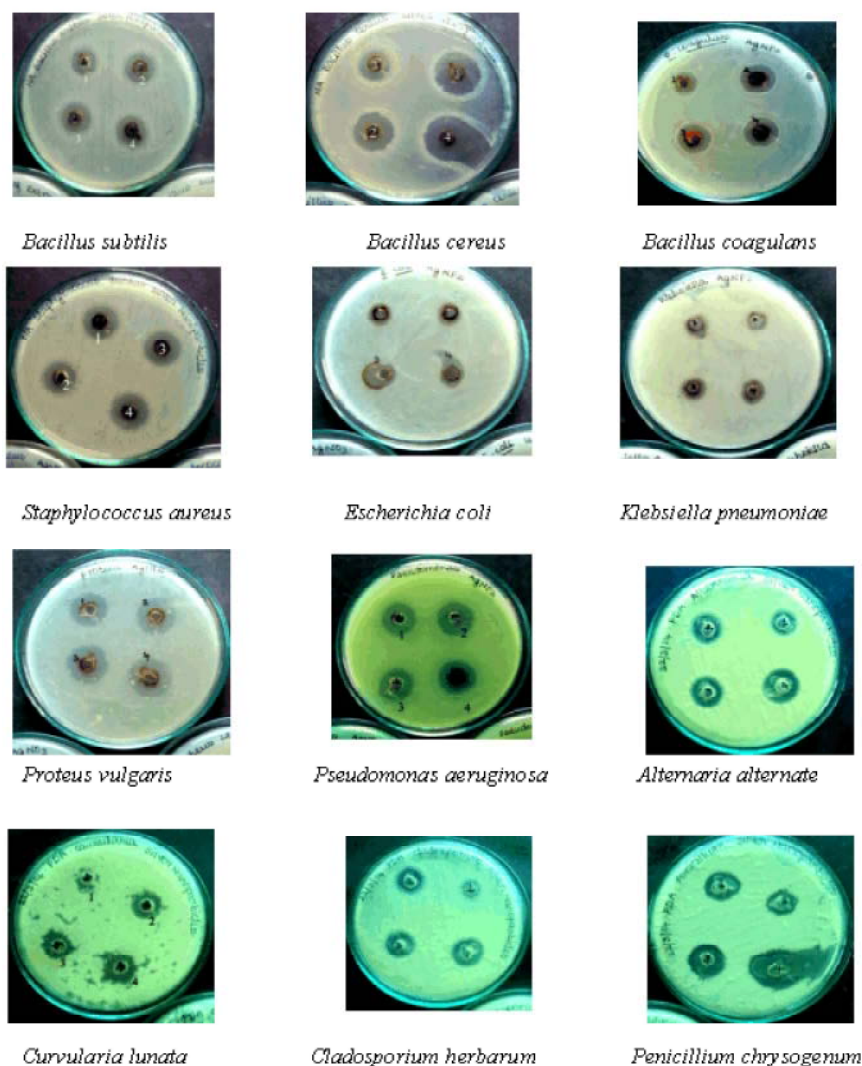


Fig. 7: Bactericidal and fungicidal activities of silver nanoparticles (1- 15 µl; 2- 30 µl; 3- 45 µl; 4- 60 µl of silver nanoparticle)

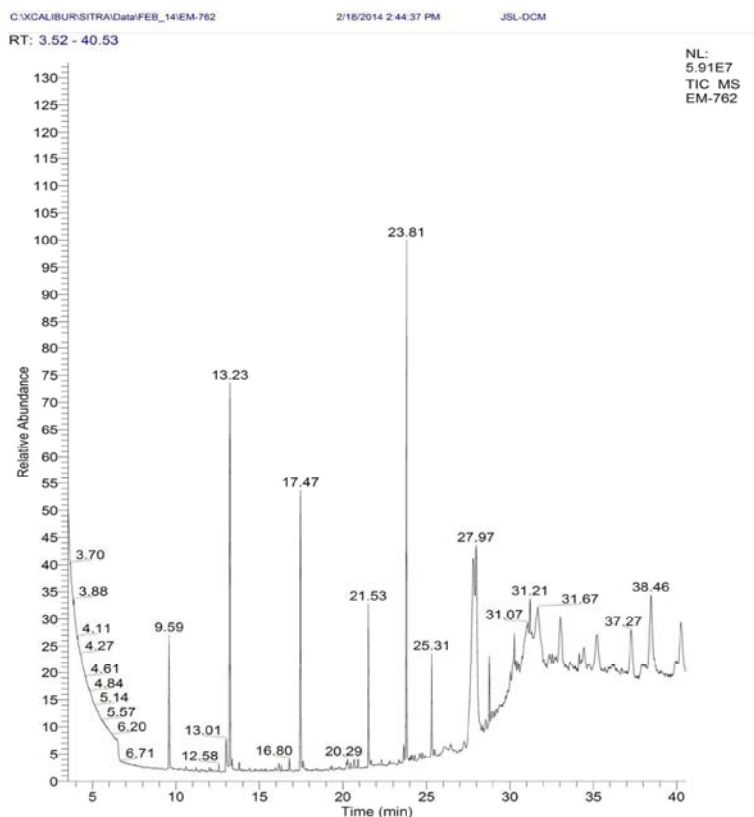
these nanoparticles can be used as a potent antimicrobial agent with broad spectrum of activity. In a recent study, the stable silver nanoparticles using the leaf extract of *J. sambac* were synthesized by microwave assisted phytosynthesis method. It was found out that silver nanoparticles were effective growth inhibitors for various bacteria (*S. aureus*, *E. coli*, *S. typhi* and *P. aeruginosa*) and fungi (*T. rubem*, *C. albicans* and *C. indicum*) [23]. The present study also provides additional evidence that *J. sambac* is a good source of silver nanoparticle synthesis with potential antimicrobial activity.

