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Bio synthesis of silver nano particles using sapota fruit extract and its antibacterial activities

G. Vasuki[1] R. Selvaraju[2] A. Abdul sardhar[3]
[1]Department of physics, Sethupathy Govt. Arts college, Ramanathapuram
[2]Department of Engineering Physics, Annamalai University, Annamalainagar-608 002, Tamil Nadu, India.
[3]Department of Chemistry, syed hameedha arts and science college, kilakarai.
[1]Corresponding author E-mail: vasurmd235@gmail.com

Abstract: *The modern research on synthesis and structure manipulation of a particle in the nanometer size is gaining importance as it finds applications in significantly addressing the problems associated with healthcare ,cosmetics ,biomedical, food and feed, drug/gene delivery environment health ,mechanics, chemical industries, electronics and catalysis. There are several chemical, physical and biochemical methods for the preparation of the metal nanoparticles (NPs. In this present work, we describe a cost effective and environment friendly technique for green synthesis of silver nano particles. Green synthesis of silver nanoparticles (Ag Nps) was carried out using pommigranate fruit extract were characterized based on the observations of UV–visible spectroscopy, Fourier transform infrared spectroscopy (FT-IR), X-RAY diffraction(XRD).*

Key Words: *Sapota fruit extract, Silver nano particles, U-V, FT-IR., XRD, antibacterial activity.*

1. INTRODUCTION:

Nanoparticles (NPs) are seen as a solution to many technological and environmental challenges. Green synthesis methods of NPs involve less chemicals and are, therefore, environment-friendly. It provides a platform for the development of ecofriendly and the green synthesis of nano particles with the help of biological sources like plants and microorganisms. Nano particles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. Metal nanoparticles have been of great interest due to their distinctive features such as catalytic, optical, magnetic, and electrical properties. Nano particles of noble metals, such as silver, gold and platinum, are widely applied in products that directly come in contact with the human body, such as shampoos, soaps detergent, shoes, cosmetic products, and toothpaste, besides medical and pharmaceutical applications. The silver nano particles have various important applications. Silver has long been recognized as having inhibitory effects on microbes present in medicinal and industrial process. Historically, silver has been known to have a disinfecting effect and has been found in applications ranging from traditional medicines to culinary items. It has been reported that silver nano particles are non-toxic to humans and most effective against bacteria, virus and other eukaryotic microorganisms at low concentrations and without any side effects.

A number of approaches are available for the synthesis of silver nanoparticles: Facile method, thermal decomposition of silver compounds, electrochemical, sonochemical , microwave-assisted process and recently via green chemistry route. Unfortunately, many of the nano particle synthesis or production methods involve the use of hazardous chemicals, low material conversions, high energy requirements, difficult and wasteful purifications. Therefore, there is a growing need to develop environmentally friendly processes for nanoparticle synthesis without using toxic chemicals. Biosynthetic methods employing either microorganisms or plant extracts have emerged as a simple and viable alternative to chemical Green nanoparticle synthesis has been achieved using environmentally acceptable plant extract and ecofriendly reducing and capping agent. Many reports are available on biosynthesis of silver nanoparticles by plant parts like Punica granatum peels, Citrus sinensis peel, lemon leaves, Myrica esculenta leaf, Wrightia tinctoria leaves and Mango peel. The nano particles produced by this method are safe, cost effective and more environmental friendly when compared to the chemical methods. Silver nanoparticles (AgNPs) have been used for antimicrobial purposes, as well as in anticancer ,anti-inflammatory and wound treatment applications. Silver has long recognized as having inhibitory effect on microbes present in medical and industrial process and also have antimicrobial properties with low toxicity especially in the treatment of burn wounds where transient bactera is prevalent and its fact is essential. The most important use of silver nanoparticles is topical ointments, to prevent infection therefore it incorporated into various medical applications, plastic catheters coated with AgNPs prevent bio film formation. The sapota fruit has its origin in the rain forests of Central America, particularly in Mexico and Belize but it is easily available in India. The fruit is a round or oval-shaped berry measuring around 10 centimeters in diameter and weighing 150 grams. It has 2-5 big, black, shiny bean-like seeds in the centre. The unripe fruit has a hard surface and white pulp due to its high content of latex.

