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Research Paper

Eco-Friendly Synthesis, Characterization, and Antimicrobial Evaluation of Silica Pomegranate Nanoparticles

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Abstract
Plant-based synthesis of nanoparticles is an advanced and sustainable approach with wide-ranging applications across industries such as food processing, pharmaceuticals, and agriculture. In this study, pomegranate leaf extract was utilized as a simple, cost-effective, and eco-friendly method for synthesizing silica nanoparticles. The biological synthesis employed the leaf extract both as a reducing and stabilizing agent. Characterization of the biosynthesized silica nanoparticles was performed using Energy-Dispersive X-ray Spectroscopy (EDX), Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), and X-ray Diffraction (XRD). The results confirmed that the nanoparticles possessed a spherical shape and an amorphous structure. Their antibacterial properties were evaluated using the well-diffusion method against four bacterial strains, revealing significant antimicrobial activity. These findings suggest that silica nanoparticles synthesized via plant extracts hold strong potential for the development of novel antimicrobial agents, especially to tackle drug-resistant bacteria, while promoting environmentally sustainable practices.

Keywords: *Antimicrobial; Nanoparticles; Pomegranate; Silica.*

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I. Introduction

The production of nanoparticles is one area of nanotechnology that is growing quickly; the global market was estimated to be worth $80 billion in 2023, and it is expected to rise by 20% annually between 2024 and 2030 [1]. This interest in nanoparticles is fueled by their intriguing surface effects, which are identified by their high surface-to-volume ratio, which indicates a high reactivity due to the large number of exposed atoms. Silicon dioxide (SiO_2), also known as silica, is one of the most widely utilized minerals to create nanoparticles. It has been used to create flexible, biocompatible hybrid materials with specialized features. Because of their great purity, large number of surface groups, colloidal stability, superior dispersibility, and low cytotoxicity, silica nanoparticles have attracted a lot of interest in a variety of scientific and technological applications [2]. Because of their huge pore volume, well-defined pore structure, and high specific surface area, mesoporous silica nanoparticles (SNPs) are especially well-suited as drug delivery vehicles and for use in pharmaceutical and biological applications [3].

Furthermore, SiNPs are frequently used to immobilize biomolecules [4]. Nevertheless, there are still problems with their usage as drug delivery systems, and further study is required to increase their efficacy, safety, and usefulness for practical uses. A wide range of naturally occurring chemical compounds known as polyphenols are created by plants as secondary metabolites and are employed as a defense against illnesses and infections brought on by bacteria, viruses, and fungi [5]. The antioxidant, anti-inflammatory, and perhaps anticancer qualities of polyphenols have drawn the interest of many scientists in recent years [6]. Their limited solubility and bioavailability, however, limit their intestinal absorption and, as a result, diminish the potential health benefits of their use [7]. Furthermore, they may oxidatively degrade and lose their bioactivity as a result of their aggregation phenomenon in aquatic environments or their sensitivity to heat and light [8].

In order to keep polyphenols from degrading and to increase their stability, bioavailability, and biological activity thereby extending their biological effects the interaction between polyphenols and

nanoparticles is frequently researched [9]. Catechins, resveratrol, curcumin, quercetin, anthocyanins, and tannins are among the polyphenols that have been effectively connected to nanoparticles, demonstrating enhanced anti-inflammatory and antioxidant properties [10]. SiNPs loaded with curcumin and resveratrol have recently demonstrated substantial cytotoxicity rates against human cancer cell lines of breast cancer and melanoma, respectively [11]. MCM 41 and MCM-48 mesoporous silica types were loaded with transferulic, p-coumaric, and caffeineic acids to provide a polyphenol-controlled release system suitable for a range of biological uses [12]. Mesoporous nanoparticles loaded with resveratrol have demonstrated cytotoxicity against human lung (A549) and breast cancer (MDA-MB-231), suggesting that they may be used to treat cancer [13]. Additionally, SiNPs have been shown to be effective in transporting polyphenol blends for application in cosmetic and nutraceutical compositions. On the NIH3T3 cell line, the hydroalcoholic extract of grape pomace loaded in silica mesopores of the MCM-41 type functionalized with organic groups demonstrated good cytocompatibility and antioxidant activity in vitro [14]. With the inclusion of a collagen scaffold, a polyphenolic extract from sage (*Salvia officinalis*) was embedded in functionalized mesoporous silica nanoparticles, which produced improved antibacterial activity and high cytocompatibility for use in skin lesions [15].

