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Plant mediated bio synthesis of silver nanoparticles: Characterization and antimicrobial activity

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Abstract
The silver nanoparticles (AgNPs) have gained attraction of Chemist and Physic's since last decade and widely accepted in electronics, engineering, nanotechnology, biotechnology, medicine and chemical industries. The green synthesis of silver nanoparticles has been known for their beneficial, eco friendly and less hazardous results as compared to other conventional methods. Based on the literature survey, here we first time report the synthesis and bioactive analysis of (AgNPs) by aqueous extract of leafs of variety of medicinally important plant belonging from family *Cupressaceae*. During the study, multiple changed was observed for different part of different plants, an intense surface resonance Plasmon band in the UV-visible region at 465 nm. The further studies of formation of AgNPs were confirmed by UV, FTIR, SEM and antimicrobial activity.

Keywords: silver nano particles, *Thuja oriental* extract, green synthesis

Introduction
Today, nanotechnology is considered as one of the swiftly emergent interdisciplinary areas of science and technology that amalgamates material science and biology. In past few years, nanotechnological research is promising as cutting-edge technology interdisciplinary related to physics, chemistry, biology, material science as well as medicine. Greek word 'Nanos' refers to things of one billionth (1029 m) in size [1]. Nanotechnology has significance in the field counting electronics, biomedical sciences, pharmaceutical industry, cosmetics preparation, water filtration, as well as catalytic systems and so on. Silver nanoparticles (AgNPs) are extensively in practices as antiseptics in health care delivery [2], oxidative stress, induce DNA damage and apoptosis-related changes [3]. Today, scientists were focus on the microorganisms and plant mediated synthesis of nanoparticles. Silver nanoparticles have been successfully synthesized using various bacteria [4-6], fungi [7-9] and plants [10-12]. The first established evidence of synthesis of silver nanoparticles was in 1984 via microorganism *Pseudomonas stutzeri* AG259, a bacterial strain isolated from silver mine [13]. Plant extracts may act as both reducing as well as capping agents in synthesis of silver nanoparticles [14]. They are widely grown as ornamental trees, and extensively used for hedges. Oil of Thuja contains the terpene, thujone (naturally occurring (−)-α-thujone and (+)-β-thujone) which has been studied for its GABA (*gamma*-Aminobutyric acid) receptor antagonizing effects, with potentially lethal properties (scheme 1) [15], Cedarwood oil and cedar leaf oil, which are derived from *Thuja occidentalis*, having different properties and uses [16].

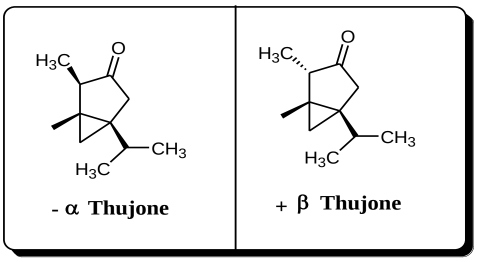

Fig 1a: (-)α Thujone and (+)β Thujone.

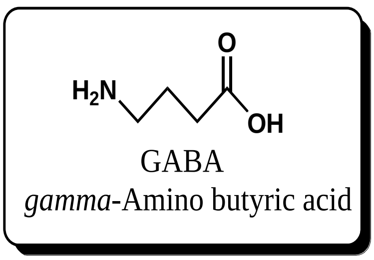

Fig 1b: *Gama* Amino butyric acid

Here, we report plant mediated synthesis of silver nanoparticles reduced by the leaf extract of *Thuja orientalis* and the particles were observed to active against bacteria *E. coli*.

Material and Methods

Preparation of Silver Nanoparticles

The synthesis course of action consists of four simple steps: (i) Preparation of leaf extract of "*Thuja orientalis*" (ii) Preparation of silver nitrate solution of 0.1M (iii) Green synthesis of AgNPs and (iv) Incubation at room temperature to allow nanoparticles formation

Preparation of *"Thuja orientalis"* leaf extract

Fresh leaves of (*Thuja orientalis*) were collected from the main campus of Jaipur National University, Jaipur and washed thoroughly under running tab water two to three times and dried in oven at 60°c, then crushed the dry leaves into fine powder using mortar Pestle. The powder was mixed with 100 ml distilled water in 250 ml conical flask and boiled for 45 minutes. The extract was then filtered through Whatman filter paper and stored at -4^{o}c.

Preparation of Silver Nitrate solutions

Silver nitrate ($AgNO_3$), a costly chemical, was purchased from local Laboratory chemical suppliers. A Silver nitrate solution was prepared by adding 1.6g of silver nitrate in 100ml deionized water in a 100ml volumetric flask. The solution were kept away from light (the containers were wrapped with carbon papers) and kept in dark.

