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Phytomediated Synthesis and Characterization of Silver Nanoparticles from the Leaf Extracts of Begonia Malabarica Lam and its Antimicrobial Activity

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ABSTRACT

Development of biologically inspired phytomediated synthesis of silver nanoparticles is evolving into an important branch of nanobiotechnology. In the present investigation, we report the phytomediated synthesis of silver nanoparticles (AgNPs) employing the leaf extract of *Begonia malabarica* Lam. (Begoniaceae). The synthesized Ag-NPswere characterized by UV-visible, X-ray diffraction (XRD), Fourier-transform infrared (FT-IR), Scanning electron microscopy (SEM), and Energy dispersive X-ray (EDX). Formation of silver nanoparticles was confirmed by the change of colour from pale yellow to dark brown in colour. These results authenticated that the appearance of AgNPs was analyzed by UV-visible spectrum around the peak 420 nm. XRD (X-ray diffractometer) demonstrated the formation of crystalline AgNPs with FCC structure having an average crystalline size of 24 nm from XRD profile. FT-IR analysis revealed the presence of different functional groups in the synthesized AgNPs. Antimicrobial activity of the synthesized silver nanoparticles was evaluated against Gram positive and Gram negative bacteria such as *Bacillus subtilis, Staphylococcus aureus*, *Streptococcus faecalis, Klebsiellapneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* and fungus *Candida albicans.* Both the leaf extract of *Begonia malabarica* and synthesized silver nanoparticles from the leaves of *Begonia malabarica* showed moderate antimicrobial activity.

Keywords: Phytomediated synthesis, characterization, *Begoniamalabarica*, silver nanoparticles, Antimicrobial activity,

Introduction

The growth of nanotechnology is rapid in the areas of research and development that holds tremendous applications for the society, industry and medicine (1). Nanotechnology mainly deals with the formulation of experimental process for the synthesis of nanomaterials using different systems with their wide applications. The use of metal nanoparticles has received extensive attention in present century due to their remarkable properties and wide range of the applications (2). They can be synthesized by several physical, chemical and biological methods (4-6).Use of toxic chemical synthesis greatly limits their biomedical applications

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particularly in clinical fields. Therefore, the focus for the green synthesis of nanoparticles is an emerging branch of nanotechnology with the help of biological resources like plant extract (7, 8), Bacteria (9), Fungi (10, 11), which provides numerous benefits of ecofriendliness and compatibility for pharmaceuticals and other biomedical applications.Green-assisted synthesis of nanoparticles using plant materials are effortless, capable and eco-friendly in comparison with chemical-mediated or microbe-mediated synthesis (12).*Begonia malabarica* Lam. (Begoniaceae) is one of the important medicinal plants whose main secondary metabolites are luteolin, quercetin and beta-sitosterol. The leaves are used for the treatment of respiratory tract infections, diarrhoea, blood cancer and skin diseases.However, the research on the biosynthesized AgNps from *Begonia malabarica* leaves for characterization and antimicrobial activity is hitherto unavailable.Hence, the present study has been carried out to characterize the biosynthesized silver nanoparticles using leaf powder extract of *Begonia malabarica* and to study its antimicrobial activity

Materials and Methods

Preparation of *Begonia malabarica* leaves extract

The AR grade of silver nitrate was purchased from Sigma- Aldrich chemicals in India. Microbial strains were procured from Department of Biology, Gandhigram Rural Institute - Deemed University, Gandhigram. Mueller–Hinton broth and agar were purchased from Hi-Media, Mumbai, India and fresh leaves of *Begonia malabarica*were collected from Pothigai hills, Tenkasi District, Tamil Nadu, India. 25g of the fresh leaf powder was mixed with 100 ml of double distilled water and transferred into the 500 ml beaker and boiled at 100°C for 40 minutes and then brought down to room temperature. Further, the extract was filtered with Whatman No.1 filter paper and stored at 4°C.