The pomegranate tree (*Punica granatum L.*) is now grown throughout the world. Because of its healthful qualities, its fruit has become more and more popular over time, to the point where it is now regarded as a new superfood. Bioactive compounds such polyphenols and polyunsaturated fatty acids, which have anti-inflammatory, antidiabetic, antioxidant, antibacterial, and antitumor effect against some types of cancer, are abundant in pomegranate fruits [16]. In accordance with the circular bioeconomy and bioeconomy principles, the pomegranate fruit's beneficial effects extend beyond its juice to include secondary components like its peel, which accounts for 50% of production chain waste. Exploiting this peel could be a sustainable way to value agri-food waste [17]. According to earlier research, pomegranate peel has a high concentration of polyphenols, including anthocyanins, catechins, tannins, gallic, and ellagic acids. These compounds have potent anti-inflammatory, anti-cancer, and antimicrobial properties that are even stronger than those of the polyphenols in the juice [18]. Pomegranate peel polyphenols, either by themselves or in combination, have been found to have potent anti-cariogenic and anti-food-borne bacterial action [19].

The purpose of this work is to offer strategic options for the production of multifunctional hybrid nanomaterials that will increase the bioavailability and advantages of pomegranate polyphenol. Because of its simplicity, scalability, and controllability, SNPs were synthesized using the sol-gel technique [20]. They were then functionalized with 3-aminopropyl triethoxysilane (APTS) to enable the efficient adsorption of pomegranate leaf extract on the silica surface. Pomegranate leaf extract was then physically adsorbed onto SiNPs as part of an extra-situ technique to merge the organic and inorganic phases. The physical adsorption method used to encapsulate pomegranate extract preserves the extract's native structure and redox characteristics, which improves the accessibility of its active ingredients and gets around the problems caused by the organic compounds inherent sensitivity to heat and light [22], in contrast to the one-pot in-situ templated synthesis strategy [21]. The pomegranate leaf extract loaded nanoparticles were characterized morphologically, physico-chemically, and by Fourier transform infrared spectroscopy (FT-IR), and transmission electron microscopy (TEM), SEM, Zeta, UV and XRD. This work's primary innovation is the incorporation of pomegranate extract, inexpensive, bioavailable resource rich in polyphenols, as an active phase within an inorganic, biocompatible support. In contrast to earlier research [23,24], this work uses an approach to create hybrid silica nanoparticles based on pomegranates and thoroughly assesses their morphological, physicochemical, and biological characteristics. By using this method, the pomegranate leaf extract's inherent qualities can be maintained, guaranteeing that its bioactive ingredients stay strong and functional.

Additionally, this approach might offer a secure and adaptable platform for encapsulating pomegranate bioactive compounds in accordance with the circular bioeconomy and sustainability principles, valuing agri-food by-products and opening the door for the creation of multifunctional nanomaterials with substantial application potential in the food and biomedical industries.

II. Methodology

Plant extract preparation

A slightly modified version of Sharma et al. (2021)'s procedure was used to prepare the plant extract [25]. To put it briefly, the leaves of pomegranate plants were repeatedly cleaned with ultrapure deionized water to get rid of dust, and then they were left to dry for six days at room temperature (25°C) until their weight stabilized. For four minutes, dried plant extract was pulverized in a grinder. After that, 5g of finely ground plant extract was boiled for 45 minutes at 55 °C in 100mL of deionized water. The heated leaves were then filtered and utilized for SiNPs' green synthesis (SiPG@NPs).

Pomegranate plants were utilized as a green Si precursor in the green synthesis of SiNPs. With minor adjustments, the procedures outlined by Mohd et al. (2017) were followed in order to synthesize SiNPs [26]. For

one hour, sodium hydroxide (NaOH; 1.0 N) was used to reflux the produced plant extract. After the plant extract was refluxed with NaOH, the following reaction was explained.

By gradually adding hydrochloric acid (HCl; 0.1 M) until the pH of the solution reached 6.0, the SiNPs were extracted from sodium silicate (Na_2SiO_3). A reaction between NaCl and SiO_2 was created when HCl was added to Na_2SiO_3. Following many rounds of washing with ethanol and water to get rid of the generated NaCl, the precipitate was recovered by centrifuging it for ten minutes. After that, the finished product was baked for 24 hours at 50 °C. The Si-PG@NPs were dried and then put in a vial for additional examination and characterization.

Characterization: Si-PG@NPs' micromorphological structures were examined using a transmission electron microscope (TEM) and a field emission scanning electron microscope (SEM) with an energy spectrum. To find the surface functional groups of Si-PG@NPs, the FTIR spectrometer (400-4000 cm^{-1}) was utilized. The Si-PG@NPs was determined by XRD wide-angle diffraction (Bruker, Germany's D8 ADVANCE X-ray diffractometer).

Antimicrobial properties:

The antibacterial activity of silica pomegranate nanoparticles was evaluated using the well diffusion method. Four bacterial strains were selected for the study: *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Escherichia coli*. Each bacterial strain was cultured in nutrient broth and incubated at 37°C for 24 hours to obtain an active culture.

