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BIOSYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES OBTAINED FROM *PORTULACA OLERACEA* CALLUS AND ITS ANTIBACTERIAL ACTIVITY

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SUMMARY

Green nanotechnology is an important and environmentally friendly technology that is applicable in various areas, such as health and food. The following study demonstrates the possibility of synthesizing silver nanoparticles obtained from *Portulaca oleracea* callus to determine their effectiveness against pathogenic bacteria. Silver nanoparticles' synthesis from *P. oleracea* callus samples used four different pH values (3, 7, 10, and 12). Samples at a pH value of 12 with the smallest particle showed the best results after conducting SEM and UV-Vis analyses. The results revealed the reaction samples with higher pH values gave effects that are more positive. Effectiveness of the nanoparticles against the bacterial activity of *Escherichia coli* (*E. coli*) was successful, and the results were favorable, with an inhibition diameter of 9 mm. The nanoparticles, in combination with plant extracts of nodes and stem callus, had the mixed samples exhibiting the best activity and a more powerful effect than the silver nanoparticles alone. The sample of node extract and the nanoparticles had the most powerful effect on the bacteria, with an inhibition diameter of 22 mm. However, the sample with the stem extract and nanoparticles had an inhibition diameter of 11 mm.

Keywords: *P. oleracea*, antibacterial activity, SEM and UV-Vis, silver nanoparticles

Key findings: Silver nanoparticles produced from *P. oleracea* callus showed that the reaction samples with higher pH values emerged with results that are more positive. Integrating nanoparticles with plant extracts revealed effectiveness against *E. coli* bacteria.

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INTRODUCTION

Nanotechnology is a most important and rapidly developing new field, as this technology can be beneficial in various fields of study (Anjum and Abbasi, 2016; Aktepe, 2021). It is a new, innovative field with modern technology that can further progress and has a greater potential in numerous engineering, medical, food, and pharmaceutical industries (Sari *et al.,* 2023). The classification of nanocomposites depended on their size, and the particles with a size less than 100 nm have become nanoparticles (Bouqellah *et al*., 2019). The use of nanosilver particles is widespread and better than gold and copper nanoparticles due to its antibacterial properties (Dakal *et al*., 2016). For the synthesis of silver nanoparticles, the different methods used are solvent reduction, chemical and photochemical reactions in reverse cycles, and thermal decomposition of silver components. However, by using toxic and hazardous solvents with high pressure and temperature, these methods are harmful to the natural environment (Nagajyoti *et al*., 2011).

The eco-friendly nanoparticles' synthesis processes are also beneficial in reducing waste in the environment, making these methods called green nanoparticle synthesis methods (Castro-Langoria *et al*., 2011). The green synthesis process is environmentally friendly, gentle, and economical for costs and production methods, with easy implementation using environmentally friendly materials, such as plant callus extracts (Jebril *et al*., 2021). Different parts of *Portulaca oleracea* contain numerous active compounds, i.e., phenolic, oxalic, malic, and ascorbic acids; soluble carbohydrates; tannins; omega-3; flavonoids; and alkaloids. Moreover, it is rich in proteins, amino acids, and glycosides, with these compounds commonly known as the best reducing agents for silver ions (Sanja *et al*., 2009). The *Portulaca oleracea* plant is one of the succulent plants belonging to the family of *Portulacaceae*, a seasonal plant growing up to 40 cm, highly sown in large quantities in China and in warm climates (Huxley, 1992).

This study sought to produce silver nanoparticles using the green synthesis from *P. oleracea* callus with different pH values. Moreover, it aimed to diagnose these particles using the UV-vis spectrophotometer and the scanning electron microscopy (SEM) to demonstrate its effectiveness in bioremediation against pathogenic bacteria. Additionally, the research hopes to discover the effectiveness of these nanoparticles on *E. coli*, as well as the possibility of producing a sample of mixtures of plant extracts and nanoparticles and knowing their therapeutic effectiveness against these bacteria.

MATERIALS AND METHODS

The biosynthesis of nanosilver commenced in the laboratory at the College of Environmental Sciences, University of Mosul, Mosul, Iraq. Figure 1 shows the methodology of the said study.

