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BIO-INSPIRED SYNTHESIS OF SILVER NANOPARTICLES USING LEAVES OF *MILLINGTONIA HORTENSIS* L.F.

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ABSTRACT

The present work explains the bio-inspired synthesis of silver nanoparticles using the leaf broth of *Millingtonia hortensis* L.f. The exposure of aqueous silver nitrate to the leaf broth of *M. hortensis* leads to the synthesis of stable silver nanoparticles within 24 hours. The mechanism of reduction of aqueous silver nitrate during the reaction with the leaf broth was analyzed by UV-Visible (UV-Vis) Spectrophotometer and the Surface Plasmon Resonance (SPR) was observed between 330nm and 550nm with a λ max at 414nm. Fourier Transform Infra-Red Spectroscopic analysis reveals that the presence of biomolecules in the reaction medium may be responsible for the reduction of silver ions. The bio-inspired synthesis of silver nanoparticles exploiting the leaves of *M.hortensis* has been elucidated by the characteristic peaks observed in the Photoluminescence spectra, XRD spectra and EDX spectra. The structural view of silver nanoparticles was documented with the help of Scanning Electron Microscope and Transmission Electron Microscope. The average size of the nanoparticle was found to be 22.7±1.72 nm. This approach of bio-inspired synthesis using the leaves of *Millingtonia hortensis* appears to be cost effective, eco-friendly and easy alternative to conventional methods of synthesis of silver nanoparticle.

Keywords: Bio-inspired synthesis, silver nanoparticles, *Millingtonia hortensis*, eco-friendly approach.

INTRODUCTION

In the recent past, silver nanoparticles have gained interest due to their distinctive properties such as good conductivity, chemical stability, catalytic, antibacterial, antifungal, anti-viral and anti-inflammatory activities [1,2]. Various chemical methods [3] and physical methods [4] are employed in the synthesis of silver nanoparticles. They are tedious, toxic, non eco-friendly and have low productivity [5]. Hence, the importance of bio-inspired synthesis is being emphasized at present and made to design a protocol for "green synthesis" in which there is no involvement of high pressure, temperature and toxic chemicals [6]. Green synthesis of silver nanoparticles has been achieved by using bacteria [7], seaweed [8], fungi and actinomycetes [9], bryophytes [10] and higher plants [11]. Among the biosynthetic processes, extracellular synthesis of nanoparticles using plants or their extracts would be more useful since it is produced in a controlled manner [12]. Bio-inspired synthesis of metal nanoparticles using plant extracts is the most favourite green method of production of nanoparticles and it is exploited to a vast extent because the plants are widely distributed, easily available, safe to handle and with a range of metabolites [10].

The leaves of higher plants have been extensively exploited for the synthesis of silver nanoparicles. They are *Polyalthia longifolia* [13], *Lawsonia inermis* [14], *Catharanthus roseus* [15], *Rauvolfia tetraphylla* and *Enicostema hysopifolium*, [16], *Cardiospermum helicacabum* [17], *Prosopis chilensis* [18], *Merremia tridentata* [19], *Tecoma stans* [20], *Hyptis suaveolens* [21] *etc*. In addition, the flowers of *Millingtonia hortensis* have been also used to synthesize silver nanoparticles [22]. The present work is therefore aimed to evaluate the ability of the leaves of *Millingtonia hortensis* in the reduction of silver nitrate into silver nanoparticles and to characterize the silver nanoparticles that are synthesized through aforesaid method.

MATERIALS AND METHODS

Fresh and healthy leaves of *Millingtonia hortensis* L.f. (Bignoniaceae), Figure 1 were collected from the campus of Ayya Nadar Janaki Ammal College, Sivakasi, Tamil Nadu, India. The collected leaf samples were air- dried for 48 hours under shade. 10g of the dried leaves were cut into fine pieces and suspended in 100 ml of distilled water and kept in a boiling water bath for 10 minutes to prepare the leaf broth. 10 ml of this freshly prepared leaf broth was re-suspended in 190 ml of 1mM aqueous solution of silver nitrate and this mixture is used as reaction medium. This reaction medium was kept in an incubator cum shaker (Orbitek) with 250 rpm at 27^0C for 24hrs for the synthesis of silver nanoparticles and it was characterized through various analyses to find out the presence of silver nanoparticles.