Agar plates were prepared using Mueller-Hinton Agar (MHA) and uniformly swabbed with 100 µL of bacterial suspension. Wells (6 mm diameter) were created in the agar medium, and 100 µL of SiO_2-PNPs at concentrations of 100, 200, 300, and 400 µg/mL were added to separate wells. Streptomycin (10 µg/disc) was used as a standard reference drug. The plates were incubated at 37°C for 24 hours, after which the diameter of the inhibition zones around the wells was measured in millimeters (mm).

The MIC was determined using the broth dilution method. Different concentrations of SiO_2-NPs (25, 50, 75, 100, 125, and 150 µg/mL) were prepared in nutrient broth. Each concentration was inoculated with 100 µL of bacterial suspension. The mixtures were incubated at 37°C for 24 hours. MIC was recorded as the lowest concentration of nanoparticles that inhibited visible bacterial growth. Streptomycin (10 µg/mL) was used as a standard reference drug.

III. Result And Discussion

Characterization of Iron Nanoparticle

Absorption peaks in the FTIR spectra ranged from 400 to 4000 cm^{-1}(Fg.1). Within the 700–1200 cm^{-1} range, several peaks were seen, including 806, and 1107 cm^{-1}. The stretching vibrations of Si–O–Si, which take place in the 1000–1200 cm^{-1} range, were represented by these peaks. Both the bending and stretching vibrations of the N–H bond are responsible for the strong signals seen at 3444cm^{-1}. A sign of C-H bending is the absorbance peaks at 1384 and 1634 cm^{-1}. The O-H bond's stretching vibrations are responsible for the wide spectral characteristic that spans between 3000 and 3600 cm^{-1}. These hydroxyls (OH) groups' existence indicated that the methoxyl groups attached to the silicon atom had not been fully hydrolyzed, which would have resulted in insufficient cross-linking between the hydrolyzed silane molecules. The generated SiNPs contained functional groups such as CH, OH, NH, and SiO, according to the FTIR study.

This XRD pattern displays a broad peak centered around 22°, typical of amorphous silica. The lack of sharp diffraction peaks confirms the non-crystalline (amorphous) nature of the material, consistent with silica-based nanoparticles like pomegranate-structured silica NPs. The broad hump in the 2θ range of 15–30° is characteristic of disordered SiO_2 networks, common in sol-gel synthesized materials(Fig. 2). According to the investigation, the produced SiNPs were mostly of the same kind and showed consistent properties. Additionally, the SiNPs' XRD spectra showed no extra peaks, suggesting that the produced material included few contaminants. An absorption peak at 297 nm was found when the UV–Vis absorption of SiNPs was evaluated in the 200–600 nm wavelength range (Fig. 3).

The negative value indicates the nanoparticles carry a net negative surface charge, typical for silica-based materials due to deprotonated silanol groups.A zeta potential of (-23.5mV) suggests moderate colloidal stability enough repulsion to prevent immediate aggregation, but not as stable as particles with zeta potentials beyond ±30 mV (Fig. 4).

