GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM POMEGRANATE (PUNICAGRANATUM) LEAVES AND ANALYSIS OF ANTI-BACTERIAL ACTIVITY

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ABSTRACT

Nano biotechnology is a rapidly growing scientific field of producing and constructing devices utilizing nanosized particles. Nowadays, the green synthesis of silver nanoparticles using plant extracts emerges as a cost efficient, eco-friendly, and exciting approach. The present work leads to the synthesis of nanoparticles from 1mM of silver nitrate ($AgNO_3$) solution through an aqueous extract of pomegranate leaveswhich act as a reducing agent as well as a capping agent. The formation of nanosized silver was confirmed by its characteristic surface plasmon absorption peak at around 437 nm in UV–visible spectra. The morphology was characterized by scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDAX) and X-ray diffraction (XRD), with the active functional groups present in the synthesized silver nanoparticles being confirmed by Fourier Transform Infra-red (FTIR) spectroscopy. Moreover, their antibacterial activity was evaluated against Pseudomonas, Bacillus cereus, staphylococcus albus and proteus pathogens. The characterised silver nanoparticles have the potential for various medical and industrial applications.

Keywords: Bacillus Cereus, Green Synthesis, Pseudomonas, Proteus and Staphylococcusalbus, Pomegranate Leaves, Silver Nanoparticle

I. INTRODUCTION

Nanoparticle are the greatest building block for health care, structural material, automation and also they possess unique electrical, optical as well as biological properties and are thus applied in catalysis, biosensing, imaging, drug delivery, nanodevice fabrication and in medicine [1]. Silver nanoparticles have unique optical, electrical, and thermal properties [2].and are being incorporated into products that range from photovoltaics to biological and chemical sensors. Additional applications include molecular diagnostics and photonic devices, which take advantage of the novel optical properties of these nanomaterials. An increasingly common application is the use of silver nanoparticles for antimicrobial coatings, textile industry, keyboards, wound dressings and biomedical devices which now contain silver nanoparticles that continuously release a low level of silver ions to provide protection against bacteria [3].There are several methods for thesynthesis of silver nanoparticles that is physical, chemical and green synthesis methods [4]. Among these three physical and chemicalsynthesishave toxic capacity [5].comparedwith green synthesis methods. So, the green synthesis method is the best method for the synthesis of silver nanoparticles sinceit is an eco-friendly approach [6].*Punicagranatum* [7].is a fruit-bearing deciduous shrub [8].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 remedies for thousands of years [9].

In this communication, we demonstrate the synthesis of silver nanoparticles using pomegranate leaf extract (Punicagranatum .L). Pomegranate belongs to the family Punicaceae[10].A Pomegranate leaf has a wide range of potential health benefits. It has been shown to aid in digestion and to help treat certain infections and illnesses. More recently, it has been studied as an appetite suppressant and weight loss aid [11]. Pomegranate leaves contain Tannins, flavones, apgenin, luteolin, glycosides [12]. They can also be taken in various ways, including teas, pastes and juices [13].

Several papers are published from pomegranate seed and peel [14-17]. However sufficient literature is not yet reported onthe greensynthesis of silver nanoparticles using leaf extract of pomegranate (punicagranatum). So, in this work, we have explored an inventive contribution for the synthesis of silver naoparticles using leaves of pomegranate.

II. MATERIAL AND METHODS

2.1 Preparation of Leaves Extract

To obtain silver nanoparticles deionized water was used as a reaction medium, the reducing agent used was pomegranate leaves extract. The reagent used for the synthesis was silver nitrate which does not evolve any toxic hazard on the atmosphere. Collection of pomegranate was from the local market and also nearby houses. The leaves of pomegranate (fig: 1) weighing 10g were thoroughly washed in distilled water and cut into small pieces. The cut pieces were ground well in 100 ml of de-ionized water usingmotar and pestle.The Extract was filtered twice by using Whatman No-1 filterpaper; this filtrate was collected for future study.

Fig: 1 Collection of Pomegranate Leaves

2.2 Synthesis of Silver Nanoparticle

90 ml of 1mM silver nitrate solution was preparedin anErlenmeyer flask. 10ml of freshly pomegranate preparedleaf extract was added to the silver nitrate solution. The90% of the bioreduction of $AgNO_3$ions occurred within minutes (fig.2). The green colored solution which turned into a dark tea brown colorslowly indicates the formation of silver nanoparticles [18-19].

