



## ECO-FRIENDLY, NON-TOXIC, AND HIGHLY ANTIMICROBIAL BIOSYNTHESIZED OF Silver NANOPARTICLES (PAgNPs) FROM POMEGRANATE (PUNICA GRANATUM) PEEL (WASTE) EXTRACT (PPE) WITH MANY APPLICATIONS IN NANO INDUSTRIES AND PATHOGEN ELIMINATION FROM WATER



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### Abstract

The green synthesis of silver nanoparticles (AgNPs) regarded as the most significant field of nanotechnology science because of the cost-effective and environmentally friendly. Methodology used in the process of the fundamentals of green nanotechnology is the use of reductive by products in the food and fruit processing industry as a cheap and dependable source of green reducing agents due to their favorable physicochemical properties and applications potential. Our study's objective was to use pomegranate to biosynthesize silver nanoparticles (PAgNPs) from (Punica granatum) Peel(waste) Extract (PPE) to explore its characterization using scanning electron microscopy and an ultraviolet-visible Spectrophotometer which revealed the development of spherical and sparsely agglomerated PAgNPs as assessed in the 200–600 nm range. Antimicrobial activities where they demonstrated positive activity to varied degrees against the majority of the tested human pathogenic bacteria and fungi. The toxicity test was conducted using a Microtox analyzer 500 in aqueous solutions, and it was found that the EC50 value of Ag<sup>+</sup> after 15 minutes of exposure was lower than the EC50 value of Ag<sub>0</sub>NPs. This showed that Ag<sup>+</sup> was more toxic to Luminescent bacteria than PAg<sub>0</sub>NPs.

**Keywords:** Antimicrobial Activity - Disc Diffusion - Medicine Industry- Green synthesis. of silver nano particles -Silver Nonmaterial – pomegranate Peel Extract - (*Punica granatum*) - Toxicity- Microtox analyzer 500.

### 1. Introduction

Nanotechnology applied in many aspects of daily living Because of the higher surface area in relation to volume. Nanomaterial's are more responded and efficient in comparison to materials as they also provide potential in surface chemistry since they may be loaded with functional groups that can target the desired molecules [1]. Everywhere in our environment including the air we breathe, medical equipment and even our food are nanoparticles (NPs). Nanotechnology currently become the most encouraging field for development medical applications. The most noticeable nano products are silver nanoparticles (AgNPs) [2]. New kinds of nanoparticles could be used in the fast expanding field of nanoscience. Silver nanoparticles are frequently employed in biomedical applications, research, solar cells, water purification systems, and optoelectronics because of their special qualities. Their potential for usage in biomedicine is greatly constrained by the utilization of expensive, unstable,

and poisonous chemicals. Therefore, biological methods continue to be the best, including medicinal plant extracts, fungi, and prokaryotic bacteria which employed for the biological synthesis of AgNPs because it's time saving, cost-effective, and environmentally benign procedure [3].

In contrast to chemical approaches, green nanoparticles are environmentally benign, cost effective, long lasting and capable of producing a variety of results. Various shapes (spheres, prisms, or plates) with different diameters between one and one hundred nanometers. Because of their small size and high surface to volume ratio, nanoparticles are helpful in a variety of fields medicine being one of them [4]. Silver nanoparticles (AgNPs) are extremely important because of their unique chemical, biological and physical properties. The bio-reduction process, which has no adverse effects on its applications, more cost effective and environmentally

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benign than chemical methods, is commonly used to create silver nanoparticles [5].

The present study was created to evaluate the antimicrobial activity (bacteria and fungi) of biosynthesized AgNPs against many microbial strains also assessing its toxicity using Microtoxic Analyzer 500 and (*Vibrio fisheri*) bacteria due to the widespread usage of AgNPs in commercial and medical items and to avoid the possibility of their potential side effects.

## 2. Materials and Methods

The present work carried out in the department of Microbiology, Sanitary and Environmental Engineering Institute, Housing and Building National Research Centre.

### 2.1 Materials

Silver nitrate ( $\text{AgNO}_3$ ) that was acquired from Sigma Aldrich and employed as a precursor. Pomegranate fruits that were certified as being (*Punica granatum*) were procured from a market in the governorate of Giza and thoroughly cleaned, followed by washings with water. then Was later chopped into small pieces, peeled and then dried in air. a (100) g of pomegranate peel was added to 250 ml of distilled water and then heat at  $80^\circ\text{C}$  for 30 minutes. pomegranate peel extract was PPE was stored at  $4^\circ\text{C}$  in refrigerator as shown in figure: (1) [(A), (B), (C) and(D)]. [6].

