International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.4, No.3, pp 1323-1331, July-Sept 2012

Biogenic Synthesis Of Gold Nanoparticles Using Leaf Extract Of Tephrosia purpurea And Study Of Their Antibacterial Effect

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Abstract: Nanobiotechnology has emerged as integration between biotechnology and nanotechnology for developing bioactive, biosynthetic and ecofriendly technology for synthesis of nanomaterials. Development of eco-friendly process for the synthesis of nanoparticles is one of the main steps in the area of nanotechnology research. The synthesis, characterization, and application of biologically synthesized nanomaterial have become an important branch of nanotechnology. Hence, there is significant current interest in preparing nano-materials of small size. This manuscript describes ways of synthesising gold nanoparticles by using leaf extract *Tephrosia Purpurea*. The synthesis of gold nanoparticles was rapid and within few hrs gold ion makes contact with the leaf extract of *Tephrosia Purpurea* was reduce AUCL4 in to fine gold nanoparticles. The appearance of reddish colloidal dispersion indicates the synthesis of Gold Nano particles. UV Spectral analysis indicated that the reduction of tetra auric chloride (AUCL4) occurred in a short reaction period and showed a peak at 540 nm due to the surface Plasmon resonance of gold nanoparticles. The gold Nano particles are characterized by FTIR, SEM. The antibacterial activities of the studied nanoparticles were tested for biological applications. The antibacterial activity of gold nanoparticle is studied with the bacteria *Escherichia coli, Enterobacter faecalis, Staphylococcus aureus* and *Klebsiella pneumoniae*. The gold nanoparticles conjugated with the Tetracycline antibiotics shows the high zone of inhibition in both the organisms. This result confirms the prepared gold nanoparticles can be used as a good antibacterial agent. The synthesized gold nanoparticle can be used in various fields as an antibacterial agent.

Keywords: Gold nanoparticle, HAUCL4, *Tephrosia Purpurea, E.coli, Staphylococcus aureus, Enterobacter faecalis, Klebsiella pneumonia.*

INTRODUCTION

Tephrosia purpurea is a species of flowering plant in the pea family, fabacea that has a pantropical distribution. It is a common wasteland weed. In many parts it is under cultivation as green manure crop. It is found throughout India and Sri Lanka in poor soils (2). The root and seed are reported to have insecticidal and piscicidal properties and also used as vermifuge (15). The roots are also reported to be effective in leprous wound and their juice is applied to the eruption on skin. Its aerial parts and roots are used in bronchial asthma, hepatic ailments, pain and inflammation. *Tephrosia purpurea* is a wild plant known as “Sarapunkha” in Sanskrit,‘Purple tephrosia’ or

'Wild indigo' in English and "Avuri" or "Kolinji" in Tamil and 'Unhali' in Gujarati. In Ayurvedic system of medicine various part of this plant are used as remedy for impotency, asthma, diarrhea, gonorrhea, ulcear and urinary disorders (11).

Nanotechnology provides the broad knowledge of applied science and technology to control the matter on the atomic and molecular scale. Nano biotechnology is an important and emerging technical tool for development of eco friendly, reliable methodology for synthesis of nanoscale materials using biological sources (7). In modern nanoscience and technology, the interaction between inorganic nanoparticle and biological structures are one of the most exciting areas of research. The biological synthesis process elucidates the importance of metal microbe interaction in several biotechnological applications including the field of bioremediation, biomineralization, bioleaching and microbial corrosion (14).

Generally, gold nanoparticles are produced in a liquid ("liquid chemical methods") by reduction of chloroauric acid (H AuCl4), although more advanced and precise methods do exist. After dissolving HAuCl4, the solution is rapidly stirred while a reducing agent is added. This causes Au3+ ions to be reduced to neutral gold atoms. As more and more of these gold atoms form, the solution becomes supersaturated, and gold gradually starts to precipitate in the form of sub-nanometer particles. The rest of the gold atoms that form stick to the existing particles, and, if the solution is stirred vigorously enough, the particles will be fairly uniform in size.

