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Article

Green synthesis of gold nanoparticles using pomegranate peel extract and investigation of their antibacterial activity

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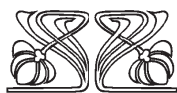
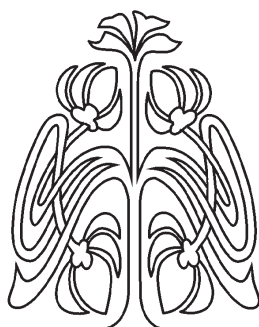
Abstract. Gold nanoparticles (AuNPs) have been created utilizing pomegranate (*Punica granatum* L.) peel extract as a natural reducing and stabilizing agent in an environmentally friendly green synthesis method. Pomegranate peel contains polyphenols and antioxidant phytochemicals that efficiently convert hydrogen tetrachloroaurate (HAuCl_4) to generate stable AuNPs, as indicated by a distinctive color change from light yellow to wine red and validated using UV-Vis spectroscopy. The biosynthesized nanoparticles have been further studied using scanning electron microscopy (SEM), atomic force microscopy (AFM), and zeta potential analysis. The SEM and AFM data showed primarily spherical AuNPs with an average particle size of around ($r = 23$ nm). The serial dilution approach has been used to investigate antibacterial efficacy against *Escherichia coli* at different nanoparticle doses. AuNPs obtained from pomegranate peels did not kill the *Escherichia coli* bacteria at tested concentration (1.7, 0.17 and 0.017 $\mu\text{g/mL}$), as confirmed by the growth of bacteria in culture conditions. This paper demonstrates the “green” production and characterization of AuNPs with an agro-waste, pomegranate peel, which exhibits low antibacterial effect vs. *E. coli* investigated conditions.

Keywords: Green synthesis, AuNPs, Pomegranate Peel, Anti-bacterial activity, scanning electron microscopy

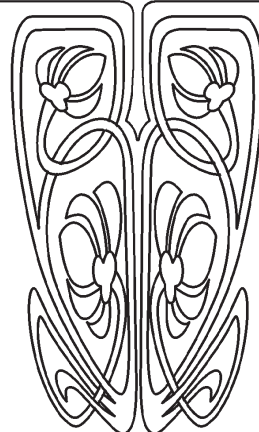
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НАУЧНЫЙ
ОТДЕЛ





Научная статья
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Зеленый синтез наночастиц золота с использованием экстракта кожуры граната и исследование их антибактериальной активности

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Аннотация. В результате проведенного эксперимента были получены наночастицы золота (AuNPs); для этого использовали кожуру граната (*Punica granatum* L., 1753) в качестве естественного восстанавливающего и стабилизирующего агента. Таким образом, предложен экологически безопасный способ синтеза наночастиц. Гранатовая кожура содержит полифенолы и антиоксидантные фитохимические вещества, которые обеспечивают эффективное восстановление ионов золота из тетрахлораурата водорода (HAuCl₄) для получения стабильных AuNPs, о чем свидетельствуют характерное изменение окраски раствора от светло-желтого до винно-красного и данные спектроскопии UV-Vis. Биосинтезированные наночастицы были дополнительно исследованы с использованием сканирующей электронной микроскопии (SEM), атомно-силовой микроскопии (AFM) и анализа дзета-потенциала. Данные SEM и AFM показали в основном сферические AuNPs со средним диаметром около 23 нм. Антибактериальную активность в отношении бактерий *Escherichia coli* оценивали методом серийных разведений при различных концентрациях наночастиц. AuNPs, полученные из кожуры граната, не проявляли ингибирующего действия в исследованных концентрациях (1,7, 0,17 и 0,017 мкг/мл), что подтверждается ростом бактерий в условиях культивирования. В статье представлен «зеленый» способ синтеза AuNPs с использованием агроотхода – кожуры граната – и охарактеризованы физико-химические свойства полученных наночастиц; показана их ограниченная антибактериальная активность в отношении бактерий *E. coli*.

