



A comparative study of chemo- and phytosynthesized silver nanoparticles using *Ceratophyllum demersum* extract: characterization and biological activities

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Abstract

The fabrication of nanoparticles (NPs) via eco-friendly methods has gained attention due to its sustainability, accessibility, and affordability. The current work aimed to establish a comparative study between the chemical and phytosynthesis of silver nanoparticles using the extract of *Ceratophyllum demersum* (Coontail), a submerged aquatic plant species from Egypt, using five volume ratios of the aqueous plant extract (1 mL, 2.5 mL, 5 mL, 10 mL, and 20 mL). The morphological and compositional properties of both phyto- and chemosynthesized NPs were performed. Electron microscopy imaging showed that the AgNPs' sizes decreased, and their shapes were more distinct and well-defined as the volume of plant extract increased. The FTIR elucidated the strategies of NP phytopreparation and stabilization of NPs through conjugation to flavonoid compounds in the extract. The zeta potential validates that the FTIR analysis shows that the plant extract can bio-stabilize the produced AgNPs via OH, C–O, and C=N bonds. The XRD data showed that the phyto- and chemo-AgNPs had face-centered cubic crystalline NPs. The antimicrobial potential of the synthesized NPs against seven human pathogenic bacteria demonstrated that the phyto-NPs had a superior bacterial inhibitory capacity than the chemosynthesized ones, especially against the Gram-negative bacteria more than Gram-positive ones. The antioxidant capacity of all synthesized AgNPs was synergistic, but the highest was that of AgNPs made using 20 mL of extract. In conclusion, *Ceratophyllum demersum* proved its efficiency as a bioactive compound to fabricate AgNPs employing five various ratios of the plant extract for developing novel therapeutics to combat biological problems with minimal side influences.

Keywords Silver nanoparticles · *Ceratophyllum demersum* extract · Electron microscopy · FTIR · The zeta potential · XRD · Antibacterial potential · Antioxidant capacity

1 Introduction

In recent years, the rise of infections caused by various severe pathogenic microorganisms, including viruses, fungi, and bacteria, has presented a complex challenge for researchers worldwide. This issue is largely attributed to the growing resistance of these microorganisms to traditional antibiotic therapies. Nanotechnology has played an essential role through the last twenty years in overcoming viral infections such as COVID-19 [1] and numerous bacterial infections [2, 3] and fatal diseases by providing new candidates of nanomaterials that have excellent anti-fungal, anti-viral, and antibacterial activities. Popular examples of these are the metal nanoparticles, including silver (Ag), gold (Au),

platinum (Pt), and copper (Cu), have a substantial importance in biomedical applications, such as drug delivery, therapeutics, and immunoassays [4, 5].

Silver nanoparticles (AgNPs) are among the most widely used nanomaterials because of their optical characteristics, chemical makeup, and biological activity. AgNPs are used in many different industries, including textiles, pharmaceuticals, medical devices, and cosmetics. It has various activities, including wound healing [6], antiviral, antibacterial [7–9], and anticancer [10] activities. AgNPs are also widely utilized to remove microorganisms from water [11, 12]. The fabrication of inexpensive nanomaterials synthesized through physical or chemical techniques is gaining popularity [13].

These processes are complicated, costly, and time-consuming and require high power, temperature, and pressure. Chemical processes' by-products are harmful to both the

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environment and humans. Consequently, the use of chemically and physically synthesized nanoparticles (NPs) in medical applications is no longer permitted [14–16]. On the other hand, green route synthesis can be achieved by using microbes (yeast, bacteria, fungi, etc.) or plant extracts. Contamination in the microbial culture poses a risk, and microbe-mediated synthesis is costly [17–19].

NPs phytosynthesis has become more commonly used because it is eco-friendliness [20, 21] and the plant precursor functions as a stabilizing and reducing agent in a single stage of the reaction. Green NPs production methods that utilize plant materials such seeds, flowers, leaves, bark, and roots have been the subject of several articles. For example, plant extracts from rose [22], ginger [23], neem [24], papaya [25], grass [26], and sachai inchi [27] were investigated. Functional groups such as hydroxyl (-OH), carboxylic (-COOH), aldehyde (-CHO), ketone (-CO), and amine (-NH₂) serve as capping and reducing agents.

Ceratophyllum demersum, sometimes known as coon-tail, is a worldwide submerged aquatic macrophyte that may aggressively overrun various aquatic habitats. It has the ability to take over water bodies, negatively impacting both navigation routes and phytoplankton growth. However, it has a lot of bioactive components of medical value. Therefore, controlling the massive amount of biomass that such plants produce is important for the environment, and rather than incinerated, its biomass might be used for other purposes. Recently, *Ceratophyllum demersum* L. was prepared in the nanoscale and proved its ability for the removal of the copper metal (heavy metal pollutant water) [28]. Additionally, the Ag-ZnO/rGO nanocomposites was successfully biosynthesized using *Ceratophyllum demersum* L. leaf extract and the produced NPs utilized for the photocatalytic degradation of Rhodamine B [29]. Both *Ceratophyllum demersum* and *Acinetobacter radioresistens* involved in the treatment demonstrated a greater interaction between the two to eliminate dangerous substances from the environment, such as methylene blue dye [30].

Ceratophyllum demersum extract is richer in natural components such lactones, polyphenols, flavonoids, and glycosides, as reported previously [31]. A recent study showed that *Ceratophyllum demersum* extract contains very potent capping agents, including polyphenols, piceatannols, resveratrols, quadrangularins, flavonoids, and triterpenoids, which may inhibit the release of Ag⁺ from the surface of bionanoparticles, resulting in limited antibacterial activity [32].

The current work presents the effect of different volumes of *Ceratophyllum demersum* leaf extract for biosynthesizing the AgNPs and compare resulting NPs with AgNPs chemically prepared, which has not been frequently discussed before. The extract contains various metabolites, including flavonoids, alkaloids, saponins, tannins, phenols, and polyphenols, that serve as stabilizing, capping, and

reducing agents, facilitating a simple, safe, and environmentally friendly approach to the green synthesis of AgNPs. The impact of use of different extract volumes on the morphology, antioxidant capacity, and antibacterial capability of NPs was investigated. We set comparative analyses between the phytosynthesized AgNPs using the *Ceratophyllum demersum* extract and those fabricated via traditional chemical reduction techniques. The synthesized NPs were characterized using various spectroscopic and microscopic techniques. The antimicrobial effect was assessed against seven human pathogenic bacteria, as well as the antioxidant activity and efficacy for degradation of the industrial MB dye was examined.

2 Materials and methods

2.1 Chemicals and reagents

The aquatic macrophyte *Ceratophyllum demersum* (*C. demersum*) was collected from Lake Mariout, a Nile Delta Lake located in the Mediterranean coastal land of Egypt. Silver Nitrate (AgNO₃), sodium borohydride (NaBH₄ (99%)), ascorbic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich. Polyvinylpyrrolidone (PVP) was purchased from Loba Chemie, India. Deionized water was used for preparing all aqueous solutions.

2.2 Preparation of plant extract

The collected specimens of *C. demersum* were rinsed several times with deionized water to get rid of any impurities and then released to dry in the air. The washed plant specimens were cut into tiny bits and then dried in an oven at 60 °C until completely dry. Afterward, they were ground in a stainless-steel mixer to obtain a fine powder. The aqueous plant extract was prepared according to El-Borady, et al., previous works [33, 34]. Five grams of the plant powder were suspended in 50 mL of deionized water; then, the mixture was heated for 20 min at °C and stirred at 400 rpm until boiling for nearly 20 min. The boiled solution was quickly filtered using a Whatman size No. 1 filter paper. The filtrate plant extract (pH = 5) was stored at 4 °C for further investigations.

