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ANTIOXIDANT AND ANTI CANCER STUDY OF GIVEN SYNTHESIZED SILVER NANO PARTICLES FROM *CARICA PAPAYA* LEAVES EXTRACT

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ABSTRACT

The synthesis of noble metal nano particles attracts an increasing Interest due to their new and different characteristics as compared with those of microscopic phase, that allow attractive applications in various fields. Silver nano particles (AgNPs) have the properties of high surface area, very small size and high dispersion. AgNPs are one of the most commonly used nano materials. The aim of the current study were to use *Carica papaya* eaves extract for the synthesized of silver nano particles. DPPH and UV- Visible spectra, XRD, HR-TEM. It reveals that nano particles are poly dispersive in nature. This study proves that silver nano particles has radical scavernging potential than the aqueous extract this may be due to the capping agent found in aqueous extract.

KEYWORDS: AgNPs, *Carica papaya*, DPPH Assay, UV- Visible spectra, HR- TEM, XRD pattern.

INTRODUCTION

Nanobiotechnology is a rapidly growing scientific field of producing and constructing devices. An important area of research in nanobiotechnology is the synthesis of NPs with different chemical compositions, sizes and morphologies, and controlled dispersities. It is a multidisciplinary approach resulting from the investigational use of NPs in biological systems including the disciplines of biology, biochemistry, physics and medicine. Nowadays, there is a growing need to develop eco-friendly processes, which do not use toxic chemicals in the synthesis protocols. *Carica papaya* belongs to family Caricaceae and commonly known as *Papaya*, Paw Paw, Kates and Papaw.

MATERIALS AND METHODS

Collection and Identification

Fresh papya leaves ***Carica papaya*** were collected from Presidency college, Chennai campus, Tamil Nadu, India and were authentically identified by Prof. P. Jayaraman, Institute of Herbal Science, Plant Anatomy Research Centre (PARC), West Tambaram, Chennai, India, as Caricaceae with voucher specimen no: PARC/2017/232.

Taxonomical Classification

Kingdom : Plantae

Order : Brassicales

Family : Caricaceae

Genus : Carica

Species : Papaya

Binomial name : *Carica papaya*

Fig. 1: *Carica papaya* leaves.

Preparation of *C. papaya* leaf extract and 1mM silver nitrate

Fresh leaves of *C. papaya* (50 g) were sliced into fine pices and then transferred to a clean and sterile 250 ml conical flask. Double distilled water 200 ml was added to the flask and heated at 50° C for 15 min and incubated on a water bath for 30 min to facilitate the formation of aqueous extract. The aqueous extract was filtered using Whatman No. 1 filter paper and the filterate was stored at -4° C for further analysis. Silver nitrate (HIMEDIA, India), 0.0421 gram was added to 100 ml of double distilled water and dissolved thoroughly. The aqueous

silver nitrate solution obtained was transferred to an brown coloured amber bottle to prevent autoxidation of silver.

Characterization of *C. papaya* leaf silver nanoparticles

Characterization of the synthesized silvernanoparticles were carried out by UV-VIS absorption spectrum was recorded in the region 200-1000 nm using Varian Caty-5 UV-VIS-NIR Spectrometer. The FTIR spectra were recorded using Perkin Elmer Spectrum -1 in the range of 4000-450 Cm^{-1} at a resolution 1.0 Cm^{-1} based on KBr pellet technique, X-Ray Diffraction (XRD) patterns were recorded with a PAN Philips X'Pert ProX-ray diffractometer using CuKα radiation low angle diffract grams were recorded in the 2 theta range 5.0-80° with a 2 theta step size of 0.02° and a step time of 20 sec at each point, The surface morphologies were characterized by transmission electron microscopy (TEM) using Philips Techani 10 and high resolution scanning electron microscope (HRSEM) with energy dispersive X-ray analyser (EDAX) using FEI Quantum 200 MK II.

RESULTS AND DISCUSSION

Antioxidant potential of *C. papaya* aqueous leaf extract and synthesized silver nanoparticles

UV- vis analysis of green phyto -synthesized solutions

The aqueous extracts of papaya leaves () when mixed in aqueous solution of silver ion complex, the reduction of pure Ag^{+} ions to Ag^{o} was monitored by measuring UV-vis spectrum of the reaction media. The reduction of silver nitrate into AgNPs during exposure to plant extract is followed by a gradual increase in colour development from clear to drak brown colour, as a result of the surface plasmon resonance phenomenon (Fig.1).

