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Green-synthesis of silver nanoparticles using Abies webbiana LEAVES and evaluation of its antibacterial activity

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Abstract
A present study reveals the efficiency of Abies Webbiana for green synthesis of silver nanoparticles (AgNPs). The synthesised silver nanomaterials were characterized by using UV-visible, FTIR, PXRD, FESEM & EDX.PXRD studies confirmed the nano size of the particles. FESEM studies showed the structure of the nanoparticles that is no other elements were present in the bio-synthesised sample. The antibacterial activity of the nanoparticles was examined against gram positive bacteria (staphylococcus aureus), gram negative bacteria (Escherichia coli & pseudomonas aeruginosa), respectively. The bio-synthesized AgNPs were great interest due to their eco-friendliness, economic prospect and feasibility and short time for synthesis may be wide range of applications in nanomedicine, catalysis medicine mainly for the pharmaceutical industry for development of few formulations against the microbial strains which are developing resistance to traditional antibiotics.

Keywords: Abies Webbiana, Silver nanoparticles, UV-Vis, FTIR, PXRD, FESEM, Antibacterial Activity.

1. Introduction
Nanoparticles are one dimensional particles, having size between 1 to 100 nm. They exhibit unique properties because of their size, distribution and morphology. Which leads to several developments in the field of nanotechnology. Silver is a metal, which are discovered more than 2000 years ago is well known for its medicinal properties [1]. Early Researches found that silver based compounds have wide variety of antimicrobial applications and it was highly toxis for micro organisms but when silver nanoparticles are exposed to the surface area, different types of microbes increases considerably [2]. Abies webbiana is a medicinal plant which is an extract, ever green perennial shrub. It is used to prepare ayurvedic formulated drugs because of aphrodisiac, rejuvenating, antiflammatory, anti tumor properties [3]. The genus Abies belongs to the division Tracheophyto, class pinopsida, order pinales and family Pinaceae [4-7]. The studies reveals that the leaves of Abies Webbiana (Talisapatra) are anti oxidant, immune modulatory and haematopoietic in nature [8, 9]. The chemical copounds like phytochemicals are biological active and can provide health benefits for humans than those attributed to macronutrients and micronutrients [10, 11]. In present study Abies webbiana leaf extract is used to synthesize silver nanoparticles and their potential is demonstrated in absorption of infrared rays. First these green nanoparticles were examined under UV-vis, FTIR, PXRD, FESEM, EDX and their shape, size were determined.

2. Material and Methods
2.1 Plant Material Sourse
Abies Webbiana (Fig 1) collected from Trichy district in Tamilnadu state India.

2.2 Preparation of leaf extract
The Abies Webbiana leaf were gathered and washed double distilled water to remove dust particles. The leaf were dried and grained with mortor. The leaf extract sample was mingled with 100 ml of double distilled water and kept at 60 ˚C in heating mantle. Using whatmann No 1 filter paper, the sample was filtered. Then the final extract of leaf was stored.

2.3 Synthesized silver nanoparticles
In a typical Synthesis of AgNPs using the decoction of Abies Webbiana leaves extract as reducing agent at room temperature.The collected leaf extract were washed double distilled water to remove dust particles. The leaf extract were dried in shade to remove the moisture content and powdered in a blender. About 10g of Abies webbiana sample was added to 100ml

of distilled water and heated to 60 ◦C with continuous stirring time for 1hr. The mixture was cooled to obtain temperature, filtered with whatmann no 1 filter paper and then centrifuged at 8000rpm for 10mins. The supernatant solution obtained after centrifugation was further used as the abies webbiana leaf extract throughout 5ml of the extract was added to 45ml of $AgNO_3$ solution for bio-reduction process at 50◦C. The synthesized silver nanoparticles were then kept for further characterization by UV-vis, FTIR, PXRD, FESEM and EDX analysis.In Abies Webbiana leaf its dark brown colour is formed which indicate the formation of silver nanoparticles. The colour change is shown in below (Fig.2).

3. Measurements

3.1. Uv-Vis Spectroscopy

Ultraviolet-visiblespectroscopy (Perkin-Elmer Lambda) or ultraviolet-visible spectrophotometer (UV-Vis) refers to absorption spectroscopy in the UV-Visible spectral region.