The utilization of silver as a disinfectant is not new and silver compounds are shown to be effective against both aerobic and anaerobic bacteria by precipitating the bacterial cellular proteins and by blocking the microbial

respiratory chain system. The exact mechanism of antimicrobial activity of nanoparticles is not clearly known but some of the hypotheses were provided by Chaloupka *et al.* [24], Prabhu and Eldho [25] and Sarsar *et al.* [26] in their review. For instance, bacterial death may be due to the attachment of silver nanoparticles on the surface and penetration into the cell which result in the destruction of peptidoglygon of bacterial cell wall that leads to lysis of the cell membrane. The interaction with DNA affects replication and binding with mitochondria affects respiratory chain. This may create free radicals and induce oxidative stress in cells. The silver nanoparticles may also interact with the thiol groups of many vital enzymes and inactivate them and may denature ribosomes, thereby inhibiting protein synthesis.

Table 4: List of phytochemicals in the leaf extract of *J. sambac* by GC-MS

S.No.	Retention time (mins)	Compound name	Molecular formula	Molecular weight
1	03.06	Cyclopropanecarboxylic acid, 2-(1,1-dimethylethyl)-1,2-dimethyl-, methyl ester, cis-	$C_{11}H_{20}O_2$	184
2	06.47	d-Leucyl-d-leucine, trimethylsilyl ester	$C_{15}H_{32}N_2O_3Si$	316
3	09.59	Tetradecane	$C_{14}H_{30}$	198
4	13.23	Hexadecane	$C_{16}H_{34}$	226
5	17.47	Octadecane	$C_{18}H_{38}$	254
6	20.29	4-tetradecyl ester dichloroacetic acid	$C_{16}H_{30}Cl_2O_2$	324
7	21.53	Eicosane	$C_{20}H_{42}$	282
8	23.81	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276
9	24.62	Dimethyl bicyclo[3.1.0] hexane-6,6-dicarboxylate	$C_{10}H_{14}O_4$	198
10	25.31	Docosane	$C_{22}H_{46}$	310
11	26.07	Synaptogenin B	$C_{30}H_{46}O_4$	470
12	27.26	5-(2-Aminopropyl)-2-methylphenol	$C_{10}H_{15}NO$	165
13	27.97	Docosane	$C_{22}H_{46}$	310
14	28.77	Tetracosane	$C_{24}H_{50}$	338
15	30.26	Bis(2-ethylhexyl)ester hexanedioic acid	$C_{22}H_{42}O_4$	370
16	31.19	Dibromoschizandrin	$C_{24}H_{30}Br_2O_7$	588
17	31.67	3,3'-(1''-benzoyloxybut-3''-en-1''-yl)-2'',7'',12'', 18''-tetramethyl-21H,23H-porphyrin-13'',17''-diyl]-dipropionate	$C_{43}H_{44}N_4O_6$	712
18	33.03	1-Hentriacontanol	$C_{41}H_{84}O$	592
19	37.90	3',4'-Dihydro-stephasubine	$C_{36}H_{56}N_2O_6$	592
20	38.46	6-bromo-4-{2-[(trifluoroacetyl) amino] phenyl}-5,8-dimethoxyquinoline	$C_{19}H_{14}BrF_3N_2O_3$	454

Fig. 8: Gas Chromatogram of *Jasminum sambac* leaf extract

In the present study, GC-MS measurements of leaf extract enabled the identification of 20 components (Fig. 8; Table 4). About 34 phytochemicals were identified

in the methanolic extract of *A. paniculata* by Suman *et al.* [27]. The leaf extract of *J. sambac* was found to have major compounds such as Tetradecane, Hexadecane,

Octadecane, Eicosane, 7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione, 5-(2-Aminopropyl)-2-methylphenol and 6-bromo-4-{2-[(trifluoroacetyl) amino] phenyl}-5,8-dimethoxyquinoline along with several other minor compounds. These compounds are suggested to mediate the metal ion reduction process and may also control the stability of thus formed nanoparticles by coating them and hindering agglomeration. The presence of a wide diversity of functional groups may be attributed to silver reduction.

Thus, the present study suggests that the *J. sambac* leaf extract is the good source for the synthesis of potential silver nanoparticles by ecofriendly manner at low cost with very good antimicrobial activity. Further detailed investigation would be helpful to obtain new pharmacological products from these silver nanoparticles.

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