Biosynthesis of Silver Nanoparticle Using Aqueous Extract of *Hibiscus Rosasinensis* Leaves

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Abstract

The present study is for the green formation of silver nanoparticles by *Hibiscus rosasinensis* leaves extract. After successful formation of silver nanoparticles, Confirmation of Ag nanoparticles was carried out by using various characterizations techniques viz. structural by FT-IR, X-Ray Diffraction (XRD), morphological by Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM) and elemental by Energy Dispersive X-ray Spectroscopy (EDX). The silver nanoparticles are also characterized for antibacterial activity. Staphylococi aures, Strepotococci pyogens, Salmonella typhy.

Keywords: *Hibiscus rosasinensis*, Silver nanoparticle, Bioactivity of silver nanoparticles.

Introduction

From the ancient Babylonians and Greeks period, silver is extensively used as an antimicrobial agent [1]. Nanoparticle possess completely new or improved properties based on specific characteristics such as shape, size etc [2]. Nanoparticles have been investigated because it has unique properties which are different than bulk material [3]. Number of green methods has been developed for the synthesis of nanoparticle such as synthesis from chemical method [4] using plant extract [5] fungi [6, 7], microbes [8], and polysaccharides [9]. But for the synthesis of silver nanoparticles using plant extracts can be advantageous over other biological process of maintaining cell cultures [10] The biosynthesis of nanosilver crystals has been successfully reported using different plant extract such as leaf extracts of *Rosa rugosa* [11], *Coriandrum sativam* leaf extract [12], *Ocimum sanctum* [13] *Argemone Mexicana* [14], *Ocimum* [15] *Aspergillus niger* [16],*Iresine herbstii* leaf extract [17] The plant extract mediated synthesized silver nanoparticles shows enhanced antibacterial properties. When nanoparticles enter into the cell, it interferes with the bacterial growth signaling pathway by modulating tyrosine phosphorylation of putative peptides substrate critical for cell viability and division [18]. These properties make them useful in topical ointment and creams to prevent infection of burns and open wounds [19]. The factor on which antibacterial property depends is the shape of nano silver, surface coating, and surface charge. The activity of nano silver is solely due to silver ion releases. A low concentration of silver ions directly absorbed as coating on silver nanoparticle with smaller size is more effective because of higher specific surface and is faster release of silver ion [20]. To the best of our knowledge till date no body have used *Hibiscus rosasinensis* for the synthesis of silver nanoparticles.

Material and method

Green synthesis of silver nanoparticles was performed as describe in the literature [21].

Result and Discussion:

IR spectra of the silver nanoparticle: The FTIR measurement is carried out to identify the possible molecules, which are responsible for the reduction of silver to silver ion. The FTIR spectrum (Fig 1) of the silver nanoparticles synthesized from aqueous extract of *Hibiscus rosasinensis* leaves shows various bands, 3900, 3772, 3691, 3544, 3251, 3193, 3116, 2950, 2865, 2356, 979,948 and 744 cm-1. Out of which 3544 corresponds to (H-bonded hydroxyl group), 2050 (-OH stretching) [22], 979 cm-1 is for secondary amines [23].

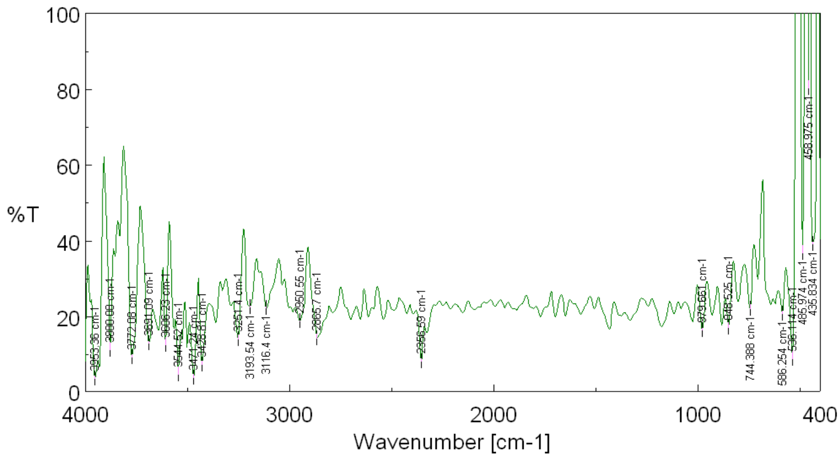

Fig1: FT-IR spectra of silver nanoparticles

X-ray diffraction

The XRD pattern of *Hibiscus rosasinensis* shows peak at (221) and (222) planes for silver. The Scherrer formula (Fig2) is used to calculate the particle size. t = 0.94 λ / β Cos θ

