Analysis of Anti-Bacterial Activity of Silver Nanoparticles from Pomegranate (Punicagranatum) Seed and Peel Extracts

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Sinnappan Jesurani
Mother Teresa Women's University

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Analysis of Anti-Bacterial Activity of Silver Nanoparticles from Pomegranate (Punicagranatum) Seed and Peel Extracts

M. Haniff Nisha, R. Tamileaswari, Prof. Sr. S. Jesurani.
PG and Research Department of Physics,
Jayaraj Annapackiam College for Women (Autonomous),
Periyakulam- 625601, Tamil Nadu, India.

Abstract - **The green process for the synthesis of nanoparticles is evolving into an important branch of nanotechnology. It has many advantages such as economical, easily available, and eco-friendly process. The present study investigated the synthesis of silver nanoparticles from 1mM of silver nitrate ($AgNO_3$) solution through aqueous extract of pomegranate seed and biowaste of the fruit of peel act as a reducing agent as well as capping agent. The silver nanoparticle is confirmed by its color changes of fruit extract and further conformed to the help of UV-Visible spectroscopy. The morphology was characterized by Scanning Electron Microscope (SEM), Energy Dispersive X-ray analysis (EDAX), X-ray Diffraction (XRD).Moreover, their anti-bacterial activity was evaluated against *Pseudomonas, Bacillus cereus, staphylococcus albus and proteus* Species of pathogens were used. The applications of silver nanoparticles have the potential for electronics, medicine, Environment, manufacturing and materials.**

Key Words - Silver nanoparticles, Green process, pomegranate seed and biowaste of the fruit of peel extract, Pseudomonas, Bacillus cereus, staphylococcus albus and proteus

I.INTRODUCTION

The field of nanotechnology is one of the most active researches nowadays in Modern material science and technology. Nanoparticles are fundamental building Blocks of nanotechnology. [1] Physical, Chemical, and green synthesis method are available for synthesis of silver nanoparticles. [2] But the physical and chemical methodologies can be toxic and highly reactive posing a risk to the environment and humans, or the procedures are too expensive to be feasible at an industrial scale. Therefore there has been a search for inexpensive, reliable, safe, and "green" approach to the synthesis of stable metal nanoparticles with controlled size and shape [3]. Silver nanoparticles have proved to be most effective as it has good anti-microbial efficacy against bacteria, Viruses and other eukaryotic micro-organisms [4]. Currently silver nanoparticles are wildely used as an antibacterial and antifungal agent in a diverse range of consumer products: air sanitizer sprays. Detergent, soaps, shampoos, toothpastes and washing machine [5]. The most important application of silver and SNPs is in medical industry such as tropical ointments to prevent infection against burn and open wounds [6].

Punica granatum is a fruit-bearing deciduous shrub or small tree growing between five and eight meters tall. In the Indian subcontinent's ancient Ayurveda system of medicine, the pomegranate has extensively been used as a source of traditional remidies for thousands of years [7]. The rind of the fruit and the bark of the pomegranate tree is used as a traditional remedy against intestinal parasites [8]. Pomegranate juice (of specific fruit strains) is also used as eyedrops as it is believed to slow the development of cataracts [9]. Pomegranate aril juice provides about 16% of an adult's daily vitamin C requirement per 100 ml serving, and is a good source of vitamin B5 (Pantothenic acid), potassium and polyphenols, such as tannins and flavinoids [7-10].
The fruit and seed are used in modern herbal medicine. And also helps overcome depression, Protect against heart ailments, provides relief from stomach disorder, Reduces risk of developing cancer, provides youthful and glowing skins, helps reduce symptoms of anemia [11]. The pomegranate peels contain compounds that especially help to support and modulate hormones and hormonal balance [12]. Pomegranate peel is primarily composed of alkaloids and poly phenols. The active be constituent that appears to be responsible for its multiple health benefits is ellagic acids. Ellagic acid is a naturally occurring compound found in several fruits and nuts. The ellagic acid effectively protects cells from damaging free radicals [13].