Microorganisms

The microbial strains such as bacteria *Bacillus subtilis*(MTCC 441), *Staphylococcus aureus*(MTCC 96), *Streptococcus faecalis*(MTCC 5383), *Klebsiellapneumoniae*(MTCC 4030), *Pseudomonas aeruginosa (*MTCC 741*),E.coli* (MTCC 443) and fungal *Candida albicans*(GRIBI 03) were collected from Department of Biology, Gandhigram Rural Institute - Deemed University, Gandhigram. Microbial strains were incubated at 37^0C in Mueller-Hinton Broth.

Synthesis of Silver nanoparticles using *Begonia malabarica*leaves extract

10 ml of leaf extract of *Begonia malabarica*was added to 1mM $AgNO_3$ (90 ml) aqueous solution. The reduction process of Ag^+ to Ag^0 was followed by the change of colour pale yellow to dark brown in colour which indicates the formation of AgNPs.

Characterization of AgNPs

The absorption spectrum of the synthesized silver nanoparticles from the leaves of *Begonia malabarica* was analysed by UV Visible spectroscopy-1800v (Shimadzu, Japan) at the wavelength ranging from 200-700 nm. The external morphology of the synthesized silver nanoparticles was observed on scanning electron microscope (SEM) at GRI and elemental composition of synthesized AgNPs was confirmed by EDAX analysis. FT-IR spectra

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revealed that perkin Elmer spectrum 400 FT-IR transmission mode 4000-400 cm^{-1}. DLS and Zeta potential measurements were carried out on same instrument HORIBA SZ-100 which was used to evaluate particle size and surface charge of the synthesized AgNPs. The average grain size was calculated by image Joint Committee on Powder Diffraction Standards (JCPDS) software. The crystalline structure of the synthesized AgNPs was illustrated that XPERT-PRO through powder X-ray diffraction using Cu Kα radiation with and theta angles at 10º C to 80º C.

Antimicrobial activity

Antimicrobial activity of the synthesized silver nanoparticles was carried out by using *Bacillus subtilis* (GRIBI 01), *Staphylococcus aureus* (GRIBI 05), *Streptococcus faecalis* (GRIBI 08), *Klebsiellapneumoniae* (GRIBI 02), *Pseudomonas aeruginosa (GRIBI04), E.coli*(GRIBI 06) and *Candida albicans* (GRIBI 03) agar well diffusion method. Mueller hinton agar medium 30.4g MH medium was mixed with 800 ml of distilled water and sterilized in autoclave at 20 minutes. The sterilized medium was allowed to pour into the Petri dishes. The solidified plates were poured to cork borer with the help of 6 mm diameter. The plates were plant extract, silver nitrate and different concentrations of synthesized AgNPs 100µg/ml, 150µg/ml, and 200µg/ml for antimicrobial studies.

Phytochemical analysis

The collected plant leaves extracts were subjected to synthesis of silver nanoparticles and synthesized particles were used in phytochemical analysis. (Table 1)

Test for Alkaloids (Mayer's Test)

One ml of synthesized AgNPs solution was mixed with 6 drops of Mayers reagent. Yellowish creamish precipitate was formed and thus indicated the presence of alkaloids (13 & 14).

Test for Tannins (Braymer's Test)

One ml of synthesized AgNPs solution was mixed thoroughly with 2ml of water. To this mixture, two drops of 5% ferric chloride solution was added. Appearance of dirty green precipitate denoted the presence of tannins (13 & 14).

Test for Steroids (Salkowski Test)

Equal volume (2 ml) of the synthesised silver nanoparticles solution and chloroform was taken and added concentrated sulphuric acid along the side of the test tube. Formation of reddish brown ring at the junction gave positive result for steroids [15].

Test for Terpenoids

About 2ml of the solution was mixed with 2ml acetic acid. Then, few drops of concentrated sulphuric acid was added. The appearance of deep red color showed the presence of terpenoids (15).