Ключевые слова: зеленый синтез, AuNPs, гранатовая кожура, антибактериальная активность, сканирующая электронная микроскопия

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Introduction

A major challenge confronting contemporary medicine is the rapid proliferation of antibiotic-resistant bacteria. Globally, *Escherichia coli* significantly contributes to bloodstream infections, gastrointestinal ailments, and urinary tract infections. The escalation of multidrug resistant (MDR) *E. coli* has drastically limited the available treatment modalities, thereby necessitating the development of novel antimicrobial approaches. One of the most promising options to traditional antibiotics offered by nanotechnology is metallic nanoparticles that have demonstrated antibacterial activity via a diversity of mechanisms. Besides, those ways of action render it less likely for resistance to be emerged [1, 2]. The main objective of their design has been the uncover of their remark-

able capabilities, their compatibility with living organisms, and their efficacy in eliminating of *E. coli* bacteria [3]. An alternative synthesis method of green fabrication which utilizes plant extracts are not only cheaper but also environmentally friendly and less harmful to living beings as compared to chemical processes [4, 5]. Moreover, phytochemicals present in plants function as organic stabilizers and reducers [6]. A significant transition from traditional chemical and physical methods to environmentally sustainable practices is the green synthesis of metallic nanoparticles. The use of pomegranate peel extract to synthesize AuNPs addresses a problem related to agricultural waste while generating therapeutically relevant nanoparticles [7, 8]. The peel of the pomegranate (*Punica granatum* L.) constitutes roughly 30 to 40% of the weight of the whole fruit. Throwing them



away as waste in the process of juice production is a very common practice. Besides, the rinds being loaded up with various bioactive compounds and in fact, they contain more polyphenols than the fruit parts that are eaten. AuNPs, manufactured using a green technique using pomegranate peels, has the capability to inhibit the development of (*E. coli*) ($r = 13$ nm) [9, 10]. In the synergistic effects of AuNPs combined with pomegranate peel extract (PPE) and AuNPs serve as potent and environmentally friendly nanotherapeutic agents for the treatment of urinary tract stones [11], exhibiting antioxidant, antibacterial, and nephroprotective properties [12–14]. The phytochemicals in pomegranate peel, responsible for its antioxidant, antibacterial, and anti-inflammatory properties, also render it a suitable choice for the environmentally friendly synthesis of AuNPs [15].

The aim of this study is to determine the effect of AuNPs combined with pomegranate peel in inhibiting the growth of *E. coli* bacteria.

Material and Methods

Pomegranate peels were obtained from a local store. Samples were collected, washed with tap water, dried in the shade, and ground into a powder using a small electric grinder. The chemicals and reagents used in this study were sodium chloride (NaCl , $\geq 99.5\%$ purity was purchased from Sigma-Aldrich, Russia.), ethanol ($\text{C}_2\text{H}_5\text{OH}$, 96% v/v analytical grade) was obtained from Chimmed, Russia), deionized water, and hydrogen tetrachloride [HAuCl_4] (1 mM stock solution was supplied by Sigma-Aldrich, Russia).

Preparation of Pomegranate Peel Extract

The extract was prepared by weighing 5 grams of pomegranate powder and mixing it with 100 ml of ethanol using a magnetic vibrator for one hour. The extract was then filtered using filter paper (Red Ribbon, MELIOR XXI, Russia; equivalent to Whatman grade 2) and centrifuged for two minutes at 2000 rpm [16].

Synthesis of AuNPs

The pomegranate peel extract (100mL) was added dropwise to an aqueous solution of HAuCl_4 (1 mM, 100 mL) while stirring at ambient temperature. The *in situ* synthesis of AuNPs was shown by a shift in solution color from light yellow to wine red as a result of bio-reduction facilitated by plant phytomolecules. The pomegranate peel ex-

tracts were separated by centrifugation (4000 rpm, 15 minutes). Put in a dark bottle and freeze [4, 3].

Confirmation of the synthesis of nanoparticles by spectrophotometric method

The absorption of the resulting nanoparticles was measured using a ULAB US-1500A spectrophotometer in automatic mode, in the visible spectrum range (315–800 nm). To examine nanoparticle nucleation, measurements were taken immediately after adding HAuCl_4 and again after an hour [16].

Study of AuNPs using SEM

The resulting AuNPs were examined using a Zeiss Supra-25 scanning electron microscope (SEM) for morphology and dimension. Sample preparation included: cleaning the conductive substrate using acetone and ethyl alcohol. Drying it in a closed Petri dish in a microbiological box. Application of a ready-made suspension of nanoparticles to a dry substrate. After applying the nanoparticles, the preparation was dried naturally in a microbiological box to minimize contamination. Then the substrate with the samples was placed in a scanning electron microscope and analyzed [17].

