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Green Synthesis of Silver Nanoparticles using Tephrosia purpurea Root Extract, Morinda tinctoria Leaf Extracts and Evaluation of their Antibacterial Activities

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ABSTRACT

The importance of Tephrosia purpurea root and Morinda tinctoria leaf as revealed by various literature resources, we planned to carry out green synthesis of Silver nanoparticles using the above two extracts. Silver nanoparticles were prepared by adopting standard procedure. The formations of Silver nanoparticles from the extracts were identified first by observing the colour changes. The extract colour changes during the formation of Silver nanoparticles from brown to intense yellow for Tephrosia purpurea root extract and black to brown for Morinda tinctoria leaf extract. Silver nanoparticle formations were characterized by UV, FT-IR, XRD and SEM. UV absorbance at 440nm and at 460nm for the Silver nanoparticles derived from the above two extracts. FT-IR stretching frequencies of these nanoparticles observed at 439-516 and at 604 respectively. XRD and SEM analysis of silver nanoparticles indicated that they exist in spherical, face centered cubic (fcc) crystalline structure with size range 21nm and 26nm for the above two extracts. Invitro bioactivity determination of the tephrosia purpurea root, morinda tinctoria leaf extracts and their Silver nanoparticles by zone of inhibition studies showed that Silver nanoparticles are highly active against Staphylococcus aureus(16,13)mm and Pseudomonas aeruginosa(11,12)mm of than their corresponding extracts.

Keywords: Tephrosia purpurea, Morinda tinctoria, Silver nanoparticles, XRD, SEM.

INTRODUCTION

The application of "green" chemistry rules to nanoscience and nanotechnology is very important in the preparation of various nanomaterials[1].Metallic nanoparticles prepared from noble metals such as Gold, Silver, Platinum and Lead exhibits antibacterial properties [2].Among the noble metals, silver (Ag) is the metal of choice in the application field of catalysts, staining pigments, solar cells surface coatings, photonics, biological sensing, electronics and surface-enhanced Raman scattering detection biological systems, living organisms and medicine[3].Silver nanoparticles are used in bone cements that are used as artificial joint replacements[4].

Tephrosia purpurea (Linn.) Pers., belong to the family Fabaceae[5].The roots are useful in inflammation, skin diseases, scrofula, elephantiasiss, dyspepsia, stomachalgia, flatulene, haemorrhoids, asthma,

bronchitis, anaemia, hepatossplenomegaly, verminosis, strangury, dysmenorrhoea, chronic fever, pimples, odontalegia & gingivitis.Various Studies reported on Tephrosia purpurea root extract were CNS depressant [6], analegesic activities[7], Antiulcer activity[8], Antimicrobial activity[9], Antioxidant activity[10]. Wound Healing activity [11], Anticarcinogenic and antilipidperoxidative effects[12].
Morinda is a genus of flowering plants in the madder family, rubiaceae[13].Morinda tinctoria has been reported to possess antithrombotic [14], antioxidant[15], analgesic, anti-inflammatory [16] ,blood pressure lowering and vasodilatory properties[17].

In our present study, we have demonstrated a suitable green method for the synthesis of silver nanoparticles using T*ephrosia purpurea root extract and Morinda tinctoria leaf* extract as reducing agent. The antibacterial activity of silver nanoparticles has been tested against various pathogens.

MATERIALS AND METHODS

Materials: The root of *tephrosia purpurea* were collected from Kovilpatti and fresh leaf of morinda tinctoria were collected from Sivakasi areas of Virdhunagar district,Tamil Nadu.Silver nitrate of Merck grade was used.

Methods:
Preparation of the root extract: Tephrosia purpurea root were washed several times with water to remove the dust particles and then aerial dried to remove the residual moisture. The T.purpurea root **extract** used for the reduction of silver ions (Ag^+) to silver nanoparticles (Ag^0) was prepared by placing 50g of washed dried roots in 250ml round bottom flask along with 200ml of distilled water. The mixture was then boiled for 2 hours until the color of the aqueous solution changes to intense yellow. Then the extract was cooled to room temperature and filtered with Whatman No.1 filter paper. The aqueous root extract was used as a reducing agent for further nanoparticle synthesis. These extracts can be stored at 4 °C for one week.