Test for Coumarins

About 2ml of the test solution was taken and mixed with 3ml of 10% sodium hydroxide. The formation of yellow colour denoted the existence of coumarins in the sample (15).

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Test for Catechins

About 2ml of alcoholic test solution was treated with few drops of Echrilich reagent and few drops of concentrated Hydrochloric acid. The formation of pink colour denoted the presence of catcehin (15).

Test for Phenols

About 1ml of the test solution was treated with 3 ml of 3% aqueous ferric chloride solution. The appearance of deep blue colour gave positive result for phenol (16).

Test for Flavonoids

About 1ml of the test solution was treated with 1ml of sulphuric acid. The appearance of orange colour showed the presence of flavonoids (17).

Test for Quinones

About 1ml of the test solution was treated with 5ml of HCL. The formation of yellow colour precipitate showed the presence of quinine (18).

Test for Saponins (Foam Test)

About 1ml of the test solution was mixed with 5ml of distilled water. The contents were mixed well and heated in a boiling water bath for 10 minutes. Frothing indicated the presence of saponins (19).

Resultsand Discussion

Visual examination and UV-vis spectroscopy

Green synthesis of silver nanoparticles was carried out by using aqueous leaf extract of *Begonia malabarica.* Inthe present study, 20 ml of aqueous leaf extract was supplemented with 80 ml of 1mM silver nitrate solution. After adding this, the mixture was turned to pale yellow to dark brown in colour. The appearance of dark brown colour indicated the formation of silver nanoparticles which was due to the excitation of Surface Plasmon Resonance (Fig 1). It is remarkable that the surface Plasmon band was registered at 420 nm. The absorption band value of 420 nm was closely related to the absorption band by the colloidal silver nanoparticles which were mainly due to the surface Plasmon resonance (Fig 2).

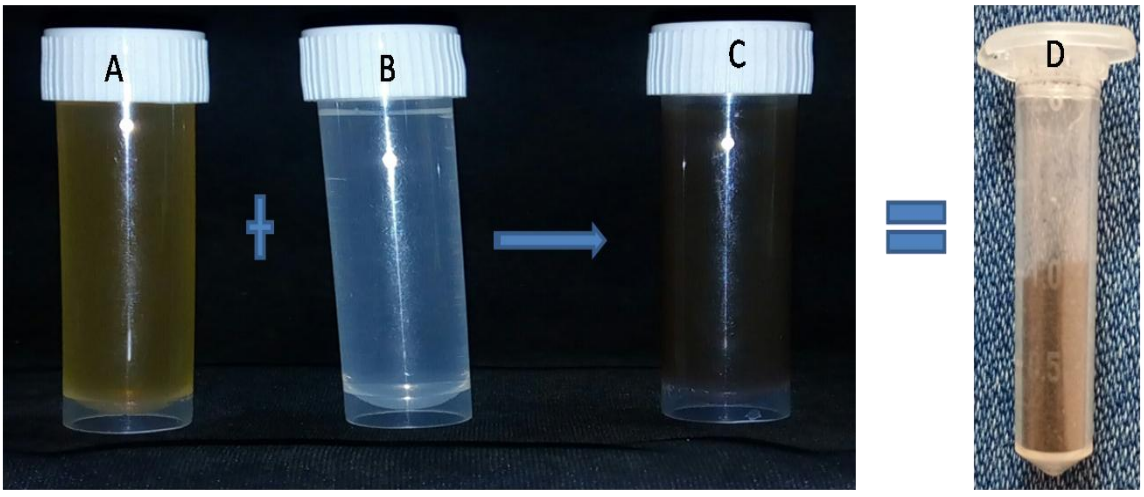

Figure 1.Change of color after the addition of silver nitrate solution in the green synthesis of silver nanoparticles.

