

Biogenic Synthesis of Intracellular Silver Nanoparticle by Endophytic Ascomycetes Fungi From *Calotropis gigantea* Seeds (L.) R. Br

Kavi Praba A

kavipraba.a.bte@sathyabama.ac.in

Sathyabama Institute of Science and Technology

V. Ramesh Kumar

Sathyabama Institute of Science and Technology

Raghul Rajah S

Sathyabama Institute of Science and Technology

Research Article

Keywords: Biosynthesis, Endophytic fungus, *Calotropis gigantea* seed, copper nanoparticles, SEM, XRD

Posted Date: February 10th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-8758115/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Biogenic Synthesis of Intracellular Silver Nanoparticle by Endophytic Ascomycetes Fungi From *Calotropis gigantea* Seeds (L.) R. Br

Abstract

The biosynthesis of monodispersed nanoparticles, along with the determination of potentially responsible biomolecules, is the major bottleneck in the area of bio-nanotechnology research. The current investigation focused to synthesize the Silver Nanoparticles (AgNPs) using an endophytic fungus isolated from *Calotropis gigantea* (L. Br) seed, located in Chennai, India. The fungus was identified for its morphological and molecular characteristics. The fungus identified as *Pichia kudriavzevii* strain based on 16SrRNA sequencing. The produced particles were spherical shaped in nature and shows the surface plasma resonances 10nm. FTIR spectra of nanoparticle showed the C=C bonding, N-compound, C-O stretching vibration denotes the presences of alkene, protein and aromatic compound on the surface of silver nanoparticle produced by *Pichia kudriavzevii*. Synthesised NanoParticle revealed the mean negative zeta potential and electrophoretic mobility of -29.8 mV and -0.000255 cm²/Vs respectively. The nanoparticles produced were proteinaceous compounds as capping agents with -29.8mV zeta potential and revealed the antibacterial activity against gram positive bacterial strain.

Keywords: Biosynthesis, Endophytic fungus, *Calotropis gigantea* seed, copper nanoparticles, SEM, XRD.

Introduction

Calotropis gigantea is a shrub that is about 10 meters tall. It grows in a dry coastal area along the sheltered shores of lagoons and has many branches at the bottom banks [1]. The plant has a source of fiber. *Calotropis gigantea* shrub has vital medicinal values and is rich in fiber. The powdered flowers are used to treat colds and coughs. Intestine worms, rheumatics, and epileptic attacks can be treated with an infused medicine [2]. In the tropics, it is cultivated as an ornamental and sometimes grown for fiber from its stem.

There are potential sources of biologically active natural products in the form of endophytes, which are symbiotically associated microorganisms of living plants (12,13). They are found in every plant species studied and are estimated to be around a million or more (14). There is a chance to find a novel and interesting microorganism with significant therapeutic efficacy (3,19). A better understanding of the mechanism of endophytes' existence and their interaction with the surroundings can be achieved by the selection of suitable higher plants (4,16). A new natural product might be revealed through this procedure. The number of useful microorganisms is larger than the amount of utilization for food and health industries (5,16,17)

A chemical synthesis process is the easiest and most convenient way to synthesize AgNPs (6,18). Several toxic reagents are used to synthesize AgNPs. Many metal particles are used in creams and cosmetics (11,15). The use of plants and microorganisms has been proposed as a way to synthesize metal nanoparticles (7,10). It requires less

effort to synthesize nanoparticles from plants than to maintain and culture them. (20) The use of plant biomolecules in the synthesis of AgNPs is beneficial because of their low toxicity to living cells. (8,9)

The purpose of the current study was to explore the use of *Calotropis gigantea* for the synthesis of AgNPs using secondary metabolites from the seed extract. The synthesis process of AgNPs compounds was characterized using different qualitative and quantitative analyses to evaluate the formation of silver nanoparticles.

Material And Methodology

Isolation, purification of fungal strain

Fungi are collected from *Calotropis gigantea* seed. The seeds were placed in PDA (Potato Dextrose Agar) and incubated at room temperature for 6-7 days. After incubation days, spores are obtained from the seed, placed in another PDA Petri plate by using sterilized wire loop, and incubated for 6-7 days at 37 degrees Celsius. For purification purposes, subculturing was performed to obtain a pure fungi culture. The purified fungi culture was stored at 4 degrees Celsius for further purposes.

Identification of Fungal Strain

Fungi sample are characterized based on morphology, and molecular characteristics. The Color, Shape, and nature of fungi colonies were observed on Potato Dextrose Agar plates for 7 days. A Cotton Blue Lactophenol test was performed to identify the Morphology structure of isolated fungi.

DNA extraction

The genomic DNA of fungal isolates was extracted. The quantity and the quality were determined using 1.5% of agarose gel.

Molecular sequencing and construction of the phylogenetic tree

Amplification of 16S rDNA was performed. For PCR amplification, the microbial 16S ribosomal RNA (16SrRNA gene) region was amplified using universal primers by PCR from the above-isolated DNA. The band of 1523bp was observed when it was resolved on Agarose Gel. The product was cleansed. The Universal forward or reverse primers were used to sequence the amplicon. The ABI 3500 Genetic Analyzer was used for the Sequencing. The NCBI National Center for Biotechnology Information database has a basic local alignment search tool that can be used for the alignment of isolated sequences. NCBI software was used to construct the tree.

Fungal biomass preparation for AgNPs biosynthesis

Loop full of inoculum was suspended in PDB (potato dextrose broth) and incubated at 27degree Celsius in shaker incubator. After 7- 10 days of incubation the pale white color fungal mycelium is observed in the PDB. Then the

biomass was separated by filtration process. The filtered biomass was resuspended in 100 ml of denoised water and kept in shaker incubator was 2 days. This cell filtrate sample is used for further analysis.

Biosynthesis of silver nanoparticles

The experiment was conducted on the cell filtrate. Initially the sample was sonicated for 20-25 mins. After sonication, the sample was centrifuge at 6000 rpm for 20 mins. The supernant was collected and it is filtered by using Whatman filter paper grade no 3. The filtered sample was used for further analysis. By using different concentration of AgNO₃ (2mM,4mM,6mM,8mM, and 10mM) the experiment was carried out.10 ml of fungal extract was mixed with 20 ml of AgNO₃ as per different concentration and kept on dark condition for 72 hrs. and observe the color change from pale white to brownish color. The synthesis of silver nanoparticle was monitored by UV-Visible Spectral in the wavelength ranges of 200- 800nm.

