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Green synthesis of silver nanoparticles and its characterization using *Cucumis melo* (L). Fruit extract

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Abstract
Nanoparticles have potential effects in life sciences and pharmaceutical applications. Among the nanoparticles, the silver nanoparticles are playing a major role in the field of biomedical nanotechnology and nanomedicine. A green method to synthesize silver nanoparticles using the *Cucumis melo* fruit extract has been developed. Biosynthesis of silver nanoparticles using aqueous extract of *Cucumis melo* fruit was prepared and its characterization by using various techniques such as UV-Visble spectroscopy, Scanning electron microscopy (SEM), Energy dispersive x-ray spectroscopy (EDX), Particle size analyzer and X-ray diffraction (XRD) methods. In fruit-mediated synthesis, after addition of *Cucumis melo* fruit extract to the aqueous solution of AgNO3, the mixture showed a gradual change in color at room temperature from orange to dark brown and the color intensified after 24 hours. The synthesized nanoparticles observed at 320nm to 380nm in UV-Visible spectroscopy revealed the presence of AgNPs. Morphology evaluation by SEM analysis showed the spherical structure of nanoparticles and XRD shows the crystalline size. Particle size was found to 35nm. The current study revealed that silver nanoparticles can be synthesized in a biological method using *Cucumis melo* fruit extract. The nanoparticles play a pivotal therapeutic importance because of their ability to cross biological barriers and stimulate self-healing cell responses augmenting biocompatibility.

Keywords: Cucumis melo (L); Silver nanoparticles; UV-Visible absorption; Particle size; SEM(Scanning Electron Microscopy); EDAX(Energy dispersive x-ray spectroscopy); XRD(X-ray Diffraction Analysis); SNPs-AECMF- Silver nanoparticles of Aqueous extract of Cucumis melo fruit

1. Introduction

Nanotechnology is a developing interdisciplinary field of research interspersing material science, bio nanoscience and technology. Remarkable advances are made in the field of biotechnology and nanotechnology to harness the benefit of life sciences, health care and industrial biotechnology. Nanoparticles are being cited as fundamental building blocks of nanotechnology. Nowadays, nanoparticles have potential effects in life sciences and pharmaceutical applications [1]. Among the nanoparticles, the silver nanoparticles are playing a major role in the field of biomedical nanotechnology and nanomedicine [2]. The processes used for nanoparticles synthesis are chemical, physical and a recently developed biological method. Chemical methods have various drawbacks, including the use of toxic solvents, generation of hazardous by-products and high energy consumption, which pose potential risks to human health and to the environment. Therefore, the synthesis of nanoparticles by biological methods, using plants or plant extracts, agricultural wastes, microorganisms, enzymes (have been suggested as possible eco-friendly and cost-effective alternatives to chemical and physical methods [3].

Nanoparticles are generally characterized by their size, shape, surface area, and dispersity. Homogeneity of these properties is important in many applications. The common techniques of characterizing nanoparticles are as follows: UV-Visible spectrophotometry, Dynamic light scattering (DLS), scanning electron microscopy (SEM), Transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), Powder X-ray diffraction (XRD) and Energy dispersive spectroscopy (EDS) [4]. Green synthesis of metal nanoparticles is a growing research area because of their potential applications in nanomedicines. The process of biosynthesis of silver nanoparticles requires the use of water as an environmentally friendly solvent and also the water is more biocompatible than other organic solvents. Avoiding the use of toxic solvents and increasing the biocompatibility of the silver nanoparticles with normal tissues for *In vivo* applications, aqueous extract was selected for the synthesis of silver nanoparticles of *Cucumis melo* fruit [5].

2. Materials and methods

2.1 Collection of plant material

Cucumis melo (L.) (Family - Cucurbitaceae) fruits were collected from the local markets of Coimbatore district, Tamilnadu, India. The specimen sample was identified and authenticated by Dr. M. Palanisamy, Scientist-C, Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamilnadu, India. The identification No. BSI/SRC/5/23/2014-15/Tech/482.