Callus induction and callus extract

Germinating *Portulaca oleracea* seedlings from seeds preceded the induction of callus culture from the different parts of *P. oleracea* seedlings. The callus at 30 days old became samples obtained from plant parts of the *P. oleracea*, grown in the laboratory by tissue culture technique following the methodology of Mude *et al*. (2009). First, washing the callus with deionized water took place to obtain the aqueous extract comprising the 20-g callus ground with a ceramic mortar with 100 ml of deionized water. The resulting extract underwent filtration through a filter paper before use in the synthesis of silver nanoparticles.

Production of AgNPs

From the extract, take 10 ml and continue to mix in 90 ml of 1 M silver nitrate solution ($AgNO_3$) prepared in advance. Before incubation, changing the pH values of three of the four samples to 3, 7, 10, and 12 pH.

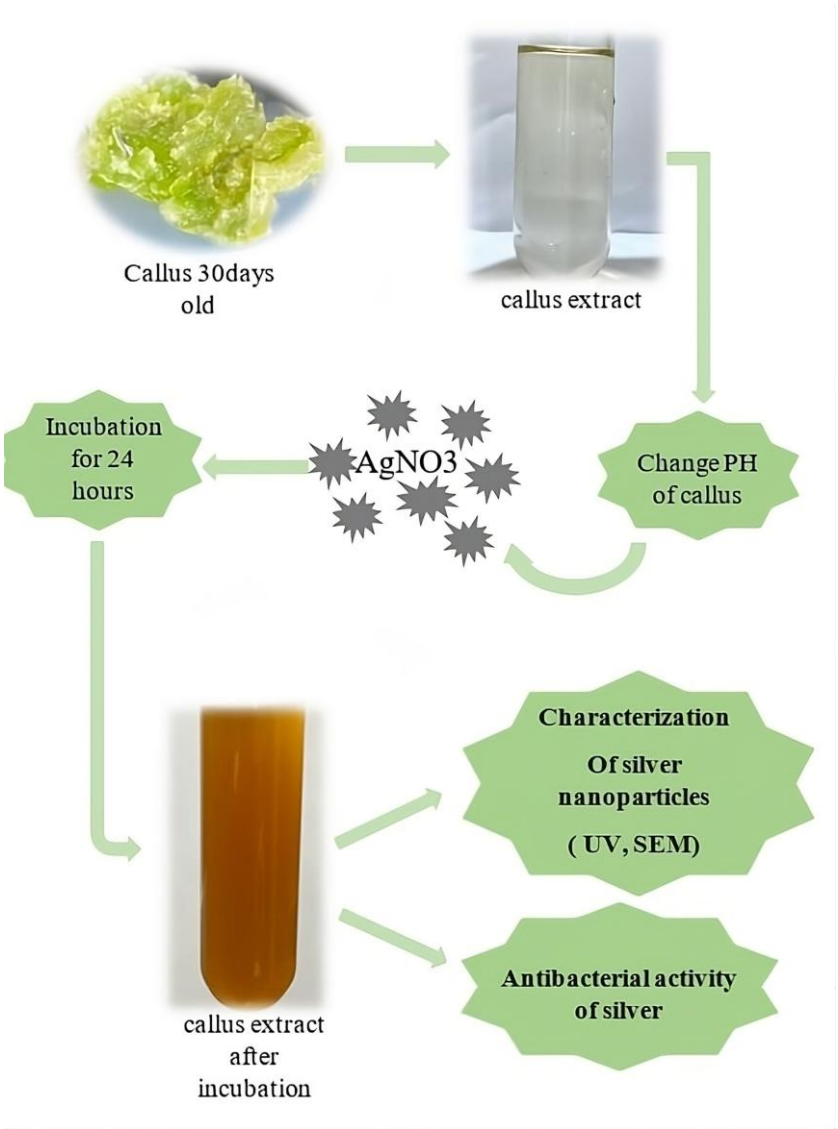

Figure 1. General diagram showing the sequence of practical steps of the research.

ensued, and the pH sample's incubation at room temperature took 24 h. After incubation, observing the samples with changed color, note if the reaction samples of the aqueous extract reacted with the silver nitrate solution. Then, the extracts continued to be centrifuged 4–5 times to isolate the silver nanoparticles using a force of 10,000× g for 10 min. For every centrifugation of the samples, replacing the upper liquid with distilled water transpired (Mude *et al.,* 2009).