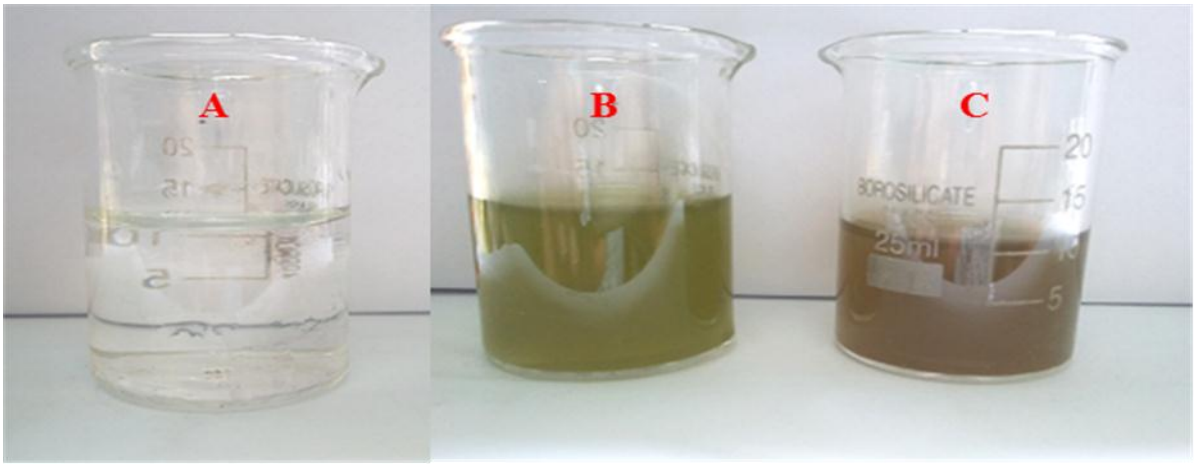


Fig. 1A. Color intensity of 1 mM $AgNO_3$ solution.

B. Aqueous extract of *Carica papaya* (pale light green colour).

C. AgNPs synthesized at different hours with pale light green colour to dark brown colour.

The UV- visible spectral analysis of (*Carica papaya*) without AgNO3 did not show any change in colour. The UV-vis spectra of all the samples exhibited distinct surface plasmon resonance bands. The position of plasmon resonance band in UV-vis spectra is sensitive to particle shape, size, its interaction with the medium and the extent of charge transfer between medium and the particles. Here, the sharp bands were centered at 445.7 nm in (*Carica papaya*) AgNPs extract clearly indicate the presence of silver nanoparticles (Fig. 2).