So sufficient literature is not reported on the green synthesis of $AgNO_3$ from seed and peel extract of pomegranate. In this work, we have explored an inventive contribution for synthesis of silver nanoparticles using seed and peel of pomegranate. The procedures for development of stable silver nanoparticles, simple and viable synthesized nanoparticles were characterized by various methods, such as UV-visible spectroscopy. The interaction between nanoparticles with functional groups was confirmed by using Fourier transform infrared spectroscopy analysis (FTIR). The morphological characterizations are performed using scanning electron microscope (SEM) with Energy dispersive analysis (EDAX), X-ray diffractometer (XRD) and also further the antibacterial activity of these biologically synthesized silver nanoparticles was evaluated against different pathogenic microorganisms [14].

II. MATERIAL AND METHODS

A. Preparation of Seed and Peel Extract.

Collection of pomegranate fruit was collected nearer shops. And separated to the pomegranate seed and peel from the pomegranate fruit (Fig: 1). The seed and peel weighing 25 g were thoroughly washed in de-ionized water. The peels were cut into small pieces, and then take it seed (don't cut into small pieces). The cut pieces of peel were ground well in 100 ml of de-ionized water using motor and pestle. Hereafter the seed of pomegranate were ground well in 100 ml of de-ionized water using motor and pestle. Both extract was filtered twice by using Whatmann No-1 filter paper, this filtrate was collected for further study.

Fig: 1 collection of pomegranate seed and peel.

B. Synthesis of Silver Nanoparticles

90 ml of 1mM of silver nitrate solution was prepared in Erlenmeyer flask. 10 ml of freshly prepared pomegranate seed and peel extract was added separately to the silver nitrate solution. The bioreduction of $AgNO_3$ ions occurred within minutes (fig: 2 and 3).The seed before its pink colored solution which turned into the brown color slowly indicate the formation of silver nanoparticles. And also peel extract its yellow color solution which turned into the tea brown color slowly indicate the formation of silver nanoparticles.

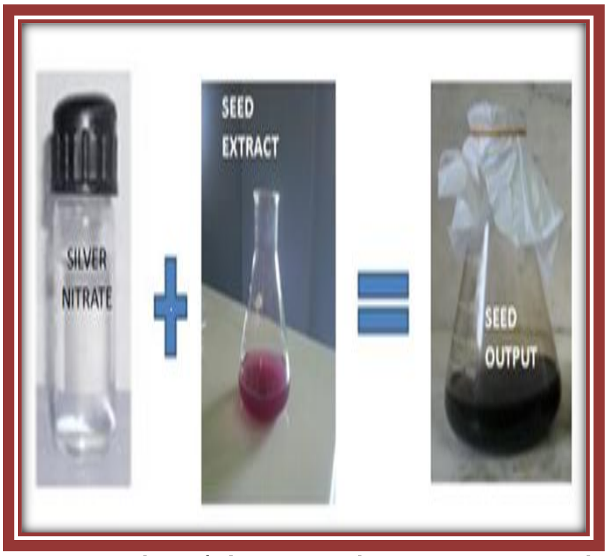

Fig: 2 synthesis of silver nanoparticles using pomegranate seed

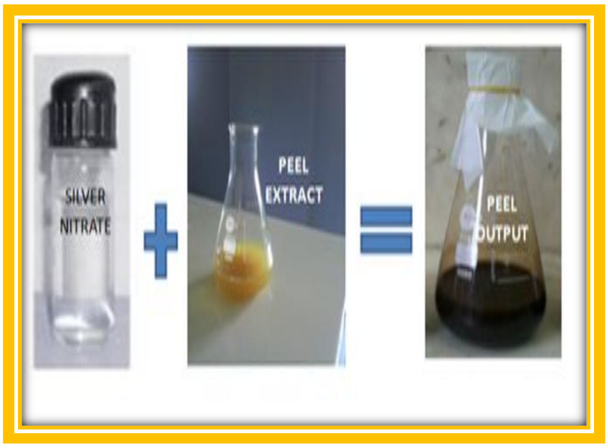

Fig: 3 synthesis of silver nanoparticles using pomegranate peel.