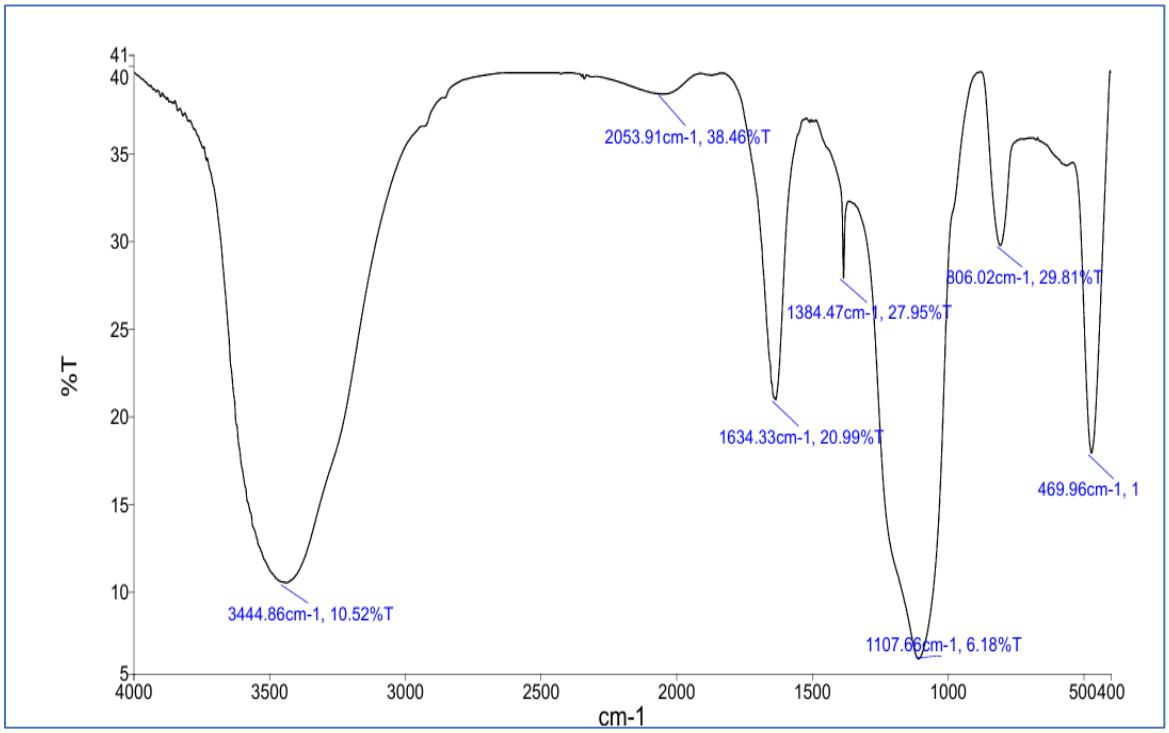

Fig. 1: FTIR spectrum of Si-PG@NPs.

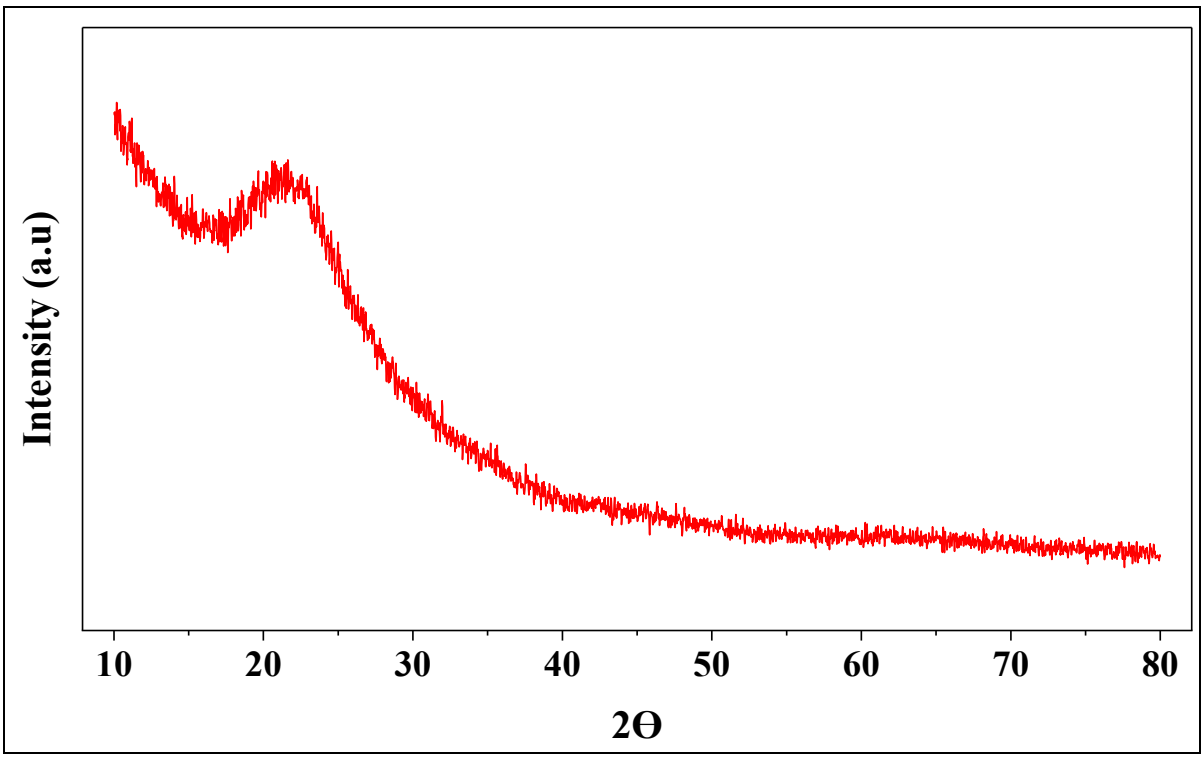

Fig. 2: XRD pattern of Si-PG@NPs.

