Comparative study of green synthesized silver nanoparticles using leaf and stem extract of *Pauzolziabennettiana* and its antimicrobial activity

Kalarani[1, 2], Parthiban[3], Divya[4], *Chandra Lekha

[1] Research Scholar, Reg.No.18222102032018 ,

Department of Chemistry, Kamaraj College,Thoothukudi.

(Affiliated to M.S University, Tirunelveli)

[2]Department of Chemistry, G.Venkataswamy Naidu College, Kovilpatti.

(Affiliated to M.S University, Tirunelveli)

[3]Department of Chemistry, Kamaraj College, Thoothukudi-628002, Tamilnadu, India

[4]Department of Chemistry, St.Mary's College, (Autonomous)Thoothukudi.

(Affiliated to M.S University, Tirunelveli)

[*]Department of Chemistry, Kamaraj College, Thoothukudi-628002, Tamilnadu, India

Abstract

Green synthesis of AgNPs was achieved by using the aqueous extract of dried leaf and stem of *Pauzolziabennettiana* and $AgNO_3$.The silver nanoparticles were characterized by using ultraviolet-visible(UV-Vis) spectroscopy and Fourier transform infrared(FT-IR) spectroscopy. The bioreduction of silver ions was observed by the colour change from pale yellow to dark brown. UV-Visible spectrum showed absorbance peak at 442nm for AgNps of leaf extract and at 428nm for AgNps of stem extract.Fourier transform infrared spectroscopy (FTIR) analysis revealed that the phenoliccompounds, tannins and other secondary metabolites in the aqueous extracts may act as capping agent for the nanoparticle synthesis. Topographies obtained from Atomic force microscopy displayed the surface morphology of the silver nano particles. The synthesized nanoparticles showed active against bacteria such as *P.aeroginosa, H.influenzae, S.aureus, S.pyogenes* and fungi like *Aspergillus sp., Fusarium sp.*

Keywords:*Pauzolziabennettiana*, green synthesis, silver nanoparticles,UV, FT-IR, AFM etc

1. Introduction:

The agency of environmental protection is green chemistry, it diminishes the perilous substances. Anastas and warner give twelve principles, among this the chemists to derive new chemical compound and new synthesis and technological processes[1, 2]. Now a day's green nano technology methodology to prepare a huge amount of metal and metal oxide NPs using biopolymers such as cellulose, chitosan, dextrans [3]. Silver nano particles have the distinctive thermal, electrical optical properties. So it is widely used in antimicrobial coatings, wound dressings textiles, biomedical devices. Now a day's low level silver ions used to protect against bacteria to compare the silver salts and the toxicity is much less in silver nanoparticles in antimicrobial application [4]. In recently every year silver nanoparticles are synthesized from various sources exorbitantly increases. Different types of nano particles such as Ag,Cu,Au,Pt,Fe,Zn,Cd synthesized by chemical, physical, photochemical, biological methods. From this, the most wildly produced NPs are likely to be TiO_2 and silver nanoparticles, because of their antibacterial and photo catalytic properties [5].

The synthesis for metal /metal oxide nanoparticles, utilization of plant extracts is a rather simple and easy process to produce nanoparticles in large scale relative to bacteria and fungi mediated synthesis and considered due to the availability of effective photo chemical in plant extracts, especially in stem, and leaves such as flavones, terpenoids [6]. These components are capable of reducing agent in to metal nanoparticles.Silver nanoparticles are used to purify the air, water and soil. In water it is used for waste water treatment plant [7]. Silver nanoparticles allows easily interact with other particles and increases their antibacterial efficiancy. In this study we evaluation of AgNp's antimicrobial properties and it is knows excellent antimicrobial agent.

So it is alternate disinfectant agent [8]. Here we have to study the synthesis of silver nanoparticle as from silver nitrate using Pouzolzia bennettiana leaf and stem extracts. Biological activities are scrutinizes from the newly prepared Silver nanoparticles by *P.aeroginosa, H.influenzae, S.aureus, S.pyogenes*and fungi like *Aspergillus sp., Fusarium sp.* Pouzozia is a genus of flowering plants in the nettle family. There are about 35 species distributed throughout the tropical world. Most are shrubs, and some are herbs. The genus was named for French botanist and plant collector.

