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Preparation and Characterization of Fruit-Mediated Silver Nanoparticles using Pomegranate Extract and Assessment of its Antimicrobial Activities

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Abstract

Today the "Green" nanoparticle synthesis has been achieved using environmentally acceptable solvent systems and eco-friendly reducing and capping agents. The present study investigated the synthesis of silver nanoparticle using pomegranate fruit extract as reducing agent to synthesize silver nanoparticles. The formation of nanosized silver was confirmed by its characteristic surface plasmon absorption peak at around 460 nm in UV–vis spectra. The morphology and crystalline nature, were characterized by Scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDAX) X-ray diffraction (XRD), and conform the active functional groups present in the synthesized silver molecule by Fourier transform infra-red (FTIR) spectroscopy. Moreover, their antibacterial activity has been tested against *Bacillus subtilis,* (Gram positive) and *Klebsiella planticolae* (Gram-negative). The approach of plant-mediated synthesis appears to be cost efficient, eco-friendly and easy alternative to conventional methods of silver nanoparticles synthesis.

Keywords *: Biosynthesis ; Bacillus subtilis ; Klebsiella Planticolae ; Pomegranate fruit and Silver nanoparticle.*

1. INTRODUCTION

Nanotechnology is a field that is developing day by day, making an impact in all spheres of human life and encouraging a growing sense of excitement in the life sciences especially biomedical devices and biotechnology (Singh et al. 2010; Prabhu et al. 2010). Nanobiotechnology describes an application of biological systems for the production of new functional materials such as nanoparticles. Nanoparticles are being viewed as fundamental building blocks of nanotechnology. The nanoparticles exhibit entirely new or improved properties based on specific characteristics such as size, distribution and morphology (Jain et al. 2009). Materials by changingtheir size from normal to nanometer range shows a great change in their physicochemical properties primarily due to their small size and large specific surface area (Prusty,2011).

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Nanometer-sized particles have shown special toxicity and are usually more toxic than the bulk material of larger size (Donaldson et al. 1999). Biosynthesis of metal nanoparticles is gaining impetus as an environment friendly technology in material science (Ingle et al. 2008). Recently research on synthesis of nanoparticles using microbes (Kathiresan et al. 2010; Natarajan et al. 2010) and plant extracts is going on and mainly cost depends on the many aspects like the metal particles, and isolate the chemicals added as the precursor for the nanoparticle synthesis. The most effectively studied nanoparticles today are those made from noble metals, in particular Ag, Pt, Au and Pd. Metalnanoparticles have marvelous applications in the area of catalysis, optoelectronics, diagnostic biological probes and display devices.

Among the above four, silver nanoparticles play a significant role in the field of biology and medicine (Leela and Vivekanandan, 2008).

Fabrication of inorganic nanoparticles by plants is rapid, cost effective and eco-friendly process (Rai et al. 2008). The synthesis can be done by both intra and extracellular methods, such as leaf broth (Shivshankaret al. 2003, 2004a, 2004; Song and Kim, 2008; Gade et al.2010), sun-dried leaves (Huang et al. 2007), fruits (Li et al. 2007; Dubey et al. 2010), seeds (Bar et al. 2009), bark (Sathishkumar et al. 2009) etc. Metal nanoparticles prepared by chemical and biological methods uses reducing agents for reduction of metal ions and a protective agents or phase transfer agents to stabilize the nanoparticles.

In this communication, we demonstrate the synthesis of silver nanoparticles using pomegranate fruit extract. *Punica granatum* L. (pomegranate) belongs to the family Punicaceae. Pomegranate arils juice is the good source of vitamin C, B_5 and natural phenols, such as ellagitannins and flavonoids. In the Ayurveda system of medicine, the pomegranate has been extensively used as a source of traditional remedies for thousands of year. The arils juice are considered a tonic for the heart, throat etc.