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a) Plant extract **b) Silver nitrate**
c) Plant extract with silver nitrate **d) silver nanoparticles**

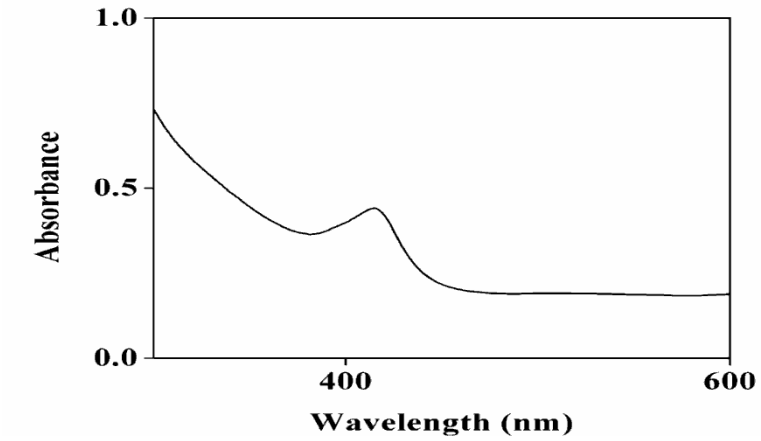

Figure 2.UV-visible spectrum of AgNPs synthesized using aqueous leaf extracts of *Begonia malabarica*.

FTIR analysis of synthesized AgNPs

The FTIR analysis of silver nanoparticles synthesized from the aqueous leaf extracts of *Begonia malabarica*revealed the presence of certain functional groups which were seen at the ranging from 500 to 4000cm^{-1}. It is cleared from the spectral data that some common absorption bands were pointed at 3436cm^{-1} are due to the presence of amine group.The absorption bands at 2929cm^{-1}is due to C-H stretching modes.1715cm^{-1} is assigned to the stretching vibration of C=O in aldehydes, ketones etc.1025cm^{-1} bands at C-O bond in alcohol, ether etc.Thus, C=O,C=C,C-O, C-N which acts as a stability of silver nanoparticles was achieved by the capping and reduction of green synthesis of silver nanoparticles (Fig.3).

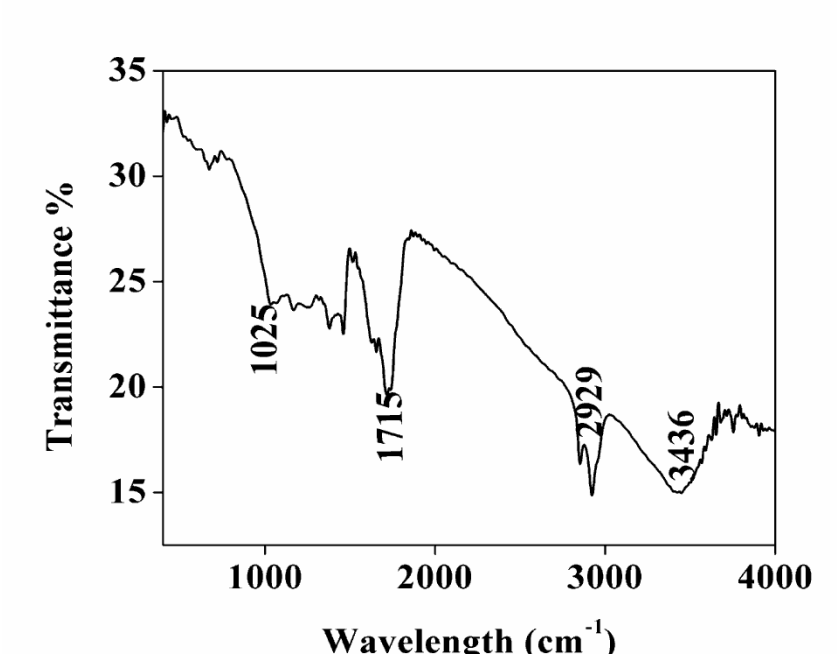

Figure 3.FTIR spectra of phytomediated synthesis of silver nanoparticles from the leaf extract of *Begonia malabarica*