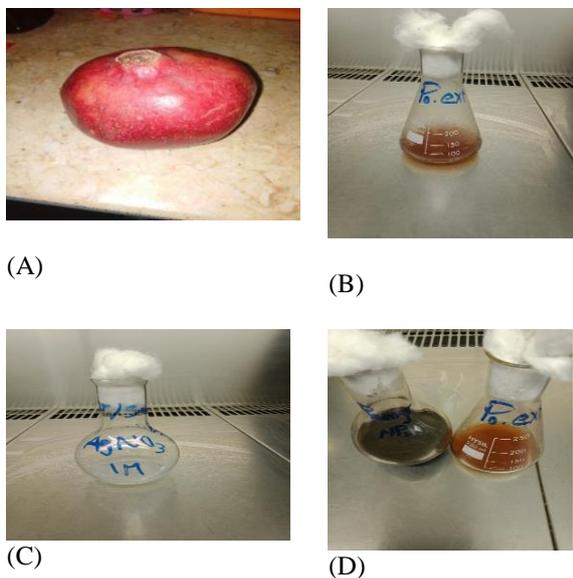


Figure (1) (A): pomegranate fruit (B) pomegranate peel extract (C) silver nitrate  $1\text{M AgNO}_3$  (D) PAgNPs

### 2. 2. Green Synthesis of Pomegranate

Pomegranate peel extract PPE(5ml) freshly produced added to 50 ml silver nitrates  $1\text{M}(\text{AgNO}_3)$  with constant stirring for 15 minutes at  $50^\circ\text{C}$ , react until a brown colored solution was obtained as a result of

reduction of AgNPs. The nanoparticles were stored for characterization [Figure: (1) (A), (B), (C), and (D)] [7].

## 2.3 Characterization.

### 2.3.1. Ultraviolet–Visible Spectroscopy (UV-Vis Spectroscopy)

Spectroscopic analysis was used to describe silver PAgNPs. The UV-visible spectrometer (Shimadzu UV-visible 2900 spectrometer) in the range 200–600 nm was used to identify PAgNPs [8].

### 2.3. 2. Scanning Electron Microscopy (SEM)

After centrifugation at 8000 rpm for 10 minutes, the cleaned silver nanoparticles were dried to eliminate undesirable material after being centrifuged at 8000 rpm to produce powder at  $100^\circ\text{C}$  for 10 minutes. examined using a scanning electron microscopy (SEM) (JSM- 6490, JEOL CO, Ltd., Japan) Includes high-resolution photographs and a chosen area for the composition, size, and structure and shape of produced PAgNPs were characterized [9].

### 3.3. X-ray diffraction (XRD) spectrum

In order to characterize the green PAgNPs made from PPE After the addition of  $\text{AgNO}_3$  to pomegranate peel extracts, the color of the mixture changed from yellow to brown (Figure 1 C). Spectral analysis from UV-visible (UV-Vis) spectroscopy revealed that the solution peaked at an average wavelength of 437 nm and 450 nm for pomegranate, after 72 h of reaction time, as presented in Figure 6 extract by X-ray diffraction (XRD) spectrum measurement using a XRD powder method (MiniFlex 600, Rigaku) [10].

## 2.4. Microorganisms

The disc diffusion method was used to evaluate the antibacterial and antifungal activity of PAgNPs in comparison to antibiotics (Ampicillin and Fluconazole 10g) respectively against different microbial (bacterial and fungal) strains in order to assess its efficacy of the PAgNPs with enhanced antimicrobial properties. The generic antibiotic discs was acquired from Sigma Aldrich Co., St. Louis, Missouri, USA.

### 4.1. Bacterial Strains

Sigma-Aldrich Co., St. Louis, Missouri, USA) was utilized as the standard reference for all bacterial Strains for this test specifically (*Bacillus subtilis* ATCC6633 ; *Staphylococcus aureus* ATCC6538 ; *Escherichia coli* ATCC 25922 ; *Enterobacter aerogenes* ATCC13048; *Enterococcus Faccalis* RCMB012B001).

**4.2. Fungal Strains**

All fungal strains were purchased from the Regional Centre for Mycology and Biotechnology(RCMB), Al-Azhar University, Cairo, Egypt. Namely, *Aspergillus flavus* RCMB 002F002(2) *Asperillus terrieus* RCMB002F009 ;*Asperillus niger* ; RCMB002F008(4);*Penicillum* *egypticum* RCMB001F042.

**5. Agar Disc Diffusion Method**

Microbial (bacterial & fungal) suspension (100 µL) was spread on (nutrient Agar (NA) & potato dextrose agar medium, respectively) with a glass rod. The sterilized Whatman No. 1 filter paper discs (6 mm) were dipped in (a) Silver Nitrates AgNO<sub>3</sub>(control) ,(b) Pomegranate Peel Extract (PPE) , and (c) PAgNPs . Then discs were placed on the media equidistantly. The same method was followed for all of tested microbial strains. Then, the plates were subjected to incubation at 35 ± 2 °C for 48 hrs. and 3-4 weeks at 28°C± 2 respectively, for bacteria and fungi . After incubation, the plates were observed, the zone of inhibition was measured compared with. Ampicillin10 mg and Fluconazole10 mg as a positive control references antibiotic from Sigma-Aldrich Co., St. Louis, MO, USA [11].

