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Research Article

GREEN SYNTHESIS CHARACTERIZATION AND ANTIMICROBIAL ACTIVITES OF SILVER NANOPARTICLES FROM *CLITORIA TERNATEA* PLANT EXTRACT

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ABSTRACT

Nanotechnology has a wide range of applications in field of medicines. Nanoparticles are the fundamental blocks of nanotechnology. Among all the noble nanoparticles, silver nanoparticles are widely studied due to their unique properties. For the synthesis of silver nanoparticle plant extract is used as it easily available and capable of producing nanoparticles in large scale. The silver nanoparticles are formed by mixing the plant extract in silver nitrate ($AgNO_3$). The present study was done in *Clitoria ternatea* belonging to family Fabaceae. The synthesized nanoparticles from the plant extract were confirmed by UV-Vis spectrophotometer, FTIR and XRD. Color changed from yellow to brown color indicated the presence of silver nanoparticles in the plant extracts. The result showed that *Klebsiella pneumoniae* shows higher zone of inhibition than the others, in which silver nanoparticles synthesized by stem part shows higher activity.

INTRODUCTION

Now a day's nanotechnology become the most dynamic and fast growing field for the research due to their unique optical, magnetic, chemical and mechanical as well as antimicrobial properties. Nanoparticles are the fundamental blocks of nanotechnology. The size of nanoparticles is 10-200 nm and they are either amorphous or crystalline in nature [13]. It is widely used in many fields such as chemical industries, energy science, food and feed, drug-gene delivery, optics, electronics, space industries, catalysis, and photo electrochemical application and so on[12].

There are number of metal nanoparticles such as silver (Ag), zinc oxide (ZnO), copper (Cu), magnesium oxide (MgO), platinum (Pt), titanium oxide (TiO2), gold (Au), and iron oxide nanoparticles. Among all the metal nanoparticles, silver nanoparticles are widely studied due to their unique properties such as chemical stability, good conductivity, anti-fungal, anti-bacterial, anti- viral, anti – inflammatory activities, nontoxic to human beings, being added to the wound dressing, and many more [22].

Zinc oxide nanoparticles are nontoxic, and are used as an alternative to molecular UV- absorbers and protects from broader UV ranges [15].Copper nanoparticles also have wide range of applications such as – heat transfer system, antimicrobial materials, sensors, catalyst, used as plaster or paint as a bactericide agent to coat hospital equipment.

The major advantage of using plant extract for the synthesis of silver nanoparticle is that they are easy to use, safe process, eco- friendly, single step technique, and they are easily available. Plant extracts contain combination of biomolecules such as proteins, amino acids, enzymes, polysaccharides, terpenoids, flavones, quinones which are responsible of reduction and stabilization of silver ions [22].

Clitoria ternatea commonly known as Asian pigeon wings, bluebell vine, blue pea, butterfly pea, cordofanpea and Sankupushpam. It belongs to the Fabaceae family. In traditional Ayurvedic medicine, it is used to enhance the memory, anti-stress, anti-depressant, eye infections, skin diseases, urinary troubles and many more. The plant is used for curing asthma, hectic fever and also as a tonic against tuberculosis[13].

METHOD AND MATERIALS

Collection of Samples- Fresh and diseased free leaves and stems of *Clitoria ternatea* were collected from nearby area of Jabalpur. Leaves and stems were washed thoroughly 2-3 times with sterile water and was sun dried. Then ground to make a fine powder.

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Preparation of Sample - 4 gram of powder was taken into a beaker and added 100 ml of sterile distilled water and boiled for 20 min at 100^0C in water bath. The whole plant extract was collected in a separate beaker by standard filtration (Whatsmann filter paper no. 1) method and store it at 4^0 C for further experiment.

Synthesis of Silver Nanoparticles- In 10 ml of leaf and stem extract in a separate beaker add 50 ml of 1 mM $AgNO_3$ solution drop wise with constant stirring at 50 -60^0C.Incubate the beaker for bioreduction process at room temperature for 24 hours. The color change of whole plant extracts indicates the presence and synthesis of silver nanoparticles from the plant extracts of *Clitoria ternatea.*

Characterization of Silver

UV-Vis Spectrophotometric analysis–The mixture was centrifuged at 10,000 rpm for 15 min. The supernatant was scan in UV-Vis spectra, between the wave lengths of 350 – 700 nm in a spectrophotometer. UV-Vis spectra were recorded at intervals of 1 minute, 15 minutes, 30 minutes, 1 hour, 24 hours, and 48 hours, respectively.