Characterization of AgNPs

UV Visible spectroscopy

The optical properties of Surface Plasmon resonance of the AgNPs and control were monitored by UV–Visible absorption. The liquid sample was diluted with distilled water and absorbance was taken in the range of 200–800 nm Jasco-UV-VIS NIR.

ATR-FTIR

ATR-FTIR analysis was carried out to find the functional groups in the fungal cell responsible for the reduction of silver ions and the formation of biogenic intracellular AgNPs. A pinch of powder sample was mixed with water and subjected to FTIR analysis in the Happ- Genzel FTIR spectroscopy device. 32 scans were taken at the range of 4000- 650 cm⁻¹ and the resolution was kept at 4cm⁻¹ for the attenuation of noise ratio.

TEM

For TEM analysis, a drop of aqueous solution containing AgNPs were placed on the copper grids coated carbon and dried by allowing alcohol to evaporate at room temperature. Micrographs were obtained using a Tecnai F20 S-Twin operating at 200 kV. The size of AgNPs were estimated from the Debye-Scherrer Equation by determine the width of the (23) Bragg reflection, and size distribution of the resulting nanoparticles was also estimated on the basis of TEM micrographs.

PSA and Zeta potential

Particle size analyser and Zeta Potential analysis was carried out at scattered angel of 25°C by scattering laser intensity, using HORIBA AZ100 zeta potential analyser based on the laser doppler electrophoresis. Measurements were obtained in the range of -400 to +500mV.

Effect of Temperature

The effect of incubation temperature (30 °C, 40 °C and 60 °C) on the biogenic AgNPs was carried out by cell filtrate with 8 mM of AgNO₃ and kept in water bath for different temperature as mentioned above. Finally colour changes observed from pale white to brownish color and AgNPs were determined by UV-Vis spectral analysis.(25)

Effect of pH

The effect of incubation pH was carried out in acidic, neutral and alkaline condition (3, 7, 10) on the biogenic AgNPs was carried out by cell filtrate with 8mM of AgNO₃ and the pH is adjusted with 1N of NaOH and 0.1 N OF HCl for the above mentioned pH. After adjustment of pH colour changes were observed from pale white to brown color and AgNPs were determined by UV-Vis spectral analysis.(24)

Antibacterial Screening of AgNPs

The antibacterial activity of AgNPs were evaluated against gram positive bacteria (Streptococcus mutants and Staphylococcus). All bacterial cultures were grown on Muller Hinton Agar, pH7.6. By using sterile pipette tip 3 wells were made and markings are done as A, B & C. Each well contain 3 different samples. The synthesized 10mM of AgNPs (30ul) was loaded on the well and 30ul of Streptomycin and 30ul of cell free without AgNPs were loaded and kept for incubation for 24 hrs to observe the zone formation (21,22).

RESULTS

Identification of fungal strain

Macroscopic and microscopic characteristics were identified. After 7 days of incubation on Potato Dextrose Agar, they reveal the appearance of white color (Fig. 2). By using the Cotton Blue lactophenol strain the fungal spores were identified by using an Electron Microscope with a 40X lens. (Fig.1) They showed that fungal strains have smooth, unbranched, long conidiophores, Metula, Phialides, Ramuli, and Sterigmata.

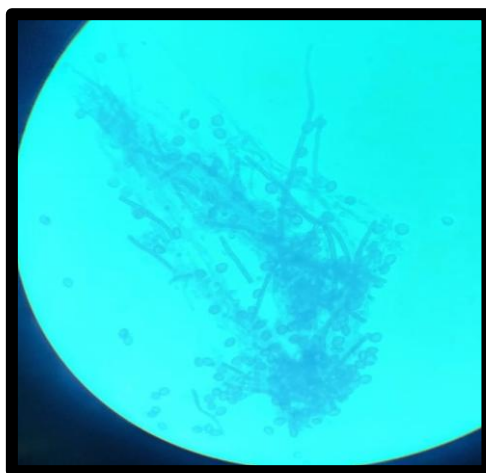


Fig. 1 Microscopic analysis of *Pichia kudriavzeii* strains F2-T427-10 was determined by bright field microscopy at 40 X magnification



Fig. 2 *Pichia kudriavzeii* strain F2-T427-10 Macroscopic characteristic of colonies of fungal strain

Molecular Characterization

16S rDNA was resulted as ITS1 forward sequence > CTTGGTCATTTAGAGGAAGTAA and ITS1 reverse sequence > GCTGCGTTCTTCATCGATGC (Fig. 3). Based on sequence homology and phylogenetic analysis, the Microbial culture was found to be, *Pichia kudriavzeii* strains F2-T427-10 complete genome with GenBank Accession Number MK329988.1