In the aqueous solution of pomegranate fruit extract by introducing solution of silver nitrate, the development of stable nanoparticle is hurried, simple and discernible. And the morphological characterizations are performed using scanning electron microscope (SEM) and X-ray diffractometer (XRD).The optical absorption properties are measured using U V-visible spectrophotometer and observed the absorption peaks in 450-480 nm regions, which are close to the characteristics surface plasmon resonance (SPR) wavelength of metallic silver. The interaction between nanoparticles with functional groups was confirmed by using Fourier transform infrared spectroscopy analysis (FTIR). Further the antimicrobial activity of these biologically synthesized silver nanoparticles was evaluated against different pathogenic microorganisms.

2. MATERIAL AND METHOD

2.1. Chemicals

$AgNO_3$ was purchased from Himedia, Mumbai, India. The bacterial cultures of *B.subtilis* and *K.planticolae* were obtained from Microbial Type Culture Collection, Chandigarh, India.

2.2. Preparation of fruit extract

Ripe fruits of pomegranate were used for the preparation of extract (fig.1). The arils of fruit weighing 25g were thoroughly washed in distilled water and dried, cut into small pieces and were crushed into 100ml of sterile water. Extract was filtered by using Whatman filter paper No-1, this filtrate was collected and second filtration done using the same type of filter paper but with small pore size. The pure form of filtrate was collected as sample.

(A) Pomegranate fruit (A) Pomegranate arils

Fig. 1 : (a) Pomegranate fruit (b) Pomegranate fruit arils.

2.3. Formation of Silver nanoparticle

1 mM of 95 ml silver nitrate (0.017 g) solution was prepared and kept in a 250 ml Erlenmeyer flask. 5ml of pomegranate fruit extract was added to the silver nitrate solution. The 95% of the bioreduction of $AgNO_3$ ions occurred within 4 hours (fig.2). The pink colored solution which it turned brown slowly, indicating the formation of silver nanoparticles.

3. APPARATUS FOR ANALYSIS

After completion of nanoparticle synthesis process, the particle containing solution was centrifuged to separate the nanoparticles and this process was repeated 2 or 3 times to remove unwanted garbage. Then it was dried in Hot air oven at 70ºC. The synthesized nanoparticles was indicated by UV-visible spectroscopy, and it was carried out in Perkin-Elmer spectrophotometer operating in a wavelengthfrom 300-900 nm. The morphology of the nanoparticles was identified by scanning electron microscope and it was performed in Philips Scanning electron microscope. The presence of elemental silver in the solution mixture was identified by energy dispersive X-ray spectrophotometer (EDX) and it was operated at the accelerating voltage of 20 kV. The crystallinity of the nanoparticles was evident from XRD measurements. Fourier transform infrared (FTIR) spectroscopic data indicate a bonding of Ag NPs with functional group of fruit extract through bridging linkage. The antimicrobial activities were done by using bacteria like *Bacillus subtilis, Klebsiela planticola* for biomedical application.

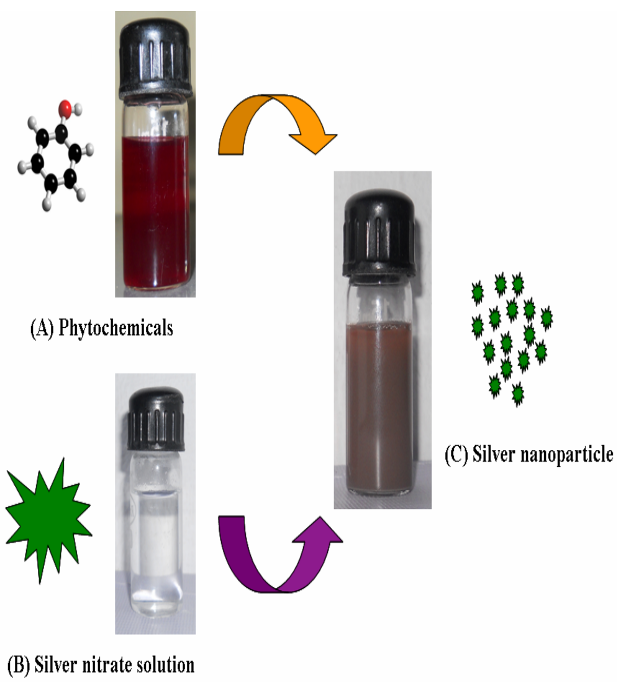

Fig. 2 : Photography scheme of the colloidal silver nanoparticles synthesized in aqueous fruit solution. The formation of silver nanoparticles and it is identified by the colour changes (a) Fruit extract without addition of silver nitrate, (b) Silver nitrate solution, (c) Fruit extract with silver nitrate solution.