Gold nanoparticles are known for their beautiful wine-red colouration and are known to have one of the highest extinction coefficients known (of the order of 109 M–1 dm–1). The bright colour of nanoparticles is ascribed to the transverse oscillations of the surface electrons of the particle on interaction with light of suitable wavelength, the surface plasmon resonance. It has also been shown that the surface plasmon band is greatly dependent on the nanoparticle size. Shape of the nanoparticle also plays an important role in deciding the position of this band. In case of gold nanorods, for example, it shifts to the near infra-red region of around 850 nm. Now methods are available to synthesize nanoparticles that are remarkably stable for many cycles of drying and redissolution (13).

Fig 1: ***Tephrosia purpurea***

Over the recent decade, gold nanoparticles have a wide variety of potential applications, for instance, an effective drug delivery in a cancerous tumour, biomedical, in a wide range of cosmetic and beauty applications, biosensors and catalysts (3 & 8). Nowadays, research efforts are being concentrated on integrating nanoparticles with biology. It has been reported that antibiotics often disturb the bacterial flora of digestive tract which may develop multiple drug-resistant isolates, hence novel ways of formulating biocide materials is an upcoming field of attraction (1, 4, 5 & 10). For this reason, there is a need for the use of an agent which does not generate resistance and presents a good bactericidal property. Gold nanoparticles have a great bactericidal effect on a several range of microorganisms; its bactericidal effect depends on the size and the shape of the particle (12). Nanoparticles can act as antibacterial and antifungal agents, due to their ability to interact with microorganisms (6 & 9).

Here in we report for the first time, an environmentally friendly and rapid method for the aqueous synthesis and stabilization of gold nanoparticles by the reduction of aqueous AuCl4. Gold nanoparticles were synthesized by using the leaf extract of *Tephrosia Purpurea* and characterized by UV–Vis spectrum and FTIR, Scanning Electron Microscope (SEM). Further, the antibacterial efficacy of drugs coated gold nanoparticles was studied against various strains *Escherichia coli*, *Enterobacter faecalis*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. The results obtained that the gold nanoparticle conjugated with the drug showed good antibacterial effect. From these results it is confirmed that it can also can be used as a good antibacterial agent and find its application in public health and various industrial fields.

MATERIALS AND METHODS

Collection of plant sources

Tephrosia purpurea were collected along with the root nodule from the Udaya School of Engineering campus, Kanyakumari District, Tamil nadu, India. Then the leaf were separated and washed with distilled water. Extract *Tephrosia purpurea* was prepared by taking 20g of thoroughly washed and finely cut leaves in 500 ml Erlenmeyer flask along with 100 ml of distilled water and then boiling the mixture for 10 min before decanting it. Further, the extract was filtered with Whatman No. 1 filter paper and stored at 4°C and used for further experiments.

Biological Synthesis of Gold Nanoparticles

For the synthesis of Au- NPs (Gold nanoparticles), two boiling tubes were taken, one containing 10ml of 1mM Hydrogen tetra chloro aurate (HAuCl4) solution as control and the second flask containing 9ml of 1mM Hydrogen tetra chloro aurate (HAuCl4) solution and 1ml of plant leaf extracts as test solution were incubated at room temperature and continue stirring for 2 hrs. The gold nanoparticle solution thus obtained was purified by repeated centrifugation at 15,000 rpm for 20 min. Supernatant is discarded and the pellet is dissolved in deionised water for further studies. The gold nanoparticules were confirmed by colour changes and qualitatively characterized by UVvisible spectrophotometer.

Characterization of Gold Nanoparticles

UV-Vis spectral analysis

UV-Visible spectrophotometer is the one of the important techniques for analysis of synthesized gold nanoparticles. After the synthesis, the pure gold nanoparticles were characterized by Microprocessor UV-Visible absorption spectrophotometer Double Beam, I-2902. The colour change in reaction mixture (metal ion solution +plant extract) was recorded through visual observation. Synthesized gold nanoparticles was confirmed by sampling the aqueous component of two hour after reaction and the absorption maxima was scanned by UV-Vis spectrophotometer at the wavelength of 400– 800 nm.

