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Green Amalgamation of Gold Nanoparticles Using *Psophocarpus tetragonolobus* Leaf Extract and its Bioassay

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Abstract

Due to their special physical and optical characteristics, gold nanoparticles have led to their recent rise in importance in the realm of biological applications. Other than the currently available methods, gold nanoparticles could be synthesized using *Psophocarpus tetragonolobus* leaf extract and examined utilizing UV-Vis and FTIR spectra, SEM and XRD. The presence of AuNPs was detected by the presence of a surface plasmon resonance band at 535 nm and peak at 601.79 cm^{-1} respectively from UV-Vis and FTIR spectra. Using SEM, the morphology of amalgamated AuNPs was found spherical in shape. XRD helped to state the crystalline nature of AuNPs. Further, AuNPs demonstrated significant anti-inflammatory activity. In addition to being tested for their antifungal properties against *Candida albicans* and *Aspergillus niger*, amalgamated AuNPs have also been tested against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* for their antibacterial activity. Overall, the findings of this study shed light on strategies for designing nano drugs which makes it a potential candidate for therapeutic applications.

Keywords: Gold nanoparticles*, Psophocarpus tetragonolobus*, Anti-inflammatoryassay, Antibacterial assay, Antifungal assay

Introduction

Nanotechnology is one of the recently emerging fields in science which offers various technological and biomedical applications. Among the various methods for the synthesis of nanoparticles, such as physical (ultrasonication, irradiation, electrochemical etc.), chemical (vapour deposition, sol-gel process, etc.) and biological methods (using plants and microbial sources), biological method is an alternative and eco-friendly approach to the synthesis of environmentally safe and less expensive nanoparticles. While carrying out traditional physical or chemical methods for the preparation of metal

nanoparticles, there must be clear constraints and drawbacks. Hence, green chemical synthesis procedure marked a shift in the direction of chemical industry approximately two decades ago[1]. Metallic nanoparticles (NPs) are considered as the most prominent nanomaterials, as their activation by incident light facilitates a wide spectrum of applications with proven performances[2]. Out of the diverse metallic nanoparticles, the unique surface plasmon resonance (SPR), along with facile synthesis, tunable size, multifunctional capabilities and well-defined characteristic properties of gold nanoparticles (AuNPs)[3,4]. Gold nanoparticles are broadly integrated into medical practices like diagnostic, sensors development, photoimaging and photothermal therapeutic applications[5-7].While AuNPs shows considerable potential in the treatment of various diseases, the absence of adequate biosafety data possess a significant barrier, underscoring the necessity for well-defined usage protocols and further investigate research [8].

In this current work, green amalgamation of gold nanoparticles is done with *psophocarpus tetragonolobus* (winged bean) leaves. Leaves of winged bean are abundant in vitamins such as vitamin C (14.5 – 128 mg/100 g), thiamine, riboflavin, niacin, vitamin B_6, folate, vitamin A (5240-20, 800 IU) and vitamin E. Diabetes, cancer and asthma can be prevented using this bean[9]. The amalgamated AuNPs were analysed by UV, FTIR, SEM, EDS and XRD.Also, the anti-inflammatory, antibacterial and antifungal activity of amalgamated AuNPs were tested.

Experimental

Materials

Psophocarpus tetragonolobus leaves were collected directly from agricultural field, Thiruvananthapuram, Kerala. Analytical grade granular Chloroauric acid was purchased from Loba cheme company.

Preparation of *Psophocarpus tetragonolobus* leaf extract

Leaves of *psophocarpus tetragonolobus* were collected, washed carefully with tap water to eliminate impurities along with dust present and dried in shade. An extract was prepared by boiling 5 g of powdered leaves in 100 mL of deionized water using a hot plate for 20 minutes. The solution was then cooled and filtered using Whatman No.1 filter paper[10].

Amalgamation of Gold nanoparticles (AuNPs)

A 40 mL of mixture of Chloroauric acid was made at a specified concentration (0.1, 0.3, 0.4mM). 10 mL of leaf extract was poured to 40 mL of chloroauric acid mixture in a 100 mL beaker. It was kept for magnetic stirring for 20 minutes. After keeping 24 hours at room temperature, the sample was centrifuged for 10 minutes. In order to avoid impurities from the sample, the residue was washed thrice with deionized water after centrifugation. The resulting gold nanoparticles were subjected to dry in a hot air oven at 60 °C for two hours.

