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Characterization of Biosynthesized Silver Nanoparticles Using Silver Nanoparticles using Peel Extracts of Citrus reticulata

Keshamma E.[1], Shivashankrappa L. H.[2], Geethanjali R.[3], Kavitha K.R.[4]

[1]Associate Professor, Department of Biochemistry, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India

[2]Associate Professor, Department of Sericulture, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India

[3]Assistant Professor, Department of Botany, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India

[4]Associate Professor, Department of Botany & PG Studies, Nrupathunga University, Bengaluru, Karnataka, India

Abstract: Nanoparticles synthesized using variety of hazardous chemical methods which are not environmentally friendly. Hence, the present study was designed to synthesize silver nanoparticles biologically using peel extract of Citrus reticulata and characterization of biosynthesized silver nanoparticle. In our study silver nanoparticles were synthesized with peel extract of Citrus reticulata fruit and aqueous solution of 1 mM silver nitrate solution and then characterized using UV-Visble, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and Scanning electron microscopic (SEM) methods. Absorption maxima for silver colloidal solution showed at 420 nm in a UV-visible spectrum. The functional biomolecules such as carboxyl groups responsible for the silver nanoparticles formation were characterized by FTIR. The x-ray diffraction results revealed that the crystallization of the bioorganic phase occurs on the surface of the silver nanoparticles or vice-versa. The broadening of peaks in the x-ray diffraction patterns was ascribed to particle size effects. In our study, presence of elemental silver was proven by EDX analysis and x-ray diffration evidenced that the silver ions have been reduced to elemental silver. In conclusion, our study demonstrated simple benign, cost-effective biosynthesis methods of silver nanoparticles using peel extract of Citrus reticulata fruits. In conclusion our findings could be explored for industrial production of nanoparticles and their use in biomedical and pharmacy applications.

Keyword: Citrus reticulata, Silver nanoparticles, Biosynthesis, Vitamin C

1. INTRODUCTION

There has been a noticeable increment in the field of manufacture of nanoparticles with controlled morphologies and exceptional highlights making it a broad zone of research. The amalgamation of nanoparticles with command over molecule size, shape and crystalline nature has been one of the fundamental targets in science that could be utilized for potential applications, for example, bio-medical, biosensor, catalyst for bacterial biotoxin end and lower cost electrode [1-3]. Distinctive synthetic techniques have been utilized for the preparation of nanoparticles with various morphology and size. In spite of the fact that these techniques have brought about high quality nanoparticles yet at the same time a key comprehension of improved manufacturing process is required which could be explored at the industrial and business level to have better fabricated, enduring, cleaner, more secure and smarter items, for example, home appliances, communication technology, medicines, transportation, agriculture and industries. Along these lines, the fundamental center is to develop nanoparticles utilizing naturally favourable methodologies. These give answers for growing challenges identified with natural issues.

Nature has given ways and knowledge into the union of cutting-edge nanomaterials. It has now been accounted for in the literature that biological systems can go about as the 'bio-research center' for the creation of unadulterated metal and metal oxide particles at the nanometer scale utilizing biomimetic approach. Different microorganisms, for example, bacteria [4], [5], fungi [6], [7], yeast [8], plant extracts [9] and waste materials, have gone about as eco-accommodating forerunners for the amalgamation of nano particles with potential applications [10]. Plants are most prominent natural chemists on the earth that start with the conversion of sunlight in to chemical energy, a premise of essential items in the

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biosphere and reach out to the amazingly shifted and mind-boggling secondary products, which orchestrated in their body [11]. Protein based products; metallothionins and phytochelators, phytochemicals; polyphenols, tannins and flavonoids are found in naturally occurring products, presumed to have vital role in various biochemical activities and for the synthesis of nanoparticles [12].

Citrus reticulata (Mandarin orange) is one of the well-known and most loving product of the world belonging to Rutaceae family. It has assorted and valuable, capping and reducing phytochemicals like Vitamin C, carotenoids and flavonoids and numerous others which may be engaged with the biosynthesis of silver nanoparticles [13], [14]. Literature study evidenced various parts of plant and their extracts possess antioxidant or reducing potential because of richness of phytochemicals like flavonoids, polyphenol and tannins which play a vital role green synthesis of silver nanoparticles by reduction of metals [15]. With this background the present study was undertaken with the objective to synthesize silver nanoparticles using peel extract of Citrus reticula and characterization biosynthesized silver nanoparticle.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material and Chemicals

The fruits of Citrus reticulata were purchased from local market of Bangalore, India. All the chemicals were obtained from Sigma-Aldrich. All the experiments were done in triplicates. Double distilled water was used for the experiments.

