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Green synthesis and characterization of silver nanoparticles using *Triumfetta Rotundifolia* plant extract and its antibacterial activities

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ABSTRACT

Development of reliable and eco-friendly green methods for the production of metallic nanoparticles have many advantages in the field of nano-technology. The aim of the present work, describes a cost effective and environment safe technique for green synthesis of silver nanoparticles from silver nitrate solution using the plant extract as reducing agent. The synthesized silver nanoparticles have been characterized by UV-Vis spectra, FTIR, XRD, HR-SEM analysis. The absorption maxima were scanned by UV-Vis spectroscopy. From FTIR spectra, the functional group responsible for silver reduction and capping for efficient stabilization of silver nanoparticles. The SEM analysis shows the synthesized nanoparticles are rod in shape. Further these biologically synthesized nanoparticles exhibit a tremendous anti-bacterial activity. Hence, the plant based route could be considered as fast and easy bio process of nanoparticles production.

Keywords: *Silver nanoparticles; UV-Vis; FTIR; XRD; HR-SEM*

INTRODUCTION

The field of nanotechnology is one of the most active areas of research in modern material science. Nanotechnology mainly deals with the fabrication of nanoparticles having various shapes, sizes and managing their physical and chemical parameters for further use in human benefits [1]. Nanoparticles can be synthesized using various approaches including chemical, physical, and biological methods. Although chemical method of synthesis requires short period of time for synthesis of large quantity of nanoparticles, this method requires capping agents for size stabilization of the nanoparticles. Currently, nanometal particles have gained significant attention (particularly silver), due to their broad uses in the areas of electronics, material science and medicine [2]. In nanotechnology, silver nanoparticles are the most prominent one. Silver nanoparticles have a particles size between 1 nm and 100 nm and have attracted intensive research interest. Silver nanoparticles can be synthesized using various methods: chemical, electrochemical [3], γ- radiation [4], photochemical [5], laser ablation [6], biobased[7] protocol etc. The bio-based protocol is the most important and eco-friendly production method [8]. Bioactive compounds are rich in plant extracts which have recently been used for the synthesis of nanoparticles. Many different plant leaves and herbs for the synthesis of nanoparticles have been reported [9]. Nano crystalline silver particles have found tremendous applications in the field of high sensitivity bio molecular detection and diagnostics, antimicrobials and therapeutics, catalysis and microelectronics. Currently there are several methods for the production of nanoparticles like chemical and physical methods. But there are evidences regarding the harmfulness of these methods to environment [10].

The various nanoparticles like gold, silver, copper, iron, palladium, zinc, quantum dots (CdS, ZnS), among these, Silver nanoparticles places a major role because it has several important properties such as optical, chemical, electronic, photo electro chemical, catalytic, magnetic, antibacterial, biological labelling and antimicrobial. Silver nanoparticle acts as antimicrobial agent which finds applications in medical field such as silver nanoparticles coated blood collecting vessels, coated capsules, band aids etc [11-14]. The silver is nontoxic to animal cells and highly toxic to bacteria, and other microorganisms (E-coli, Pseudomonas aeruginosa, Staphylococcus aureus). Due to these phenomena it is considered to be safe and effective bactericidal metal [15-17]. The synthesis of silver nanoparticles using green methods which are non-toxic, less usage of chemicals and environmental friendly and low cost. The most promising approach for generating new fields in biomedical sciences is the pharmaceutical application of nanoparticles [18]. Silver nanoparticles have potential in treating a variety of diseases, including retinal neovascularization, immunodeficiency syndrome [19], infection [20] and cancer [21].

The present study describes the process of synthesis of silver nanoparticles using the plant extract of *Triumfetta Rotundifolia*. The synthesized silver nanoparticles were further characterized by using UV-Vis absorption spectroscopy, FTIR, XRD, HR-SEM analysis and its anti-bacterial activities. The *Triumfetta Rotundifolia* plant was come under Tilliaceae family, its native was Myanmar and throughout in India. Its height up to 2m tall, younger parts stellately hairy. Leaves orbicular- rhomboid. Flowers yellow, in interrupted racemes. Fruit capsule, ovoid or globose, densely pubescent, spine dilated at base. The plant is used as a demulcent. The alcoholic extract of roots shows anti-inflammatory activity and the whole plant exhibited antibacterial activity. It completely inhibited the growth of Pseudomonas aeruginosa, *Salmonella paratyphi, Bacillus subtlis, Staphylococcus albus, Staphylococus aureus.*

Fig.1. Image of *Triumfetta Rotundifolia* plant

MATERIALS AND METHODS

Silver nitrate was purchased from Sigma Aldrich chemicals India. Double distilled water was taken for the whole process; Whatmann no.1 filter papers were used. Glass wares used for the complete reactions were washed well and rinsed with double distilled water and dried in hot air oven before use.