2.3 Synthesis of silver nanoparticles using plant extract

In the present research, five AgNPs samples were synthesized using various plant extract amounts (1 mL, 2.5 mL, 5 mL, 10 mL, and 20 mL). The samples are denoted to as AgNPs (A), AgNPs (B) and AgNPs (C), AgNPs (D), and AgNPs (E), respectively (Fig. 1). Briefly, 20 mL of the filtered plant extracts and AgNO₃ solution (AgNO₃

Fig. 1 *C. demersum* powder (**A**), aqueous extract (**B**), the five phytosynthesized AgNPs samples using various plant extract amounts (1 mL, 2.5 mL, 5 mL, 10 mL, and 20 mL) (**C**)



Table 1 Comparison of colour and reaction parameters of silver nanoparticles synthesized from plant extract and chemically synthesized

Type of synthesis	Extract volume (mL)	Initial colour	Final colour	Final temperature °C	Time in minutes (heating duration)	pH
<i>Ceratophyllum demersum</i> extract (phytosynthesis)	1	Light brown	Pale yellow	100	5	4.5
	2.5		Dark yellow	100	5:30	4.65
	5		Light brown	100	4	4.65
	10		Medium brown	100	6	4.7
	20		Dark brown	100	9:30	4.75
NaBH ₄ and PVP (chemosynthesis)	0	Coloureless	Brown	10	30	6

concentrations were kept at 0.001 M) were subjected to heating at 100 °C and stirring adjusted at 600 rpm (Table 1); the first color change in the solution was observed that was taken as an indication of the colloidal AgNPs formation, each solution complete stirring at room temperature to 30 min after color changes. The formed colloidal solutions were cooled and stored in the refrigerator (10 °C) for further analysis. The plant extract in the current experiment has a dual action, as both a reducing agent (that reduces Ag^{1+} ions to Ag^0) and a capping agent to prevent particles aggregation.

2.4 Synthesis of silver nanoparticles using chemical method

AgNPs were chemosynthesized, in an aqueous medium, following the reduction method provided previously [35]. In an ice bath, 10 mL of one mM AgNO_3 solution was added dropwise to an iced solution of 100 mL NaBH_4 (2 mM), including 3% of PVP, until a color change was observed from colorless to yellow. In this method, the NaBH_4 served as a reducing agent, while the PVP was used as a capping material that inhibited the formed particles' aggregation and additional growth. Table 1 represents the different conditions for the current synthesized AgNPs.

2.5 The silver nanoparticle characterization

The morphology of the synthesized NPs, including the nanoscale size, shape, and particle distribution, was conducted on transmission electron microscopy (TEM), JEOL model 1400EX microscope with an accelerating voltage of 80 kV, implemented with a Gatan Digital Camera (Model Erlangshen ES500). The NP's zeta potential (surface charge), hydrodynamic size distributions, and polydispersity index (PDI) were achieved by dynamic light scattering spectroscopy (DLS) using a Malvern Zetasizer Nano ZS90

analyzer at 25 °C. Energy-dispersive X-ray spectroscopy (EDX) identified powdered samples' elemental composition using a JEOL model JSM-IT100 scanning electron microscope (SEM). The FTIR analysis using palletized samples containing KBr was carried out to demonstrate the plant extract's probable functional groups that force participants to reduce the Ag^+ ions and enclose the formed AgNP's good stability. The FTIR measurements employed an FTIR-4100-JASCO spectrometer covering the 4000 to 400 cm^{-1} . Furthermore, to investigate the AgNPs crystalline nature, the samples were subjected to the XRD measurements of the AgNPs powder samples were performed on a powder X-ray diffractometer (PXRD-6000 Shimadzu) worked at a current of 30 mA and a voltage of 40 kV in the angle range of 10 to 80 °C. The antioxidant data were recorded on a Fluorescence Microplate Reader & Autosampler, model FP-8000 Series (JASCO).

2.6 The antioxidant efficiency assessment

The free radical scavenging efficiency of different prepared NPs and the plant extract was assessed using the DPPH assay and evaluated according to the method described by Guntur et al. [36]. In the current process, approximately 1 mL of the tested freshly prepared solutions was mixed with 1000 μL of DPPH (0.2 mM). A control DPPH solution without NPs was also examined for comparison (blank and positive control). Furthermore, vitamin C (ascorbic acid) is used as a reference. In addition, tests were also made on 1- and twofold diluted solutions for each sample. Then, the DPPH mixtures were blended for 20 min at ambient temperature in dark conditions. After passing the incubation period, the reduction in absorbance percentage of the mixture at 517 nm wavelength reflected the radical concentration. The free radical scavenging (%) of the AgNPs and vitamin C was calculated through the equation:

$$\text{Free radical scavenging (\%)} = \frac{(\text{Control absorbance} - \text{sample absorbance}) \times 100}{\text{control absorbance}} \quad (1)$$

2.7 The antibacterial activity assessment

The in vitro antibacterial potential comparison of the five bio-inspired AgNPs (A), AgNPs (B), AgNPs (C), AgNPs (D), and AgNPs (E) solutions and the chemosynthesized AgNPs was evaluated using the agar well diffusion method against seven pathogenic bacteria: three gram-positive pathogenic bacterial strains (*Bacillus thuringiensis*, *B. licheniformis* (plant pathogen), and *Enterococcus faecalis*

ATCC29212 (human pathogen)) and four gram-negative human-pathogenic bacterial strains; (*Pseudomonas aeruginosa* ATCC10145, *Enterobacter ludwigii* ATCC13047, *P. putida* ATCC49128, and *Klebsiella pneumonia* ATCC1705 [37]. Briefly, the obtained pure cultures of this organism were subcultured in Mueller–Hinton broth and incubated at 37 °C on a rotary shaker at 120 rpm for 24 h. After the sub-cultured strains were washed with 0.9% saline solution until the intensity of strain was obtained at 0.5 optical

density at 570 nm, then each strain was swabbed uniformly on the individual Mueller–Hinton agar plates using sterile cotton swabs, and a well was made on agar plates using gel puncture. Then, the 100 μ L of NPs under investigation were added into the respective well. The zone of inhibition was measured after 24 h incubation at 37 °C as evidence of the NP's antibacterial activity.

2.8 The photocatalytic activity

The photocatalytic activity for methylene blue (MB) dye degradation via both the green and chemosynthesized AgNPs was performed under ultraviolet (UV) light irradiation at 25 °C without adding any additional catalyst. Briefly, 20 mL of dye solution (2×10^{-6} M) was mixed with 100 μ L of each tested sample of AgNPs. The solution was subjected to UV light to allow degradation to occur, while the degradation efficiency was measured by using a UV–Vis spectrophotometer to measure the UV–Vis spectra of the solution after different time intervals. The removal efficiency was calculated using the following equation:

$$\text{Percentage of dye removal (\%)} = \frac{A_0 - A}{A_0} \times 100 \quad (2)$$

where (A_0) denotes starting the initial absorbance, and (A) denotes the final absorbance.

3 Results and discussion

The current work describes the successful fabrication of the AgNPs phytosynthesized using five different volumes of *C. demersum* aqueous extract. The resulting green synthesized AgNPs were characterized, and their antimicrobial and antioxidant potentialities were conducted and compared with the AgNPs prepared via the traditional chemical method.

3.1 The silver nanoparticle characterization

The *C. demersum* was used to reduce the AgNO₃ solution to Ag⁰, leading to rapid color variations in a few minutes, from light yellow to dark brown, depending on the plant extract volumes joined. The NPs' colors are related to the production of NPs exhibiting different shapes and sizes due to using the different volumes of extract, which was also confirmed previously in other studies [38].