Characterisation of Gold Nanoparticles

The characterisation of amalgamated AuNPs was carried out through techniques such as Ultraviolet – Visible spectroscopy in which surface plasmon resonance band for AuNPs was identified, Fourier Transform Infrared Spectroscopy(FTIR) in which the functional groups present in AuNPs was

identified.X-ray diffraction (XRD) technique in which size of AuNPs was determined, Scanning Electron Microscope (SEM) in which morphology was analyzed and Energy Dispersive Spectroscopy (EDAX) which helped to indicate the element present in AuNPs.

Anti-Inflammatory Assay

For the anti-inflammatory analysis, protein denaturation inhibition assay was done. In this assay, the reaction mixture of 0.5 mL solution consists of 0.4 mL bovine serum albumin (3% aqueous solution) along with test sample of various concentrations. Then, it was incubated at 37^0C for 20 min followed by the addition of 2.5 mL phosphate buffered saline (pH 6.3). Finally, the sample was heated at 80^0C for 10 min and the absorbance was taken using spectrophotometer at 660nm.

The percentage of inhibition of protein denaturation was calculated as follows[11-12]:

Percentage of Inhibition = [(Abs Control – Abs Test)/ Abs Control] ×100

Antibacterial and antifungal assay

The amalgamated AuNPs was effectively tested for its antimicrobial activity by agar well diffusion method. The possible antibacterial activity of AuNPs was assessed against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus subtilis*. Muller-Hinton agar was prepared. Sterilization was done with the help of autoclave by autoclaving at 15 lbs pressure at 121^0Cfor 15 minutes.This step was followed by the thorough mixing of culture media and then, it was streamed into sterile petri plates followed by the incubation at 37^0C for 24hrs. The positive and negative control employed was Gentamycin (40µl from 4mg/ml stock) and methanol[13]. And the antifungal activity was tested against *Candida albicans and Aspergillus niger*. Mueller-Hinton agar and Potato Dextrose Agar MH096 Himedia was synthesized. Sterlization was carried out by autoclaving at 15 lbs pressure at 121^0C for 15 minutes. The culture media was blended thoroughly and it was streamed into sterile petriplates. The positive and negative control employed was clotrimazole (40µl from 300 mcg/ml stock) and methanol respectively. Finally, the plates were incubated for 48 hours at 27^0C[14].

Results and Discussion

Visual inspection

As a preliminary confirmation, violet colour formed after stirring revealed that the gold nanoparticles were formed. As time period increases, intensity of violet colour increases. The emergence of dark violet colouration specifies that the biogenically synthesized AuNPs was completely formulated. The excitation of surface plasmon vibration in biogenic gold nanoparticles, the solution's colourshifts from light yellow to violet colour [15].

Fig. 1 – Colour change of the solution from light yellow to violet colour

UV-Visible Spectroscopy

The reduction of pure Au^{+} ions was recorded by performing the UV-Vis spectrum of the samples. Fig.2 displays the UV-Vis spectrum of the AuNPs. The scale of wavelength was selected from 450nm to 750 nm. The absorption spectra of AuNPs showed a characteristic maximum absorbance around 535nm which clearly indicates the surface plasmon absorption of gold nanoparticles and formation of nanoparticles. The free electrons in the conduction band get collectively oscillated and hence,

characteristic peak is observed. The result is same with that obtained from the AuNPs using *P. Salicifolia* aqueous extract. When AuNPs synthesized using *P. Salicifolia* aqueous extract, the SPR observed at 535nm by UV-Vis Spectra along with a noticeable colour of the synthesized AuNPs were consistent across all samples, indicating no significant differences among them[16]. This result is also in good accordance with AuNPs synthesized using *Camellia Sinesis* where the absorption intensity of SPR band obtained at 534nm [17].