2. MATERIALS AND METHODS

2.1. Plant Material and Preparation of Dry Biomass

Figure: 1 Pouzolzia *bennettiana*

Pouzolzia bennettiana plants were collected from the Jagathala village, Aravankadu in Niligiris District. The collected leaves and stem were washed twice with distilled water and dried for ten days, and then the leaves were ground to a fine powder.

2.2. Preparation of extracts

About each 20g of *Pauzolzia bennettiana*leaf and stemwere weighed separately and transferred into 500ml beaker containing 300 ml of distilled water and boiled for 20 minutes. The extracts were then filtered thrice through Whatmann No.1 filter paper to remove particulate matter and to get clear solution and stored in dark place used for the further analysis.

2.3. Silver Nano particle Synthesis

0.01M aqueous solution of silver nitrate was prepared and used for the synthesis of silver nanoparticles. Sample and silver nitrate was added in 1:4 ratio kept at dark place at 48 hours inhibition time. The extracts were added for reduction of silver ions into silver nanoparticle. In the mean time, color change of the mixture from greenish yellow color to dark brown.

2.4. Characterization of AgNPs

UV-absorption spectra of synthesized AgNPs by using leaf and stem extracts of *P. bennettiana* were measured using UV-visible spectrometer (JASCO variant 630 spectrometer). Fourier transform infrared (FTIR) spectral measurements were carried out on the Thermal science-Nicolet Si5, ATR-iD1 spectrometer to identify the potential phytochemical constituents of plant *P. bennettiana*.

2.5. Antimicrobial activity of silver nanoparticles

Microorganisms

The bacteria were collected from Department of Microbiology, Kamaraj College, Thoothukudi, Tamilnadu, India. The test organisms used for assay are *Pseudomonas aeroginosa, Hemophilus influenzae, Staphylococcus aureus and Streptococcus pyogenes*. The fungal cultures such as *Aspergillus sp*. and *Fusarium sp.* were used for antifungal activity.

Preparation of media

3.8g of Muller Hinton agar medium was weighed correctly and dissolved in 100ml of sterile distilled water, pH was adjusted to 7.2 and was autoclaved at 121^0C for 15 minutes. 20ml of molten agar was poured in to the sterile Petri plate and allowed to solidify.

The antibacterial activities of Ag nanoparticles were carried out by agar well diffusion method. Muller Hinton agar plates were prepared, sterilized and solidified. After solidification, 100µl of the suspension containing10^6 CFU ml^{-1} of the test microorganisms were swabbed on the Petri plates uniformly. Wells of 6mm in diameter and about 2 cm apart were punctured in the culture media using sterile cork borers to make four uniform wells in each Petri plates. The Ag nanoparticles extract concentration was 100µl, 200 µl, 300 µl and 400 µl for each well respectively and allow diffusing for 45 minutes. The antibacterial activities of Ag nanoparticles extract were determined after 24 hours at 37^0C incubation in the incubator. The zone of inhibition (in millimeters) produced by the Ag nanoparticles extract against bacterial pathogens were measured.

Antifungal assay was also done by Agar well Diffusion Technique. The fungal cultures were grown on Rose Bengal broth (Hi media). The cultures of 7^{th} day old culture was washed, suspended in normal saline solution and then filtered through glass wool aseptically. The colony forming units (CFU/ml) of 0.1ml suspension of the test fungus was adjusted to 3×10^5 CFU/ml. These conidia were used for antifungal assay tests. Inocula were applied on the surface of the Rose Bengal agar plates and spread by using sterile glass spreader. Wells of 6mm in diameter and about 2 cm apart were punctured in the culture media using sterile cork borers to make four uniform wells in each Petri plates. The Ag nanoparticles extract concentration was 100µl, 200 µl, 300 µl and 400 µl for each well respectively and allow diffusing for 45 minutes. The antifungal activities of Ag nanoparticles extract were determined after 48 hours at 27^0C incubation in the

incubator. The zone of inhibition produced by the Ag nanoparticles extract against fungal pathogens were measured in millimeter.

3. RESULTS AND DICUSSION:

3.1.UV-Visible spectroscopy of synthesized AgNPs:

This work inspects the bio synthesis of AgNPs using leaves and stem extract of *Pouzolzia bennettiana*. The color changes observed pale yellow to brown solution indicate the formation AgNPs in the reaction mixture was acquired by the UV-Vis analysis at the range between 300-800nm. [9, 10] The AgNPs of leaf and stem extract has sharp absorbance with highest peak at 442nm and 428. The occurrence of the peak at 442nm and 428nm is due to the phenomenon of surface Plasmon resonance, which due to the excitation of the surface plasmons present on the outer surface surface of the silver nanoparticles. The high OD of the solution suggests a high conversion of Ag^{+} toAg^{0} as nanoparticles [11].