3.2. Fourier Transmission Infra-Red Spectroscopy (FTIR)

The FTIR spectrum of Abies Webbiana extract, silver nanoparticles and amine functionalized silver nanoparticles were recorded using were obtained using a RX 1000 PERKIN ELMER typeFTIR spectrophotometer.

3.3. Powder X-Ray diffraction(PXRD)

The silver nanoparticles solution was centrifuged at 2000 g for 15 min. The pellet was washed three or four times with 25 mL of deionized water. The structure of prepared silver nanoparticles has investigated and powdered XRD patterns of the silver nitrate were recorded by X PETRO PRO – X-ray diffractometer.

3.4 Fesem and Edx

Field emission scanning electron microscopy (FESEM) has used to visualize the as-synthesized nanoparticles. On a glass slide having 10 mm by 10 mm dimensions, one drop of purified nanoparticles solution was dried for 24 hours. The film which forms on the glass surface was made use of to examine the morphology of the nanoparticles using the model were performed on FEI QUANTA-250 FEG equipped with an EDX instrument.

3.5 Anti-bacterial activity

Anti-bacterial activity of Abies webbiana leaf extract and silver nitrate solution was determined by discdiffusion method. The testing moicro-organisms are three bacterial strains.one strains of gram positive (Staphylococcus aureus) and two strains of gram negative (Escherichia Coli & Pseudonas aeruginosa).The plates were then incubated for 24 hours at 37 °C.

4. Result and Discussion

4.1 Uv – Visible Analysis

Uv -vis spectrum of colloidal silver NPs for different time periods (or) intervals of $AgNO_3$ (1hr, 2hrs). The respective Surface Plasmon Resonance (SPR) peak is observed at 438 respectively for 1hr of 1mm $AgNO_3$ concentration (Fig.3). Which results with formation of AgNPs [12, 13]. During the synthesis, the colour of suspention has changed from brown to dark brown which shows the formation of nano sized silver metals.

4.2 Fourier Transmission Infra Red Spectroscopy (FTIR)

Generally, Fourier transform infrared spectroscopy (FT-IR) is employed as a special tool to study the structural information. In our study, it was used to identify the possible bio-molecules capping on the surface of silver nanoparticles. The FT-IR spectra for the as-prepared silver nanoparticles are depicted in (Fig.4). An absorption peak that appears at 3421 cm^{-1} is attributed to the stretching vibrations of the hydroxyl group (-OH). The IR peaks located at 2927 cm^{-1} and 2867 cm^{-1} are correspond to the bending vibration of C-H group.The peak in 2427 cm^{-1} can be allocated to the stretching vibration of the carboxyl group (-OH). The peak located at 1629 cm^{-1} is attributed to the propyl stretching vibration. The peak appeared at1576 and 1364 cm^{-1} are related to C-C bending vibration and C-O-C stretching vibration. The peak in 1072 cm^{-1} can be allocated to the stretching vibration of the C-OH bond. The FT-IR analysis indicated the existence of molecules on the surface of silver nanoparticles.

4.3 Powder X-Ray diffraction (PXRD)

PXRD analysis showed five distinct diffraction peaks at 38.2, 44.5, 64.7, 77.5, 81.8 and can be indexed the angle values of (111), (200), (220), (311) and (222) respectively, confirming that the samples were metallic silver nanoparticles with a face-centered cubic (FCC) crystal structure (JCPDS NO.04-0873). The high peaks in the analysis indicate the active silver composition with the indexing (Fig 5). Thus the PXRD confirms the crystalline nature of the silver nanoparticles and from the angle value it is clear that the compound is stable.

4.4 FESEM And EDX Analysis

In order to study the morphology, size and elemental composition of the green synthesized silver nano particles FESEM and EDX was used (Fig.6) shows the FESEM image of the as-synthesized silver nanoparticles. It is clear that the silver nanoparticles are homogeneous and relatively spherical particles. The elemental composition of green prepared silver nanoparticles was confirmed by EDX (Fig.7) reveals the results of EDX analysis. The strong elemental peak at around 2.5-3.5 kev is found which is in congruence with the major emission peaks specified for metallic silver, indicating the formation of silver nanoparticles. This result is consistent with the literature values [14, 15]. Along with this, small peak of oxygen and carbon were also observed because of the capping of silver nanoparticles with bio-molecules of Abies webbiana extract. Apart from these peaks, no other peak can be formation of pure silver nanoparticles.

