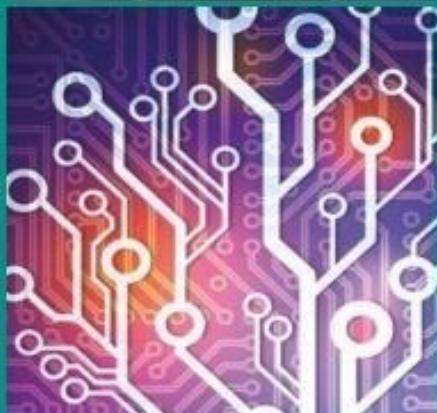
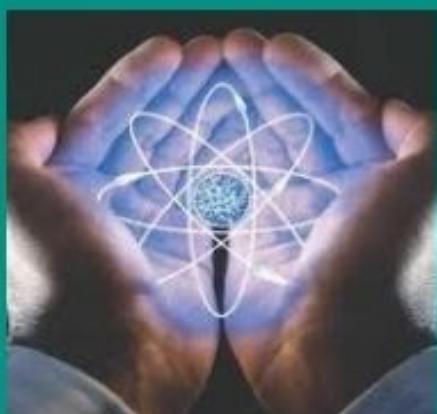


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# Academia Open



*By Universitas Muhammadiyah Sidoarjo*

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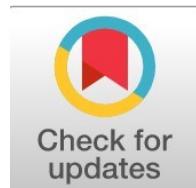
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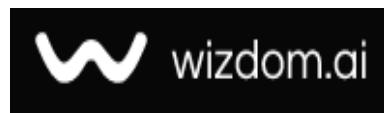
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# Green Synthesis of *Polypogon monspeliensis*–Based Silver Nanoparticles and Their Multifunctional Biomedical Potential

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## Abstract

**General Background:** Green synthesis of silver nanoparticles has gained attention as a sustainable approach to produce bioactive nanomaterials for biomedical use. **Specific Background:** *Polypogon monspeliensis* contains diverse phytochemicals that can function as reducing and stabilizing agents in plant-mediated nanoparticle synthesis. **Knowledge Gap:** Its potential as a green bioresource for fabricating multifunctional silver nanoparticles remains insufficiently explored. **Aims:** This study aimed to synthesize, characterize, and evaluate the antibacterial, antioxidant, and anticancer activities of *P. monspeliensis*–derived silver nanoparticles (Pol-AgNPs). **Results:** Pol-AgNPs were successfully synthesized, exhibiting crystalline, quasi-spherical particles ( $\approx 51$ – $69$  nm) with notable dose-dependent antibacterial activity, strong DPPH radical scavenging capacity, and selective cytotoxicity against A549 lung cancer cells. **Novelty:** The work demonstrates the dual functional role of *P. monspeliensis* extract in nanoparticle synthesis and bioactivity. **Implications:** Pol-AgNPs represent promising eco-friendly nanomaterials for future biomedical applications.

**Keywords :** Green Synthesis, *Polypogon Monspeliensis*, Silver Nanoparticles (AgNPs), Antibacterial And Antioxidant Activity, Anticancer Nanomaterials

## Highlight :

- Plant extract reduced Ag<sup>+</sup> yielding quasi-spherical, crystalline materials sized 51–69 nm.
- Dose-dependent antibacterial action strongest against *Pseudomonas aeruginosa* among tested pathogens.
- High radical scavenging and selective cytotoxicity toward A549 cells with limited normal fibroblast toxicity.

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## Introduction

Nanotechnology is a quickly progressing field that designs, manipulates, and applies materials at nano-size (1–100 nm), where materials have peculiar physicochemical properties that are significantly different from the bulk state [1]. These have led to the broad use of nanoparticles in biomedical, pharmaceutical, environmental, and industrial applications [2]. AgNPs, of all nanomaterials, have been highly appreciated for their potent antimicrobial activity against a wide range of bacteria, viruses, and other pathogenic microorganisms as well as their antioxidant and anticancer activities [3].

AgNPs are widely used in medical apparatuses, wound dressings, drug delivery systems, food packing materials, water disinfectants, textile coating, and sensor devices [4]. Their bioactivity is largely due to their high surface area, greater reactivity, and their interaction with cellular membranes and biomolecules [5]. Nevertheless, most of the traditional chemical and physical synthesis methods required harsh conditions and toxic reagents as well as high energy consumption, limiting their practical application due to environmental and biocompatibility issues.

In an attempt to address these drawbacks, green synthesis methods using biological sources have been developed as eco-compatible and up-scaled processes. Plant-based synthesis is particularly appealing because it is simple, cost effective, scalable, and non-pathogenic [6]. The plant extracts are rich sources of phytochemicals such as (phenolics, flavonoids, terpenoids, alkaloids, and glycosides) which have the capability to act as reducing, stabilizing and capping agents at the same time for synthesis bioactive AgNPs in the absence of any extra chemical entity [7].

*Polypogon monspeliensis* (L.) Desf (*P. monspeliensis*), commonly known as rabbit's foot grass, is an annual monocotyledonous plant belonging to the Poaceae family [8]. It is found from Europe and North Africa to the Middle East and some parts of Asia, often in saline or xeric habitats such as coastal wetlands, inland sabkhas and semi-arid landscapes. Due to its halophytic character, *P. monspeliensis* shows a high ability of salinity and water deficit tolerance, which justifies maintaining plant productivity even at high NaCl levels. Aside from its ecological toughness, *P. monspeliensis* is receiving greater scientific attention for multi-utilization, including as ornamental, forage, and food resource [9].

