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Employing the aqueous extract obtained from the Salicornia plant to facilitate the production of silver nanoparticles and to assess their efficacy against bacteria

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ABSTRACT

The advancement of the most dependable and environmentally friendly methods for nanoparticle production represents a pivotal progression in the realm of nanotechnology. Numerous traditional methodologies applied in the nanoparticles synthesis are costly, hazardous, and not eco-friendly. To address such issues, natural sources which act as capping and reducing agents, emerge as suitable candidates for the fabrication of valuable nanoparticles. In this study, the bio fabricated silver nanoparticles (Ag-NPs) were synthesized utilize aqueous extract of *Salicornia europaea* L. leaves (SEL), characterized, and used to inhibit pathogenic bacteria with high efficiency. The characterization fabricated Ag-NPs were performed using FTIR, TEM, EDX, zeta potential and biological activity evaluated against different pathogenic bacteria (gram negative and positive). In conclusion, the phyto-synthesis of Ag-NPs employing extract of SEL could give a simple, non-expensive, and green technique for synthesizing Ag-NPs that could be used efficiently as an eco-friendly antibacterial operator with broad spectrum.

Key words: *Salicornia europaea*, aqueous extract, silver nanoparticles, antimicrobial activity.

1. Introduction

Egypt has a year-round mild climate especially in northern Egypt that is ideal for the cultivation and growth of Wild numerous fragrant and medicinal plants that are used in a variety of agricultural and medical applications (Mahrous *et al.*, 2022; Arief and Ahmed, 2023).

Wild a lot of plants are among the most significant natural antioxidants,that enable their presence in the medical and nutritional areas, appropriate for the health of both humans and plants ((Nurzyńska-Wierdak, 2023; Teterovska *et al.*, 2023; Simsek and Whitney, 2024). It contain a large number of primary and secondary components and genetic markers (Ozturk *et al.*, 2022). These substances preserve plant health and increase the vitality of different plant and crop sections (Llauradó Maury *et al.*, 2020; Salam *et al.*, 2023), apart from that function, these chemicals are also used in the synthesis of other substances that are currently highly regarded in scientific circles, the most significant of which is the creation of green nanocomposites (Ameta *et al.*, 2021; Mushtaq *et al.*, 2023). The fundamental tenets of green chemistry have experienced considerable progression to integrate the synthesis and enforcement of nanoparticles (NPs), consequently leading to the emergence of the connotation of green NPs via the methodologies of biomass conversion techniques (El-Sayyad et al., 2024). Number of applications in agriculture in many specialties including bacterial, fungal, and insect control as well as fertilization have been reported (Negi *et al.,* 2024; Elshaer et al., 2024). Numerous of wild plants are harmful to the ecosystem and are not being appropriately used because of its haphazard, disorganized, and improperly capitalized expansion (Pawera et al., 2020).

Utilizing these plants for the green nanosynthesi s of minerals is a safe practice for the environme nt and public health. These days, a variety of plants rich in primary and secondary chemical compounds are utilized to manufacture nano-mineral compounds, including iron, silver, zinc, selenium, and others (Samynathan et al., 2023, Singh et al., 2023; Khan and Khan, 2023). These materials include leaves, fruits, bark, and roots. portions of plants whose extracts include secondary substances like flavonoids and phenols as well as primary substances like carbohydrates. These substances are appropriate for the reduction of salts and the produce of green nanoparticles (Shafey, 2020; Habeeb Rahuman et al., 2022; Antonio-Pérez et al., 2023).

Salicornia genus of succulent plants is typically found near saltwater bodies of water, such as coastal regions. Because of their potential applications and capacity to flourish in arid environments, they are significant in many places, Egypt included (Ozturk et al., 2018; Ragab et al., 2020; Alfheeaid et al., 2022; Puccinelli et al., 2024). Salicornia is indeed rich in various bioactive compounds, including phenolic compounds, Tannins, Saponins and Amino acids (Renna and Gonnella, 2020; Lyra et al., 2022; Limongelli et al., 2022; Hulkko et al., 2023). It explains Salicornia capacity to create green nanocomposites and its antibacterial qualities (Manikandan et al., 2009; Khatami et al., 2018). Hence, the outer of this research is to produce silver nanoparticles through an environmentally friendly process, utilizing the aqueous extract derived from the aerial portion of the *Salicornia europaea* (SE) plant. Subsequently, the intention is to assess their efficacy as antimicrobial versus various strains of Gram-positive and Gram-negative bacteria.