FTIR analysis of Gold nanoparticles

The interaction between protein and gold nanoparticles was analyzed by Fourier transform infrared (FTIR) analysis. After the reaction the supernatant was again centrifuged at 10000 rpm for 60 min and the pellet was obtained. This is followed by redispersion of the pellet of Au-NPs into 1 ml of deionized water. Thereafter, the purified suspension was freeze dried to obtain dried powder. The FTIR spectrum of the dried sample was carried out by using Prucker Instrument (model Tensor 7) in the range of 500-4000 cm^{-1} at a resolution of 4 cm^{-1}.

Scanning Electron Microscopy of Gold Nano Particles

SEM is the powerful tools to study the morphological characterization of synthesized nanomaterials. The SEM has a large depth of field, which allows more of a specimen to be in focus at

one time. The SEM is also widely used to identify phases based on qualitative chemical analysis and crystalline structure and also can be used to examine micro fabric and crystallographic orientation in many materials. SEM analysis of dried gold nano particles powder was carried out by using Jeol, model JSM 6390 made in Japan.

Antibacterial activity of Gold Nanoparticles

Antibacterial activity of synthesized gold nanoparticles was done to detect any antagonistic character against some human pathogens.

Test microorganisms

The strains of *Escherichia coli, Enterobacter faecalis, Staphylococcus aureus* and *Klebsiella pneumoniae* were procured from CMFRI, Trivandrum, used for the antibacterial activity. Nutrient broth was used for cultivate the bacteria and the cultures were grown overnight in nutrient broth on a rotary shaker (200 rpm) at 37 ºC for 24 hrs. Then the bacterial cultures is maintained and used for antibacterial activity.

Antibacterial testing

Antibacterial activity was evaluated by using agar well diffusion techniques in petri dishes. The Muller Hinton agar is the suitable media for the growth of many pathogens and antibacterial activity. Each micro organism was spread on Muller Hinton Agar (MHA) plates with the help of sterile cotton swabs. Wells are made on Muller Hinton Agar plate by using sterile well borer. Commercially available Tetracycline antibiotic 10mg/ml was used as standard for this analysis. 100 µl of Gold nanoparticles, Tetracycline antibiotic coated with gold nanoparticle, and Antibiotic was loaded in to the wells which were cut using well borer. Four petri plates was prepared in similar way for the four bacteria namely of *Escherichia coli, Enterobacter faecalis, Staphylococcus aureus* and *Klebsiella pneumonia.* After incubation for overnight at 37^{o}C, a clear zone of inhibition around a well was the evidence of antibacterial activity. Diameters of the zone of inhibitions were measured in millimeter.

RESULTS AND DISCUSSIONS

The plant *Tephrosia purpurea* was collected from the Udaya School of Engineering campus, Kanyakumari District, Tamil Nadu, India. The *Tephrosia purpurea* was given in the fig.1 and the extract was prepared. The *Tephrosia purpurea* plant extract was mixed in the aqueous solution of the to10^{-3} M aqueous HAuCl4 solution led to the appearance of reddish colloidal dispersion indicates the synthesis of Gold Nano particles fig.2.

Characterization of Gold Nanoparticles

UV-Vis spectral analysis

The UV-Visible spectrophotometer proved to be very useful technique for the analysis of some metal nanoparticles. The color change showed the presence of gold nanoparticles in the *Tephrosia purpurea* leaf extract and it was characterized by UV-Visible spectrophotometer. The formation of gold nanoparticles was confirmed by color changes followed by UV-Visible spectrophotometer analysis and monitored by taking readings at regular time intervals in a UV-Visible absorption spectrophoto meter Double Beam, I-2902. The UV- visible spectra (shown in Graph 1) indicated a strong broad peak located at 540nm was observed for gold nanoparticles.