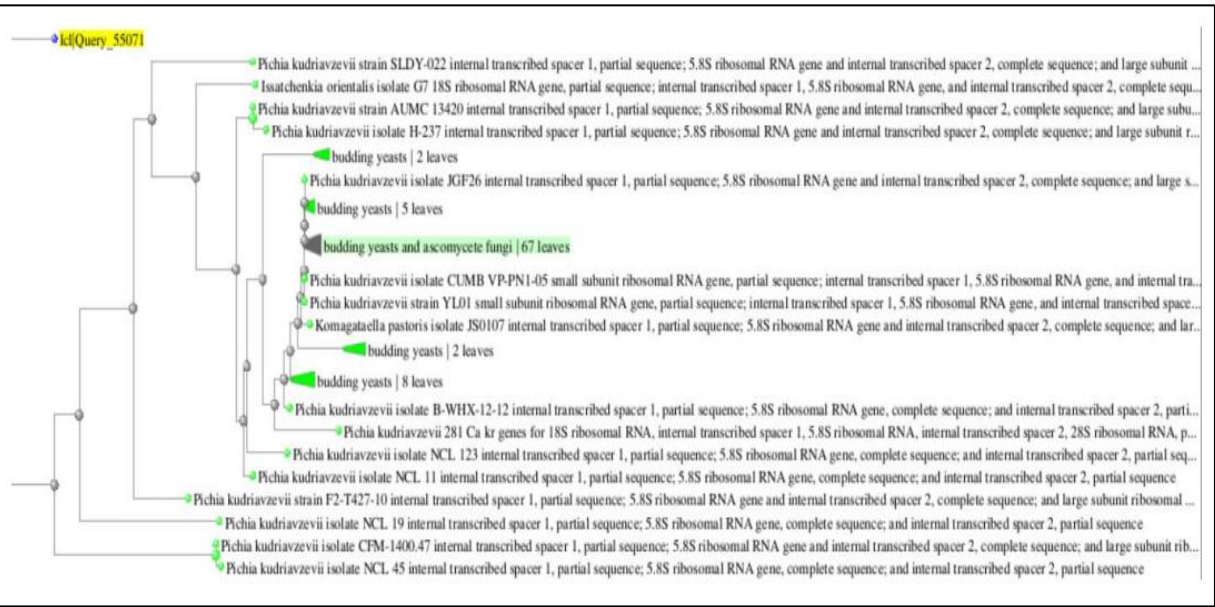


Fig. 3 Phylogenetic tree of Sample A using NCBI software

Sequences producing significant alignments

Download

Select columns

Show

100

☒ select all 100 sequences selected

GenBank

Graphics

Distance tree of results

MSA Viewer

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per Ident	Acc Len	Accession
☒	Pichia kudriavzevii strain F2-T427-10 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	Pichia kudriavzevii	540	540	54%	2e-148	88.33%	481	MK329988.1
☒	Pichia kudriavzevii isolate CFM-1400.47 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	Pichia kudriavzevii	540	540	54%	2e-148	88.30%	473	MZ677198.1
☒	Pichia kudriavzevii strain SLDY-922 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	Pichia kudriavzevii	527	527	54%	1e-144	87.86%	475	MH740627.1
☒	Pichia kudriavzevii isolate NCL 45 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	Pichia kudriavzevii	523	523	52%	2e-143	88.48%	440	FJ231424.1
☒	Pichia kudriavzevii isolate NCL 19 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	Pichia kudriavzevii	523	523	53%	2e-143	88.18%	440	FJ231421.1
☒	Issatchenkia orientalis isolate G7 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	Pichia kudriavzevii	518	518	53%	7e-142	87.81%	516	FJ315753.1
☒	Pichia kudriavzevii strain AUMC 13420 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	Pichia kudriavzevii	512	512	54%	3e-140	87.22%	466	MH534936.1
☒	Pichia kudriavzevii isolate H-237 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	Pichia kudriavzevii	508	508	57%	4e-139	86.29%	502	MG015957.1
☒	Pichia kudriavzevii 281 Ca kr genes for 18S ribosomal RNA, internal transcribed spacer 1, 5.8S ribosomal RNA, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	Pichia kudriavzevii	501	501	54%	8e-137	86.78%	664	LC567131.1
☒	Pichia kudriavzevii isolate LV17 ITS1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	Pichia kudriavzevii	490	490	60%	2e-133	82.41%	1002	ON262420.1
☒	Pichia kudriavzevii isolate NCL 11 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	Pichia kudriavzevii	490	490	53%	2e-133	86.85%	440	FJ231418.1
☒	Pichia kudriavzevii strain UMY534 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	Pichia kudriavzevii	486	486	55%	2e-132	86.03%	468	KP171557.1
☒	Pichia kudriavzevii 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	Pichia kudriavzevii	483	483	56%	3e-131	85.26%	497	JQ083432.1
☒	Pichia kudriavzevii isolate CC-5 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	Pichia kudriavzevii	481	481	54%	1e-130	85.93%	501	MG015961.1
☒	Pichia kudriavzevii internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	Pichia kudriavzevii	481	740	57%	1e-130	85.20%	1370	KP998095.1
☒	Pichia kudriavzevii isolate NJ60 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	Pichia kudriavzevii	479	479	54%	4e-130	85.90%	515	MK215802.1
☒	Pichia sp. QAUPK03 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	Pichia sp. QAUP...	479	479	69%	4e-130	82.02%	633	KJ870986.1
☒	Issatchenkia orientalis isolate G18 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	Pichia kudriavzevii	479	479	54%	4e-130	85.90%	517	FJ315756.1
☒	Pichia sp. strain PP5 isolate Y117 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	Pichia sp.	477	477	57%	1e-129	85.05%	476	MG752902.1
☒	Pichia kudriavzevii isolate NCL 123 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	Pichia kudriavzevii	477	477	52%	1e-129	86.47%	437	FJ231419.1

Fig. 4 Based on the maximum identity score first ten sequences were selected for further analysis.

Phylogenetic tree

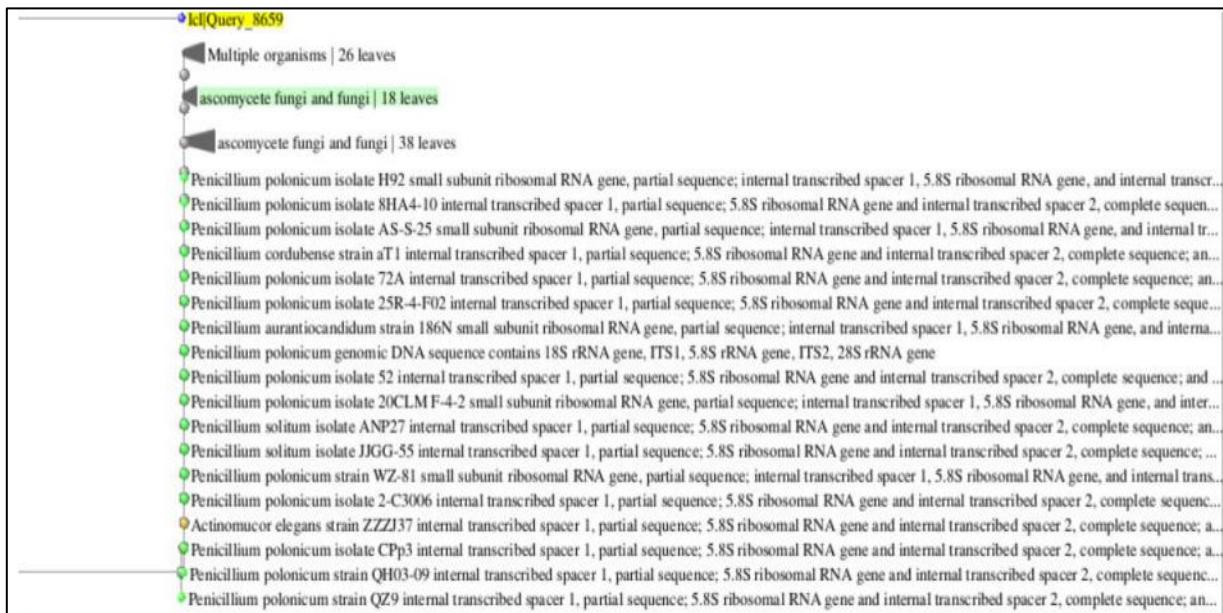


Fig. 5 Phylogenetic tree of Sample B using NCBI software

Based on sequence homology and phylogenetic analysis, the Microbial culture was found to be Pichia kudriavzevii strain F2-T427-10, complete genome with GenBank Accession Number MK329988.1