The particle size of *Hibiscus rosasinensis* is found to be 11.80 nm. Some unexpected peaks are also obtained in the XRD of *Hibiscus rosasinensis* . It is also reported that a single peak corresponding to (222) plan has been observed at in the diffraction pattern of silver nanoparticle synthesized by using aqueous solution of ficcus benghafensis leaf extract [24]. The extracts from plants may act both as reducing agent and capping agent in silver nanoparticle synthesis [25]. The water-soluble compounds present in the aqueous extract of plant materials were found to be responsible for efficient stabilization of nanoparticles and reduction of metal ions [26].

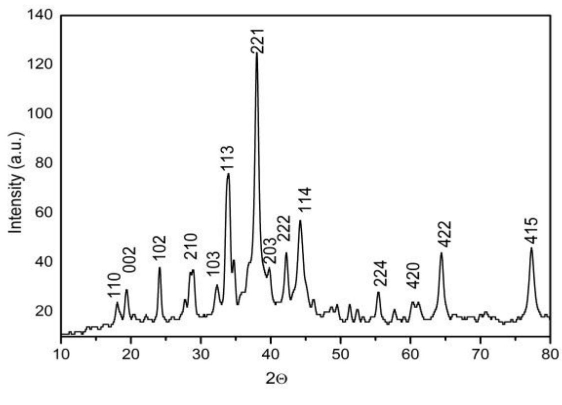

Fig2: X-ray Diffraction of Silver nanoparticle

Scanning Electron Microscope (SEM) and Atomic Force Microscope (AFM)

SEM images are used to study the morphology of silver nanoparticles (fig 3 & 4). It is observed that the size differences, size distribution, and capacity for aggregation depends on experimental conditions, stabiles, etc [27]. The shapes of silver nanoparticles, synthesized using plant extract, are like spheres, rods, prisms, plates, needles, leaf or dendrites. It depends on concentration of plant extract, part of the plant used, rate of addition or mixing of plant extract and metal salts, time taken for reaction, pH, temperature, etc [28]. In our case we are getting the shape of the nanoparticle like rods. The atomic force microscope is used to find out morphology of the surface. Experiments are also designed to evaluate surface tension of nano-material [29]. There is slight different between surface energy and surface tension. The surface energy is as reversible work per unit area needed to create a new surface, whereas surface tension or surface stress is the reversible work per unit area needed to elastically stretch a pre-existing surface. Hence surface topology of living endothelial cells can be imaged by AFM [30].