2.2 Preparation of aqueous extract of *Cucumis melo* fruit

The 10 g of the *Cucumis melo* fruit powder was weighed and mixed in 100 ml of distilled water and the mixture was boiled in heating mantle at 60ºC for 10 to 20 minutes. The extract was cooled to room temperature filtered through Whatmann No.1 filter paper (Pore size 25 µm). The filtrate was further filtered through 0.6 µm sized filters.

2.3 Synthesis of silver nanoparticles
The extract was cooled to room temperature, filtered through Whattman No.1 and used for further analysis. Aqueous solution of 1mM $AgNO_3$ was prepared and used for the synthesis of silver nanoparticles. 5 ml of aqueous extract of *Cucumis melo* was mixed to 95 ml of $AgNO_3$ solution and incubated at room temperature in dark condition for 24 hours for the synthesis of silver nanoparticles. The yellow coloured solution was changed into reddish brown after 3 hours under room temperature. The appearance of reddish brown colour after 3 hours indicates the formation of silver nanoparticles. The synthesis of silver nanoparticles was characterized and confirmed by UV-Visible Spectrophotometer, SEM, EDAX, PSA and XRD analysis.

Separation of silver nanoparticles
The synthesized silver nanoparticles were separated by centrifugation (Spectrofuge 7M) at 13,000 rpm for 15 mins. The process was repeated by dispersion of pellets in water, to obtain coloured supernatant solutions. The sample was then stored at -4°C for further use.

Characterization of silver nanoparticles
2.4 UV-VIS spectra analysis
The reduction of pure Ag+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 10-12 hours. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer UV- 2450 (Shimadzu).

2.5 Scanning Electron Microscopy (SEM)
Scanning Electron Microscope (SEM) analysis was done using Hitachi S – 4500 SEM 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, extra solution was removed using a blotting paper and then the films on the SEM grid were allowed to dry by putting (placing) them under a mercury lamp for 5 minutes.

2.6 Energy Dispersive X-ray Spectroscopy (EDAX)
Energy dispersive X-ray analysis, the aqueous extract of *Cucumis melo* were dried and drop coated on to carbon film and performed on JEOL-MODEL 6390 SEM instrument equipped with a Thermo EDX attachments.

2.7 X-Ray diffraction (XRD) Analysis
The silver nanoparticle solution obtained was purified by repeated centrifugation at 5000 rpm for 30 minutes followed by redispersion of the pellet of silver nanoparticles in to 10 ml of deionized water. After freeze drying of the purified silver particles, the structure and composition were analyzed by XRD and SEM. The dried mixture of silver nanoparticles was collected for the determination of the formation of Ag nanoparticles by an X' Pert Pro X-ray diffractometer (PAN analytical BV, The Netherlands) operated at a voltage of 40 kV and a current of 30 mA with Cu K α radiation in a configuration.

3. Results and Discussion
The biosynthesized silver nanoparticles have been used for various drug delivery and drug carrier systems. Silver nanoparticles can be used for both active and passive targeting of drugs. The silver nanoparticle carrying small drug molecules or large biomolecules, like proteins, DNA or RNA. Efficient release of these therapeutic agents to a target site for effective therapy. In the present research work, biosynthesis of silver nanoparticles from silver nitrate is one of the most widely used methods in the field of nanotechnology.

3.1 Visual Observation
In fruit-mediated synthesis, after addition of *Cucumis melo* fruit extract to the aqueous solution of AgNO3, the mixture showed a gradual change in color at room temperature from orange to dark brown and the colour intensified after 24 hours is represented in Figure 1. It is an indication of silver nanoparticles formation as the color change observed is due to the excitation of surface plasmon vibrations in silver nanoparticles. The silver nanoparticles exhibit dark brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles reported by Thirumurgan [6]. The intensity of color is increased when the time of incubation was increased. The color exhibited by metallic nanoparticles is due to the coherent excitation of all "free" electrons give rise to surface plasma resonance within the conduction band, leading to an in-phase oscillation [7]. There was no significant change observed in *Cucumis melo* fruit-synthesized silver nanoparticles, beyond 24 hours, indicating the completion of the reduction reaction. At the end of the reduction process, there is a dark brown color of silver nanoparticles that settled at the bottom of the conical flask.