Synthesis of Silver Nanoparticles from root extract: 5mM aqueous solution of Silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 10ml of **Tephrosia purpurea root extract** and was added into 90 ml of aqueous solution of 5mM Silver nitrate for reduction to Ag+ ions and kept at room temperature for 6 hours. As a result, a intense yellow solution was formed, indicating the formation of silver nanoparticles and it was further confirmed by UV-Vis spectrum analysis.

Fig. 1A

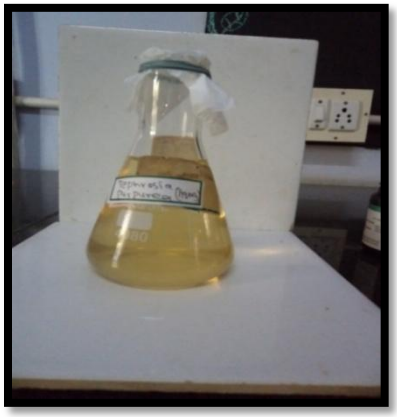

Fig. 1B

Photographs showing A) Pure T.Purpurea root Extract B) Colour changes after adding root Extract with $AgNO_3$ solution.

Another 10ml of **Morinda tinctoria leaf** extract was added into 90 ml of aqueous solution of 5mM Silver nitrate for reduction into Ag+ ions and kept at room temperature for 6 hours. As a result, a dark brown

solution was formed; indicating the formation of silver nanoparticles and it was further confirmed by UV-Vis spectrum analysis

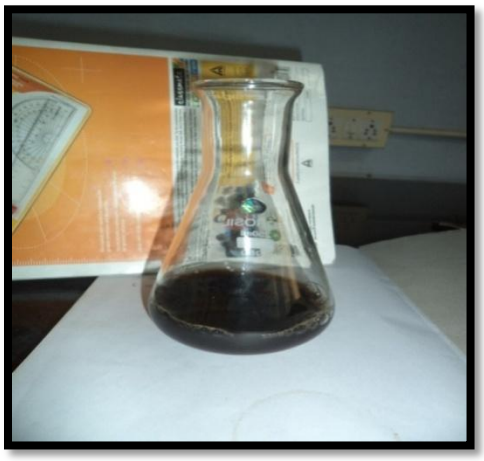

Fig. 2A

Fig. 2B

Photographs showing A) Pure Morinda tinctoria leaf Extract
B) Colour changes after adding Leaf Extract with AgNO3 solution.

Separation of silver nanoparticles: The synthesized AgNP's was separated by means of centrifugation (Spectrofuge 7M) at 10,000 rpm for 30 mins. The pellets was redispersed and again centrifuged for 30 mins. The supernatant solution thus obtained was stored at -4℃.

Characterization of AgNPs: Characterisation of silver nanoparticles was first carried out using UV-visible absorption spectrophotometer 2400PC with a resolution of 1 nm between 300 and 900 nm possessing a scanning speed of 300 nm/min. FT-IR measurements were carried out on a Shimadzu FT-IR 8400S Model and the spectra was scanned in the range of 4000-400cm^{-1}range at a resolution of 4 cm^{-1}. The sample were prepared by dispersing the AgNPs uniformly in a matrix of dry KBr, compressed to form an almost transparent disc. KBr was used as a standard analyze the samples. The particle size and nature of the AgNPs were determined using XRD PW3050/60 X-pert PRO operating at a voltage of 45 kV, a current of 40 mA with Cu K α radiation at 2θ angle ranging from 10^0 to 80^0. A thin film of the silver nanoparticle was made by dipping a glass plate in a solution and carried out for X-ray diffraction studies. SEM analysis was done by using a JSM 6701F – 6701Model.

Antibacterial Assay: Several loopful of microorganisms were inoculated into 5 ml of sterilized peptone water and the turbidity was compared and adjusted with 0.5 McFarland Nephalometric standard (Baron and Finegold, 1990) [18]. Mueller Hinton Agar surface was inoculated and uniformly spread by using a swab impregnated with standardized inoculum. After 15 minutes of inoculation, wells (6 mm diameter) were made by well puncture and the wells were filled with 50 ml of each different extract and AgNP's.