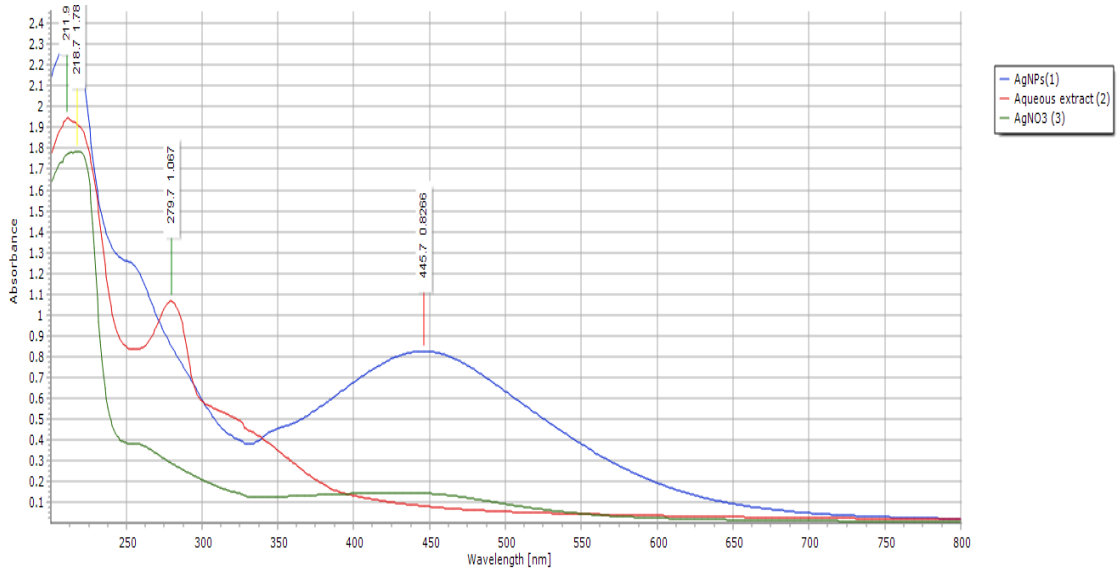


Fig. 2: UV-Vis spectral image of *Carica papaya* aqueous extract based synthesized silver nanoparticles.

XRD pattern of *Carica papaya* aqueous extract based synthesized silver nanoparticles

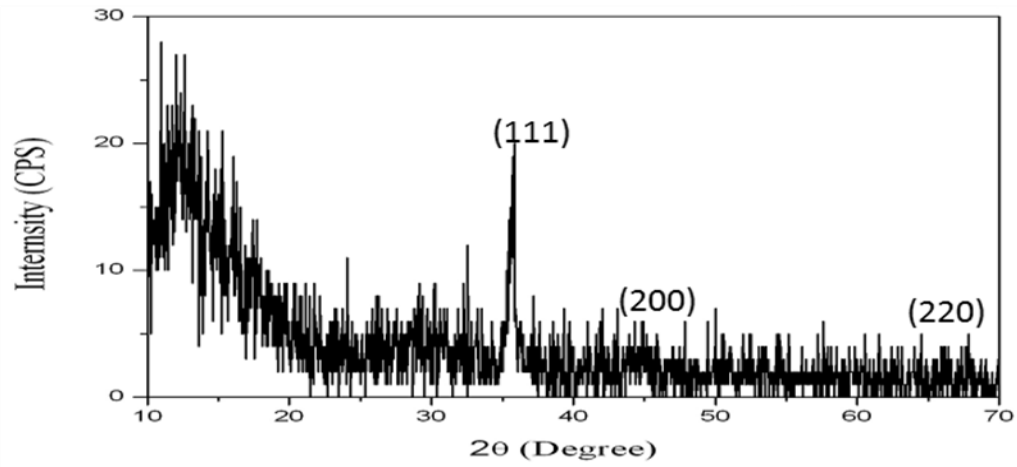
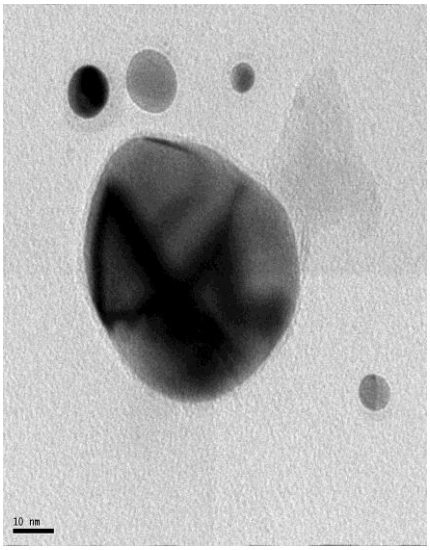
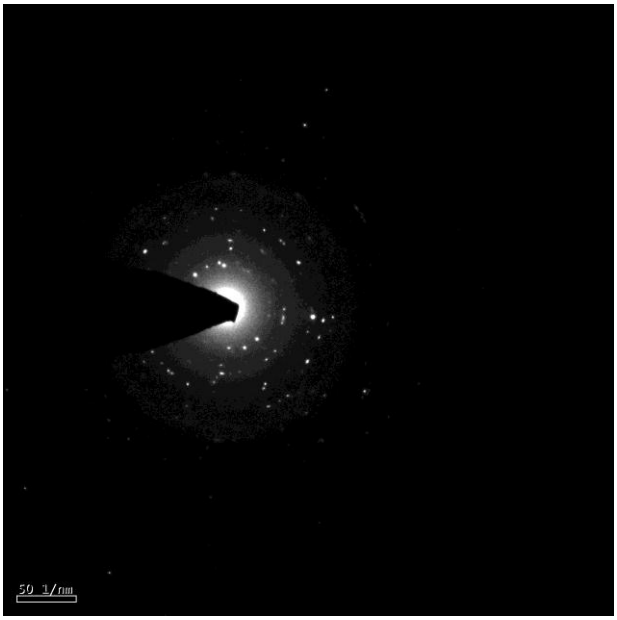