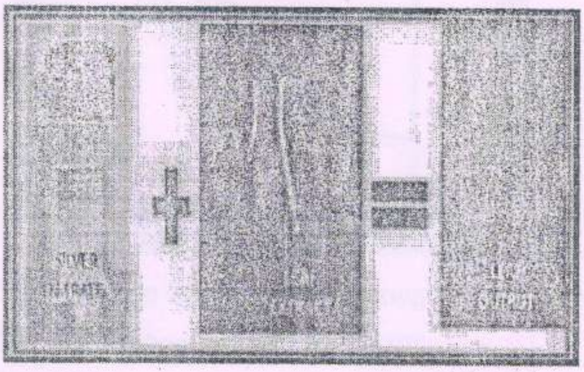

Fig: 2 Pomegranate Leaves Extract with Silver Nitrate.

2.3 Characterizations of AgNPs

The extract was centrifuged 1 or 2 times to separate the nanoparticles to remove unwanted garbage. It was dried ina hot air oven for 15 minutes at temperature of100^0C and silver nanoparticles were obtained in a powder form. Synthesized nanoparticles were confirmed by UV-visible spectroscopy carried out in a Perkin-Elmer spectrophotometer operating in a wavelength range from 200-1100 nm. The morphology of the nanoparticles was studied in a Philips Scanning electron microscope (SEM). The presence of elemental silver in the solution mixture was identified by an Energy Dispersive X-ray spectrophotometer (EDAX) at the accelerating voltage of 30 kV. The crystalline nature of the nanoparticles was evident from XRD measurements. Fourier Transform Infrared (FTIR) spectroscopic data indicated the bonding of silver nanoparticles with afunctional group of leaf extract through a bridging linkage. The antibacterial activities were done by using disc diffusion methods.

Analysis of Antibacterial Activity

Antibacterial activity of silver nanoparticles against *Pseudomonas, Bacillus cereus, staphylococcus albus and proteus* was evaluated using the disc diffusion method and the zone of inhibition was measured.

III. RESULTS AND DISCUSSION

3.1 UV-Spectrum Analysis

A mass of different substances was blended together which was an aqueous silver nitrate solution and Punicagranatum leaf extract. First the extract was at green color; after that it turned into the dark tea brown color .The sample was observed by auv-visible spectrophotometer for its maximum absorbance, confirming the reduction of silver ions. The maximum peak was found at 437nm.

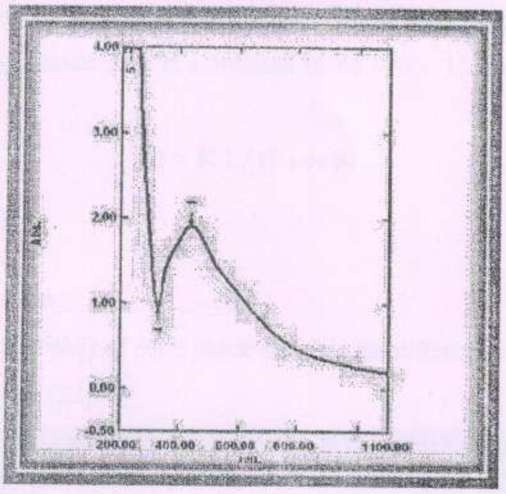

Fig: 3UV-Visible Spectrum of Pomegranate Leaves

A Similar,UV output was obtained by Shalini chauhan et al (2012) [20].

3.2 Fourier Transform Infrared Spectroscopy (FTIR)-Analysis

The FTIR measurements were carried out to identify the natural products present in pomegranate leaves extractfor the reduction of Ag^+ ions to silver nanoparticles. The FTIR spectrum of leaves extract is given in Fig: 4 peaks appearat 3433.49, 2075.98, 1638.74, 1400 and 688.67 cm^{-1}while some intensity peaks like 2930.18, 1114.87, and 1638.74. The band at 3433.49 cm^{-1} indicates Phenolic OH. The band at 2075.98 cm^{-1} is due to –C≡C- Stretching vibrations of alkynes. The band at 1638.74 cm^{-1} indicates C=O stretching vibrations of carbonyles. The band at 1400 cm^{-1} is due to C-C Stretching vibrations of Aromatics. The band at 688.67 cm^{-1} indicates C-H bondingto aromatics. The band at 2930.18 cm^{-1} corresponds to C-H stretching vibrations of alkanes. The peak 1114.87 cm^{-1} indicates –C-H bond to alkyle halides and the band at 1638.74 cm^{-1}corresponds to –C=C- stretching vibrations of alkanes.