3.1. UV-Spectrophotometer

At the very beginning of the biosynthesis, reduction of silver ions present in the aqueous solution of silver complex during the reaction with the ingredients present in the pomegranate fruit extract was seen by the UV-Vis spectroscopy. After mixing the pomegranate fruit extract in the aqueous solution of the silver ion complex, solution slightly started to change colour from pink to brown. Colloidal solutions of Ag nanoparticles showed a very intense colour, which is absent in the bulk material as well as formation of individual atoms. It was an indication of silver nanoparticles formation as the color change observed is due to excitation of surface plasmon vibrations in the silver nanoparticles (Darroudi et al. 2011). The surface plasmon resonance band observed around 450-470nm (fig.3).

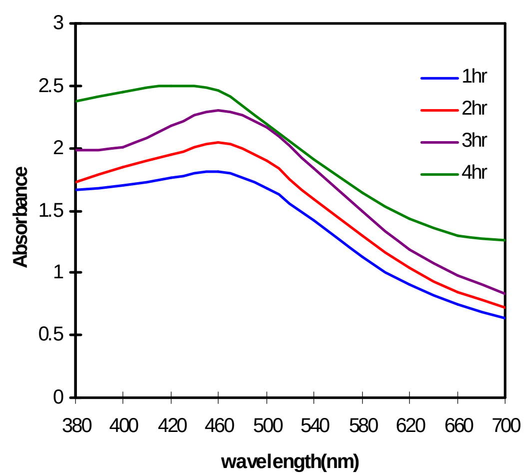

Fig. 3 : UV-Spectra recorded after the exposure of 5 ml fruit broth to 1mM silver nitrate solution at different time intervals and observed maximum absorbance at 460 nm

3.2. Scanning electron microscope (SEM)

SEM technique was employed to visualize the size and shape of Ag nanoparticles. This scaning electron micrograph was taken using a Philips Scanning electron microscope. Dried powder of the silver was placed on carbon-coated copper grid. The SEM characterizations of the synthesized Ag nanoparticles are shown in Fig. 4. Image of SEM showed relatively spherical shape nanoparticle formed with diameter range 30-40nm. The nanoparticles were examined under various magnifications such as X 25,000, 37,000, 60,000 and 70,000. The particle aggregation was clearly viewed in the X 60,000 and it indicates the completion of the nanoparticle synthesis process and no action of stabilization of nanoparticles.

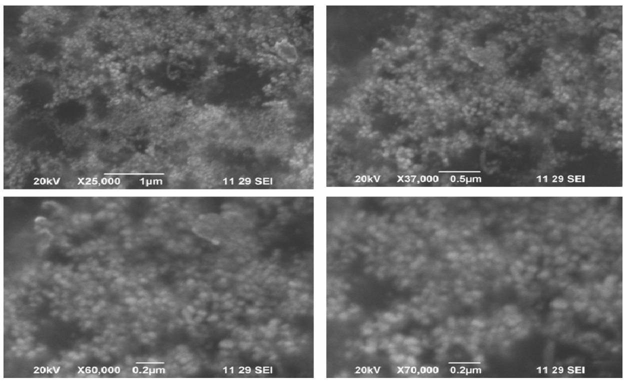

Fig. 4 : SEM image of synthesized Ag nanoparticles.