3.1.1 HR-TEM findings

The morphological characteristics of the chemically synthesized AgNPs and those made with different volumes of *C.*

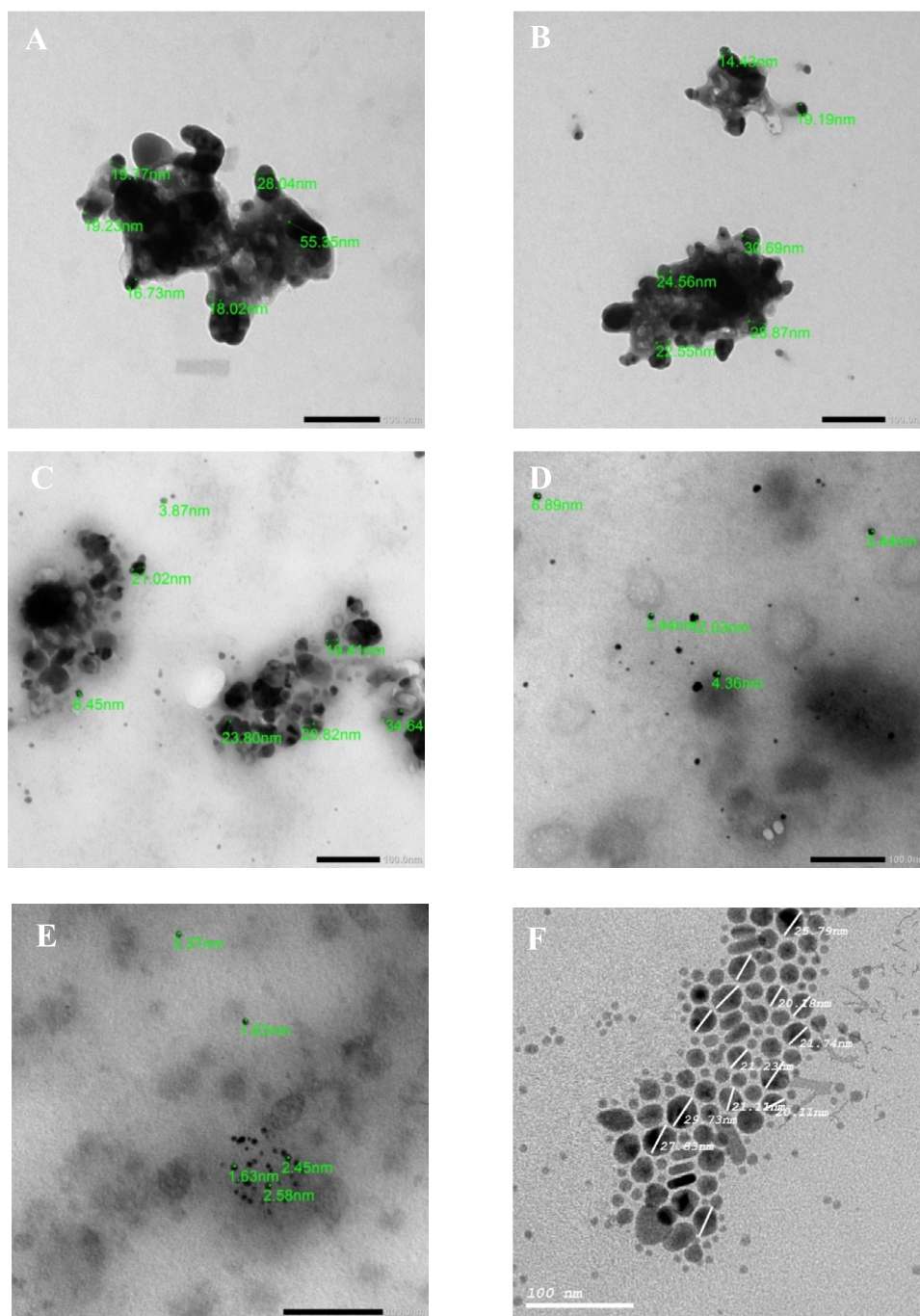
demersum extract were shown in the high-resolution transmission electron microscope (HR-TEM) images in Fig. 2. As shown in Fig. 2A, the phytosynthesized AgNPs (A) held irregular shapes that looked like flowers with a 16–55 nm size range. However, AgNPs (B) exhibited few spherical accompanied by undefined agglomerated AgNPs were observed with sizes ranging from 14 to 30 nm (Fig. 2B). Many semispherical to spherical shapes of AgNPs with sizes ranging from 4 to 35 nm in AgNPs (C) (Fig. 2C). Furthermore, the TEM image of AgNPs (D) confirmed frequent spherical NPs with sizes ranging from 2 to 7 nm (Fig. 2D). The TEM image of AgNPs (E) revealed the creation of distinct spherical NPs with tiny sizes (clusters) ranging from 2 to 4 nm (Fig. 2E). On the other hand, the TEM image of the chemically synthesized AgNPs proved the appearance of spherical NPs with a size less than 30 nm. Additionally, few rod-shaped NPs were recognized (Fig. 2F).

In the current study, the variation in the plant extract volume exerted a difference in the resultant AgNPs' shape and size. The size of the AgNPs decreased, and their shapes became more discrete and well-defined, with increasing the plant extract volume. Using small extract volumes (1–2.5 mL) produced large AgNPs sized with irregular shapes. Semispherical to spherical AgNPs with a size of 4–35 nm were achieved using 5 mL extract. While using higher volumes (10–20 mL), well-defined spherical tiny AgNPs, around 3 nm, were obtained. The use of large volumes of plant extract may lead to the formation of particles of small sizes due to the presence of a very large amount of reducer (plant extract). Also, it leads to the preservation of the particle size from aggregation and consequently, the formation of homogenous NPs.

Table S1 (Supplementary Information) presents a comparison of spherical AgNPs of varying sizes synthesized from different plant extracts. The findings indicate the remarkable effectiveness of the plant extract from *C. demersum* in the fabrication of small-sized spherical AgNPs. Previous studies have shown that AgNPs with fine or ultrafine dimensions are of great scientific interest due to their distinct characteristics and properties when compared to bulk silver metals. Furthermore, these colloidal particles, which exhibit quantum size effects, possess a high surface area and demonstrate excellent antimicrobial and antiviral properties, as well as chemical stability and catalytic activity [39].

Zachar et al. [40] mentioned the possibility of applying AgNPs to suppress bacterial and viral respiratory infections, as the effective dosage was highly sensitive to the NP size. Also, they flashed out that the optimal AgNP size utilized to treat viral respiratory infections, such as COVID-19, ranged from 3 to 7 nm. Therefore, the currently studied phytosynthesized AgNPs may act as promising candidates for several viral infections.

Fig. 2 Microscopic images for five phytosynthesized silver nanoparticles **A**, **B**, **C**, **D**, and **E**, respectively. **F** is the microscopic image of the chemically synthesized. Magnification par: 100 nm



3.1.2 EDX analysis

Energy-dispersive X-ray spectroscopy (EDX) analysis was used to identify a solid sample's elemental composition and provide the elemental percentage and distribution. The EDX spectra of dried free plant extract, the AgNPs (D) sample as an example of the others prepared, and chemically prepared AgNPs are presented in Fig. 3A, B, and C.

The plant EDX chart displayed several peaks attributed to Carbon, Nitrogen, Oxygen, Potassium, Magnesium

elements, and other traces of minerals, as reported earlier for *C. demersum* [41]. On the other hand, the AgNPs EDX chart detected several strong lines due to the Ag element as significant emission energies along with additional weaker signals identified to C, O, Si, S, Cl, N, Na, K, and Cu signals those originated from residual plant's biomolecules bounded to the surface of AgNPs [42] and serve as a stabilizing agent for the phytosynthesized AgNPs as previously mentioned [43–46]. All the EDX results confirm the existence of Ag with a suitable dispersion to the plant's active molecules.