Notably, *P. monspeliensis* has been found to have significant bioactive components such as antioxidant, antimicrobial, cytotoxic, hepatoprotective, allelopathic and phytoremediation effects [10]. Phytochemical studies have indicated the existence of active metabolites among flavonoids, phenols, alkaloids, terpenes, saponins, tannins and coumarins; formononetin and linarin are compounds that show importance from biological point of view. Despite these favorable characteristics, the prospect of using *P. monspeliensis* as a green bioresource for NPs fabrication has not been extensively investigated.

AgNPs were successfully green synthesized by the aqueous extract of *P. monspeliensis* in this research. Characterization of synthesized AgNPs was performed systematically, and their antibacterial activity, antioxidant potential, and in vitro anticancer effect were fully surveyed. This work highlights the dual role of *P. monspeliensis* as a reducing and stabilizing agent for AgNPs with promising biomedical applications.

## Materials and Methods

This study integrates the methodology of green synthesis, physicochemical characterization and biological evaluation into a single experimental cascade. Aqueous extraction of freshly aerial parts of *Polypogon monspeliensis*. Fresh aerial parts of *Polypogon monspeliensis* were collected from the surroundings of Olomouc, Czech Republic, washed with water, dried in the shade and grounded to fine powder before extraction with water. Preparation of the plant extract The mechanism of this plant extract preparation is flowing two legs, refluxing of a weighed quantity of the dried powdered material in deionized water (conductivity-0.03  $\mu$ S) and filtering the suspension using a filter paper, then recollect by using other deionized water at 4 °C until the bioactive constituents. For synthesis of nanoparticles, the aqueous extract was added dropwise to a solution of silver nitrate at controlled conditions, and the reaction mixture was left in the dark until the color change indicated the reduction of Ag<sup>+</sup> ions to metallic silver nanoparticles. The produced nanoparticles were subsequently isolated through centrifugation, washed several times with pure water in order to remove any unbound phytochemicals, air-dried, and stored for further analysis. Complementary analytical techniques were used to characterize biosynthesized silver nanoparticles and to confirm their formation and properties. Surface plasmon resonance was monitored using UV-visible spectroscopy, while FTIR spectroscopy was utilized to determine the functional groups proposed to be involved in either reduction or stabilization of the NPs. X-ray diffraction analysis was used to evaluate crystallinity and scanning electron microscopy with energy-dispersive X-ray for particle morphology, size distribution and elemental composition. Standardized in vitro assays were used to assess biological activities. The antibacterial activity of the synthesized silver nanoparticles was evaluated using agar well diffusion method against choice Gram-positive and Gram-negative bacterial pathogens at various concentrations of the nanoparticles. DPPH free radical scavenging assay was used to evaluate antioxidant potential and ascorbic acid was used as a standard. Cytotoxicity and anticancer activity were assessed on human lung adenocarcinoma cells normal fibroblast cells to investigate selectivity using the MTT assay. All experiments were carried in triplicate and the statistical analysis was conducted using suitable, so that the data were reliable and significant

## Results and Discussion

### A. *P. monspeliensis* extracts preparation

Fresh shoots of *P. monspeliensis* extracts were collected from the campus of University of Basrah during flowering stage and taken to laboratory, where they were well washed carefully by tap water then deionized distilled water in order to remove dust and surface impurities. Plant material was shade-dried at room temperature and subjected to aqueous extraction through reflux using an electric grinder, with minor modification according to the method of Alassadi. In brief, 5.0 gram (g) of powdered shoots were mixed with 100 milliliter (mL) of deionized distilled water (DDW), and refluxed for 5 hour (h), after cooling to room temperature (r.t), the solution was filtered by Whatman No.1 filter paper, focusing on filtrate which was concentrated at ambient temperature and maintained at 4 °C until used.

### B. The green synthesis of Pol-AgNPs

The biosynthesis of Pol-AgNPs was performed by a plant-mediated green synthesis method using hot aqueous extract of the *Polypogon monspeliensis*. In short, a 1 mM AgNO<sub>3</sub> solution was added to DDW and slowly stirred at low temperature with plant aqueous extract in an optimal volume ratio [11]. The reaction mixture was left in the dark at r.t until a color change was observed, indicating the formation of AgNPs. Afterwards, the suspension was centrifuged to collect the nanoparticles, and they were washed several times with deionized water to eliminate any unreacted materials, further air-dried at r.t, and stored in dark bottles at 4 °C until ready for characterization and biological studies.

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### C. Cytotoxicity of Pol-AgNPs

The cytotoxic potential of Pol-AgNPs was determined against A549 human lung cancer cells as described previously, with slight shifts by MTT assay [12]. A549 cells were incubated in RPMI 1640 with 10% fetal bovine serum and 1% penicillin/streptomycin at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub>. For 24 h, the cells were then treated with different concentrations of Pol-AgNPs (25–400 µg/ml), and following that, the standard protocol was performed for 48 h. Following incubation, MTT solution was added, and the formazan crystals formed were solubilized in Dimethyl sulfoxide (DMSO). The absorbance was read at 570 nm with a microplate reader. All biological assays were carried out in triplicate, and the antiproliferative effect of Pol-AgNPs was expressed as half maximal inhibitory concentration (IC<sub>50</sub>).

### D. Antioxidant properties of Pol-AgNPs

The antioxidant potential of Pol-AgNPs was assessed by DPPH free radical scavenging assay according to a previously published method with slight modifications. The principle of the assay depends on the reduction of a purple-coloured DPPH radical to its yellow colloidal hydrazine stable form by anti-oxidant compounds. All experiments were implemented in the Biotechnology Research Center, Al-Nahrain University [13].