2.2 Preparation of Peel Extract

Fresh peels of Citrus reticula fruit were washed thoroughly with double distilled water and incised into small pieces. Finely cut peels (20 g) was weighed and transferred to 500 mL flask containing 100 mL of deionized water, mixed well, and boiled for 5 min. The extract obtained was filtered through Whatman No. 1 filter paper and the filtrate was collected in 250 mL Erlenmeyer flask and stored at 4°C for further use [14].

2.3 Synthesis of Silver Nanoparticles Using the Peel Extract

To synthesize silver nanoparticles, 5 mL of peels of Citrus reticulata fruit was mixed with 50 mL of an aqueous solution of silver nitrate (AgNO3) (1 mM) and stirred for 10 min at 30°C. Reduction occurs rapidly as indicated by a reddish-brown color after 40 mins. indicating the formation of the silver nanoparticles. The silver nanoparticles obtained were purified by centrifuging at 10,000 rpm for 20 mins. and dispersing the pellets obtained in deionized water three times to remove water soluble.

2.4 Characterization of Silver Nanoparticles

2.4.1 UV–Vis spectral analysis:

The reduction of pure Ag+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium after 30 min. A small aliquot of the sample was taken for UV-Vis spectrum analysis (350-750 nm).

2.4.2 Fourier Transform Infra-Red Spectroscopy (FTIR)

FTIR measurements were carried out using Agilant Microlab, Carry 630 FTIR in the range from 4000 cm-1 to 400 cm^{-1}. The silver nanoparticles pellet obtained using peen extract of Citrus reticula fruits was air dried. The dried nanoparticles were mixed with the potassium bromide (KBr) to make thin pellets and were used for FTIR analysis in transmittance mode.

2.4.3 X-Ray Diffraction (XRD) Analysis

Resulting solution of the developed nanoparticles of silver was centrifuged at 10,000 rpm for 30 min. The solid residues of silver nanoparticles were washed twice with double distilled water and then dried at 80ºC to obtain powder of silver nanoparticles used for X-ray diffraction measurements. The X-ray diffraction (XRD) patterns were recorded on (Shimadzu XRD-6000) with copper radiation (Cu Kα 1.5406 Å) at 40 kV and 30 mA.

2.4.4 Scanning electron microscopic (SEM) Analysis of AgNPs:

SEM analysis was done using Inspect S 50 machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid.

3. RESULTS

3.1 Ecofriendly Synthesis of Silver Nanoparticles

Ecofriendly synthesis of silver nanoparticles was carried out by mixing 5 mL of peels of Citrus reticulata fruit with 50 mL of an aqueous solution of 1mM silver nitrate (AgNO3) and stirred for 10 min at 30°C. Reduction occurs rapidly as indicated by a reddish-brown color after 40 mins. indicating the formation of the silver nanoparticles.

3.2 Ccharacterization of Silver Nanoparticles

3.2.1 UV–Vis spectral analysis

The formation of metal nanoparticles by reduction of the aqueous metal ions during exposure of peel extract of Citrus reticulata fruits was followed by UV–Vis spectroscopy. The surface plasmon resonance peaks in absorption spectra for

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silver colloidal solution showed an absorption peak at 420 nm in a UV-visible spectrum, suggesting that the nanoparticles were dispersed in the aqueous solution with no evidence for aggregation in UV-Vis absorption spectrum. (Figure 1).