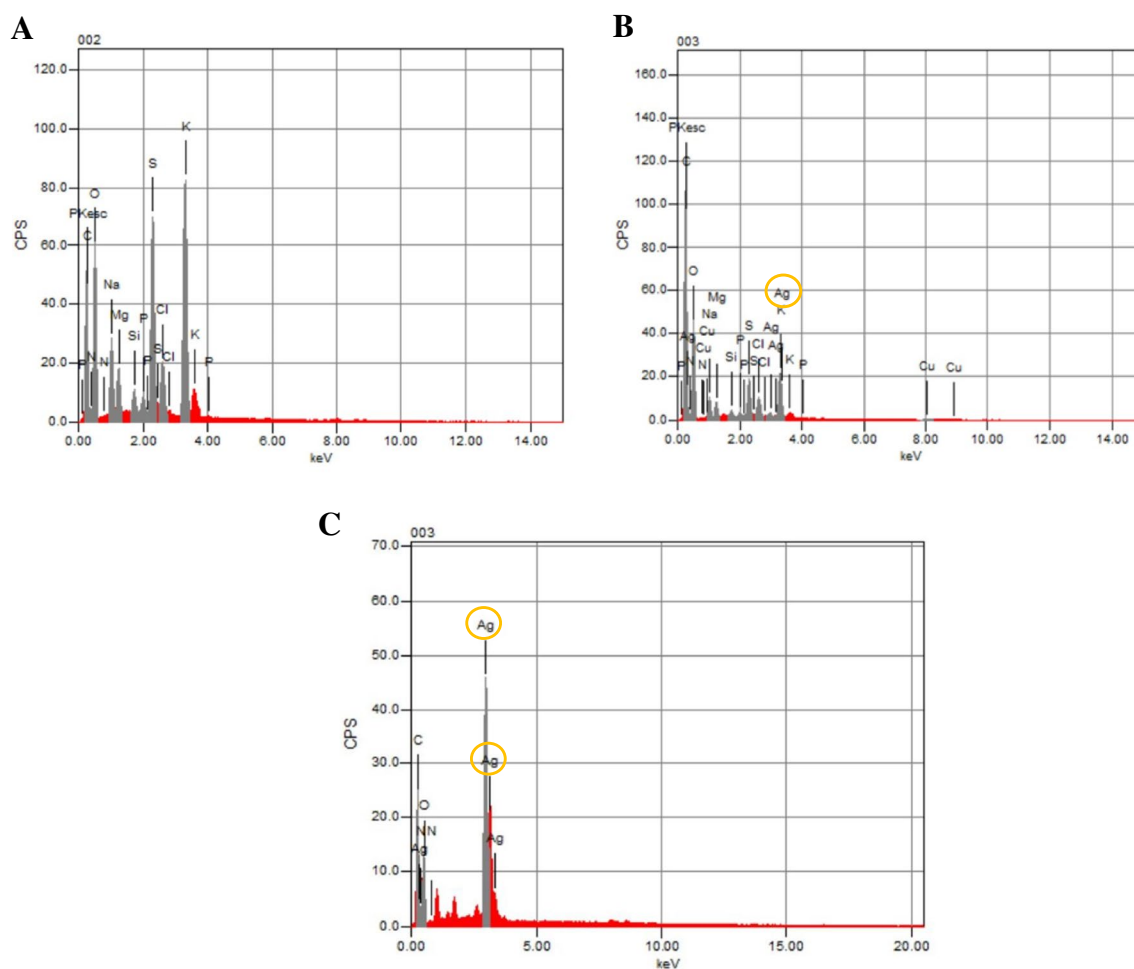
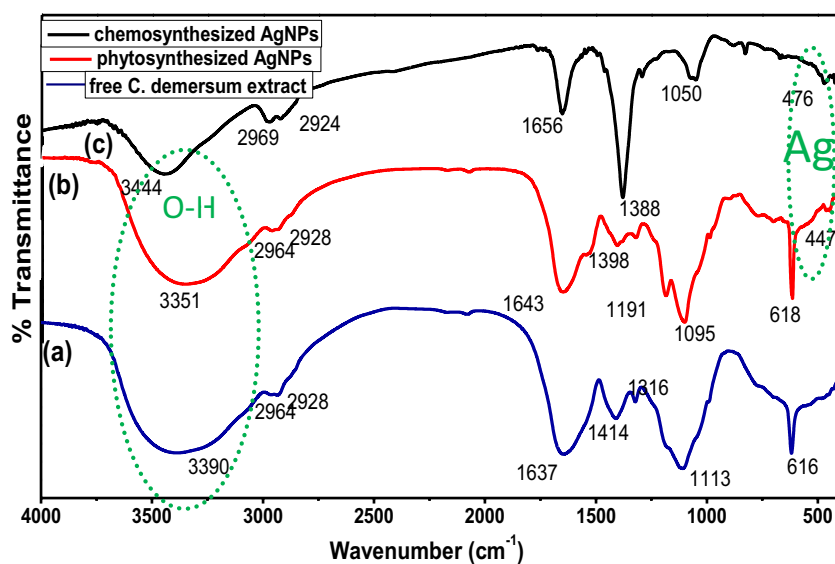


Fig. 3 Energy-dispersive X-ray spectroscopy chart of **A** the free-dried plant extract, **B** phytosynthesized silver nanoparticle sample D, and **C** chemosynthesized silver nanoparticles

Fig. 4 The Fourier transformed infrared spectra for the free plant extract, the phytosynthesized silver nanoparticles sample D, and the chemosynthesized silver nanoparticles



EDX chart of chemosynthesized AgNPs (Fig. 3C) showed Ag signals with other signals recorded for C, N, and O.

The Fourier transformed infrared spectroscopy The Fourier transformed infrared (FTIR) spectroscopic analysis was applied to recognize the functional groups and biomolecules available in the plant extract that have a major role in reducing Ag ions and serve as binding sites to contact the AgNPs surface (bio-stabilizing agent). The free *C. demersum* extract, phytosynthesized AgNPs, and chemosynthesized AgNPs are demonstrated in Fig. 4. In the free *C. demersum* FTIR spectrum, a broad and strong peak at 3390 cm^{-1} due to O–H stretching vibration was observed; this peak was slightly shifted to lower wavenumber 3351 cm^{-1} in the phytosynthesized AgNPs. The C–H stretch at 2964 cm^{-1} in the extract spectrum was observed without change in its position in the AgNPs FTIR spectrum. While the peaks presented at 1637 cm^{-1} (C–O symmetric stretch) [47], 1414 cm^{-1} , and 1113 cm^{-1} (C–N stretch vibration of aliphatic amines) [48] in the extract FTIR were presented at 1634 cm^{-1} , 1398 cm^{-1} , and 1095 cm^{-1} , respectively for AgNPs. The presence of O–H, C–N, and C–O functional groups suggests the presence of some moieties, such as flavonoids and alkaloids, in both the plant extract and its mediated AgNPs (Fig. 5). Furthermore, an additional peak located at 447 cm^{-1} due to the presence of the Ag metal [44] has appeared in the AgNP FTIR spectrum, proving the existence of Ag between the plant molecules.

In our previous investigations [33, 34], it was confirmed that the presence of absorption peaks for the O–H, C–N, and C–O moieties with a slight difference from their original

position in the plant extract plays a significant role in the bio-reduction process of Ag ions to form AgNPs, and also can develop protective layers on the AgNPs surface. Accordingly, it was concluded from the FTIR analysis that silver ions were reduced via plant extract and bound to it through OH and/or C=O groups. The chemosynthesized AgNPs FTIR spectrum (Fig. 4) showed vibrations reflecting the presence of the O–H, C–H, C–O, and C–N functional groups located at 3444 , 2969 , 1656 , and 1388 cm^{-1} , respectively. Additionally, the existence of Ag metal was confirmed by the presence of an Ag-ligand peak at 476 cm^{-1} .

