

Oxalis pes-caprae L. Extract Conferred Its Biological Activities to Silver Nanoparticles

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Abstract

This study used *Oxalis pes-caprae* L. (OP) to develop a low-budget, low-maintenance, and environment-friendly production method for silver nanoparticles (AgNPs) and evaluated their antibacterial, antifungal, and antioxidant abilities. Ultraviolet–visible (UV–Vis) spectroscopy results revealed a maximum of absorbance at 431 nm, confirming the formation of AgNPs. Moreover, Fourier transform infrared spectroscopy (FTIR) analysis of synthesized AgNPs indicated the presence of various important functional groups, such as O–H, CH, H–C–H, and C–O–C, which are involved in the synthesis and stability of the OP-AgNPs. X-ray diffraction (XRD) analysis confirmed the crystalline nature of the synthesized AgNPs, which had an average particle size of 46.31 nm and four Bragg reflections at 38.2°, 44.32°, 64.4°, and 77.1°. The Nanomeasurer software confirmed that 89% of the particles had an average size of 50 nm, and the scanning electron microscope analysis also confirmed spherical shaped, monodispersed, high-density AgNPs formation. Energy dispersive X-ray analysis confirmed that silver was the main element with a highest sharp peak at 3.0 keV. We assessed the antibacterial and antifungal activities of the AgNPs and OP extract on the bacteria strains *Escherichia coli*, *Acetobacter*, and *Staphylococcus epidermidis*, and fungi strains *Aspergillus niger* and *Aspergillus flavus*. The AgNPs displayed more potent antibacterial and antifungal inhibition abilities than the plant. Similarly, in 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azobis,3-ethylbenzothiazoline-6-sulphonic acid (ABTS), and hydrogen peroxide (H₂O₂) assays, AgNPs displayed better reduction capacities than the plant extract.

Keywords: *Oxalis pes-caprae*; silver nanoparticle (AgNPs); nanotechnology; biological activities

Introduction

Nanotechnology is the study of the synthesis, manipulation, and imaging of nanostructures with a size of 1–100 nm [1]. Due to their unique properties and applications, nanoparticles have recently gained attention [2, 3]. At present, many nanoparticle manufacturing processes exist, including physical methods (plasma, chemical vapor deposition,

microwave irradiation, and pulsed laser), biological methods (plants, bacteria, fungi, and alga), chemical methods (chemical reduction), and hybrid methods mixing two of these methods [4–6].

Initially, nanoparticles were manufactured through physical and chemical routes, but these processes involved toxic materials and by-products. Therefore, researchers turned towards safe, simple, economical, and environmentally clean nanoparticle synthesis

routes involving plants and microorganisms, as these processes do not yield toxic by-products and are user-friendly [7–9]. Even though using microorganisms like bacteria [10, 11] and fungi [12, 13] is a good way of manufacturing nanoparticles, these processes involve careful culturing, isolation, and media preparation techniques, making the process sensitive and increasing the overall cost of the procedure. To make the process cost-effective, eco-friendly, and readily available, using plants is a better option, as it is easily scaled up and requires no special treatments such as high pressure and temperature or toxic chemicals [14, 15]. Hence, using plants to synthesize nanoparticles has very few disadvantages compared with physical, chemical, and microbial methods [8, 16].

Nanoparticles are either metallic or non-metallic. Metallic nanoparticles have been studied in more depth due to their unique characteristics, including their catalytic, optical, electronic, magnetic, and antimicrobial properties [11, 17]. Notably, silver, copper, gold, cobalt, nickel, and some semiconducting materials are extensively used in research [18]. Among them, silver nanoparticles (AgNPs) are widely studied because of their tremendous applications and unique electrical, optical, drug delivery, nano-device fabrication and biosensing properties [19–21]. They have been utilized in the industry as antibacterial and antifungal agents, as well as domestic and health care products, medical device coatings, orthopedic treatments, and anticancer medications. They have also been used to improve the lump-slaying properties of already existing anticancer medications [17]. They also have applications in textile, wound dressing, and device wrapping [22, 23]. The large volume-to-surface area is their most sought-after property [24].