The latex content reduces as the fruit ripens and its flesh acquires a brown color. It has a smooth and grainy texture with a sweet and musky flavor. It is rich in calories and its sweet flavor can be attributed to the presence of simple sugars like fructose and sucrose that replenish energy and revitalize the body. Sapota fruit is rich in antioxidants. Therefore, it acts as an anti-ageing agent by eradicating free radicals in the body which are responsible for enhancing the ageing process. Sapota fruit is rich in antioxidant. Antioxidants, dietary fiber and nutrients found in sapota ,fights against certain cancers.

2. MATERIALS AND METHODS :

The Sapota fruit was collected from the market at Ramanathapuram. Silver nitrate was purchased from Sigma–Aldrich and used as received. Double distilled water was used for the experiments. All glass wares were properly washed with distilled water and dried in oven. **Preparation of sapota fruit extract**

Sapota fruit was used as a reducing agent for the development of silver nano particles. The fresh Sapota fruit was washed repeatedly with distilled water to remove the dust and organic impurities present in it. Then, the peels were removed. About 25 g of fruit was taken in a 250 ml conical flask containing 50 ml double distilled water and then the fruit was boiled at 80 for 10 min and filtered through Whatman No. 1 filter paper twice. The resultant filtrate was stored at 4°C and used as reducing and stabilizing agent.

2.1 Synthesis of silver nano particles

To optimize the synthesis route for producing the AgNPs, Fruit extract concentrations were varied on precursor solution. The various concentrations (2.5– 5 ml) of aqueous fruit extract were added to the 100 ml aqueous solution of 0.1 mM AgNO3. The reduction of silver ions takes place within 30 min at room temperature. The color change of the solution viz., dark brown color was observed, indicating the formation of silver nano particles.

Fig.1 Colloidal AgNPs by bioreduction of Sapota fruit extract

3. RESULT AND DISCUSSION:

The nano particles were primarily characterized by UV–Visible spectroscopy, which has proved to be a very useful technique for the analysis of nanoparticles. Ultraviolet–Visible spectra were obtained using a UV- 1800 Shimadzu. XRD analysis was carried out on an X-Ray diffractometer (X'Pert-PRO). The high resolution on XRD patterns measured at 3 KW with Cu target using a scintillation counter (k = 1.5406 A °) at 40 kV and 40 mA was recorded in the range of 2h = 10–80. The changes in the surface chemical bondings and surface composition were characterized using Fourier Transform Infrared (FT-IR) spectroscopy (Nicolet Avatar series 330) ranging from 400 to 4,000 cm^{-1}.

3.1 UV–Visible analysis

The preliminary detection of AgNPs was carried out by visual observation of the color changes of the reaction solutions. These changes were attributed to the excitation of surface plasmon resonance (SPR) in the metal nanoparticles. Typically, UV–visible absorption is used to investigate SPR. Characteristic surface plasmon absorption band was observed at 450 nm for the dark brown colored silver nanoparticles synthesized from 0.01M silver nitrate with fruit extract concentration (2.5- 5 ml). It has been found that the optimum concentration for the synthesis of AgNPs is 5 ml extract. There is small increase in the intensity of SPR band from 2.5 to 5 ml. However, when the concentration is increased further, there is a decrease in the intensity of SPR band. The increase in SPR band intensity is due to the formation of more AgNPs because of high initial concentration of Ag ions. The regular decrease in SPR band intensity from curve (i and ii) supports the formation of large sized AgNPs. The AgNPs prepared from 5 ml concentration of Ag are used for other characterizations. According to Mie's theory, only a single SPR band is expected in the absorption spectra of spherical metal nano particles, whereas anisotropic particles could give rise to two or more SPR bands, depending on the shape of the particles.