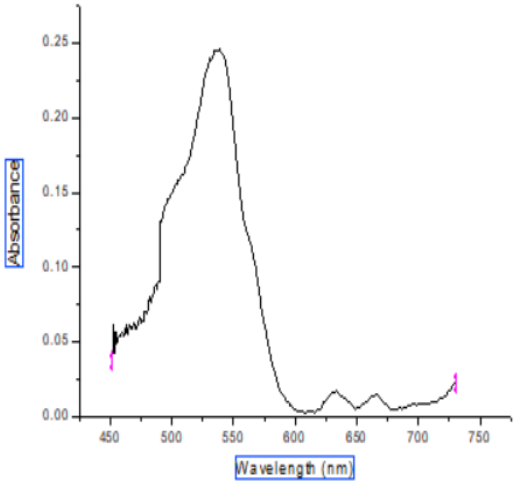


Fig.2 – UV-Visible spectra of gold nanoparticles

Fourier Transformation Infrared Spectroscopy

The biogenically synthesized AuNPs using *Psophocarpus tetragonolo*bus leaves extract showed the presence of Au at the peak around 601.79 cm^{-1}. The characteristic peak around 1643.25 cm^{-1} denotes the carbonyl stretching vibration of AuNPs synthesized using *Ananas Cosmosus* whereas here a characteristic peak obtained around 1643.36 cm^{-1}[18]. The peak at 1519.91 cm^{-1} represents N-O asymmetric stretch of nitro compounds), 1411.80 cm^{-1} (amine-II) and 1072.42 cm^{-1}(C-OH vibration)[19].

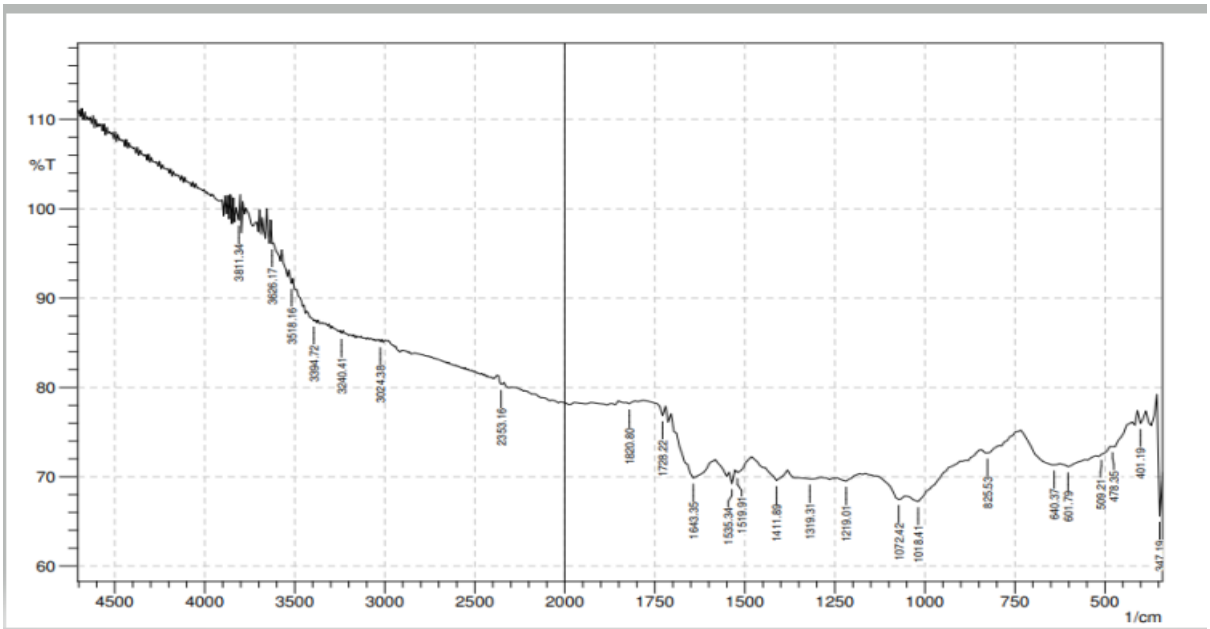


Fig. 3 - FTIR spectra of gold nanoparticles

X-ray diffraction

X-ray diffraction pattern of biogenically synthesized AuNPs was taken using Bruker D8 advance X-ray Diffractometer (XRD).The average particle size of synthesized AuNPs was calculated using Debye Scherrer equation. The biogenically synthesized AuNPs have an average size of 15 nm[20]. The sharp spectral peak confirms the crystalline nature of the amalgamated AuNPs [21].The peak at 38.3, 44.2, 64.28 and 77.32 indexed to (111), (200), (220), (311) clearly determines it as a face-centered cubic (FCC) lattice crystalline plane[22].