Test Microorganism- For antimicrobial study the strains of bacteria and fungus were procured from MTCC, Chandigarh. The cultures were maintained in their respective media and broth.

Antibacterial activity (disc diffusion method) - Antibacterial assay was done on *Staphylococcus aureus* (gram positive), *Kleibsiella pneumoniae* (gram *negative),* and *Mycobacterium luteus (*gram-positive) by disc diffusion method. In Disc Diffusion method the discs were soaked with plant extracts, nanoparticles containing solutions (Ag^+), and 1 mM $AgNO_3$ respectively. Then, the discs were air dried in sterile condition. The diameter of sterile disc is 0.5 cm. The plate containing nutrient agar media (NAM) were prepared and microbial cultures were inoculated by spread plate method. The plates containing media as well as cultures were divided into 4 equal parts and placed the sterile discs and antibiotic disc in their respective place [3]. Nalidixic acid (Antibiotics) was used as standard control. The plates were incubated at 37^0Cfor 24 hours. Next day the diameter of inhibition zone was measured.

Antifungal activity (disc diffusion method) - Antifungal assay was done on *Colletotrichum*, *Aspergillus niger*, and *Candida albicans* by disc diffusion method. All the three cultures were inoculated in the potato dextrose agar media (PDA) aseptically. Then, the discs were soaked with plant extracts, nanoparticles containing solutions (Ag^+), and 1 mM $AgNO_3$ respectively. The plates containing media as well as cultures were divided into 4 equal parts and placed the sterile discs and antibiotic disc in their respective place. Nystatin (Antifungal) was used as standard control. Then, plates were incubated and observed next day.

FTIR (Fourier transform infrared) spectroscopic studies- FTIR analysis were carried out in order to identify the presence of various functional groups in biomolecules, which is responsible for the bioreduction of Ag^+ ions and capping/stabilization of nanoparticles. For FTIR, the synthesized silver nanoparticles solution was centrifuged at 10,000 rpm for 15 minutes. The pellet obtain was dried at 100^0C for 24 hours. Then, synthesized silver nanoparticles were grounded with KBr (Potassium Bromide) pellet. Then it was analyzed by FTIR. The sample and pellet was taken in the ratio of 1:3.

XRD (X- ray diffraction) -X-ray Diffraction analysis is the most useful method to identify the crystalline structure of sample [16]. For XRD analysis, the precipitate of silver nanoparticles obtained using plant extracts of *Clitoria ternatea* were dried in an oven at 100^0C for 24 hours. Then, the dried silver nanoparticles were then grounded using a mortar and pestle into fine particles and XRD pattern was then recorded using X-ray diffractometer. The XRD data were obtained in the 2θ value range of 4^0 to 80^o at 0.002 min^{-1} and at 1 second time constant. By using Debye- Scherrer equation the size of the nanoparticles was calculated. The Debye- Scherrerequation:

$$\mathbf{D = k\lambda /\beta cos\theta}$$

Where, D= thickness of the nanocrystal, k= constant (0.94), λ= wavelength of X-rays (1.5406 x 10^{-10}), β is the annular FWHM (full –width at half maximum) of the XRD peak at the diffraction angle θ and θ= Bragg angle [11].

RESULT AND DISCUSSION

The reduction of silver nitrate using the plant extract was determined by colour change in the reaction solutions (figure 1). When the leaf extract mixed with silver nitrate, its colour changes from deep yellow to deep brown, which indicate the formation of silver nanoparticles while when, stem extract mixed with silver nitrate, its changes from light yellow to drown colour. Similarly, Ravindra *et.al* (2012) reported that the colour change of the plant extracts from yellow to brown indicates the presence of nanoparticles and the change in colour is due to the Plasmon vibration in silver nanoparticles [13]. Ahmed *et.al* (2011) mentioned different ways for the reduction of silver in plant extracts. The secondary metabolites present in plant system may be responsible for the reduction of silver and synthesis of nanoparticles. During glycolysis process, electron release which converts the NAD to NADH, due to this, silver nitrate transformed to form the nanoparticles. [2].