Fig 1: Biosynthesis of silver nanoparticles using aqueous extract of *Cucumis melo* fruit

3.2 UV-Visible Spectroscopy
The UV-Visible spectroscopy is a commonly used technique. It was proven to be a good technique for the confirmation of silver nanoparticles in the solution. The absorption maxima for the biosynthesized silver nanoparticles were noted on UV-Vis spectrophotometer in the visible range of 200-800nm [8]. The silver nanoparticles were characterized by UV-Vis spectroscopy, one of the most widely used techniques and to confirm AgNPs formation by showing the plasmon resonance. The absorption spectrum of the silver nanoparticles depicts in figure 2. The typical absorption maxima for silver nanoparticles using *Cucumis melo* fruit extract was obtained around 320 to 380 nm indicating the presence of Ag nanoparticle.

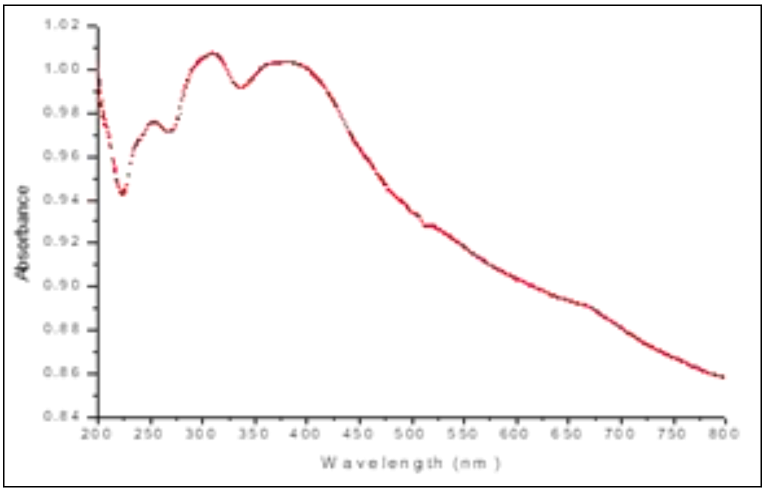

Fig 2: UV-Visible absorption spectra of biosynthesized silver nanoparticles of *Cucumis melo* fruit.

3.3 Scanning Electron Microscopy (SEM) Analysis

Scanning electron microscopy is used for morphological characterization at the nanometer to micrometer scale. The synthesized silver nanoparticles were further characterized by SEM analysis under various magnifications of ×20,000, ×30,000, 40,000 and ×60,000. SEM characterizations of the synthesized silver nanoparticles from *Cucumis melo* fruit extract are shown in Figure 3.

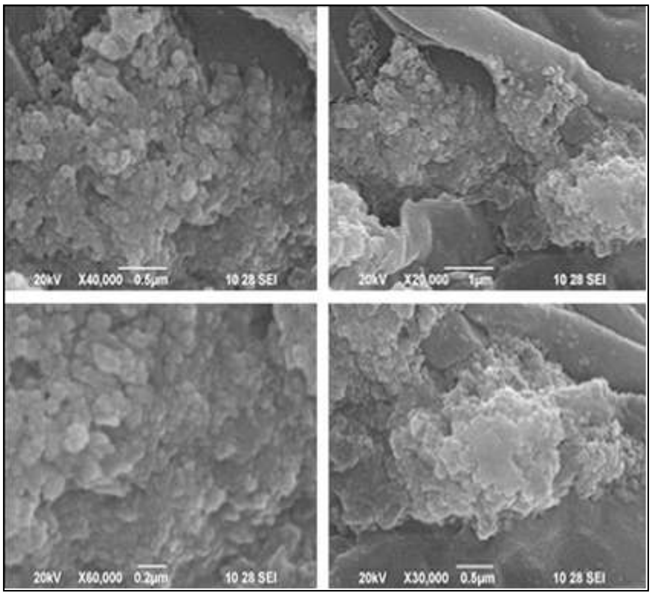

Fig 3: SEM images of silver nanoparticles synthesized from *Cucumis melo* fruit.