2. Materials and Methods

Preparation of Plant extract

An aqueous extract was intended by heating the combination at 60-70 ˚C for 120 min, using 10 g of ground dry leaves of *Salicornia europaea* L. (SEL) (Collected from om El-Mamoura Agricultural area, Alexandria, Egypt) and added to a vial containing 100 mL distilled water (figure 1). The prepared extract was then cooled and filtered using Whatman filter paper no.1. according to Santhoshkumar *et al*., 2017 with some modifications, to eliminate any existing impurities, owing to the utilization of SEL extract to produce silver nanoparticles, which was selected due to its perpetual nature, ease of accessibility, and exceptional ecological characteristics. Following filtration, the extract was stocked at a temperature of 4 ˚C for posterior analysis.

Total phenolic and total flavonoid content

The aqueous solutions originating from the leaves of SE were employed in the spectrophotometric placement of phenolic compounds and flavonoids. The assessment of the total phenolic content (TPC) was executed through the utilization of a slightly adjusted Folin-Ciocalteu method as elucidated by Prabhavathi et al. (2016) and Kupina et al. (2019). As for the total flavonoid content (TFC), it was measured using a spectrophotometric technique with minor adaptations, following the procedures outlined by Pękal et al., (2014) and Shraim, et al., (2021).

Synthesis of silver nanoparticles

The synthesis of silver nanoparticles using SEL, which was accomplished by adding the SEL extract to a freshly prepared and heated aqueous silver nitrate (obtained from El-Gamhouria Trading Chemicals and Drugs Company, Egypt) solution at a ratio of 1:9. The mixture was heated to either 90 °C (2 mM) or 80 °C (5 mM) and incubated at room temperature or with gentle stirring for several hours. The pointing of Ag-NPs, indicated by a color change from yellow to reddish-brown, resulted from the reduction of silver ions. Following incubation, the mixture was centrifuged at high speed for 30-60 minutes to precipitate the nanoparticles, after which the supernatant was ostracized, and the nanoparticles were washed with distilled water to strip any unreacted materials. The nanoparticles were then dried either at room temperature or in an oven and stored for further characterization (Salayová et al., 2021).

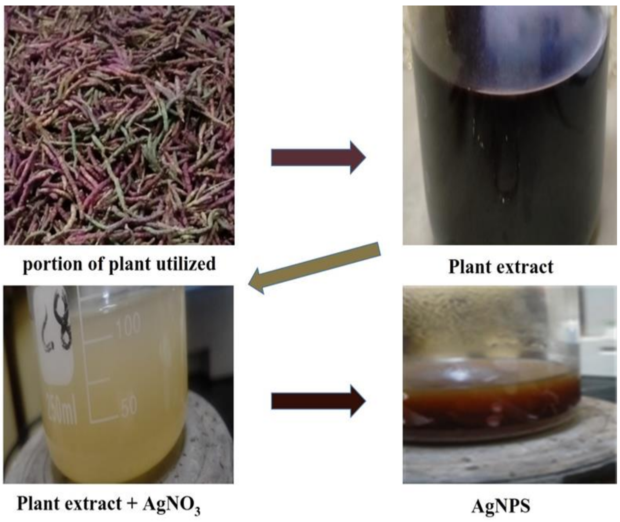


Fig. 1. Stages of collection and synthesis of Ag-NPs from aqueous extract of *Salicornia europaea* L. leaves.

FTIR analysis

FTIR Spectroscopy is a technique commonly utilized for the examination of polymers, inorganic, and organic compounds in various studies such as those on aqueous SEL extract and green Ag-NPs (Prasanna et al., 2020). Experimental procedures involved the use of a JASCO FT-IR 3600 infrared spectrometer, with the KBr pellet method being applied prior to the commencement of experiments. The spectral data obtained covered a floppy range of wave numbers from 400 to 4000 cm-1 (Izzo et al., 2020).