### E. Antibacterial activity of Pol-AgNPs

The antibacterial potential of the biosynthesized Pol-AgNPs against *P. aeruginosa*, *S. aureus*, *S. epidermidis*, and *K. pneumoniae* was evaluated following standard procedures with slight modifications using the agar well diffusion method [13]. Bacterial cultures, grown on nutrient agar plates for 24 h, and bacterial suspensions adjusted to the 0.5 McFarland standard were spread onto Mueller–Hinton agar medium. Agar medium was punched in wells (6 mm diameter), and 100 µL of AgNPs at various concentrations were loaded, while DMSO was used as the negative control. The plates were incubated at 37 °C for 24 h, and the diameters of the inhibition zones (ZOI mm) were measured to evaluate antibacterial activity.

### F. Characterization Pol-AgNPs

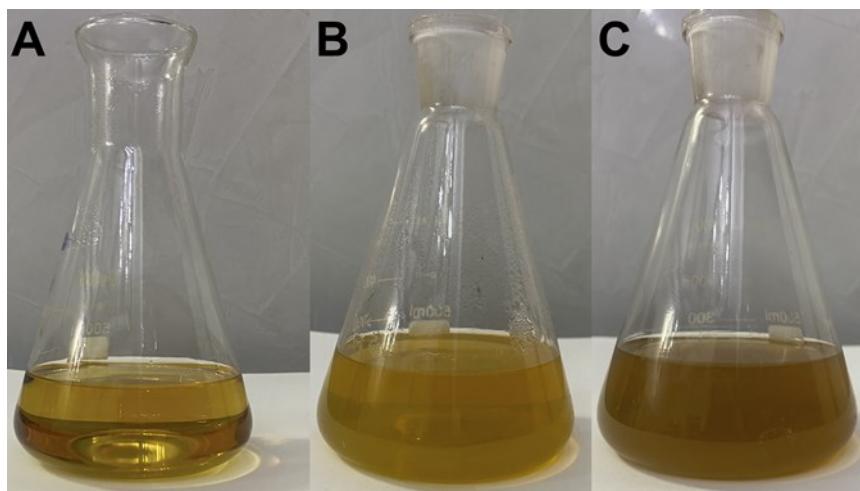
The green synthesized Pol-AgNPs were analyzed through standard analytical methods. The formation of nanoparticles was verified using UV–vis spectroscopy by monitoring the surface plasmon resonance (SPR) band in the range of 200–700 nm. Groups responsible for the reduction and stabilization of AgNPs were determined by FTIR spectroscopy in KBr pellets ranging from 4000 to 400 cm<sup>-1</sup> wavenumber. The crystalline nature of the nanoparticles was studied by XRD examination with the help of Cu K $\alpha$  radiation over a 2 $\theta$  range between 20 and 80°, and then results were compared with standard JCPDS data. The morphology and size distribution of the samples were studied by SEM, and EDX.

## Statistical Analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) to evaluate the antibacterial activity of the synthesized silver nanoparticles (SPSS v22). Cytotoxicity and antioxidant activity data were analyzed using two-way ANOVA with GraphPad Prism 8, and the results were presented graphically.

### A. Green synthesis of Pol-AgNPs.

In the process of green synthesis of Pol-AgNPs, a visual color change occurred, and a clear sign for qualitative indication of formation of such NPs was observed (Khoiri *et al.*, 2024). The plant extract showed a pale-yellow color, indicating the presence of bioactive compounds, including flavonoids and phenolics, among other secondary metabolites. The reaction mixture immediately became yellow-brown when silver nitrate was added (Figure 1), indicating the reduction of Ag<sup>+</sup> ions to metallic Ag<sup>0</sup> nanoparticles. The continuing brown color after 5 days of dark incubation indicates the presence of stable AgNPs. Such a stable state is ascribed to the presence of phytochemicals in *P. monspeliensis*, acting as reducing and capping agents thereby inhibiting aggregation and providing colloidal stabilization. The relatively rapid and stable color transition suggests a high content of efficient bioreductants in the extract, supporting its suitability as an eco-friendly resource for nanoparticle synthesis [14].



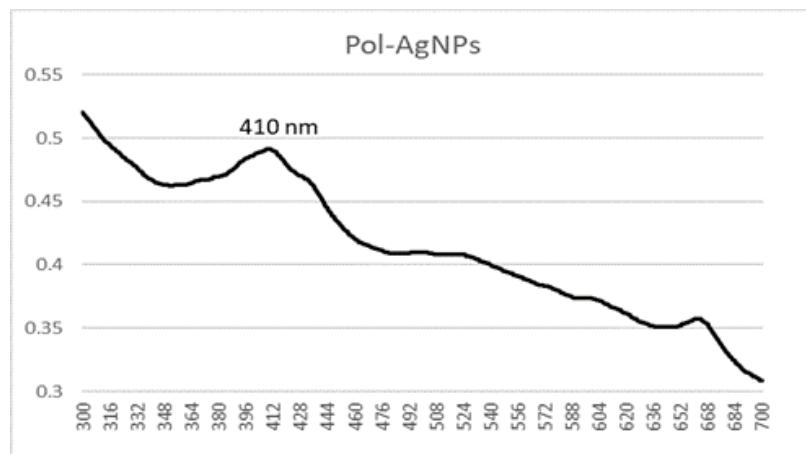
**Figure 1:** (A) Color change of silver nanoparticles solution (Pol-AgNPs) synthesized from the hot aqueous extract of *P. monspeliensis*; (B) AgNPs solution immediately after synthesis; (C) stability of the color change after 5 days of incubation in the dark.