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X-ray diffraction study

The crystalline nature and purity of phytomediated synthesis of silver nanoparticles was confirmed by X-ray crystallography. The XRD pattern of the phytomediated synthesis of silver nanoparticles from the leaf extract of *Begonia malabarica* was shown in Fig.4. The XRD data confirmed that the phytomediated synthesis of silver nanoparticles from the leaf extract of *Begonia malabarica* was crystalline in nature. In this study, four strong Bragg's reflections were noticed at 2θ of 38.03°, 44.10°, 64.49° and 77.63° which were corresponding to the lattice planes of (111), (200), (220), and (311)respectively. The data was well matched with the standard diffraction data by Joint Committee on Powder Diffraction Standards (JCPDS) file number 87-0720. The average size of phytomediated synthesis of silver nanoparticles from the leaf extract of *Begonia malabarica*was 35.73nm.

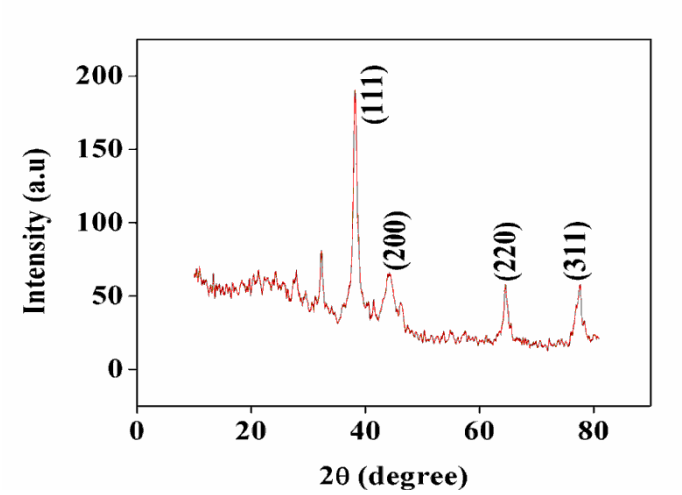

Figure 4.X-ray diffraction profile of phytomediated synthesized silver nanoparticles from leaf extract of *Begonia malabarica*

Scanning Electron Microscopy (SEM)

The size and morphology of the synthesized AgNPs was investigated by SEM. Figure 5 shows that the synthesized nanoparticles consist of large number of spherical like structures with the length ofmicrometer (24µm).

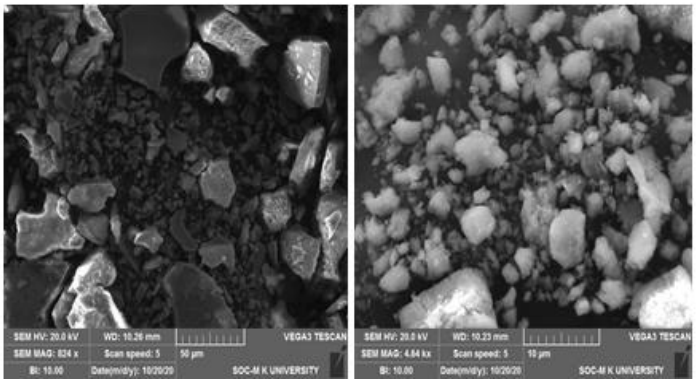

Figure 5.SEM image of phytomediated synthesis of Silver nanoparticles from the aqueous leaves extract of *Begoniamalabarica*

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Energy Diffraction X-ray (EDX)

Energy dispersive analysis by X-rays (EDAX) spectrum of phytomediated synthesis of silver nanoparticles by using aqueous leaf extracts of *Begonia malabarica*was shown in Fig.6.The elemental silver peak was noticed at 3 keV which meant for metallic silver. This highest peak also proved the purity of the silver nanoparticles. In addition to this, the peaks for carbonand oxygen were also visualized.