FTIR analysis of Gold nanoparticles

FT-IR dimensions were carried out to recognize the possible biomolecules accountable for the reduction of the Au+ ions and capping of the bio reduced gold nanoparticles synthesized by *Tephrosia purpurea* plant extract. The FTIR spectra of synthesized gold nanoparticles show in graph. 2. The spectrum shows lot of absorbance band indicates the active functional group in the synthesized gold nanoparticles. The intensity peaks are slightly increased for the period of gold nanoparticle synthesis like 555, 1014, 1416, 1691, 2974 and 3421cm^{-1} as well as some intensity peaks decreased like 1553, 2821 cm^{-1}. By comparing other research data (16 & 17), the peak at 555 cm^{-1} corresponds to C- Cl, C-Br stretching vibrations to alkyl halides. The peaks at 1014 cm^{-1} indicate C-O single bond. The peak at 1416 cm^{-1} and 1553 cm^{-1} may be assigned to symmetric stretching vibrations of –COO- (carboxyl ate ion) groups. The peak at 1691 cm^{-1} indicates the stretching vibration of alkenes and amide. The peak at 2974 cm^{-1} and 2821 cm^{-1} indicates the C-N, amine and amino methyl stretching. Then the last peak at 3421 cm^{-1} indicates the N-H, O-H Stretching vibrations of alkanes, alcohol and H–bonded to phenols. To obtain good signal to noise ratio of silver nanoparticle were taken in the range 500–4000 cm^{-1}.

Scanning electron microscopy (SEM) of gold nanoparticles

The Scanning Electron Microscope (SEM) has been employed to characterization of size, shape and morphology of synthesized gold nanoparticles.

The scanning electron microscope of pure gold nanoparticles at 10000X magnification is shown in fig 3. SEM photograph of gold nanoparticles clearly indicates that in the room temperature synthesized gold nanoparticles relatively spherical and uniform arrangement and size of the nanoparticles is ~100 nm. The SEM image showed the high density and spherical shape gold nanoparticles synthesized from *Tephrosia purpurea* leaf extract for further development of gold nanomaterials.

Antibacterial activity of Gold Nanoparticles

The antibacterial activities of synthesized gold nanoparticles were studied against the human pathogens such as *Escherichia coli*, *Enterobacter faecalis*, *Staphylococcus aureus* and *Klebsiella pneumonia* by using the agar well diffusion method. The result of antibacterial activity of gold nanoparticles was shown in fig4. Anti bacterial study indicates that the gold nano particles synthesized by *Tephrosia purpurea* leaf extract conjugated with Tetracycline antibiotic exhibit more zone of inhibition compared to standard Tetracycline antibiotic used (Table 1). The zone of inhibition on *E.coli* , *Enterobacter faecalis*, *k.pnemonea*, were more when compared with the zone of inhibition exhibited on *S.aureus*. The biosynthesized gold nanoparticles with the Tetracycline antibiotic [31mm, 34mm, 27mm and 20mm] exhibit high zone of inhibition compared with only Tetracycline antibiotic [27mm, 31mm, 24mm and 16mm]. Hence the antibacterial activity of gold nanoparticles has confirmed that the synthesized gold nanoparticles conjugated with the Tetracycline antibiotic has high activity and determined high zone of inhibition against the human pathogens *Escherichia coli*, *Enterobacter faecalis*, *Staphylococcus aureus* and *Klebsiella pneumonia*.

Fig 2: Biogenic synthesis of Gold nanoparticles

A- 1 mM Hydrogen Tetra Chloro Aurate (HAuCl4) solution before adding extract (*Tephrosia purpurea* leaf extract)
B- After adding extract to the HAuCl4 solution.