Determination of antibacterial activity of AuNPs

In the first case:

Antibacterial activity was determined using the serial dilution method. To do this, 0.9 ml of pre-cooked and thrice sterilized nutrient broth was placed in sterile test tubes. From the stock solution of nanoparticles reconstituted with pomegranate extracts, the subsequent dilutions (0.5, 0.75, 0.876) in the broth were prepared by adding 0.1 ml of nanoparticles to each of these test tubes. Then 0.1 ml of the bacterial suspension under study (*Escherichia coli*) was added to each dilution. The crops were incubated at 37°C until the next day, after which the results of the experiment were noted by the turbidity of the nutrient medium. To assess the minimum bactericidal concentration, they were sown on a solid nutrient medium, on Petri dishes pre-filled with triple sterilized agar, which until this point had been monitored for overgrowth for 24 hours. Place the dishes with the test culture in a thermostat for a day.

In the second case:

Antibacterial properties were determined using the serial dilution method. To do this, 0.5 / 0.75 / 0.876 ml of pre-cooked nutrient broth was placed in sterile test tubes. Additionally, 1 / 0.5 / 0.75 / 0.876 ml of distilled water was added to sterile



test tubes for the control culture. Sterilized in a Koch apparatus four times. At the same time, the enrichment culture (*Escherichia coli*) was revived. For this purpose, 50 ml of nutrient broth was added to 100 ml flasks and sterilized 4 times. Then, an enrichment culture from the slanted agar was added to the flask and incubated in a thermostat for 24 hours. A day later, the purity of the culture was checked by preparing a fixed preparation and viewing ten fields under a microscope. The optical density was measured and was 0.3.

Then, 1 ml of bacterial suspension was added to test tubes with distilled water and 1 / 0.5 / 0.250.125 ml of nanoparticles, respectively, and 1 ml of bacterial suspension were added to test tubes with broth. Incubated in a shaker for 3 hours. Then they were sown on a dens nutrient medium, on Petri dishes that had been previously filled four times with sterilized agar, which until that moment had been monitored for overgrowth for 24 hours. The culture plates were incubated in an incubator

at the appropriate temperature for 24 hours. The culture plates were incubated in an incubator at the appropriate temperature for 24 hours [14].

Result and Discussion

UV-Visible data

The ultraviolet-visible spectroscopic data confirm the successful green synthesis of AuNPs from *Punica granatum* extract. The surface plasmon resonance position and spectral shape indicate the formation of stable, nanoscale AuNPs suitable for further physicochemical characterization and potential applications in biomedical, catalytic, or sensing fields.

The absorption spectrum recorded in the wavelength range of approximately 315–800 nm (Fig. 1) displays a distinct and intense absorption band centered around ~400–420 nm, characteristic of the surface plasmon resonance (SPR) of colloidal AuNPs [18].

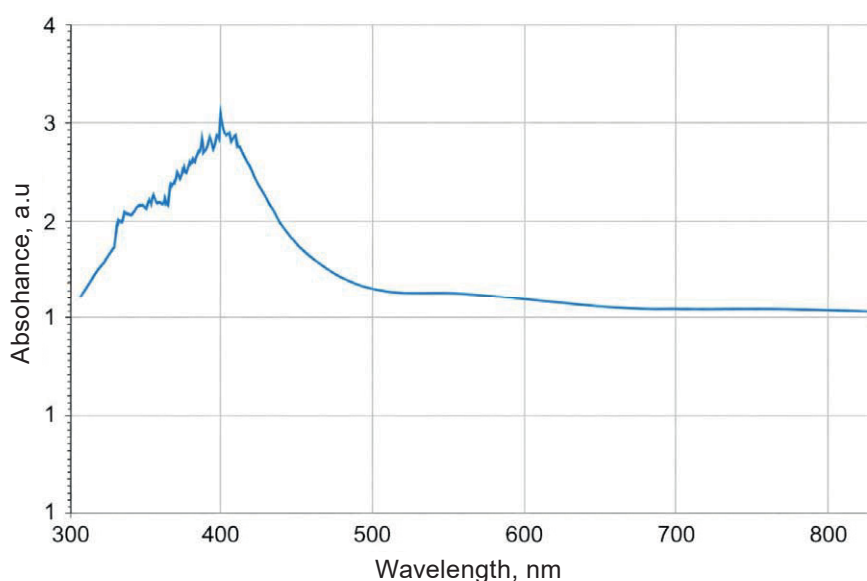


Fig. 1. UV-Visible Spectrophotometric Analysis of AuNPs Synthesized Using Pomegranate (*Punica granatum*) Extract Absorption spectra are representative when the optical density does not exceed 1.1