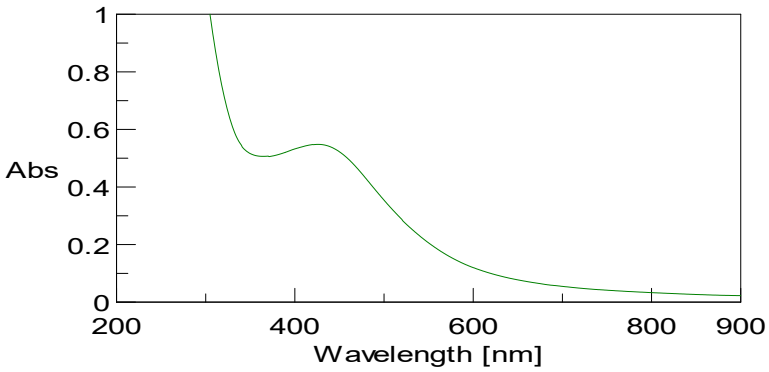


Figure: 2 UV spectra of Silver nanoparticles synthesized from *Pouzolzia bennettiana* stem extract

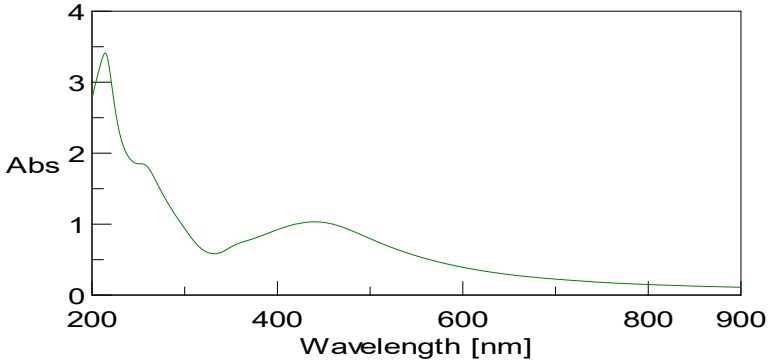


Figure: 3 UV spectra of Silver nanoparticles synthesized from *Pouzolzia bennettiana* leaf extract