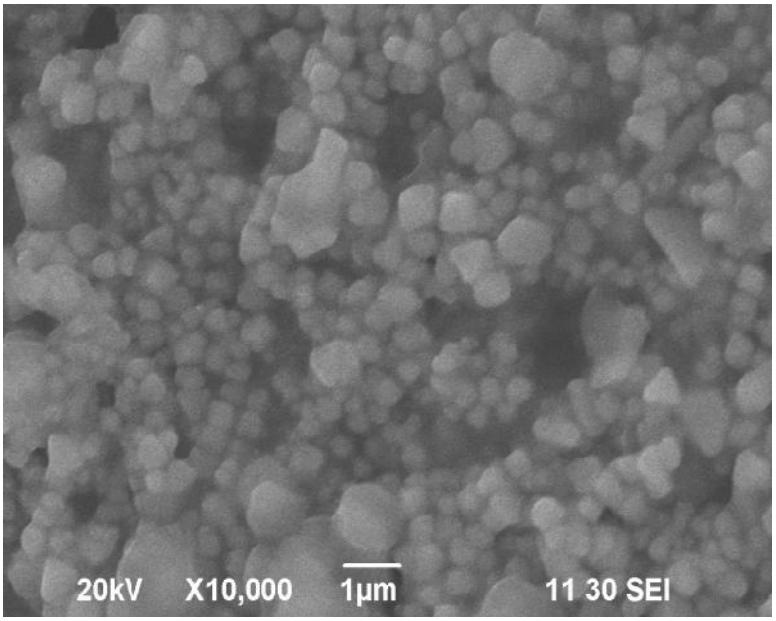


Fig 3: SEM Image of gold nanoparticles.

Table 1: Determination of zone of inhibition of gold nanoparticles

Pathogens	Tetracycline antibiotic (mm)	Gold nanoparticles coated with Tetracycline antibiotic (mm)
Escherichia coli	27	31
Enterobacter faecalis	31	34
Klebsiella pneumonia	24	27
Staphylococcus aureus	16	20