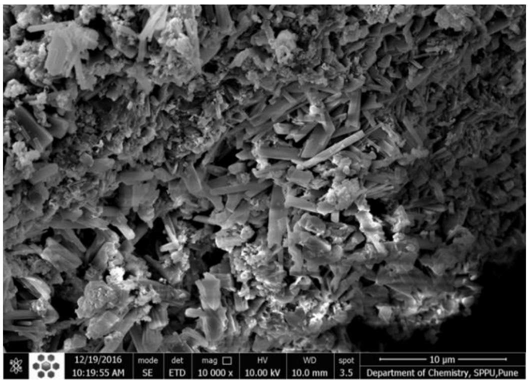

Fig3: SEM image of silver nanoparticle

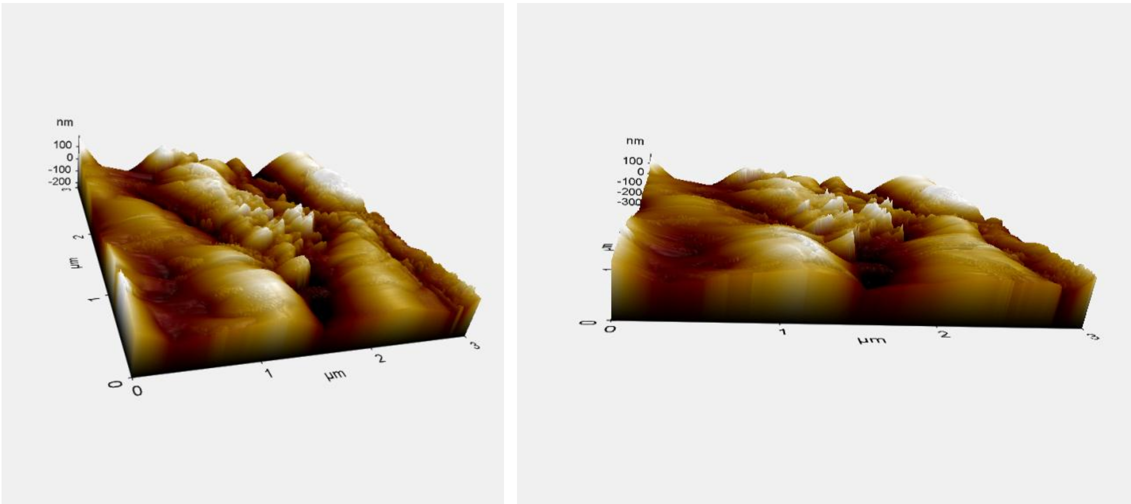

Fig4: AFM images of nanoparticle

Energy dispersive X-ray analysis EDX

Energy dispersive X-ray analysis shows the percentage of silver nanoparticle. EDX data shows the presence of carbon, sodium, and oxygen. The presence of carbon may due to the phytochemicals which was added for the formation of silver nanoparticles and presence of sodium and oxygen may be from sodium carbonate which was added during the experiment.

Table 1: Percentage of atoms

Sr. No.	**Atoms**	**% of atoms**
1.	**% of silver**	51.90
2.	**% of C**	5.28
3.	**% of Na**	22.83
4.	**% of O**	56.90

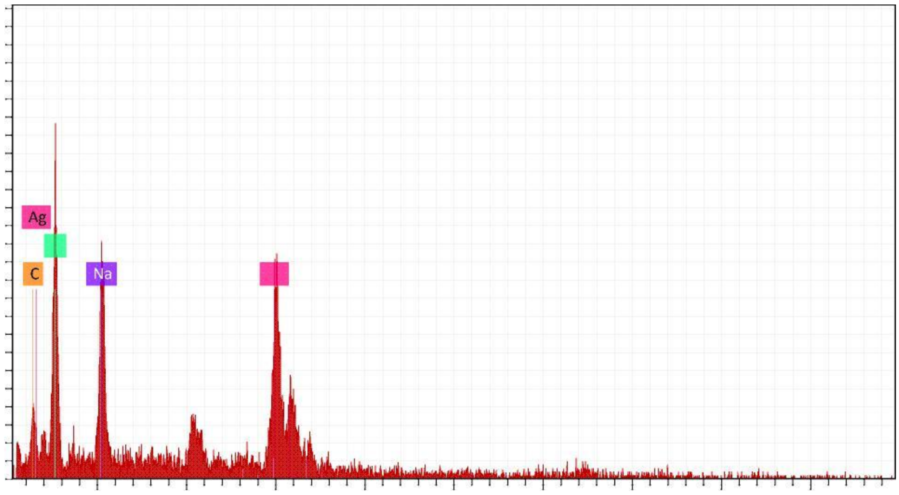

Fig 5: EDX spectra of silver nanoparticles

Antibacterial activity

The antimicrobial activity is measured by different ways, it include (i) zone of inhibition by disk diffusion and agar well diffusion method. (ii) minimum inhibitory concentration (MIC) by both macro-dilution and micro-dilution assay (iii) minimum bacterial concentration (MBC) (iv) growth pattern and (v) time kill curve [31]. The antibacterial activity is shown in table 2.

Table 2: Anti-bacterial activity of silver nanoparticles

	Bacteria	Activity
GM +ve Bacteria	*Staphylococi aures*	Highly active
	Streptococci pyogens	Highly active
	Diplococci sp.	Highly active
GM –ve Bacteria	*Escherichia coli*	Highly active
	Pseudomonas fluroscens	Highly active
	Salmonella typhy	Highly active

Conclusion

In conclusion, we succeed in the biosynthesis of silver nanoparticle by *Hibiscus rosasinensis* leaves, which have tremendous applications. The nanoparticles are then confirmed by instrumental analysis such as Fourier transformed infrared spectroscopy which shows the peaks for the compounds which are responsible for the reduction of silver to silver ions and act as capping agent. Scanning electron microscope and atomic force microscope gives the topological image of nanoparticle. EDX gives the percentage of the silver present, and XRD also confirms the formation of silver nanoparticles. The synthesized nanoparticles were investigated for the antibacterial activity, and it shows high activity against all tested bacteria, i.e., *Staphylococi aures, Streptococci pyogens, Diplococci sp., Escherichia coli, Pseudomonas fluroscens, Salmonella typhy*

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