**2.6. Toxicity Test**

Pomegranate peel extract PPE, PAgNPs, and Silver Nitrates(AgNO<sub>3</sub>) as the control were each added to1L of autoclaved distilled water individually. The test was then conducted , and the results were reported in accordance with (ISO 11348-3:2018). Employing the tested Microbes *Vibriofischeri* and Microtox analyzer 500. During testing, the bacteria were exposed to different Solutions, Along with standard solutions and control samples. The bacteria's reduction in light emission was assessed using the Microtox Omni Azur software,the data were recorded and the EC<sub>50</sub> (concentrations generating a 50% reduction in light) is computed [12].

Table 1: Toxicity degrees and EC<sub>50</sub> according to ISO 11348-3:2018 for (a) Silver Nitrates (AgNO<sub>3</sub>) as control ;(b) pomegranate peel extract PPE and(c)PAgNPs

Degree of toxicity	Extremely Toxic	Very Toxic	Toxic	Moderate Toxic	Non-Toxic
EC50 Value	0-19	20-39	40-59	60-79	80≥100

EC<sub>50</sub>: The effective concentration causing 50% luminescence inhibition Bacteria. Storage temperature: -25 °C Reference substances used: 3,5-Dichlorophenol, Zinc sulfate hydrate, Potassium dichromate.

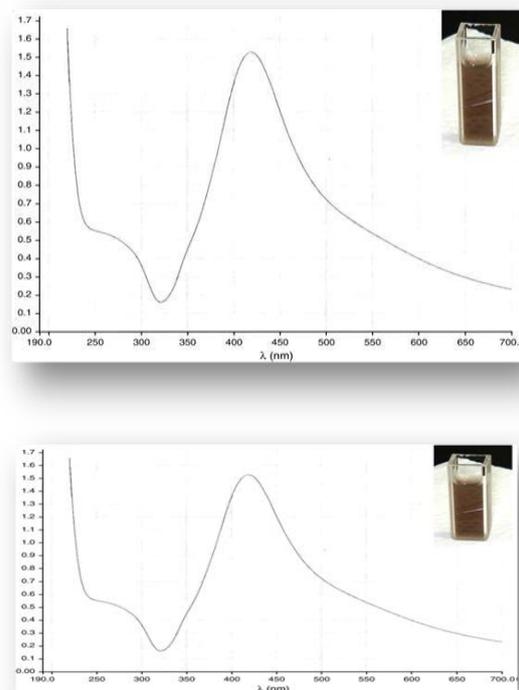
**2. Results and discussion**

Although the process of chemical reduction is frequently employed to produce AgNPs , it produces

hazardous compounds that are harmful to the environment .Because of this ,chemical methods are worthless in medical settings. As a result, green synthesis of AgNPs is extremely advantageous because it is cost effective and sustainable. PAgNPs created using a variety of techniques, including chemical and biological. The use of plant resources in the synthesis of AgNPs is growing in popularity today. In our study, the colour of the reaction media quickly changed from colorless to brown when pomegranate peel extract is added to the aqueous solution of the silver nitrate. The control AgNO<sub>3</sub>solution did not exhibit any change it Figure[ (1) (A) ,(B) ,(C) and (D)].These findings in agreement [13].

**3.1. Characterization Ultraviolet–Visible Spectroscopy (UV-Vis Spectroscopy)**

Figure( 2) showed the UV-Vis spectrum from PAgNPs synthesized with the addition of pomegranate peel extracts as a reducing agent. While no absorbance peak was observed in control. Color change from pale yellow to brown indicated the reduction of AgNO<sub>3</sub> to form PAgNPs was confirmed by recording their UV absorption spectrum in the 200–600 nm range Appearance of new broad surface plasmon resonance peak at 421 nm in the UV-Vis absorption spectral[14].

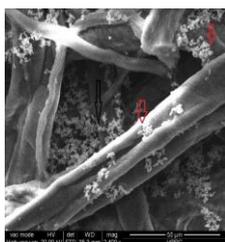


Figure(2):UV-Vis absorbance spectra of Silver nanoparticles PAgNPs synthesized using (a) 1M AgNO<sub>3</sub> as control with PPE

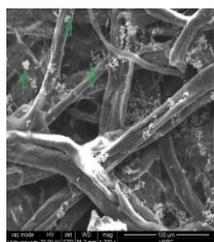
The formation of PAgNPs using pomegranate peel extract confirmed by the results of UV-vis spectroscopy. It displayed surface Plasmon band of PAgNPs between 200 and 600 nm demonstrated PAgNPs development. Due to a significant proportion of  $\text{Ag}^+$  being transformed to  $\text{Ag}^0$ . The creation of Plasmon bands increases as reaction time grows. A decrease in absorption Intensity and wavelength, which is assign of some agglomeration of silver nanoparticles supported by the maximum absorption in the UV-Vis spectra . A color change from yellow to brown indicated reduction of  $\text{AgNO}_3$  and PAgNPs was confirmed by recording their UV absorption spectrum in the 200–600 nm range [15].