A Similar output was obtained by C.Kannan et al [21].

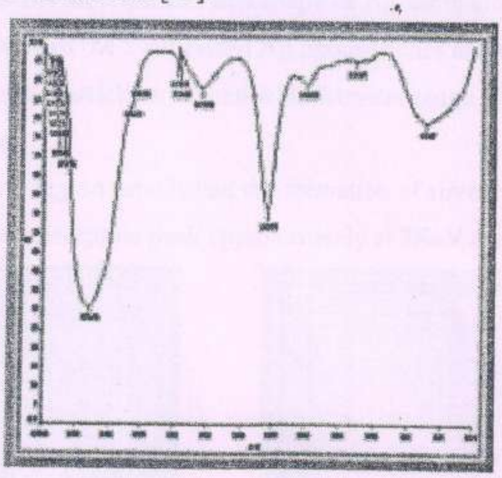

Fig: 4 FTIR Spectrums of Pomegranate Leaves.

3.3 X-Ray Diffraction Analysis

The X-ray diffraction (XRD) pattern clearly shows that synthesized Ag nanoparticles formed are crystalline in nature compared with the standard powder diffraction pattern (fig: 5) (PDF# card No: 87-0720). The broadening of the Bragg's peaks indicates the formation ofnano metal size silver. The major planes corresponding 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 leaves can be estimated to be ~10 – 15 nm, from the X-ray peak broadening using Scherrer's formula.

$$D = K \lambda / (\beta \cos \theta)$$

where,

K is a constant taken as 0.9,

$\lambda$is the wavelength of the X-ray radiation.

$\beta$is the full width at half maximum (FWHM) of each phase and θ is the diffraction angle and

D = average particle size of crystallites [22].

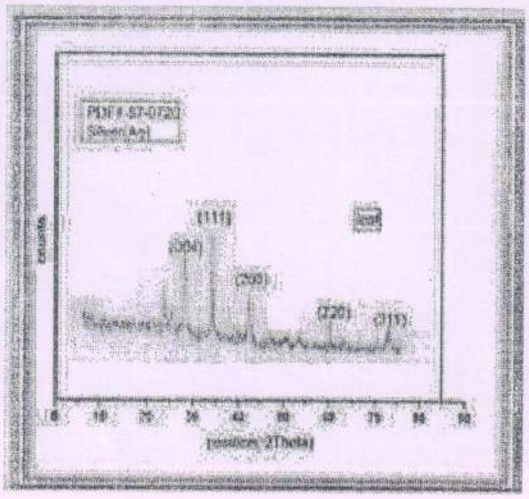

Fig: 5 XRD of Silver Nanoparticle Using Pomegranate Leaves.

3.4 Scanning Electron Microscopy (SEM) With EDAX Analysis

The SEM technique was engaged to envision the size and shape of Ag nanoparticle synthesized using pomegranate leaves extract. The SEM characteristics of the synthesized Ag nanoparticles are shown in Fig. 6. Illustration of SEM showed somewhat nanorod shaped nanoparticlesproduced with diameter range 10-30 nm. It indicates the completion of the nanoparticle synthesis process.

The EDAX strong signal in the silver region established the formation of silver nanoparticles (fig.7). Metallic silver nanocrystals generally show a typical absorption peak approximately at 3KeV due to surface plasmon resonance.