Characterization of silver nanoparticles

Visible color change

In the process of detecting the presence of nano silver, the first and easiest step was the color change from yellow to brown that could be visible to the naked eye. The composition of the nanosilver reached estimation after six hours of incubation.

UV-Vis Spectrophotometer

UV-Vis technology is one of the easiest and most reliable methods for the initial examination of silver nanoparticles. Silver nanoparticles can also interact with a specific wavelength of light. This technology can also verify the structure and stability of nanoparticles (Tomaszewska *et al*., 2013). The UV-Vis spectrophotometer wavelength remained between 350 and 800 nm (Rajasekharreddy *et al*., 2010).

Scanning electron microscopy (SEM)

Scanning electron microscopy is a particle morphology technique that determines the particle size, its distribution, and surface morphology ***(***Pal *et al*., 2007; Dada *et al*., 2017a). This analysis ran at the University of PSL, Paris, France, utilizing a scanning electron microscope (SEM) (model a JEOL JSM-7001F ultra-high resolution FESEM).

Preparation of alcoholic extracts

The study used 1 g of callus of stem and nodes at the age of 50 days. The ground samples had their volume completed to 10 ml of ethyl acetate solution and attained incubation at room temperature for three days.

Efficacy of the extract against pathogenic bacteria

The efficacy of *P. oleracea* callus extracts continued for testing with four different treatments (nanoparticles, node extract, stem extract, stem extract with nanoparticles, and node extract with nanoparticles) against *Escherichia coli* using the disk diffusion method. Sterile swabs served to impose the bacterial cultures on Muller Hinton medium and spread them over the entire plate. The earlier soaked callus extracts in paper discs (sterile filter paper cut into small discs) went on to be distributed on the plate at the rate of five discs per plate, with the plates incubated at 37 °C for 24 h. Later, calculating the diameters of inhibition determined the effectiveness of the *P. oleracea* callus extracts against *E. coli* bacteria.

RESULTS AND DISCUSSION

Visible color change

Preliminary results showed reaction samples changed color after the incubation period, and the color of the sample with pH 12 was dark yellowish-brown; the sample with pH 3 became dark gray; the sample with pH 7 was light yellowish-brown; and the sample with pH 10 was dark brown. The color change of the reaction samples with the change of pH values appears in Figure 2. Changes in the samples' color from clear to shades of brown provide an initial indication of the formulation of silver nanoparticles. Notably also, the increased pH value caused a darkening in the color of the solution to dark brown and raised the speed of its formation. The increasing pH greatly affects the speed of the reaction, and these results were consistent with past findings that reported similar observations (Labulo *et al*., 2016; Jalab *et al*., 2021).

The appearance of color in the reaction samples to form silver nanoparticles was due to the stimulation of surface plasmon vibrations in the silver nanoparticles (Karahan *et al*., 2023). Botcha and Prattipati (2019) also reported a color change of the aqueous extract of *Hyptis suaveolens* callus from pale yellow to brown and dark brown. During the formation of silver nanoparticles from Ag+ to Ag°, the color change was because of the reducing agent found in the extract. Additionally, the phenolic compounds in the extract may play an important role in the reduction of Ag+ (Urnukhsaikhan *et al*., 2021). The differences in the resulting color depend on the compound present in the callus extract. These results were greatly analogous to past findings where the *Aloe vera* callus extract color turned brown (Chandran *et al*., 2006).