### Scanning Electron Microscope analysis (SEM)

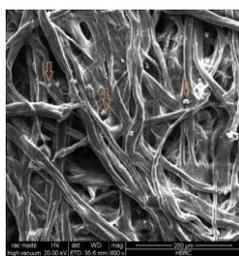
According to the Surface morphology, shape and elemental composition of PAgNPs were analyzed through Scanning electron microscope (SEM; JOEL JSM-7800F) operated at 15 kV with a working distance of 10 mm findings were present in Figure (3) [ E ,F ,G and H)] with various magnification powers. It seemed to be spherical [16].



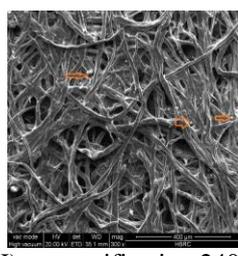
(E) at magnification 2400x



(F) at magnification 1200x



(G) at magnification 600x



(H) at magnification 2400x

Characterization of PAgNPs using SEM as shown in Figure : ( 3) [(E, F G, and H)] with different Magnification power.

### 3.1c. X-ray diffraction (XRD)spectrum

PAgNPs crystal plane showed 20 angles at 28.57, 29.34 ,33.46, 38.28 these angles corresponding to 111, 200 and 22 sets of lattice planes. the XRD results are similar to standard peaks and confirmatory with finding in [17].

### 3.2. Antimicrobial (antibacterial & antifungal) activity

To show the antibacterial efficacy of the produced PAgNPs, the zone of inhibition for each tested bacterial and fungal strain that was exposed to green biosynthesized nanoparticles (PAgNPs), pomegranate peel extract (PPE), and silver nitrates ( $\text{AgNO}_3$ ) as controls was determined by measuring the diameter of inhibition zone of bacterial growth in (millimeters) mm.

#### 3.2.a. Antibacterial activity

Table (2): Inhibition zone (mm) of bacterial growth tested with PAgNPs synthesized using pomegranate peel extract (PPE) which examined against different certified bacterial strains

Antibacterial activity of PAgNPs against tested certified bacterial strains were recorded by measuring the inhibition zone for bacterial growth in millimetres.

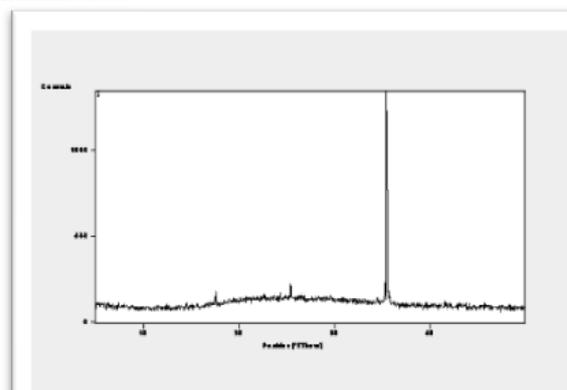


Figure 4: The XRD pattern Characterization of biosynthesized PAgNPs

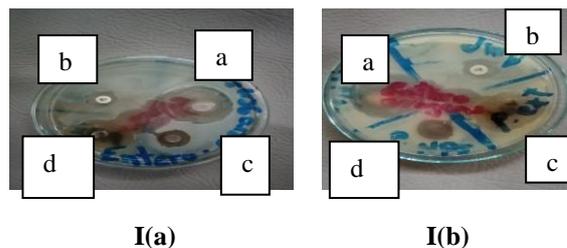


Figure (5) [I: a &b] Antibacterial activity of PAgNPs against *Enterobacter aerogenes* ATCC13048 in comparison to PPE and  $\text{AgNO}_3$  where a: Control: disc saturated with  $\text{AgNO}_3$  b: Antibiotic disc : Amp c: disc saturated with PPE d: disc saturated with PAgNPs

Table (2) and Figure (5) [I: a &b] Antibacterial activity of PAgNPs against *Enterobacter aerogenes* ATCC13048, Figure (6) [(J): a&b]

Antibacterial activity of PAgNPs against *Escherichia coli* ATCC25922 and Figure (7) [K, L & M] Antibacterial activity of PAgNPs against *Enterococcus faecalis* RCMB012B001, *Bacillus subtilis* ATCC6633 and *Staphylococcus aureus* ATCC 6538 Demonstrated antibacterial activity of a: Control: disc saturated with AgNO<sub>3</sub> b: Antibiotic disc : Amp c: disc saturated with PPE d: disc saturated with PAgNPs.

The inhibition zone obtained from PPE tested against certified bacterial strain was arranged in order of *Bacillus subtilis*ATCC6633, *Escherichia coli* ATCC 25922, *Enterobacter aerogenes*ATCC13048, *Staphylococcus aureus*ATCC6538, and *Enterococcus faecalis* RCMB012B001, where PAgNPs showed positive activity against most of the tested human pathogenic bacteria with varying degrees. With

values (28, 19, 17, 13, and 11 mm) respectively.