Oxalis pes-caprae L. (OP) is a shade-loving herbaceous plant with an underground bulbiferous stem, indigenous to South Africa, Europe and Mediterranean regions, Southwest Asia, and North Africa [25]. Several cultures consumed it as food, a source of acid, or a medicine. The bulb of the plant can be used for treating tapeworm and other worm-related diseases, or as a diuretic. The underground stems are also eaten with milk as a folk medicine. According to Li et al. [26], OP has potent anti-inflammatory, analgesic, heart clearing, and wound-healing effects. Its sour taste of the plant is due to

high vitamin C content, which is very efficient against cancer. The whole plant and especially the leaves contain oxalic acid and can be formulated into a locally applied paste to treat swellings [27]. Various OP family members have been used as traditional medicinal plants and were screened for pharmacological activities and for biosynthesis of AgNPs, including *Oxalis adenophylla*, *O. corniculata*, *O. griffithii*, *O. debilis*, *O. stricta*, *O. barrelieri*, *Averrhoa bilimbi* fruit, *Averrhoa carambola*, and *Biophytum sensitivum* leaf [28–35]. This prompted us to use OP for the synthesis of AgNPs, as it had never been done before. We first evaluated the use of this plant for the synthesis of AgNPs. Then we characterized the synthesized AgNPs using different techniques and evaluated their antibacterial, antifungal, and antioxidant effects.

Materials and Methods

Materials

We collected the whole plant from a guava garden in the Bannu District of Khyber Pakhtunkhwa (KPK) Province of Pakistan. Furthermore, Professor Tahir Iqbal of the Department of Botany at the University of Science and Technology in Bannu confirmed the botanic identity of the collected plant. We deposited the plant specimen with voucher No. OP-C #01 in the herbarium of the Department of Botany UST-Bannu KPK. We obtained the chemicals (silver nitrate, sulfuric acid, nutrient agar, NaOH, dimethyl sulfoxide (DMSO), sodium phosphate, ascorbic acid, ammonium molybdenum, sodium acetate buffer, 2,2-diphenyl-1-picrylhydrazyl (DPPH), H₂O₂, 2,2-azobis,3-ethylbenzothiazoline-6-sulphonic acid (ABTS), K₂S₂O₈, and methanol from the Department of Biotechnology and Department of Chemistry, University of Science and Technology, Bannu KPK.

Plant extraction

After identification, we washed the whole plant with tap water to remove debris and dust and let it completely dry. Next, we ground the whole plant to powder. To prepare the extract, we soaked about 600 mg of powdered plant in 80% methanol on a shaker for 12 h at 120 r/min. After 7 days we filtered the solution with a Whatman filter paper No. 1 and evaporated the methanol using a rotary vacuum evaporator; we then stored the crude methanolic

extract at 4 °C in a refrigerator until use.

Synthesis of *Oxalis pes-caprae* AgNPs

We synthesized the AgNPs from plant extract as described in Ref. [36]. We dissolved silver nitrate in 50 mL of deionized water to make a 10 mmol/L solution, which was then diluted 10 times in deionized water to make a 1 mmol/L solution. Next, we raised the solution's pH value to 11 by adding NaOH because at low pH values (5–8) no band was obtained, but as the pH increased, the band became sharp [37]. Most nanoparticle solutions use a pH buffer [38] but in the case of AgNPs that is not necessary because they are stable in alkaline water solution; therefore, most of the literature suggests using deionized water for the synthesis of AgNPs. We produced a similar solution by adding 0.8 g of methanolic plant extract into 100 mL of deionized water, which was then stirred with a magnetic stirrer for approximately 30 min. Next, we mixed the two solutions (450 mL of silver nitrate + plant extract). As AgNPs began to form, the solution's color changed from milky white to brownish. We then transferred the solution into a 15 mL falcon tube and centrifuged it for 10 min. We discarded the supernatant, resuspended the precipitate, centrifuged it again, and dried it over 10 days. After complete drying, we used the AgNPs powder in the different assays and characterized them by scanning electron microscopy, X-ray diffraction, Energy dispersive X-ray (EDX), Fourier transform infrared (FTIR), and ultraviolet-visible (UV–Vis) spectroscopy analysis.