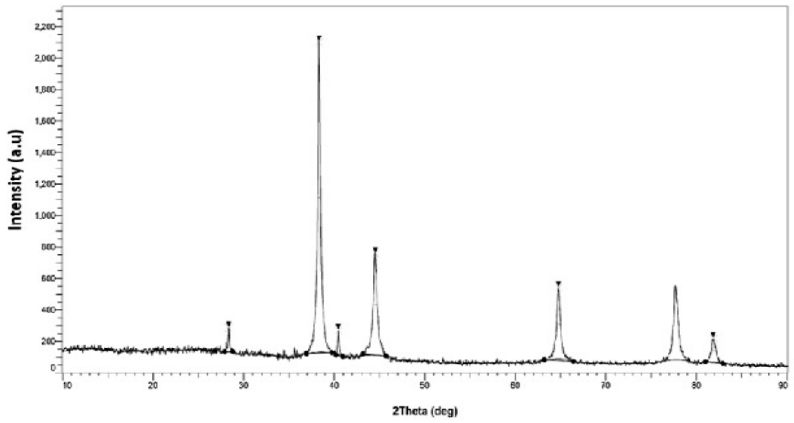
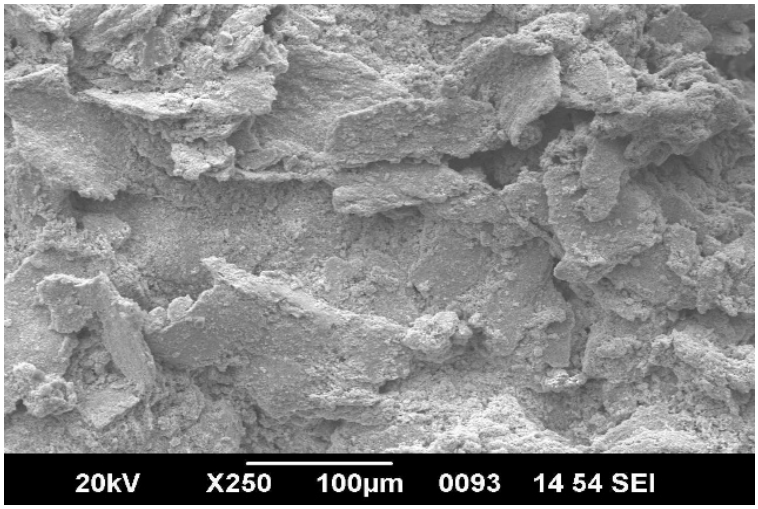


Fig. 4 - XRD spectra of gold nanoparticles

Scanning Electron Microscope

The morphology of AuNPs was examined using a model Joel's 5800 LV SEM. The SEM analysis of AuNPs was done at a magnification of 2.45 kx with an accelerating voltage of 20 kV. The sample was placed in a vaccum chamber and it was scanned using a focussed beam to get the image of the surface. The SEM image reveals that the amalgamated NPs are spherical in shape[23].

Fig. 5 - SEM image of gold nanoparticles



Energy – dispersive Spectroscopy

The fig.5 shows the EDAX spectrum of AuNPs prepared using winged bean leaves. The EDAX analysis confirmed that the biosynthesized AuNPs using *Psophocarpus tetragonolobus* leaves extract have the gold element in it with a typical peak approximately around 2 keV which is in accordance with the EDS spectrum of Cit-AuNPs and BH-AuNPs [24]. Also, the presence of biomolecules in the extract available for the preparation of gold nanoparticles may be the reason for feeble signals of oxygen and carbon element [19].Also, metal Iron is present which may be come from the leaf extract and the presence of Chlorine (Cl) may be due to the precursor salt solution.