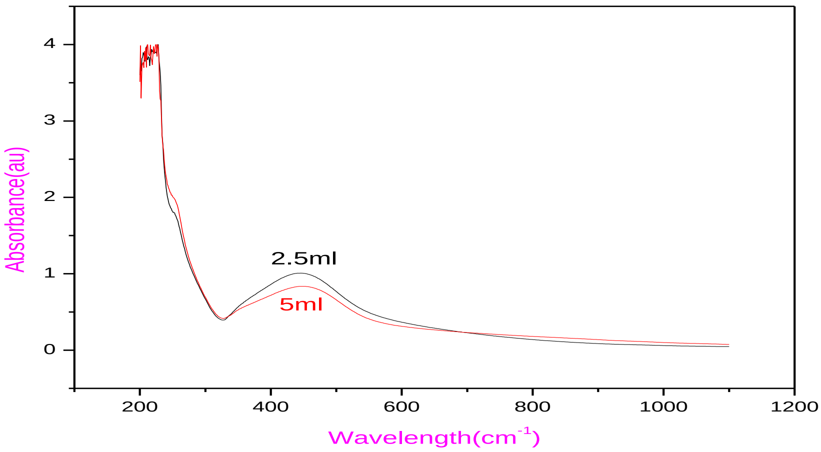


Fig.2. UV Vis spectra for the AgNPs prepared with 0.1 M aqueous $AgNO_3$ solution with sapota fruit extract concentration 2. 5 ml and 5ml

3.2 FT-IR ANALYSIS

To determine the functional groups on Sapota fruit extract and predict their role in the synthesis of silver nano particles, FTIR analysis was performed. The band intensities in different regions of the spectrum for the Sapota fruit extract and AgNPs (before and after reaction with silver nitrate, respectively) were analyzed and are shown in Fig.4. There was a shift in the following peaks: 3,865–3278, 2,894- 2338, 1,641, 1314-1203, 1160, 895, 667. The shift from 3,865 to 3278 cm^{-1} may indicate the involvement of O–H functional group in the synthesis of nano particles. The peak located at around 2,929- 2338cm^{-1} was attributed to the N–H stretching or the C = O stretching vibrations. The peak shift from 1641 cm^{-1} implicated that these groups may be involved in the process of nano particle synthesis. The peak located at 1,641cm^{-1} to the amide stretching bond which can be associated with the residual protein molecules involved in the reduction of silver. The bands at 667 and 1425 cm-1 may be ascribed to vibrational energy of aromatic hydrocarbons and amide linkage those have possessed their energy changed due to interaction with nanoparticles.