Characterization of synthesized AgNPs

We determined the concentration of the OP extract by UV spectroscopy at 300–800 nm. For the morphological characterization at the nano level, we took advantage of scanning electron microscopy.

We identified the functional groups using FTIR spectroscopy [28]. To understand the crystalline structure and phases of the silver nanoparticles, we analyzed X-ray diffraction [39]. The bending pattern of the rays entering the nanoparticle are acquired and correlated with a standard to reveal structural information about the particles. Besides, we identified compounds present in the solutions by EDX spectroscopy. This method also allows the detection of harmful substances present within the extract.

Bio-assays of synthesized silver

nanoparticles

We tested the antibacterial activity of the plant extract and synthesized AgNPs on *Staphylococcus epidermidis*, *Acetobacter*, and *Escherichia coli* and their antifungal activity on *Aspergillus niger* and *Aspergillus flavus*. Similarly, we assessed the antioxidant scavenging potential of the AgNPs using DPPH [40–42].

Determination of the optimal OP extract concentration for AgNP synthesis

To determine the optimal OP extract concentration for the synthesis of AgNPs, we performed the reaction with OP extracts at different volumes (0.3, 0.5, 0.8, 1.5, and 2.0 mL) and recorded the UV absorbance spectra of each resulting solution to find the one with the highest absorbance peak.

Statistical Analysis

We expressed all data as mean and standard error. After confirming the normal distribution of the data, we compared groups using two-way ANOVA followed by Tukey's HSD test ($P \leq 0.05$). We conducted all statistical analyses with OriginPro 8.5.

Results

UV–Vis spectroscopy

Due to the surface plasmon resonance, AgNPs have a dark brownish color. Upon mixing, the silver nitrate and OP extract solution quickly turns from milky white to metallic brown (Fig. 1). This indicates that the Ag^+ concentration has decreased. Solutions produced with OP extract at volumes of 0.3, 0.5, 0.8, 1.5, and 2.0 mL had absorbance maximums at 419, 421, 410, 421 and 429 nm, respectively (Fig. 2). The increasing plant extract volume sharpened the surface plasmon resonance bands. At the lowest volume (0.3 mL), the peak was at 419 nm with an absorbance of 1.23; meanwhile, at the highest volume (2.0 mL), the solution had a surface plasmon absorption band with an absorbance of 3.22.

Scanning electron microscopy analysis

We observed the morphology and distribution of nanoparticles on the surface by scanning electron microscopy. The current voltage varied from 5 to 20 kV, and the resolution of the microscopic lens was between 5 000× and 50 000×, indicating high-density

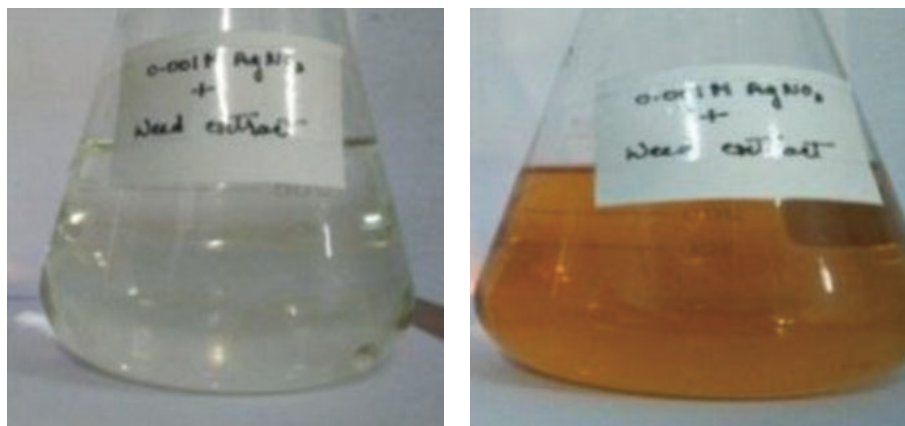


Fig. 1 The color of an OP extract + AgNPs solution after 0 min and 24 h.