3.2. FTIR spectroscopy of AgNPs synthesized from *Pouzolzia bennettiana* stem and leaf extract:

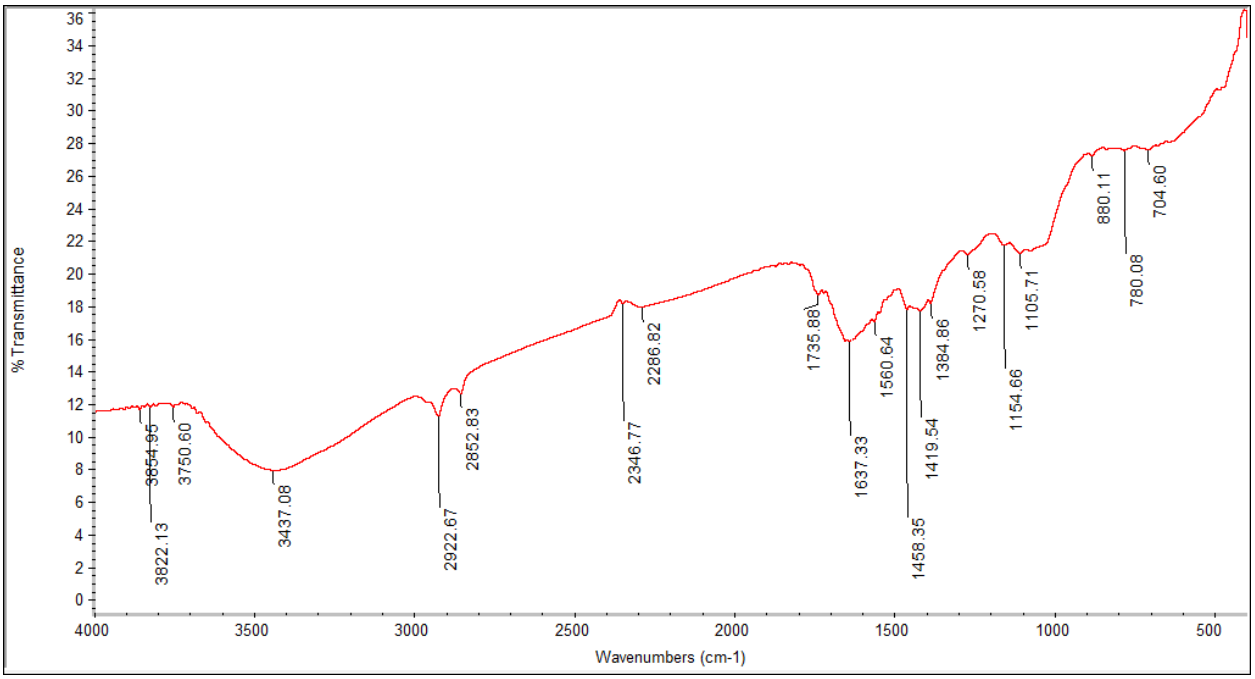


Figure: 4 FTIR spectra of AgNPs from *Pouzolzia bennettiana* stem

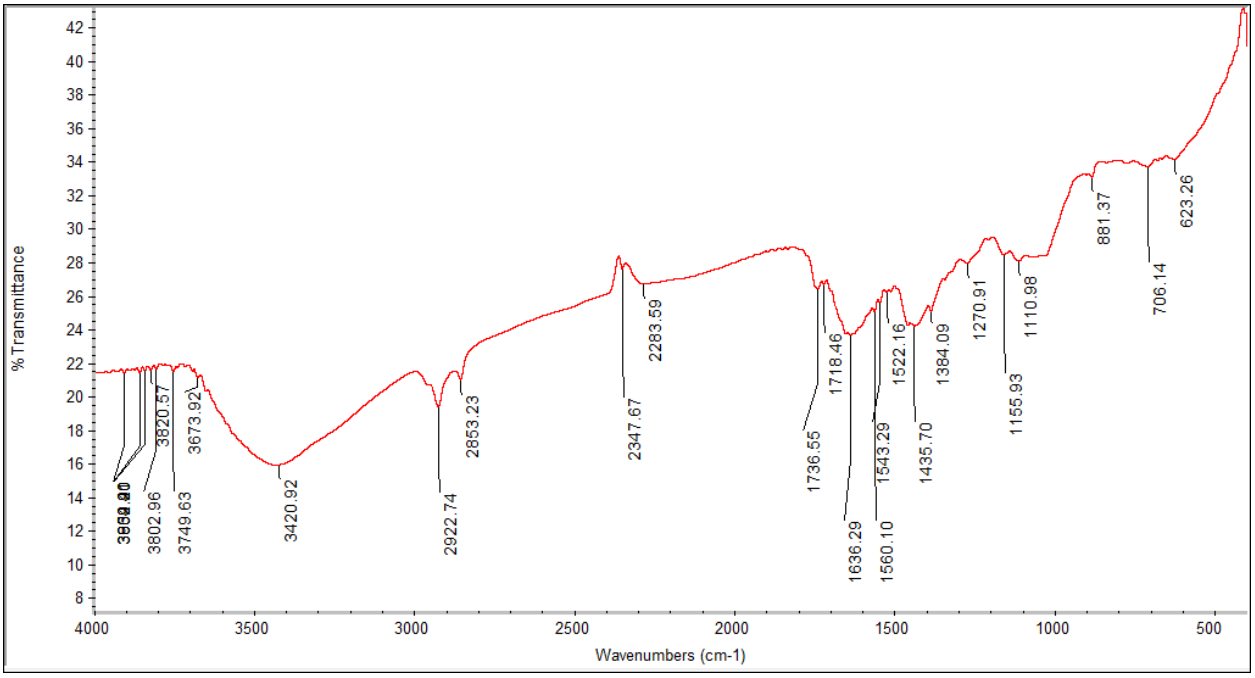


Figure: 5 FTIR spectra of AgNPs from *Pouzolzia bennettiana* leaf

FTIR measurements were done to identify the presence of various functional groups in biomolecules, this functional groups are important for the bio reduction of Ag^{+} and capping/stabilization of silver nano particles[12]. In both *Pouzolzia bennettiana* leaf and stem silver nano particles were showed absorption bands in the range at 3854.95 cm^{1}, 3822.13 cm^{1}, 3750.60 cm^{1},3437.08 cm^{1}, 2922.67 cm^{1}, 2852.83 cm^{1}, 2346.77 cm^{1},2286.82 cm^{1}, 1735.88 cm^{1}, 1637.33 cm^{1}, 1560.64 cm^{1}, 1458.35 cm^{1}, 1419.54 cm^{1}, 1384.86 cm^{1}, 1270.58 cm^{1}, 1154.66 cm^{1}, 1105.71 cm^{1}, 880.11 cm^{1}, 780.08 cm^{1}, 704.60 cm^{1}[13].

The absorption bands at 3854.95 cm^{1} to 3750.60 cm^{1} in the spectra corresponding to the O-H, indicating the presence of alcohol and phenol. The bands at 3437.08 cm^{1} to 2852.83 cm^{1} was assigned N-H it shows the presence of alkaloids. The bands at 2346.77 cm^{1}, 2286.82 cm^{1} represents the O=C=O it indicates the presence of carbon dioxide. The bond at 1735.88 cm^{1} was assigned the C=O indicates the presence of Carboxylic acid. The bands at 1637.33 cm^{1} and 1560.64 cm^{1} in the spectra