The UV-Visible spectral measurements were carried out on UV-1800 SHIMADZU UV- spectrophotometer instrument. FTIR experiment was done on SHIMADZU-8400S model instrument. The X-ray diffraction analysis was done by using BRUKER, ECO D8 Advance model instrument. The SEM images of silver nanoparticles was analysed by using CARL ZEISS, EVO 18 model instrument. An EDS image was carried out on BRUKER, X flash 6130 model instrument.

1. Preparation of plant extract

The Whole plant of *Triumfetta Rotundifolia* was collected from Kovilpatti, Tuticorin District, Tamil Nadu and India. Taxonomic identification was made from Central Council for Research in Ayurveda & Siddha, Government of India. The plant was dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve. 5g of the collected plant powder was mixed with 100 ml of double distilled water and boiled to 60°-70° C for about 10-15 minutes. Then the crude extract was filtered using Whatmann No.1 to get clear solution. The filtrate was stored at 4°C over night for further studies.

2. Synthesis of silver nanoparticles

5 ml of stock solution of leaf extract was slowly added with 20 ml of 1mM solution of silver nitrate solution under room temperature. After the complete addition of leaf extract, the colourless solution changed from pale yellow colour, after 30 min colour changed from pale yellow to dark brown colour. The colour change indicates the formation of silver nanoparticles. Then the solution was centrifuged for 10 min in 10,000 rpm for 15 min [22], consequently dispersed in double distilled water to remove any heavy biological materials present in synthesized silver nanoparticles [23].

RESULTS AND DISCUSSION

1. UV-Vis spectroscopy analysis

The UV-Visible spectroscopy measurements were used to confirm the formation of silver nanoparticles. The reduction of silver ions to silver nanoparticles by using UV-Vis spectrum was shown in fig.2. It is well known that the silver nanoparticles shows brown colour in water. These colours occur due to the observable fact of surface Plasmon excitations in the metal nanoparticles [24]. When the plant extract was added into the 1mM silver nitrate solution, the colourless solution turned into dark brown colour solution. The absorption spectra of silver nanoparticles formed in the reaction mixture was obtained by the UV-Vis analysis at the range between 200-600 nm, the silver nanoparticles has sharp absorbance with highest peak at 437 nm and progressively decreased while nanometre increased [25].

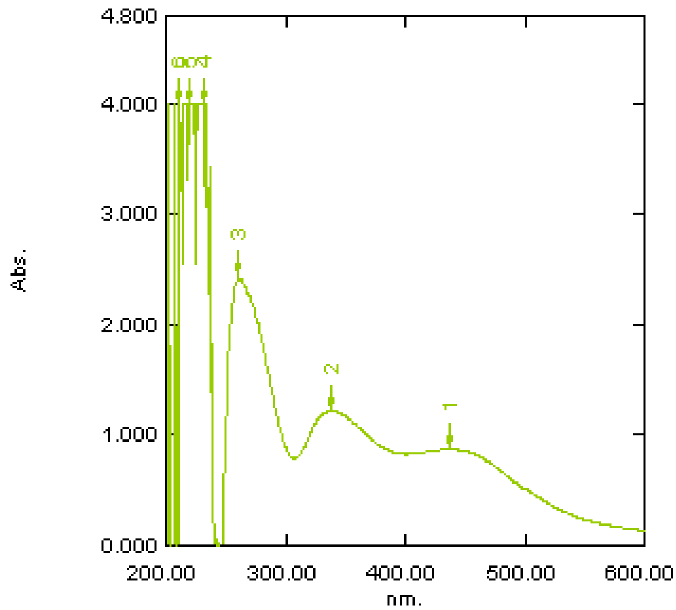

Fig.2. UV-Vis spectra of silver nanoparticles using *Triumfetta Rotundifolia* plant extract.