Biosynthesis and characterization of Silver nanoparticle

After 3 days of incubation in dark condition the diverse color change was observed in the aqueous silver nitrate solution from pale white to reddish brown, this indicated the rapid synthesis of silver nanoparticles in the reduction of AgNO_3 ions to Ag^+ ions. (Fig. 6)



Fig. 6 A) *Pichia kudriavzeii* extract and AgNO_3 . B) synthesized silver nanoparticles

UV-Visible spectral is an instrument that determine the accurate characterization metal NPs. From the above-mentioned different concentration 6mM,8mM and 10Mm shows the highest color differences and these samples were given for uv vis spectrophotometry analysis. From the (Fig. 7) 8mM shows the UV-Vis absorption peaks at around 456 nm. The λ_{max} for the as-prepared biogenic synthesis of Ag nanoparticles exhibits the absorbance at 456 nm, also morphologically indicated by brownish colour formation of the solution from precursor state blackish colour state. This indicates the maximum surface plasmonic resonance at the particular wavelength. Further flattening of the peaks in the UV-Vis range (200-700 nm), indicating agglomeration of the precursor molecules in the solution. There is a gradual increase in the steepness and bumping at the 456 nm wavelength (More pronounced for 6mM and 10mM), indicating good formation of Ag nanoparticles after repeated dilution and reaction conditions of the bottom-up synthesis of Ag nanoparticles from biogenic sources. It is also understood from the figure that, owing to the different reactants involving the biogenic precursors like some functional groups, keto groups present in the biogenic (fungus) source, it is contributing in the reductional process of the Ag^{2+} to Ag^0 to form Ag nanoparticles in the aq. solution.

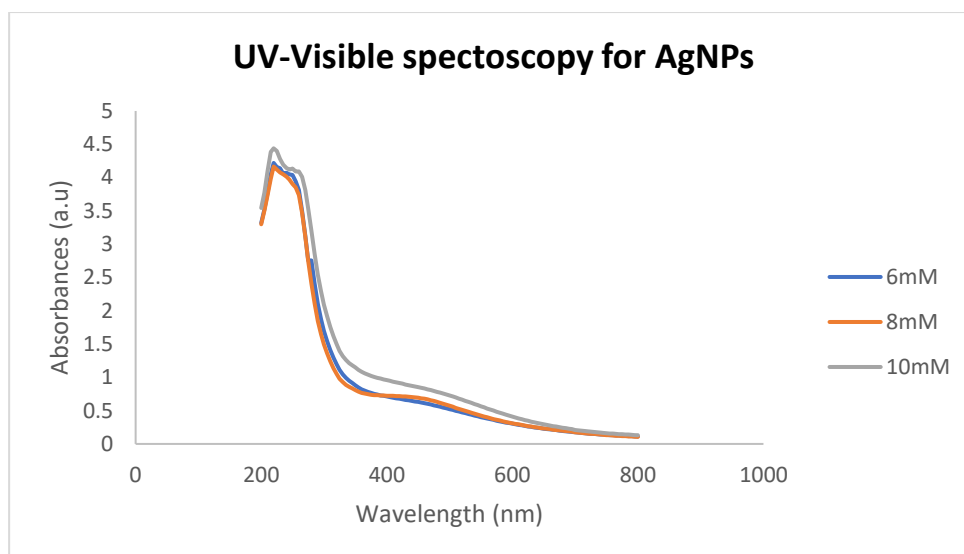


Fig. 7 A) Ultraviolet-visible spectrophotometer for 6,8 and 10mM AgNPs.

By using the vibration and frequency the FTIR spectroscopy shows the atomic level of compound with specific chemical bonds that represent the molecular fingerprint for sample. (Fig. 8), shows the FTIR spectral bands of the fungal extract and AgNPs synthesized using *Pichia kudriavzeii* extract. Sharp transmittances peak was observed at 1149 and 993 cm^{-1} in freshly synthesized AgNPs. (Table 1) contain FTIR spectra of fungal extract obtained in wavelength range from 3500-650 cm^{-1} and primary functional group found in fungal extract.

A broad spectrum found between 3500-2500 cm^{-1} due to the OH, -CH stretching vibration of alkene and carboxylic acid functional group, which involves hydrogen bonding as the broad band of OH & -CH group in this region indicates presences of hydrogen bonding in the molecules (Fig. 8). A set of strong peaks observed in 1149 and 993 cm^{-1} , which generally represent CO stretching and C=C bending that associate the presences of aromatic amine and alkene. From the (Fig. 8) it clearly observed that functional group associate with fungal extract have imprinted their functional group on synthesized AgNPs. Thus, FTIR experiment, shows the bioactive compounds identified as alkene, nitro compound (protein) and carboxylic acid present in *Pichia kudriavzeii* play a vital role in covering agent and stabilizing AgNPs.

