Biosynthesis of Silica Nanoparticles Using the Leaf Extract of *Punica granatum* and Assessment of Its Antibacterial Activities Against Human Pathogens

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**Abstract**

Plant-mediated nanoparticle synthesis is a revolutionary technique with numerous applications in fields, such as agriculture, food processing, and medicine. This study reports that *Punica granatum* leaf extract is capable of the green and eco-friendly synthesis of silica nanoparticles that provides a simple, cost-effective, and efficient methodology. *P. granatum* leaf extract was employed as a capping and stabilizing agent for the formation of silica nanoparticles, which were synthesized by a biological method using tetra ethyl ortho silicate. Biosynthesized silica nanoparticles are characterized by X-ray diffraction analysis, scanning electron microscopy, energy-dispersive X-ray spectroscopy, and Fourier transform infrared spectroscopy. All the analyses and characterization determined that the particles were spherical in shape and amorphous in nature with an average size of 12 nm. *P. granatum*-assisted silica nanoparticles were tested for antibacterial activity by a well-diffusion method against two-gram negative bacterial pathogens (*E. coli* and *Salmonella* sp.). The antibacterial studies prove that *P. granatum*-assisted silica nanoparticles have good antibacterial properties. These studies will help us find a new nano-drug or medicine against multidrug-resistant bacteria.

**Keywords**  Green synthesis · *Punica granatum* · Silica nanoparticle · Antibacterial activity

**Introduction**

Nanotechnology refers to an emerging field of science that includes the synthesis and development of various nanomaterials. Nanoparticles can be defined as objects ranging in size from 1 to 100 nm that, due to their size, may differ from the bulk material [1]. Nanoparticles are very essential in developing sustainable technologies for the future, for humanity, and for the environment. Synthesis of nanoparticles by plants is a green chemistry approach that interconnects nanotechnology and plant biotechnology [2]. Nanotechnology has emerged as one of the leading fields of science and has tremendous
application in diverse disciplines including biotechnology and agriculture, owing to the physicochemical properties of nanoparticles, such as the large surface area to volume ratio of nanoparticles (NPs), which provide an enormous reactive interface between the particle and its local environment. Although, in general, nanoparticles are considered a discovery of modern science, they have a very long history [3]. Predominantly the nanoparticles’ extremely small size and large surface area to volume ratio lead to significant differences in properties (e.g., biological, catalytic activity, mechanical properties, melting point optical absorption, thermal and electrical conductivity) not seen in the same material at larger scales in their bulk form. Because of these unique physicochemical and optoelectronic properties, nanoparticles are of particular use in several applications ranging from catalysts, chemical sensors, electronic components, medical diagnostic imaging, pharmaceutical products, and medical treatment protocols [4]. Nanotechnology has become one of the most promising technologies applied in all areas of science. Metal nanoparticles produced by nanotechnology have received global attention due to their extensive applications in biomedical and physicochemical fields [5, 6]. Nowadays, the synthesis of metal nanoparticles using microorganisms and plants has been extensively studied and has been recognized as a green and efficient way for further exploiting microorganisms as convenient nano factories [7]. In recent years, biological synthesis has emerged as an attractive alternative to traditional synthesis methods for producing nanoparticles [8]. Biosynthesis is environment-friendly, and biosynthesis of nanoparticles by microorganisms is a green and eco-friendly technology [9]. Both prokaryotes and eukaryotes are used for the synthesis of metallic nanoparticles, viz. silver, gold, platinum, zirconium, palladium, iron, cadmium, and metal oxides such as titanium oxide and zinc oxide. These microorganisms include bacteria, actinomycetes, fungi, and algae [8, 10]. Nowadays, there is a growing need to develop eco-friendly processes that do not use toxic chemicals in the synthesis protocols. Recent studies have shown that green biologically based methods using microorganisms and plants to synthesize nanoparticles are safe, inexpensive, and an environment-friendly alternative [11]. Green synthesis approaches include mixed-valence polyoxometalates, polysaccharides, Tollens, and biological and irradiation methods, which have advantages over conventional methods involving chemical agents associated with environmental toxicity. Selection of solvent medium and selection of eco-friendly non-toxic reducing and stabilizing agents are the most critical issues which must be considered in the green synthesis of NPs [12].