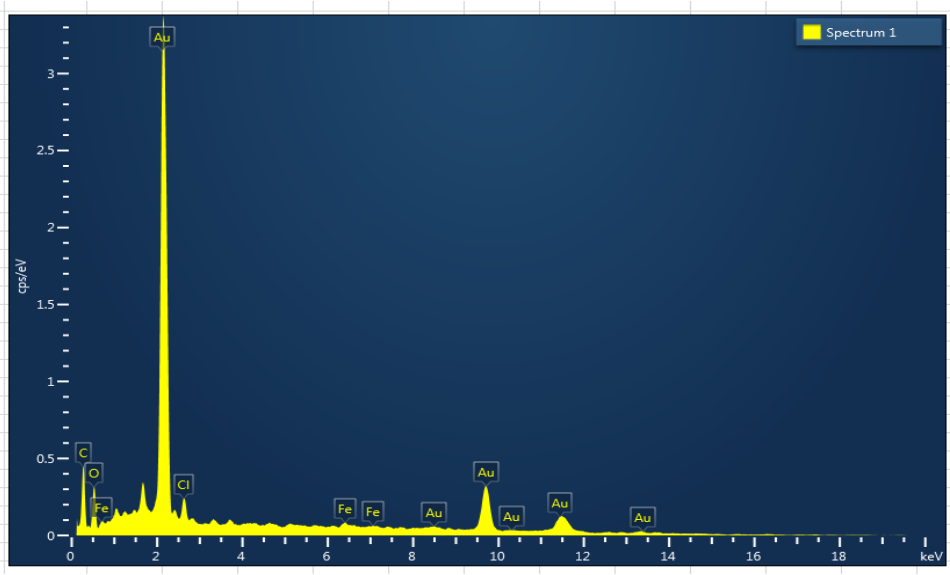


Fig. 6- EDAX spectrum of gold nanoparticles

Table 1 – Line type and weight % of elements in the sample

Element	Line Type	Weight %
C	K Series	22.58

O	K Series	7.29
Cl	K Series	1.81
Fe	K Series	0.37
Au	M Series	67.95

Anti-inflammatory Activity

In this, the anti-inflammatory activity of AuNPs was assessed using a protein denaturation inhibition assay. In this method, the absorption was spectroscopically measured at 660nm. The results indicated that the AuNPs, at various concentrations (6.25, 12.5, 25, 50,100), showed significant anti-inflammatory activity. Anti-inflammatory activity exhibits a positive correlation with increasing concentration.