Our results are well correlated with a previous report obtained using papaya fruit extract [9]. The SEM micrographs of SNPs-AECMF obtained showed that they are spherical shaped, well distributed in solution. The synthesized silver nanoparticles were well dispersed without aggregation, possessing spherical shapes are confirmed by scanning electron microscope.

3.4 Energy Dispersive X-Ray Spectroscopy (EDX)

Energy dispersive X-ray spectroscopy or EDX is a technique that is mainly used to identify the presence of different elements in a sample. It is necessary to verify the presence of desired element in a sample. Elemental composition of metal nanoparticles is commonly established using energy dispersive spectroscopy (EDS) [10]. The EDX analysis of silver nanoparticles synthesized from fruit extract of *Cucumis melo* is shown in Figure 4.

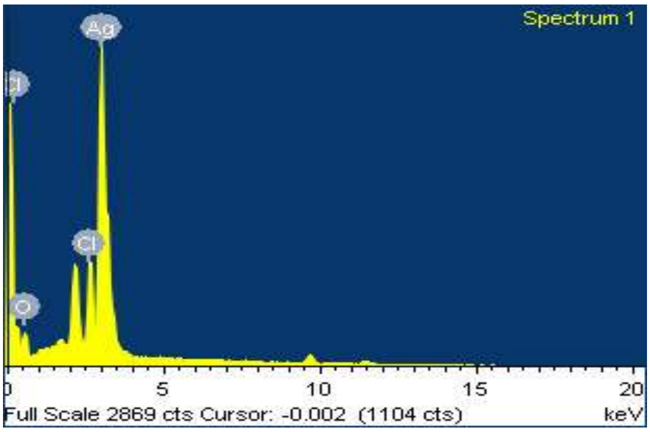

Fig 4: EDX spectrum analysis for biosynthesized silver nanoparticles from *Cucumis melo* fruit.

In the present study, Energy Dispersive Analysis of X-ray (EDAX) gives qualitative as well as quantitative status of elements that may be involved in the formation of AgNPs. The fruit extract of *Cucumis melo* synthesized silver nanoparticles also produces a strong signal at 3 keV which confirms the presence of silver nanoparticles. It is well known that silver nanocrystals show typical optical absorption peak approximately at 3 keV due to surface plasmon resonance [11]. Figure 4 shows the absorption peak at 3 keV regions revealing that nanoparticles were formed exclusively of silver with crystalline nature. The presence of the strong signal from silver (72.30 %) atoms in the nanoparticles and weaker signals from oxygen (17.24 %) and chlorine (10.46 %) atoms was confirmed. The weaker signals (Cl and O) are recorded possibly due to X-ray emission from organic moieties like enzymes or proteins present within the extract of the *Cucumis melo* fruit. Similar observations were found with pomegranate extract [12].

3.5 Particle Size Analyzer

The size distribution of the silver nanoparticles was characterized by particle size analyzer (PSA). PSA provides information regarding the size of aggregates rather than the diameter of individual particles. Particle size distribution was plotted using the results obtained from DLS [13].

Dynamic Light Scattering (DLS) is a technique that is based on the scattering of light by moving particles. DLS has been popularly employed to determine the particle size distribution in a fluid. Figure 5 depicts that the SNPs-AECMF was composed of a large quantity of well dispersed spherical AgNps with an average particle size of 35 nm.