In the past few years, several studies have been made to prepare silica from natural resources and its effects on purification and characterization studies. Silica nanoparticles have been the subject of research because of their unique properties (e.g., their size and shape depending on optical, antimicrobial, and electrical properties) [13].

 Punica granatum belongs to the family Punicaceae (commonly known as pomegranate) and is one of the oldest known edible fruit. It is widely cultivated throughout Indis [14] and is an important ethnomedicinal plant with ameliorating therapeutic value. It is used in the treatment of various diseases, such as cardiovascular diseases, diabetes, dental conditions, allergic dermatitis, diarrhea, and in the treatment and prevention of cancer [15]. Pomegranate juice, peel, and oil have anticancer properties that can interfere with tumor cell proliferation, cell cycle, invasion, and angiogenesis, according to studies [16, 17]. These could be linked to pomegranate’s anti-inflammatory properties. The extract of P. grantum has medicinal value with its antioxidant, antibacterial, antidiabetic, cardioprotective, and anticarcinogenic activities [18].
The present investigation aimed to study the green synthesis of silica nanoparticles using *P. granatum* leaf extract. The biosynthesized silica nanoparticles were characterized by different analytical techniques. In addition, the antibacterial activity for *P. granatum*-leaf-mediated nanoparticles was investigated against the selected gram-negative and pathogenic bacteria.

**Materials and Methods**

**Preparation of Plant Extract**

*P. granatum* leaves were collected from the campus of the Karpagam Academy of Higher Education, Coimbatore, Tamil Nadu, India. Ten grams of the fresh leaves of *P. granatum* were taken and thoroughly washed. An extract was prepared by adding 200 ml of distilled water, and the mixture was boiled for 30 min at 50–65 °C. Further, the extract was filtered with Whatman No. 1 filter paper and stored at 4 °C and used for further experiments [19].

**Synthesis of Silica Nanoparticle**

Silica nanoparticles were synthesized by adding 12.5 ml of tetra ethyl ortho silicate as a precursor, and 17.5 ml of plant extract was added to the mixture. The reaction was allowed for 10 min of continuous stirring. Then 12.5 ml of 1 M HCl solution was added, and the mixture was stirred continuously for 15 min. As a result, a jelly-like formation was obtained, and it was placed in a hot air oven overnight at 100 °C. Finally, a white powder was obtained, and it was stored in an airtight container.

**Characterization of Synthesized Silica Nanoparticle**

The characterization was done for finding the physicochemical properties using UV–visible spectrophotometer, X-ray diffraction analysis, SEM analysis, EDAX analysis, and FTIR analysis.

**UV–Visible Spectra Analysis**

The green-synthesized silica nanoparticle was dissolved in distilled water and sonicated for 5 min at room temperature. The nanoparticle solution and plant extract were then transferred to a quartz cuvette, and, after being placed in the cell, the absorption maxima were determined from 200 to 800 nm using a UV–visible spectrophotometer. The bioreduction was monitored by UV–visible spectra of the supernatant solution as a function of time at room temperature using a double beam spectrophotometer [20].

**X-ray Diffraction Analysis**

Silica nanoparticles were characterized by Shimadzu XRD6 X-ray diffractometer using Cuk(alpha) radiation. The lattice parameter was calculated by the least-square fitting method using DOS computer programming. The theoretical density of the powder method...
was calculated with XRD data. The crystallite size of that powder was calculated by Scherrer’s formula Shimadzu IR prestige-21 model [21].

Scanning Electron Microscopy (SEM Analysis)

SEM was mainly used to obtain two types of information, namely the size (diameter distribution average) and morphology of nanomaterials. Silica nanoparticles were observed for their morphology and size using scanning electron microscopy (JEOL Model JSM 6360) [20].