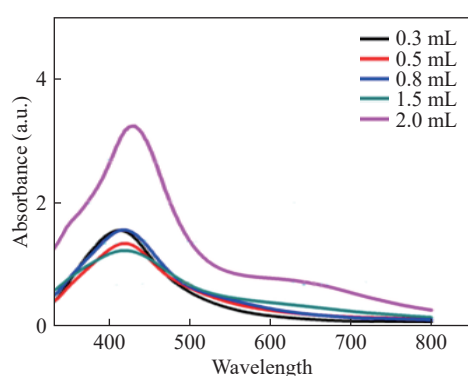


Fig. 2 UV-Vis spectra of AgNPs solutions obtained from OP extract at various volumes.

monodispersed, face-centered cubic rounded AgNPs, (Fig. 3). Marking 10 particles in the Nanomeasurer software confirmed that 89% of the particles have an average size of 50 nm.

FTIR analysis

We performed FTIR analysis of the OP-AgNPs, identified and analyzed the peaks for the various functional groups in AgNPs, and compared with the

OP extract. We then contrasted the resulting FTIR peaks with the common functional group value chart. Figure 4 shows the peaks at 1 041, 1 399, 1 597, 1 728, 2 853, 2 926, and 3 289 cm^{-1} , which correspond to different functional groups that were synthesized with the biological material. The peak at 2 926 cm^{-1} corresponds to CH functional groups, the one that at 1 728 cm^{-1} indicates aldehydes, that at 1 399 cm^{-1} indicates H-C-H hexagonal vibration, that at 1 041 cm^{-1} indicates C-O-C (ether) stretching, and all other peaks represent various functional group vibrational bands. The peak at 3 289 cm^{-1} indicates that phenolic compounds will exhibit O-H stretching.

X-ray diffraction

We investigated the form of the AgNPs using an X-ray diffractometer with fixed $\lambda \sim 1.54 \text{ \AA}$ radiation wavelengths. We recorded diffracted intensities within 20–70 nm. Figure 5 shows four Bragg reflections at 38.2°, 44.32°, 64.4°, and 77.1°, which correspond to planes (101), (200), (220), and (311), respectively. In accordance with Ag's face-to-face centered cubic crystal structure, the relevant index

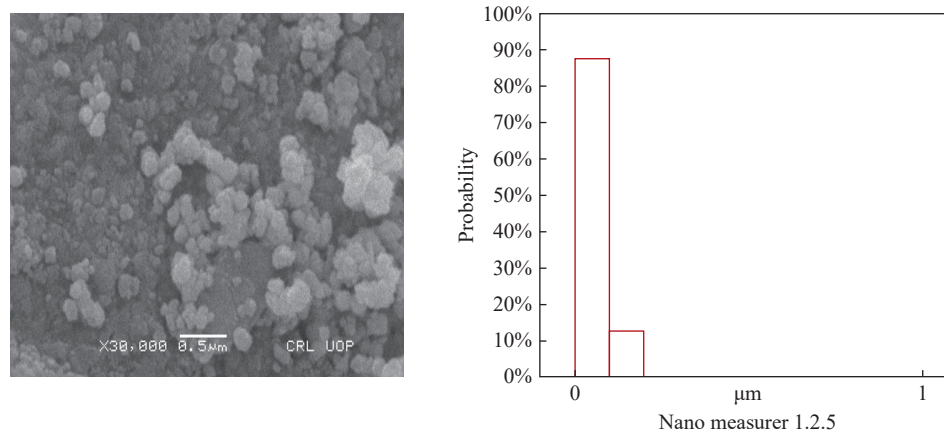


Fig. 3 Scanning electron microscopy image of AgNPs at 30 000× and calculated size of OP-AgNPs.