The appearance of the surface plasmon resonance SPR band is strong proof that Au⁺ ions (Au⁺ ions are gold atoms in the +1 oxidation state, meaning they have lost one electron. They are commonly found as intermediates during reduction, such as when chloroauric acid is converted into AuNPs have been reduced to metallic Au nanoparticles) [19, 20]. In noble metal nanoparticles,

the SPR phenomenon is caused by the collective oscillation of conduction band electrons triggered by incident electromagnetic radiation. The observed SPR position in the visible region is similar to previously reported biosynthesized AuNPs that use plant-derived polyphenols, flavonoids, and organic acids as reducing and stabilizing agents [21–23].



The relative sharp increase in absorbance toward the SPR maximum (with absorbance values exceeding 3.0 near ~ 400 nm) following a gradual decrease toward longer wavelengths indicates the formation of predominantly nanoscale, quasi-spherical gold particles. This suggests the formation of predominantly nanoscale, quasi-spherical gold particles with moderate size dispersion [24]. The absence of multiple SPR peaks or significant band splitting indicates minimal anisotropy. This suggests that rod-like or highly irregular morphologies are not dominant in the sample.

Beyond the SPR region, the absorbance gradually decreasing and stabilizing in the 500–800 nm range, indicating the limited aggregation and reasonable colloidal stability of the synthesized AuNPs. A pronounced red-shift or broad tail extending deep into the near-infrared region – often associated with extensive particle aggregation or larger particle sizes – was not detected. The results of the study show that the extracts of pomegranate peel and juice have a significant effect on the growth and agglomeration of AuNPs.

Pomegranate peel and juice extracts are recognized for being rich in ellagitannins, phenolic acids, and flavonoids, that can attach to the nanoparticle surface and affect the dielectric environment around the AuNPs, which slightly changes the SPR profile [25].

Scanning electron microscopy data

We obtained images of nanoparticles reduced by pomegranate extract. SEM micrographs show the nanoparticles created by green reduction utilizing pomegranate extract, where phytochemicals in the extract function as both stabilizing and reducing agents. The emergence of AuNPs as brilliant, quasi-spherical structures scattered over the upper half of the photograph indicates successful nucleation and development of the particles. Despite some clumping, the individual particles exhibit a moderate size dispersion, which is typical of biosynthesized nanomaterials. Synthesis with reaction variables such as extract concentration, temperature, and pH impacting particle shape and size distribution respectively. Due to its high atomic number, which encourages electron scattering in SEM imaging, gold has a unique particle brightness and background contrast. The partial aggregation seen at the surface may be the result of drying during sample preparation or inadequate stabilization by organic molecules from the extract. Overall, the micrograph shows that pomegranate extract promotes the creation of nanosized gold particles with a somewhat uniform shape and is an eco-friendly reduction system for the synthesis of AuNPs. Additionally, it raises the possibility of applications in antibacterial, catalytic, and biological research [26] (Fig. 2).