EDAX Analysis

The JEOL Model JSM 6360 was used to conduct EDAX analysis on the synthesis of nanoparticles to determine the atomic weight percentage of elements in the samples [22].

Fourier Transform Infrared Spectroscopy (FTIR Analysis)

FTIR is a valuable device for the identification and characterization of functional groups (chemical bonds) and molecules present in the compound. The dried powder of green-synthesized silica and plant extract was placed on the quartz slide, and then FTIR spectrometer (Shimadzu IR Prestige-21 model) was employed to record the FTIR spectra of materials in the range of 4000–400 cm\(^{-1}\) [23].

Antibacterial Activity

The synthesized silica nanoparticles using \textit{P. granatum}-assisted silica nanoparticles were tested for antibacterial activity by agar well-diffusion method against the gram-negative strains (\textit{E. coli} and \textit{Salmonella}). The human pathogens (\textit{E. coli} and \textit{Salmonella}) were collected from the Department of Microbiology, Karpagam Academy of Higher Education, Coimbatore. Then the bacteria were swabbed uniformly on the individual plates using sterile cotton swabs on the nutrient agar. Five wells were made on 5 (in mm) on nutrient agar with the help of well puncture. Kanamycin and four different concentrations (5 μg/ml, 10 μg/ml, 15 μg/ml, and 20 μg/ml) of synthesized silica nanoparticles were used. The plates were incubated for 24 h at 37 °C to observe the formation of a zone of inhibition [24].

Results and Discussion

Characterization of Nanoparticles

UV–Visible Spectroscopy Analysis

UV–visible absorption spectra of the green-synthesized silica nanoparticles were recorded at a different wavelength from 200 to 800 nm, as shown in Fig. 1. The absorption spectra of silica nanoparticles were found to be 300–370 nm. Patil et al. [25] reported that the
UV–visible spectrum of silica nanoparticles displayed the maximum absorption band edge of 310 nm.

**XRD Analysis**

XRD (Fig. 2) was used to determine the nature of nanoparticles. XRD is an effective characterization to confirm the amorphous nature of the synthesized silica nanoparticles. The XRD of the silica nanoparticles revealed the characteristic peaks at 101 planes and planes at a diffraction angle of $2\Theta = 20^\circ$. The average size was calculated by using the Scherrer equation,

$$D = \frac{K\lambda}{\beta \cos \theta}$$

*D—Size of nanoparticles*
**K**—Scherrer constant with a value from 0.9 to 1λ is the wavelength of the X-ray source used in XRD

**B**—Full width at half maximum of the diffraction peak

**Θ**—Bragg’s angle

The average size of silica nanoparticles was found to be 12 nm. Similarly, Sankarreswaran et al. [26] reported 2Θ values of the (101) plane at 20° for silica nanoparticles. The XRD patterns also revealed that the obtained silica nanoparticles were amorphous. Kerry et al. [27] determined the nature of silica nanoparticles using the XRD analysis.

**SEM Analysis**

Scanning electron microscopy was used to recognize the morphology and size of silica nanoparticles of *P. granatum* as shown in Fig. 3. The silica nanoparticles are found as aggregates with spherical morphology. Similarly, Maroušek et al. [28] reported on the spherical shape of silica nanoparticles, and it was confirmed by scanning electron microscopy. Patil et al. [25] also confirmed the spherical shape of the as-synthesized silica nanoparticles using the SEM analysis.

**EDAX Analysis**

EDAX measurement result of the green-synthesized silica nanoparticles is shown in Fig. 4. The spectra of all the samples have shown the signal peaks corresponding only to Si (28.98%) and O (71.02%) elements. From the results, it was confirmed that no other impurity elements were present in the samples. Similarly, Yadav and Fulekar [29] reported

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**Fig. 3** SEM analysis of silica nanoparticles
that purified final silica nanoparticles had peaks for Si, O, and C. This indicates the purity of silica nanoparticles of ~94–97%.