TEM analysis

It is an exceedingly potent methodology within the field of materials science, which can be elucidated as the passage of a high-energy electron beam through exceedingly thin specimens. The characteristics of silver nanoparticles in relation to their size and style were investigated utilizing Transmission Electron Microscopy (TEM) technology (Images were analyses by the ImageJ software (GEOL, GEM- 1400$^+$ Transmission Electron Microscope). A quantity of the dehydrated residue containing nano silver was resolve in ethanol, following which the mixture was subjected to ultrasonic treatment for a duration of 15 minutes; subsequently, a droplet was extracted from the solution and deposited onto a copper grid coated with carbon. It is worth mentioning that post the desiccation of the specimen leading to the formation of a partially clear coating, the sample is scrutinized, and the resultant image is generated through the depiction of the electron beam's shadow cast onto the specimen (Er, 2019).

Zeta potential analysis

The determination of the superficies electric charge of Ag NPs involved measuring the optimal stability of particles amidst electrostatic discord. The Zeta potential was assessed through the utilization of Malvern Instruments Inc., USA, employing a Zeta sizer that relies on photon correlation spectroscopy (Kamble et al., 2022).

EDX analysis

The samples underwent analysis using Energy Dispersive X-ray (EDX) with the identical instrument utilized for Scanning Electron Microscope (SEM) analysis, to validate the elemental composition of the sample (Awwad and Salem 2012).

Bacterial strains

The effectiveness of SEL extracts against six pathogenic bacterial strains, comprising three gram-negative (*Pseudomonas aeruginosa, Escherichia coli, Listeria monocytogenes*) and three gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) strains, as well as *Bacillus subtilis,* a food spoilage bacterium, was evaluated. These bacterial isolates were stored at 4°C on nutrient broth slants. The criterion inoculum commentary were adjusted to a McFarland turbidity of 0.5 and a density of 1×10^6 cells or spores per milliliter (Odongo *et al*., 2023).

Sterilization and equipment

The glassware, nutrient media, and distilled water utilized in the investigations evaluating antibacterial efficacy were subjected to sterilization within an autoclave at a temperature of 121°C for a period of 15 minutes prior to their usage. The bacteriological wire loop and cork puncture were sterilized through exposure to flame utilizing a Bunsen burner. All laboratory methodologies involving the manipulation of microorganisms were performed within a biosafety level 1 (BSL-1) cabinet, whereas a freezer incubator-1 was employed for the incubation of microorganisms (Atwaa et al., 2022; Omosa et al., 2014).

Inoculums preparation

The bacteria were prepared according to the CLSI guidelines: M02-A11. In summary, a stock culture of microorganisms was cultivated on Muller-Hinton agar (MHA) at a temperature of 37°C for a duration of 12-24 hours. Two to three colonies of microorganisms were inoculated into 1 mL of Mueller-Hinton broth (MHB) utilizing a sterile cotton swab and subsequently subjected to vortex mixing for a period of 15 minutes. Following the vortex mixing, the bacterial suspension was incubated at 37°C for an additional 12-24 hours (Rukayadi et al., 2013; Hasanien et al., 2023), and 10 µL of the suspension was transferred into 10 mL of MHB. The turbidity of the inoculum was standardized to 10^5-10^6 colony-forming units (CFU) per milliliter before the test using the standard broth micro dilution method (Odongo et al., 2023; Abobaker et al., 2023).