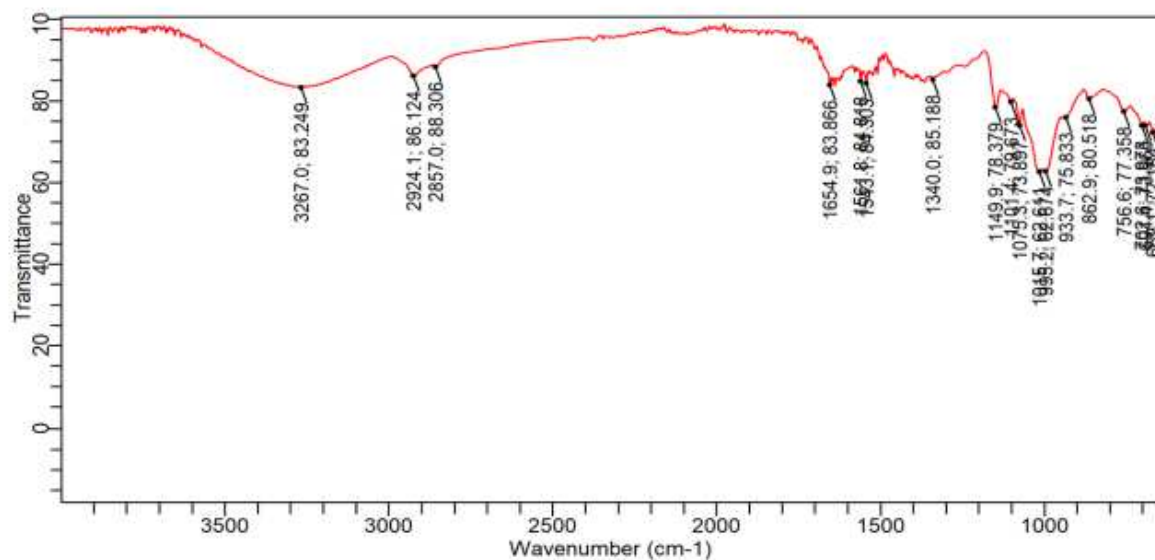


Fig. 8 ATR-FTIR spectroscopy analysis if AgNPs from aqueous extract of *Pichia kudriavzeii*

Table 1 FTIR tabulation AgNPs from aqueous extract of *Pichia kudriavzeii*

Wavelength ranges	Transmittances	Functional group
3500-3000	3267	O-H stretching
3000-2500	2924	C-H stretching
	2857	C-H stretching
1670-1600	1654	C=C stretching
	1561	C=C stretching
1600-1300	1543	N-O stretching
1400-1000	1340	O-H bending
	1149	C-O stretching
	1101	C-O stretching
	1075	C-O stretching
	1015	
1000-650	993	C=C bending
	862	C=C bending
	756	C=C bending
	702	C=C bending
	669	C-Br stretching

The accurate size and charges play a major role in the structural and functional properties of any particle used in biological application. In current investigation, the size and surface charge of AgNPs were calculated based on the laser diffraction using an Aqueous solution of AgNPs were Z- Average (nm): 33.84, Pdl: 0.487, Intercept: 0.871.(Fig. 9)

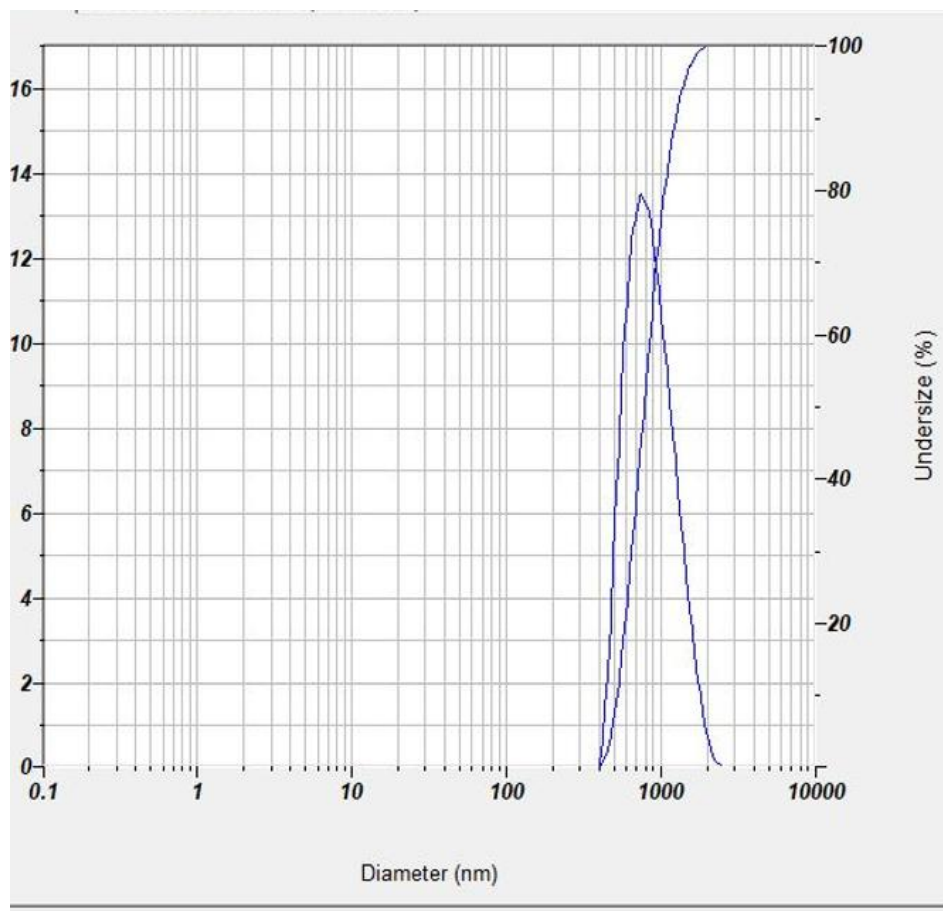


Fig. 9 Graphical representation of Particle Size Analyser from aqueous extract of *Pichia kudriavzeii*

From this investigation, it is proven that particle size 0.487 shows to be good formation of AgNPs formation from our aqueous solution. (Table 2)

Table 2 Particle size analyser for AgNPs formation aqueous extract of *Pichia kudriavzevii*

Peak No.	Size(nm)	% Intensity	Width(nm)
1	63.71	85.3	30.75
2	9.698	14.2	3.189

3	2.987	0.5	0.5155
---	-------	-----	--------

Zeta potential analysis is used to determine the surface charges and stability of the colloidal suspension of nanoparticle, is important in determining the interaction of cell membranes. The zeta potential of the biosynthesized AgNPs was found as sharp peak at -29.8mV (Fig. 10) (Table 3).