2. FTIR analysis

The Fourier transform infrared spectrum (FTIR) measurement was done to identify the reducing, capping and stabilizing capacity of biomolecules in synthesized silver nanoparticles using *Triumfetta Rotundifolia* plant extract. The presence of polyphenolic biomolecules in *Triumfetta Rotundifolia* plant extract and their interaction with the surface of the silver nanoparticles was confirmed by FTIR spectra was shown in fig.3. The peak at 3338 cm^{-1} assigned to O-H group of alcohols and N-H band of secondary amines. The peak at 1585 cm^{-1} indicates N-H bend of primary amines, N=O stretch of nitro group, C-C stretch (in ring) of aromatics. The peak at 1384 cm^{-1} shows the presence of nitro group. The peak at 1120 cm^{-1} assigned to C-O group of aliphatic esters. These biomolecules reduced silver ions to silver.

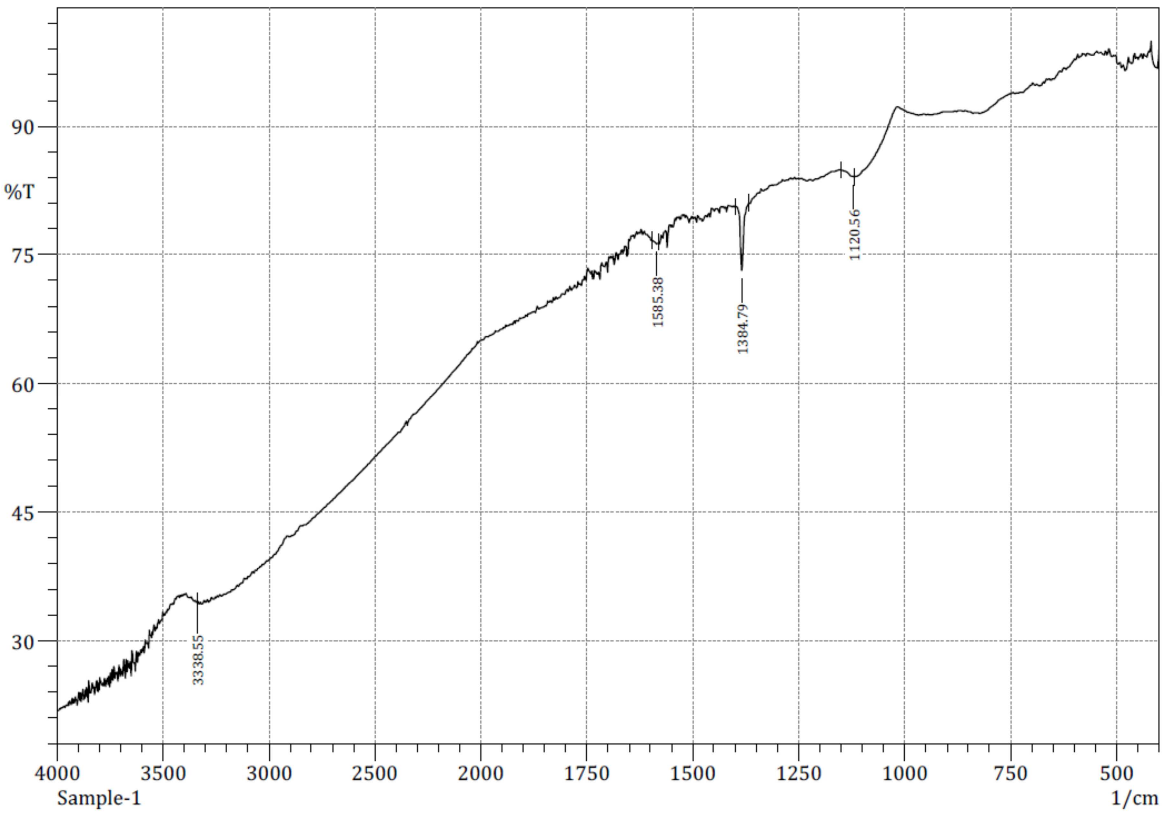

Fig.3. FTIR spectrum of silver nanoparicles using *Triumfetta Rotundifolia* plant extract.

3. XRD analysis

The X-Ray Diffraction (XRD) spectrum analysis indicated different diffraction peaks at 27.40°, 32.52°, and 76.92° was shown in fig 4. The XRD analysis reveals that the crystalline structure of silver was face centred cubic in nature. The diffracted intensities were recorded from 20° to 80° at 2 theta angles which corresponding to the planes (122), (220), and (311). The synthesized crystalline silver nanoparticles were calculated from the width of the XRD peaks, the Debye-Scherrer equation is used to determine the average grain particle size of the nanoparticles. $D = K\lambda / \beta \cos\theta$ Where, D is the crystalline size of nanoparticles, λ is the wavelength of the X ray source (1.54 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 and θ is the Bragg angle. Using Debye- Scherrer equation the average particle size was determined for the silver nanoparticles formed in the bio reduction process was 17 nm [21].