UV-Vis spectrophotometer analysis

The results demonstrated the possibility of producing silver nanoparticles from the aqueous extract of *P. oleracea* callus. Figure 3 showed the UV-visible spectrophotometer of silver nanoparticles synthesized from samples of different pH values. The presence of silver nanoparticles gained validation by the appearance of peaks ranging from 300 to 800 nm. In this study, the absorption spectrum of the wavelength of the reaction samples, prepared at pH 3, 7, 10, and 12; the silver nitrate sample; and a sample of aqueous extract of callus tissue had the following results:

The silver nitrate sample and the aqueous extract of the callus sample showed no absorption peak, which authenticated the non-formation of silver nanoparticles. As for the reaction samples with different pH, the absorption peaks appeared clearly at 300–450 nm, indicating the formation of silver nanoparticles by reducing negative silver ions (Ag-) to metallic silver particles. The pH value played a vital role in the difference of absorption peaks, as it reached 390 nm for the reaction sample of pH 12 and 380 at pH 10. The wavelength of the reaction sample at pH 7 reached 440 nm, and the pH 3 sample reached 420 nm. A slight increase in the absorption intensity was evident with an elevation in pH. These results agree with past findings of Anju *et al*. (2021), who also reported observing a peak in the UV-Vis absorption spectra at around 300–400 nm.

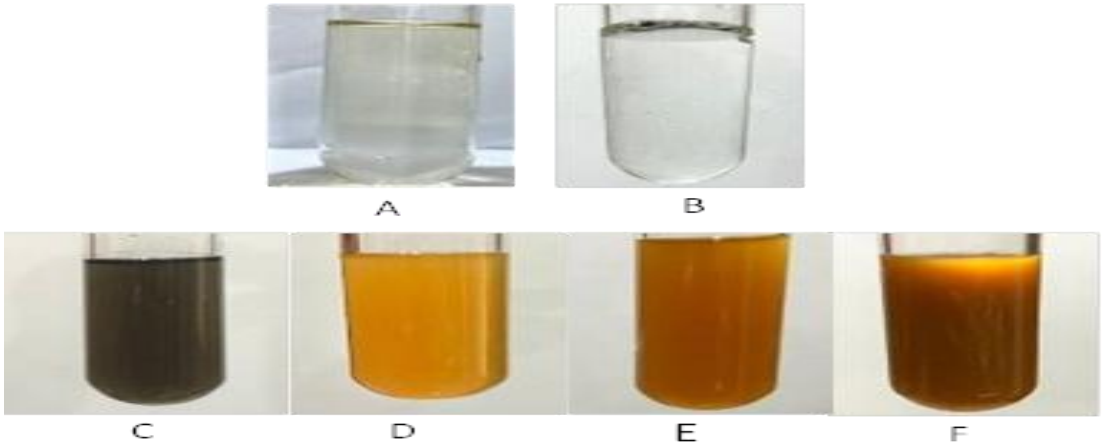

Figure 2. Reaction solutions of different PH values. A: callus extract, B: AgNO3, C: PH = 3, D: PH = 7, E: PH = 10, and F: PH = 12.

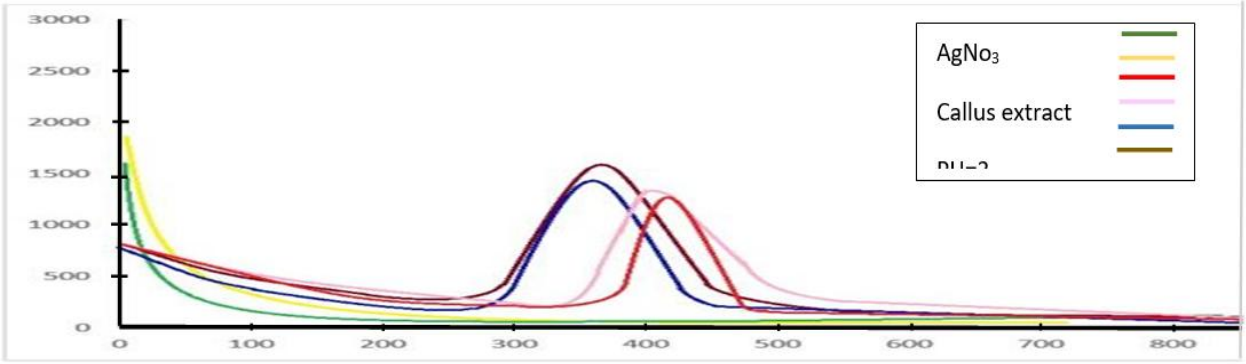

Figure 3. UV–Vis spectra of AgNPs synthesized with different PH value of AgNO3.