C. Characterizations of Silver Nanoparticles

The pomegranate seed and peel extract was centrifuged (1 or 2 times) to separate the nanoparticles & to remove the unwanted garbage. It was dried in hot air oven for 15 minutes and silver nanoparticles were obtained in a powder form at temperature 100^0 C. synthesized nanoparticles were confirm by UV-visible spectroscopy, and it was carried out in Perkin-Elmer spectrophotometer operating in a wavelength from 200-1100 nm. The morphology of the nanoparticles was studied in Philips Scanning Electron Microscope (SEM - Bruker Company). The Presence of elemental silver in the solution mixture was identified by Energy Dispersive X-ray Spectrophotometer (EDAX) and which was operated at the accelerating voltage of 129 KV. The crystalline nature of the nanoparticles was evident from XRD measurements. Perkin Elmer of Fourier Transform Infrared (FTIR) spectroscopic data was used to indicate the bonding of silver nanoparticles with functional group of seed and peel extract through bridging linkage. The antibacterial activities were done by using disc diffusion methods.

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III. RESULTS AND DISCUSSION

A.UV-visible spectrum analysis

Addition of seed and peel extract of pomegranate fruit to aqueous silver nitrate solution. The seed extract before its pink colored solution which turned into the brown color. And the peels extract its yellow color solution which turned into the dark tea brown color. Reduction of silver ions into silver nanoparticles during exposure to fruit extracts was observed as a result of the color change. The color change is due to the Surface Plasmon Resonance phenomenon. The samples were observed by UV-visible spectrophotometer (Fig: 4).The sharp bands of silver nanoparticles were observed around at 472 nm for seed and 448 nm for peel.

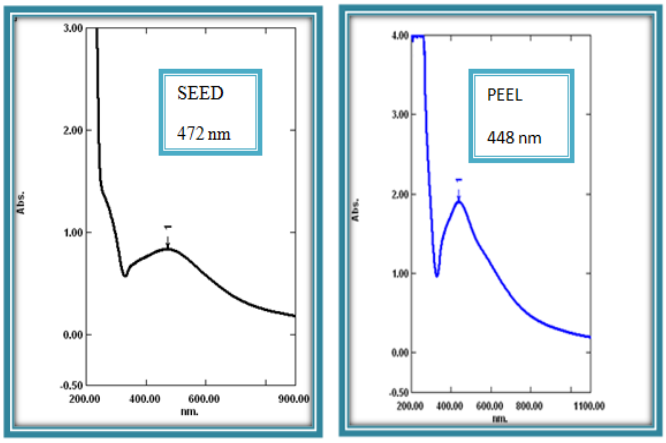


Fig: 4 UV-visible spectrums of pomegranate seed and peel.

B.Fourier Transform Infrared Spectroscopy

The FTIR measurements were carried out to identify the natural products for the reduction of silver ions to silver nanoparticles present in pomegranate seed and peel extract. The FTIR spectrum of seed extract is given (fig: 5) peaks appear at 3449, 2073, 1400, 1638, 1053, 675 cm^{-1} respectively. The band at 3449 cm^{-1} indicates phenolic OH. The band at 2073 cm^{-1} is due to stretching vibration of alkynes. The band at 1638 cm^{-1} is due to N-H bend of 1^{o} amines. The band at 1400 is corresponds to C-C stretching vibration to aromatics. The band at 1053 cm^{-1} is due to C-N stretching vibration to aromatic amines. The band at 675 cm^{-1} is due to C-H bending of alkynes.

Similar output was obtained J.Sivakumar et al (2011) [15]

The FTIR spectrum of peel extract (Fig: 6) peaks appear at 3364, 2931, 1639, 1090, 1639 cm^{-1} respectively. The band at 3364 cm^{-1} is due to phenolic OH. The band 2931 cm^{-1} C-H plane bends to alkenes. The band at 1639 cm^{-1} corresponds to N-H bends to 1^{o} amines. The band at 694 cm^{-1} is due to C-H bend to alkynes. The band corresponds to 1090 cm^{-1} corresponds to C-H Wag to alkyl halides.