RESULTS AND DISCUSSION

UV-Vis spectral analysis: Green synthesis of silver nanoparticles using Tephrosia purpurea root and Morinda tinctoria leaf extracts were confirmed by UV-Visible Spectroscopy, Fourier Transform Infrared Spectroscopy, X-ray Diffraction and Scanning Electron Microscopy studies.The formation of silver nanoparticles can be observed by the change in the color of the solution from brown to intense yellow color for Tephrosia purpurea root extract and black to brown for Morinda tinctoria leaf extract after six hours of incubation. Color of silver colloid is attributed to surface Plasma resonance (SPR) arising due to the collective oscillation of free conduction electrons induced by an interacting electromagnetic field. **Fig.**

3 & 4 showed that the UV absorption spectra of the silver. Surface Plasmon Resonance bands of the colloids are centered at 440 nm for T.purpurea and M.tinctoria for 460nm. The bands are broad and the intensity increases, indicated the formation of silver nanoparticles.

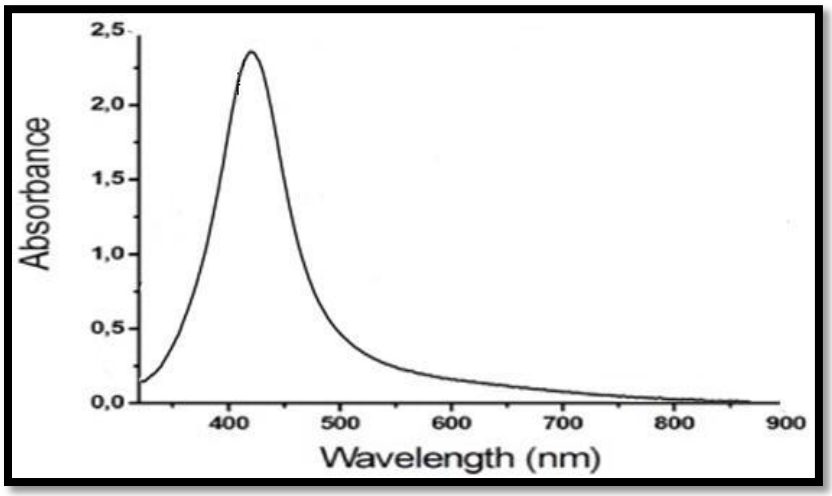

Fig. 3 UV-Visible Spectra of Silver nanoparticles synthesized using T.purpurea root extract

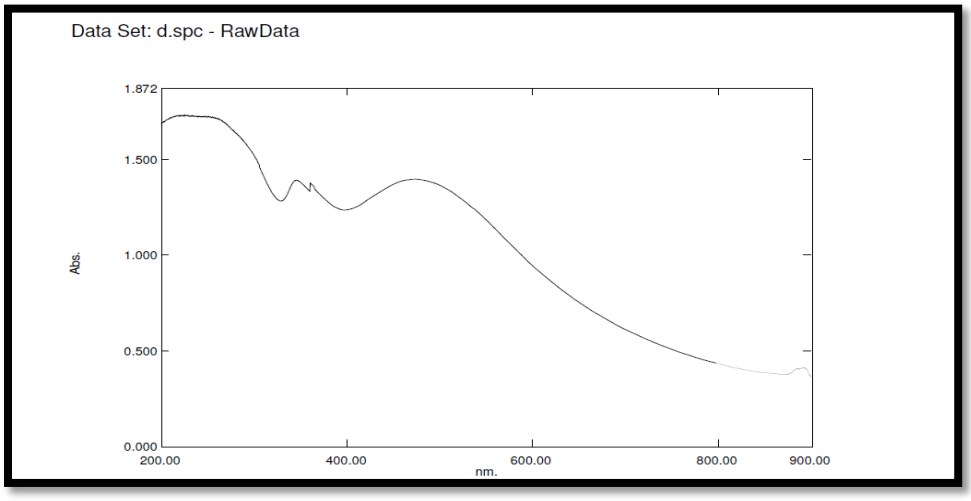

Fig. 4 UV-Visible Spectra of Silver nanoparticles synthesized using M.tinctoria leaf extract

FT-IR spectroscopy: The FT-IR spectra of T.purpurea root extract,showed a broad band at 3402 cm^{-1} bonded O-H and at 1604 cm^{-1} band indicated bonded carbonyl. The FT-IR spectra of silver nano particles at 3448cm^{-1}(V.W) indicated the bonded O-H in the extract broken and involved in binding with silver nano particles. The formation of strong peak at 1735cm^{-1}indicated that during the formation of silver nanoparticles the carbonyl group which was found to be weakly bonded in the extract ,was found to be freed during the formation of silver nanoparticles. Formation of the silver nano particles was confirmed by the appearance of stretching band at 439 and 516cm^{-1} corresponds to (M-O) stretching vibrations **Fig. 5A & B.**