SEM analysis of produced silver nanoparticles

The outcomes revealed the nanoparticles were circular and oval-shaped, and the scanning electron microscope (SEM) images showed the distribution of silver nanoparticles in the form of spherical particles resulting from *P. oleracea* callus (27.5-21 nm) (Figure 4). This study proceeded on four samples with different acidic functions to produce silver nanoparticles, where the best results recorded were at pH 12, with the particle size at 0–34 nm and an average size of 21 nm. Following it are the pH 3 sample with a particle size of 15–50 nm and an average size of 27.5 nm and the pH 7 sample with the particle size of 10–40 nm, and its average size was 25 nm. Meanwhile, the pH 10 sample had a particle size of 10–40 nm and an average size of 22.3 nm (Table 1). The results further revealed the average dimensions of the nanoparticles differed according to the pH of the samples.

The related results considerably align with past findings of Lashin *et al*. (2021), who produced silver nanoparticles with an average size of 31.1 nm from the callus extract of *Solanum incanum*. Grace *et al*. (2019) stated in their study the possibility of forming biosynthetic silver nanoparticles from the *Gleichenia pectinata* plant extract with an average size of 7.51–2.88 nm.

Antibacterial analysis of AgNPs

The results in Figure 5 detailed that the nanoparticle sample No. 5 had an inhibitory effect on *E. coli* bacteria, while the plant extracts alone (stem extract 1 and node extract 2) did not show any inhibitory activity against the bacteria. The mixed sample between the nanoparticles and plant extract (sample No. 4) and the mixture sample between nanoparticles and node extract exhibited limiting activity with an inhibition area of 22 mm. The mixture sample between nanoparticles and the stem extract (sample No. 3) showed less inhibitory activity as compared with sample No. 4. Several studies have proven the activity of silver nanoparticles against *E. coli* bacteria was low. According to Yulia *et al*. (2023), the *E. coli* bacteria

