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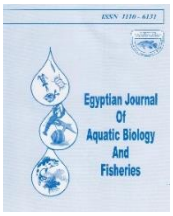

Biosynthesis of Silver Nanoparticles Using Ethanolic Extract of the Marine Alga *Enteromorpha intestinalis* and Evaluation of their Antibacterial Activity

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ABSTRACT

The current study explored the use of the ethanolic extract of *Enteromorpha intestinalis* to determine its capability to synthesize silver nanoparticles. The GC-mass technique was utilized to identify the active compounds in the algal ethanolic extract. The extract's potential in fabricating silver nanoparticles was tested, and their formation was inferred through the color change of the mixture from light green to dark brown. The synthesized nanoparticles were characterized using various techniques, (FTIR) spectroscopy, a scanning electron microscope (SEM), and an energy-dispersive X-ray spectroscopy (EDX). In addition, they were analyzed using X-ray diffraction (XRD). The antibacterial effectiveness was tested through the agar well diffusion method. The GC-mass results showed that the ethanolic extract contained several active compounds, including n-hexadecanoic acid (37.40%), neophytadiene (11.08%), 9,12,15-octadecatrienoic acid, (Z, Z, Z)-(17.32%), and oleic acid (10.28%). A peak at 435nm in the UV-vis absorption spectrum confirmed the formation of silver nanoparticles. The XRD technique determined the crystalline nature of the silver nanoparticles, and SEM results showed that they had spherical shapes with sizes ranging between 47- 78nm. EDX analysis revealed that the synthesized silver nanoparticles comprised silver, carbon, oxygen, and chlorine. The efficacy of the synthesized silver nanoparticles was tested against four types of pathogenic bacteria, namely *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The results demonstrated that the silver nanoparticles had a significant inhibitory capability against these pathogens.

INTRODUCTION

Nanotechnology is a science that deals with the use and studying of the molecule's and particle's applications at the nanoscale, with dimensions not exceeding 100 nanometers. The term nano is a Greek word derived from the word nano which means dwarf, and this unit represents a part of a billion **(Madkhour, 2019)**. Nanoparticles are among the most significant in biomedical applications at the early stage of scientific development, especially in the field of nanomedicine **(Zhang *et al.*, 2016)**. Silver nanoparticles are renowned for their effective properties, which afford them high antimicrobial activity compared to other nanoparticles due to their exceptionally large

surface area that facilitates interaction with microorganisms **(Logeswari *et al.*, 2015)**. The antimicrobial activity of silver nanoparticles against microbes is attributed to the binding of silver ions to cell walls, inhibition of membrane-associated enzymes, interference with essential biomolecules of bacterial cells, alteration of the cell wall, and formation of reactive oxygen species, all of which are detrimental to bacterial cells **(Kim *et al.*, 2011)**.

A study conducted by **Gholami-shabani (2014)** elucidated that silver nanoparticles are effective even at very low concentrations, as they induce disturbances in the cell wall, suggesting their potential as an effective alternative treatment for infections caused by antibiotic-resistant bacteria. The field dealing with the fabrication of nanoparticles using algae, known as phyconanotechnology, is a relatively recent branch of nanotechnology. Algae are utilized in the synthesis of nanoparticles due to various capabilities, including their high metal absorption and ion reduction properties, low production costs, and, most importantly, their ability to produce nanoparticles in large quantities. Additionally, algae are easy to handle and cultivate under laboratory conditions. They are more effective in dealing with environmental conditions than other organisms, have short life cycles, and are mainly non-toxic to the environment **(Negi & Singh, 2018)**. Dried and dehydrated biomass can be utilized in the biosynthesis of nanoparticles. Thus, they are called bionanofactories **(Omar *et al.*, 2017)**. Bacteria and fungi may cause diseases in people and other species. Despite the discovery of several medicines to combat these microorganisms, they have gradually developed resistance to these drugs, posing challenges in the treatment of such diseases **(Komolafe, 2003)**. Therefore, alternative treatments and methods that could overcome the antibiotic resistance of these bacteria have been sought **(Kim *et al.*, 2011)**. Silver nanoparticles are among the most important nanomaterials owing to their antimicrobial properties **(Grosse *et al.*, 2013)**. A study was conducted to investigate the antibacterial efficacy of silver nanoparticles against *Staphylococcus aureus*. The findings revealed that the presence of silver nanoparticles at a concentration of 4µg/ ml resulted in a total inhibition of bacterial growth. Transmission electron microscope (TEM) results confirmed the damage to the bacterial cell wall caused by silver nanoparticles, as well as their accumulation in the bacterial cell membrane **(Mirazajani *et al.*, 2011)**. **Rai *et al.* (2012)** postulated that the antibacterial activity of silver nanoparticles is among the most effective treatments, capable of eliminating many multidrug-resistant (MDR) pathogens and drug-susceptible strains, such as ampicillin-resistant *E. coli* and *Pseudomonas aeruginosa*, erythromycin-resistant *Streptococcus pyogenes,* and vancomycin-resistant *Staphylococcus aureus.* Additionally, a study on the antimicrobial properties of nanoparticles synthesized from the brown algae *Padina tetrastomatica* showed an effectiveness against several bacterial strains, including *Bacillus subtilis*, *Klebsiella planticola*, and *Pseudomonas aeruginosa* **(Rajeshkumar *et al.*, 2012)**.