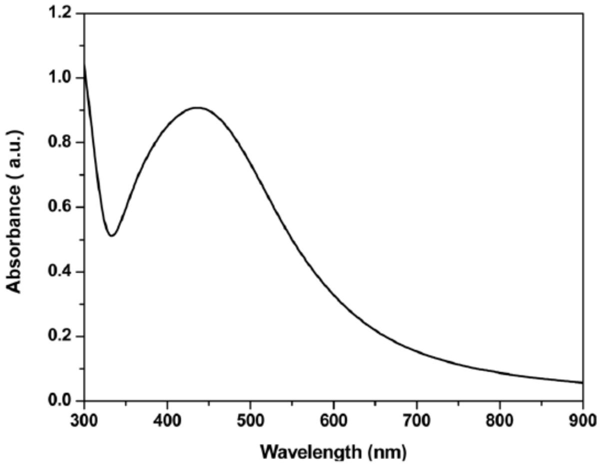

FIGURE 1: UV-Vis Spectra of silver nanoparticles synthesized with peel extracts of Citrus reticulata and 1 mM silver nitrate solution.

3.2.2 Fourier Transform Infra-Red Spectroscopy (FTIR)

The results of FTIR analysis of our study shown different stretches of bonds shown at different peaks; 3327.21—N– H stretch, 1641.42 —C=C, and 1211.30—C=O. Peaks near 3440 cm^{-1}, and 2968 cm^{-1} assigned to OH stretching and aldehydic C–H stretching, respectively. The weaker band at 629 cm^{-1} corresponds to amide I arising due to carbonyl stretch in proteins. The peak at 1051 cm^{-1} corresponds to C–N stretching vibration of the amine. The peak near 1743 cm^{-1} corresponds to C=C stretching (non-conjugated). The peak near 866 cm^{-1} assigned to C=CH2 and the peaks near 678 cm^{-1} and 638 cm^{-1} assigned to CH out of plane bending vibrations are substituted ethylene systems –CH=CH. FTIR spectra of silver nanoparticles exhibited prominent peaks at 1641, and 1382 cm^{-1}. The spectra showed sharp and strong absorption band at 1641 cm^{-1} assigned to the stretching vibration of (NH) C=O group. (Figure 2).

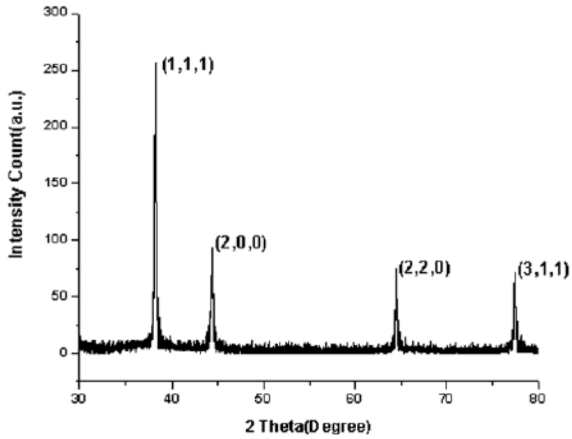

FIGURE 2: Fourier transform infrared (FTIR) spectroscopy image of silver nanoparticles synthesized with peel extracts of Citrus reticulata and 1 mM silver nitrate solution.

3.2.3 X-Ray Diffraction (XRD) Analysis

The X-ray diffraction patterns revealed that all the reflections correspond to pure silver metal with face centered cubic symmetry. The reflections were indexed as (111), (200) and (220) with the corresponding 2θ values of 39.118, 43.205 and 63.358 respectively. The intensity of peaks reflected the high degree of crystallinity of the silver nanoparticles. (Figure 3).

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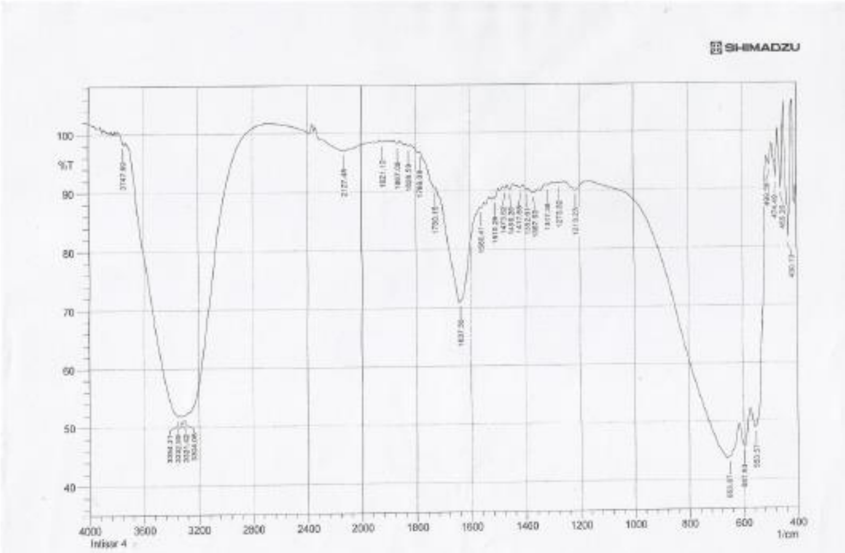