### B. Characterization of AgNPs

#### 2.1 UV–Vis spectroscopic analysis.

To ascertain the complete stabilization of the silver nanoparticles, UV–visible spectroscopy was also used to confirm the successful biosynthesis. The UV–Vis absorption spectrum of the biosynthesized Pol-AgNPs showed a characteristic SPR peak at 410 nm (Figure 2), which is due to the collective vibration of conduction electrons in presence of nanosized AgNPs. This SPR band pattern is typical for spherical homogeneously

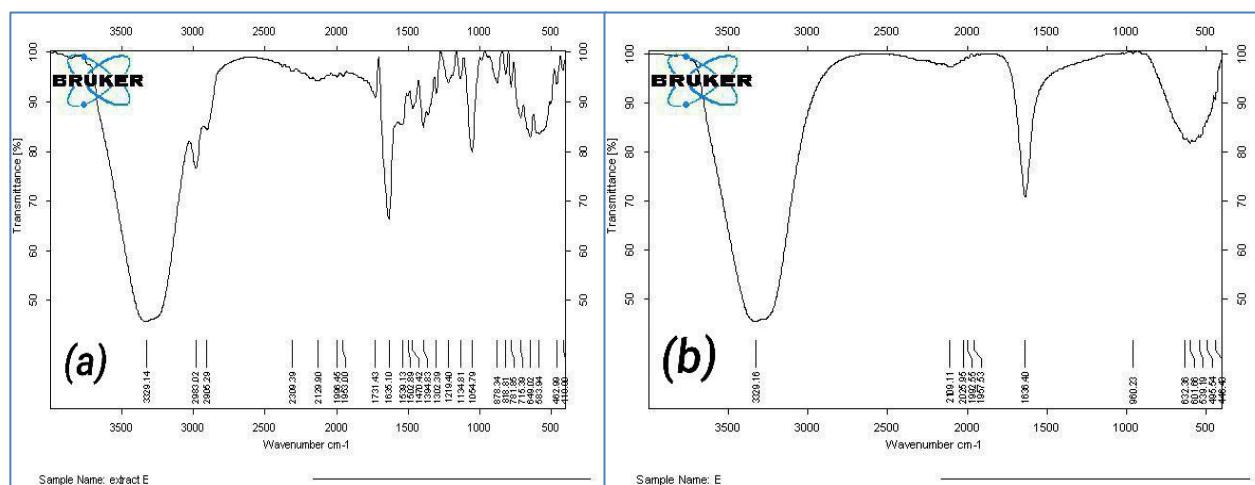
dispersed AgNPs and in accordance with literature reports on plant-based AgNPs production [15]. The lack of other absorption bands also demonstrates that little to no aggregation and bulk silver formation was observed, demonstrating AgNPs stability. These results clarify that *P. monspeliensis* extract can serve as a reducing, capping, and stabilizing agent toward NPs, supporting its promise as environmentally benign for resource nanotechnology and biomedicine applications.



**Figure 2 :** UV-Vis spectroscopic analysis of Pol-AgNPs.

### C. FTIR spectroscopy analysis

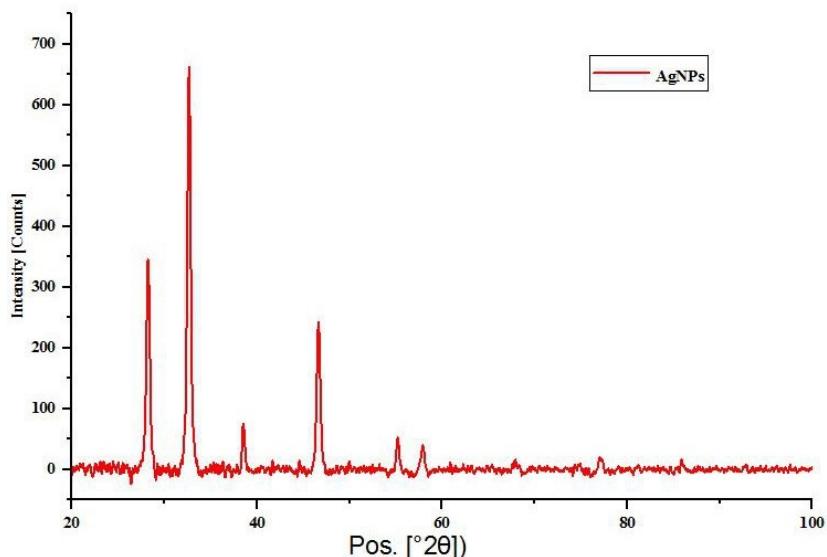
FTIR spectroscopy was employed to identify the functional groups of *P. monspeliensis* extract involved in the biosynthesis and stabilization of AgNPs. The FTIR spectrum of the crude plant extract (**Figure 3a**) displayed multiple characteristic absorption bands, confirming the presence of various phytochemicals such as polyphenols, flavonoids, and proteins. A broad band at  $3329\text{ cm}^{-1}$  was attributed to O-H stretching vibrations of hydroxyl groups, while bands at  $2933$  and  $2895\text{ cm}^{-1}$  corresponded to C-H stretching of aliphatic chains. The strong absorption band observed at  $1731\text{ cm}^{-1}$  was assigned to C=O stretching of carbonyl groups associated with secondary metabolites [16]. In contrast, the FTIR spectrum of the biosynthesized AgNPs (**Figure 3b**) showed noticeable shifts and intensity changes, indicating the involvement of specific biomolecules in nanoparticle formation. The O-H stretching band shifted to approximately  $3229\text{ cm}^{-1}$ , suggesting the participation of hydroxyl groups in the reduction of  $\text{Ag}^+$  ions, while the carbonyl band became sharper and more intense at around  $1636\text{ cm}^{-1}$ , reflecting strong interactions with the nanoparticle surface that contribute to effective capping and stabilization. The disappearance or weakening of several fingerprint-region peaks further indicates that only selected phytoconstituents were involved in nanoparticle binding. Overall, these spectral changes confirm that hydroxyl- and carbonyl-containing biomolecules in *P. monspeliensis* play a crucial role in both the reduction and stabilization of AgNPs [17].



