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Biosynthesis of Silver Nanoparticles from Three *Opuntia sps*

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Abstract

In present work, the rapid biosynthesis of silver nanoparticles was investigated in three different *Opuntia* sps using aqueous extract. The biosynthesised silver nanoparticles were confirmed by visual observation and UV-Vis spectroscopy. Formation of dark brown colour indicated the synthesis of silver in the reaction mixture. The silver nanoparticles were found to be spherical, rod and round in shape with variable size ranging as evident by X-ray diffraction studies, TEM. The X-ray diffraction studies, energy dispersive X-ray analysis and TEM analysis indicate that the particles are crystalline in nature. The nanoparticles appeared to be associated with some chemical compounds which possess amine, hydroxyl and carbonyl groups, confirmed by FTIR. This is first novel report of silver nanoparticles synthesised from *Opuntia* plant extract.

Keywords: *Opuntia*, Cladode, Fruit, Silver nanoparticle

1. Introduction

Nanotechnology signifies one of the key directions in growth of science and technology, which yield materials with unique physical, chemical and biological properties [Feynman R 1991]. Increased interest in nano sized silver nanoparticles is dictated by their antibacterial applications. Silver nanoparticles (AgNP) have been recognized as having inhibitory effect towards many bacterial strains and microorganisms [Prashant M, *et al*.2008]. These nanoparticles can offer extraordinary interactions with bio molecules both on the surface and inside the body cells [Whitesides G M, *et al*. 2003],[Yong P *et al*.2003], which may bring revolution in diagnosis and treatment of many dangerous diseases [Ferrari M 2005].

In addition, silver nanoparticles (AgNPs) have been used as superior disinfectants in water treatment plants, food packaging, wound healing ointments, cosmetics, bandages etc. because of their mutation-resistant antibacterial, antiviral and anti-inflammatory properties [Zangh W *et al*.2003][Klaus-joerger T *et al*. 2001][Willems *et al*. 2005][Krishanaraj C *et al*. 2010]. The biological methods of nanoparticles synthesis using microorganisms [Jebali A *et al*. 2011], enzymes [Ahmad A *et al*.2002], mushroom [Bhat R *et al*. 2011][Philip D 2009] and plant extract [Shankar S *et al*. 2004][Ankamwar B *et al*.2005] [Philip D *et al*. 2006] have been recommended as possible environmental friendly alternatives to chemical and physical methods. Various plant extracts sourced from leaves, bark, seeds, flowers, fruits, latex etc. which act as both reducing and capping agents, have shown many advantages [Dubey S P *et al*.2013][Bar H *et al*.2009][Philip D 2009]. It is well know that the bioactive molecules in the plant extract, phytochemicals, are responsible for the reduction of silver ions. Nevertheless, there are only few reports [Raghunandan D *et al*.2011] on the identification of these bioactive molecules involved in the reduction and stabilization process. Hence, it is important to demonstrate the role of phytochemicals isolated from these plant extracts.

The *Opuntia* belonging to the family Cactaceae is a xerophyte represented with about 200 – 300 species worldwide mainly grown in arid and semi-arid zones. Due to their remarkable genetic variability, the plant shows a high ecological adaptivity and hence encountered in places of virtually all climatic conditions North, Central and South America, the Mediterranean, North, Central and South Africa, the Middle East, Australia, and India [Mohamed-Yasseen Y *et al*.1996][Nobel P S *et al*. 1995]. Traditionally, cactus plants serve as the sources of fruits and vegetables, medicine and cosmetics, forage, building material, and natural colours. However, their uses are still mainly restricted to the countries of origin [Cruse R R *et al*. 1973][Donguez Lpez A *et al*.1995][Hamdi M *et al*. 1997],[Meyer B N 1981][Vigueras G A *et al*. 2001]. *Opuntia* fruits also known as cactus pears or prickly pears are

regionally consumed in the form of fruit or juice and also exported to the European market [Mizrahi Y *et al*. 1997][Senz-Hernndez, C *et al*.2002,]. Recent investigations anticipate, the use of fruit juice as the functional ingredient for the soft drink market and betalainic coloring foodstuff [Castellar R *et al*. 2003] [Stintzing F C *et al*. 31].
The reports also reveals the presence of natural cactus molecules have a high potential interest in human health and medicine [Stintzing F C *et al*. 2003][Alimi H2010].

2. Materials and Methods

2.1 Collection of material

Synthesis of Silver nanoparticles

Selected plant parts were collected from from Gulbarga university campus, Gulbarga. Silver nitrate ($AgNO_3$) is procured from High Media Laboratories. Solutions were prepared with triply distilled water.