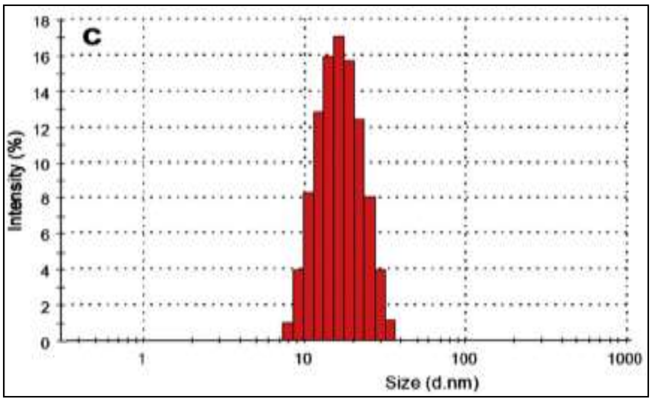

Fig 5: Particle size distribution histogram of silver nanoparticles from *Cucumis melo* fruit.

3.6 X-Ray Diffraction Analysis

The X-ray diffraction (XRD) analysis is used for the phase identification and characterization of the crystal structure of the nanoparticles. X-rays penetrate into the nanomaterial and the resulting diffraction pattern is compared with standards to obtain structural information [14]. The crystalline nature of silver nanoparticles was confirmed by the X-ray diffraction analysis.

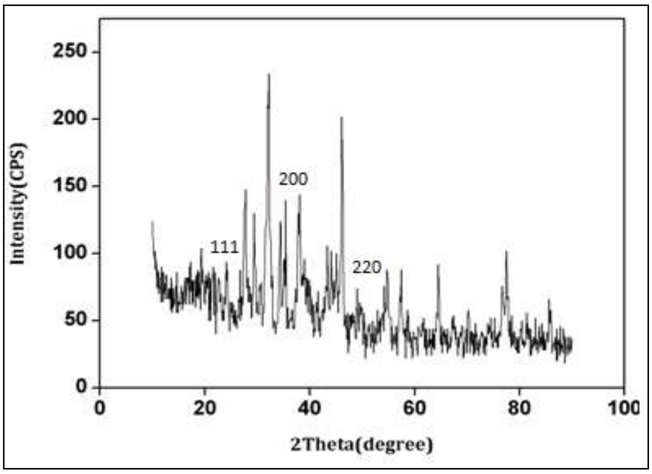

Fig 6: XRD pattern of synthesized silver nanoparticles from *Cucumis melo* fruit.

The fruit mediated synthesized (*Cucumis melo*) silver nanoparticles have shown three intense peaks in the whole spectrum of 2θ values ranging from 20 º to 80 º. Figure 6 shows the XRD pattern with the diffraction peaks at 27.95, 32.42 and 46.39 corresponding to the (111), (200) and (220) facets of the face centred cubic (FCC) crystal structure. The data obtained was matched with the database of Joint Committee on Powder Diffraction Standards (JCPDS) file No. 04-0785. The broadening of the Bragg peaks indicates the formation of nanoparticles. In this XRD pattern (Figure 6) showed the conformation of the existence of peaks belonging to silver nanoparticles in the sample.

The Braggs reflections were observed in XRD pattern at around 2Ѳ = 270, 320. Hence the XRD pattern thus clearly illustrated that the silver nanoparticles formed in this present synthesis. Peak obtained at around 330 indicated that the prepared samples were crystalline in nature. The crystalline size (D) was calculated using the Scherer's Debeye formula from the full width half maximum (FWHM) given in the table 1. Where D is the average crystallite domain size perpendicular to the reflecting planes. λ is the X ray wave length, k is Dimensionless shape factor, β is the full width at half maximum and θ is the diffraction angle.