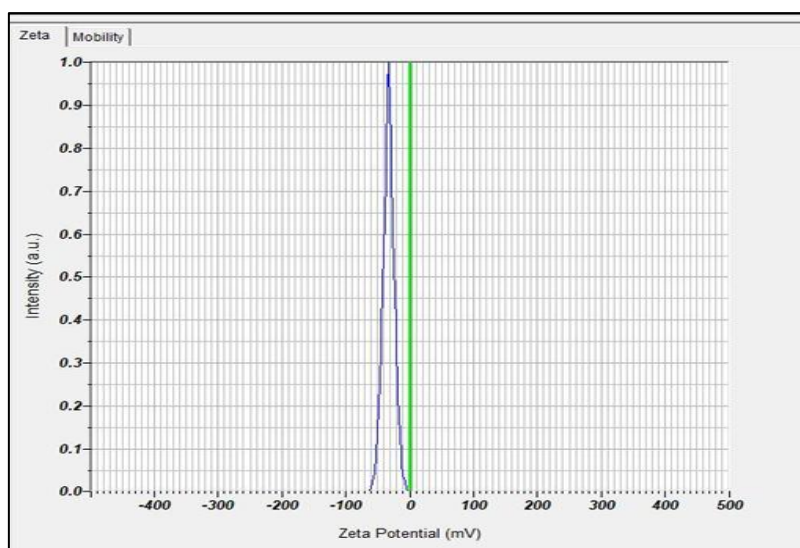


Fig. 10 Graphical representation Of Zeta Potential analyser for AgNPs formation aqueous extract of *Pichia kudriavzevii*

Table 3 Tabulation of Zeta Potential analyser for AgNPs formation aqueous extract of *Pichia kudriavzevii*

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-29.8 Mv	-0.000255 cm ² /Vs
2	--mV	--cm ² /Vs
3	--mV	--cm ² /Vs

Its suggested that there is Slight interaction between the anionic and cationic in the dispersed medium. The negative value Confirms the repulsion among the particle and proves that they are more stable.

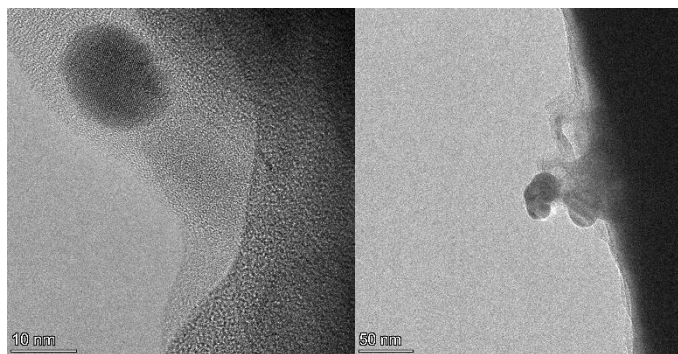


Fig. 11 shows the shape of AgNPs formation by *Pichia kudriavzevii* strain

The size and shape of the intracellular AgNPs were determined by TEM (Fig. 11). Measurements of biosynthesized nanoparticles diameters (600 AgNPs) showed that the average diameter was 16.3 ± 9.01 . Most of the intracellular nanoparticles synthesized were homogeneous and spherical in shape (Fig. 11). It was clear that the biogenic nanoparticles formed under the aerobic condition was in the range of 10 nm and displayed polydispersity.

Effect of Temperature on AgNPs formation

The diverse color change was observed in aqueous AgNO_3 solution was turns to Ag^+ ions after heating process and confirms the synthesis of AgNPs (Fig. 12).

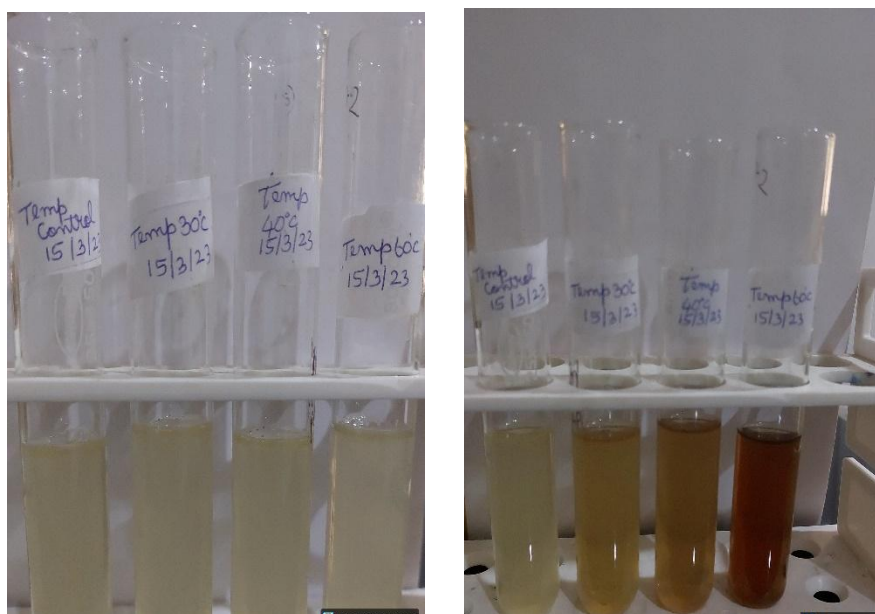


Fig. 12 Different temperature on AgNPs formation

(Fig. 13) Shows the effect of temperature on the AgNPs by *pichia kudriavzeii*. It is noticeable that the optimum temperature for the production of biogenic nanoparticles is between 30 °C - 40 °C. However, the thin peak is developed in higher wavelength region (475 nm at 60 °C), which indicates the formation of higher nanoparticles, whereas at lower temperature blunt peaks observed with smaller wavelength region of (420nm at 40 °C) and (415

nm at 30 °C) indicates the formation smaller NPs. These results show that if temperature increases the reactant rate rapidly produce silver nuclei and prevent the reduction of secondary metabolites on surface of AgNPs.

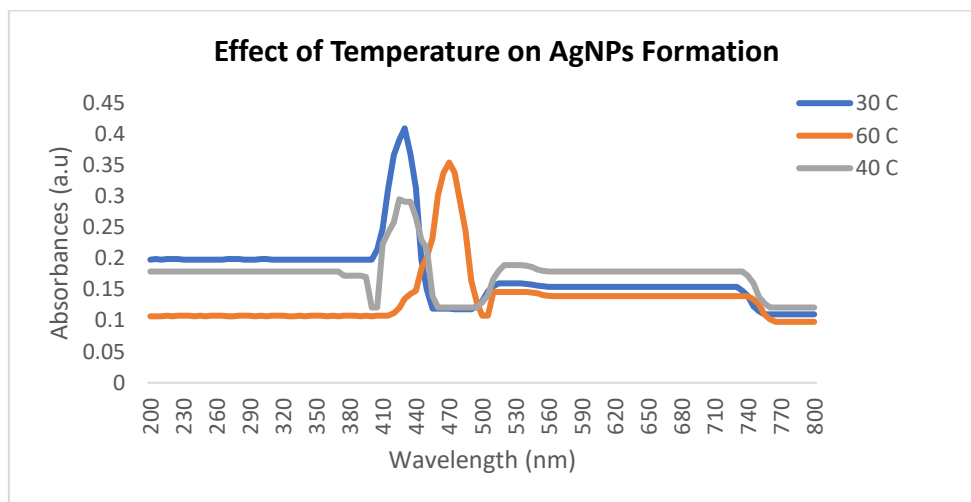


Fig. 13 UV- Vis Spectral for the effect of temperature on AgNPs formation by *Pichia kudriavzeii*.