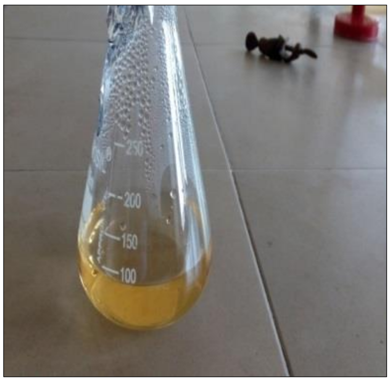

Fig 2a: leaf extract

Fig 2b: leaf extract and $AgNO_3$ Sol.

Fig 2c: Comparison of color change

Green synthesis of AgNPs

10 ml of leaf extract was mixed with 100ml of $AgNO_3$ solution. The color changes were observed from pale yellow to brown due to surface Plasmon resonance (Fig.1a and Fig.1b). After 12 hours, dissimilar change in the color of experimental sample was observed. The color of experimental sample (mixture of silver nitrate and leaf extract) turned dark-brown, (Fig 1c) then became deeper after incubation for 24 hr. The color change indicated formation of silver nanoparticles and then the sample was centrifuged by using research centrifuge (Remi) at 9000 rpm for 20 minutes at 0-4^{o}C. Thereafter, the supernatant was aspirated out from the centrifuge tube and the loose precipitate formed at the bottom was dispersed in a small volume of deionized water. The precipitate was filtered washed and air dried, brown color powder was obtained. It was used in further spectral analysis for determining various parameters like size, shape, chemical composition.

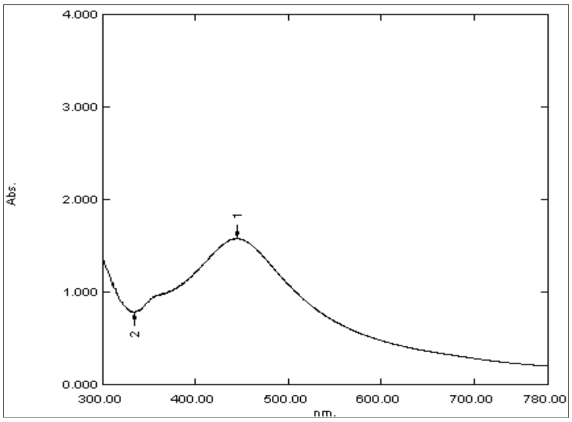

Fig 3: UV-VIS spectra of silver nanoparticles

Result and Discussion

UV–Vis Spectroscopy

UV–Vis spectroscopy is the most important technique and the simplest way to confirm the formation of nanoparticles. The formation of plant mediated silver nanoparticles was monitored by the spectral analysis. The UV spectra of the biosynthesized silver nanoparticles were recorded using an Elico SL- 159 UV Spectrophotometer by continuous scanning from 300nm to 700nm.

The aqueous leaf extract of *Thuja orientalis* was used as the standard for baseline correction. The result of UV-Vis analysis revealed that the sample with 0.1M silver nitrate concentration exhibited maximum absorbance at 434 nm, confirms the silver nanoparticles formation which is shown in figure.

FTIR Analysis

FTIR analysis was used for the characterization of the *Thuja orientalis* extract and the resulting Nanoparticles. The IR spectra of silver nanoparticles were recorded by Perkin Elmer Spectrum Version 10.4.00.

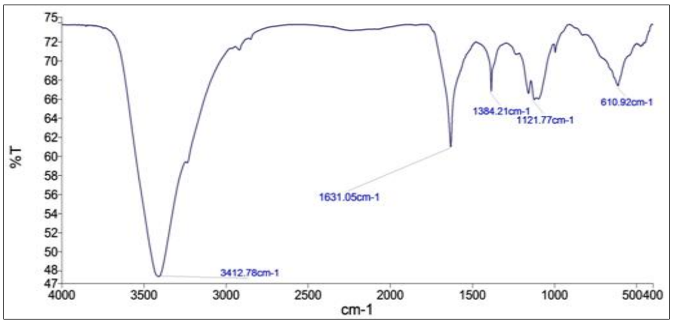

Fig 4: FTIR spectra of silver nanoparticles

FTIR absorption spectra of *Thuja orientalis* after reduction of Ag ions. The band at 3412.78 cm^{-1} corresponds to O-H stretching, H-bonded alcohols and phenols showing the behavioral characteristic absorption of hydroxyl groups. The peak at 1631.05 cm^{-1} is assigned to CH out of plane bending vibrations of substituted ethylene system. The peak at 1384.21 cm^{-1} corresponds to C-H bend of alkane. The peak observed at 610cm^{-1} for silver nanoparticles.

Scanning electron microscopy

The Fig. 4 shows the scanning electron microscopic (SEM) image for the characterization of AgNPs synthesis using leaf extract of *Thuja orientalis*, where the scale bar is 1 µm (Fig 4a) and 500 nm (Fig 4b) shows that the nano particles formed are agglomerated with each.