PAgNPs clearly inhibited Gram-positive *Bacillus subtilis* ATCC6633 with largest inhibition zone 40 mm and Gram-negative *Escherichia coli* ATCC 25922 with inhibition zone 30 mm bacteria. This might be the result that PAgNPs entering the cells and inducing intracellular loss that results in cell death. Due to the high levels of lipopoly saccharide and thick peptidoglycan layer, bacteria lyses became extremely sensitive to PAgNPs . [18]. PAgNPs are widely employed in biomedical production, wound healing, house hold filters, baby bottles, food preservation, cosmetics and water purification due to their effective antibacterial qualities [19].

**Table (2)** Zone of certified bacterial growth inhibition (mm)

Certified Bacterial strains	Zone of certified bacterial growth inhibition (mm)				
	AgNO <sub>3</sub> (control)	Pomegranate extracts(PPE)	Peel	Antibiotic	PAgNPs
<i>Enterobacteraerogenes</i> ATCC13048	23±1	17±1.56		20±2	25±2.8
<i>Escherichia coli</i> ATCC 25922	27±0.57	19±0.88		28±2.4	30±3
<i>Enterococcusfaecalis</i> RCMB012B001	15±1.52	11±0.72		19±1.16	23±2
<i>Bacillus subtilis</i> ATCC6633	32±1.16	28±1.95		35±3	40±3
<i>Staphylococcus aureus</i> ATCC 6538	19±1	13±0.79		25±2.2	28±1.85

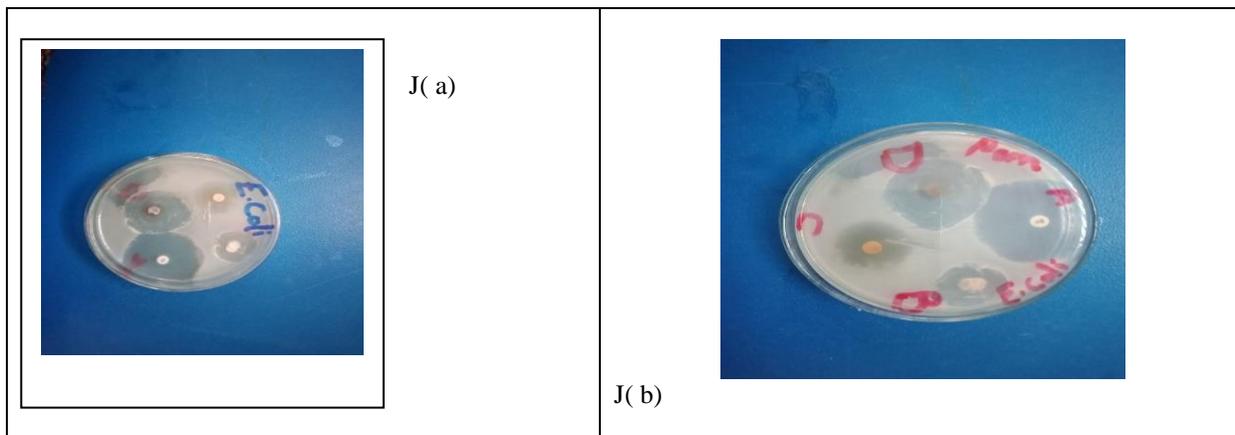


Figure (6) [(J): a&b] Antibacterial activity of PAgNPs against *Escherichia coli* ATCC25922 in comparison to PPE and AgNO<sub>3</sub> where a: Control: disc saturated with AgNO<sub>3</sub> b: Antibiotic disc : Amp c: disc saturated with PPE d: disc saturated with PAgNPs

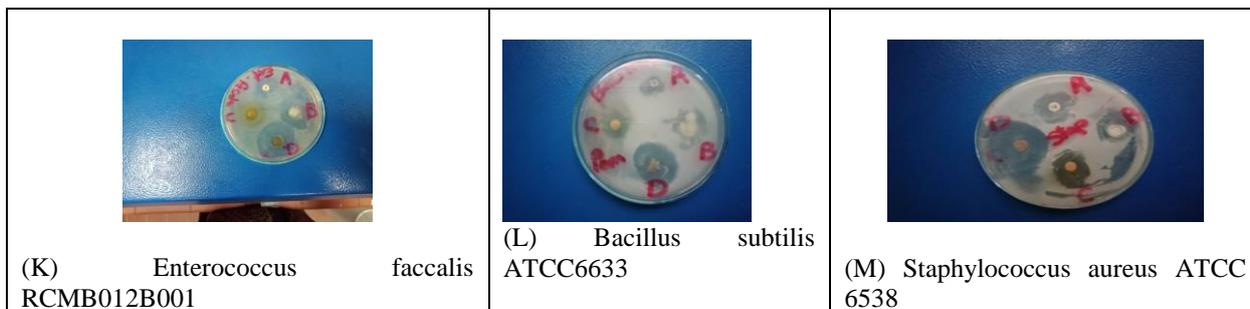


Figure (7) [K, L & M] Antibacterial activity of PAgNPs against *Enterococcus Faecalis RCMB012B001*, *Bacillus subtilis ATCC6633* and *Staphylococcus aureus ATCC 6538* in comparison to PPE and  $AgNO_3$  where a: Control: disc saturated with  $AgNO_3$  b: Antibiotic disc : Amp c: disc saturated with PPE d: disc saturated with PAgNPs.