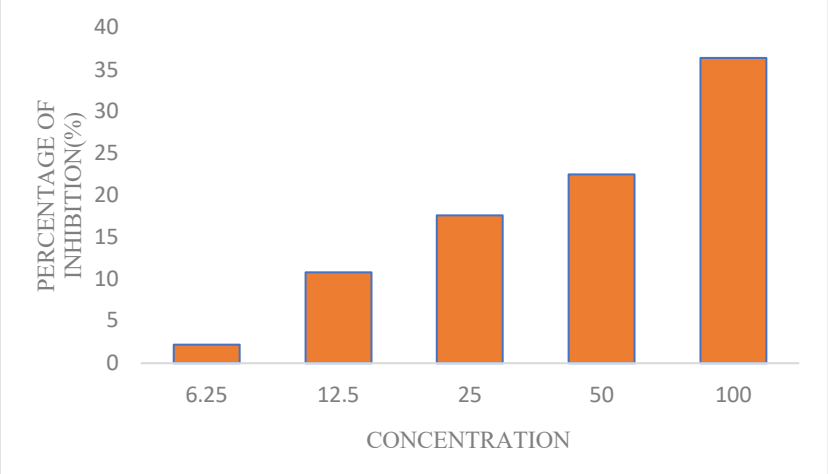


Fig.7 – Anti-inflammatory assay of gold nanoparticles

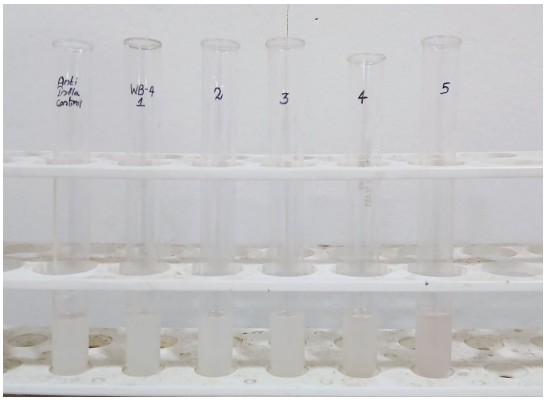


Fig. 8– Anti-inflammatory activity of gold nanoparticles

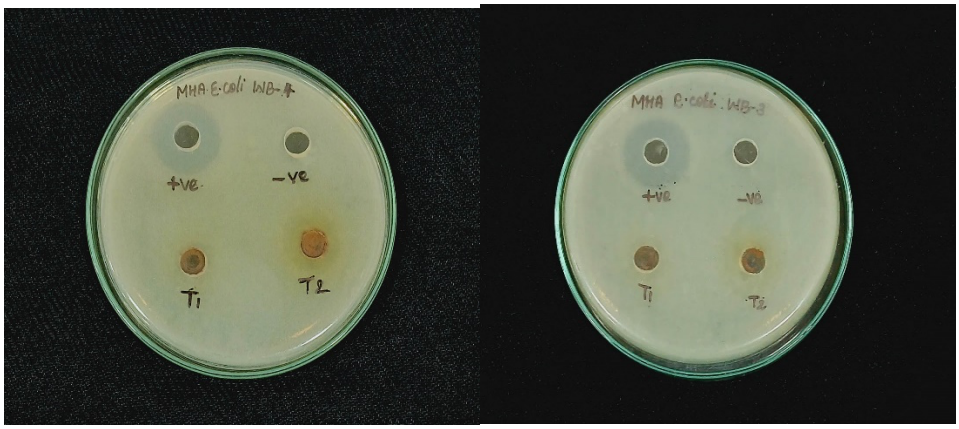
Antibacterial assay

The antibacterial activity was evaluated by agar well diffusion method against bacteria like*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus subtilis*. Table 2 demonstrates the

variation of zone of inhibition of amalgamated AuNPs at two different concentrations 0.3mM and 0.4mM respectively effectively against four bacterium.Increase in the concentration of the sample corresponds to an enhancement in the zone of inhibition. Here, the biosynthesized AuNPs displayed maximum antibacterial against *Bacillus Subtilis*. Similar results were obtained when AuNPs synthesized using *Spirulina platensis green alga* since it also showed antibacterial activity against *Staphylococcus aureus* and *Bacillus Subtilis*[25]. AuNPs prepared using *Gambir Roxb.*leaf extract also demonstrated antibacterial against *Staphylococcus aureus*[26].

Table 2 – The variation in the zone of inhibitions against different bacterial pathogens by gold nanoparticles

Bacterial name	**Zone of inhibition (mm)**		
	Standard	Concentration level	
		0.3mM	0.4mM
Escherichia coli	21	8	8
Staphylococcus aureus	26	8	9
Pseudomonas aeruginosa	20	8	8
Bacillus subtilis	26	11	20