FIGURE 3: X-ray diffraction of Silver nanoparticles synthesized with peel extracts of Citrus reticulata and 1 mM silver nitrate solution.

3.2.4 SEM Analysis of AgNPs

The scanning electron micrographic study of silver nanoparticles synthesized using peel extracts of Citrus reticulata revealed that silver nanoparticles seem to be spherical in morphology and particles form cluster. It is easy to notice that the examined particles consist of a number of smaller objects of a few micrometers in size. (Figure 4). The EDX spectrum of biosynthesized silver nanoparticles revealed that the middle part of the spectrum shown a strong peak located at 3 KV. This maxima is directly related to the silver characteristic. The maxima located on the left part of the spectrum at 0.2 kV clearly comes from carbon. (Figure 5). Quantitative analysis proved high silver contents (100%) in the examined samples. (Table 1).

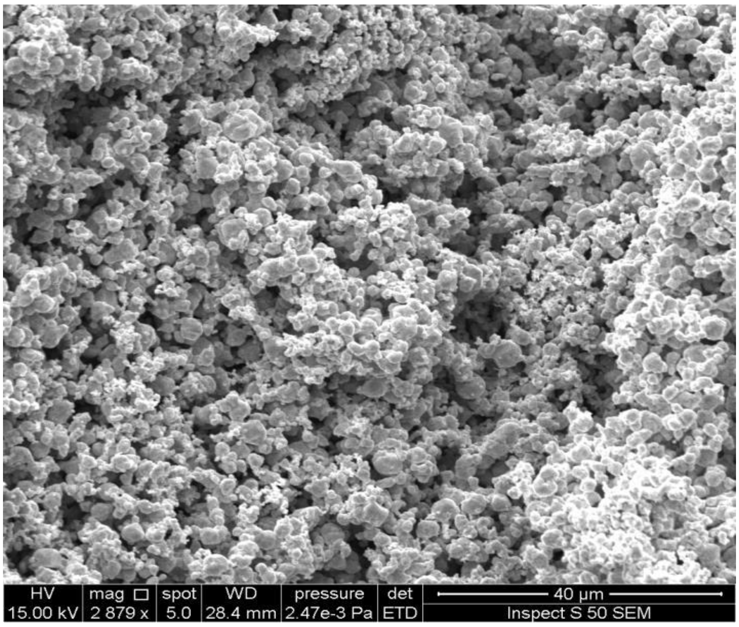

FIGURE 4: SEM micrographs of silver nanoparticles synthesized with peel extracts of Citrus reticulata and 1 mM silver nitrate solution.

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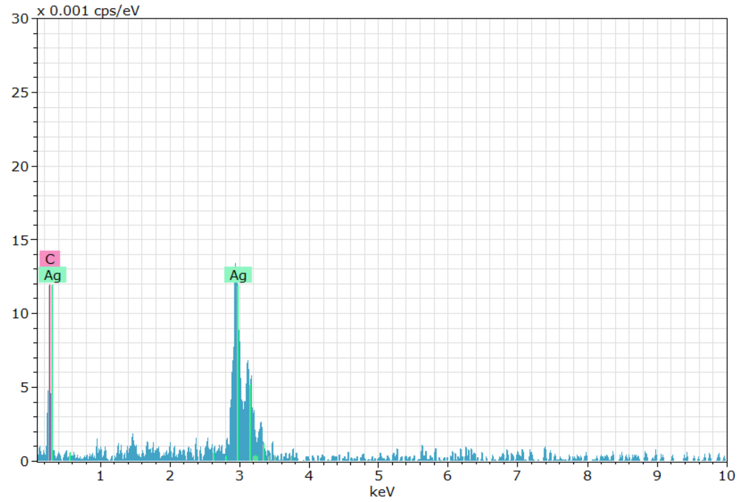

FIGURE 5: EDX characteristic spectrum obtained for silver powder synthesized with peel extracts of Citrus reticulata and 1 mM silver nitrate solution.