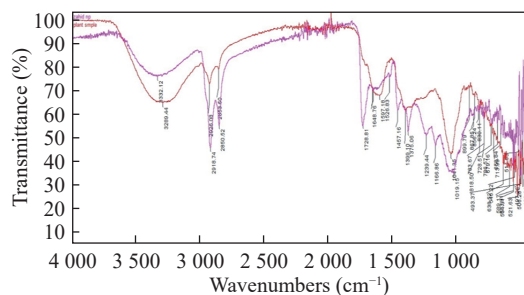


Fig. 4 FTIR spectra of OP plant extract (red) and OP-AgNPs (pink).

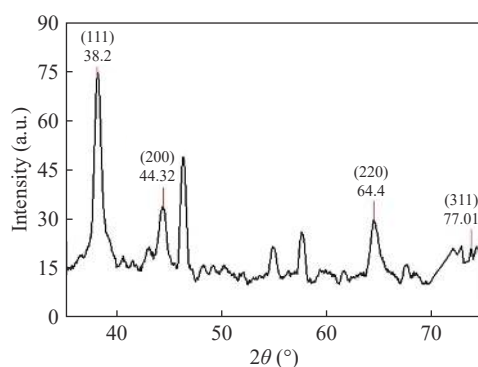


Fig. 5 X-ray diffraction of synthesized OP-AgNPs.

can be given. The average size of synthesized AgNPs is 46.31 nm.

EDX measurements

The EDX measurements indicated that the OP extract contained various inorganic ions in addition to carbon and oxygen, both of which were initially derived from the plant's organic components. In addition to magnesium, the OP extract also contained calcium, chlorine, silica, and perhaps a significant amount of chlorophyll, indicated by the presence of magnesium

in the extract. The elemental silver signal confirmed the presence of AgNPs. On EDX spectra (Fig. 6), the horizontal axis shows the energy in keV, and the vertical axis shows the X-ray counts, indicating the purity and complete chemical make-up of AgNPs. The other elements served as organic capping agents that were affixed to the surface of AgNPs.

DPPH scavenging investigation

We assessed the ability of OP-AgNPs, OP extract, and ascorbic acid at various concentrations (50, 75, and 100 µg/mL) to reduce DPPH using a colorimetric assay. AgNPs and the OP extract showed minimal DPPH reduction compared with ascorbic acid. The maximum scavenging capacity of the AgNPs, OP extract, and ascorbic acid were $46\% \pm 0.04\%$, $39\% \pm 0.31\%$, and $88\% \pm 0.21\%$, respectively, at 100 µg/mL. As shown in Fig. 7, DPPH reduction was dose-dependent.

In vitro H₂O₂ scavenging effect of AgNPs

We tested the H₂O₂ scavenging ability of OP-AgNPs and OP extract (50, 75, and 100 µg/mL). Figure 8 shows that the AgNPs, OP extract, and control exerted their maximum scavenging effect ($50\% \pm 0.032\%$, $42\% \pm 0.21\%$, and $91\% \pm 0.3\%$, respectively) at the highest concentration.

In vitro ABTS scavenging effect of AgNPs

We compared the ABTS scavenging ability of OP-AgNPs, OP extract, and ascorbic acid (standard) at 50, 75, and 100 µg/mL. Figure 9 shows that the AgNPs, OP extract, and ascorbic acid exerted their maximum scavenging effect ($80\% \pm 0.042\%$, $61\% \pm 0.03\%$, and $91\% \pm 0.05\%$, respectively, at 100 µg/mL).