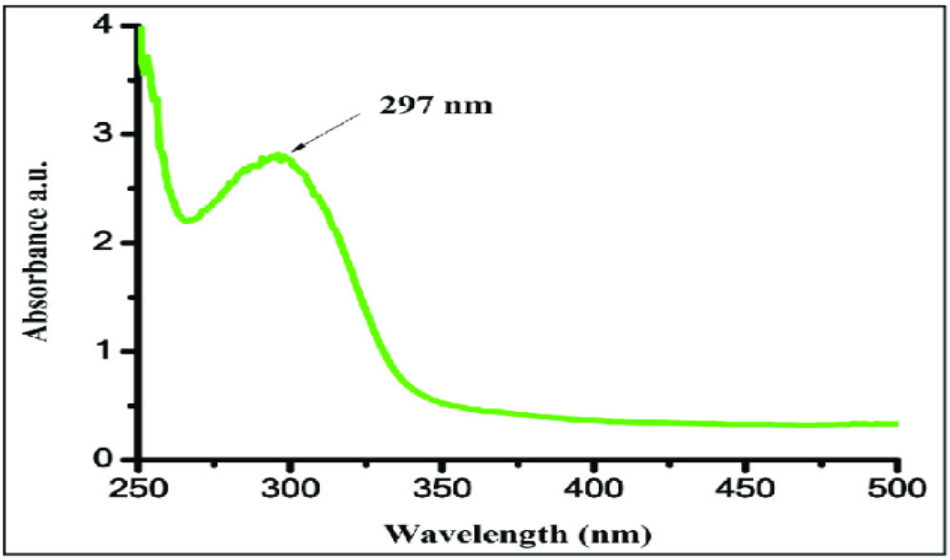

Fig 3 UV-Vis spectrum of Si-PG@NPs.

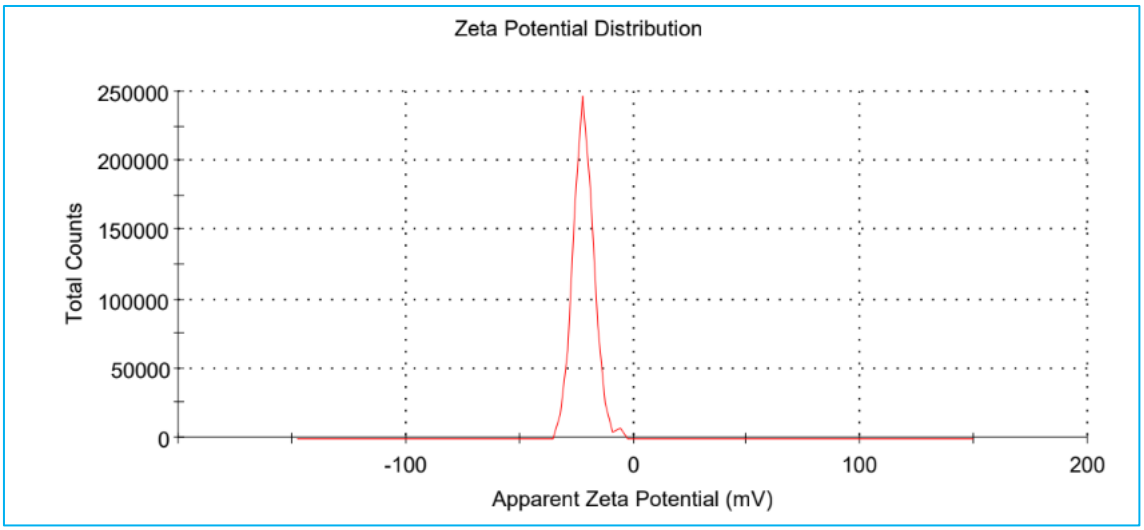

Fig. 4 Zeta potential of synthesized Si-PG@NPs.

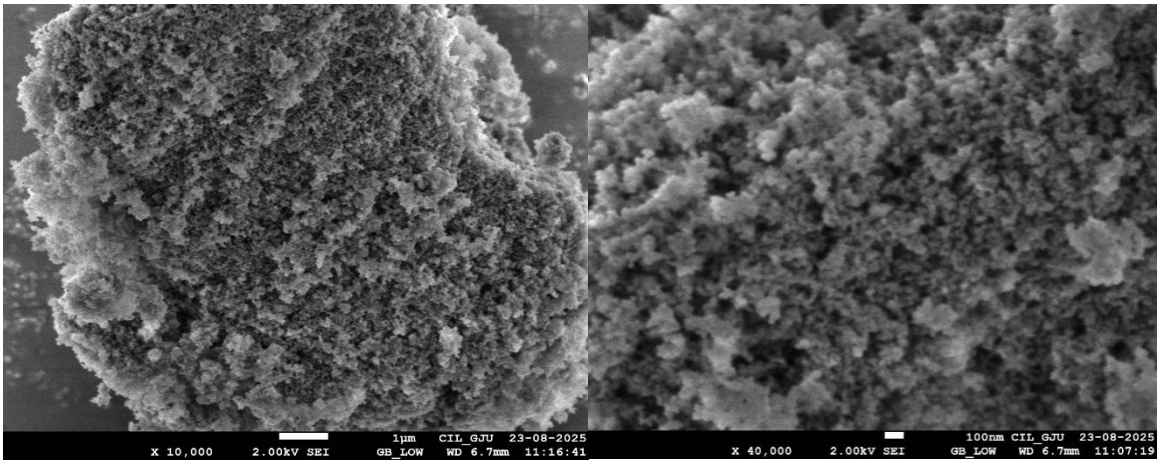

Fig. 5: FESEM of synthesized Si-PG@NPs.