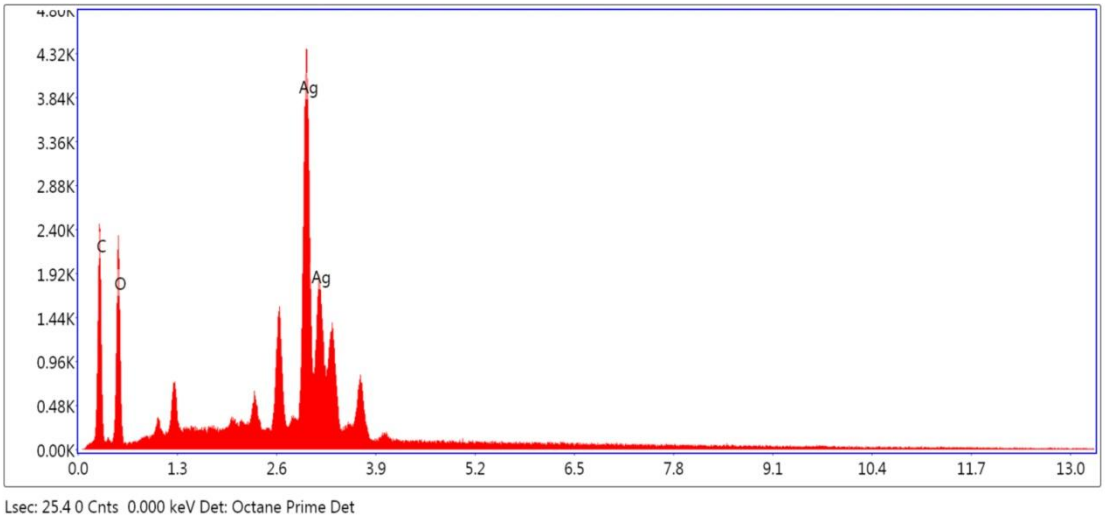

Figure 6EDX analysis of silver nanoparticles from the aqueous leaf extract of *Begonia malabarica*

Qualitative Phytochemical Screening andAntimicrobial activity

The preliminary phytochemical screening of phytomediated synthesis of silver nanoparticles when compared to the aqueous leaf extract of *Begonia malabarica*revealed the presence of various phytoconstituents. The synthesized silver nanoparticles and aqueous leaf extract of *Begonia malabarica*indicated the presence of the various phytochemicals such asalkaloids, flavonoids,saponins andtannins. It is remarkable that the phytochemicals such as phenols,terpenoids,quinonesand steroidswere not reported from the aqueous leaf extract of *Begonia malabarica*and synthesized AgNPs. (Table 1)

Phytochemical tests	**Synthesized AgNPs**	**Aqueous fruits extract**
Alkaloids	+	+
Flavonoids	+	+
Phenols	-	-
Quinones	-	-
Saponins	+	+
Steroids	-	-
Tannins	+	+
Terpenoids	-	-

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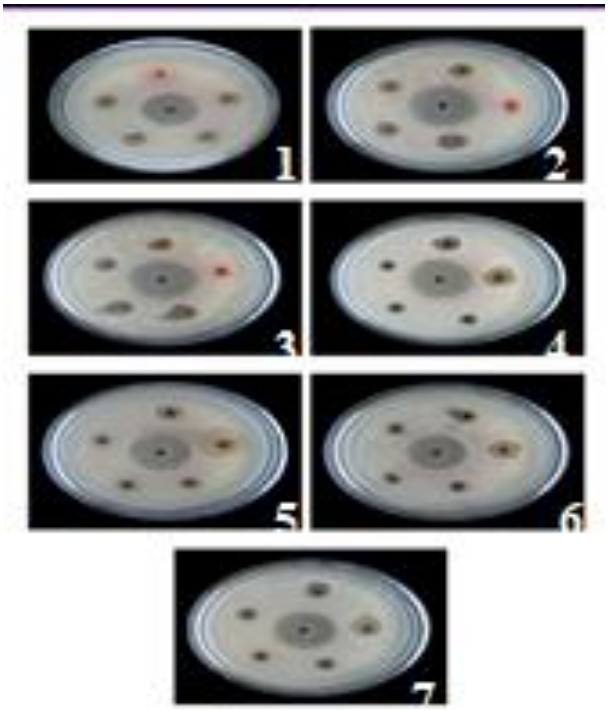
+ indicates presence of phytochemicals; - indicates absence of phytochemicals