Effect of pH on AgNPs formation

The medium supplement with AgNO₃ solution changed from white to brown color based on different pH mixtures. UV-Vis spectral reading was recorded after the reaction at different pH in acidic, neutral and alkaline as pH 3, pH 7 and pH10 (Fig. 14). The maximum AgNPs bioproduction was at pH 10, of which narrow peak developed, which indicates the formation of highly dispersed bio nanoparticles. At acidic condition, the polysaccharides and protein were reduced its function. Therefore, in present study, pH 10 was recorded for biogenic synthesis of AgNPs.

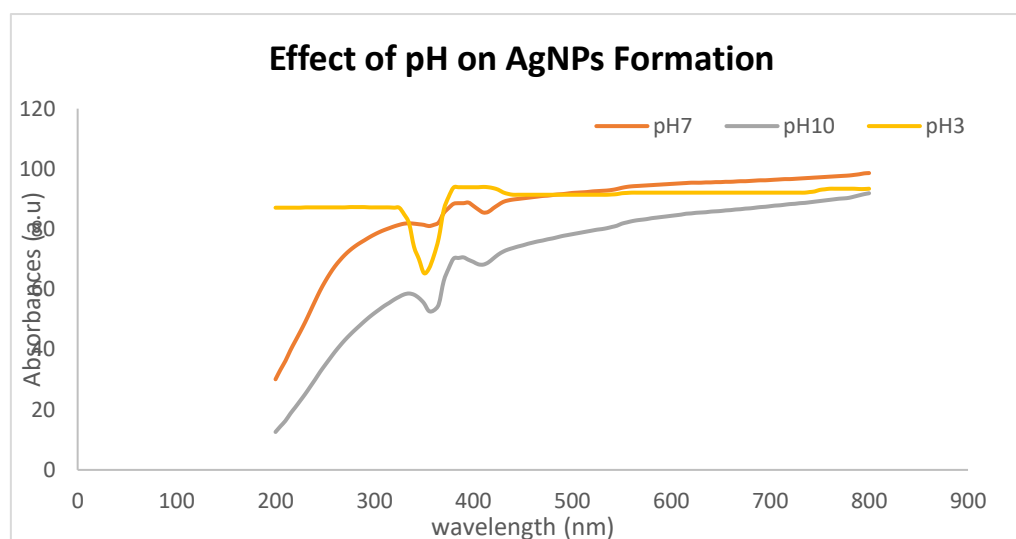


Fig. 14 Shows the effect of pH on AgNPs formation by *Pichia kudriavzeii*

Antibacterial activity

The antibacterial characteristics of silver have been well documented since Roman times; nevertheless, with the development of nanoparticles, this bactericidal property has been rediscovered. The antimicrobial property of nano silver and their able to walk surfaces has a lot of potential to fight microbes, providing a different way to lessen microbial colonisation and device-associated infections, such as pneumonia caused by ventilators, central venous catheter infections, and urinary tract infections caused by catheters. However, the exact mechanism is still unknown.

The antibacterial activity of isolated *P. kudriavzevii*-synthesized nanoparticles was assessed using two Gram-positive (*Streptococcus* mutants and *Staphylococcus*) bacterial strains by the well-diffusion method (Fig. 15). A control (cell-free fermentation broth without AgNO₃ addition) was also maintained in each plate along with 30 µl of standard antibiotic gentamycin (30 µg/ml) in a separate well as positive control. The diameter of inhibition zones around each well with AgNPs is represented in (Table 4). It was noticed that the produced nanoparticles revealed antibacterial activity against selected Gram-positive (Table 4). The highest antimicrobial activity was observed against *Streptococcus* mutants (12 mm), followed by the least was noticed against *Staphylococcus* (10 mm). The negative control (culture broth without AgNO₃) did not show any activity (Fig. 15), which further confirms the antibacterial activity of silver nanoparticles.



Fig. 15 Shows the antibacterial activity for AgNPs produced by *P. kudriavzevii*. 1) *Streptococcus* mutants, 2) *Staphylococcus*. C- cell free filtrate (negative control); B- Gentamycin antibiotic (positive control); A- *Streptococcus* mutants, *Staphylococcus*.

Further comparative evaluation of the antibacterial property of silver nanoparticles to that of standard gentamycin suggested that the antibacterial property of produced silver particles is almost comparable with gentamycin (Fig. 15). The observed variation in zone of inhibition against *Streptococcus* mutants and *Staphylococcus* by biologically produced silver nanoparticles by and in the present study may be attributed to use of more than 50% less particles in the present investigation.

Table 4 Antibacterial Activity of AgNPs produced by *Pichia kudriavzevii*

Microorganism	Zone Of Inhibition(mm)	
	AgNPs	Gentamycin
Streptococcus mutants	3	28
Staphylococcus	1	25

Conclusion

For the first time, the potential use of newly isolated yeast strain (*P.kudriavzevii*) to synthesize AgNPs using biogenic method is demonstrated in this study. The silver nanoparticles were found to have the spherical structure with an average crystalline size of 10nm. The reaction duration was found to play a critical role in the shape, size and distribution of AgNPs. 10mm of concentration of AgNPs shows the best colour formation and this step was further for the parameter studies. The synthesized AgNPs revealed stretching vibration of Nitro compound and alkene along with N-O stretching, C-O stretching and C=C bending. The metabolically produced protein compounds are involved in size regulation of reduced AgNPs. An average negative zeta potential value was observed -29.8mV was observed when characterizing the electrophoretic mobility. And the particle size was valued as 0.4 observed from the laser diffraction. The Parameters were performed for silver nanoparticles by effect of temperature, and pH by which it concludes that the pH 10 (alkaline) and temperature 40 °C shows the best formation of AgNPs. The synthesized AgNPs were performed for antibacterial activity studies against gram positive bacteria such as Streptococcus mutants and Staphylococcus. The biosynthesized nanoparticles revealed the antibacterial activity shows that streptococcus mutants show the highest zone of inhibition when compared to Staphylococcus.