Fig. 6A & B shows a strong absorption peak at 3296 cm^{-1} indicates presence of carboxylic groups. The broad absorption band was observed between 3436- 3220 cm^{-1} due to the O-H stretching and H- bonded

alcohols and phenol groups .A weak band was observed at 1634 cm^{-1} corresponding to N-H bending primary amines. It was modified into 1672 cm^{-1} indicates presence of C=O stretching vibrations of carbonyls groups, respectively. The weak bands at 724 cm^{-1} were disappeared in the synthesized silver nanoparticles. A small peak was formed at 604 cm^{-1} due to the occurrence of alkyl halides. Moreover, the functional biomolecules are hydroxyl, carboxylic, phenol and amine groups in *M. tinctoria* leaf extract involved in the reduction of silver ions which was confirmed by FTIR Spectrum.

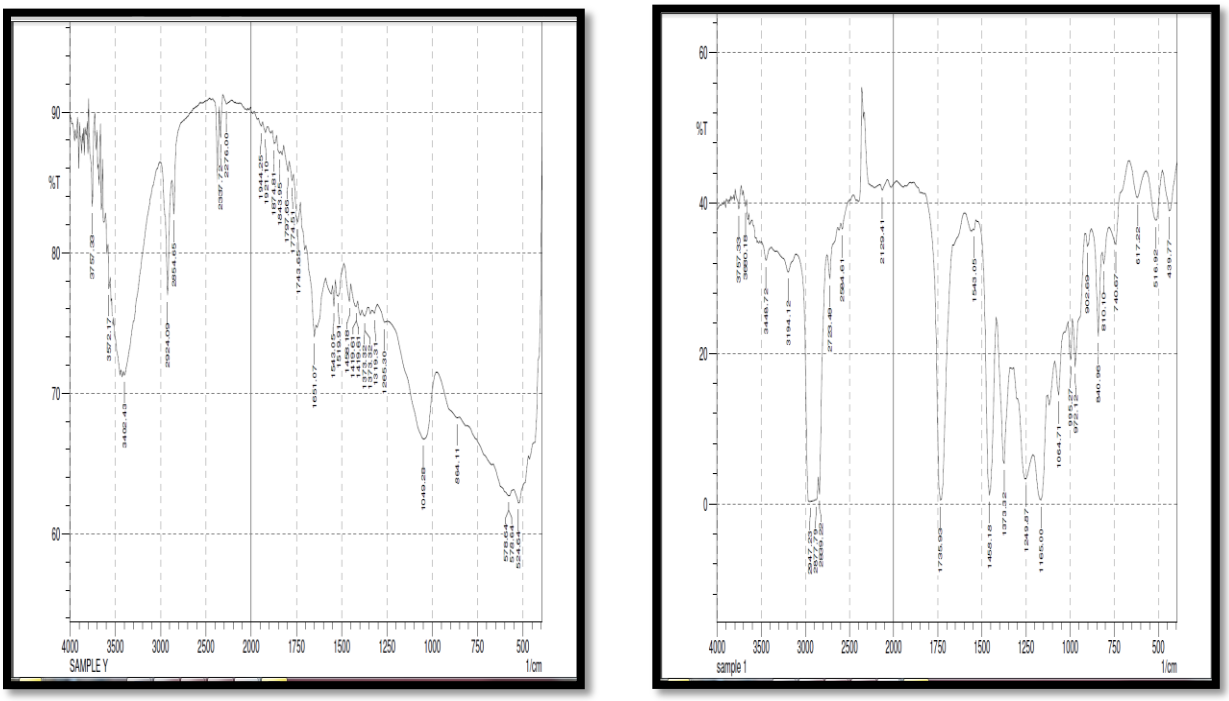

Fig. 5A & B FT-IR Spectrum of T.purpurea root extract mediated silver nanoparticles