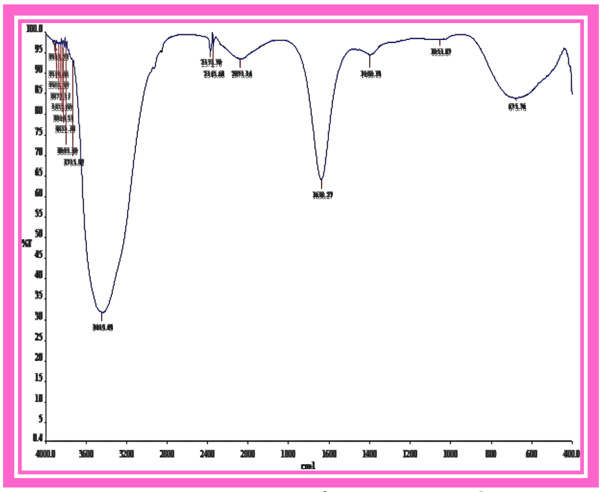


Fig: 5 FTIR spectrums of pomegranate seed.

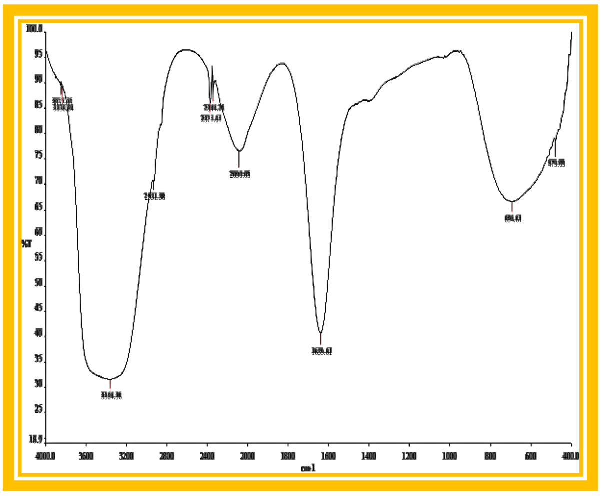


Fig: 6 FTIR spectrums of pomegranate peel.

C.X-Ray Diffraction Analysis

The experimental powder diffraction (XRD) pattern of prepared silver nanoparticles (Fig: 7). In the XRD pattern of silver nanoparticles diffraction peaks at 32^{o}, 39^{o}, 49^{o}, 65^{o}, and 79^{o} can be assigned to face-entered cubic. The broadening of the Bragg's peaks indicates the formation of silver. The major planes correspond to (004), (110), (200), (220), (311), were found to be matched which confirmed the presence of Cubic Silver. The average crystallite sizes of the silver nanoparticle using seed can be estimated from ~10 – 30 nm using peel the range was~15-35 nm from the X-ray peak broadening using Scherer's formula.

$$\mathbf{D = K \lambda / (\beta \cos \theta)} \qquad \mathbf{(1)}$$

In which, where *K* is a constant taken as 0.9, θ is the diffraction angle; λ is the wavelength of the X-ray radiation. β is the full width at half maximum (FWHM) of each phase and θ is the diffraction angle, D = Average particle size of crystallite. [16]