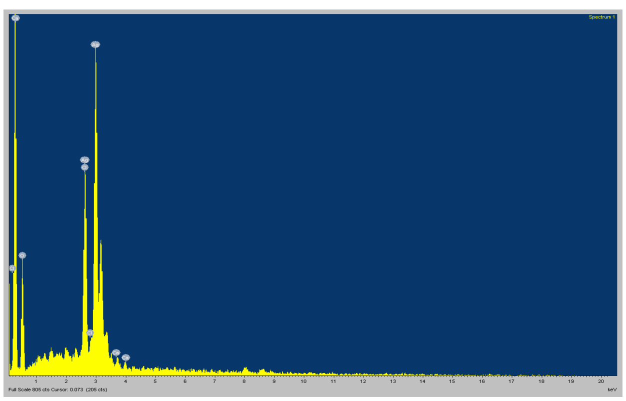

Fig. 5 : EDAX Spectrum of silver nanoparticle prepared from pomegranate fruit extract

3.3. EDAX

EDAX exposed strong signal in the silver region and confirmed the formation of silver nanoparticles (fig.5). Metallic silver nanocrystals generally show typical absorption peak approximately at 3KeV due to surface plasmon resonance (Magudapatty et al. 2001) . And also a strong signal for Ca and some other week signals are recorded possibly due to elements (Cl, O) from enzymes or proteins present within the extract of pomegranate fruit. These results are consistent with other reports on the EDAX analysis of silver structures synthesized by using extracts derived from the leaves of *Catharanthus roseus* (Mukunthan et al. 2011).

3.4. XRD

The XRD pattern thus clearly shows that the Ag nanoparticles formed by the reduction of Ag ions by pomegranate fruit broth are crystalline in nature. Purified silver nanoparticle casted onto the glass substrate was carried out using Phillips PW 1830 instrument operating at a voltage of 40 kV (fig.6). The broadening of the Bragg's peaks indicates the formation of silver nanoparticle (Smitha et al. 2009). The average size of Silver nanoparticles was calculated by using Scherrer's equation.

$$D = \frac{K\lambda}{\beta_{1/2} COS \ \theta} \quad \text{(1)}$$

D- Average grain size of crystallite, λ- Incident wavelength, θ- Bragg angle, β- Diffracted Full Width at Half maximum (in radians) respectively. The XRD patterns displayed are consistent with earlier reports on microstructures (Ghodake et al. 2010).

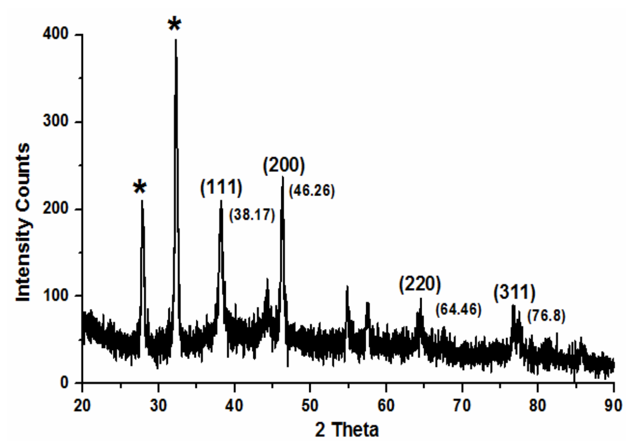

Fig. 6 : XRD spectrum of silver nanoparticles synthesized by Pomegranate fruit extract

3.5. FTIR

FTIR measurements were to identify some of the possible biomolecules responsible for the reduction of the

Table 1. Antibacterial activity of various concentrations of Ag nanoparticles against selective bacterial pathogens (*Bacillus subtilis. Klebsiella planticola*).

Bacterial *sp*	Silver nanoparticle concentration (µl)					Negative control (mm)
	10 µl	20 µl	30 µl	40 µl	50 µl	
Bacillus subtilis (Zone of inhibition-mm)	7	7	8	9	9	0
Klebsiella planticola (Zone of inhibition-mm)	8	9	9	10	11	0

Ag^+ ions and the capping of the bioreduced silver nanoparticles synthesized by the broth (Ahmad et al. 2010). The broth after complete reduction of Ag^+ was centrifuged at 12,000 rpm for 20 minutes to isolate the silver nanoparticles free from proteins or other compounds present in the solution.