Figure 1: *Millingtonia hortensis* L.f. Leaves

Formation of silver nanoparticles in the aqueous solution was analysed by periodic sampling, after diluting a small aliquots of sample with sterile distilled water using UV-visible spectrophotometer (Labomed Model UV-D3200) and its optical property was evaluated by recording emission and excitation spectrum with an aid of Spectrofluorimeter (ELICO). After the complete reduction of Ag+ ions by the leaf broth, the reaction medium was centrifuged at 5000 rpm for 10 min and purified by repeated centrifugation using sterile water. Thereafter the purified suspension was completely dried and analyzed by FT-IR spectrophotometer (Shimadzu) using KBr pellet method from the range of 4000- 400 Cm^{-1}. The dried powder of silver nanoparticles was further analyzed under X'Pert Pro X-ray diffractometer operated at a voltage of 40 kV and a current of 30 mA with Cu Kα radiation in θ- 2 θ configurations and its crystalline domain size was calculated from the width of the XRD peaks using the Scherrer's formula, $D= 0.94 \lambda / \beta \cos \theta$, where D is the average crystalline domain size perpendicular to the reflecting planes, λ is the X-ray wavelength, β is the full width at half maximum (FWHM) and θ is the diffraction angle. Hitachi S-4500 Scanning Electron Microscope (SEM) coupled with Energy Dispersive X-ray Spectroscopy (EDX) was used to assess the shape and percentage of the synthesized silver nanoparticles respectively. TEM measurements were performed on a Philips-Techno 10

Transmission Electron Microscope (TEM) operated at an acceleration voltage of 200 kV with a resolution of 0.3nm to find out the exact size of the synthesized silver nanoparticles.

RESULTS AND DISCUSSION

Visual observation and UV-Visible spectroscopic analysis

The colourless solution of silver nitrate Figure 2A was exposed to the leaf broth of *M.hortensis* (reaction medium) which was pale brown in colour Figure 2B. Immediately after this exposure, during the zero hour reaction it became yellowish-brown in colour Figure 2C. The intensity of the colour gradually changed to light brown then to dark brown within 24 hours Figure 2D-H. This colour change may be ascribed to the reduction of silver nitrate into silver nanoparticles [23, 24]. The time taken for the change in colour of the reaction medium varies from plant to plant [19]. *Lawsonia inermis* [14], *Dioscorea batatus* [25] and *Epipremum aureum* [26] made the colour change within two hours, while, *Coleus amboinicus* [27] took 28 hrs for the formation of brownish colour. This may be because of the variation in the biomolecules and their quantity involved in the reduction mechanism.

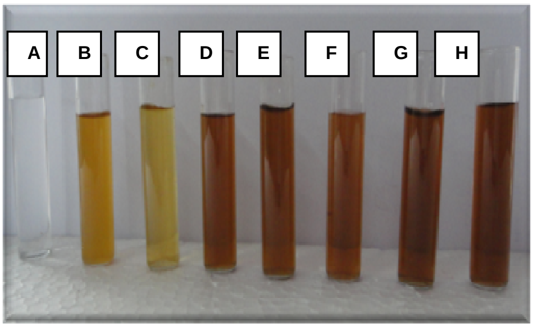

Figure 2: Colour change of the reaction medium (Leaf broth of *Millingtonia hortensis* L.f. and 1mM aqueous silver nitrate during the Bioinspired synthesis of silver nanoparticles). A - Control (Aqueous silver nitrate); B- leaf broth; C- 0 hr; D-30 mts; E-1 hr; F-3 hrs; G - 6 hrs; H - 24 hrs.

The formation of metal nanoparticles and their stability in aqueous solution was ascertained by UV-visible spectroscopy [28]. UV-visible spectra of the reaction medium taken at different time intervals explicit that surface plasmon resonance (SPR) vibrations are found between 330nm and 550nm with the λ_{max} at 414nm which is blue shifted. The absorbance at 414nm was raised up to 0.459 a.u. Figure 3**.** It was found to be at 405 and 480 nm for the reaction media of silver nitrate with the leaf broth of *Enicostema hysopifolium* and *Rauvolfia tetraphylla* respectively [16], whereas the reaction media prepared by the leaf extract of *Catharanthus roseus* showed a peak at 440nm [15]. The variation in the frequency and width of the surface plasmon absorption was due to the variation in size, shape, dielectric constant of the metal and the surrounding medium [29, 30].