Table 1: Determination of crystalline size of SNPs-AECMF fruit by using Debye-Scherrer's equation

S. No	2θ (degrees)	FWHM	B =π*FWHM/180 (radians)	θ	Cos θ	D = k λ / β. Cos θ (nm)
1	27.95	1.0675	0.018621	13.97	0.970	7.67
2	32.42	1.0412	0.018163	16.21	0.960	7.95
3	46.39	1.1125	0.019406	23.19	0.919	7.77

4. Conclusion

The current study revealed that silver nanoparticles can be synthesized in a biological method using *Cucumis melo* fruit extract. The synthesized nanoparticles from *Cucumis melo* fruit observed at 320nm to 380nm in UV-Visible spectroscopy revealed the presence of AgNPs. Morphology evaluation by SEM analysis showed the spherical structure of nanopartciles and XRD shows the crystalline size. Particle size was found to 35nm. The biological method of synthesis of silver nanoparticles from *Cucumis melo* has proved to be an eco-friently method than the chemical methods since the physical and chemical method involves use of hazardous chemicals and highly expensive approaches. However, further *In vivo* studies are needed to fully characterize the potential of the biosynthesized AgNPs using *Cucumis melo* fruit. The outcome of the present study has opened up several promising avenues of possible research may be extended to elucidate the mechanism of action of *Cucumis melo* at molecular level and the potential of silver nanoparticles can be further probed using cancer cells lines as well as other animal models.

5. References

1. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv, 2009: 27:76-83.
2. Gnanajobitha G, Kanniah P, Mahendran V, Shanmugam R, Chelladurai M, Gurusamy A, *et al*. Fruit-mediated synthesis of silver nanoparticles using *Vitis vinifera* and evaluation of their antimicrobial efficacy. J Nano Chem, 2013; 3:67-73.
3. Prabhu S, Poulose EK. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. Int Nano Lett, 2012; 2:32-42.
4. Shalini C, Mukesh KU. Fruit based synthesis of silver nanoparticles an effect of temperature on the size of particles. Recent Res Sci Technol. 2012; 4(5):41-44.
5. Park Y. A New Paradigm Shift for the Green Synthesis of Antibacterial Silver Nanoparticles Utilizing Plant Extracts. Toxicol. Res. 2014; 30(3):169-178.
6. Thirumurgan A, Tomy NA, Jai Ganesh R, Gobi Krishnan S. De Phar Chem. 2010; 2(6):79-284.
7. Ankanna S, Prasad TK, Elumalai EK, Savithramma N. Production of biogenic silver nanoparticles using

Boswellia ovalifoliolata stem bark. Dig J Nanomater Bios, 2010; 5:369-372.

8. Pal S, Tak YK, Song JM. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gram-negative bacterium *Escherichia coli*. Appl Environ Microbiol, 2007; 73:1712-20.
9. Schaffer B, Hohenester U, Trugler A, Hofer F. High-resolution surface plasmon imaging of gold nanoparticles by energy-filtered transmission electron microscopy. Phys Rev B, 2009, 79-82.
10. Jain D, Daima HK, Kachhwaha S, Kothari SL. Synthesis of plant mediated silver nanoparticles using papaya fruit extract and evaluation of their anti-microbial activities. Dig J Nanomater Bios, 2009; 4:557-563.
11. Strasser P, Koh S, Anniyev T, Greeley J, More K, Yu C, *et al*. Lattice-strain control of the activity in dealloyed core–shell fuel cell catalysts. Nat Chem, 2010; 2:454-60.
12. Magudapatty P, Gangopadhyayrans P, Panigrahi BK, Nair KGM, Dhara S. Electrical transport studies of Ag nanoparticles embedded in glass matrix. Physica, 2001; 299:142.
13. Xu H, Kall M. Modeling the optical response of nanoparticle-based surface plasmon resonance sensors. Sensors and Actuators B: Chemical, 2002; 4:244-249.
14. Devi JS, Bhimba BV. Anticancer activity of silver nanoparticles synthesized by the seaweed *Ulva lactuca in vitro*. Open access Sci Rep. 2012; 1(4):1-4.