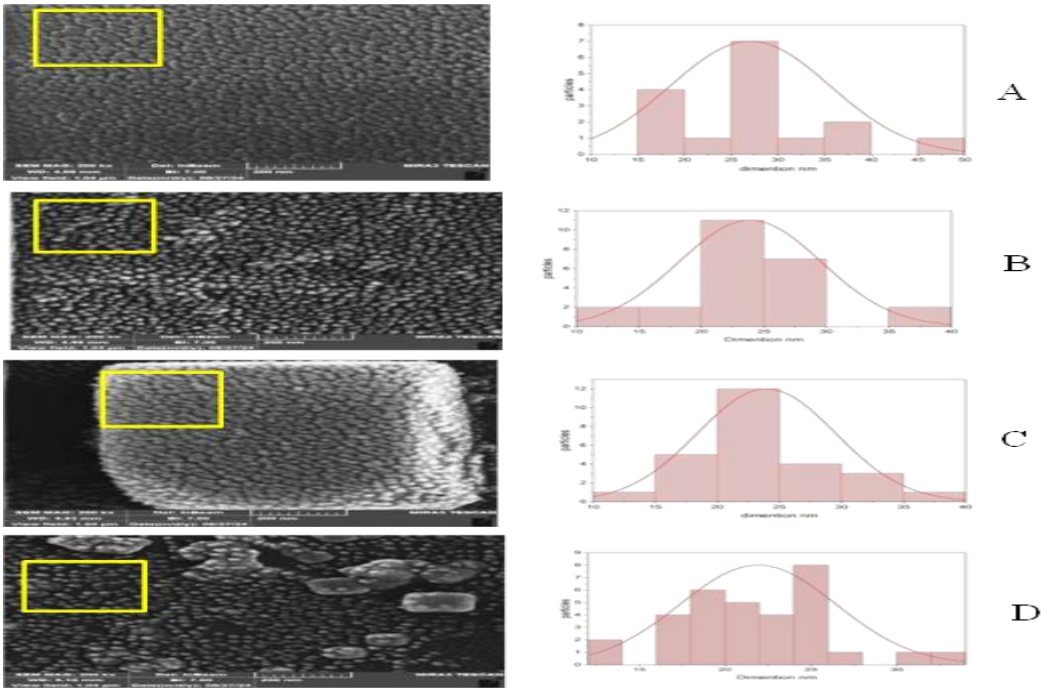

Figure 4. SEM analysis images and histogram of the spectrum of nanoparticles' dimensions for reaction samples with different pH values. A: PH = 3, B: PH = 7, C: PH = 10, and D: PH = 12.

Table 1. Size and numbers of nanoparticles based on the nanoparticle dimension distribution spectrum diagram.

PH values	Particles' Size	Number of Particles	Average
3	15-20	4	27.5
	20-25	1	
	25-30	7	
	30-35	1	
	35-40	2	
	40-45	-	
	45-50	1	
	(15-50)	16	
7	10-15	2	25
	15-20	2	
	20-25	11	
	25-30	7	
	30-35	-	
	35-40	2	
	(10-40)	24	
10	10-15	1	22.3
	15-20	4.5	
	20-25	12	
	25-30	4	
	30-35	3	
	35-40	1	
	(10-40)	25.5	
12	0-10	2	21
	16-18	4	
	18-20	6	
	20-22	5	
	22-24	4	
	24-26	8	
	26-28	1	
	28-30	-	
	30-32	1	
	32-34	1	
	(0-34)	32	

displayed little effect on nanoparticles produced from the callus of *Aristolochia manshuriensis* compared with other bacteria (Shkryl *et al*., 2018; Yugay *et al*., 2020, 2023). Studies have shown the difference in sizes and shapes of silver nanoparticles affected the bactericidal efficacy (Menichetti *et al*., 2023), and the antimicrobial potency of AgNPs depends on the size and its dose (Prasad and Elumalai, 2011). Therefore, it is necessary to clarify that in the context of the disc diffusion method, the method of distributing silver nanoparticles essentially supports the toxicity, efficacy, and effects of particles regardless of their dimensions and morphology (Kourmouli *et al*., 2018). This may explain the negligible effects of silver nanoparticles on *E. coli* bacteria in this study.

CONCLUSIONS

The synthesis of silver nanoparticles from *P. oleracea* callus tends to produce silver nanoparticles in a green and environmentally safe way. This study succeeded on four samples with different acidic functions to produce silver nanoparticles, and the best results emerged at the pH of 12, as per the readings and analyses of UV-Vis, SEM, and

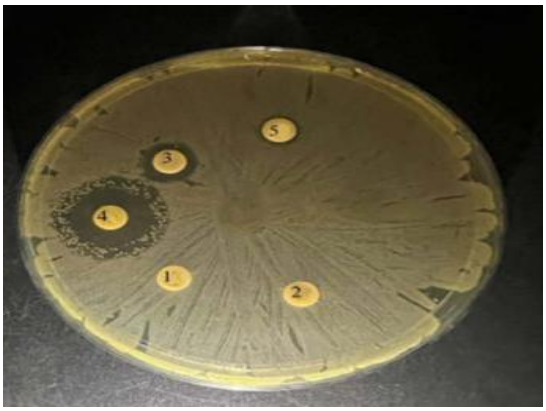

Figure 5. Antibacterial effect of AgNPs and AgNPs mixed with stem extract, nodes extract of the *Portulaca oleracea* callus. 1: stem extract, 2: nodes extract, 3: nanoparticles with stem extract, 4: nanoparticles with nodes extract, and 5: silver nanoparticles.

color change. The study also showed the efficiency of treating *E. coli* and the effectiveness of combining plant extracts with nanoparticles to treat these bacteria. The mixture of plant extract with nanoparticles was more effective than nanoparticles alone, with an inhibition diameter of 22 mm.

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