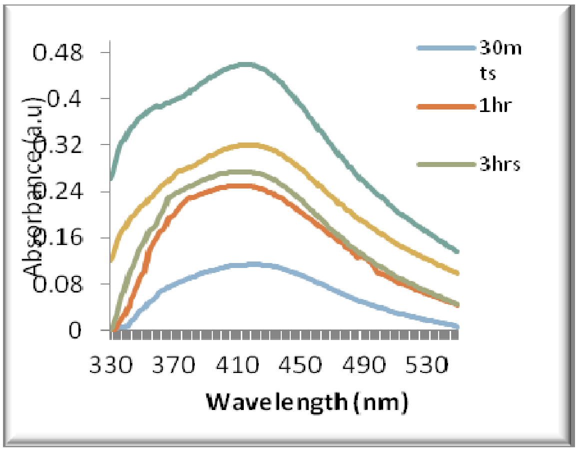

Figure 3: UV-visible spectra of silver nanoparticles synthesized by leaf broth of *Millingtonia hortensis* L.f. as a function of time

PHOTOLUMINESCENCE(PL) SPECTRA ANALYSIS

The silver nanoparticles synthesized using the leaf broth of *M.hortensis* were found to be photoluminescent. The PL spectra obtained from the synthesised silver nanoparticles Figure 4 shows an excitation peak at 425 nm and an emission peak at 428 nm. However, the PL spectra of silver nanoparticles synthesized using the leaves of *Parthenium hysterophorous* [31] and *Epipremnum aureum* [26] showed peak at 480nm and 473.21 nm respectively. It is due excitation of electrons from'd' orbital into the state above the Fermi level and it is responsible for the visible luminescence property of the silver nanoparticles [32, 31].

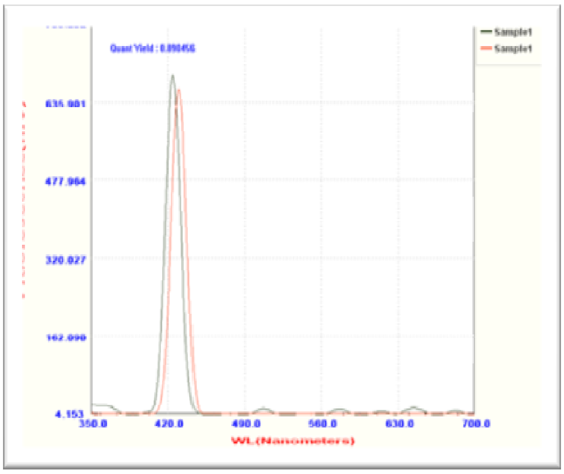

Figure 4: Photoluminescence spectra of synthesized silver nanoparticles

FT-IR SPECTROSCOPIC ANALYSIS

FT-IR measurements were performed to identify the potential biomolecules in the leaf broth of *M.hortensis* responsible for the formation of silver nanoparticles. The FT-IR spectrum of leaf broth before reaction, showed several absorption peaks at 464, 513, 603, 651, 750, 1116, 1193, 1338, 1400, 1620, 1668, 1747, 2881, 2931, 2970, 3124, 3201, 3315, 3390, 3417, 3440 and 3525 cm^{-1} Figure 5a**.** The FT-IR spectrum of purified and dried silver nanoparticles Figure 5b showed absorbance peak at 603, 1112, 1195, 1396, 1670, 2090, 2885, 2975, 3195 and 3313 cm^{-1}. The total disappearance of the bands at 1338, 1400, 1620, 1747, 2931 and 3124 cm^{-1} after bio-reduction may be ascribed to the reduction of silver ions into silver nanoparticles. It indicates that the stretching vibrations such as -C-N of amide III band, -C=O of ketones and -C-H of ketones were responsible for the reduction. Terpenoids, flavones, ketones, aldehydes, amides and carboxylic acids were the identified phytochemicals responsible for the formation and stabilization of silver nanoparticles [33]. Our study also revealed that ketones and amides as a key compound involved in the reduction process. The fabrication of silver nanoparticles using the leaf extract of *Nicotiana tobaccum* indicated the role of carboxyl (-C=O), hydroxyl (-OH) and amine (-NH) in the synthesis of silver nanoparticles [34].

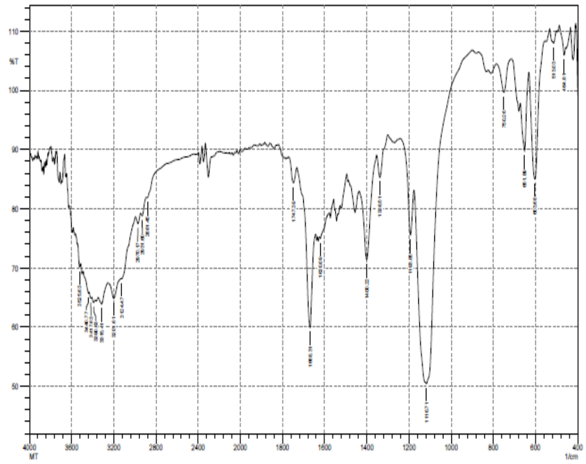

Figure 5a: FT-IR Spectrum of leaf broth of *Millingtonia hortensis* L.f.