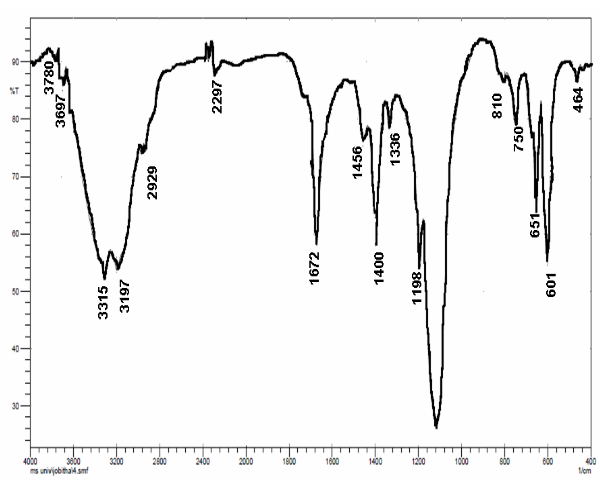

Fig. 7 : FTIR adsorption spectra of silver nanoparticles synthesized by Pomegranate fruit extract after bioreduction of silver ions.

The representative spectra of nanoparticles obtained obvious absorption peaks located at about 3315, 3197, 2929, 1672, 1400, 1193, 651 and 601, as well as some intensity peaks decreased like 3780, 3697,2297,1456,1336,810,750 and 464 . Fig.7 shows the band at 3315 correspond to N-H, O-H Stretching vibrations of 1°, 2° amines, amides, alcohol and H–bonded to phenols. The peak at 3197 indicates to O-H stretching vibrations to carboxylic acids. The peak at 2929 represents to C-H in plane bend to alkenes. The peek at 1672 corresponds to C=O stretching vibrations to carbonyls. The band at 1400 corresponds to C–C stretching vibration to aromatics. The peak at 1193 indicates C-O stretching vibrations and it corresponds to the presence of alcohols, carboxylic, acids, ethers, esters.

The band at 651 corresponds to C–H bend to alkenes. The peak at 601 indicates to C–Cl stretching vibration to alkyl halides. The weak band at 3780 corresponds to N%H stretching to primary amines. The peak at 3697 corresponds to C – OH stretching to alcohol. The peak indicates to P-H stretching to Phosphines. The peak at 1456 corresponds to C-H stretching to alkanes. The peak at 1336 indicates to C-F stretching to alkyl halides. The band at 810 corresponds to C–Cl stretching to alkyl halides .The weak band at 750 corresponds to C –H to aromatic group in the fruit extract. The presence of active functional groups in seed extract results in the swift reduction of silver ions to silver nanoparticles. To obtain good signal to noise ratio of silver nanoparticles were taken in the range 500–3400 cm.$^{-1}$

3.6. Antibacterial activity

Antibacterial activity of the synthesized Silver nanoparticles was determined using agar disc diffusion assay method (Mahitha et al. 2011). This was confirmed by the inhibitory effect on bacterial growth as reflected by the inhibition zone compared to known antibiotics. The sterile Muller hinden agar medium (20 ml) in Petri dishes was uniformly smeared using sterile cotton swabs with test pure cultures of pathogenic bacteria, *Bacillus subtilis, Klebsiela planticola.*. The sterile discs (HiMedia -Diameter 5mm) were impregnated with different concentrations (1:5 diluted) of the pellets like 10µl, 20µl, 30µl, 40µl and50µl of the diluted compounds and dried for 10-15minutes. The dried discs were placed on MH agar surface. The plates were incubated at 37ºC for 24 hr and the resulting zone of inhibition was measured (Table:1). The used fruit of pomegranate as a source which is easily obtainable and extensively useful in biomedical applicationis deduced from this.

4. CONCLUSION

"Green" synthesis of metallic nanoparticles has received increasing attention due to the development of eco-friendly technologies in materials science. The present study demonstrated the bioreductive synthesis of nano-sized Ag particles using pomegranate fruit extract. The small and spherical shaped Ag nanoparticles were formed by fruit broth. The reduction of silver ions and stabilization of the silver nanoparticle were formed by the precipitation of fruit broth consist of proteins and other molecules. Most importantly, the reaction was simple and convenient to handle; and it is believed that the *in vitro* phytosynthesis of silver nanoparticles has more advantages over other biological synthesis.

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