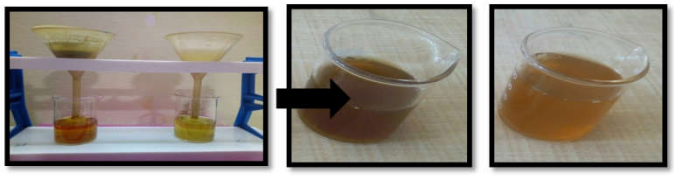


Figure 1 Color change of leaf and stem extract indicate the synthesis of nanoparticles

Characterization of Silver

UV- Vis analysis- the maximum absorbance and wavelength of the sample confirm the reduction of silver nitrate ($AgNO_3$) when observed under UV-Vis spectrophotometer. The maximum absorbance peak of silver nanoparticles synthesized by leaf was seen at 400- 450 nm while maximum absorbance peak of silver nanoparticle synthesized by stem was seen at 400-500 nm respectively. Thus, the UV-Vis spectra showed that the most rapid bioreduction was achieved using leaf extract as reducing agent followed by stem extract. The result of UV-Vis spectra also revealed that the nanoparticles start forming within 15 minutes and remain stable even after 48 hours of

completion of reaction. Banerjee *et.al* (2014) also supports the formation of silver nanoparticles within 15 minutes [3]. The absorbance peaks of silver nanoparticles in leaf and stem of *Clitoria ternatea* was plotted in graph given below

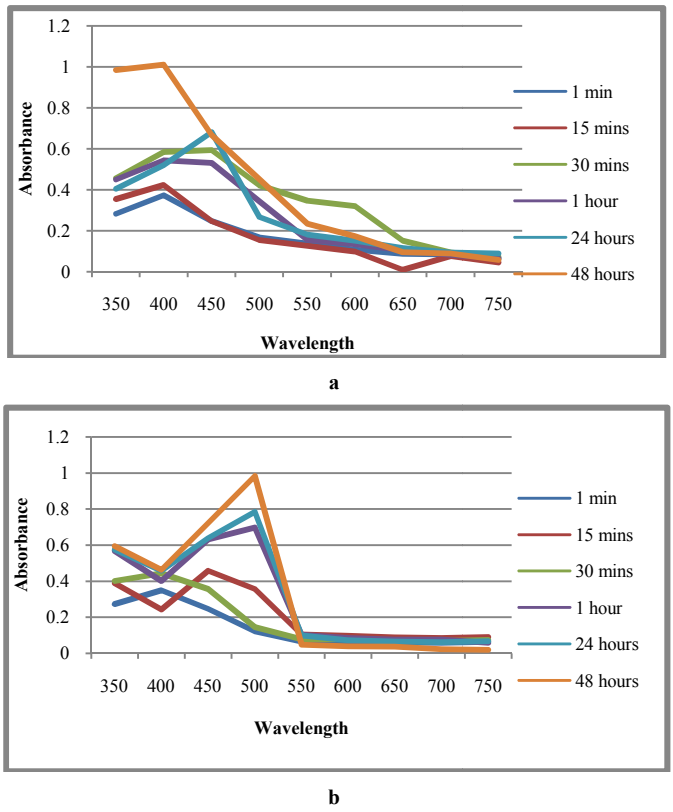


Figure 2 UV-Vis spectra of silver nanoparticles from (a) leaf and (b) stem

Antibacterial Activity- In this study, the antibacterial activity of silver nanoparticles was investigated by *Staphylococcus aureus, Mycobacterium luteus* and *Klebsiella pneumoniae*. Shakeel *et.al* (2014), reported that the antimicrobial activities of silver nanoparticles depend on-

- Size and environmental conditions (size, pH, ionic strength)
- Capping agent [22].