**Figure 3:** (a) FTIR spectrum analysis of *P. monspeliensis* extract, and (b) Pol-AgNPs.

### D. XRD analysis of the synthesized Pol-AgNPs

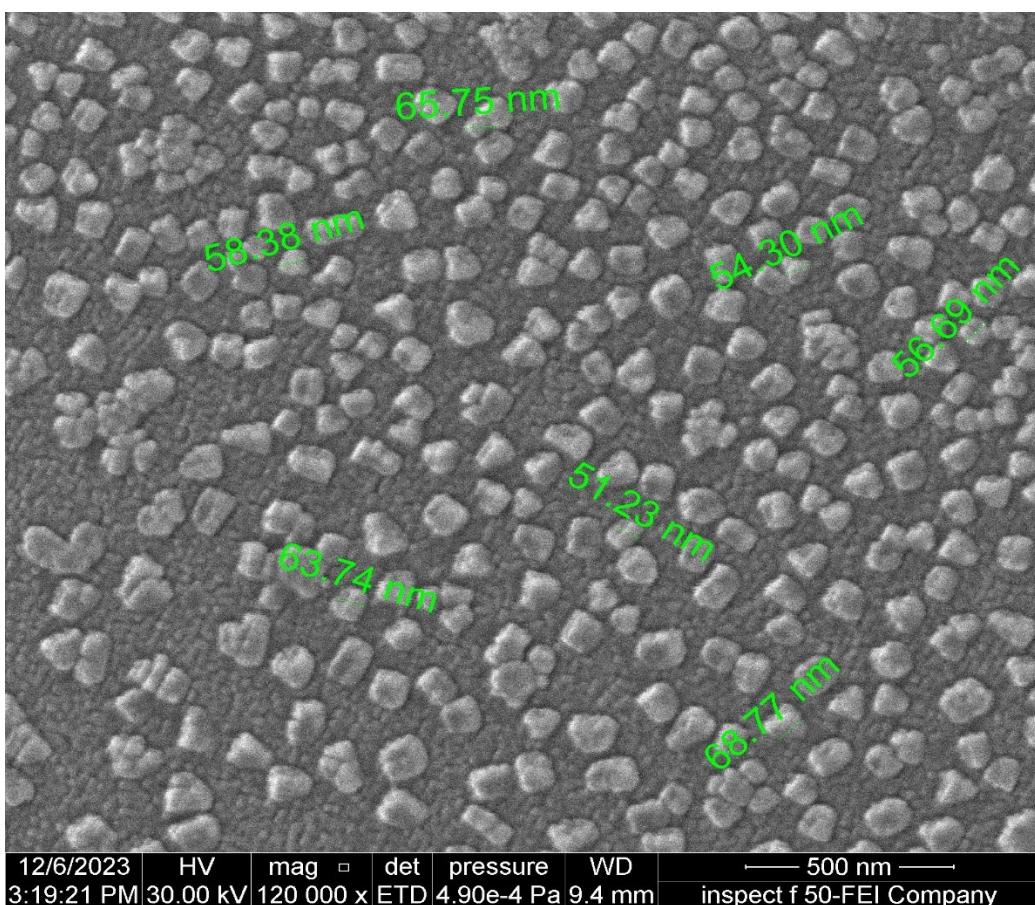
XRD was used to assess the crystallinity of the Pol-AgNPs (**Figure 4**). The diffractogram shows prominent reflections at  $2\theta$ :  $28.8^\circ$ ,  $32.9^\circ$ ,  $46.9^\circ$ ,  $55.4^\circ$ ,  $58.3^\circ$ ,  $68.5^\circ$ , and  $78.4^\circ$  which correspond to the (111), (200), (220), (311), (222), (220), and (311) planes, respectively [18], confirming the formation of a well-crystalline phase. In addition to these major peaks, several low-intensity reflections are also observed at higher  $2\theta$  values, which may arise from minor crystalline contributions, nanoscale-induced lattice strain, or the presence of phytochemical residues capping components associated with green synthesis. Overall, the sharpness and intensity of the main reflections indicate good crystallinity, while the additional weak peaks suggest that the sample may not be a single perfectly phase-pure crystalline component, which is commonly reported for plant-mediated nanoparticle systems [19].