show the presence of alkynes. The bands at 1458.35 cm^{1} to 1105.71 cm^{1} could be attributed the O-H indicating the presence of alcohols. The bands at 880.11 cm^{1} to 880.11 cm^{1} were assigned the C=C indicates the presence of alkenes.

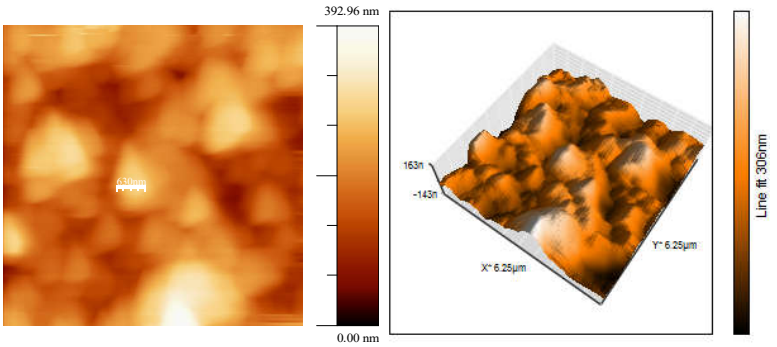
3.4. Phytochemical analysis of stem and leaf extracts of *P.bennettiana*

The extracts of stem and leaf extracts of *P.bennettiana* were screened for the presence of phytochemical constituents by following the method of Sofowora (1982) [14] and Kepam (1986) [15]. Preliminary phytochemical analysis revealed the presence of eight compounds such as tannins, steroids, terpenoids, alkaloids and cardiac glycosides.

3.5. AFM studies

AFM images were taken using NanoSurf easyscan 2 AFM (BT02218).

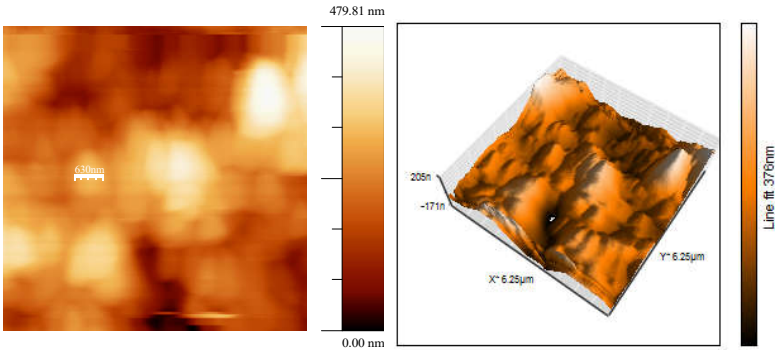
Topography of the silver nano particles synthesized from Pauzolzia bennetianna stem extract were given in the Fig.6. Triangular shapes of different sizes were seen in the topography. Spherical shape was reported for the silver nano particles synthesized using aqueous solution of propolis.[16]



(a) (b)

Figure: 6 Topography of AgNps from *P.bennettiana* stem in a) normal view and b) 3D view

Topography of the silver nano particles synthesized using Pauzolzia bennetianna leaf extracta were given in the Fig.7. Triangular shapes of different sizes were seen in the topography. Some elongated oval shaped particles were present in the topography.