Table 1.Preliminary phytochemical screening of aqueous leaves extract and synthesized AgNPs of *Begonia malabarica*

Antimicrobial activity

The antimicrobial activity of the phytomediated synthesized silver nanoparticles of *Begonia malabarica*leaves had powerful antimicrobial activity against gram-positive, gram-negative and one fungal pathogens. The silver nanoparticles of three different concentrations (100µg/ml, 150µg/ml, and 200µg/ml) treated have antimicrobial activity against gram positive and gram negative bacteria, with varying various size of inhibitory zone. Moreover, the leaf extract of *Begonia malabarica*showed low antimicrobial activity when compared to $AgNO_3$ and synthesized AgNPs. The results showed that AgNPs are effective antibacterial activity against *E.coli*(27.03 mm), *Candida albicans*(27.05 mm)*Staphylococcus aureus* (24.03mm) and*Klebsiellapneumoniae* (25.05mm) respectively. The remaining bacterial strains *viz. Pseudomonas aeruginosa*(23.06mm),*Bacillus subtilis* (23.05mm) and *Streptococcus faecalis*(22.05 mm)are fairly susceptible against various concentration of silver nanoparticles. The AgNPs synthesized from the leaves of *Begonia malabarica*exhibited greater significant antimicrobial activity against human pathogenic bacteria. (Plate 1).AgNPs, which are filled with polyphenolic compounds, disrupt the cell walls of bacteria, which make gramnegative bacteria specifically sensitive. Polyphenolic compounds generate free radicals and other oxygenbased reactive species, which can induce considerable damage and toxicity [17].The antimicrobial properties of silver nanoparticles are due to the release of silver ions from the particles, which confers the antimicrobial activity [18].



1.*Bacillus subtilis*2.*Streptococcus faecalis*3.*Staphylococcus aureus*4.*Klebsiellapneumoniae*5.*E.coli* 6. *Pseudomonas aeruginosa*7.*Candida albicans*

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Plate 1 Antimicrobial activity of various concentration of synthesized Silver nanoparticles from the leaves of *Begonia malabarica* against selective pathogens

Conclusions

The present study illustrated a simple and convenient way approach for the phytomediated synthesis of AgNPs at room temperature using *Begonia malabarica*leaves extract. The biogenic synthesis of AgNPs confirmed by UV-Visible spectroscopy and SEM. The crystalline nature and property of the synthesized AgNPs was carried out by XRD and the multifunctional group was illustrated by *Begonia malabarica*leaves extract and synthesized AgNPs confirmed by FTIR studies. The antimicrobial activity of the synthesized AgNPs was evidenced by different microbial strains such as *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Klebsiellapneumoniae*, *Pseudomonas aeruginosa*, *E.coli* and fungal *Candida albicans*.Antimicrobial studies revealed that small size, spherical shapednanoparticles have potential activity against different microbial strains and serves as eco-friendly antimicrobial agents.Therefore, this phytomediated synthesis of silver nanoparticles is more economic and beneficial to produce promising and compatible metal nanoparticles using biological method at commercial level and to explore their potentials in drug delivery system using surfacecapping activities of medicinally useful plant metabolites. .

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