Table 3: Inhibition zone of certified fungal strains measured in (mm) tested against PAgNPs .

Zone of inhibition of certified fungal strains measured in (mm)				
Certified Fungal strains	Control $AgNO_3$	Pomegranate Peel extracts (PPE)	Anti biotic	PAgNPs
<i>Aspergillus flavusRCMB002F002(2)</i> .	50± 1.1	18± 1.12	20	50± 1.61
<i>Aspergillus terriusRCMB002F009</i>	38±1.19	12± 0.76	30	35± 1.2
<i>Penicillium egypticumRCMB001F042</i> .	52±1.83	24± 0.16	35	49± 1.71
<i>Asperillus nigerRCMB002F008(4)</i> .	60± 1.3	21± 0.61	30	50± 2

### Anti-fungal Activity

PAgNPs were tested as efficient antifungal against many certified fungal strains *Aspergillus flavusRCMB002F002*, *Penicillium egypticum RCMB001F042*, *Asperillus terrius RCMB002F009*, and *Asperillus niger RCMB002F008 (4)*. Were eco-friendly. PAgNPs demonstrated more antifungal activity than PPE where the fungal inhibition zones were (50± 1.61, 50± 2, 49± 1.71 and 35± 1.2) for PAgNPs for *Aspergillus flavusRCMB002F002*, *Asperillus niger RCMB002F008 (4)*, *Penicillium egypticum RCMB001F042* and *Asperillus terrius RCMB002F009*, respectively whereas fungal inhibition zones for PPE were recorded in order of *Penicillium egypticum RCMB001F042*, *Aspergillus nigerRCMB002F008(4)*, *Aspergillus flavusRCMB002F002(2)* and *Aspergillus terriusRCMB002F009* with values (24± 0.16, 21± 0.61, 18± 1.12 and 12± 0.76 mm). Pomegranate contains a wide range of antibacterial and antifungal properties, including an inhibitory effect on both Gram-positive and Gram-negative bacteria (Table2) Fungi, mold (Table3). However, numerous studies conducted by researchers have revealed that PPE has antibacterial activity and was correlated with the substance's overall flavonoid and tannin concentration. The antibacterial action of PPE against bacterial and fungi infections is well recognized [20-21].

The food borne pathogens *Escherichia coli*, *Fusariumsambucinum*, *Penicillium notatum*, and *Bacillus Subtilis* are among those that are affected by PPE and PAgNPs [22-23]. The antibacterial effectiveness of pomegranate extracts (peel, seeds, juice, and entire fruits) against seven microorganisms were investigated (*Bacillus coagulans*, *Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus*, *E. coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*). All bacteria are most effectively inhibited by pomegranate peel extracts and it was discovered to enhance the antibacterial activity [24].

The antibacterial activity of PPE can be considerably influenced by the extraction technique. PPE has also

been reported to drastically reduce Gram-positive bacteria. According to our study, PPE has significant antibacterial action against gram-negative bacteria such *E. coli*, *B. subtilis*, *Enterobacter aerogenes*, *Staphylococcus aureus*. PAgNPs might be interacting with both membrane and cell wall components, impeding bacterial growth. This indicates that the PAgNPs induce bacterial cell permeability by the disruption of cell membrane integrity. Thus, this can be one potential antibacterial mechanism linked to the effective antimicrobial properties exhibited by PAgNPs. In this context, alterations in permeability and membrane structure have been mentioned as one mechanism of antimicrobial activity of PAgNPs [25]. The PAgNPs have been reported to exert bactericidal activity by multiple mechanisms of action such as by attacking the respiratory chain and cell division leading to cell death, alteration of cell membrane permeability, release of polysaccharides, and membrane potential. [26].



N(a)



N(b)

Figure (8) [N(a)& (b)] antifungal activity of PAgNPs against *Aspergillus flavus RCMB002F002(2)*. in comparison to PPE and  $AgNO_3$  where a: Control: disc saturated with  $AgNO_3$  b: Antibiotic disc : Fluconazole 10g c: disc saturated with PPE d: disc saturated with PAgNPs.

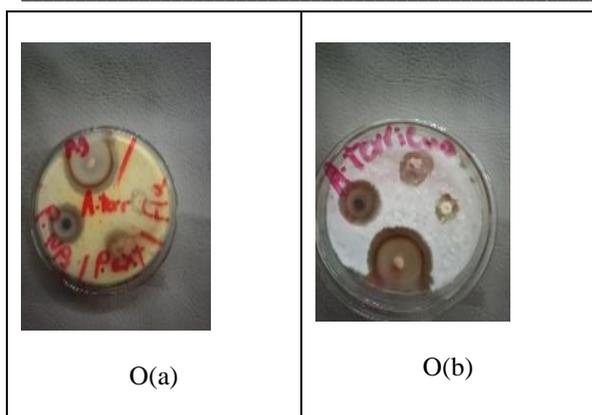


Figure (9) [O(a)& (b)] antifungal activity of PAgNPs against *Aspergillus terreus* RCMB002F009 in comparison to PPE and  $AgNO_3$  where a: Control: disc saturated with  $AgNO_3$  b: Antibiotic disc : Fluconazole 10g c: disc saturated with PPE d: disc saturated with PAgNPs