Preparation of the extract

25 g of plants parts were washed repeatedly with distilled water, so as to remove any organic impurities present on it and cut into fine pieces. The pieces of selected plant parts were taken into 1000 ml beaker containing 500 ml double distilled water and were exposed to microwave for 180^0C to suppress the enzymes present in the solution. The raw extract obtained was filtered twice with Whatman filter paper No. 42 (pore size 0.45 µm and 0.22 µm sized). The resultant filtrate is nothing but extract of the selected plant extract used for the reduction of Ag^+ to Ag^0. The extract was treated with silver nitrate solution of concentration 10^{-3}.

Synthesis of Silver nanoparticles using selected plant extracts

The aqueous solution of 1mM silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 2-5 ml of plant extract was added into 250 ml of aqueous solution of 1 mM silver nitrate for reduction into Ag+ ions and kept for incubation for 5-30 min at room temperature. The filtrate acts as reducing and stabilizing agent for 1mM of $AgNO_3$.

2.2 Characterization

UV- Visible spectra analysis

The formation of AgNPs is verified by using UV–visible 5704SS ELICO spectrophotometer operated at with 1 nm resolution with optical length of 10 mm. UV–visible analysis of the reaction mixture was observed for a period of 300s.

X-ray-diffraction (XRD) analysis

This analysis carried out for crystalline, films of colloidal AgNPs formed on Si (III) substrates by drop coating were used for X-ray-diffraction (XRD) study. The data were obtained using Ricago X-Ray Diffractrometer (Japan), operated at 30 kV and 20 mA electric power with Cu Ka (I = 1.54 A °).

Fourier Transforms Infra-Red Spectroscopy (FTIR) analysis

The powder sample of AgNPs was prepared by centrifuging the synthesized AgNPs solution at 10,000 rpm for 20 min. The solid residue formed is then washed with deionised water to remove any unattached biological moieties to the surface of the nanoparticles, which are not responsible for biofunctionalization and capping. The resultant residue was then dried completely and the powder obtained was used for FTIR measurements carried out on a Nicolet iS5 FTIR with diamond ATR (Attenuated Reflectance Technique).

Transmission Electron Microscopy (TEM) analysis

The transmission electron microscopy (TEM) images were obtained using Technai-20 Philips instrument operated at 190 keV. Sample for this analysis was prepared by Biosynthesis of Silver Nanoparticles using selected plant extracts 109 coating of aqueous AgNPs drops on carbon coated copper grids, kept for 5 min; the extra solution was removed using blotting paper. The film of TEM grid is exposed to IR light for drying.

3. Results

3.1 Visible colour change

A visible colour change was observed from transparent to brown within time indicating the formation of silver nanoparticles, which was confirmed by UV-visible analysis. r, the colour change to dark orange-brown is due to increased concentration as well as growth of silver nanoparticles. After a particular time there was no significant colour change, which is evidence for the completion of reduction reaction (Fig. 1).

3.2 UV study

On mixing the *Opuntia* plant extract with aqueous solution of the Ag ion complex (100mM silver nitrate, in v/v), solution turned colourless to brown within the particular time. The characteristic surface Plasmon resonance of silver nanoparticles ranges between 300 nm to 400 nm due to excitation of Surface Plasmon vibrations and this is responsible for the striking yellow brown

colour of silver nanoparticles. The specific band of silver nanoparticles was observed around 413 nm for 24 hrs reaction mixture with plant A extract. And 379 nm absorbance peak for plant B, D and F extract, similarly 381 nm and 405 nm peak for plant extract C and E respectively. This band was a signal for formation of spherical nanoparticles in a reaction solution (Fig 2).

3.3 XRD pattern

The average size and crystalline nature of the green synthesized silver nanoparticles were confirmed by X-ray diffraction pattern. AgNPs, is obtained by Powder X-ray diffraction peaks (Fig.3). Three prominent peaks obtained at 2θ angle (Bragg reflections peaks) = 38.19°, 44.22° and 77.7° (X-axis), corresponding fraction between the intensity of (111), (200) and (311) diffraction peaks (Y-axis). Intensity of the (111) facets for the very sharp diffraction peak at 38.19° is considered for the face centred cubic structure. The (111) facet is extremely reactive and stable due to high rate of electron transfer. The XRD facets of the Bio-AgNPs (Ag, JCPDS card No. 04-0783) compared and indexed with standard, which were published by JCPDS file (International centre of Diffraction Data). The average crystallite size (D) of AgNPs were calculated using the Debye-Scherer equation by determining the width of the (111) and the similar Bragg reflection was found to be around 84.23 nm.