Figure shows the XRD pattern of *Carica papaya* aqueous extract based synthesized silver nanoparticles which exhibits strong and broad diffraction peaks at and= θ2 24.9 41.30. The four other characteristic peaks showed in the case of Ag^0 nanocomposite hydrogel indicates the face-centered cubic (fcc) structure. The diffraction peaks at and= θ2 38.9, 44.62 and 64.9 corresponding to the reflections of crystal planes (1 1 1), (2 0 0) and (2 2 0) respectively. This face- centered cubic (fcc) structure indicates that Ag0 nanoparticles were dispersed in the *Carica papaya* aqueous extract based synthesized silver nanoparticles by the bioreduction method.

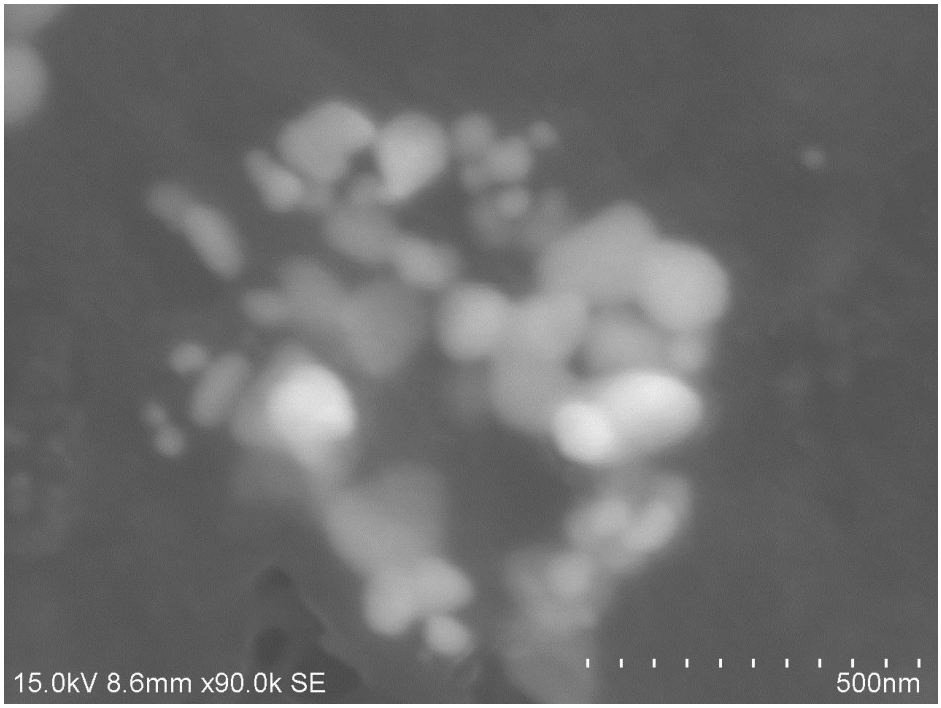




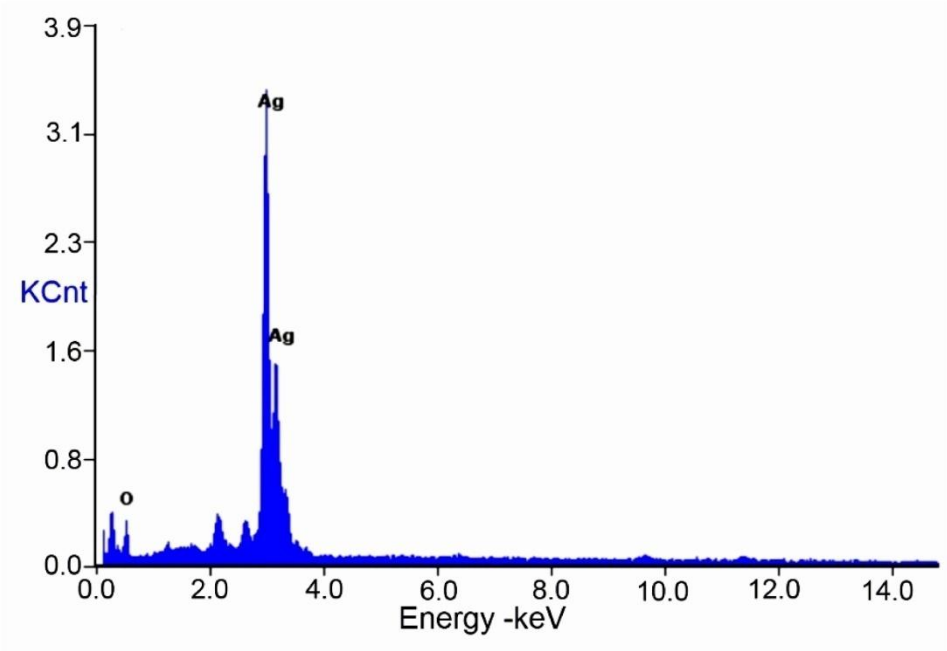
A **B**

A- HR-TEM image of *Carica papaya* aqueous extract based synthesized silver nanoparticles
B- SAED pattern image of *Carica papaya* aqueous extract based synthesized silver nanoparticles.