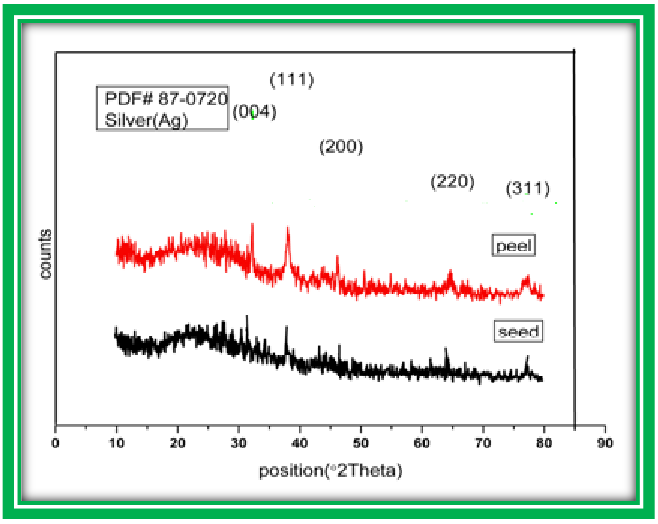
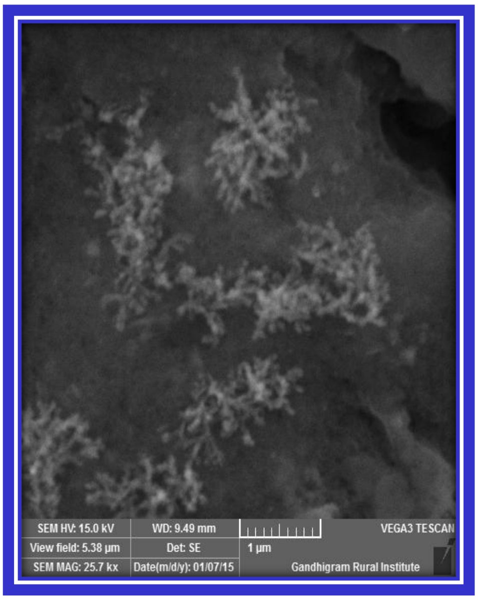


Fig: 7 XRD of silver nanoparticles using pomegranate seed and peel.

D.Scanning Electron Microscopy (SEM) with (EDAX)

The SEM technique was employed to visualize the size and shape of the silver nanoparticles using pomegranate seed and peel extract. The SEM characterization of the synthesized silver nanoparticles are shown in (fig: 8 and fig: 9). The SEM image shows that flower shape and spherical shape nanoparticles formed with diameter range approximately 10-30 nm. EDAX exposed strong signal in the silver region and confirmed the formation of silver nanoparticles (fig.10 and fig: 11). Metallic silver nanocrystals generally show typical absorption peak approximately at 3KeV due to surface plasmon resonance (Magudapatty et al. 2001)



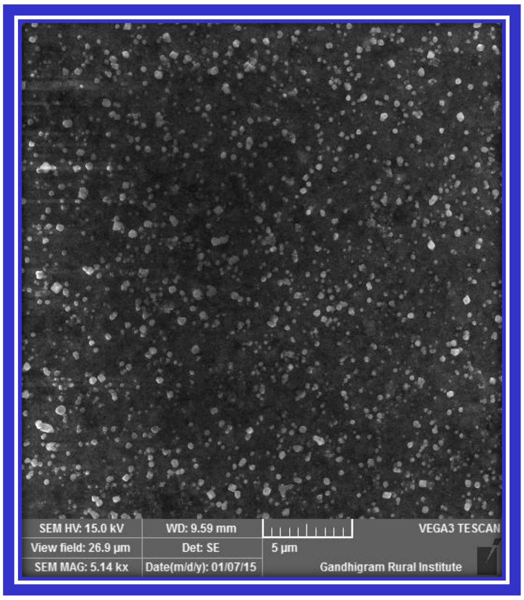
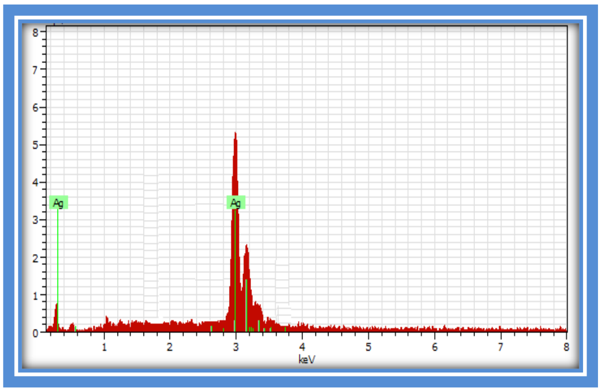


Fig: 8 & Fig: 9 SEM image for synthesized silver nanoparticles



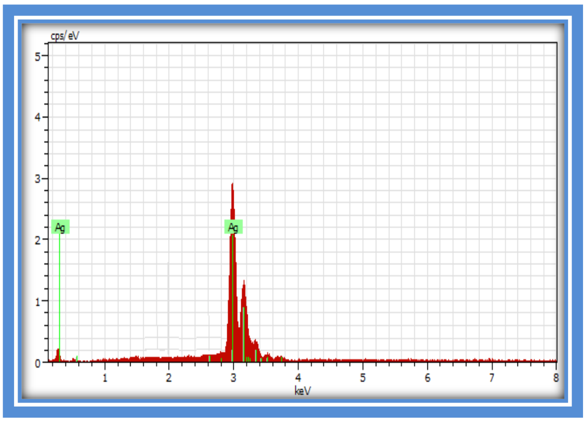


Fig: 10 & Fig: 11 EDAX spectrums of silver nanoparticles prepared from pomegranate seed and peel.