3.4 FTIR

FTIR molecular spectrum was obtained in the wavelength range from 500 to 4000 cm^{-1} used to identify the possible functional groups involved in the bioreduction of ions. The spectra were observed for plant extract A, B, C, D, E and F after reacting with reactant molecules (Silver nitrate for the synthesis of AgNPs) by extracellular method. Generally, peaks at 3847 cm^{-1} was assigned to O-H stretching vibration of alcohols and phenolic group of compounds. The strong peaks at 3406 - 3430 cm^{-1}, 1470-1350 cm^{-1} and 725cm^{-1} were attributed to the stretching and bending vibration of C–H in alkanes. In case of AgNPs, synthesized from plant extract A, B, C, D, E and F was attributed to the O–H stretching vibrations of alcohols and phenolic and stretching and bending vibration of C–H in alkanes (Fig.4).

It seems that the FT-IR spectrum for silver nanoparticles synthesized from all six different type of plant extract, the presence of functional groups, such as amide linkages and –COO–, is common and possibly between amino acid residues in protein and the silver nanoparticles. This FT-IR spectrum supports the idea of protein type of compound on the surface of green synthesized silver nanoparticles.

3.5 Tem analysis

In the present investigation, plant extracts A, B, C, D, E and F was found notable in producing silver nanoparticles of different size ranging from 1-100 nm in distribution. TEM analysis reports the presence of green synthesized nanoparticles from *plant* extract A with core shell morphology of size between 19.96 -38.75 nm (Fig 5 A) 26.92-32.17, (plant extract 2, Fig.5 B) size and spherical in shape, silver nanoparticle synthesized from plant extract C is having the size 8.25-19.41 nm , plant extract D ranging from 21.40-47.21 (Fig. 4. C, D) ranging from nm with marginal variation and aggregate form and ranging from 50 nm (plant E and F 15.88-31.47,and 18.11-54.54 and F Fig 4 E & F) in size and approximately spherical and few are in aggregated form.

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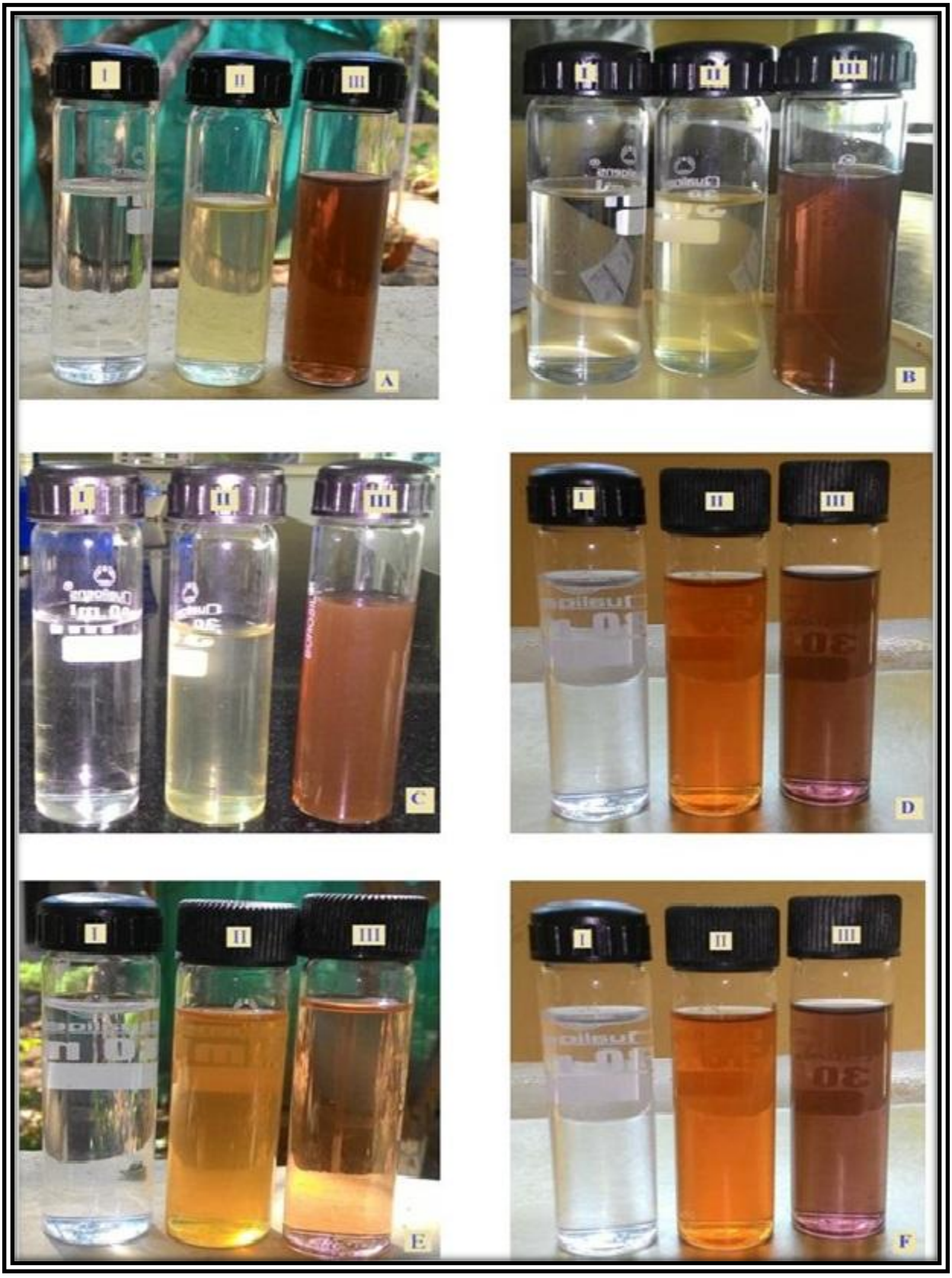