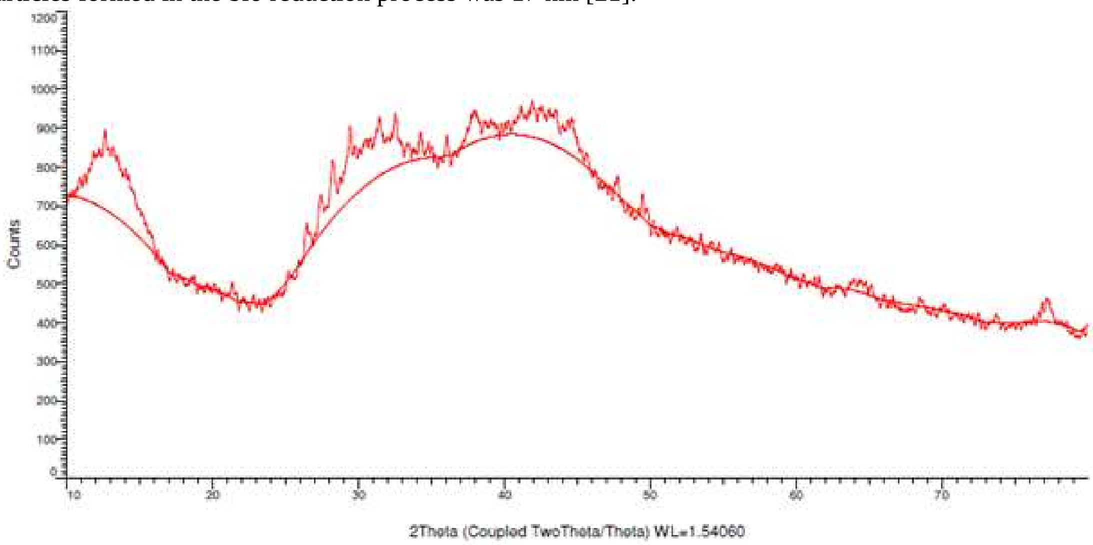

Fig.4. XRD image of silver nanoparticles using *Triumfetta Rotundifolia* plant extract

4. SEM analysis

Scanning Electron Microscopy (SEM) analysis provided the size and shapes of the nanoparticles. The SEM image of silver nanoparticles using *Triumfetta Rotundifolia* plant extract was shown in fig.5. The interactions such as hydrogen bond and electrostatic interactions between the bio-organic capping molecules bond are the reason for synthesis of silver nanoparticles using plant extract [26]. It was shown that rod and cylindrical shapes and the shape of the silver nanoparticles in the range 20-100 nm. The direct contacts even within the aggregates of nanoparticles were not found, indicating the stabilization of the nanoparticles by a capping agent [11, 27].

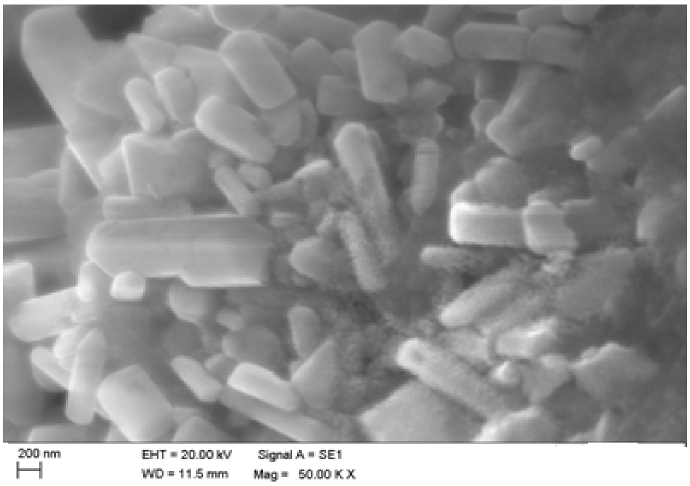

Fig.5. SEM image of silver nanoparticles using plant extract of *Triumfetta Rotundifolia*

5.EDS analysis

The qualitative and quantitative position of elements that may be concerned in the formation of silver nanoparticles was analyzed by Energy dispersive spectroscopy (EDS) analysis which shows a strong signal in the silver region and confirms the formation of silver nanoparticles. The elemental profile of silver has been confirmed from the sample using plant leaf extract shown in Fig 6. It confirms bio reduction of silver ions to silver. Due to the Surface Plasmon Resonance the silver nanoparticles shows a sharp peak at 3 Kev[28].