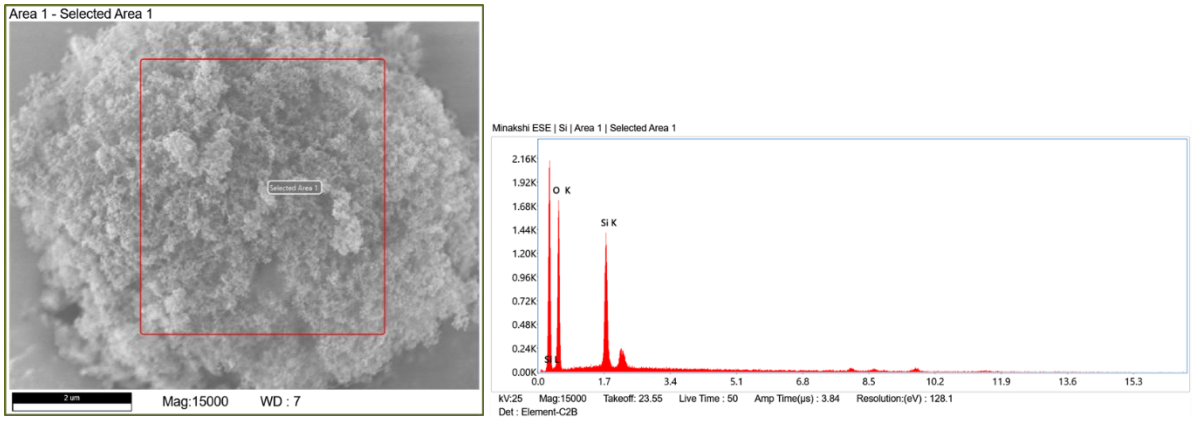

Fig. 6: EDS of synthesized Si-PG@NPs.

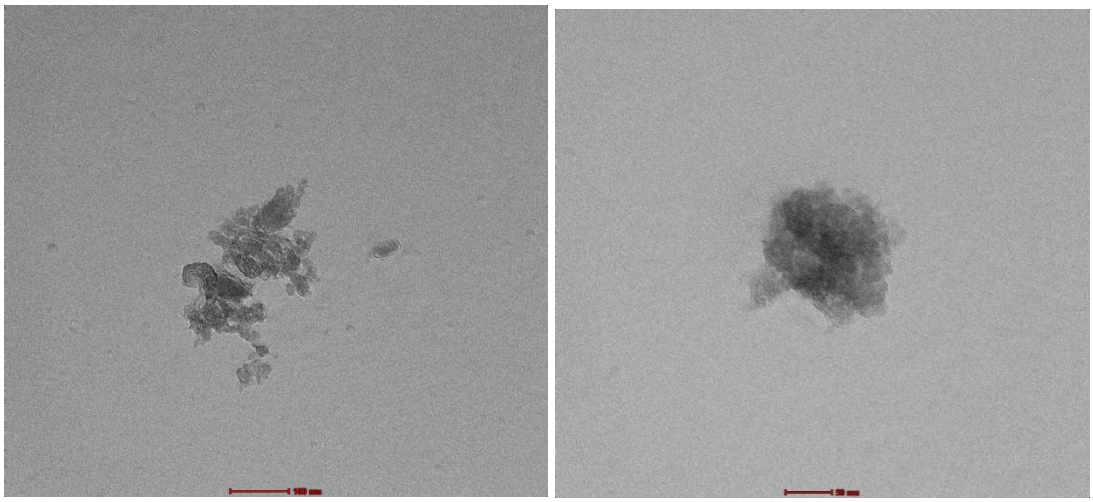

Fig. 7: TEM of synthesized Si-PG@NPs.