Fig.1: Visible colour change profile of biosynthesised AgNPs

I: silver nitrate solution, II: plant extract, III: synthesised silver nano particle

A: *O. cochenillifera* cladode, B: *O. ficus indica* cladode, C: *O.elatior* cladode, D: *O. cochenillifera* fruit, E: *O. ficus indica* fruit, and F: *O.elatior* fruit

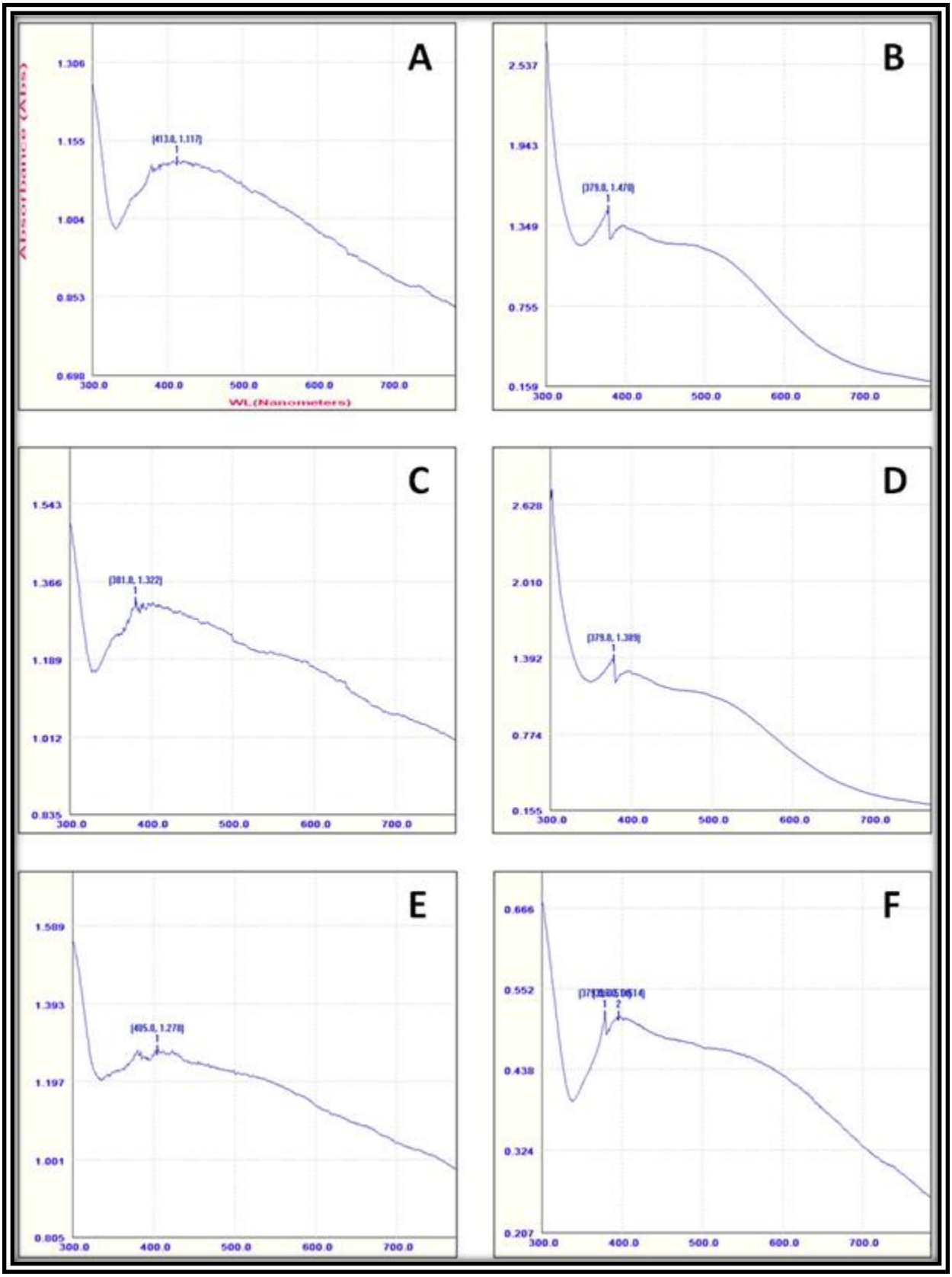

Fig.2: UV-Spectral profile of biosynthesised AgNPs

A: *O. cochenillifera* cladode, B: *O. ficus indica* cladode, C: *O.elatior* cladode, D: *O. cochenillifera* fruit, E: *O. ficus indica* fruit, and F: *O.elatior* fruit