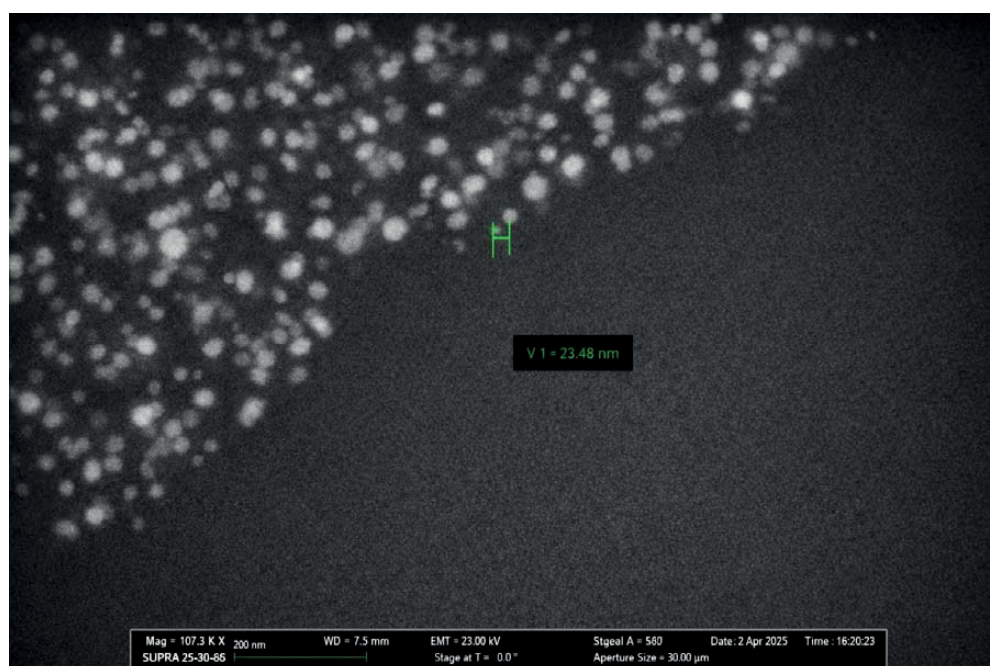


Fig. 2. SEM image of AuNPs biosynthesized using *Punica granatum* pomegranate extract



The grayscale intensity histogram derived from the SEM image shows the distribution and contrast features of the as-obtained AuNPs. As can be seen from Fig. 3, a unimodal distribution

histogram with a sharp peak at the intensity of about 85 was observed, suggesting that the as-obtained AuNPs composed of the largest number of pixels within the short grayscale intensity range.

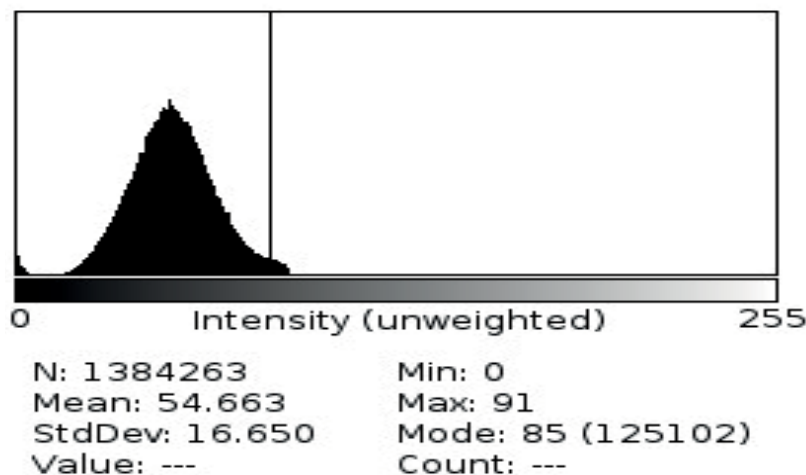


Fig. 3. Grayscale intensity histogram of SEM image of biosynthesized AuNPs revealed unimodal distribution of pixel intensity with sharp peak at around 85 and mean intensity of 54.66 ± 16.65 with small coefficient of variation (CV) indicating relatively uniform surface morphology with slight heterogeneity in AuNPs distribution

The computed mean intensity is 54.66 and the standard deviation of the intensity distribution is 16.65, indicating a relatively moderate scatter of the intensity values. This suggests that the surface topography of the film is moderately complex and that the nanoparticles are possibly arranged in some specific way. The relatively low maximum intensity value (91) in relation to the whole dynamic range of the grayscale image (0–255) indicates that most of the image area is occupied by darker shades, suggesting that there is a high degree of nanoparticle agglomeration or a poor contrast between the surface topography and the EBSS. Besides the higher number of pixels ($N = 1,384,263$) in the case of the nanocomposite varnishes investigated here, there are further reasons for this analysis. The restricted width around the mode is an indication of a homogeneous texture. Such a homogeneous texture could be caused by a homogeneous distribution of nanoparticle sizes as well as a homogeneous coating on the particle surface. The small peak broadening in the histogram only indicates small differences in the particle size, form and structure of possible clusters. Histogram of the histogram

analysis further confirmed the SEM results that the synthesized AuNPs are uniform but having some variation in size which is also a common feature for bio-synthesized nanoparticles [27].