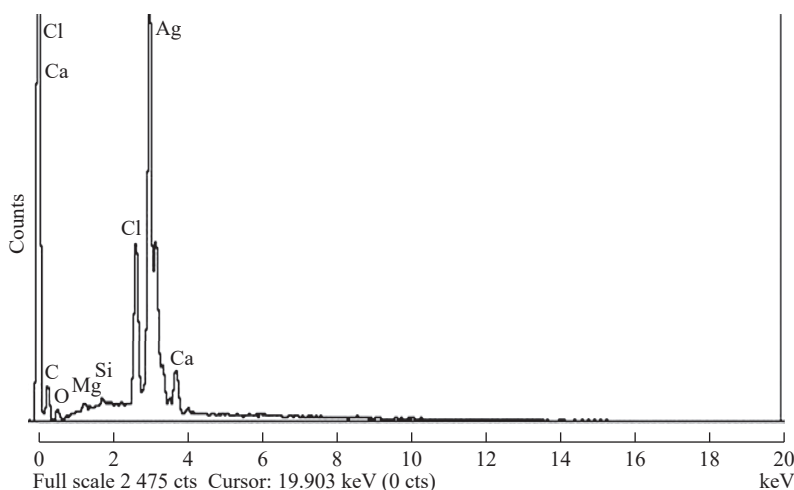


Fig. 6 EDX spectrum of synthesized OP-AgNPs showing silver element as a chief element (highest peak) along with some impurities.

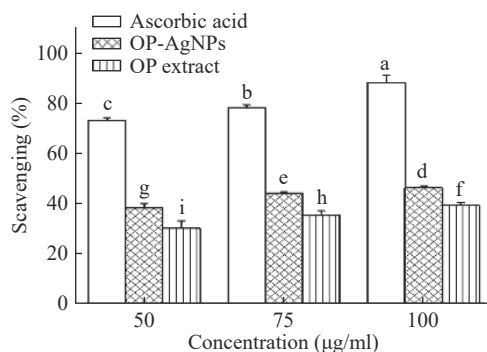


Fig. 7 DPPH reduced by OP-AgNPs, OP extract, and ascorbic acid (standard) in a dose-dependent manner (Alpha 0.05 standard error for comparison 0.081 6, critical T values 2.101, critical value for comparison 0.171 5, error term used: error, 18 DF. All 9 means were significantly different from one another).

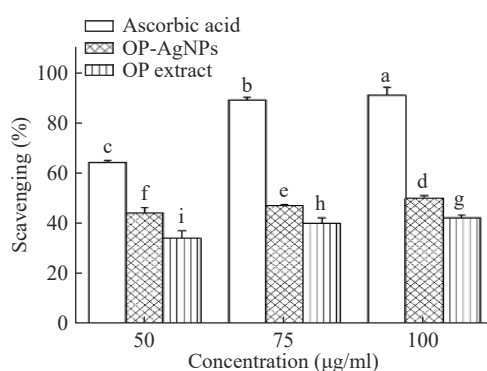


Fig. 8 The H₂O₂ scavenging effect of OP-AgNPs, OP extract, and ascorbic acid (standard) is dose-dependent. (Alpha 0.05 standard error for comparison 0.081 6, critical T values 2.101. Critical value for comparison 0.171 5, error term used: error, 18 DF. All 9 means are significantly different from one another).

Antifungal activities

In this assay, we tested OP extract and OP-AgNPs (50 and 100 µg/mL) against selected fungi strains and compared their effect with standard controls. AgNPs showed a (31.7 ± 0.4) mm *A. niger* growth suppression at 50 µg/mL and a (39.0 ± 0.3) mm *A. niger* growth inhibition at 100 µg/mL. At 50 and 100 µg/mL, they inhibited *A. flavus* growth by (32.7 ± 0.4) and (39.4 ± 0.6) mm, respectively (Table 1). This finding implies that significant results may be reached