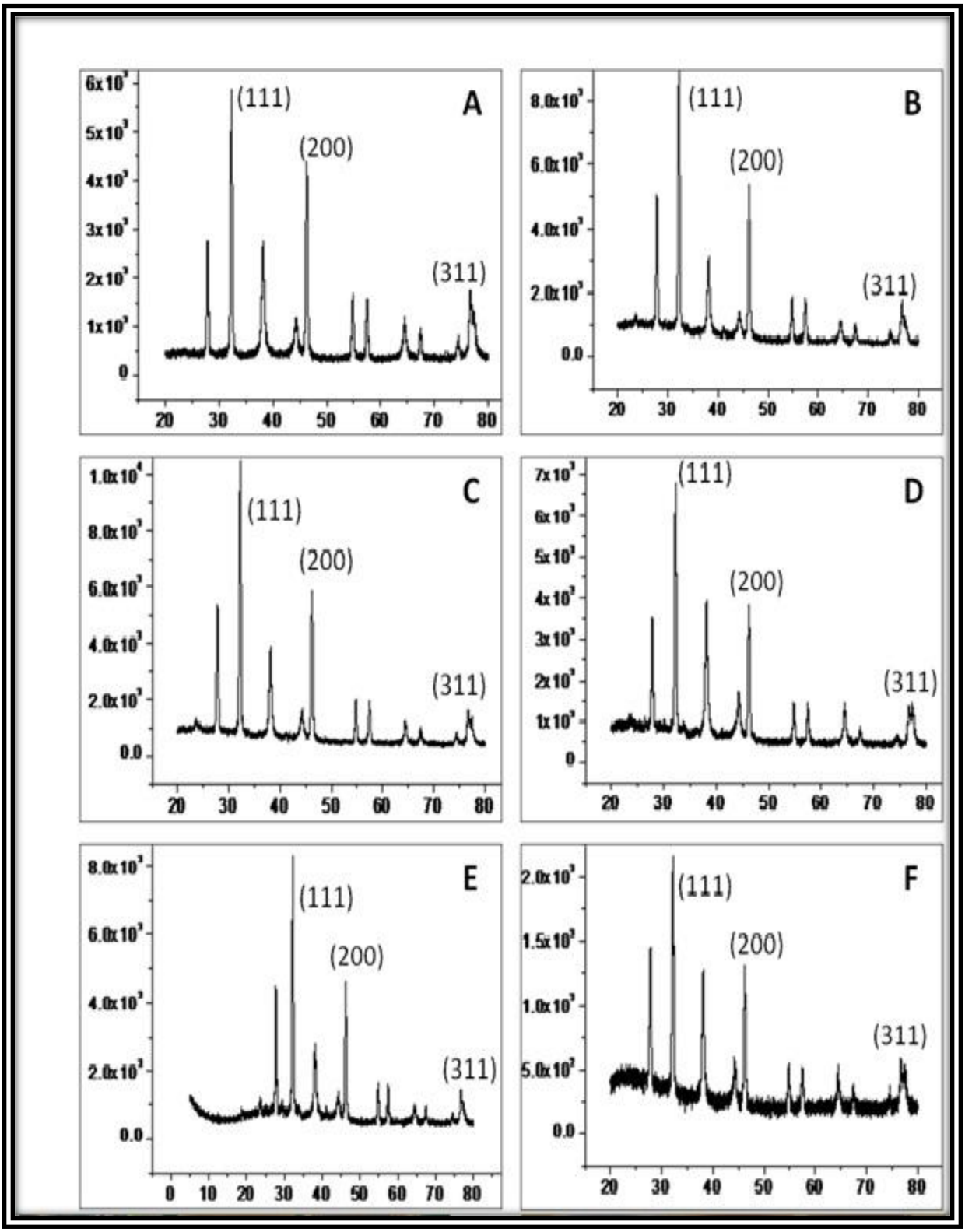

Fig.3: XRD-Spectral profile of biosynthesised AgNPs

A: *O. cochenillifera* cladode, B: *O. ficus indica* cladode, C: *O. elatior* cladode, D: *O. cochenillifera* fruit, E: *O. ficus indica* fruit, and F: *O.elatior* fruit