Antimicrobial susceptibility testing (Agar Well Diffusion Method)

The antimicrobial activities of the silver nanoparticles were determined using the well diffusion method according to the Clinical and Laboratory Standards Institute (Kazmierczak *et al*., 2015). MHA plates previously inoculated with 24 h old broth cultures of the bacterial strains were used for antibacterial activity. The agar was inoculated uniformly with the standardized test organisms. Wells with a diameter of 10 mm were produced using a sterile cork borer (Munir et al., 2020). In each petri dish, the designated wells were individually supplemented with 70 µl/well of the botanical extracts and 70 µl/well of nano silver synthesized from the examined plant extract. The inoculated petri dishes containing the test solutions in the wells were permitted to equilibrate for 30 minutes prior to a subsequent overnight incubation period of 18 hours at a controlled temperature of 37 °C. (Perera et al., 2022). All determinations were done in triplicate. The diameter of the inhibition zone around the well, metrical in millimeters, was used as a positive antimicrobial activity (Altaweel et al., 2021).

4. Results

Table 1 presents a universal overview of the total phenols and flavonoid concentrations found in aqueous extract of SEL. The mean total phenols content was determined to be 8.45 ± 0.68 mg/ml. Conversely, the mean flavonoid content was found to be 7.99 ± 0.39 mg/ml.

Table 1: Average total phenolic and total flavonoid content in aqueous leaves extract of *Salicornia europaea* L.

Compounds	Quantity
Phenols (mg gallic acid equivalent /ml of extract)	**8.45 ± 0.68**
Flavonoids (mg of quercetin equivalent /ml of extract)	**7.99 ± 0.39**

FTIR analysis

FTIR revealed the existence of multiple functional groups in aqueous extract of SEL and Ag-NPs. Furthermore, the functional groups identified in Ag-NPs specimens, potentially due to the diverse phytochemicals enveloping the silver nanoparticles present in of SEL. The prominent characteristic peaks at approximately 3421.67 cm^{-1} , 2939.24 cm^{-1}, 2339.13 cm^{-1}, 2090.50 cm^{-1}, 1630.33 cm^{-1}, 1490.87 cm^{-1}, 1473.32 cm^{-1} , 1401.03 cm^{-1}, 1362.48 cm^{1}, 1338.08 cm^{-1}, 1239.28 cm^{-1}, 1101.70 cm^{-1}, 933.71 cm^{-1}, 899.70 cm^{-1}, 804.10 cm^{1}, 722.03 cm^{-1}, 617.90 cm^{-1} and 541.43 cm^{-1} in SEL extract and 3912.65 cm-1, 3890.38 cm^{-1}, 3842.87 cm^{-1}, 3826.90 cm^{-1}, 3808.97 cm^{-1}, 3785.92 cm^{-1}, 3719.30 cm^{-1}, 3698.58 cm^{-1}, 3660.29 cm^{-1} , 3637.38 cm^{-1} , 3598.14 cm^{1}, 3576.37 cm^{-1}, 3319.97 cm^{-1}, 2350.71 cm^{-1}, 2093.34 cm^{-1}, 1630.69 cm^{-1}, 1384.41 cm^{-1}, 1105.97 cm^{-1}, 934.39 cm^{-1}, 901.07 cm^{-1} , 829.32 cm^{-1} and 618.96 cm^{-1} observed in Ag-NPs.