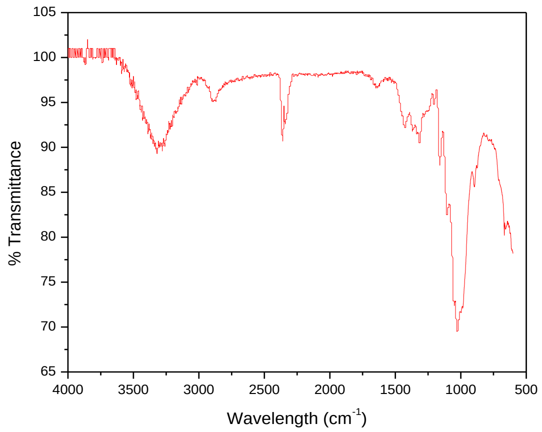


Fig.3 FT-IR spectra for Sapota fruit extract

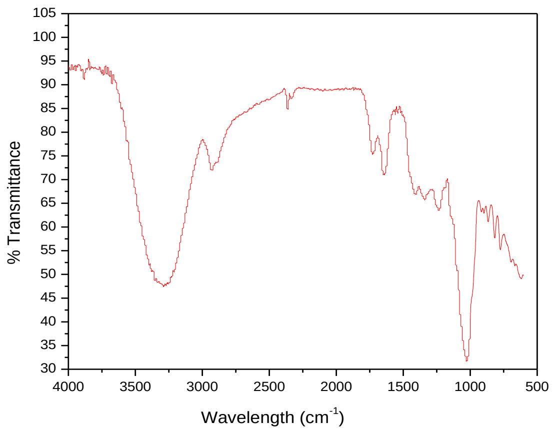


Fig.4 Bio-reduced by Sapota fruit extract silver nano particles

3.3 X-ray diffraction analysis (XRD)

The crystalline nature of AgNPs was further confirmed by X-ray diffraction (XRD) analysis. X ray diffraction is used to characterize crystallographic structure, grain size, and preferred orientation in polycrystalline or powder solid samples. The XRD patterns of synthesized AgNPs are shown in Fig. 5. Silver nanoparticles synthesized from Sapota fruit extract showed Bragg Reflection peaks at 31.99, 38.00, 43.89,46.00,64.40, 77.22 in the 2h range between 10–80 which can be indexed to the (122),(111),(200), (103), (220),(311) planes of face centered cubic (fcc) crystal, respectively. They have a match with the standard JCPDS No. 89-3722, 04-783 revealing that the synthesized silver nanoparticles are composed of pure crystalline silver and the particle size is approximately 34 nm. Only one peak value is observed because maximum particles are reduced by the fruit extract**.** The peaks observed around 27° may due to the fruit extract. These Braggs peak might have resulted due to capping agent, stabilizing the nanoparticles, The crystallite sizes of the silver nanoparticles using various concentrations of Sapota fruit extract are calculated using the Debye–Scherrer's equation.

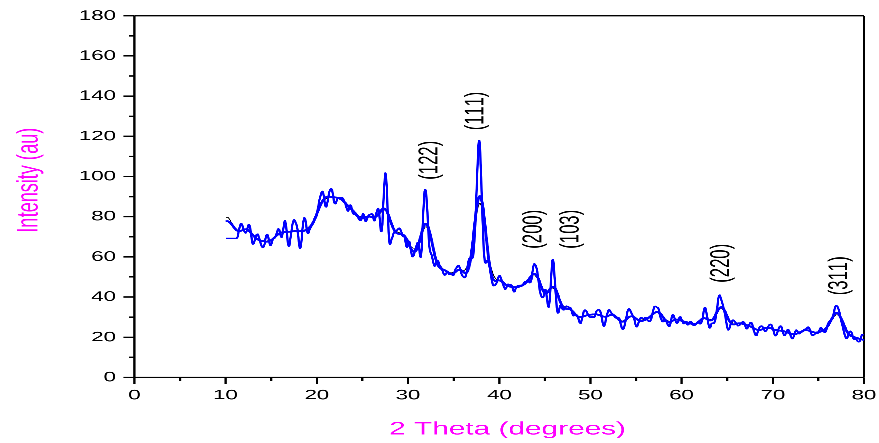
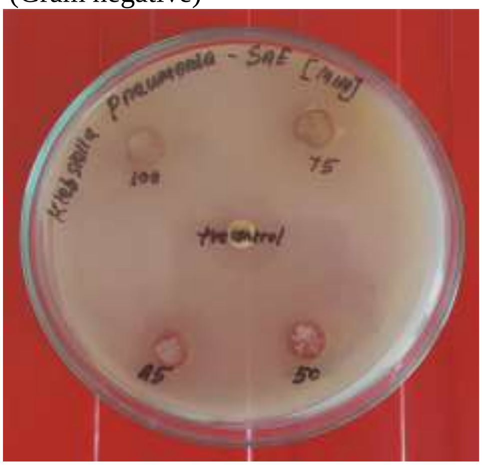


Fig. 5 XRD spectra for the AgNPs prepared with 0.1 M aqueous AgNO3 Synthesized from aqueous extract of sapota Fruit