The FESEM image of silica–pomegranate nanoparticles shows highly porous, irregular, and agglomerated structures with rough surface morphology. The nanosized clusters indicate successful silica nanoparticle formation, while aggregation results from hydrogen bonding and phytochemical capping. The individual particle size range is approximately 50–150 nm, though aggregation makes them appear larger in some regions (Fig.5).The EDS spectrum confirms that the silica pomegranate nanoparticles are primarily composed of oxygen (78.4 wt%) and silica (21.6 wt%) (Fig 6). The high oxygen and silicon peaks indicate successful synthesis of silica. No other elemental peaks suggest purity, and the atomic ratio aligns with stoichiometric silica composition.To further examine the morphological characteristics of the SiNPs, TEM was performed in addition to SEM. The produced SiNPs' recognizable spherical shape and high degree of monodispersity were shown in the TEM images (Fig. 7). The particles' exceptional homogeneity suggested a carefully regulated manufacturing procedure. Interestingly, despite their nature, the SiNPs were seen to be grouped together in tiny aggregates, creating bunches. Overall, the spherical shape of the produced SiNPs was validated by both SEM and TEM investigations. These findings demonstrated the effective synthesis of SiNPs with a distinct structure and monodispersity, which may lead to a number of potential uses for them.

Antimicrobial activity

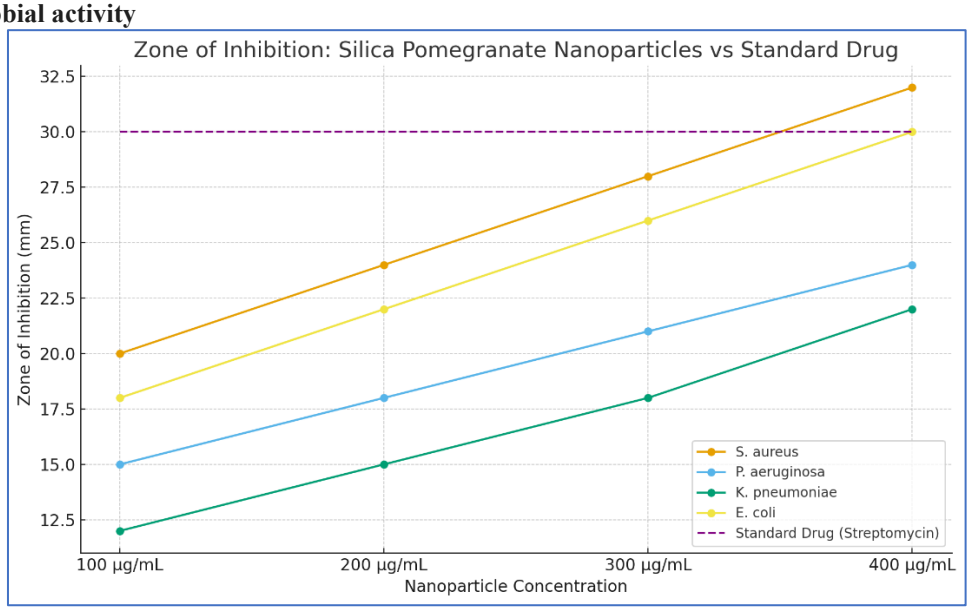

Fig. 8: ZOI at different concentration of Si-PG@NPs.

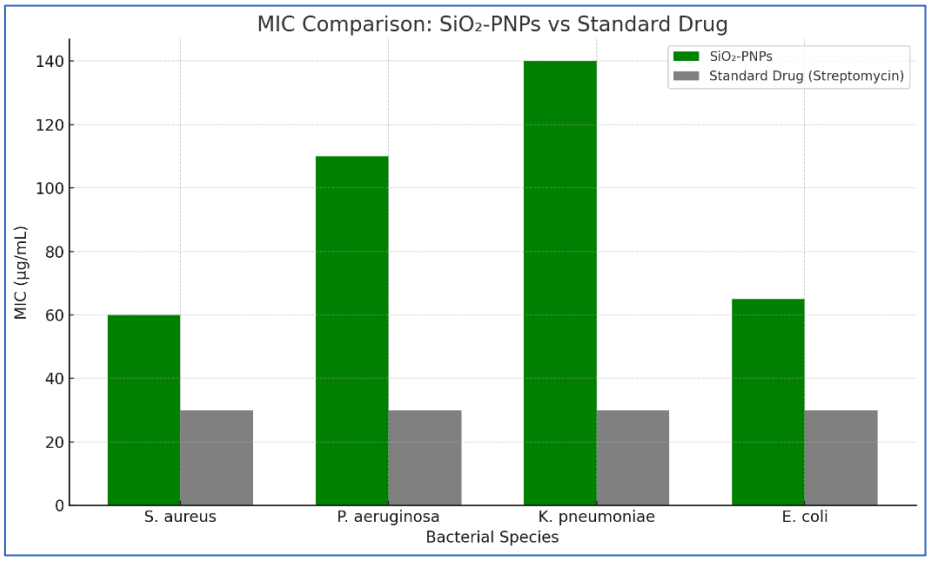

Fig. 9: MIC of synthesized nanoparticleSi-PG@NPs and standard drug.