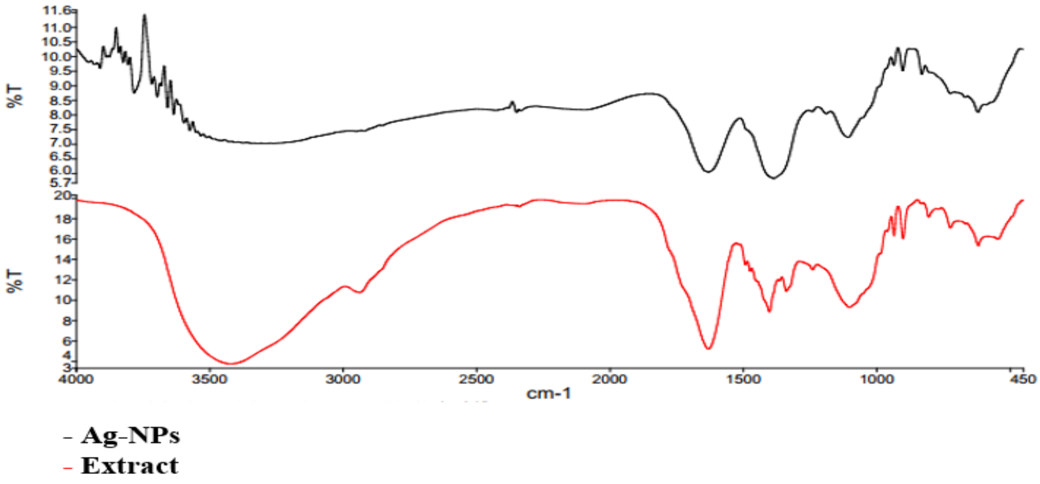


Fig. 2. FTIR analysis of *Salicornia europaea* L. leaves extract and Ag-NPs

Transmission electron microscopy

Transmission electron microscopy **(**TEM) gives resolutions that reach the atomic level and yields important details on structural characteristics including grain size, crystal flaws,

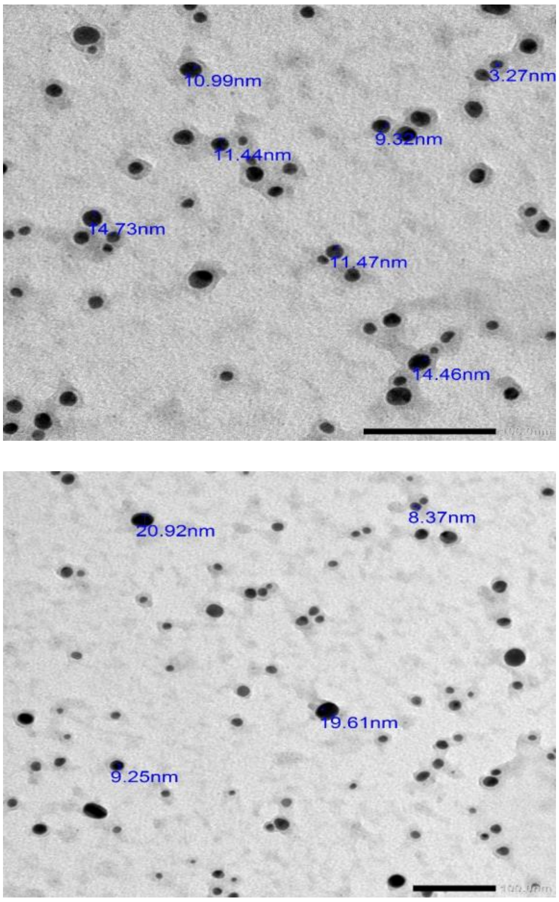


Fig 3. TEM values for Ag-NPs synthesized by *Salicornia europaea* leaf aqueous extract.

Zeta potential values

Silver nanoparticles created by SEL aqueous extract had a Zeta potential measurement of -23.1 mV (Figure 4) and the stability of a shape or structure is directly correlated with the zeta potential, or surface potential (Sharma et al., 2014).

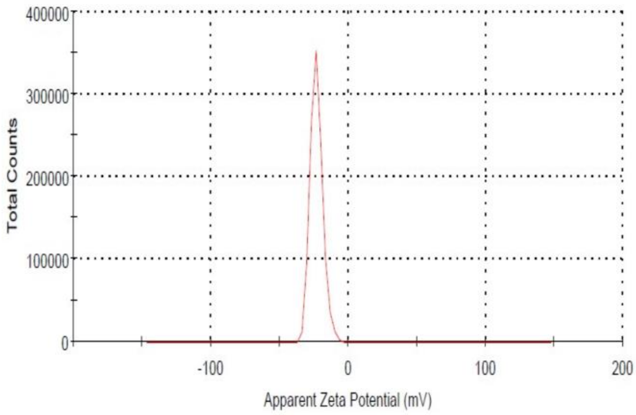


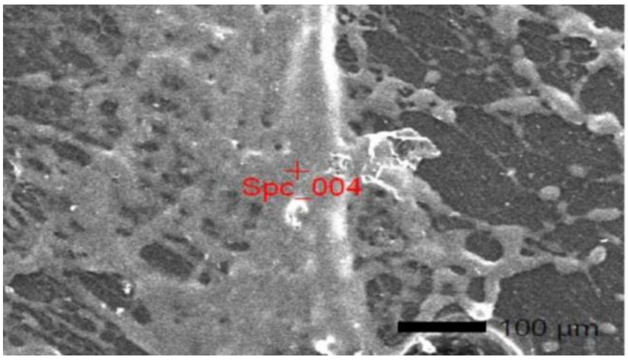
Fig 4. Zeta potential values for the synthesized Ag-NPs by SEL aqueous extract