HR-TEM analyses showed the particle size 10 nm with a spherical morphology and the SAED pattern reveals that nanoparticles are polydispersive in nature. The FESEM image of synthesized silver nanoparticles also showed the sperical shaped nanoparticles with the aggregation. The EDX spectrum also confrims the bioreduction of silver nanoparticles with the peaks of silver and oxygen and this may be due to the surface plasmon resonance (SPR).



FE-SEM image of *Carica papaya* aqueous extract based synthesized silver nanoparticles



EDX Characterization spectrum obtained for *Carica papaya* aqueous extract based synthesized silver nanoparticles.

DPPH Assay

The antioxidant potential of aqueous extract and aqueous exctract based synthesized silver were assessed by DPPH and it reveals that 50% inhibition *i.e.* was IC50 was obtained 57.22

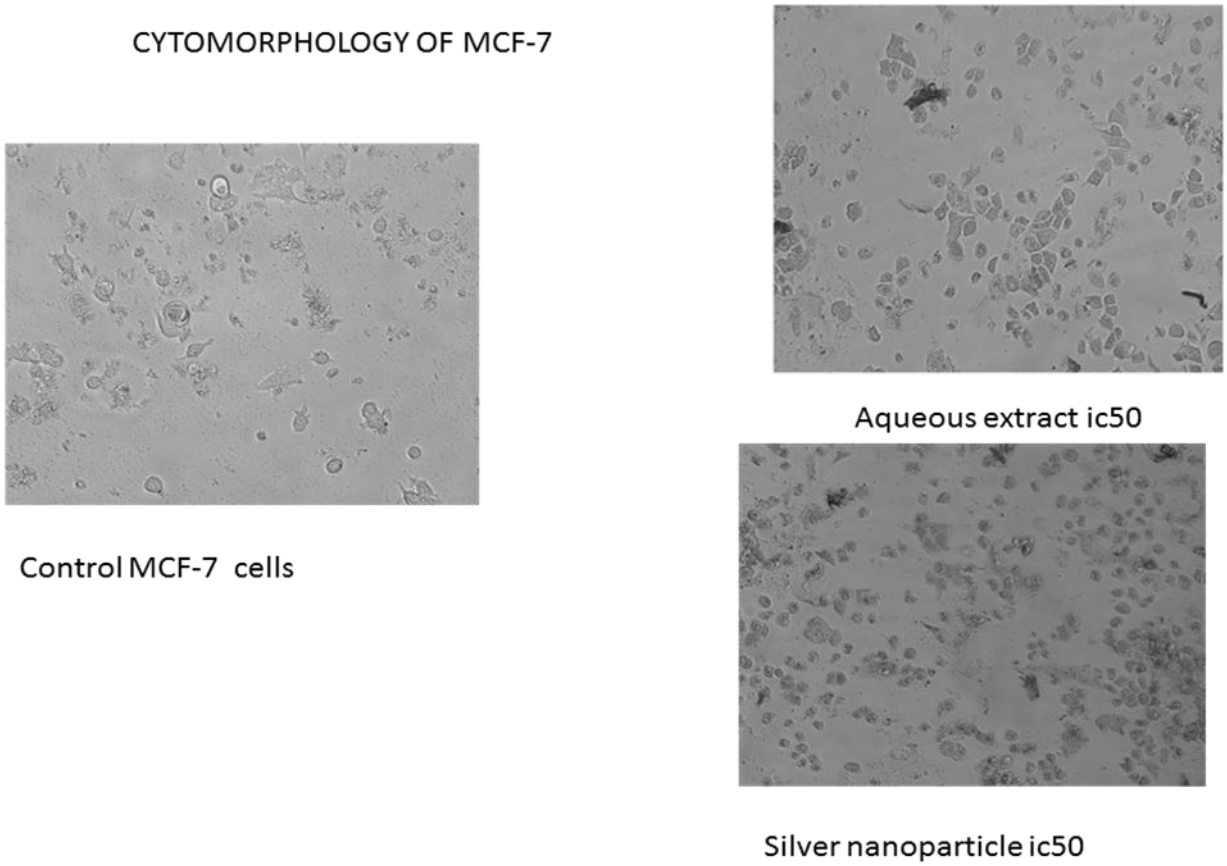
µg/ml in silver nanoparticles than aqueous extract at 75.18 µg/ml and this study proves that silver nanoparticles has radical scavernging potential than the aqueous extract this may be due to the capping agent found in aqueous extract.