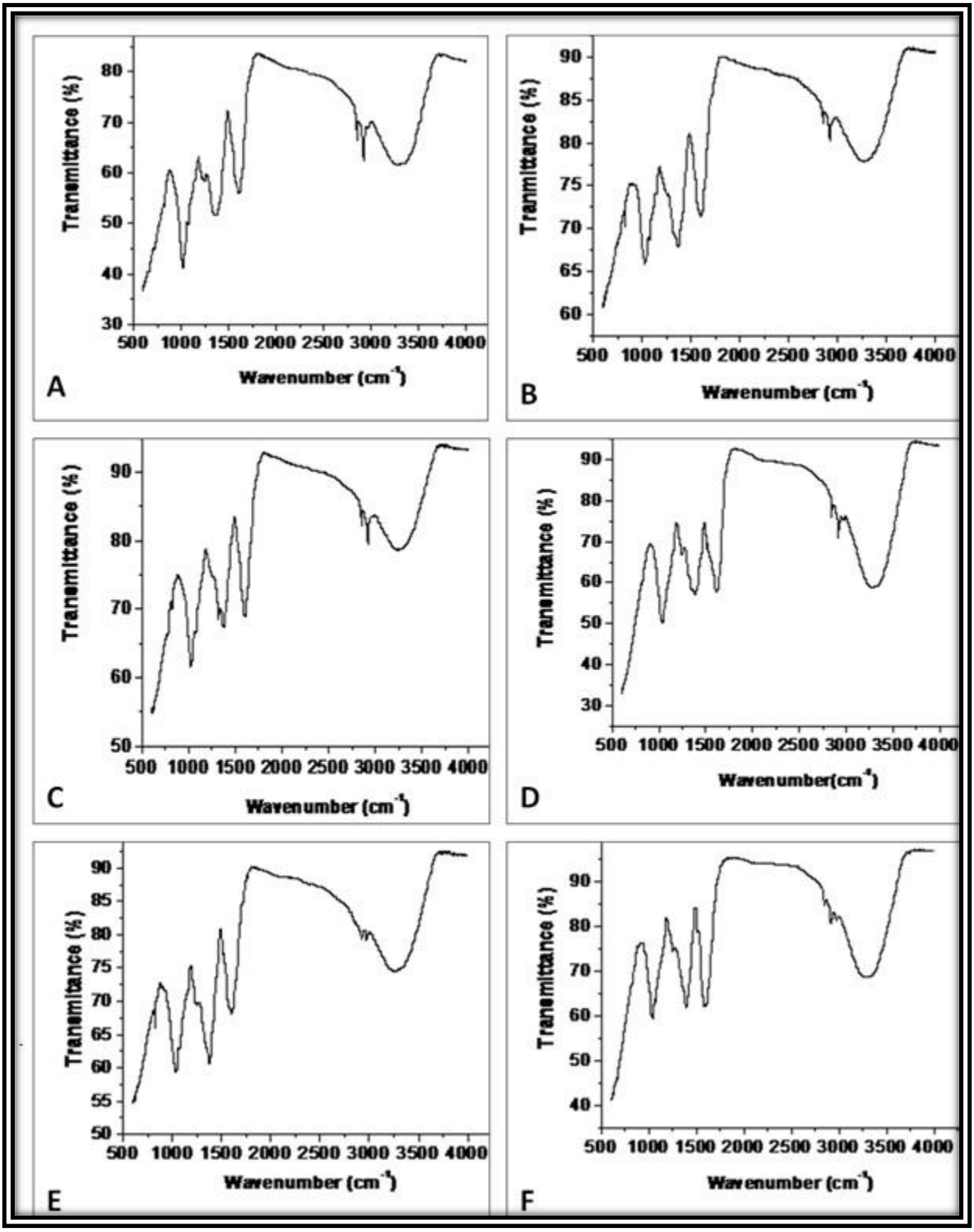

Fig.4: FTIR-Spectral profile of biosynthesised AgNPs

A: *O. cochenillifera* cladode, B: *O. ficus indica* cladode, C: *O.elatior* cladode, D: *O. cochenillifera* fruit, E: *O. ficus indica* fruit, and F: *O.elatior* fruit