Table.1. Elements in biosynthesized silver nanoparticles using peel extracts of Citrus reticulata and 1 mm silver nitrate solution.

Element	AN	Series	[Wt.%]	[Norm. wt.%]	[Norm. at.%]
Carbon	6	K-series	0	0	0
Silver	47	L-series	75.13675	100	100
		Sum	75.13675	100	100

4. DISCUSSION

Nanotechnology is one of the growing areas of research in the life sciences especially biotechnology to improve the human health. For the delivery of therapeutic agents and other activities against different diseases, synthesis of stable and versatile silver nanoparticles (AgNPs) depends on the size and shape. Nanoparticles are prepared by using variety of hazardous chemical methods which are not environmentally friendly. Our study reported, convenient and extracellular method for the synthesis of silver nanoparticles by reducing silver nitrate with the help of peel extracts of Citrus reticulata. The nanoparticles were characterized using UV-Visble, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and Scanning electron microscopic (SEM) methods.

The results of UV-Vis spectral analysis in the current study are comparable to findings of Darroudi et al wherein gelatin mediated spherical AgNPs showed a characteristic peak at around 400 nm [16]. literature reports suggest that the absorption wavelength could be used to depict the estimated nanoparticles size. Hence, in our study hypothetically estimated size of silver nanoparticles synthesized using peel extracts of Citrus reticulata might be in the range of 30-50 nm as absorption spectra was recorded maximum at 420 nm [17]. According to Caroling et al noble metal silver shows its characteristic absorbance in the range of 410-430 nm, and reported that that phytochemicals polyol components, flavonoids and terpenoids plays pivotal role in reduction of silver ions [18]. Furthermore, Rajakannu et al also proposed that phytochemicals present in the aqueous extracts of G. mangostana could reduce the silver ions [19].

The findings of FTIR spectroscopy in our study are in agreement with the previously reported findings by Jiang et al [20]. Thin layer chromatography analysis of peel extract of Citrus reticulata depicted phytoactives flavonoids are mainly responsible for the bioreduction of test of aqueous silver ions (Ag+) [14]. According to Shah et al broad peak at 1645 cm-1 was mainly due to bioreduction Ag+ ions by polyols and phenols and oxidation of unsaturated carbonyl groups [21]. The bands between 1200 cm-1 to 1000 cm-1 are attributed to C-O (esters, ethers and polyol) and C-N stretching vibrations (for aliphatic amines), -CH2, -CH3 vibrations, and –C-O-H (for alcohols) and –C-O-R (for ethers or esters) vibrations as shown by literature [22]. The similarity in plant extract spectra and their respective silver nanoparticles could be ascribed to the coating of the phytochemicals present in extract on the surface of silver nanoparticles [23].

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Metallic silver nanoparticles generally show characteristic absorption peak approximately at 3 keV due to surface plasmon resonance and some weak signals in spectra were due to atoms of molecules attached to the nanoparticles surface [24]. The other signal obtained at 8 keV was derived from the Cu TEM support grids [25]. All the reflections in the X-ray diffraction pattern corresponds to pure silver metal with face centered cubic symmetry. The reflections were indexed as (111), (200) and (220) with the corresponding 2θ values of 39.118, 43.205 and 63.358 respectively. The intensity of peaks reflected the high degree of crystallinity of the silver nanoparticles. However, the diffraction peaks were broad indicating that the crystallite size is very small. Similar characteristic peaks have been reported by other researchers [26-28]. In our study, presence of elemental silver was proven by EDX analysis and XRD results showing that silver ions had been reduced to elemental silver.

5. CONCLUSION

Our study findings established simple benign, cost-effective biosynthesis methods of silver nanoparticles using peel extract of Citrus reticulata fruits. These findings could be explored for industrial production of nanoparticles and their use in biomedical and pharmacy applications.

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