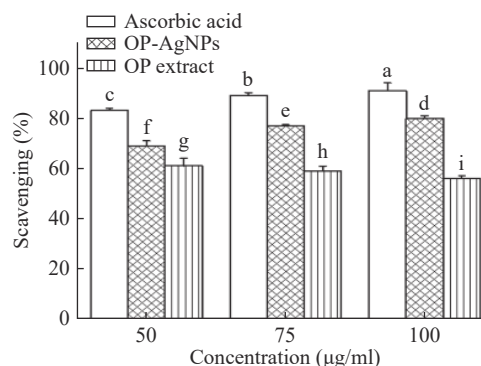


Fig. 9 The ABTS scavenging effect of OP-AgNPs, OP extract, and ascorbic acid (standard) is dose-dependent (Alpha 0.05 standard error for comparison 0.078 6. Critical T values 2.101, critical value for comparison 0.165 1. Error term used: error, 18 DF. All 9 means are significantly different from one another).

by raising the concentration of AgNPs. The negative control for this experiment was dimethyl sulfoxide (DMSO, Sigma Aldrich, purity 99.9%), which does not inhibit growth at all. Similarly, terbinafine (dilution method) was used as positive control; as shown in Table 1, it results in a 100 mm growth inhibition. All samples were analyzed in 100 mm slanted test tubes.

Antibacterial activity

We assessed the antibacterial activity of AgNPs and OP extract at 500 and 1 000 µg/mL and a standard control against the bacterial strains *E. coli* (Gram-negative), *Acetobacter* (Gram-negative), and *S. epidermidis* (Gram-positive) using the agar well diffusion method. As shown in Table 2, at 500 µg/mL AgNPs significantly outperformed OP extract in inhibiting the growth of *E. coli*, *S. epidermidis*, and *Acetobacter* (6.0, 6.13, and 6.5 mm inhibition, respectively). At 1 000 µg/mL, the effect of OP-AgNPs was even more potent, and, once again, more than that of the OP extract at the same concentration. This finding demonstrated that OP-AgNPs were more active than plant extract in all situations, and that AgNPs exerted a dose-dependent effect. DMSO served as the negative control (Sigma Aldrich, anhydrous, 99.9%), as it does not inhibit growth,

Table 1 Antifungal activity of OP-AgNPs in comparison to plant extract

Sample No.	Growth inhibition (mm)						
	Strains	OP-AgNPs		OP extract		Terbinafine	DMSO
1	<i>A. niger</i>	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL	100 ^a	0 ^f
		31.7 ± 0.4 ^c	39.0 ± 0.3 ^b	24.4 ± 0.2 ^c	28.7 ± 0.3 ^d		
2	<i>A. flavus</i>	50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL	100 ^a	0 ^f
		32.7 ± 0.4 ^d	39.4 ± 0.6 ^b	27.0 ± 0.3 ^c	31 ± 0.31 ^d		

Note: The least significant difference was 2.994 5 for *A. niger* and 3.911 1 for *A. flavus*. The superscript symbols indicate significant differences.

Table 2 Antibacterial efficiency of OP-AgNPs and OP extract

Sample No.	Strains	Zone of inhibition (mm)					
		OP-AgNPs		Plant extract		Levofloxacin DMSO	
		500 µg/mL	1 000 µg/mL	500 µg/mL	1 000 µg/mL	100 µg/mL	
1	<i>E. coli</i>	6.0 ± 0.6 ^c	8.6 ± 0.4 ^b	6.0 ± 0.6 ^c	4.0 ± 0.4 ^d	18 ± 0.6 ^a	0 ^e
2	<i>S. epidermidis</i>	6.13 ± 0.4 ^c	9.16 ± 0.5 ^b	3.0 ± 0.3 ^c	4.8 ± 0.6 ^d	21 ± 0.3 ^a	0 ^f
3	<i>Acetobacter</i>	6.5 ± 0.3 ^c	10.16 ± 0.6 ^b	4.3 ± 0.5 ^d	6.0 ± 0.3 ^c	21 ± 0.4 ^a	0 ^e

Note: The least significant difference was 0.859 4 for *E. coli*, 1.0525 for *S. epidermidis*, and 1.120 5 for *Acetobacter*. The superscript symbols indicate significant differences.

while levofloxacin was used as the positive control.