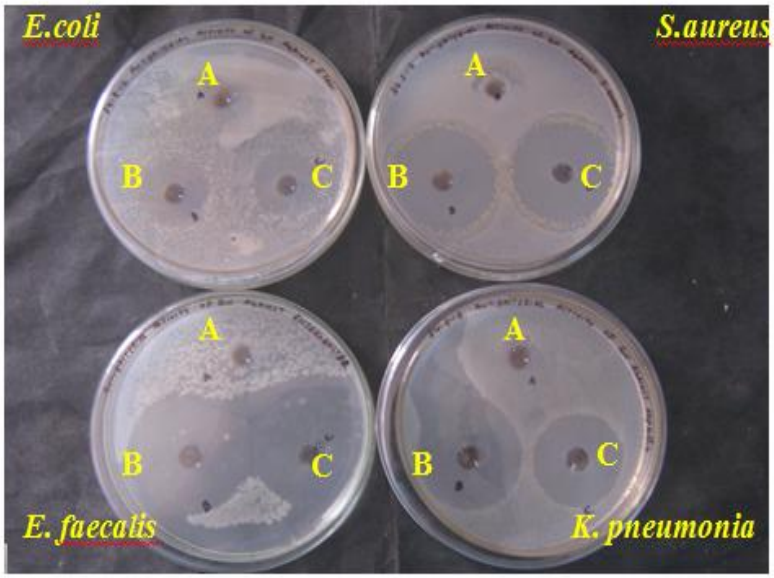
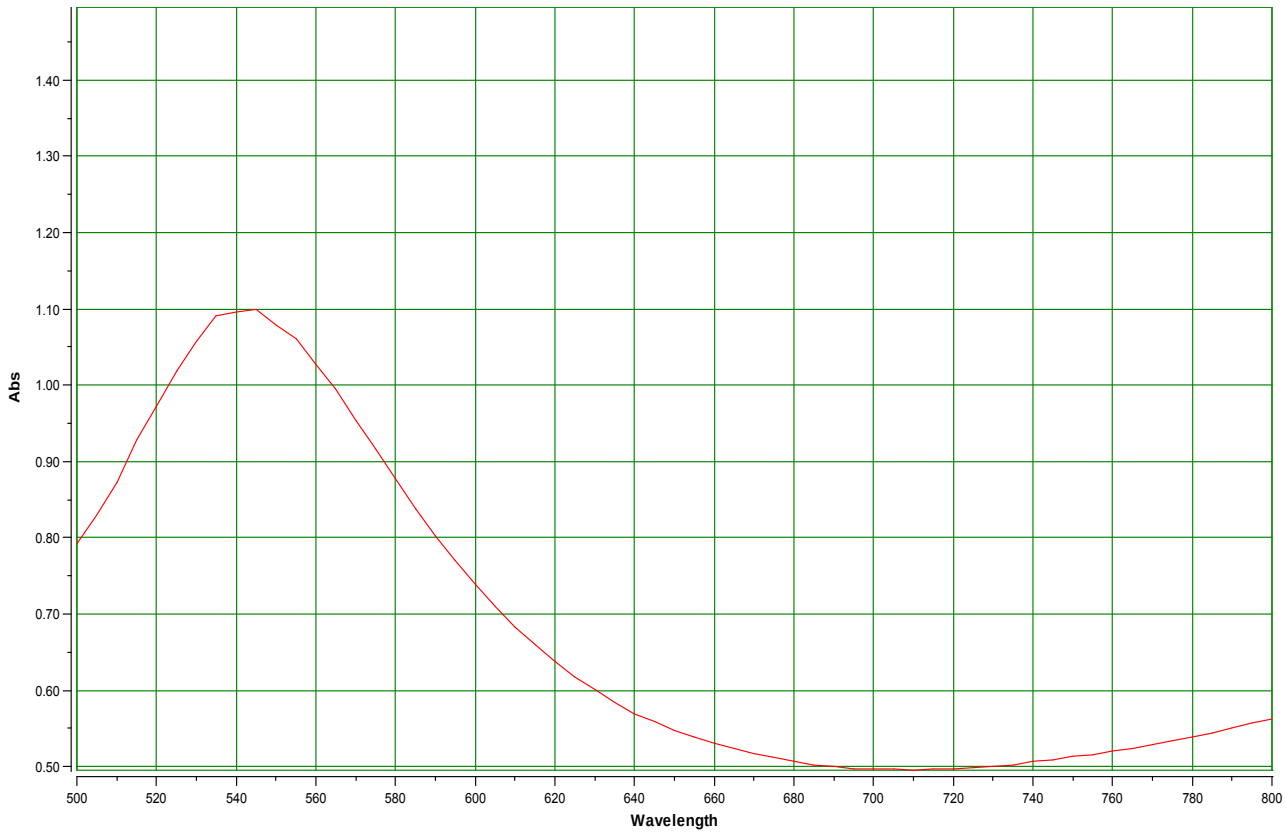
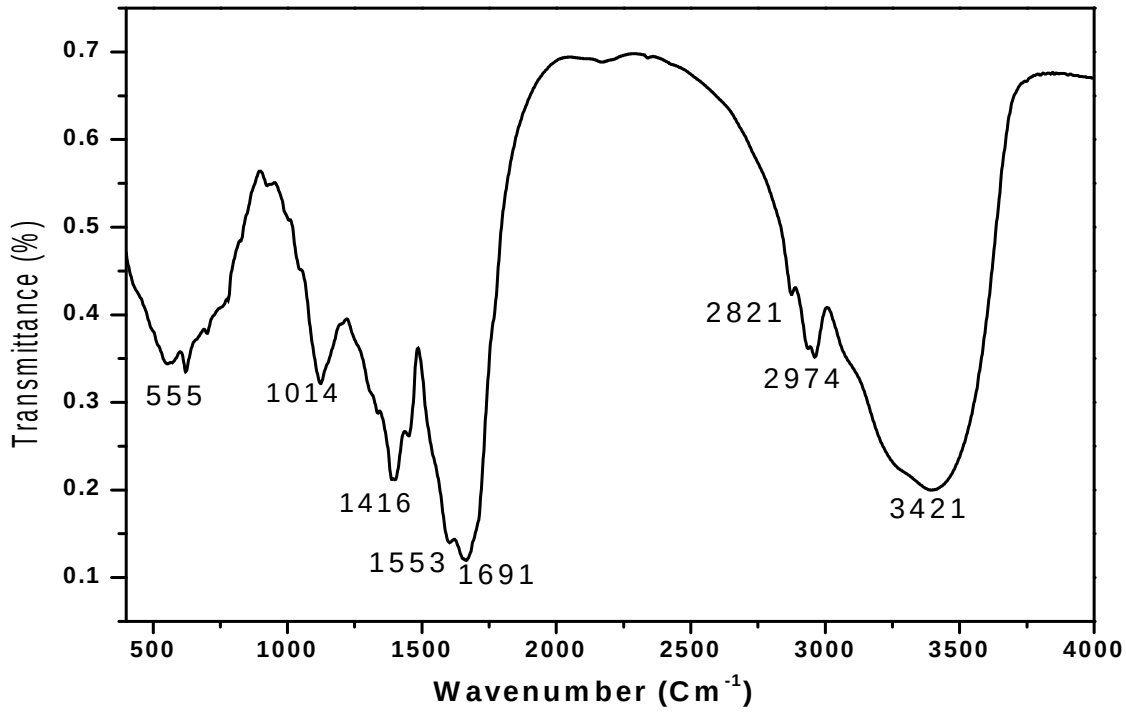