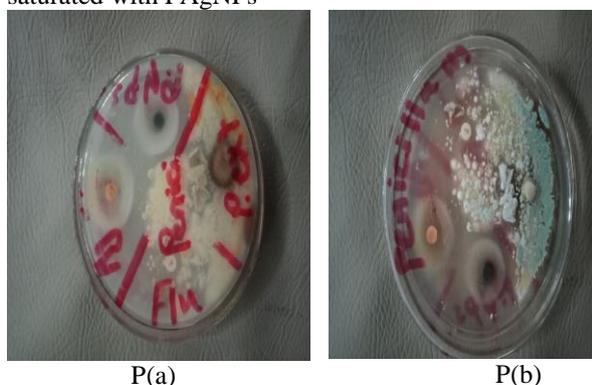


Figure (10) [P(a)& (b)] antifungal activity of PAgNPs against *Penicillium egypticum* RCMB001F042. in comparison to PPE and  $AgNO_3$  where a: Control: disc saturated with  $AgNO_3$  b: Antibiotic disc : Fluconazole 10g c: disc saturated with PPE d: disc saturated with PAgNPs

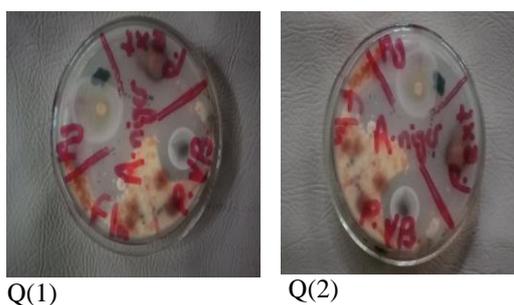


Figure (11) [Q(a)& (b)] antifungal activity of PAgNPs against *Aspergillus niger* RCMB002F008(4). in comparison to PPE and  $AgNO_3$  where a: Control: disc saturated with  $AgNO_3$  b: Antibiotic disc : Fluconazole 10g c: disc saturated with PPE d: disc saturated with PAgNPs

Being free of hazardous compounds, offering natural capping agents for the stability of AgNPs and

preventing nanoparticles aggregation make plant extracts more beneficial than microorganisms. AgNPs have been produced using a variety of plant extracts because of these benefits [27].

Phenolic compounds are aromatic benzene rings with one or additional hydroxyl groups made by plants mostly for protection against anti-stress. Furthermore, phenolics are the major unit of secondary metabolites, which are extensively dispersed in most of the plants and show a significant function in plant resistance versus many diseases and fungal disease [28].

#### Toxicity

Table 4: Effective concentrations and toxicity degrees for the three tested solutions, The toxicity effective concentration (EC) of the solutions that result in 50% inhibition in Bioluminescence of *Vibrio Fischeri* ( $EC_{50}$ ) values were calculated after 30 min exposure time and at 15°C.

The Microtox equipment includes a self-calibrating analyzer which incorporates a photomultiplier tube, a data collection and reduction system, and software. The temperature-controlled analyzer maintains the test organisms and samples at a standard temperature of 15°C. It also detects the light intensity at 490 nm, the wavelength emitted by the bacteria [29].

*Vibrio fischeri* is a bioluminescent, Gram-negative marine bacterium that can be found free living and in a mutualistic association with certain squids and fishes. This is a luminescent bacteria *Vibrio fischeri* used for in vitro test system Microtox®, which is applied for toxicity identification of pure or mixed chemicals and environmental samples [30].

*Vibrio fischeri* are nonpathogenic, marine, luminescent bacteria which are sensitive to a wide range of toxicants. The organisms are supplied for use in a standard freeze-dried (lyophilized) state, which serves to maintain the sensitivity and stability of the test organisms. Disruption of the respiratory process, by exposure to a toxicant affects the metabolic pathway that converts chemical energy via the electron transfer system of the bacteria to visible light [31].

According to *Vibrio fischeri* bacteria who was more sensitive to the toxic materials, Silver ions were more harmful than PAgNPs.

This resulted from microbial cells resistance to the solubility of PAgNPs compared to Ag ions. Silver ions diffuse more readily into bacterial cells than PAgNPs. The  $EC_{50}$

indicated that the toxicity declined as Silver ions converted to silver nanoparticles by biological reduction mechanism. This can be explained as that the bacteria *V. fischeri* can be employed as a biosensor for quick and inexpensive detection of acute nonmaterial toxicity due to its sensitivity to chemical

toxicants. Our findings were in agreement with those founding where the  $EC_{50}$  for  $AgNO_3$  was 19 with an extremely poisonous degree compared with  $EC_{50}$  for PAgNPs 98 and toxicity unit (nontoxic) [32].