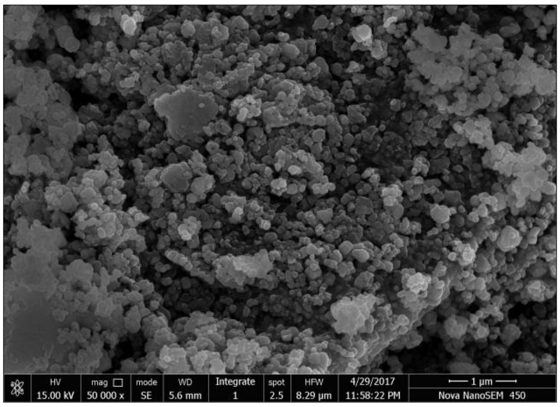

Fig 5a: SEM at 1µm

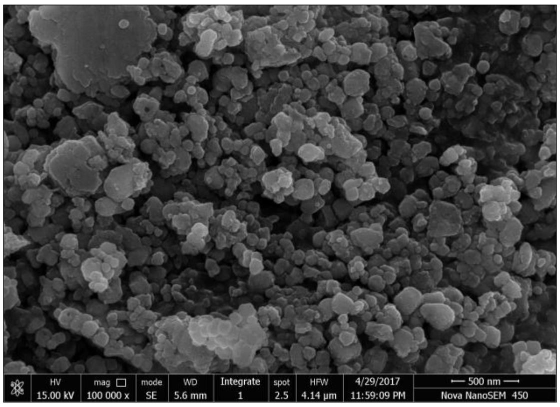

Fig 5b: SEM at 500nm

Other forming clusters. Silver nanoparticles were spherical in shape with particle size range from 5 to 40 nm. The larger size of silver nanoparticles may be due to the aggregation of the smaller ones, due to the SEM measurements.

X-Ray Diffraction (XRD) Analysis

X-ray diffraction analysis identifies the phase and crystalline nature of the silver nanoparticles. The observed and standard XRD pattern showed the face-centered cubic structure of silver nanoparticles. It is found that silver particles were formed in the form of nanocrystals, and showed the strong absorption peaks at 2θ values of 38. 32°, 44.44°, 64.58° and 77.28° corresponding to crystal planes (111), (200), (220) and (311). X-ray diffraction results undoubtedly showed that the silver nanoparticles formed by the reduction of Ag+ ions by the Thuja occidentalis leaf extract are crystalline in nature. It was found that the average size from XRD data and using the Debye-Scherrer equation was approximately 54 nm. The average particle size of silver nanoparticles synthesized can be calculated by using the Debye-Scherer equation:

$$D = K\lambda/\beta \cos\theta$$

Where D is the crystallite size of AgNPs, λ is the wavelength of the X-ray source (0.1541 nm) used in XRD, β is the full width at half maximum of the diffraction peak, K is the Scherrer constant with a value from 0.9 to 1, and θ is the Bragg angle.

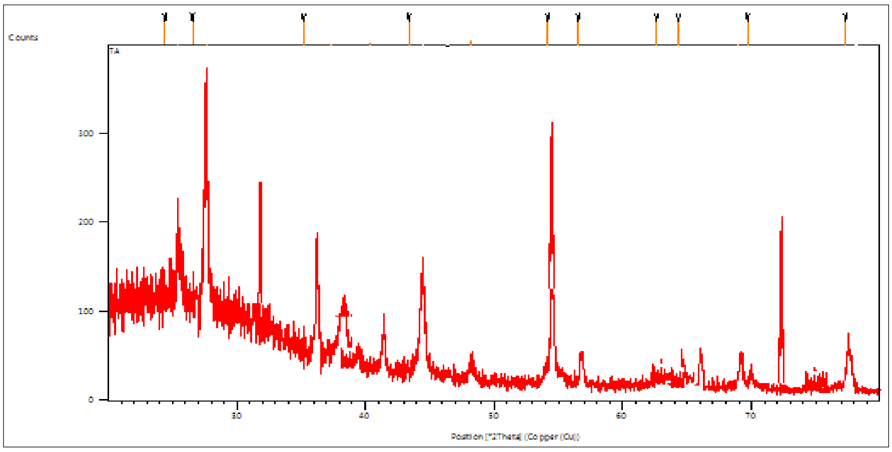

Fig 6: XRD Diffraction pattern of AgNPs

EDX (Energy dispersive X-Ray)

Energy-dispersive X-ray (EDX) analysis was carried to confirm the presence of silver as well as to detect other elementary compositions of the particles. The vertical axis displays the number of x-ray counts and the horizontal axis displays energy in KeV. In this spectrum, the peak for silver is shown between 2KV and 4KV. The peak located on the left side of the spectrum at 0.2KV indicated the carbon. The hardly observable peak located at 0.5KV indicated the Oxygen. The carbon and oxygen spots in the samples confirmed the presence of stabilizers which are composed of alkyl chains. The spectra obtained from EDX were used for carrying out the quantitative analysis. Quantitative analysis proved that high silver contents (74%) are present in the sample. we also show the presence of carbon (21%) and oxygen (5%), except silver.