**Figure 4:** XRD analysis of Pol-AgNPs.

#### E. SEM analysis

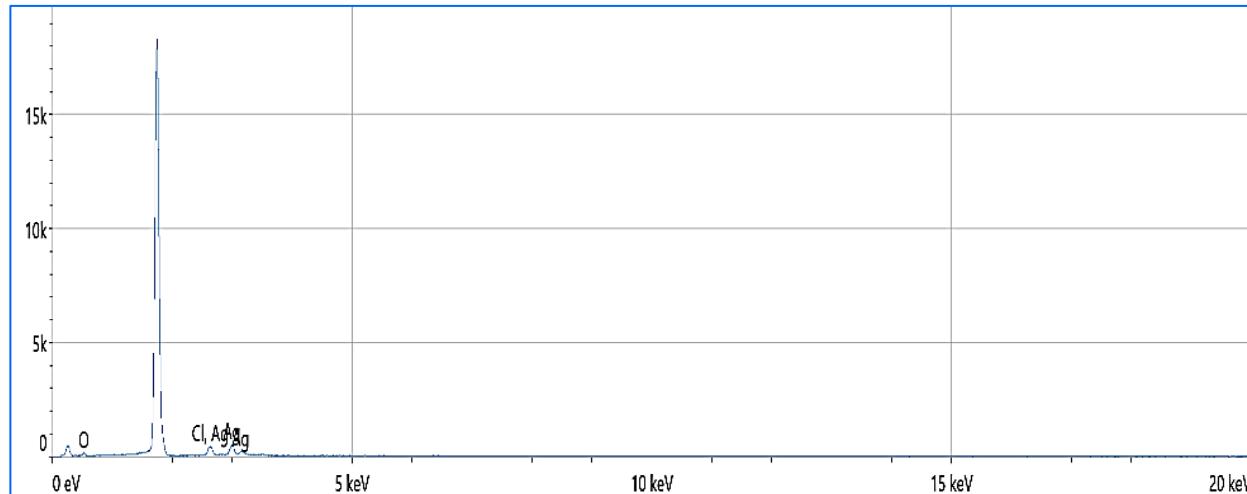
The surface morphology of the Pol-AgNPs was examined by SEM (Figure 5). The micrograph reveals a high population of nanoparticles distributed across the surface, with most particles exhibiting a quasi-spherical to slightly irregular morphology and good dispersion. The Pol-AgNPs are within the nano-size range of approximately 51.23–68.77 nm, with an estimated average diameter of 59 nm. Such nanoscale dimensions provide a high surface-to-volume ratio, which is favorable for biological interactions, as it facilitates close contact with microbial cell membranes and cancer cells. Moreover, the observed surface features and partial aggregation may promote silver ion release and reactive oxygen species generation, contributing to the antibacterial and anticancer activities of the synthesized nanoparticles. There are many studies that have indicated silver nanoparticle sizes similar to those obtained in our current study, such as the study by Echegaray-Ugarte in which silver nanoparticles were synthesized from the *Punica granatum* peel waste at an average size of 59 nm [20].



**Figure 5:** SEM analysis of Pol-AgNPs.

#### F. EDX analysis

The elemental composition of the biosynthesized Pol-AgNPs was confirmed by EDX analysis (**Figure 6**). The EDX spectrum exhibits a dominant and sharp peak at around 3 keV, which is characteristic of elemental silver and unequivocally confirms the successful formation of AgNPs. Besides silver, minor signals corresponding to oxygen (O) and chlorine (Cl) are also observed at low intensities. These elements are commonly detected in green-synthesized nanoparticles and are attributed to plant-derived biomolecules adsorbed on the nanoparticle surface, acting as stabilizing and capping agents. The absence of strong additional elemental peaks further indicates that silver is the principal component of the synthesized nanoparticles, supporting the effectiveness of *P. monspeliensis* extract in producing silver-rich, biologically stabilized nanostructures [21].



**Figure 6:** EDX analysis of Pol-AgNPs.

#### G. The Antibacterial Activity of Pol-AgNPs

As shown in **Table 1**, and visually confirmed by the agar well diffusion assay (**Figure 7**), Pol-AgNPs showed a dose-dependent antibacterial activity against all bacterial strains tested. An increase in the concentration of Pol-AgNPs from 25 to 400  $\mu\text{g}/\text{ml}$  led to a gradual rise in the diameters of inhibition zones (ZOI) and established a correlation between AgNPs content and antibacterial properties [22]. Of the tested microorganisms, *P. aeruginosa* was the most susceptible with a mean ZOI of ca 16.97 mm at 400  $\mu\text{g}/\text{ml}$ , followed by *K. pneumoniae* and *S. epidermidis* that exhibited similar and moderate sensitivities ZOI (13.7 - 13.27 mm) respectively. Conversely, *S. aureus* was the least susceptible at concentrations tested 6.0 mm ZOI at 25  $\mu\text{g}/\text{ml}$ . The table 1 shows statistically significant differences between bacterial strains and concentrations ( $p \leq 0.0001$ ).

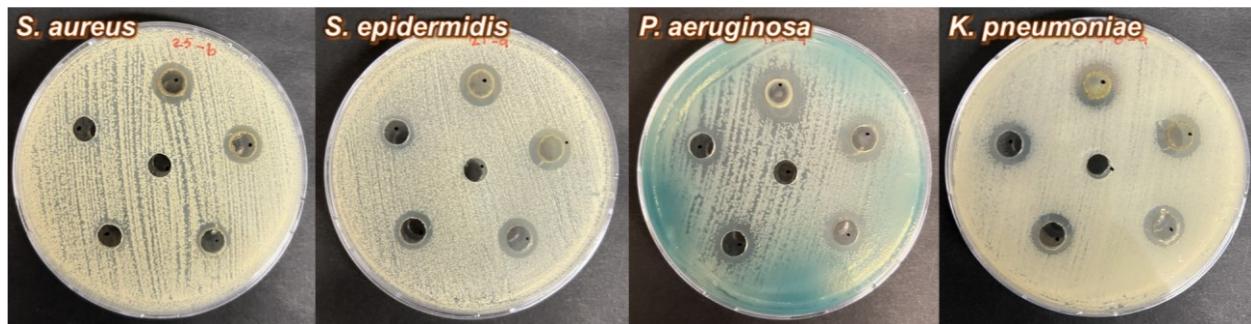
Compared to earlier plant-synthesized AgNPs, Pol-AgNPs also exhibited similar or even better antibacterial activity under similar or comparable concentrations. For instance, the AgNPs in *Zataria multiflora* had an inhibition zone of ca. 17 mm for *S. aureus*, and those biosynthesizing in *Periploca hydaspis* AgNPs were 13.83 mm towards *S. aureus* and 16.66 mm against *K. pneumoniae*. The AgNPs exhibited a concentration dependent increase against different strains [23]. Similarly, AgNPs synthesized from the root extract of *Premna integrifolia* revealed zones of inhibition 14.18 mm against *P. aeruginosa* and 12.18 mm against *S. aureus*. In contrast, *Delphinium uncinatum* stem and root extract AgNPs exhibited equal or higher efficiency with a range of inhibition zone diameters varying between 7.0 and 14.9 mm for all tested bacteria depending on the bacterial strain and plant part used [24]. An overall comparison of these values clearly demonstrates that Pol-AgNPs possess great antibacterial action compared to a large number of green-prepared AgNPs reported until now, underlining the beneficial effects of *P. monspeliensis* phytochemicals on potential application against pathogens.

