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Green Synthesis of Silver Nanoparticles by Using Pomegranate and Their Antimicrobial Activity-A Revolution

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Abstract The Pomegranate, punica granatum belongs to the family lythraceae is an ancient fruit. The present study has attempted to analyze the work done by different researchers on green synthesis of silver nanoparticles and their characterization by different techniques like UV-Visible spectroscopy, Fourier transform infrared spectroscopy(FTIR), Scanning electron microscopy (SEM), Tunnling electron microscopy (TEM) and X-ray diffraction method (XRD). The antimicrobial activity were also analyzed in the present paper.

Keywords Antimicrobial activity, Green synthesis, Silver nanoparticles, Pomegranate

1. INTRODUCTION

Nanotechnology is making an impact in all spheres of human life and it is developing day by day [1]. Nanotechnology is the application of science to control matter at nanoscale level [2]. This field is creating a lot of excitement in biotechnology and biomedical devices [3]. Besides these nanoparticles are playing important role in drug delivery, diagnostics imaging cancer detection, sensing, artificial implants, t issue e ngineering, H IV i nhibition a nd water filtration [4].

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Metallic nanoparticles are mostly prepared from nobel metals such as Gold, Silver, Platinum and Lead. Among the nobel metals, silver (Ag) is the metal of choice in the field of biological systems, living organisms and medicine [5]. In nanotechnology, silver nanoparticles are the most prominent one due to its applications.

Generally, silver does not adversely affect viable cells and does not easily provoke microbial resistance. Hence silver containing materials were also employed in textile fabrics, as food additives, and in package and plastics to eliminate microorganisms. Because of such a wide range of applications, numerous methods concerning the fabrication of silver nanoparticles, as well as various silver-based compounds containing ionic silver (Ag^+) or metallic silver (Ag) have been developed [6].

2. WHY GREEN SYNTHESIS OF SILVER NANOPARTICLES?

A number of approaches is available for the synthesis of silver nanoparticles viz., chemical and phototchemical reactions in reverse micelles, thermal decomposition of silver compounds, radiation assisted, electrochemical, sonochemical, microwave assisted process and via green chemistry route [5]. Chemical synthesis methods lead to presence of some toxic chemical absorbed on the surface that they may have adverse effect in the medical applications [7] and also necessary to use of high pressure, energy, temperature and toxic chemicals [8–9].Biosynthesis of nanoparticles is advantageous over chemical method as it is cost effective and environment friendly method . 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 bacteraemia is prevalent and its fact is essential. The most important use of silver nanoparticles are topical ointments, to prevent infection therefore it incorporated into various medical applications, plastic catheters coated with AgNPs prevent bio film formation [10].

Many researchers have done work on the green synthesis of silver nano particles by using various plant extracts. There are several reports on the use of Azadirachta indica(Neem) [11],Aloe vera[12],Emblica officinalis(Amla) [13],Coriandrum sp.[14]. Herbal medicines are gaining interest because of their cost effective and eco friendly attributes [15].

Pomegranate peel, fruit and seed have so many applications and is very useful in curing many diseases and it is very important in human life. The word pomegranate comes from the latin for “fruit of many seeds”. It is cultivated extensively in Iran, India, some parts of the U.S.A.(California and Arizona), Afghanistan, Turkey, China, Japan and Russia [16–18].

Pomegranate fruit extract is a rich source of highly potent antioxidants and is widely used in several traditional medicinal systems for the treatment of arthritis and other diseases [19].

In this paper, we have reviewed the synthesis of silver nano particles with pomegranate and their antimicrobial activity.

3. PHYTOCOSITITUENTS IN POMEGRANATE

The medicinal value of plants lies in some chemical substances that produce a definite physiologic action on the human body. The most of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds [20]. Phytochemicals, which are non-nutritive plant chemicals, constitute a heterogeneous group of substances [21]. Phytochemicals are naturally occurring biologically active chemical compounds in plants. More than 4000 of these compounds have been discovered to date and it is expected that scientists will discover many more. Any one serving of vegetables could provide as many as 100 different phytochemicals. Phytochemicals are protective and disease preventing particularly for some forms of cancer and heart diseases. The phytochemical research based on ethno-pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants [22, 23].