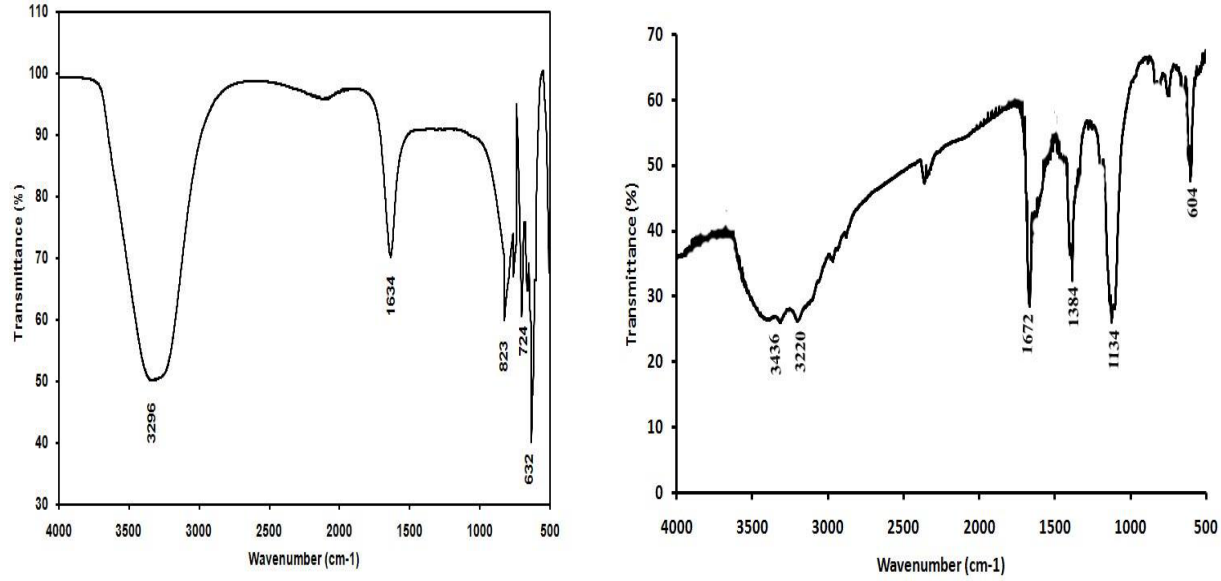

Fig. 6A &B FT-IR Spectrum of M.tinctoria leaf extract mediated silver nanoparticles

XRD analysis: The biosynthesized silver nanostructure was further confirmed by the characteristic peaks observed in the XRD pattern. The particle size and nature of the AgNPs were determined using XRD PW3050/60 X-pert PRO operating at a voltage of 45 kV, a current of 40 mA with Cu K α radiation at 2θ angle ranging from 10^0 to 80^0. A thin film of the silver nanoparticle was made by dipping a glass plate in a solution and carried out for X-ray diffraction studies. The XRD spectrum of silver nanoparticles was given in **Fig. 7& 8**. All diffraction peaks correspond to the characteristic face centered cubic (FCC) silver lines. These diffraction lines observed at 2θ angle 32.55,37.76,54.48 and 64.92 respectively, have been indexed as (111), (200), (220) and (311) planes respectively(JCPDS 01-076-1393) for T.purpurea root and M.tinctoria leaf containing four main characteristic diffraction peaks for Ag were observed at 2θ angle 38.11,44.27,77.47 and 81.53 which correspond to the (110), (200), (311) and (222) crystallographic planes of face-centered cubic (fcc) Ag crystals, respectively (JCPDS 00-004-0783). The typical XRD pattern revealed that the sample contains a cubic structure of silver nanoparticles. The average particle size of silver nanoparticles formed in the process was estimated from Debye-Scherrer's equation (d = (kλ×180)/ β Cos θβ Π) by determining the width of the (111) Bragg's reflection .The average size of silver nanoparticles was found to be 21nm and 26nm corresponding to T.purpurea and M.tinctoria respectively.