DECLARATIONS

ETHICAL APPROVAL

This study does not involve human participants or experimental animals. All experiments were conducted using microbial cultures and plant-derived materials; therefore, ethical approval was not required.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analysed during this study are included within the article. Additional datasets supporting the findings are available from the corresponding author upon reasonable request.

FINANCIAL SUPPORT

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICTS OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Bailey, Clinton; Danin, Avinoam (1981). "Bedouin Plant Utilization in Sinai and the Negev". *Economic Botany*. Springer on behalf of New York Botanical Garden Press. 35 (2): 157.
2. Harvey Brown, Y (2022). "Calotropis procera". IUCN Red List of Threatened Species. 2022: e.T19181123A212816097.
3. Li, J. L., Sun, X., Chen, L., & Guo, L. D. (2016). Community structure of endophytic fungi of four mangrove species in Southern China. *Mycology*, 7(4), 180–190. 11.
4. Litwin A., Nowak M., Różalska S. (2020). Entomopathogenic fungi: Unconventional applications. *Rev Environ Sci Biotechnol*. 19 23–42. 10.1007/s11157-020-09525-1
5. Govindappa M & Lavanya M & Aishwarya P & Kavya Pai & Prathiksha Lunked & B Hemashekhar & BM Arpitha & YL Ramachandra & Vinay B. Raghavendra Synthesis and Characterization of Endophytic Fungi, *Cladosporium perangustum* Mediated Silver Nanoparticles and their Antioxidant, Anticancer and Nano-toxicological Study, *BioNanoScience* (2022)
6. Dwivedi, A.D., Gopal, K. 2010. Biosynthesis of silver and gold nanoparticles using *Chenopodium album* leaf extract. *Colloids Surf. A* 369: 27–33.
7. Mouxing, F. U., Qingbiao, L. I., Daohua, S. U. N., Yinghua, L. U., Ning, H. E., Xu, D. E. N. G., ... & Huang, J. (2006). Rapid preparation process of silver nanoparticles by bioreduction and their characterizations. *Chinese Journal of Chemical Engineering*, 14(1), 114-117.
8. Mukherjee, P., Ahmad, A., Mandal, D., Senapati, S., Sainkar, S.R., Khan, M.I., Parishcha, R., Ajaykumar, P.V., Alam, M., Kumar, R., Sastry, M., 2001. Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: a novel biological approach to nanoparticle synthesis. *Nano Lett.* 1 (10), 515–519.
9. Patra, Sujata, *et al.* "Green synthesis, characterization of gold and silver nanoparticles and their potential application for cancer therapeutics." *Materials Science and Engineering: C* 53 (2015): 298-309
10. Rauwel, Protima, *et al.* "A review on the green synthesis of silver nanoparticles and their morphologies studied via TEM." *Advances in Materials Science and Engineering* 2015 (2015).
11. Remya, V. R., Abitha, V. K., Rajput, P. S., Rane, A. V., & Dutta, A. (2017). Silver nanoparticles
12. Sastry M., Ahmad A., Khan I. and Kumar R., Biosynthesis of metal nanoparticles using fungi and actinomycete, *Curr Sci*, 85(2),162-70, (2003)
13. Schulz B, Boyle C, Draeger S, Rommert AK, Krohn K. Endophytic fungi: A source of novel biologically active secondary metabolites. *Mycol Res*. 2002;106:996–1004
14. Senapati S, Mandal D, Ahmad A, Khan MI, Sastry M, Kumar R Fungus mediated synthesis of silver nanoparticles: a novel biological approach. *Indian Journal of Physics A*, 2004; 78: 101-105.
15. Soni, N., Prakash, S. 2011. Factors affecting the geometry of silver nanoparticles synthesis in *Chrysosporium tropicum* and *Fusarium oxysporum*. *American J. Nanotechnol.* 2(1): 112–121

16. Strobel, G. (2018). The emergence of endophytic microbes and their biological promise. *J. Fungi* 4:57. doi: 10.3390/jof4020057
17. Bansal V, Rautaray D, Bharde A, Ahire K, Sanyal A, Ahmad A, Sastry M Fungus mediated biosynthesis of silica and titania particles. *J. Mat. Chem.* 2005; 15: 2583-89.
18. Bawaskar, M., Gaikwad, S., Ingle, A., Rathod, D., Gade, A., Duran, N., Marcato, P.D., Rai, M. 2010. A new report on mycosynthesis of silver nanoparticles by *Fusarium culmorum*. *Curr. Nanosci.* 6: 376–380
19. Abdel-Azeem AM, Zaki SM, Khalil WF, Makhoulf NA, Farghaly LM. Anti-rheumatoid Activity of Secondary Metabolites Produced by Endophytic *Chaetomium globosum*. *Front Microbiol.* 2016;7 (1477):1–11.
20. Aziz, W.J., Jassim, H.A. 2018. A novel study of pH influence on Ag nanoparticles size with an antibacterial and antifungal activity using green synthesis. *World Sci. News* 97: 139–152
21. Gopinath, K., Karthika, V., Gowri, S., Senthilkumar, V., Kumaresan, S., & Arumugam, A. (2014). Antibacterial activity of ruthenium nanoparticles synthesized using *Gloriosa superba* L. leaf extract. *Journal of Nanostructure in Chemistry*, 4(1), 1-6.
22. Kaur P, Thakur R, Chaudhury A (2016) Biogenesis of copper nanoparticles using peel extract of *Punica granatum* and their antimicrobial activity against opportunistic pathogens. *Green Chem Lett Rev* 9:33–38
23. Rauwel, Protima, *et al.* "A review on the green synthesis of silver nanoparticles and their morphologies studied via TEM." *Advances in Materials Science and Engineering* 2015 (2015).
24. Aziz, W.J., Jassim, H.A. 2018. A novel study of pH influence on Ag nanoparticles size with an antibacterial and antifungal activity using green synthesis. *World Sci. News* 97: 139–152
25. Liu, H., Zhang, H., Wang, J., Wei, J. 2017. Effect of temperature on the size of biosynthesized silver nanoparticle: Deep insight into microscopic kinetics analysis. *Arabian J. Chem.* doi: 10.1016/j.arabjc.2017.09.004