(a) (b)

Figure: 7 Topography of AgNps from P.bennettiana leaf in a) normal view b) 3D view

Average roughness and root mean square roughness for line and area was given in table 1.The silver nano particle which was synthesized by stem extract showed large agglomerated particles compared to the nano particles synthesized from leaf extract.

Surface roughness of the silver nano particles synthesized from leaves of *Pauzolzia bennettiana*was more than the nano particles synthesized from the stem. This increase in roughness may be due to the formation of less agglomerated particles.

Table 1 Roughness values of Silver nano particles

Ag nano particles from	Line roughness for line of length 6.25µm		Area roughness for area of 39.37pm^2	
	Average roughness(nm)	Root mean square roughness(nm)	Average roughness(nm)	Root mean square roughness(nm)
Stem	35.846	45.848	38.748	49.509
Leaf	49.343	59.869	48.548	61.939

3.6. Antimicrobial activity of AgNPs:

The Antimicrobial activities of synthesized AgNPs were determined against bacterial suspension such as *P.aeroginosa, H.influenzae, S.aureus, S.pyogenes* and fungal suspension *Aspergillus sp., Fusarium sp.*using agar diffusion method. The results were recorded by measuring the zones of growth inhibition. The leaf and stem extract of *P.bennettiana* which showed a promising antimicrobial activity against all the pathogens (Table 2,3 and 4). *P.aeroginosa, H.influenzae, S.aureus, S.pyogenes*and fungi like *Aspergillus sp., Fusarium sp.*

Table: 2 Antibacterial activity of AgNPs *P.bennettiana* stem extract

Bacterial pathogens	Concentrtion of SNPs of *P.bennettiana* stem extract			
	100µl	200 µl	300 µl	400 µl
	Zone of inhibition(mm)			
Pseudomonas aeroginosa	16	19	22	24
Haemophilus influenza	19	21	24	26
Staphylococcus aureus	16	19	22	24
Streptococcus pyogenes	17	20	24	27

Table: 3 Antifungal activity of AgNPs *P.bennettiana* stem extract

Fungal pathogens	Concentration of SNPs of *P.bennettiana* stem extract			
	100 µl	200 µl	300 µl	400 µl
	Zone of inhibition (mm)			
Aspergillus sp.	3	5	7	10
Fusarium sp.	5	7	9	11

Table: 4 Antibacterial activity of AgNPs of *P.bennettiana* leaf extract

Bacterial pathogens	Concentrtion of SNPs *P.bennettiana* leaf extract			
	100µl	200 µl	300 µl	400 µl
	Zone of inhibition(mm)			
Pseudomonas aeroginosa	21	24	30	37
Haemophilus influenza	11	17	25	28
Staphylococcus aureus	9	13	20	24
Streptococcus pyogenes	12	17	19	22

Table: 5 Antifungal activity of AgNPs *P.bennettiana* leaf extract

Fungal pathogens	Concentrtion of SNPs of *P.bennettiana* leaf extract			
	100 µl	200 µl	300 µl	400 µl
	Zone of inhibition (mm)			
Aspergillus sp.	5	7	9	12
Fusarium sp.	6	9	11	14

Among the four concentrations tested for antimicrobial effect the silver nanoparticles of 400µl effective against all the tested pathogens. The silver nanoparticles synthesized via green route are less toxic towards fungal species when compared to bacterial species. The high bactericidal activity is certainly due to big changes in the membrane structure of bacteria as a result of the interaction with silver cations lead to the increased membrane permeability of the bacteria [17]. The ionic silver strongly interacts with thiol group of vital enzymes and inactivates the enzyme activity.

4. Conclusion:

This rapid, simple single-step "green" biosynthesis of silver nanoparticles is attractive as it is environmentally sound and safe. The leaf and stem extracts of *P.bennettiana* showed great capability to synthesis AgNPs at optimum temperature conditions. The UV absorption peak at 442nm and 428nm clearly indicates the synthesis of AgNPs. FTIR studies confirmed the bio fabrication of the AgNPs by the action of different phytochemicals with its different functional groups present in the extract solution. These biosynthesis silver nanoparticles can potentially be used for different medical applications.

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