3.1.3 Zeta potential and dynamic light scattering analysis

Lastly, the zeta potential measurement was utilized to assess the surface state and the long-term stability of bio-synthesized NPs. Earlier studies reported that the NPs with highly positive or negative zeta potentials (more than -25.0 and $+25.0\text{ mV}$) would have efficient repulsion forces between individual NPs that preserve their stability and prevent NP aggregation [49]. As shown in Figure S1 (Supplementary Information), the zeta potentials of the free extract, AgNPs (A), AgNPs (B), AgNPs (C), AgNPs (D), AgNPs (E) and chemosynthesized AgNPs are 14.1 , -9.31 , -9.35 , -27.6 , -20.9 , -25.4 mV , and -21.1 mV , respectively, exhibiting a high degree of stability.

Specifically, AgNPs produced using extract volumes of 5 mL , 10 mL , and 20 mL demonstrate greater stability compared to those made with less than 5 mL . This trend suggests that larger volumes of extract contain more biomolecules,

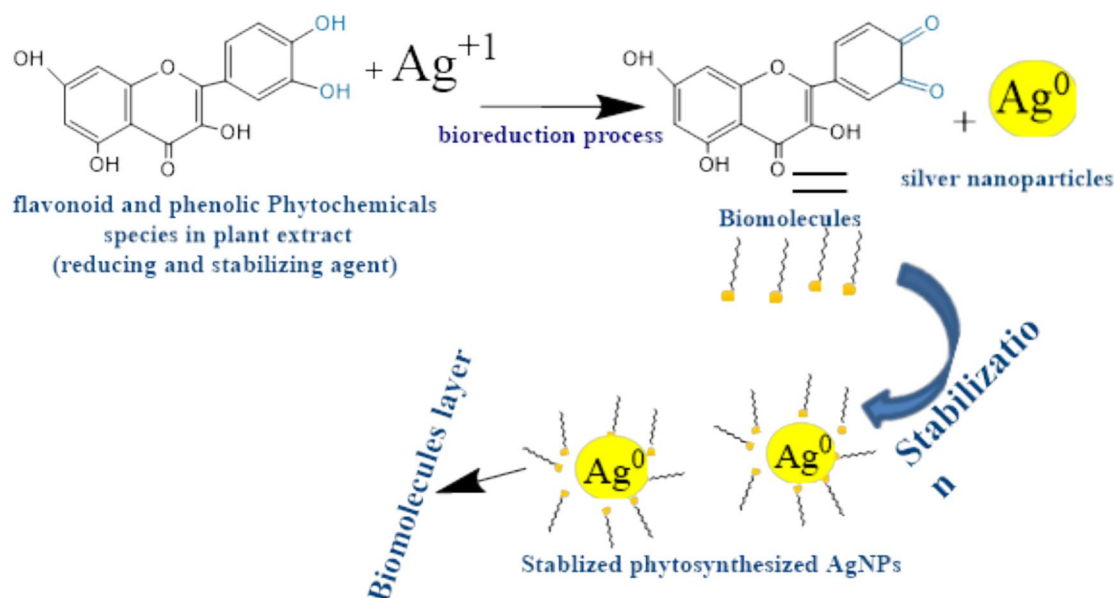


Fig. 5 The suggested mechanism concerning the silver nanoparticles bio-reduction and stabilization via the plant extract

which envelop the AgNPs more effectively. This observation aligns with recent research on the negative zeta potentials found in the phytosynthesis of AgNPs [50–52], attributed to the presence of capping biomolecules in the extract, such as flavonoids, phenolic compounds, carbohydrates, tannins, and proteins. These biomolecules stabilize the AgNPs, which is also supported by our infrared spectroscopy findings [53]. Furthermore, the zeta potential values of the AgNPs we synthesized are higher than those reported for AgNPs prepared using *Pedaliu murex* extract, which had a zeta potential of -7.66 mV [54].

The NP size distributions in a colloidal solution were determined by dynamic light scattering (DLS) measurements (Figure S2; Supplementary information). The DLS diagrams recorded on AgNPs (A), AgNPs (B), AgNPs (C), AgNPs (D), and AgNPs (E) indicate a hydrodynamic average size of 280 nm, 162 nm, 603 nm, 43 nm, and 78 nm, respectively, and 68 nm for the chemosynthesized AgNPs. The results indicate that using large amounts of extract (10 and 20 mL) in the AgNPs fabrication leads to the formation of particles exhibiting a size less than 100 nm. This result is consistent with the TEM findings, as large particles are observed for the chemosynthesis AgNPs (A), while smaller particle sizes are obtained for samples prepared using larger amounts of extract. Furthermore, the polydispersity (PDI) showed that the highest (PDI) value (0.89) was for the AgNPs (E) sample, displaying a mono dispersion behavior.

The X-ray diffraction analysis The X-ray diffraction (XRD) examination was carried out to establish the phase structure of synthesized NPs plus their crystallinity [34]. The XRD patterns of the phytosynthesized and chemosynthesized AgNPs are displayed in Fig. 6. The XRD pattern of phytosynthesized AgNPs was observed at 37.54° , 44.16° , 64.34° , and 77.34° , corresponding to the planes 111, 200, 220, and 311, respectively. However, the XRD pattern of the

chemosynthesized AgNPs showed peaks occurring at 37.4° , 44.22° , 64.12° , and 77.16° attributed to 111, 200, 220, and 311 planes.

The results confirmed the crystalline nature of the face-centered cubic (FCC) AgNPs matched with the standard powder diffraction card of Joint Committee on Powder Diffraction Standards (JCPDS) file number 04–0783 for crystalline silver. Also, in this investigation, the (220) plane was the preferred growth direction of both the phytosynthesized AgNPs and the chemosynthesized ones. Furthermore, the peaks' sharpening and their high intensities indicate the presence of AgNPs in high crystallinity degrees. The XRD results are matched with the obtained from TEM imaging. These results were in line with those investigated previously with green synthesized AgNPs using other plant extracts, such as *Pedaliu murex* leaf extract [54] and Chen and Kimura [55].

3.2 The antioxidant activity

Free radicals generated during biological processes can be harmful to human cells and contribute to pathogenicity [56, 57]. Therefore, significant efforts have been made to identify various antioxidant agents that can inhibit the oxidation process and prevent these damaging oxidative effects [58]. Antioxidants protect cells from the harmful impacts of reactive oxygen species by initiating chain reactions, breaking down peroxides, and scavenging free radical [59]. Natural antioxidants play a crucial role in reducing DNA damage, cell damage, malignant transformation, and the onset of diseases such as cancer, heart disease, and oxidative stress by neutralizing free radicals [60]. Oxidative stress is a fundamental cause of several diseases, including high blood pressure, diabetes, and atherosclerosis [61]. Recently, silver