**FTIR Analysis**

The FTIR characterization is used to find the molecules and their functional group present in the synthesized nanoparticles. Figure 5a represents the FTIR spectra of silica nanoparticle displaying different peaks that were observed at 2978 cm$^{-1}$, 2885 cm$^{-1}$, 1689 cm$^{-1}$, 1519 cm$^{-1}$, 1388 cm$^{-1}$, 1072 cm$^{-1}$, 956 cm$^{-1}$, and 416 cm$^{-1}$, whereas the FTIR spectrum of the plant extract (Fig. 5b) produce successive absorption peaks at 3302 cm$^{-1}$, 2978 cm$^{-1}$, 2138 cm$^{-1}$, 1635 cm$^{-1}$, 1388 cm$^{-1}$, 1248 cm$^{-1}$, 1072 cm$^{-1}$, and 671 cm$^{-1}$. A strong peak of 956 cm$^{-1}$ refers to Si–O groups (Fig. 5a). Pieła et al. [30] confirmed the presence of siloxane bonds and O-Si–O bonds in silica nanoparticles using the FT-IR analysis. Functional groups of C–N stretch of aliphatic amines were observed at 1072 cm$^{-1}$ in both the plant extract and biosynthesized silica nanoparticles. The absorption peak at 1388 cm$^{-1}$ corresponds to C–H bending vibrations (aromatic tertiary amine group). CH stretching was identified by the presence of a peak at 2978 cm$^{-1}$. Alves et al. [3] also reported similar FTIR signals for silica nanoparticles.

**Antibacterial Analysis**

The antibacterial activity of *P. granatum*-mediated silica nanoparticles was summarized in Table 1. The antibacterial activity of silica nanoparticles was tested against two different gram-negative organisms (*E. coli* and *Salmonella*) (Fig. 6a and b). Kanamycin was used as a positive control. The maximum zone of inhibition on *E. coli* (25 mm) and *Salmonella* (26 mm) was observed. Similarly, Gopinath et al. [31] reported that the antibacterial activity of copper nanoparticles was studied against disease-causing five bacterial pathogens like *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Salmonella typhi*, and *Bacillus subtilis* and the resultant maximum zone of inhibition on *Staphylococcus*
Fig. 5  a FT-IR analysis of silica nanoparticles.  b FT-IR analysis of *P. grantum* extract

**Table 1**  Antibacterial activity of *Punica granatum*-mediated silica nanoparticles  

| Microorganisms    | Control (kanamycin) | 5 μg/ml | 10 μg/ml | 15 μg/ml | 20 μg/ml |
|-------------------|---------------------|---------|----------|----------|----------|
| *E. coli*         | 33                  | 20      | 21       | 22       | 25       |
| *Salmonella* sp.  | 30                  | 19      | 20       | 22       | 26       |
 aureus (21 mm) followed by Salmonella typhi (20 mm), Klebsiella pneumonia (18 mm), Bacillus subtilis (15 mm), and Escherichia coli (10 mm).

**Conclusion**

The present investigation aimed to study the green synthesis of silica nanoparticles using *P. granatum* leaf extract. The biosynthesized silica nanoparticles were characterized; they are spherical in shape and amorphous in nature, and the XRD results show that the most predominant peak occurs at 2θ = 20° corresponding to 101 planes. The purity of the samples clearly showed the presence of Si and O elements, and no other impurities were present in the synthesized silica nanoparticles. *P. granatum*-leaf-mediated nanoparticles revealed good antibacterial activity against the selected pathogenic bacteria (*E. coli* and *Salmonella*). The antibacterial studies prove that *P. granatum*-assisted silica nanoparticles have good antibacterial properties. These studies will help us find a new nano-drug or medicine against multidrug-resistant bacteria.

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**Author Contribution** PR: Supervision, funding acquisition, and project administration
MPS: Investigation
PC: Methodology
RP: Data curation and writing—original draft preparation
GRS: Data curation and writing—original draft preparation
JD: Data curation and writing

**Data Availability** Not applicable.

**Declarations**

**Ethical Approval** Not applicable.

**Consent to Participate** Not applicable.
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Research Involving Human Participants and/or Animals  Not applicable.

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