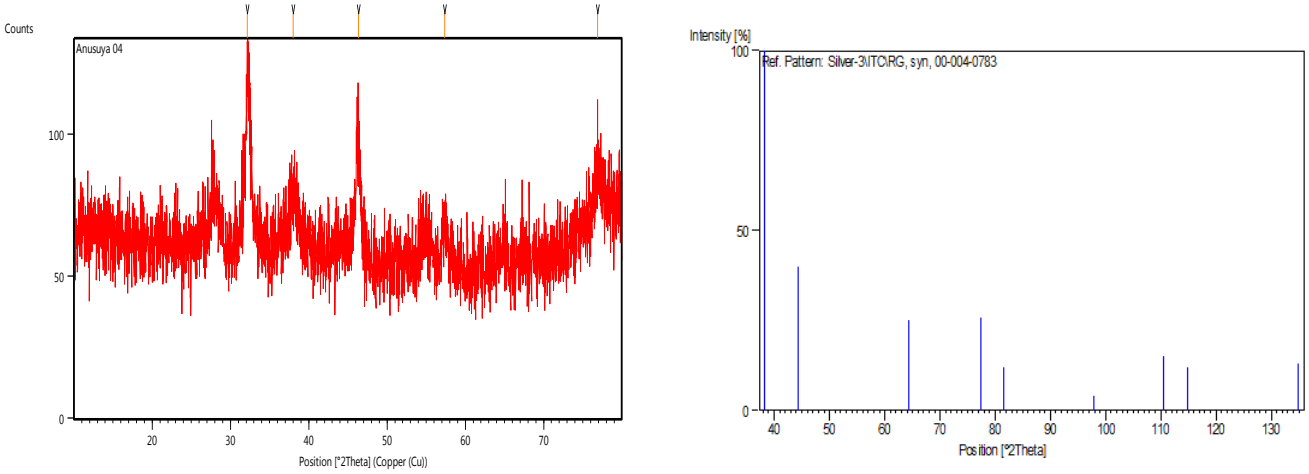

Fig. 7 XRD Spectrum of Silver anoparticles using T.purpurea root extract

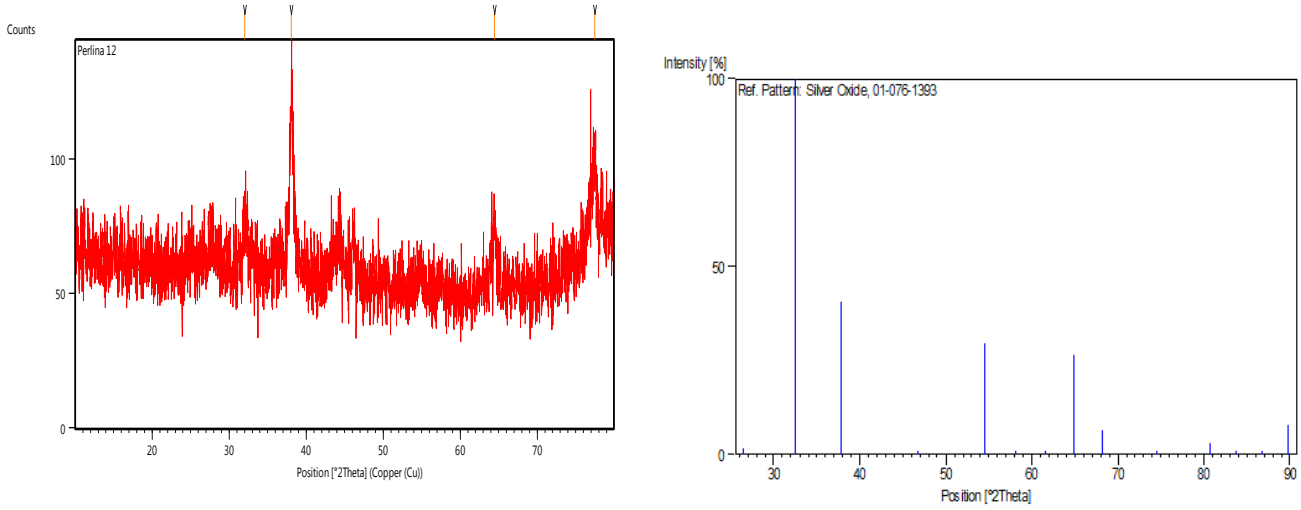

Fig. 8 XRD Spectrum of Silver nanoparticles using M.tinctoria leaf extracts

SEM analysis: Scanning electron microscopy analysis was carried out to understand the morphology and the size of the silver nanoparticles. The result showed that the synthesised silver nanoparticles exist polydisperse spherical in shape and in the range 10 to 80 nm with average size of 46 nm and 40nm corresponding to T.purpurea(**Fig. 9A)** and M.tinctoria**(Fig. 9B)** respectively. SEM image using T.purpurea root & M.tinctoria leaf extracts proved the formation of silver nanoparticles in a greener way.