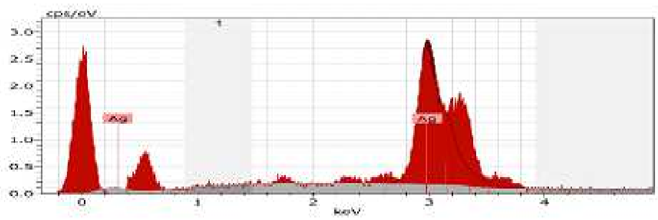

Fig.6. EDS spectrum of silver nanoparticles using plant extract of *Triumfetta Rotundifolia*

6. Antibacterial Activity

Green synthesised silver nanoparticles were analyzed for their antibacterial activity against pathogenic bacteria such as Gram-positive bacteria, Staphylococcus aureus, and Gram negative bacteria Escherichia coli. Antibacterial assay was carried out by agar Well Diffusion disc Method [21]. Plates were incubated for 16 to 18 hours at 35° to 37°C aerobically or in CO_2 atmosphere for fastidious organisms. The synthesized silver nanoparticles for the concentration of 1mM solution showed inhibition zone against Staphylococcus aureus and Escherichia coli Bacteria. Maximum zone of inhibition was found to be 10 mm in Staphylococcus aureus was shown in Fig.7 (A) and minimum of in Escherichia coli 7(B). Among these bacteria, Staphylococcus aureus was found to be more active than Escherichia coli.

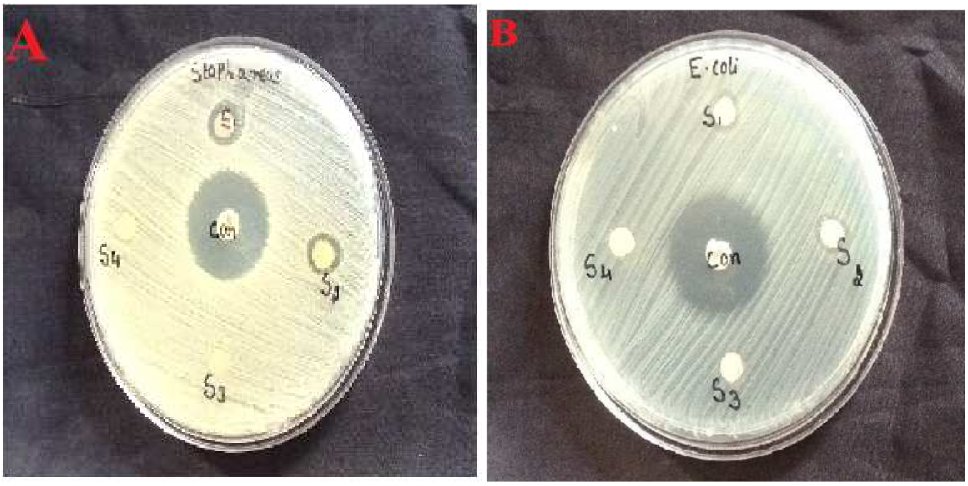

Fig.7. Antibacterial activities of silver nanoparticles against (A) Staphylococcus aureus (B) Escherichia coli

CONCLUSION

We have established an eco-friendly, rapid biological approach for the synthesis of silver nanoparticles by using *Triumfetta Rotundifolia* plant extract, which provides easy, cost effective, proficient way for the synthesis of silver nanoparticles. The simplest and efficient method to synthesize silver nanoparticles without adding any harmful chemicals as reducing agent. The synthesis of silver nanoparticles using green method can be used in various biological applications. In this study, the development of silver nanoparticles was observed by appearance of the solution and UV-Vis spectroscopy. The FTIR analysis was used to identify the functional group present in the plant extract which are responsible for the reduction of silver nanoparticles. The XRD studies were used to confirm the synthesized silver nanoparticles are found to be highly stable and crystalline in nature. The SEM studies confirmed that the synthesized silver nanoparticles were rod in shape with the range between 20-100 nm. Antibacterial analysis showed Staphylococcus aureus was found to be more active than Escherichia coli. These results concluded that even though the reduction process is slow, the green chemistry approach has many advantages such as eco-friendly, cost effective and easily scaled up to large scale synthesis.

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