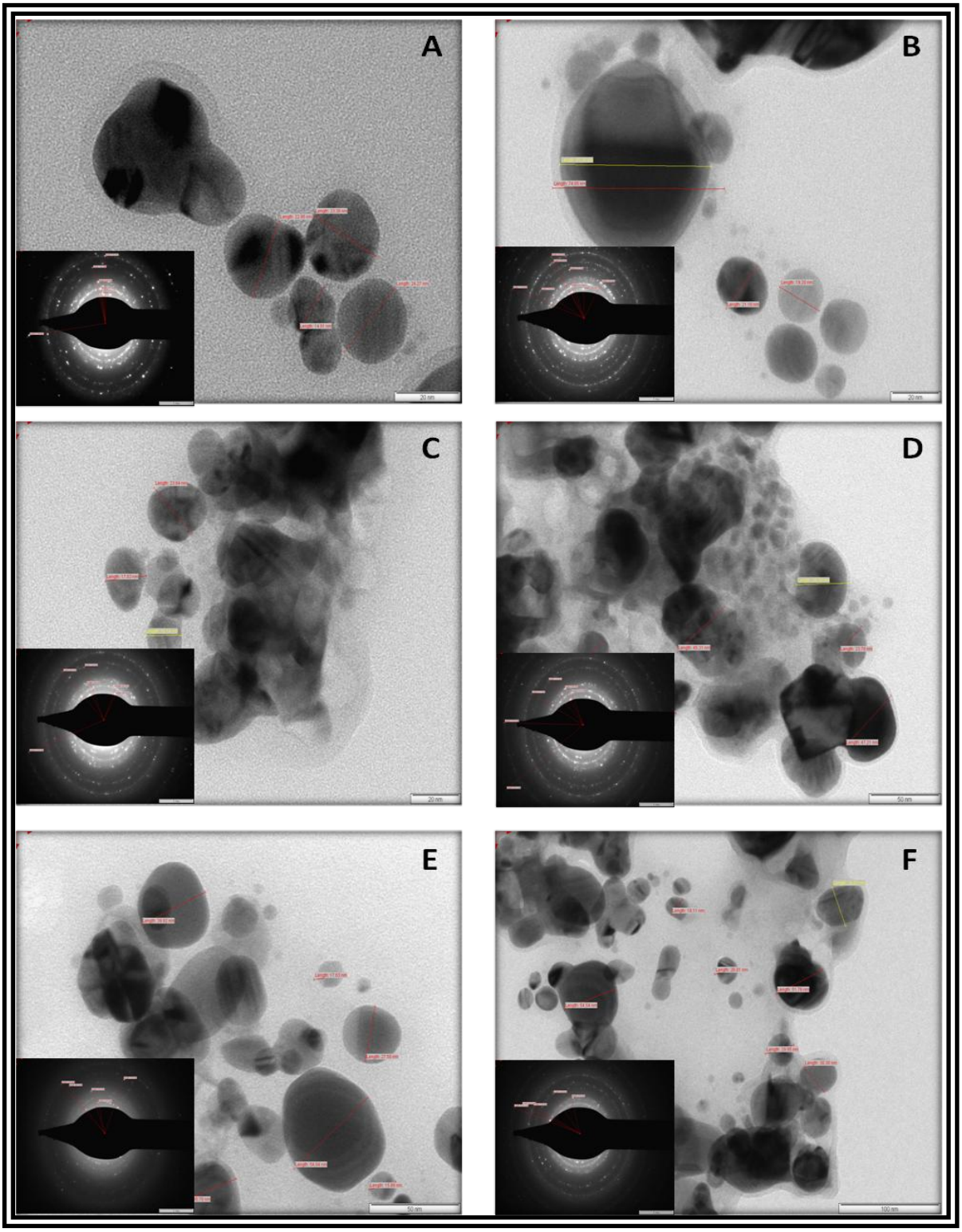

Fig.5: TEM-Profile and SHADE Pattern of biosynthesised AgNPs

A: *O. cochenillifera* cladode, B: *O. ficus indica* cladode, C: *O.elatior* cladode, D: *O. cochenillifera* fruit, E: *O. ficus indica* fruit, and F: *O.elatior* fruit