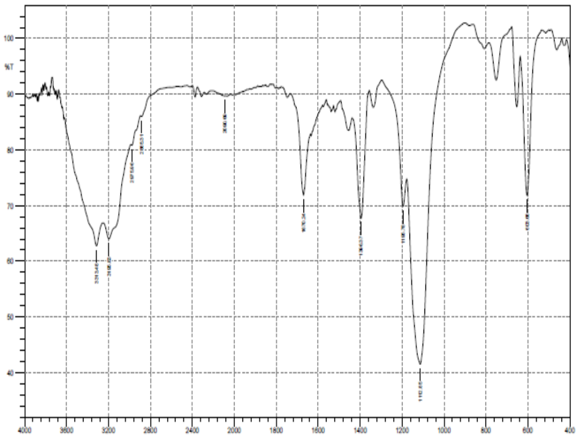

Figure 5b: FT-IR Spectrum of synthesized silver nanoparticles using leaf broth of *Millingtonia hortensis* L.f.

XRD ANALYSIS

The XRD pattern Figure 6 shows 2θ value ranging from 10 to 80 with four intense peaks specific for silver nanoparticles at 38.08°, 46.19°, 64.5° and 77.4°. These peaks correspond to 111, 200, 220 and 311 planes for silver nanoparticles indicate that the particles are crystalline in nature [35]. The sharp bands of Bragg's peak authenticate that the particles are in the nanoregime and are stabilized by the reducing agents present in the leaf broth [36]. The XRD pattern also showed peaks for additional and yet unassigned bio-organic phase present on the surface of the silver nanoparticles [37]. The calculated average size of the particles was 44nm with size range from 20 to 64nm.

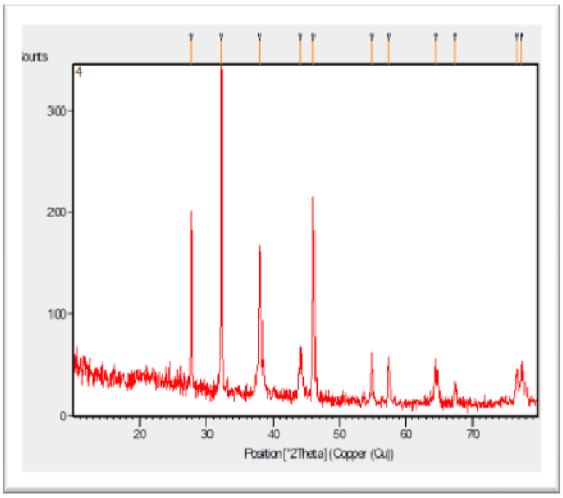

Figure 6: XRD Pattern of synthesized silver nanoparticles

SEM AND EDX ANALYSES

SEM image Figure **7** shows the surface morphology of the silver nanoparticles. The particles obtained are mostly spherical in nature. The size of the spherical shaped silver nanoparticles that were synthesized using *Merremia tridentata* [19], *Cardiospermum helicacabum* [17] and *Euphorbia hirta* [38] was ranging from 30-50nm [19]; 5-50 nm [17] and 40-50 nm [38] respectively.

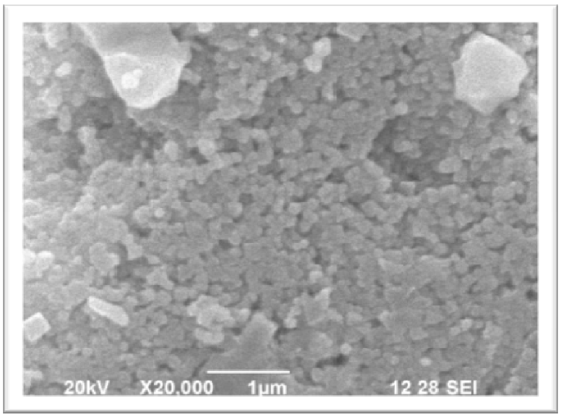

Figure 7: SEM images of silver nanoparticles synthesized using *Millingtonia hortensis* L.f. leaf broth (at 20,000X Magnification).

The presence of elemental silver can be observed from the EDX spectrum Figure 8 of synthesized silver nanoparticles. A strong elemental silver signal along with week Cl, O and Si atoms were also recorded suggesting that they are mixed precipitates present in the plant extract [39]. Metallic silver nanoparticles generally show typical absorption peak approximately at 3 KeV due to SPR [40, 19].