4.5 Antibacterial Activity

Disc Diffusion Assay

The Disk Diffusion method is performed using Muller Hinter Agar (MHA), which is the best medium for routine susceptibility test. The bio-synthesis AW-AgNPs Leaf extract, $AgNO_3$ and standard were subjected to evaluate the antibacterial activity. Against three different bacterial strains.Gram positive (Staphylococcus aureus) and gram negative (Escherichia coli & pseudomonas aerugonisa) by using disk diffusion method.The fabricated AW-AgNPs were showed remarkable shown of inhibition (ZOI). When compared to the effect of AW leaf extract and $AgNO_3$ (Table 1 &Fig 8).

Fig 1: Abies Webbiana leaf

Fig 2: Colour changed at brown to dark brown

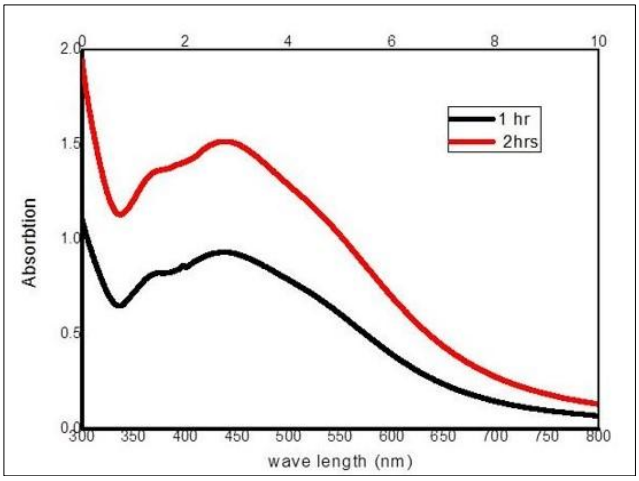

Fig 3: UV-Vis spectrum of AgNPs Using Abies Webbiana leaf extract

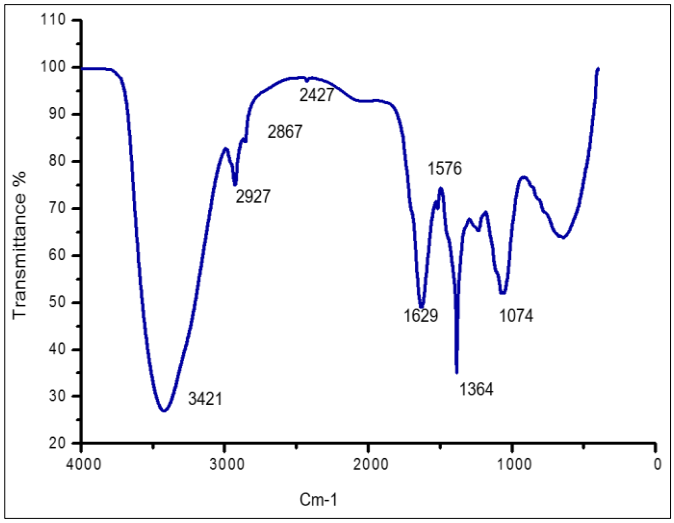

Fig 4: FT-IR spectrum of AgNPs Using Abies Webbiana leaf extract

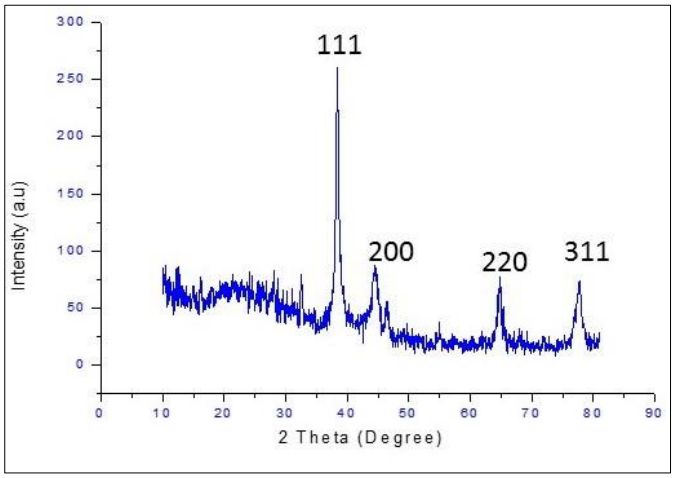

Fig 5: XRD pattern of AgNPs synthesized using supernatant of Abies Webbiana leaf

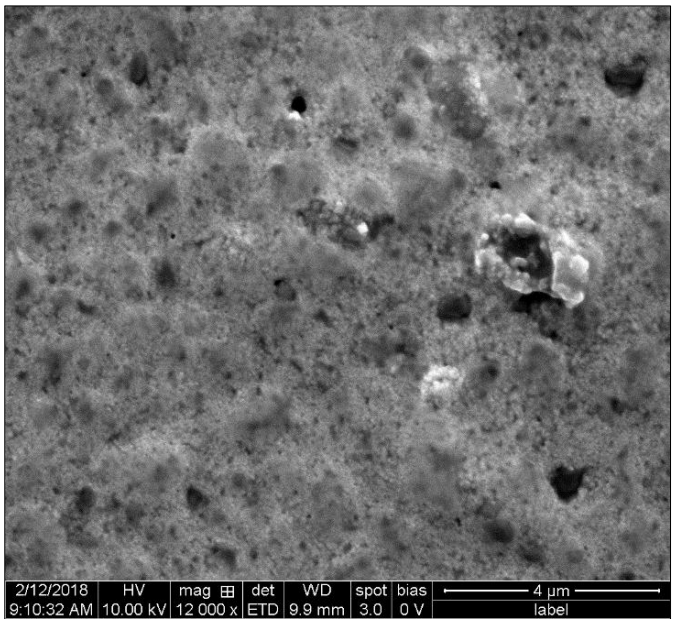

Fig 6: The typical FESEM image of the silver nanoparticle of Abies Webbiana leaf

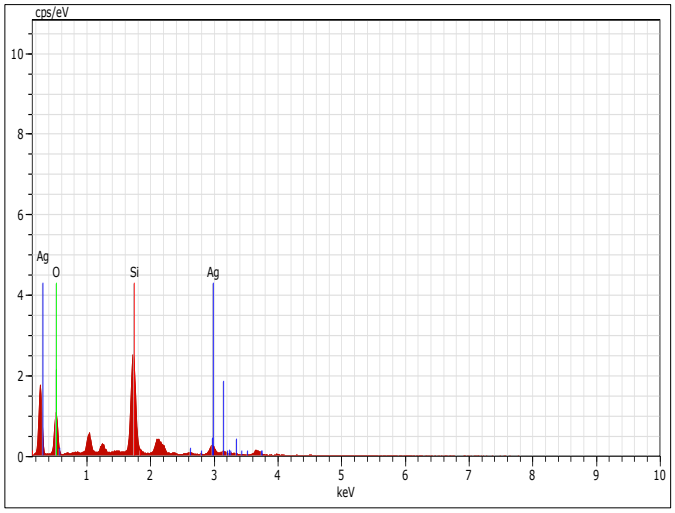

Fig 7: The EDX of silver nanoparticles of Abies Webbiana leaf

Fig 8: Anti-Bacterial Activity disc diffuce method

Table 1: Antibacterial activity of Tested compounds against Bacterial pathogens

Organism	(Diameter of zone of inhibition in mm)		
	$AgNo_3$	AgNps	AW Extract
Staphylococcus aureus	8	12	--
Escherichia coli	10	16	--
Pseudomonas aerugonisa	10	16	--

5. Conclusion

The present study demonstrates the synthesis of AgNPs by Abies Webbiana leaf extract. The formation of silver nanoparticles was observed by the change of colour from brown to dark brown. A green eco-friendly technique has been reported here (i) UV-Visible spectra has confirmed the reduction of Ag^+ ions at 438nm. (ii) FT-IR analysis conforming the presence of functional group. (iii) The PXRD analysis confirms the crystalline face centered cubic structure. (iv) The morphology and grain size of the nanoparticles range was strongly produced by Field emission Scanning Electron Microscopy (FESEM) and Energy Dispersive X-ray Spectroscopy (EDX) was confirmed the presence of absorption peak at 5kev. (v) As-synthesized AgNPs showed excellent bacterial activity against pathogenic gram positive and gram negative micro-organisms. Which can be well applied in drug delivery system. Hence, the biological approaches appears is cost efficient and eco friendly to conventional physical and chemical methods of AgNPs.

6. Reference

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