Fig 4: Antibacterial activity of Gold Nanoparticles

A- Gold Nanoparticles
B- Gold Nanoparticles coated with Tetracycline antibiotic
C- Tetracycline antibiotic



Graph: 1. UV-vis absorption spectra of gold nanoparticles.



Graph: 2. FTIR spectra of gold nanoparticles.

CONCLUSION

The rapid biogenic synthesis of nanomaterials is an important aspect of current nanotechnology research. In the present investigation, a facile, environmentally benevolent biogenic method is used for synthesis of Gold Nanoparticle by using the leaf extract of *Tephrosia purpurea.* Initially the synthesis of gold nanoparticles was confirmed by color changes reddish colloidal dispersion and Characterized by UV-visible spectroscopy. The UV-visible spectra showed a broad peak located at 540nm for synthesis of gold nanoparticles. FT-IR spectrum were carried out to recognize the possible biomolecules accountable for the reduction of the Au+ ions and capping of the bio reduced gold nanoparticles synthesized by *Tephrosia purpurea* leaf extract. The surface, spherical and uniform arrangement of gold nanoparticles was confirmed by SEM analysis. Gold nanoparticles are harmful to bacteria and fungi. They bind closely to the surface of the microorganisms causing visible damage to the cells with complete destruction of flagella. The results verified that Tetracycline antibiotic coated with gold nanoparticles had more hindrance activities than the pure Tetracycline antibiotic. They were more effective against human pathogens such as *Escherichia coli, Enterobacter faecalis, Staphylococcus aureus* and *Klebsiella pneumonia* due to the peptidogycan layer in the cell wall. Hence, we conclude that the synthesized gold nanoparticles are more efficient in the drug delivery process. From a technological point of view, these obtained gold nanoparticles have potential applications in the biomedical field and this simple and biogenic procedure has several advantages such as cost-effectiveness, compatibility for medical and pharmaceutical applications as well as large scale commercial production.

ACKNOWLEDGEMENTS

Authors gratefully acknowledge the Udaya School of Engineering and Department of Biotechnology for their guidance, support and encouragement to complete this investigation. Authors wishes to thank Bharathiyar University and Karunya University for helping in analysis of FTIR and SEM analysis.

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