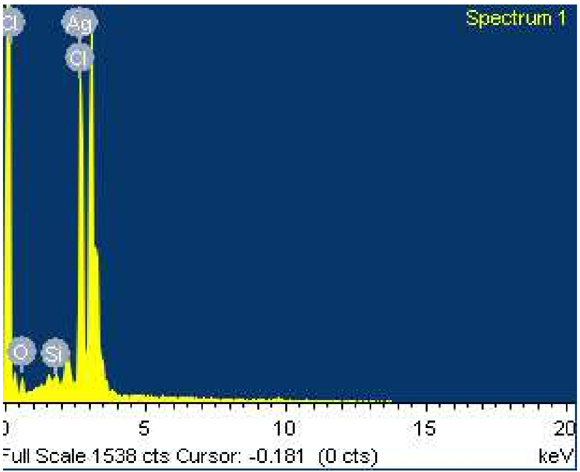

Figure 8: EDX image of silver nanoparticles synthesized using *Millingtonia hortensis* L.f. leaf broth

TEM ANALYSIS

TEM image Figure 9 clearly indicate that the shape of the silver nanoparticles synthesized using the leaf broth of *M.hortensis* are spherical and their dimensions ranging from 12 to 56nm with an average size of 22.7±1.72nm Figure 10. The leaves of *Eucalyptus hybrida* (Safeda) produced silver nanoparticles of 50nm size [41] and the leaves of *Pongamia pinnata* were employed to synthesize silver nanoparticles of 20nm size [42].

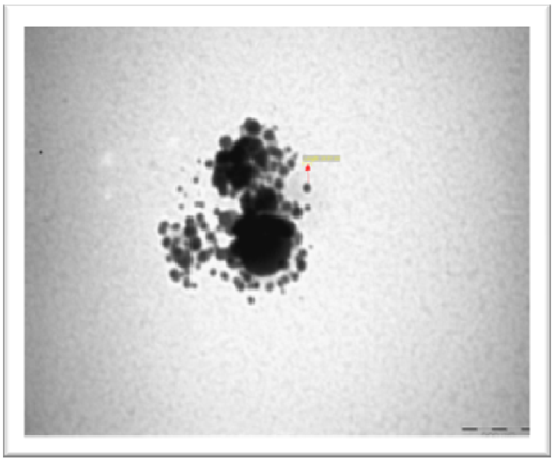

Figure 9: TEM image of silver nanoparticles synthesized from the *Millingtonia hortensis* L.f. Leaf broth

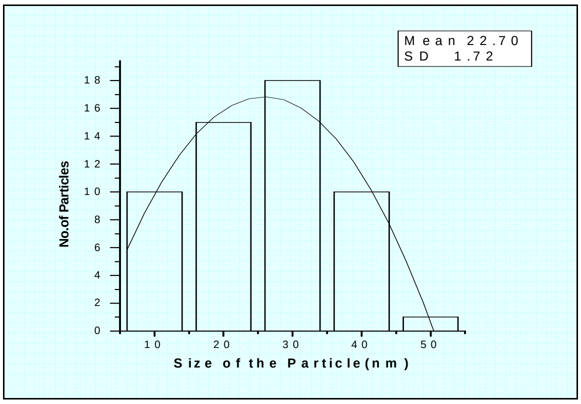

Figure 10: Histogram of silver nanoparticles synthesized from *Millingtonia hortensis* L.f. leaf broth

CONCLUSION

The present study divulges the reduction of Ag+ ions into silver nanoparticles using the aqueous leaf broth of *M.hortensis*. The formation of silver nanoparticles was visually authenticated by the appearance of brown colour and the reaction was completed within 24 hours of incubation. The characterization of silver nanoparticles was achieved with UV-Visible spectroscopy, PL spectra, FT-IR spectroscopy, XRD, SEM, EDX and TEM analyses. The UV-visible spectrum of the reaction medium showed λ_{max} at 414nm and the absorbance increased upto 0.459 a.u. The FT-IR spectrum proved the possible role of ketones and amides in the formation and stabilization of silver nanoparticles. The presence of nanosized silver with an average of 44nm was observed through XRD analysis and the synthesis of 68.15% of spherical elemental silver nanoparticle was confirmed by SEM and EDX analyses. TEM analysis elucidated the well-defied spherical nanoparticles with a diameter of 22.7±1.72nm. This biogenic approach of silver nanoparticle synthesis becomes cost effective, simple and green method, as it overcomes the adverse effects of various physical and chemical methods usually applied for the synthesis of silver nanoparticles.

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