MTT assay

The cell viability was assessed using anti-proliferation activity by MTT assay for 24 hrs and 48 hrs. The anti-proliferation increased with increase in concentration. The anti-proliferation activity was noted in the *Carica papaya* leaves extract and aqueous extract synthesized silver nanoparticles which is directly proportional to the concentration. The MTT results showed that 50% cell viability loss at the dose of in 5.647µg/ml at 48 hrs of incubation in silver nanoparticles and the treated cells showed distinct cellular morphological changes indicating unhealthy cells, whereas the control appeared normal in shape. Control cells were irregular confluent aggregates with rounded and polygonal cells. Whereas aqueous showed 50% cell viability loss at 5.986 µg/ml at 48 hrs and this result proves that silver nanoparticles has more cytotoxic property than the aqueous extract and in future the synthesized silver nanoparticles can be used to many dreadful cancers.

Nuclear Staining and Apoptotic Morphology (DAPI Staining Method)

The morphological investigations on aqueous extract and aqueous extract based synthesized silver nanoparticles nanoparticles with MCF-7 cells are depicted with broken up cells leading to apoptotic bodies. Morphological evolution of the MCF-7 cell lines treated with aqueous extract and aqueous extract based synthesized silver nanoparticles at IC_{50} concentration of 5.989 µg/ml for 48 hrs (aqueous extract) and silver nanoparticles nanoparticles (5.647 µg/ml) at 48 hrs using phase contrast microscopy revealed morphological features of apoptotic cells, membrane blebbing and shrinkage of the cytoplasm and DNA fragmentation of the nuclear chromatin in comparison with control. ethanolic extract of *Carica papaya* induced MCF-7 cells apoptosis was confirmed by using DAPI, which easily penetrate to the apoptotic cells. The apoptotic cells loose the membrane phospholipid, which leaves phosphatidylserine on the outer surface the plasma membrane.



CONCLUSION

The green synthesis of AgNPs using *Carica papaya* leaf extract was shown to be rapid, eco-friendly and produced nanoparticles are fairly uniform in size and shape. AgNPs began to form within 10min and higher formation yield at 70min after addition of leaf extract to silver nitrate as shown by the UV-vis spectrum at 445.7 nm. It was found that the formation of AgNPs was increased with time. The EDX spectrum also confrims the bio-reduction of silver nanoparticles with the peaks of silver and oxygen and this may be due to the surface plasmon resonance (SPR).

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