These variances in antibacterial efficacy may result from differences in the architecture of bacterial cell envelopes because it has been shown that Gram-negative bacteria are more susceptible to NPs interaction as compared with Gram-positive bacteria, which have thicker peptidoglycan layers that can limit penetration. Taken as a whole, the results indicate that Pol-AgNPs are effective against Gram-negative and Gram-positive strains, highlighting their potential as environmentally friendly antimicrobial agents.

**Table 1:** Antibacterial activity of Pol-AgNPs against multidrug-resistant bacteria.

Bacterial isolates	Concentrations ( $\mu\text{g}/\text{ml}$ )					<b>Average</b> $p \leq 0.0001$ <b>LSD= 1.30</b>
	400	200	100	50	25	
<i>P. aerigenosa</i>	16.97 $\pm$ 1.00	14.40 $\pm$ 0.56	13.07 $\pm$ 0.25	12.20 $\pm$ 0.44	11.07 $\pm$ 0.25	13.54 $\pm$ 2.16 <sup>a*</sup>
<i>K. pneumoniae</i>	13.37 $\pm$ 0.25	13.00 $\pm$ 0.20	12.20 $\pm$ 0.36	12.00 $\pm$ 0.26	12.53 $\pm$ 0.45	12.62 $\pm$ 0.61 <sup>ab</sup>
<i>S. epidermidis</i>	13.27 $\pm$ 0.32	13.17 $\pm$ 0.12	12.17 $\pm$ 0.42	11.27 $\pm$ 0.21	10.57 $\pm$ 0.67	12.09 $\pm$ 0.96 <sup>b</sup>

<b><i>S. aureus</i></b>	13.03±0. 38	11.23±0.7 5	10.13±0.2 5	8.47±0.47	6.00±0.00	9.77±2.45 <sup>c</sup>
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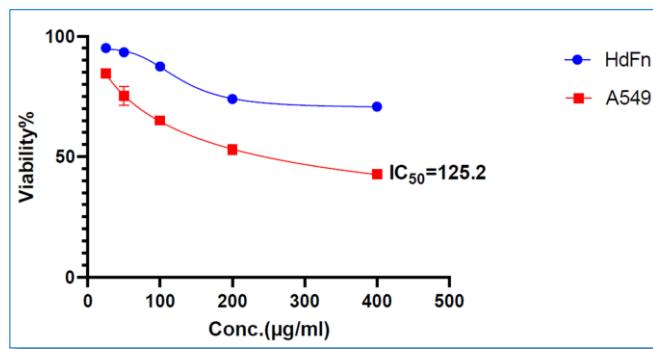
**Figure 7:** Antibacterial activity of Pol-AgNPs antibacterial efficacy against Gr<sup>+</sup> and Gr<sup>-</sup> bacteria

#### H. Cytotoxicity of Pol-AgNPs

Cytotoxicity and anti-cancer characteristics of the Pol-AgNPs using the MTT assay, the bio-efficacy of AgNPs was screened against human lung adenocarcinoma A549 cell line as targeted cancer cells **Table 2**. From the viability results and dose-response curves, it is clear that Pol-AgNPs caused a significant reduction in cell viability of both cell types in a dose-dependent manner after 48 h exposure time; however, such inhibition was substantially higher for targeted cancer cells. At the maximum concentration (400 µg/ml), A549 cells showed decreased viability to 42.67 ± 1.04%, and HdFn cells showed a significantly higher viability at 70.80 ± 1.09%, which indicates selective cytotoxicity for lung cancer cells. This selective course of action remained significant at all concentrations tested, with A549 cells consistently showing lower viability results than the normal fibroblasts. This specificity is also corroborated by the graphic analysis (**Figure 8**), showing that A549 cells exhibit a relatively low IC<sub>50</sub> value (IC<sub>50</sub> = 125.2 µg/ml), reflecting greater sensitivity of Pol-AgNP treatment in cancer cells. Compared to other plant-mediated AgNPs reported in the literature, the IC<sub>50</sub> of Pol-AgNPs on A549 lies within the wide range reported for biogenic AgNPs, however, depending upon the plant extract source, nanoparticle physicochemical characteristics, and conditions of exposure, lower values of IC<sub>50</sub> were reported. For example, AgNPs synthesized from aqueous seed extract of *Derris trifoliata* revealed an IC<sub>50</sub> value of 86.23 ± 0.22 µg/ml on A549 cells [25], whereas AgNPs prepared with *Piper nigrum* extract presented an IC<sub>50</sub> of 50 µg/ml against A549 cells; likewise, *Syzygium aromaticum*-mediated green AgNPs demonstrated an IC<sub>50</sub> of 70 µg/ml on A549 [26]. Similarly, AgNPs derived from *Albizia adianthifolia* extract showed a swift loss of A549 cell viability in the absence of long exposure 6 h exposure also signifying significant role played by duration effects on associated apparent cytotoxicity end point outcomes [27]. These inter-study differences are anticipated and could be attributed to variations between the reported nanoparticle size/shape, capping phytochemicals, colloidal stability, dose metrics, and duration of assay [28]. The discrimination of the response to Pol-AgNPs between cancer and normal cells in this study could possibly relate to the intrinsic metabolic activity, high basal level of oxidative stress, and enhanced sensitivity of cancer cells to silver ion release and reactive oxygen species generation [30]. Together, these data suggest that Pol-AgNPs preferentially exert anticancer effects on lung cancer cells but exhibit a relatively high safety profile to normal cells, and therefore they have great prospects in the development of anticancer nanomaterials [31].