Table 1: Pomegranate different parts and their constituents

S. NO.	PARTS OF PLANT	CHEMICAL CONSTITUENTS
1.	Roots and bark	Piperidine alkaloids [29], ellagitannins, punicalin and punicalagin [30]
2.	Flower	Gallic acid, ursolic acid[31],triterpenoids, including maslinic and Asiatic acid [32]
3.	Leaves	Tannins(punicalin and punicafolin),flavones glycosides including luteolin and apigenin[33]
4.	Juice	Amino acids [34], Minerals particulary iron[35],anthocyanins [36], glucose, ascorbic acid, ellagic acid, gallic acid, caffeic acid [37], Catechin, EGCG [38], quercetin, rutin [39]
5.	Peel and rind	Catechin, EGCG [38], phenolic punicalagins, galic acid and other fatty acids [37], quercetin, rutin and other flavonols [39], flavones, flavonones[33], anthocyanidins [40]
6.	Seed	Cyanidine, delphinidin, caffeic acid, chlorogenic acid, gallic acid, ellagic acid, luteolin, quercetin, kaempferol, naringenin and 17 α estradiol, estrone, estriol, testosterone, β sistosterol, coumestrol, γ-toccpherol, punicic acid, campestrol and stigmasterol [41,42]

The several chemical constituents were identified and isolated from different parts of the plant such as Juice, fruit, leaves and seeds. The chemical composition is affected by various factors like area of growing, storage condition, Cultural practice and climate[24-28].

4. GREEN SYNTHESIS AND CHARACTERIZATION OF SILVER NANO PARTICLES

Here, we reviewed the work done by many researchers on the green synthesis of silver nano particles by use of pomegranate's different parts. Silver nanoparticles are prepared from the peel, leaf, seed and fruit of pomegranate and their characterization is done by UV-visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), Scanning electron microscopy(SEM),Transmission electron microscopy(TEM), X-ray diffraction(XRD) spectra.

Peel of pomegranate was washed, dried and powdered for the making the extract of peel. Powder of peel with distilled water were placed on water bath for 15 minute at 65-70°C and cooled. Silver nanoparticles were synthesized by using 1mM $AgNO_3$ and peel extract at room temperature. The color change from yellow to brown color showed the formation of nanoparticles. Characterization was done by UV-visible spectrocscopy, FTIR, X-ray diffraction and SEM [43] and result are as shown in table 2.

The use of extracts for the synthesis of silver nanoparticles has an advantage in terms of rapid formation of stable nanoparticles. There is great effect of temperature and volume of extract used on the synthesis of nanoparticles and many other factors like pH etc. also affect the synthesis of nanoparticles.

Table 2: Biosynthesis of silver nanoparticles by different parts of Pomegranate.

S. No.	Part of pomegranate used	Temperature for synthesis	λ max.	Shape and size of nano particles	Reference
1.	Peel	Room temperature	427–440 nm	Polydisperse, Spherical, up to 5nm	[19]
2.	Peel	Room temperature	390–480 nm	Spherical,21 nm	[43]
3.	Seed	Room temperature	430 nm	Spherical, up to 30 nm	[44]
4.	Leaf	Room temperature	430 nm	Polydisperse, Spherical, 10-15 nm	[10]
5.	Fruit	Room temperature	460 nm	Spherical,30-40 nm	[45]