Atomic force microscopy data

The images showed that the particle size was ($r = 23$) nanometers for the nanoparticles reduced by pomegranate extract (Fig. 4).

AFM measurements reveal an average diameter of around 23 nm, a rather narrow size distribution, and a predominantly quasi-spherical shape. Except for minor focused areas of enhanced aggregation caused by drying interactions between nanoparticles, nanoparticles are evenly dispersed across the scan area's surface. Rough surface profiles and nanoscale protrusions (features) indicate severe surface heterogeneity. These properties indicate that the phytochemical components of plant extract were responsible for the bio-organic capping molecules. The Z-axis displays the scan height in nanometers, while the X-Y axes display the scanning surface's lateral dimensions. As a result, nanostructures generated from an aqueous solution of *E. floribunda* root extract exhibit both vertical surface feature nanoscale dimensions [28].

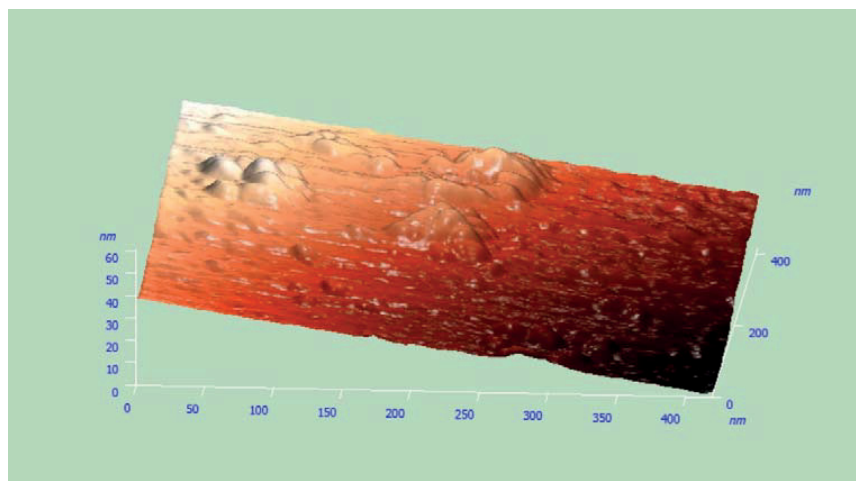


Fig. 4. Show a 3D Atomic Force Microscopy (AFM) topography image of AuNPs prepared by a simple green method using *P. granatum* peel extract. The image shows clearly the surface topography and spatial distribution of AuNPs, which are predominantly quasi-spherical in shape with an average diameter of 23 nm

Anti-bactericidal concentration

The purity of the culture was checked. We made a fixed preparation, stained with water fuchsin. We examined 10 fields under a microscope. AuNPs reduced with a solution

of pomegranate extract at concentrations of 1.7, 0.17 and 0.017 $\mu\text{g}/\text{ml}$ did not have a bactericidal effect on *Escherichia coli*, which was accompanied by continuous lawn growth (Fig. 5).

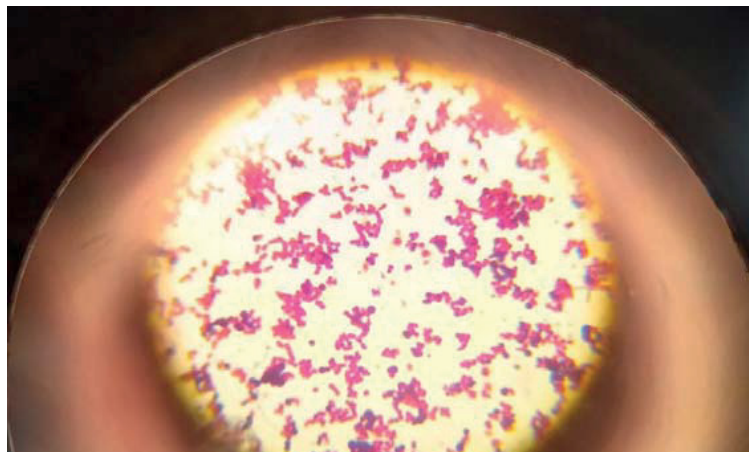


Fig. 5. An optical microscope picture of *E. coli* culture treated with AuNPs biosynthesized and shows the distribution of bacterial cells on treatment with nanoparticles. AuNPs were derived from pomegranate peel extract and tested for antibacterial applications