4. Discussion

The development of easy, reliable and eco-friendly method helps to increase the interest in the synthesis and application of nanoparticles that are beneficial for mankind. Reduction of silver ion into silver nanoparticles during exposure to the plant extracts could be followed by colour change. In this study within 15 to 60 minutes varying from plant to plant extract the colour change was observed at room temperature. In the present report Silver nanoparticles exhibited brown (A), dark brown (B), light brown (C), light orange (D), orange (E), slight brown (F), colors in aqueous solution due to the surface plasmon resonance phenomenon. The synthesized silver nanoparticles using plant extract at 24 h of incubation were reported to have alkaloids, flavonoids, phenols, tannins and terpenoids constituents from the selected plant part extracts and they might be the surface active molecules stabilizing the nanoparticles.

In the present study biosynthesis of AgNPs were synthesized at room temperature it was compared with chemical and physical method of synthesis green synthesis method provides a low cost, environment friendly, easily scale up for large scale synthesis. The previous report by [Sivakumar MVK *et al*. 2011] given as green synthesis method there is no need to use high pressure, energy, temperature and toxic chemicals.

In the present report the production of the silver nanoparticles synthesized from the aqueous extract of 6 plants was evaluated through spectrophotometer at a wavelength range of 400-500 nm and observed characteristic peaks for AgNPs in A,-413, B- 379, C-381, D-378, E-405 & F-378 nm for the extract and AgNO3 mixture, which confirmed the formation of silver nanoparticles. This is similar to the characteristic peaks of the silver nanoparticles prepared by *Geranium* leaf [Shankar SS *et al*.2003]

The frequency and width of the surface plasmon absorption are depend on the metal nanoparticles detecting the presence of silver nanoparticles in plants extracts. This can be achieved by using XRD to examine the diffraction peaks of the plant. In present study the X-ray pattern of synthesized silver nanoparticles matches the FCC structure of the bulk silver and there was no obvious other phases found in the XRD patterns. The X-ray diffraction results clearly show that the silver nanoparticles formed by the reduction of Ag+ ions by all three *Opuntia* cladode and fruit extracts are spherical in nature [Mulvaney P 1996].

TEM analysis reports the presence of green synthesized nanoparticles from *plant* extract A with core shell morphology of size between 19.96 -38.75 nm 26.92-32.17 nm (plant extract B, spherical in shape, silver nanoparticle synthesized from plant extract C is having the size 8.25-19.41 nm , plant extract D ranging from 21.40-47.21 nm with marginal variation and aggregate form and ranging from 50 nm plant E and F 15.88-31.47,and 18.11-54.54 respectively in size and approximately spherical and few are in aggregated form. Plant extract C is synthesized very small size nano particles compare to other plant extract and large size silver nano particle is synthesized by plant extract B.

5. Conclusion

The characterization of AgNPs was performed using UV, XRD, FTIR and TEM spectra's.and shown rapid biosynthesised nano particles from all three different sps of *Opuntia*. Nature has elegant and ingenious ways of creating the most efficient miniaturized functional materials. An increasing awareness towards green chemistry and use of green route for synthesis of metal nanoparticles lead a desire to develop environment-friendly techniques. Benefit of synthesis of silver nanoparticles using plant extracts is that it is an economical, energy efficient, cost effective; provide healthier work places and communities, protecting human health and environment leading to lesser waste and safer products. Green synthesized silver nanoparticles have significant aspects of nanotechnology through unmatched applications. For the syntheses of nanoparticles employing plants can be advantageous over other biological entities which can overcome the time consuming process of employing microbes and maintaining their culture which can lose their potential towards synthesis of nanoparticles. Hence in this regard; use of plant extract for synthesis can form an immense impact in coming decades.

This is the first research report of silver nano particle using *opuntia* plant species. Furthermore application of silver nano particle is needed in the field of medicinal science.

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