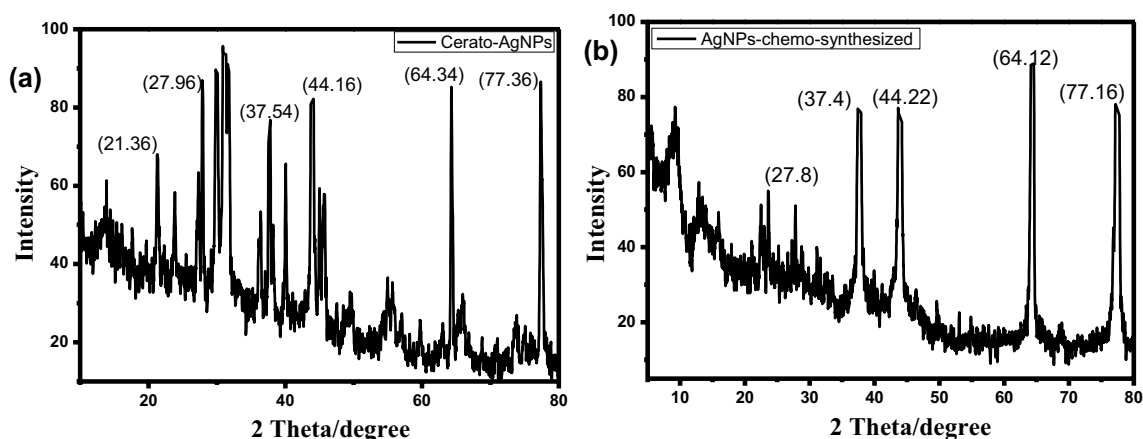
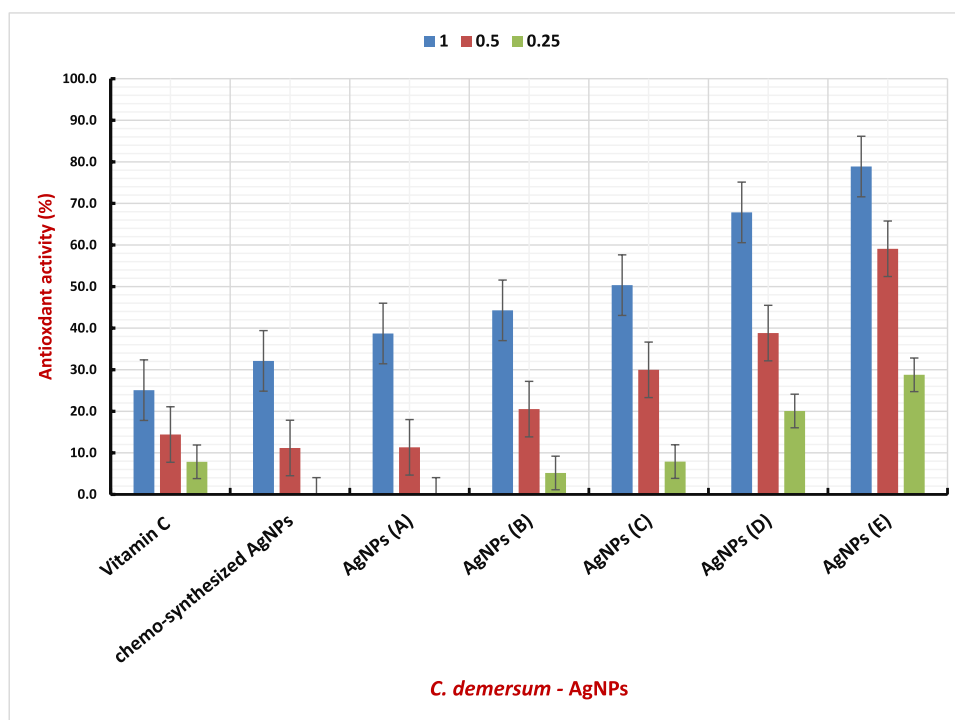


Fig. 6 The X-Ray Diffraction patterns of **a** phytosynthesized silver nanoparticle sample D, and image **b** is the chemosynthesized silver nanoparticles

Fig. 7 The antioxidant potential results of the chemosynthesized, and five phytosynthesized silver nanoparticles ((A), (B), (C), (D), and (E)). 20 refers to the initial concentration applied (50 µg/ml), 2–1 refers to the concentration after onefold dilution, and 2–2 refers to the concentration after twofold dilution



nanoparticles (AgNPs), particularly those synthesized using green and biological methods, have shown significant antioxidant potential [62, 63]. In this study, the in vitro antioxidant efficiency of phytosynthesized AgNP samples was evaluated using the DPPH assay at a concentration of 50 µg/mL, along with onefold and twofold dilutions. The results were compared to those of traditionally used vitamin C. The scavenging capacities results obtained from the experiments were tabulated in Tables S2 and S3 (Supplementary Information) and illustrated in Fig. 7.

The results demonstrated that all the AgNPs examined samples displayed good DPPH quenching when used by the initial concentration or with 1- and twofold dilutions. Additionally, as the volume of the mediated plant increased, the antioxidant effect increased. AgNPs (E) at its initial concentration had the highest scavenging percentage of 78.87%, followed by AgNPs (D), 10 mL (67.84%); AgNPs (C), 5 mL (50.34%); and AgNPs, 2.5 mL (44.28%). The lowest scavenging activity percentage was recorded for the AgNPs- 1 mL (38.71%). The highest scavenging percentage for vitamin C was 25.08%.

Hence, the antioxidant activity increases as the particle size decreases, following the use of a higher amount of plant extract according to scavenging percentage results. On the other hand, the chemosynthesized AgNPs presented the highest scavenging of 32.12%. The results indicate that the antioxidant activities achieved for the five phytosynthesized AgNPs samples are higher than that of chemosynthesized AgNPs; this may be due to secondary metabolites, including

phenolic and flavonoids compounds, essentially oil moieties found in the plant extract [64, 65]. As explained before, those substances may bind to the AgNPs' surface as a capping agent, donating hydrogen from their hydroxyl groups (-OH) to the free radicals to form stable phenoxyl radicals, consequently providing an excellent antioxidant effect [66].

3.3 The antimicrobial activity

Pathogenic bacteria cause diseases, such as endocarditis, urinary tract infections, intra-abdominal infections, cellulitis, respiratory system infections, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections, and wound infections. The traditional treatment with antimicrobials is the use of antibiotics that over time leads to antibiotic resistance [67]. Overcoming antibiotic-resistant bacteria is an ongoing challenge in various research aspects, so an alternative drug invention is necessary. Utilizing AgNPs could be an alternative solution to facing many microbial-resistant pathogens. Accordingly, the in vitro antibacterial activity of the five fabricated *C. demersum*-mediated samples AgNPs (A), AgNPs (B), AgNPs (C), AgNPs (D), and AgNPs (E), and free plant extract compared to chemosynthesized AgNPs was examined against seven pathogenic bacterial strains including three gram-positive bacterial strains (*Bacillus thuringiensis*, *Bacillus licheniformis*, and *Enterococci faecalis*) and four gram-negative bacterial strains; (*Pseudomonas aeruginosa*, *Enterobacter ludwigii*, *Pseudomonas putida*, and *Klebsiella pneumonia*). The AgNPs'