Discussion

In this work, we synthesized silver nanoparticles from a novel plant material, *Oxalis pes-caprae* L. After mixing silver nitrate with the OP extract, the synthesis of the particles can be visually confirmed by observing the color change of the solution, which turns from pale yellow to dark brown. Besides, an intense peak can be observed by UV spectroscopy at 460 nm. When we mixed the OP extract with silver nitrate salt, the color changed over time and reached its maximum darkness after about 24 h, indicating the reduction of silver ion to AgNPs, as confirmed by UV spectroscopy. A low-concentration plant extract would have low concentrations of likephenols, saponins, and flavonoids [43], so we used a highly concentrated plant extract. pH is the most important factor affecting AgNPs production and stability, and alkaline conditions improve AgNPs formation; therefore, we added NaOH to keep the pH value high [19]. After centrifugating and drying the AgNPs, we examined the powder by scanning electron microscopy, revealing an uniform distribution of spherical and mono-dispersed particles. We determined the the crystalline structure and chemical make-up of the particles using X-ray diffraction. We spread a small amount of sample across a plate, coated it lightly with aluminum, and let it dry. Finally, a thick coat of sample is formed [44] for further investigation. We calculated the diameter of the AgNPs produced from peaks obtained in the graph using the Debye–Scherrer method. To determine elemental constituents and impurities, we used EDX. We first centrifuged the silver nitrate salt centrifuged for about 10–15 min at 10 000 r/min for FTIR study. We also combined KBr with the plant powder and AgNPs for the FTIR analysis. Based on

Refs. [24, 45], we examined the spectra produced between 400 and 4 000 cm⁻¹ with a diffuse reflectance mode operating at a resolution of 4 cm⁻¹. We calculated the DPPH scavenging abilities of the OP extract and the OP-AgNPs at different concentrations. We found that OP-AgNPs had a good reducing ability compared with OP extract and ascorbic acid (standard). OP-AgNPs were more efficient than OP extract at high concentrations [46]. H₂O₂ is an inorganic compound. Here, we examined the ability of OP-AgNPs to scavenge H₂O₂. The OP-AgNPs had reasonable, dose-dependent antioxidant radical activity [47]. Similarly, the chemical compound ABTS is frequently utilized in antioxidant assays. We found that OP-AgNPs were more effective at scavenging ABTS than the OP extract. Next, we tested the antifungal efficacy of the OP-AgNPs and OP extract at two concentrations on two strains of fungi. AgNPs displayed a better growth inhibition than the OP extract in test tubes placed in slanted condition for about 2 weeks. Similarly, we tested the antibacterial efficacy on three bacterial strains and found that AgNPs inhibited their growth more potently than the OP extract did.

Conclusion

Although there are many ways to synthesize silver nanoparticles, green synthesis is the most attractive in terms of budget, low environment toxicity, and human health. Among green routes, the most sophisticated one is using plants for synthesizing AgNPs, which can enhance the development of nanotechnology. This paper documents the optimization of the green synthesis of AgNPs using OP and the characterization of these nanoparticles. The synthesized AgNPs were characterized by UV–Vis spectroscopy, scanning electron microscopy, FTIR, X-ray diffraction, and EDX. These bio-derived AgNPs present good antioxidant, antimicrobial, and

antifungal properties relative to the crude OP extract. These results demonstrate that the plant is rich in active molecules with potential for medicinal purposes. Thus, the synthesis of AgNPs-encapsulated drugs combined with OP extract can enhance this potential, opening up unprecedented opportunities.

CRediT Author Statement

Zahid Khan: writing, review, and editing; **Rahmat Ali Khan:** supervision. **Mushtaq Ahmed:** data curation. **Naila Sher:** methodology. **Nadia Mushtaq:** editing.

Conflict of Interests

The authors do not have any conflict of interest regarding this article and its publication.

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