According to Narayanaswamy *et.al* (2015), different parameters such as time, plant sources, the synthesis method, all create a wide range of different nanoparticles [11]. The result showed that the synthesized nanoparticles have a potential to show the antibacterial activity against *Staphylococcus aureus, Mycobacterium luteus* and *Klebsiella pneumoniae.* The result also revealed that *Klebsiella pneumonia* shows higher zone of inhibition than the others, in which silver nanoparticles synthesized by stem shows higher (figure 3). According to Shakeel *et.al* (2014), Gram positive bacteria are less susceptible to Ag+ than Gram negative bacteria, as the cell wall of Gram positive bacteria is made up of peptidoglycan molecule and has more peptidoglycan than Gram negative bacteria [22]. Thus, Gram positive bacteria may allow less Ag to reach cytoplasmic membrane than Gram negative bacteria. Siva Kumar *et.al* proposed that the oxygen associated with silver reacts with the sulphydral (-S-H-) group, which is present in the cell membrane of bacteria, form R-S-S-R bonds causing inhibition of respiration resulting in cell death [21]. The mode of action of silver nanoparticles and silver ions were reported to be similar. Although, the nanoparticles are significantly more effective at lower concentration that that of silver ions.

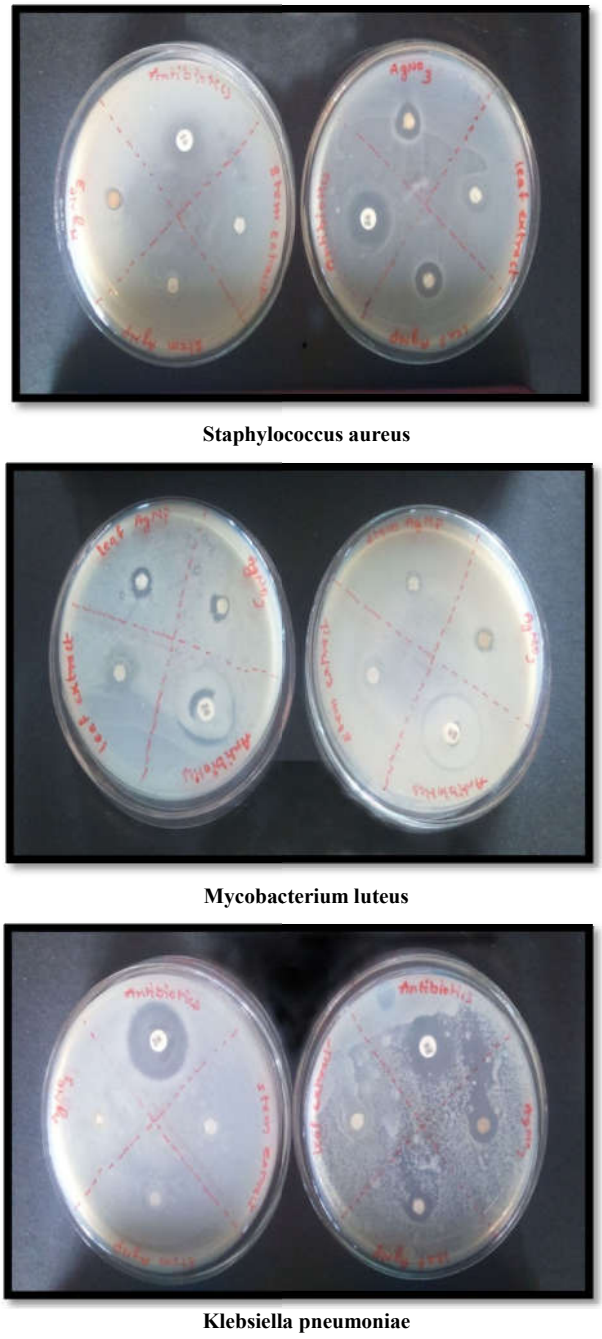
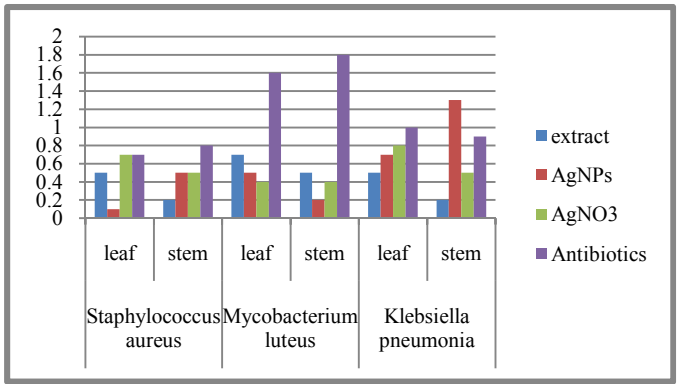