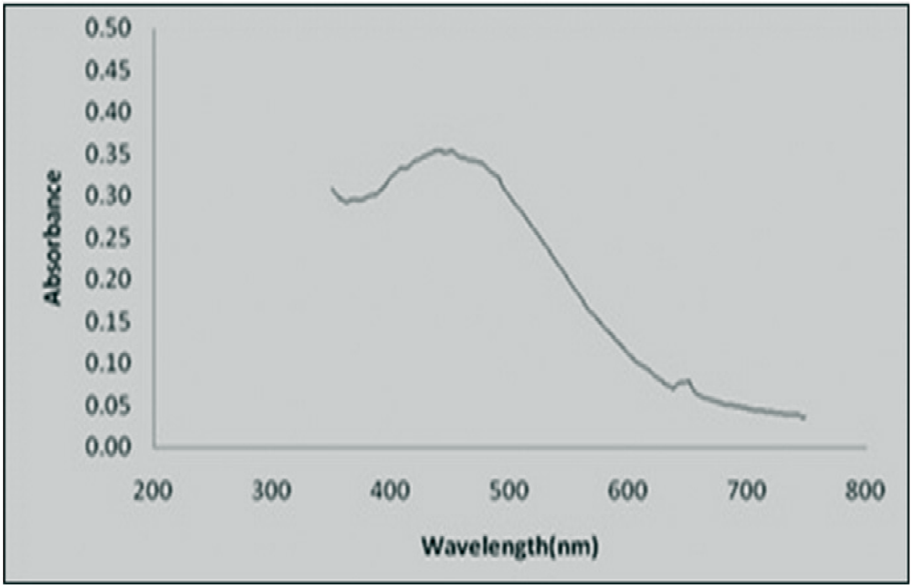

Figure: 1 UV–Visible absorption spectra of colloidal solution synthesized AgNPs as a function of the wavelength in the range of 300–800 nm[46].

Transmission electron microscopy, Scanning electron microscopy and atomic force microscopy techniques can be used for morphological studies of nanoparticles, which were used by different researchers to know the shape and size of nanoparticles but before the morphological studies, there is need to standardize the synthesis of nanoparticles using plants or their extracts. The formation of nanoparticles can be characterized by the use of UV-visible spectroscopy. As shown in table 2, silver nano particles synthesized by the use of various parts of pomegranate showed the absorption Peaks (λmax. Value) in the range of 390-480 nm. Figure 1 shows how the absorption peak come in the UV-visible spectra for silver nanoparticles[46].With the increase in time and concentration of plant extract with the salt ions clearly indicate the formation of nanoparticles.

FTIR spectrum analysis was also carried out to find out the possible reducing and stabilizing biomolecules in pomegranate extract of different parts. The FTIR spectra of peel extract taken before and after synthesis .The extract and synthesized nanoparticles showed absorption bands at 3036, 2928, 1734, 1033 and at 3379, 2939, 1730, 1028 respectively. These absorbance bands are associated with –OH, aliphatic –CH stretching or C triple bond C, C=O stretching and C-O stretching frequency. The hydroxyl group of these compound have a sronger ability to bind silver ions and may be involve in biosynthesis of silver nanoparticles and act as a reducing agent for the reduction of Ag^{+} ions to Ag(0) [43].

After that morphological studies showed that the nanoparticles formed were polydisperse, spherical and size varies from 5-40 nm from different parts of pomegranate. Figure 2 shows size and shape of silver nanoparticles synthesized by the green method [46].

Anti microbial activities were also studied by different researchers by disc diffusion method. The nanoparticles synthesized from seed extract of pomegranate were tested against multidrug resistant human pathogens E. Coli. by food poisoning and disc diffusion method and was found that 50ppm was