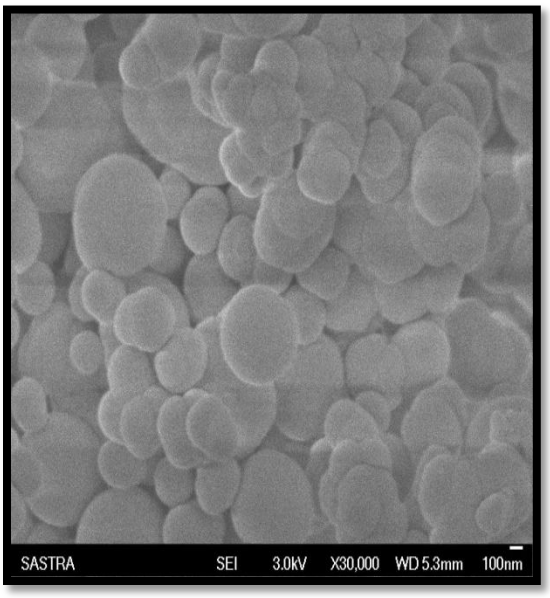

Fig. 9A

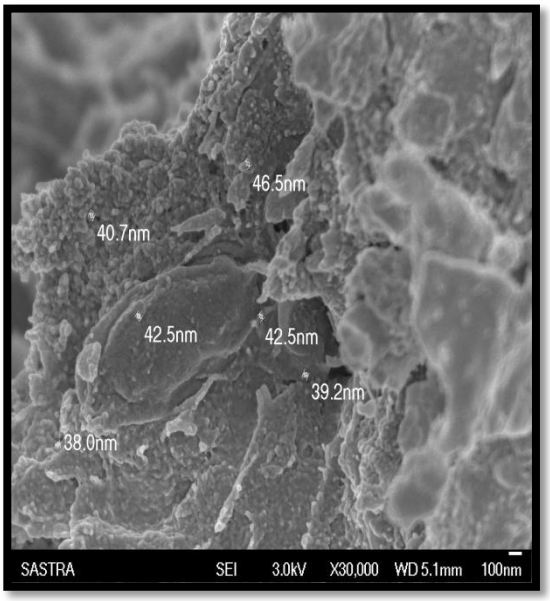

Fig. 9B

Fig. 9A &B SEM Spectrum of Silver nanoparticles using T.purpurea root & M.tinctoria leaf extract

Antimicrobial studies: In vitro antimicrobial activity of silver nanoparticle synthesized using T.purpurea root and M.tinctoria leaf extract was tested against the microorganisms like *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* by well puncture method . On comparing the T.purpurea root extract and M.tinctoria leaf extract and their Silver nanoparticles by zone of inhibition studies proved that Silver nanoparticles are highly active against Staphylococcus aureus and Pseudomonas aeruginosa with the zone of inhibition in 16&11mm and 13&12mm for both the particles.

Table. 1 Antibacterial activities of silver nanoparticles using T.purpurea root & M.tinctoria leaf Extracts

Microorganism	Tephrosia purpurea		Morinda tinctoria	
	Zone of inhibition			
	Root Extract	Ag nanoparticle	Leaf Extract	Ag nanoparticle
Escherichia coli	11	9	-	10
Staphylococcus aureus	11	16	-	13
Pseudomonas aeruginosa	-	11	-	12
Bacillus subtilis	10	12	-	10

CONCLUSIONS

Green synthesis of Silver nanoparticles using Tephrosia purpurea root extract and Morinda tinctoria leaf extract by adopting standard procedure were characterized by UV–vis, FT-IR, XRD and SEM studies. Due to surface plasmon resonance during the reaction with the ingredients present in the flower extracts Color changes which result in the formation of silver nanoparticles. The typical XRD pattern revealed that the average size of silver nanoparticles was found to be 21nm and 26nm corresponding to T.purpurea root and M.tinctoria leaf extract mediated AgNPs respectively and exist in face-centered cubic (fcc) Ag crystals. SEM anaysis showed that the synthesised silver nanoparticles existed in polydisperse spherical shape and in the range of 10 to 80 nm with average size of 46 nm and 40nm corresponding to T.purpurea and M.tinctoria respectively. In vitro antimicrobial activity of silver nanoparticle showed that Silver nanoparticles are highly active against Staphylococcus aureus and Pseudomonas aeruginosa with the zone of inhibition in 16&11mm and 13&12mm than their mediated extracts.

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