The AuNPs, which were reduced with pomegranate extract at concentrations of 17 $\mu\text{g}/\text{mL}$, 1.7 $\mu\text{g}/\text{mL}$, 0.17 $\mu\text{g}/\text{mL}$, and 0.017 $\mu\text{g}/\text{mL}$, did not show any inhibitory action on *E. coli* culture, with continuous lawn-like growth. At 17 $\mu\text{g}/\text{mL}$ (a), 1.7 (b), 0.17 (c), and 0.017 (d) of AuNPs, *M. luteus* exhibited a continuous lawn, with no

observable inhibition of growth at these concentrations. The number of times the antibacterial experiments were repeated is three replicates per treatment. The results indicate that the AuNPs reduced with pomegranate extract did not demonstrate measurable antimicrobial efficacy against *E. coli* at the concentration range studied.

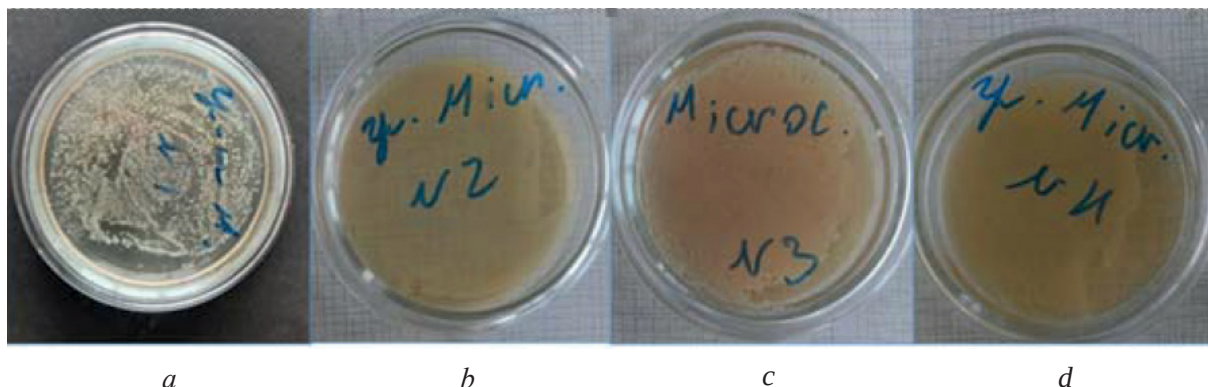


Fig. 6. Study of the minimum inhibitory concentration ($\mu\text{g/mL}$) of AuNPs reduced with pomegranate extract using the serial dilution method on *E. coli*: a – 17; b – 1.7; c – 0.17; d – 0.017

Conclusion

This study found that pomegranate peel extract works well as a green, cost-effective, and ecologically friendly reducing agent for gold nanoparticle production. SEM, AFM, and zeta potential investigations revealed the creation of stable, evenly dispersed AuNPs with an average size of ($r = 23 \text{ nm}$). The utilization of pomegranate peel, an agricultural waste product high in bioactive polyphenols, demonstrates the promise of plant-based resources in nanomaterial production while also encouraging waste repurposing. However, the produced AuNPs exhibited no antibacterial activity against *Escherichia coli* at the quantities studied, suggesting that greater concentrations, different plant extracts, or nanoparticle surface changes may be required for effective antimicrobial action. Atomic Force Microscopy (AFM) tests showed that the green production of AuNPs with *Punica granatum* peel extract was successful. Although all the structural parameters determined in previous sections have been of great utility for establishing a characterization scheme for these sustainable materials synthesized through a green chemistry approach, it is also of great interest determining topography surface and thus evaluating surface roughness together with height differences between the main constituent nanostructures in order to fully assess such a complex heterogeneous material distribution. Overall, data show the viability of using natural plant extracts for nanoparticle synthesis and provide a foundation for further exploration into their biomedical and technological applications.

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