Energy Dispersive X-ray (EDX)

Certain elements are present during the Ag NPs' synthesis; an EDX analysis can determine the weight ratio of the Ag NPs' main constituents made from SEL aqueous extract. The information on the nano-element and its composition was then discovered through the use of an energy dispersal spectrometer. where the following ratios were noted: 37.38±0.19 carbon, 40.44±0.41% oxygen, and 13.92±0.19% silver On the other hand, the lowest percentages of sodium, magnesium, phosphorus, sulfur, chlorine, potassium, calcium, and copper were seen in the sample (table 2 and figure 5).

Table 2: EDX analysis of the racist installation of the synthesized Ag-NPs by SEL.

Element	Mass%	Atom%
C	37.38±0.19	51.76±0.27
O	40.44±0.41	42.05±0.43
Na	1.04±0.06	0.75±0.04
Mg	0.84±0.04	0.57±0.03
P	0.48±0.03	0.26±0.02
S	0.67±0.03	0.35±0.02
Cl	1.53±0.05	0.72±0.02
K	2.24±0.07	0.95±0.03
Ca	0.39±0.04	0.16±0.02
Cu	1.08±0.08	0.28±0.02
Ag	13.92±0.19	2.15±0.03



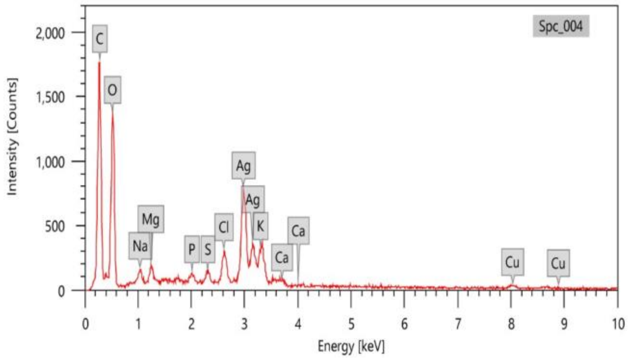


Fig. 5. EDX analysis of the biosynthesized Ag-NPs by SEL aqueous extract.

Antibacterial activities

The antibacterial properties of SEL and Ag-NPs were evaluated using six pathogenic bacteria, comprising three Gram-positive bacteria (*S. aureus*, *B. cereus*, and *B. subtilis*) and three Gram-negative bacteria (*P. aeruginosa, E. coli*, and *L. monocytogenes*). *P. aeruginosa* was the most sensitive to AgNPs (2.5±0.1), followed by *B. cereus* (2.1±0.06) and *E. coli* (2±0.08). As depicted in table (1), *B. subtilis* showed the greatest resistance to AgNPs (1.6 ±0.1), followed by *L. monocytogenes* (1.7 ± 0.05) and *S. aureus* (1.8 ±0.2) (table 3).

Table 3: Antibacterial activity of *Salicornia europaea* extract and AgNPs

Compound	**Inhibition zone diameter (Cm)**					
	Gram -ve			**Gram +ve**		
	P. aeruginosa	***E.coli***	***L. monocytogenes***	***S. aureus***	***B. cereus***	***B. subtilis***
SEL extract	-ve	-ve	-ve	-ve	-ve	-ve
AgNPs (2mM)	2.5±0.1	2±0.08	1.7 ± 0.05	1.8 ±0.2	2.1±0.06	1.6 ±0.1

-ve : No effect on bacteria

5. Discussion

The importance of SEL extract and it's AgNPs in terms of their ability as antimicrobial agent have been demonstrated. Prior findings indicate that the aqueous leaves SEL extract is rich in phenols and flavonoids, rendering it a popular choice for the synthesis of nanoparticles using green methods. Several previous studies have highlighted the abundance of phenolic compounds and flavonoids in the SEL extract (Zare-maivan et al., 2016; Cristina et al., 2018; Puccinelli et al., 2024).