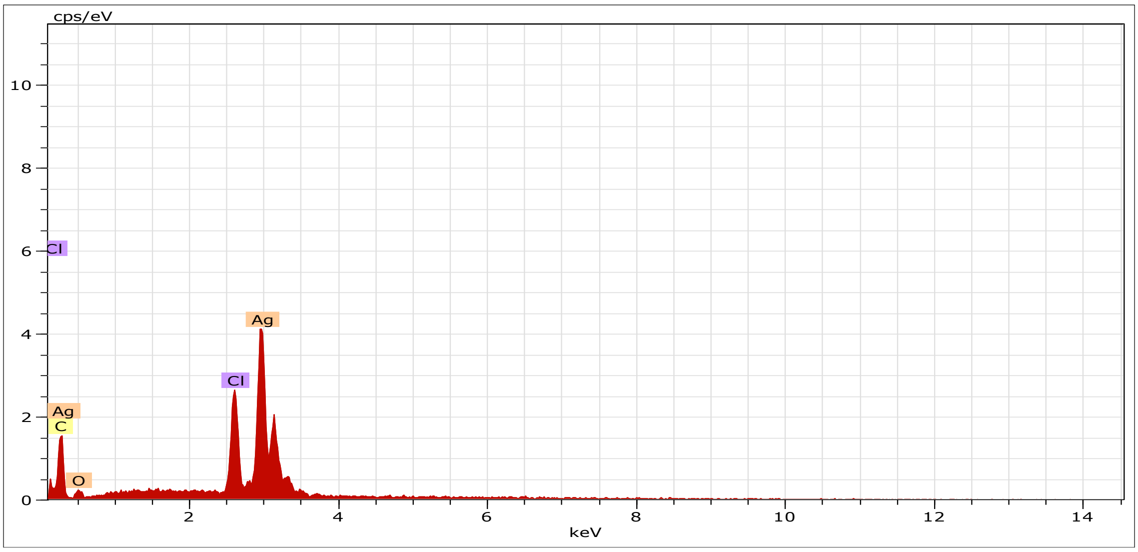

Fig 7: EDX Pattern spectra for elements direction

Antimicrobial Activity of AgNPs

The silver nanoparticles were synthesized by *Thuja orientalis* plant extract was examined for antimicrobial activity using agar as media by well diffusion method against *E.coli* bacteria by formatting the Minimum inhibitory concentration (MIC). Briefly Luria Bertani (LB) broth was used to plant-up the bacteria. These were cultured and maintained at 40^0C on nutrient agar slants.

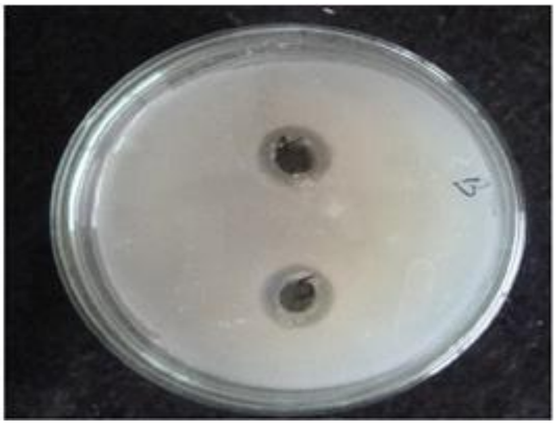

Fig 8: Shows the antimicrobial activity of silver nanoparticles

Pure cultures were sub cultured in nutrient agar broth for 24 h at 32^0C. Each strain was swabbed homogeneously into the individual plates using sterile cotton swabs. 2 wells of equal diameter were made on the agar plates using gel puncture fig 4. Using sterile micropipette 10, 20,30,40,50 and 100 µl of the sample of nanoparticles solution were loaded onto each of the wells at the centre in all the plates. After incubation at 32^0C for 24 h, the different levels of zone of inhibition were measured for determination of minimum inhibitory concentration.

Conclusion

Green synthesis of silver nanoparticles is eco friendly and less hazardous as compared to other conventional methods. Here, we report plant mediated synthesis of silver nanoparticles reduced by the leaf extract of *Thuja orientalis* and the particles were observed to active against bacteria *E. coli*. During the study, multiple changed was observed for different part of different plants, an intense surface resonance Plasmon band in the UV-visible region at 465 nm. The further studies of formation of AgNPs were confirmed by UV, FTIR, SEM and antimicrobial activity

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