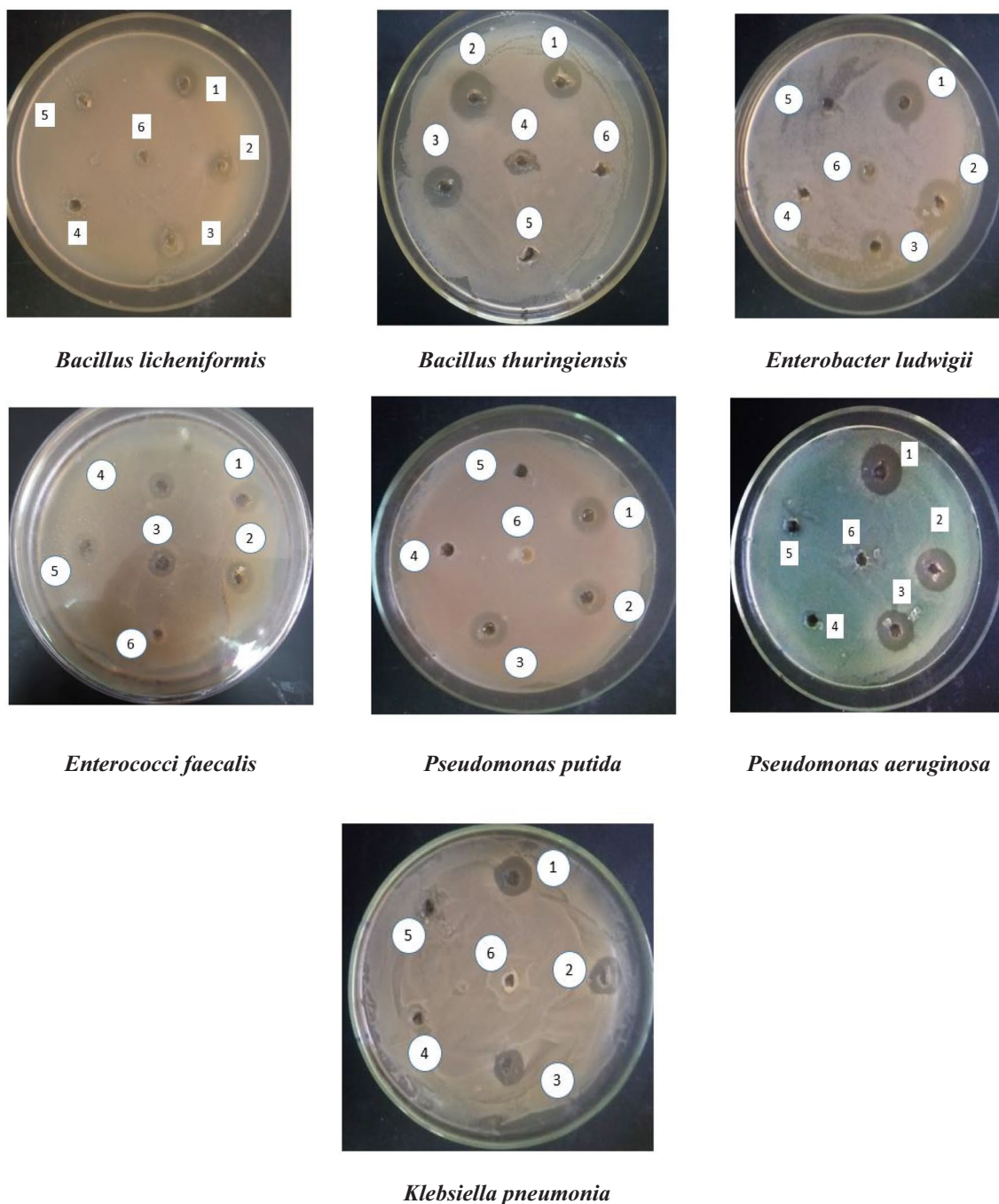
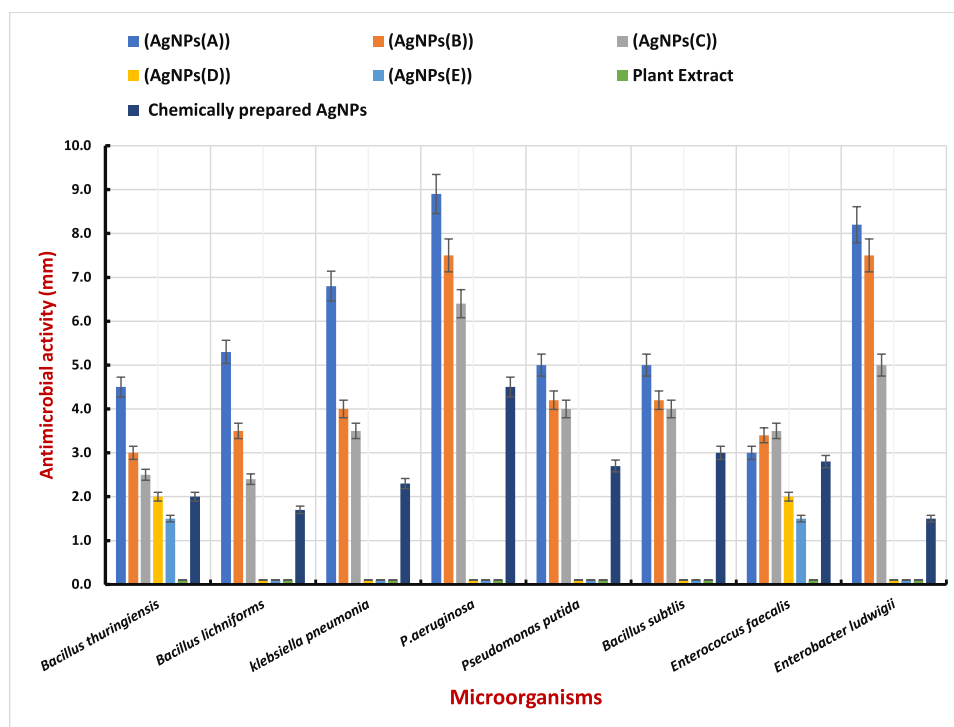


Fig. 8 1, 2, 3, 4, 5 are the antimicrobial activity of the five phytosynthesized silver nanoparticles (A), (B), (C), (D), and (E), respectively, and (6) for the plant extract against 7 pathogenic bacterial strains as evaluated by well diffusion method

antibacterial activities were determined by evaluating the mean inhibition zone (mm) (Figs. 8 and 9) and summarized in Table S4 (Supplementary Information). Several previous studies mention that AgNPs exhibit high anti-candida and antimicrobial potential, especially those synthesized using a green process. They can severely inhibit many pathogenic strains by interfering constructively with bacteria via different mechanisms.

Our results showed that the individual plant extract was inert and did not affect any tested bacterial strains. On the other hand, all the phytosynthesized samples, AgNPs (A), AgNPs (B), and AgNPs (C) provided antibacterial activities against all the examined bacterial strains. However, their effect on the gram-negative bacteria was more pronounced than on the gram-positive bacteria. The highest activity for the AgNPs (A) was against *P. aeruginosa*, and the lowest

Fig. 9 Antibacterial effect of the plant extract, chemosynthesized, and five phytosynthesized silver nanoparticles (A), (B), (C), (D), and (E) against different bacterial strains, as revealed by inhibition zones diameter



inhibition was recorded for AgNPs (C) against *Bacillus licheniformis*. Furthermore, AgNPs (D) and AgNPs (E) were active against *Bacillus thuringiensis*, and *Enterococcus faecalis*. Additionally, the inhibition observed for AgNPs (D) was higher than that recorded for AgNPs (E).

The chemosynthesized AgNPs showed bacterial activity when used in the same concentration as phytosynthesized (LC) only against *Enterococcus faecalis* with an inhibition zone of 1.5 mm. Nevertheless, all bacterial strains under investigation displayed inhibitory zones when the concentration of AgNPs was twofold (HC), with the highest inhibition observed toward the inhibition zone of 1.5 mm as shown in Figure S3 (Supplementary information).

According to the current antibacterial results, the investigated gram-negative bacteria were more affected by the treatment of AgNPs than gram-positive ones, matching the declared earlier studies [68]. That may be ascribed to the diversity in the cell wall structures of these two bacterial species. In contrast, gram-negative bacteria possess thinner cell walls than gram-positive bacteria, thereby facilitating the diffusion of AgNPs inside the bacterial cell [69]. Additionally, the gram-positive bacteria exhibit a thick and rigid peptidoglycan sheet composed of linear polysaccharide chains cross-linked through many short peptide moieties, forming a more rigid composition, making it hard for AgNPs to penetrate. Moreover, Ramadan et al. [70] explored the pathway of AgNPs within the bacterial cell, and they noticed through the TEM investigation that the

AgNPs were localized and aggregated above the cell wall, the cell membrane, and inside the cytoplasm, creating cell damage. The antimicrobial properties achieved were more efficient than those prepared recently [9].

The antimicrobial properties of AgNPs are attributed to several mechanisms, including their ability to penetrate cells, generate reactive oxygen species (ROS), and release free radicals. In this study, we observed that different sizes of AgNPs exhibited varying levels of bactericidal activity, which can be linked to their effective surface area. Therefore, the size of the nanoparticles is an important physicochemical property that influences their antibacterial effectiveness. Kumar et al. (2016) [71] found that AgNPs less than 50 nm in size can interact with the DNA of *Klebsiella pneumonia*, inhibiting cell division. Based on the results of our antibacterial activity tests, the phytosynthesized AgNPs appear to be highly suitable for treating infectious diseases caused by the pathogens we examined.