Figure 3 Plate showing zones of inhibition against different Gram positive and Gram negative bacteria's in response to the impregnated sample



Graph 1 Antibacterial activity of leaf and stem extracts of *Clitoria ternatea*

Antifungal Activity- In this study, the antifungal activity of silver nanoparticle was investigated by *Colletotrichum, Aspergillus niger* and *Candida albicans*. The antifungal activity revealed that the silver nanoparticles also have a potential to show the antifungal activity against *Colletotrichum, A. niger and Candida albicans* (figure 4). The result also revealed that the silver nanoparticle shows higher zone of inhibition than silver nitrate while plant extract shows no zone of inhibition. Nanoparticles synthesized by leaf shows higher zone of inhibition against *Colletotrichum* while nanoparticles synthesized by stem shows higher zone of inhibition against *Aspergillus niger.*

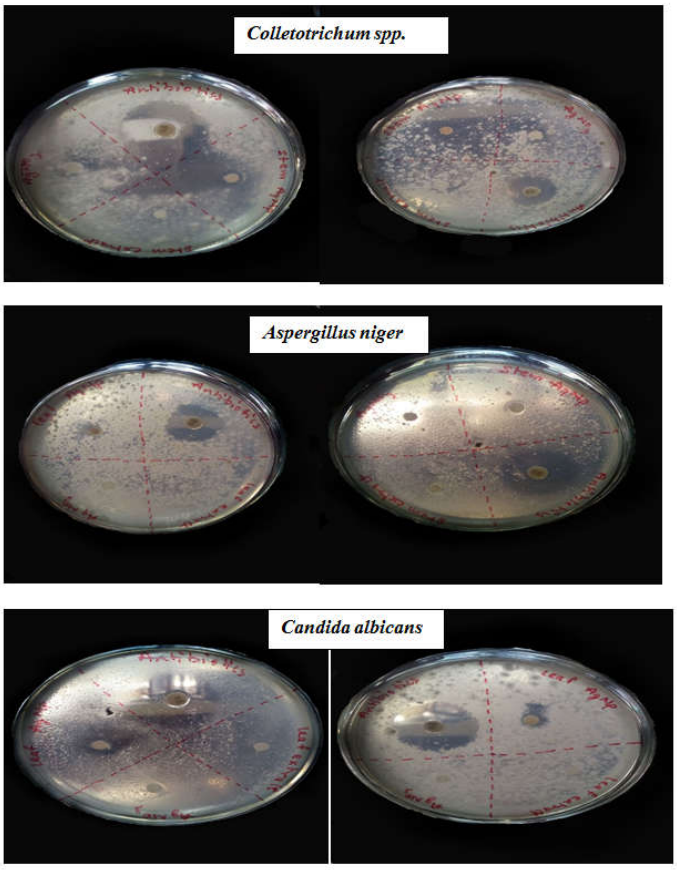
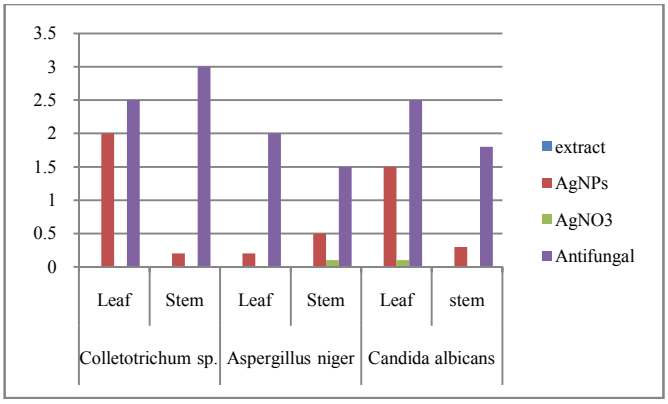