The examination of the functional groups capping silver nanoparticles synthesized utilizing SEL is conducted through FTIR spectroscopy. The identification of diverse organic compounds within the plant results in the observation of numerous peaks in contrast to the chemical approach, which exhibits only a limited number of intense peaks (Chauhan et al., 2016; Abimbola, 2017). 3598.14 cm^1 to 2939.24 cm^{-1} are linked to the stretching of O–H/N–H in amides and C≡C stretching at 2350.71 cm-1 to 2090.50 cm^{-1}. While, 1630.33 cm^{-1} to 1101.70 cm^{-1} have been linked to the aromatic compounds. Moreover, the faint peaks at around 933.71 cm^{-1} to 804.10 cm^{-1} were associated with carbohydrates and –C = O. our results were in agreement with several previous studies (Alyamaç et al., 2022; Sharma et al., 2022; Elshaer *et al*., 2024; Nazneen et al., 2024).

Generally, Silver nanoparticles synthesized using SEL aqueous extract had oval to spherical shapes and ranged in size from 3.27 to 20.92 nm and according to numerous publications, this is thought to be better for the synthesis of green silver nanoparticles (Essien et al., 2019; Fatimah et al., 2022; Velgosova et al., 2024).

The study's zeta potential result is in line with numerous other researchers' findings, which ranged from -30 to +30 in negative values (Mohamed et al., 2022; Abdelaziz et al., 2023; Abd-Elraheem et al., 2024).

Using energy dispersive spectroscopy, a sample's elemental composition and surface analysis are examined. The fundamental idea is to examine the various energy X-rays that are released from the sample when an electron beam hits its constituent parts. It is simple to determine the quantity and make-up of metal nanoparticles on the surface of the provided sample (Girão et al., 2017; Scimeca et al., 2018). The components that are adjacent to the nano silver are a result of the plant extract that was used to make the nano silver particles (Nguyen et al., 2023).

The presence of an inhibition zone or clear zone around the wellbore in a petri dish was indicative of positive results. Annisa et al., (2023) indicated that the turnout of an inhibition zone or serene zone around the wellbore in a petri dish comprise Ulin fruit extract (Eusideroxylon zwageri) and positive controls Amoxicilin and Fosfomycin, whilst in the negative control sterile distilled water, no inhibition zone was formed around the wells in the petri dish. According to the results, SEL extract demonstrated no inhibition activity against the tested bacteria (all tested isolates showed resistance to the extract), while AgNPs produced by the extract exhibited varying degrees of inhibition against the tested bacteria. Altaweel *et al*., (2021) also found that *E. coli* and *S. aureus* were resistance to all examined compounds. Ag-NPs have been found to effectively inhibit the growth of bacteria like *E. coli*, *S. aureus*, *P. aeruginosa*, *S. mutans*, and *Klebsiella pneumoniae*. They work by disrupting bacterial cell membranes, increasing permeability, inhibiting biofilm formation, and demonstrating bactericidal effects that are concentration dependent. Additionally, when combined with antibiotics, Ag-NPs show enhanced antibacterial activity, particularly against multidrug-resistant strains. The synthesized Ag-NPs are stable, with well-defined physicochemical properties, making them promising for developing antibacterial agents to combat antibiotic resistance in biomedical applications (Hanafiah et al., 2023; Xie 2024; Idris *et al*., 2024; Ma et al., 2024; Ghaffar et al., 2024).

It was be concluded that the investigations involved the utilization of SEL plant, thriving in high salinity regions of the Egyptian landscape, could be used for eco-friendly production of silver nanoparticles. Subsequently, these nanoparticles were employed to impede the proliferation and functions of specific strains of Gram-positive and Gram-negative bacteria, yielding highly favorable outcomes.

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