EDAX:

EDAX Showing strong signal in the silver region and established the formation of silver nanoparticles (Fig: 10 & Fig: 11). Metallic silver nanocrystals generally show typical absorption peak approximately at 3Kev due to Surface Plasmon Resonance.

E. ANTI-BACTERIAL ACTIVITY

The disc diffusion method was used to screen the antibacterial activity.

Antibacterial activity of biogenic AgNPs was evaluated by using standard Zone of Inhibition (ZOI) microbiology assay. The nanoparticles showed inhibition zone against almost all the studied bacteria (**Table-I)**.

Compound Seed exhibited potential antibacterial Maximum ZOI was found to be 14 mm *for Bacillus Cereus.* Whereas, the other three bacterial strains of *Pseudomonas, Staph.Albus , proteus* showed ZOI of 13,13,0 mm.

Compound Peel exhibited potential antibacterial Maximum ZOI was found to be 17mm for *Bacillus Cereus* Whereas, the other three bacterial strains of *Pseudomonas,Staph.Albus. proteus* showed ZOI of 16,13,0 mm.

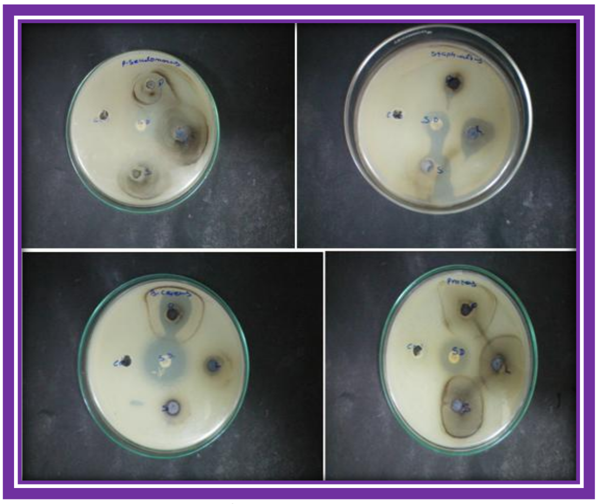


Fig: 12 Anti-bacterial image for pomegranate seed and peel with silver nanoparticles (from figure S, L – seed & peel).

Table: I

SAMPLE CODE	PSEUDO MONAS	BACILLU S CEREUS	STAPH.ALBU S	PROTEU S
PEEL	16	17	13	R
SEED	13	14	13	R
CONTROL	R	R	R	R
STANDARD DISC (AMIKACIN)	16	19	16	16

IV.CONCLUSION

Green methods for silver nanoparticles synthesis using eco-friendly and non-toxic compounds are possible. Bio-synthetic methods employing naturally occurring reducing agent as well as capping agent such as polyphenol, Tannic acids or plant extract. The systematic techniques such as UV-visible spectroscopy, FTIR, XRD, and SEM with EDAX are applied to characterize the synthesized nanoparticles. The phase formation of Ag was confirmed by X-ray diffraction pattern. The nanoparticles size range of Ag was10-30nm measured by SEM and confirmed the presence of Ag by EDAX. Nanoflower and spherical shaped Ag nanoparticles were formed by seed and peel extract. The silver nanoparticles synthesized using pomegranate seed and peel extract showed the anti-bacterial activity not in favor of psedomonus, bacillus cereus, and staph.albus. From the technological point of view these obtained silver nanoparticles have potential applications in the biomedical field and simple procedure has several advantages such as cost effectiveness, compatibility for medical and pharmaceutical applications as well as large scale commercial production.

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