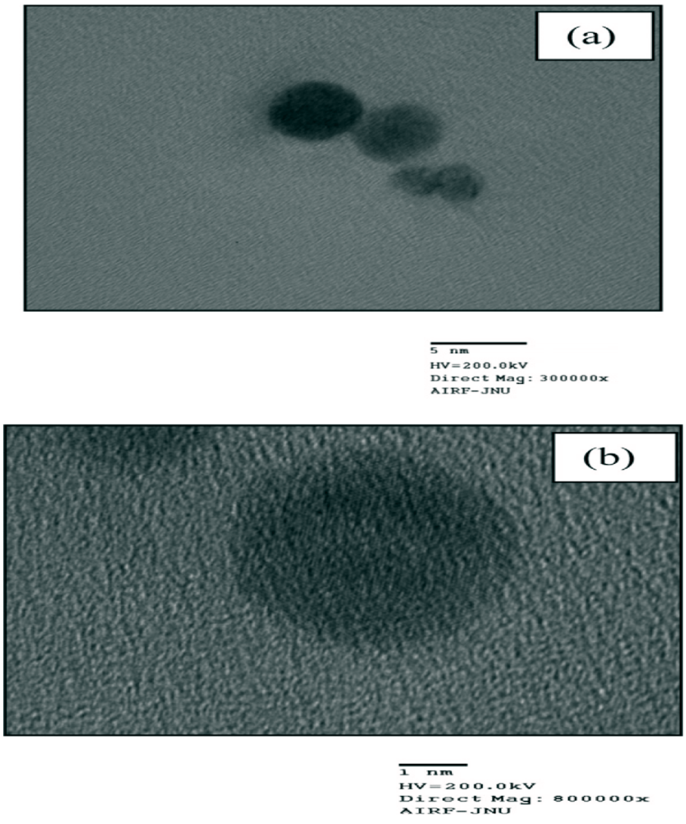

Figure 2: TEM images of silver nanoparticles at (a) 5 nm scale and (b) 1 nm scale [46].

minimum inhibitory concentration. The zone around the disc was compared with the inhibition zone of two standard antibiotic kanamycin and penicillin. The antifungal activity was tested against Aspergillius Flavus MTCC277 and gave significant result [44].

This formula can be used to find out the %of inhibition zone:

% inhibition = (C-E) X 100/C

Where, C= diameter of fungus mycelium on control plate.

E=Diameter of fungal mycelium on the experimental plate.

% inhibition of Aspergilius flavus was found 50%[44]

The silver nanoparticles synthesized from fruit extract of pomegranate were tested against bacillus subitilis(Gram positive) and Klebsiella planticolae(Gram negative) by disc diffusion method.[45].

It was found that silver nanoparticles have more inhibition zone which means they are effective in antimicrobial activities.

5. XRD

XRD was used to find out crystalline domain size. For XRD, first nanoparticles were purified by centrifugation at 10,000-15,000 rpm for15-20 minutes followed by redispersion of the pellet in deionized water and centrifuge again[44].

XRD pattern clearly showed that nanoparticles are crystalline in nature[45]. The broadening of the Bragg's peaks indicate the formation of silver nanoparticles[47].The average size of silver nanoparticles was calculated by Scherrer's equation:

$D = k \lambda / \beta_{1/2} \mathrm{COS}\ \theta$

D= Average grain size of crystallite

λ = Incident wavelength

θ = Bragg angle

β = Diffracted full width at half maximum (in radians).

The XRD pattern found to be consistent with earlier reports on microstructures [48].

6. PROPOSED MECHANISM

Some researcher tried to purpose the mechanism for stabilisation and formation of nanoparticles. It is reported earlier that proteins can bind to the nanoparticles either through free amine groups and therefore, stabilization of the AgNPs by protein is a possibility[49-51].The biological molecule perform dual function of formation as well as stabilization of nanoparticles in the aqueous medium.

Polyphenols and alkaloids are present in pomegranate extract. Ellagic acid is a naturally occurring phenolic compound found in many fruits and nuts. The esterification of the carboxyl and hydroxyl groups of ellagic acid make the ortho –phenolic hydroxyl loose the hydrogen much easily, forming a steadier semi-quinone structure. Thus, ellagic acid has an easy electron loosing capacity which results in the formation of H^+ radical which reduces the size of silver particles to nanosize.[19].

CONCULSION

The green synthesis of silver nanoparticles by the pomegranate helped out in avoiding the use of hazardous solvents and formation of waste product. It is simple and rapid method as compare to another mode of synthesis of nanoparticles. Fruit extract both act as a reducing as well as stabilizing agent. The UV-visible, FTIR, SEM, TEM and XRD characterization confirmed the formation of spherical nanoparticles in the size range 5-40 nm and face centred cubic lattice of AgNPS. Silver nanoparticles synthesized by pomegranate extract also showed good antimicrobial activity. This antimicrobial activity is one of the major role of silver nanoparticles as a antimicrobial agent and such nanoparticles synthesized by using palnts have been various applications for human benefit.

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