3.4. Antibacterial activity

Antibacterial property of silver nanoparticles of sapota fruit extract against *Bacillus subtilis* (Gram positive) and two different human pathogens are shown in Figure 6. The labels a,b represent the inhibition zones for standard antibiotic, distilled water, pure pomegranate fruit extract, 50 AgNPs and 100 AgNPs respectively. It is observed that, the antimicrobial activity of silver nanoparticles with different pommigranate seed extracts is found to be significant against both the bacterial strains. The inhibition zones for the silver nano particles with pomegranate seed extract are listed in Table 1. Chloramphenicol is used as a standard antibiotic to compare the results with the zone obtained using silver nanoparticles It is observed from Figure 6 that, increase in the concentration of silver nanoparticles will result in the formation of a well defined inhibition zone. The bactericidal activity of the green synthesized sapota fruit extract AgNPs has potent antibacterial activity against both Gram-negative and Gram-positive human pathogens. AgNPs displayed antibacterial activity against Gram positive and Gram negative bacteria, with varying degrees, as suggested by the diameter of inhibition zone, while sapota fruit extract show low antibacterial activity compare to AgNPs (Table.1).The results showed that AgNPs are effective antibacterial activity against Bacillussubtilis (Gram positive) and *K.Pneumoniae* (Gram negative)



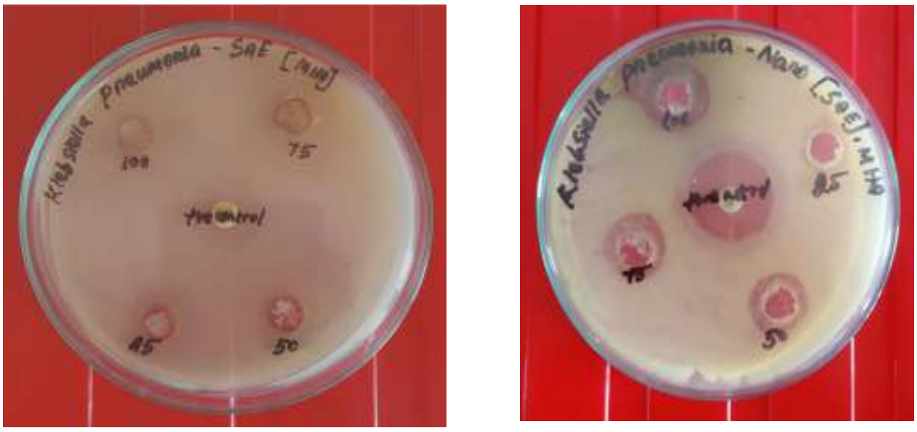


Fig.6.Antibacterial activities of silver nanoparticles against (a) *Bacillus subtilis* (b) *K.Pneumoniae*

Table.1 Inhibitory action of SFE and AgNPs against human pathogenic bacteria

S. NO	MICROORGANISMS	EXTRACT	ZONE OF INHIBITION (MM)			
			100 µl	**75 µl**	**50 µl**	**25 µl**
GRAM POSITIVE BACTERIA						
1	*Bacillus subtilis*	*SE*	12	10	09	07
2	*Bacillus subtilis*	*N.SE*	19	16	14	12
GRAM NEGATIVE BACTERIA						
1	*K.Pneumoniae*	*SE*	14	12	10	09
2	*K.Pneumoniae*	*N.SE*	18	16	14	12

CONCLUSION :

Silver nanoparticles were produced by the direct interaction of silver nitrate with Fruit extract in aqueous media without the intervention of any external man-made chemicals. Therefore, this reaction pathway satisfies all the conditions of a 100 % green chemical process. The amount of plant material is found to play a critical role in controlling the size and size dispersity of nanoparticles in such a way that smaller silver nanoparticles and narrow size distribution are produced when more Sapota fruit extract is added in the reaction medium. The fruit extract of Sapota reduced the silver ions and produced metallic silver nanoparticles with well-defined stability. These nanoparticles were closely spherical in shape and the average particle size was found to be 34 nm. The green synthesis of nanoparticles has more advantages over physical and chemical synthesis therefore; it might be used for exploitation of fruit extract for production of nano particles in nanotechnology industries.

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