3.4 The photocatalytic potential

These days, treating water that has been contaminated by organic dyes is a hard task. Due to the fact that MB is one of the most widely used organic dyes in numerous industries, and the residue it leaves in wastewater is regarded as harmful pollution. The industry made widespread use of the MB as a redox indicator and the conventional methods for extracting it from wastewater involved the use of a reducing

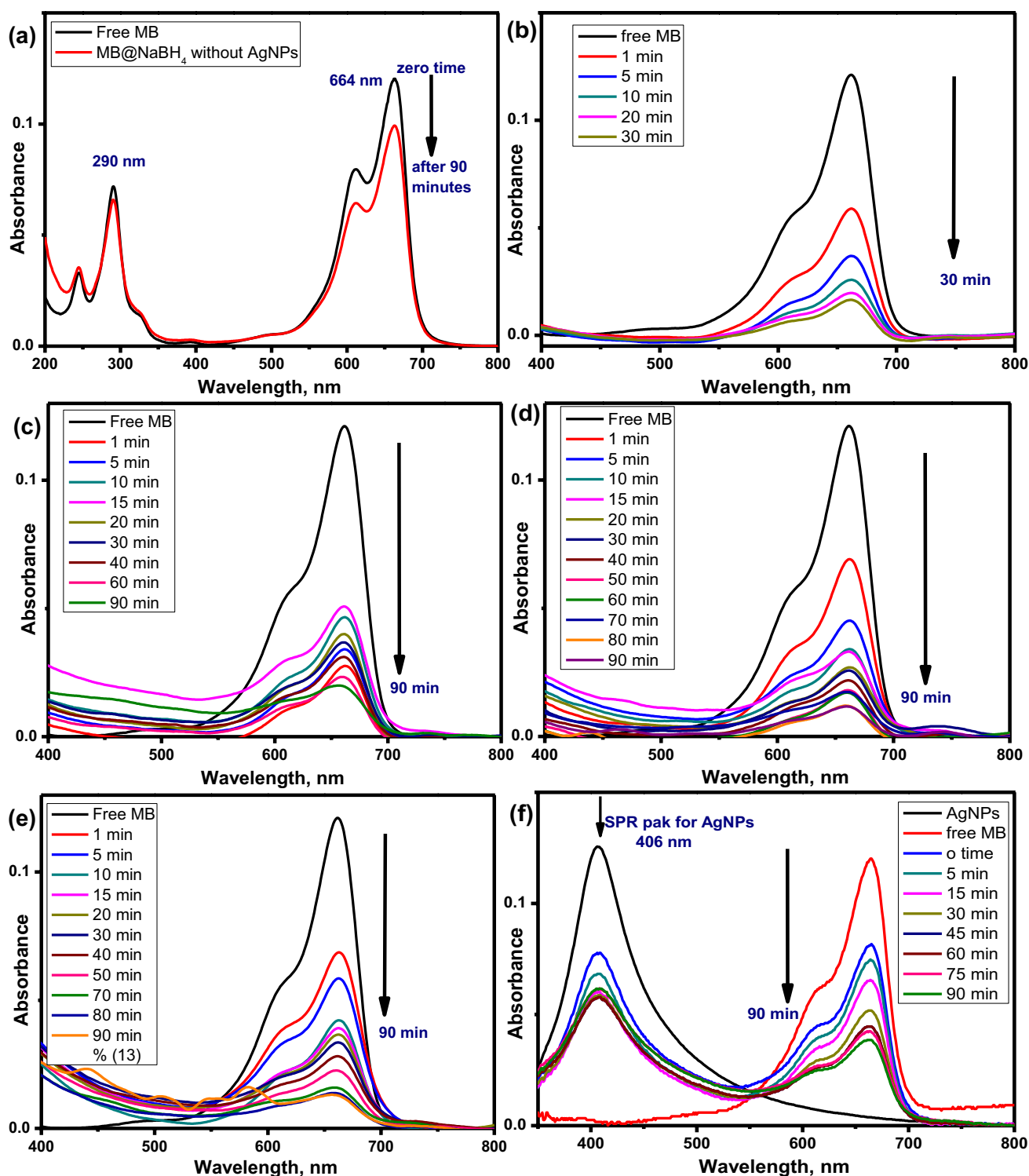


Fig. 10 a, b, c, d, e, f are the ultra-visible spectra of methylene blue reduction by sodium borohydride, and four photosynthesis silver nanoparticle samples (B), (C), (D), and (E) and chemosynthesis silver nanoparticles, respectively

agent, like NaBH_4 , which produces hydrogen gas through an aqueous hydrolysis reaction. The current investigation looks at AgNPs' catalytic activity when NaBH_4 is present.

The catalytic activity of phytosynthesized and chemosynthesized AgNPs was examined against methylene blue dye (MB), a common industrial dye (Fig. 10b, c, d, e, and f). The

absorption spectrum of dye has a prominent visible peak at 664 nm due to the $n - \pi^*$ transition [72]. An additional sample was examined using NaBH_4 without AgNPs to compare it with the currently prepared AgNPs (Fig. 10a). After treating the MB solution with chemosynthesized AgNPs, the dye removal efficiency reached 57%, 62%, and 68% after 30 min, 60 min, and 90 min, respectively. After 90 min, the treated sample using NaBH_4 alone in the absence of AgNPs had a degradation efficiency of 17%. Also, the phytosynthesized AgNPs (B) showed degradation of 87% after 30 min, and AgNPs (C) revealed degradation of 70%, 81%, and 84% after 30 min, 60 min, and 90 min, respectively. Besides, sample AgNPs (D) revealed efficiencies of 78%, 85%, and 90% after 30 min, 60 min, and 90 min, respectively. Finally, the sample AgNPs (E) efficiency reached 72%, 80%, and 89% after 30 min, 60 min, and 90 min, respectively. The results revealed a significant increase in the degradation efficiency percentage using both phytosynthesized and chemosynthesized AgNPs over NaBH_4 that could be attributed to the large surface area induced by the tiny particle size of AgNPs. On the other hand, the AgNPs (B) showed the best efficiency than the other phytosynthesized samples.

The first proposed photocatalytic mechanism of phytosynthesized AgNPs is adsorption, which may occur due to electrostatic attraction forces between the phytoconstituents, which surround NPs as stabilizing agents, and the MB dye surface. However, the supposed photocatalytic mechanism for removing MB using NaBH_4 used previously as a traditional catalyst, is via electron transfer from the negatively charged BH_4 groups (as the donor group) to the MB (as the acceptor group). Since the result of the dye removal using phytosynthesized AgNPs exceeded both the NaBH_4 catalyst and chemosynthesized AgNPs that were also prepared using NaBH_4 , therefore we assumed the adsorption mechanism was the one recommended to eliminate the MB dye. Moreover, chemosynthesized AgNPs accelerate the electron transfer between NaBH_4 and MB, resulting in MB catalytic removal [73].

4 Conclusion

Morphological analysis using transmission electron microscopy (TEM) revealed variations in the shape and size of the AgNPs as the amount of plant extract was altered. Specifically, as the volume of the extract increased, the size of the AgNPs decreased, resulting in particles that were more distinct and well-defined in shape. The zeta potential measurements indicate that the plant extract can stabilize the synthesized AgNPs through the formation of OH, C–O, and C=N bonds, which is further confirmed by Fourier transform infrared (FTIR) analysis. Moreover, all samples of phytosynthesized AgNPs exhibited superior free radical scavenging properties compared to those synthesized through chemical methods. Both phytosynthesized and chemosynthesized

AgNPs showed a more potent effect against gram-negative bacteria than against gram-positive bacteria.

In conclusion, this research focuses on a plant that is commonly found in our lakes, presenting new opportunities for the development of nanoparticles (NPs) beyond just silver nanoparticles. The plant *Ceratophyllum demersum*, found in the Nile Delta Lake, has demonstrated its effectiveness as a bioactive compound for the fabrication of AgNPs using five different volumes of the plant extract. This development paves the way for developing novel therapeutics aimed at addressing biological challenges with minimal side effects.

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Data availability All the data and materials are available in the submitted manuscript and supplemental information file.

Declarations

Ethical approval Not applicable.

Competing interests The authors declare no competing interests.

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