**Table 2:** Cytotoxic activity of Pol-AgNPs against the normal cell line HDFn and the cancer cell line A549.

Conc. of Pol-AgNPs (µg/ml)	HDFn		A549	
	Mean	SD	Mean	SD
400	70.79	1.09	42.67	1.03
200	73.99	0.88	52.89	1.97
100	87.42	1.31	65.04	1.33
50	93.40	0.53	75.19	3.82
25	95.10	0.29	84.52	1.38



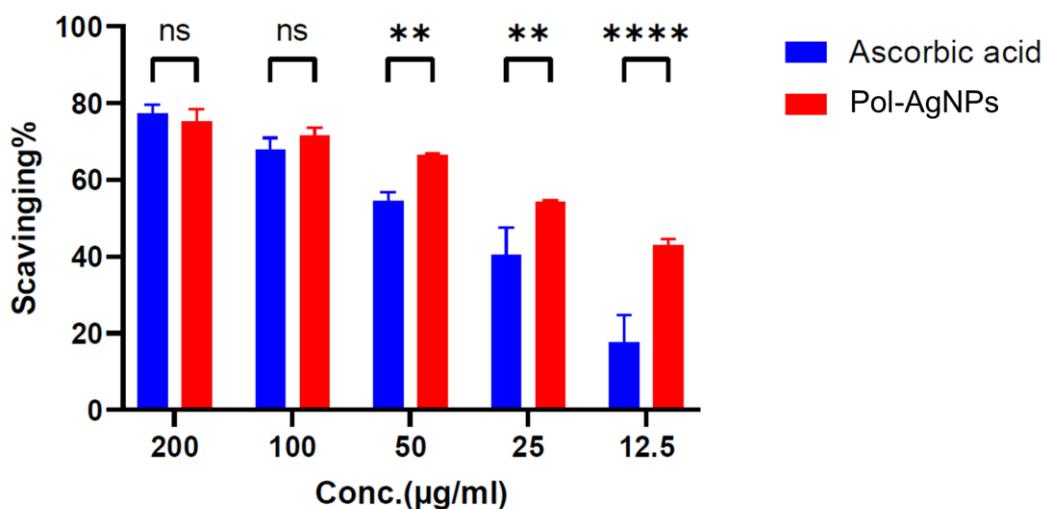
**Figure 8:** Cytotoxic activity of Pol-AgNPs against the normal cell line HDFn and the cancer cell line A549.

### I. Antioxidant activity of Pol-AgNPs

The antioxidant activity of the synthesized Pol-AgNPs was assessed by DPPH free radical scavenging assay at concentrations 12.5–200 µg/ml using ascorbic acid as a standard. As listed in **Table 3** and **Figure 9**, Pol-AgNPs showed significantly concentration-dependent enhancement in the scavenging activity from the lowest of  $42.94 \pm 1.70\%$  for 12.5 µg/ml to the highest of  $75.12 \pm 3.36\%$  for 200 µg/ml, which were nearly equivalent to that of ascorbic acid ( $77.24 \pm 2.40\%$ ) at the same tested concentration (200 µg/ml). It is important to report that antioxidant activity of Pol-AgNPs was more efficient at low concentration (50–12.5 µg/ml) in comparison with the standard, indicating enhanced radical-scavenging efficiency at reduced doses. On the other hand AgNPs synthesized with *Paeonia japonica* root extract showed greater DPPH scavenging activity 96.5% at 200 µg/ml, and *Salvia aethiopis* derived AgNPs showed moderate ABTS scavenging potential even though they acted against DPPH scavenging activity with an IC<sub>50</sub> of 24.37 µg/ml [32][33]. Such differences are due to phytochemical composition of the plant and characteristics of surface NPs. In total, the marked antioxidant effect of Pol-AgNPs, and especially at lower concentrations is very likely related to surface-functionalized phenolic compounds from *P. monspeliensis*, suggesting that they might be used as bioactive nanomaterials in oxidative stress-related biomedicine formulations [34][35].

**Table 3:** Comparison of the Antioxidant Activity of a Nano-extract from *Polypogon monspeliensis*. and Ascorbic Acid using the DPPH Assay

Conc. (µg/ml)	Ascorbic acid		Pol-AgNPs	
	Mean	SD	Mean	SD
<b>200</b>	77.23	2.40	75.11	3.36
<b>100</b>	67.97	3.01	71.52	2.09
<b>50</b>	54.47	2.41	66.35	0.52
<b>25</b>	40.43	7.08	54.20	0.46
<b>12.5</b>	17.63	7.19	42.93	1.70



**Figure 9:** Activity of Pol-AgNPs using DPPH free radical scavenging assay.

## Conclusion

In the present investigation, an eco-friendly synthesis of stable silver nanoparticles (Pol-AgNPs) has been achieved by utilizing the aqueous extract of *Polypogon monspeliensis* as a green reducing and stabilizing agent. The prepared nanoparticles displayed decent crystallinity, with nanosized and good physicochemical stability as evidenced by extensive characterization. Pol-AgNPs exhibited broad-spectrum antibacterial activity against Gram-negative and Gram-positive bacteria in concentration-dependent manner, as well as a strong antioxidant activity which was comparable to or higher than that of ascorbic acid. Significantly, the NPs possessed preferential cytotoxicity and anticancer effects against human lung adenocarcinoma A549 cells, with a relatively low toxicity to normal HdFn fibroblasts. Taken together, these findings demonstrate the promising and sustainable nanoplatform of *P. monspeliensis* mediated AgNPs in biomedical applications, and further studies are warranted to elucidate its molecular mechanisms, evaluate the efficacy and safety in vivo, as well as its potential use as a targeted drug delivery system.

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