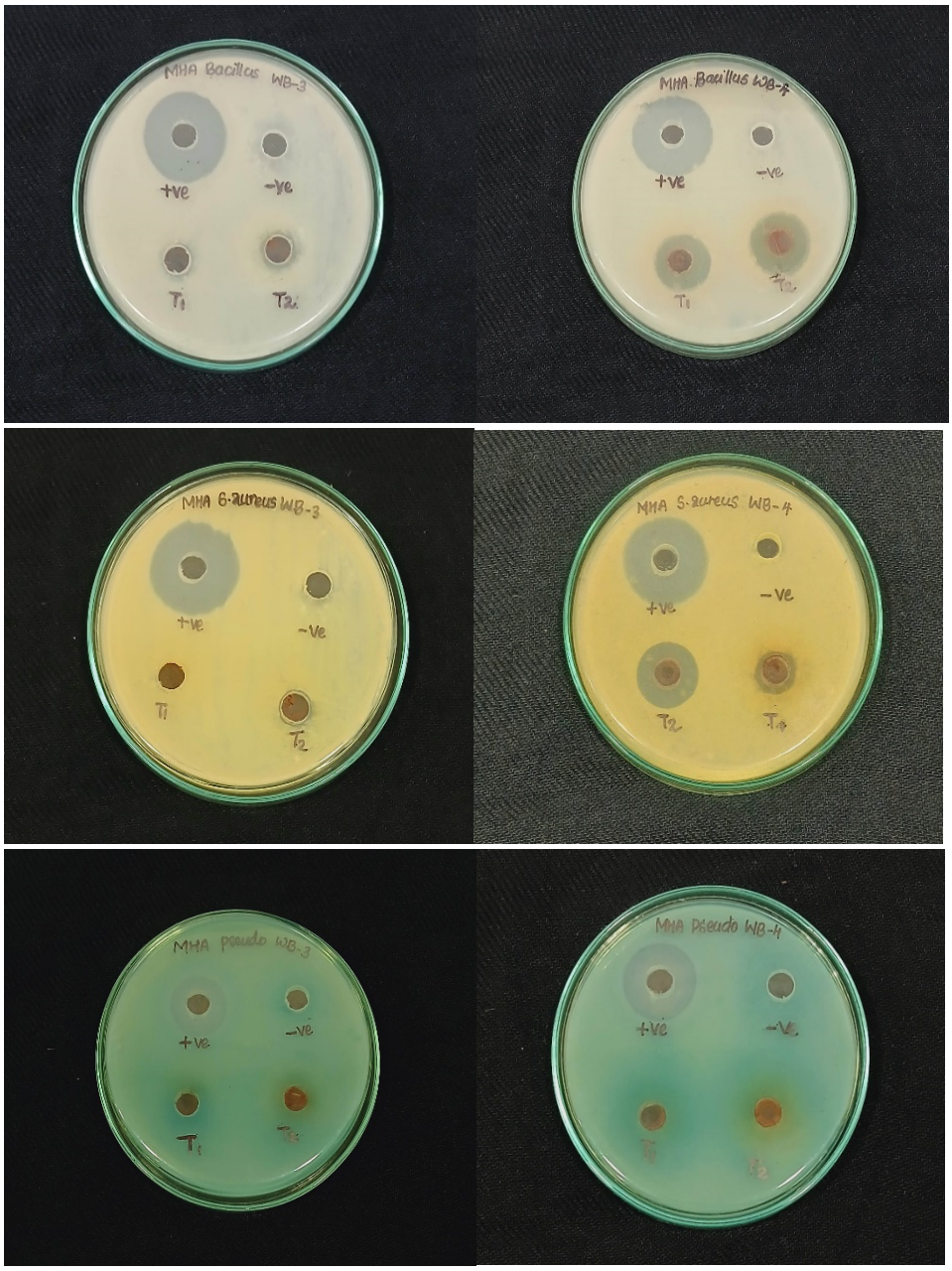


Fig. 9 – Antibacterial activity of the synthesized gold nanoparticles against four bacterium namely *Escherichia Coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*

Antifungal activity

The antifungal activity was evaluated by agar well diffusion method against *Candida albicans* and *Aspergillus niger*.The zone of inhibition of each sample against two fungi is illustrated in below table3. As the concentration of gold increases, AuNPs shows an increase in their antifungal activity against *Candida Albicans*. Since an increase in seen in their antifungal activity, there is a chance to reach the value to standard value.As similar to these results, plant mediated AuNPs using *Alpinia nigra* leaf

demonstrated antifungal activity against *Candida albicans*[27]. Similarly, AuNPs synthesized using *Azima tetracantha* leaf also shows antifungal activity against *Candida albicans*[28].

Table 3 – Variation in the Zone of Inhibition against Different Fungal Pathogens by Gold Nanoparticles

Fungi name	**Zone of inhibition(mm)**		
	Control	Concentration level	
		0.3mM	0.4mM
Candida Albicans	21	8	9
Aspergillus Niger	18	8	8

Fig. 10 – Antifungal activity of synthesized gold nanoparticles against *Candida albicans* and *Aspergillus niger*

Conclusion

Gold nanoparticles were successfully amalgamated using *Psophocarpus tetragonolobus* leaf extract at room temperature in an ecofriendly and cost-effective manner without any additional chemicals and

energy. The spectral analyses of amalgamated gold nanoparticles were carried out and hence, confirmed the presence of AuNPs. Moreover, its anti-inflammatory, antibacterial and antifungal activity makes these amalgamated AuNPs eligible for biomedical applications.

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Conflicts of interest

No potential conflict of interest was reported by the authors.

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