The antibacterial potential of biosynthesized silica pomegranate nanoparticles (SiO_2-NPs) was systematically assessed using the Zone of Inhibition (ZOI) method against four bacterial strains: *Staphylococcus aureus* (Gram-positive), *Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Escherichia coli* (all Gram-negative). The study aimed to evaluate the concentration-dependent antimicrobial efficacy of SiO_2-PNPs and to compare their activity with that of a standard antibiotic (Streptomycin).

The experimental results demonstrated a clear dose-dependent antibacterial activity of SiO_2-NPs. At the lowest concentration (100 µg/mL), the inhibition zones were measured as 20 mm for *S. aureus*, 15 mm for *P. aeruginosa*, 12 mm for *K. pneumoniae*, and 18 mm for *E. coli*. With increasing concentrations (200, 300, and 400 µg/mL), the inhibition zones significantly increased for all bacterial strains. At the highest concentration (400 µg/mL), *S. aureus* exhibited the largest inhibition zone of 32 mm, followed by *E. coli* at 30 mm, *P. aeruginosa* at 24 mm, and *K. pneumoniae* at 22 mm (Fig-8).

This differential susceptibility highlights the stronger efficacy of SiO_2-NPs against Gram-positive bacteria (*S. aureus*) compared to Gram-negative strains. The structural differences between these bacterial classes provide a plausible explanation: Gram-positive bacteria possess a thick peptidoglycan layer but lack an outer membrane, making them more vulnerable to nanoparticle interaction and membrane disruption. Conversely, Gram-negative bacteria are shielded by a more complex outer membrane, which acts as a barrier to nanoparticle penetration, reducing antibacterial effectiveness.

When compared to the standard antibiotic (Streptomycin), which consistently exhibited an inhibition zone of approximately 30 mm across all strains, SiO_2-PNPs showed comparable performance against *S. aureus* and *E. coli* at higher concentrations. Although Streptomycin generally displayed superior antibacterial action, the SiO_2-PNPs' performance is notable given their green synthesis and eco-friendly origin.

The mechanism of antibacterial action is likely multifactorial. Nanoparticles may attach to bacterial surfaces via electrostatic interactions, destabilizing the membrane. Additionally, SiO_2-PNPs could generate reactive oxygen species (ROS), causing oxidative damage to bacterial DNA, proteins, and membranes. The high surface area of nanoparticles also increases contact points with bacterial cells, enhancing their inhibitory effects.

To quantify the lowest concentration at which SiO_2-NPs effectively inhibit bacterial growth, the MIC was determined using the broth dilution method. The MIC values for the four strains were:*S. aureus*: 60 µg/mL, E. coli: 65 µg/mL, *P. aeruginosa*: 110 µg/mL and *K. pneumoniae*: 140 µg/mL (Fig. 9).

These results again confirm that *S. aureus* was the most susceptible strain, aligning with ZOI findings. The relatively low MIC values for *S. aureus* and *E. coli* indicate the high antimicrobial potential of SiO_2-NPs, while the higher MIC values for *P. aeruginosa* and *K. pneumoniae* reflect their additional structural defenses.

Compared to the MIC of Streptomycin (30 µg/mL for all strains), the SiO_2-NPs required a higher concentration to achieve inhibition. Nevertheless, the MIC values demonstrate significant antibacterial capability, especially considering the nanoparticles' biosynthesized and eco-friendly nature.The MIC study complements the ZOI results by providing quantitative evidence of nanoparticle efficiency and the concentration thresholds necessary to inhibit bacterial growth. The consistent trend of lower MIC for *S. aureus* emphasizes its higher vulnerability, supporting the hypothesis of nanoparticle-driven membrane disruption and protein interaction.

Several studies have demonstrated the antimicrobial activity of biosynthesized silica nanoparticles. For example, recent research reported that silica nanoparticles synthesized using plant extracts exhibited significant antibacterial effects against *E. coli* and *S. aureus*, consistent with our findings. The observed stronger action against *S. aureus* aligns with previous reports attributing this to the absence of an outer membrane in Gram-positive bacteria, facilitating easier nanoparticle interaction.

IV. Conclusion

In conclusion, this study successfully demonstrates the green synthesis of silica nanoparticles using pomegranate leaf extract as a natural capping and stabilizing agent. The biosynthesized nanoparticles were thoroughly characterized by Energy-Dispersive X-ray Spectroscopy (EDX), Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), and X-ray Diffraction (XRD), confirming their spherical morphology and amorphous nature. Antibacterial activity assessed through the well-diffusion method showed significant inhibition against multiple bacterial pathogens, highlighting the strong antimicrobial potential of the silica nanoparticles. These findings suggest that plant-mediated silica nanoparticles can serve as promising candidates for developing novel nanomedicines or antibiotics, particularly to address the growing challenge of multi-drug-resistant bacteria, while offering an eco-friendly and cost-effective synthesis route.

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