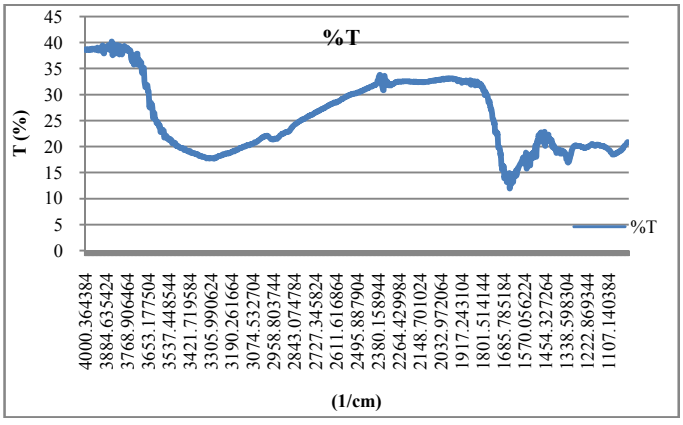
Figure 4 Zone of inhibition against fungus's in response to the impregnated sample



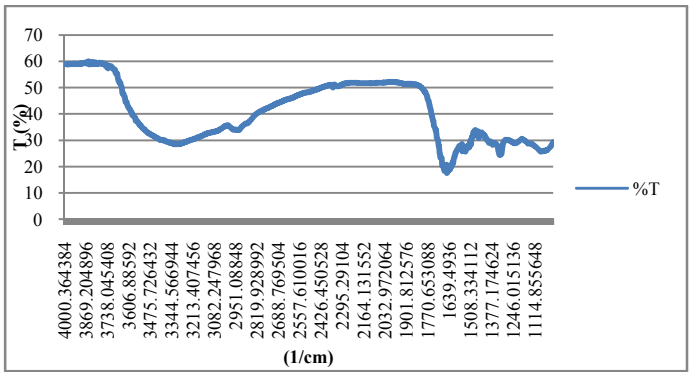
Graph 2 Antifungal activity of leaf and stem extracts of *Clitoria ternatea*

FTIR (Fourier transforms infrared spectroscopy)- FTIR analysis of silver nanoparticles in plant extract showed the absorption bands, which is corresponding to the functional groups found in secondary metabolites. The result revealed that for the synthesis of nanoparticles secondary metabolites are involved. The absorbance bands of leaf were observed at 1454.33, 1504.48, 1556.55, 1651.07, 2351.23, and 3730.33 indicate the presence of capping agent with the nanoparticles. The band at 1454.33 cm^{-1} in the spectra corresponds to aromatic C=C stretch. Bands at 1504.48 cm^{-1} correspond to carbonyl C=O stretch. Bands at 1556.55 cm^{-1} correspond to amide N-H bending. Bands at 1651.07cm^{-1}correspond to amide C=O stretch. Bonds at 2351.23cm^{-1}correspond to phosphine P-H. Bands at 3730.33 cm^{-1} correspond to alcohol O-H stretching.

The absorbance bands of stem were observed at 3332.994, 3504.657, 1809.229, 1651.066, 1055.062, 3431.364, 1321.239, 3381.214, and 1053.134 cm^{-1} indicate the presence of capping agent with the nanoparticles. Bands at 3332.994and 3381.214 cm^{-1} corresponds to Amine N-H stretch. Bands at 3504.657 cm^{-1} correspond to Amide N-H stretch. Bands at 1809.229 cm^{-1} correspond to anhydride C=O stretch. Bands at 1651.066 cm^{-1}correspond to C=O stretch. Bands at 3431.364 cm^{-1} correspond to alcohol/ phenol O-H stretch. 1055.062 and 1053.134 cm^{-1} correspond to carboxylic acid O-C stretch.1321.239 cm^{-1} correspond to S=O sulfoxide.