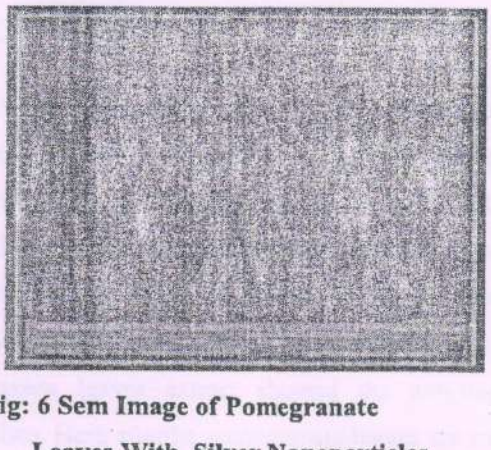

Fig: 6 Sem Image of Pomegranate Leaves With Silver Nanoparticles

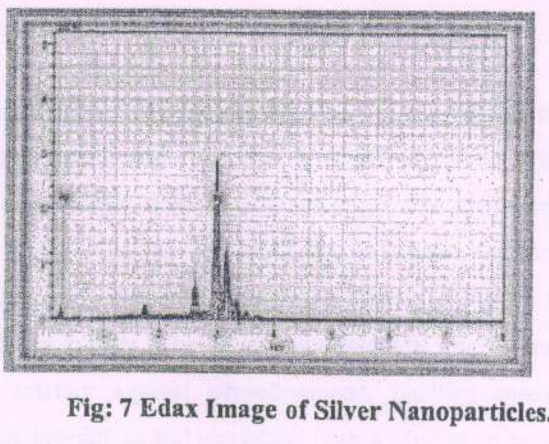

Fig: 7 Edax Image of Silver Nanoparticles.

3.5 Antibacterial Activity

antibacterial activity of biogenic silver nanoparticles was evaluated by using disc diffusion method. The nanoparticles showed an inhibition zone against almost all the tested human pathogens (Table: 1& Fig: 8).

Table: 1 Antibacterial activity of Ag nanoparticles against Pseudomonas, Bacillus.cereus, Staph.Albus, Proteus.

The disc diffusion method was able to detect the inhibitory effects of the Ag nanoparticles using different bacteria tested against the human pathogens. Compound leaves exhibited a potential antibacterial Maximum ZOI found to be 15mm,15mm,15mm for *Bacillus Cereus* ,*Pseudomonas* ,*Staphylococcus Albus* whereas, the other bacterial strains of*proteus* showed a ZOI of 0 mm. (Fig:8)[23]

SAMPLE CODE	PSEUDOMONAS	BACILLUS CEREUS	STAPH.ALBUS	PROTEUS
LEAVES	15	15	15	R
CONTROL	R	R	R	R
STANDARD DISC (AMIKACIN)	16	19	16	16

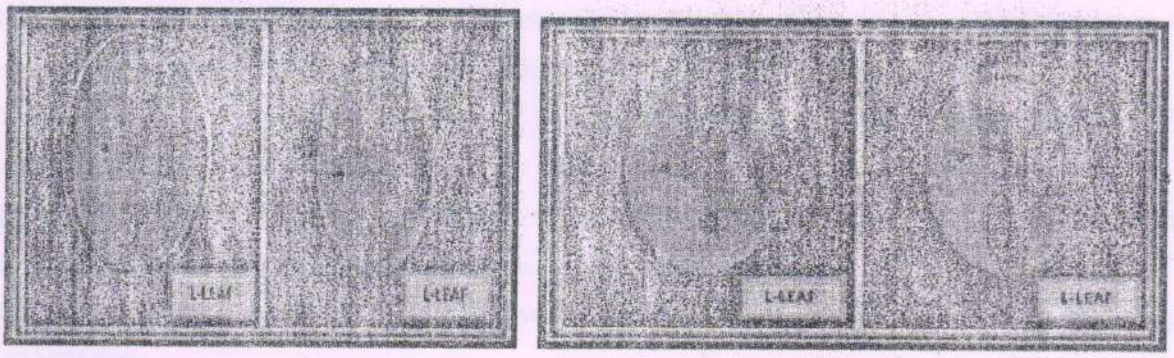

Fig: 8 Anti-Bacterial Images for Pomegranate Leaves with Silver Nanoparticles

IV. CONCLUSION

In this work pomegranate leaves extract was used as a reducing and capping agent. This green synthesis of silver nanoparticle was established to be an eco-friendly, economical, and harmless method, etc. 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 an X-ray diffraction pattern. The nanoparticles size range of Ag was10-30nm measured by SEM and confirmed the presence of Ag by EDAX. Nanorod shaped Ag nanoparticles were formed by the leaves extract. The silver nanoparticles synthesized using pomegranate leaves extract showed the anti-bacterial activity against ofpsedomonus, bacillus cereus, and staph.albus. Here afterthe pomegranate leaves are sometimes ground up and combined with a fluid to create a paste.

This paste can then be used as an antibacterial agent for minor infections. It has also been used in the treatment of an infection of the eye.

V. ACKNOWLEDGEMENTS

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