Based on the Microtox bioassay the microbiological toxicity of PAgNPs suspension ( $Ag^0$  NPs) and ionic silver solution ( $Ag^+$ ) after 15 and 30 minutes of exposure to aqueous solutions spiked with a dilution series of  $Ag^+$  and  $Ag^0NP$ , the light inhibition of luminescent bacteria was evaluated. Consequently, the dose response curves were utilized to calculate the effective concentration ( $EC_{50}$ ) that resulted in a 50% suppression of luminescence  $Ag^+EC_{50}$  value in aqueous solutions was found to be significantly lower than  $Ag^0NPs$   $EC_{50}$  value after a 15min exposure.  $Ag^+$  was shown to be more harmful to luminescence bacteria than  $Ag^0NP$ , according to this longer exposure times resulted in Lower ECs values or  $Ag^+$  and  $Ag^0NP$ , which exacerbated their toxicity. Depending on the form of silver that was already present ( $Ag^+$ ,  $Ag^0$ ), distinct silver nanoparticles had different hazardous properties. Silver nanomaterials were different depending on existing form of Ag ( $Ag^+$ ,  $Ag^0$ ), reaction medium and exposure time. [33].

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As a powerful antibacterial, antifungal, antiviral, and anti-inflammatory agent, AgNPs have been produced. in addition to being employed for their beneficial physical, chemical, and biological qualities, AgNPs were also used as medicines. The toxicity AgNPs is higher for microbes and lower for mammalian cells. It has numerous uses in the medical and pharmaceutical disciplines, including biosensors, the treatment of infected wounds, and the production of antimicrobial gel for topical antimicrobial medicines, particularly for the treatment of burns [34].

The bactericidal action of AgNPs is mediated by a number of different mechanisms. (1) AgNPs may adhere to the cell membranes surface, disrupting the cells permeability and metabolic pathways. (2) Has the ability to pierce bacterial cell membranes. (3) Can bind to the DNA found inside bacterial cells, stopping it from being replicated or interacting with the ribosome. The structure of the bacterial cell membrane can be harmed by AgNPs, and this can limit the activity of several membrane-bound enzymes, causing *E. coli* bacteria to eventually perish [35].

Between Gram-positive bacteria and Gram-negative bacteria ,there are different in the cell structure. Peptidoglycan and teichoic acid make up the thick cell wall of Gram-positive bacteria. Five glycines are used to cross-link the peptide chain between peptidoglycans. The cell wall of Gram negative bacteria, however, has a multilayer structure that is composed of a thin peptidoglycan, lipoprotein, cortical, phospholipid and lipopoly saccharide from

the inside out. Gram-negative bacteria have a different peptidoglycan structure than Gram-positive bacteria because their peptidoglycans directly cross-linked. Due to differences in their cell wall structures, Gram-positive and Gram-negative bacteria respond differently to certain antibiotics [36].Due to electrostatic contact, negatively charged bacterial cells can interact with positively charged AgNPs (1)cause bacteria to develop "pits" in their cell walls, (2) these pits allow bacteria to penetrate the periplasm. (3) rip the cell membrane apart. (4) DNA condensing; (5) combining and coagulating with bacterial cytoplasm; (6) cytoplasmic component leaking. (7) Induce DNA condensing, resulting in DNA breakdown and lack of replication, which inhibits bacterial growth. [37]

### Conclusions

(1) Recent years have seen a decline in the efficacy of many antibiotics as a result of the emergence of bacterial and fungal strains that are resistant to treatment, mostly as a result of the expression of resistance genes. Antibiotics can sometimes have negative side effects, such as hypersensitivity, immunological suppression, and allergic reactions, in addition to causing resistance. As a result, new antimicrobial medications are required for the treatment of infectious disorders.

(2) Due to its antibacterial action against both gram-negative and gram-positive bacteria and fungi. In traditional medicine, (*PUNICA GRANATUM*) waste (high-tannin peel) has been used as an astringent, diuretic, natural dye, and is also advised for the treatment of diarrhea, dysentery, gastric ulcer, and bleeding.

(3) Color change from pale yellow to brown indicated the reduction of  $AgNO_3$  to form PAgNPs was confirmed by recording their UV absorption spectrum in the 200–600 nm range Appearance of new broad surface plasmon resonance peak at 421 nm in the UV-Vis absorption spectral.

(4) shape and elemental composition of PAgNPs were analyzed through Scanning electron microscope (SEM; JOEL JSM-7800F) operated at 15 kV with a working distance of 10 mm findings were present in Figure (3) [ E ,F ,G and H] with various magnification powers. It seemed to be spherical.

(5) our study investigated the green production of PAgNPs using pomegranate peel extract as reducing agents. The Prepared PAgNPs were extremely effective as antibacterial, antifungal agents, non-toxic agents, and have the potential to be used in many applications:

(a) biomedical applications

(b)water treatment to eradicate pathogenic bacteria and fungi from water.

- (c)pesticides against fungal pathogens  
 (d) food industry  
 (6) These agents are also thought to be inexpensive, eco-friendly and low cost.  
 (7) it is good and safe way to reuse waste resulted from food industry keeping in greener production for greener Environments.

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