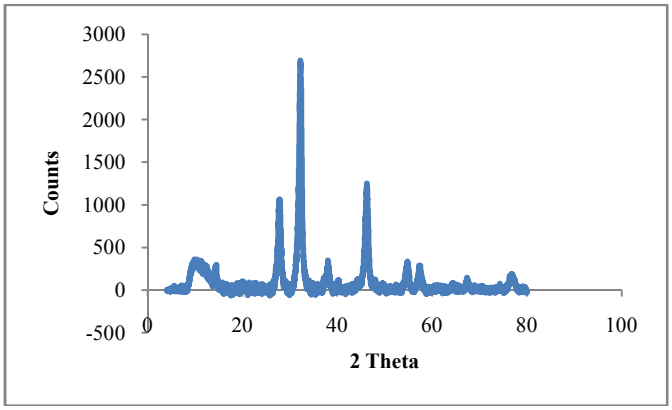


Graph 3 FTIR analysis of silver nanoparticles synthesized by leaf extract.

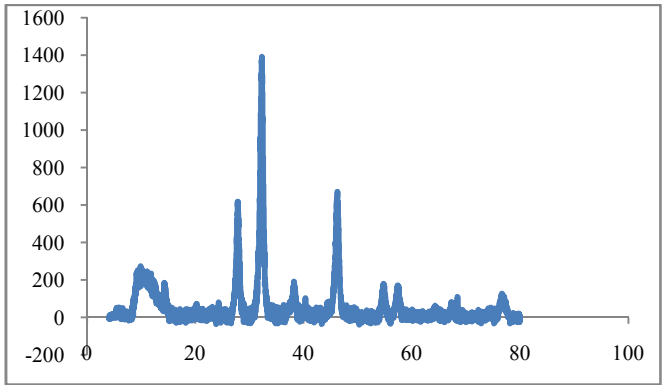


Graph 4 FTIR analyses of silver nanoparticles synthesized by stem extract

XRD (X-RAY Diffraction) Analysis- The X-ray detector moves around the sample and measures the intensity and position of these peaks. The diffraction pattern given by XRD indicates that the sample is silver nanoparticles. The intense peaks of nanoparticle synthesized by leaf are 32.209, 27.78, 46.272, 45.995, 46.204, and 54.732and while the intense peaks of nanoparticle synthesized by stem are 32.502, 28.078, 46.356, and 57.617. In graph of *Clitoria ternatea's* leaf and stem, the sharpening of peak indicates that the particles are the crystalline nanoparticles. The crystalline size of silver nanoparticle synthesized by leaf is 135.0 A^0while the crystalline size of silver nanoparticle synthesized by stem is 139.3 A^0.



Graph 5 XRD pattern of silver nanoparticles of *Clitoria ternatea*'s leaf



Graph 6 XRD pattern of silver nanoparticles of *Clitoria ternatea*'s stem

CONCLUSION

Silver nanoparticles were successfully obtained from bioreduction of silver nanoparticle solution using *Clitoria ternatea's* leaf and stem. Silver nanoparticles have been characterized using UV-Vis spectroscopy, FTIR, XRD, and antimicrobial activity.

The UV-Vis analysis confirmed the formation of nanoparticles. The UV-Vis spectroscopy showed that the absorption band of silver nanoparticle synthesized by leaf is at 400-450 nm and stem at 400-500 nm.

The antibacterial activity showed that silver nanoparticles are very active against *Klebsiella pneumonia t*han the other pathogens. And it also showed that silver nanoparticle synthesized by stem extract showed highest antibacterial activity.

FTIR analysis showed the presence of functional groups in the plant extract. The functional groups found in leaf extract are aromatic C=C stretch, carbonyl C=O stretch, amide N-H bending, amide C=O stretch and alcohol O-H stretch. While the functional groups found in stem extract are amine N-H stretch, amide N-H stretch, anhydride C=O stretch, alcohol/phenol O-H stretch, carboxylic acid O-C stretch.

XRD analysis of silver nanoparticles showed average particle size of stem extract (13.74 nm) is larger than the leaf extract (13.20).

The study concluded that stem of *Clitoria ternatea* show higher antibacterial activity against *Klebsiella pneumoniae* whereas *Clitoria ternatea*'s leaf shows higher antifungal activity against *Colletotrichum*. Due to their antimicrobial property, silver nanoparticles can be used as bactericidal, fungicidal, in wound healing, water purification and also in the field of medicine. This green synthesis method is alternative to chemical method, since it is cheap, pollutant free and eco-friendly.

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