MATERIALS AND METHODS

1. Collection of algal samples

Algal mass was directly collected from the aquatic environment in different water areas of Kutiban-Shatt al-Arab in Basrah Governorate in southern Iraq. Nets were used for collection, and the algal samples were placed in sterile plastic containers, after which they were brought to the laboratory. Algal samples were washed with tap water to remove impurities. The samples were washed several times with distilled water to ensure cleanliness. Moreover, they were examined under a light microscope to determine the type of the isolated alga, then morphologically identified based on taxonomic sources.

2. Identification and purification of algal isolates

Enteromorpha intestinalis was phenotypically identified by preparing temperorary glass slides, and the sample was examined under light microscope to determined its morphological characteristics and identification, based on taxonomic sources of **Prescott (1975)** and **Bourrely (1980)**. A sonicator apparatus was used to isolate diffierent microorganism (bacteria and fungi) attached to the alga, thereby obtaining a pure isolated alga (axenic calture).

3. Preparation of the ethanol extract

3 grams of the freeze-dried algal material were mixed with 100ml of 70% ethanol solvent, and the extraction process was carried out using a magnetic stirrer for two hours. The extract was then filtered using Waltman NO1 filter paper with a 0.2 diameter and transferred to large petri dishes. The solvent was evaporated at room temperature, and the extract was stored in the refrigerator at -20°C until further use.

4. Biosynthesis of silver nanoparticles

The biosynthesis of silver nanoparticles was performed according to the method of **Kumar *et al.* (2012)** by adding 10ml of the extract to 90ml of 1mM silver nitrate (AgNO3) solution and adjusting the pH to 11. The mixture was then heated at 60- 70°C for 30 minutes under dark conditions by wrapping the flasks with aluminium foil to prevent light exposure. The color change was monitored for 20- 30 minutes. Then, the mixture was left at room temperature (25°C) for two days. The prepared nano solution was centrifuged at 6000rpm for 30 minutes; the precipitated nanoparticles were collected, washed three times with distilled water, dried in an electric oven at 40°C, and stored in clean, sterilized glass bottles, then transferred to the refrigerator at 4°C.

Isolation and identification of compounds in algal extracts using gas chromatography-mass spectrometry

An Agilent Technologies 7890B G.C. system coupled with an Agilent Technologies 5977A MSD mass spectrometer was used to separate the components of the ethanol extract of the algae and identify the isolated chemical compounds. The operational conditions of the device included setting the oven temperature to 40°C for five minutes, then raising it to 300°C for twenty minutes. The flow rate of the carrier helium gas was 1ml/ min; the injection temperature was 290°C; the injection system was pulsed, and the injected sample volume was 0.5 microliters. The mass spectrometer temperature was 230°C, with a scan speed of N2 1562 and a mass range of 44- 750m/ z. The data were confirmed through the INST 2014, 2020 database library as an additional tool to confirm the identities of the compounds.

5. Characterization of silver nanoparticles

The synthesized silver nanoparticles, created using the ethanolic extract of *E. intestinalis*, were characterized through several techniques. UV-vis spectrophotometry was employed using a Shimadzu spectrophotometer, with the absorbance range recorded between 300- 700nm. The dried AgNPs were analyzed using a Jasco FTIR 4200 machine (Japan) in the region of 500- 4000cm^{-1} at room temperature; these spectra were recorded at the University of Basrah / Polymer Research Center. Scanning electron microscope (SEM) analyses were conducted using a TE-SCAN microscope (Czech Republic). X-ray diffraction analysis (XRD) was utilized to determine the crystalline nature of the synthesized AgNPs using a paralytical machine (Holland). SEM-EDX and XRD analyses were recorded at the University of Tehran, Islamic Republic of Iran.

6. Study of the antibacterial activity of silver nanoparticles

The antibacterial activity of the manufactured silver nanoparticles was evaluated against human pathogenic bacterial species, including *E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Staphylococcus aureus,* using a modified well diffusion technique. A 10^{-6}CFU/ ml concentration of bacteria was spread on petri dishes containing Mueller-Hinton agar using a cotton swab. Wells with a diameter of 6mm were punched into the agar, and 70 microliters of the nanoparticle solutions at concentrations of 62.5, 125, 250, 500, and 1000µg/ ml were applied to the agar. The plates were incubated at 37C^{o} for 24 hours. The control treatment included adding the same volume of the solvent DMSO and the algal extract to the agar wells instead of the silver nanoparticle solutions. DMSO was also used as a negative control. The diameter of the bacterial growth inhibition zone was measured in millimeters **(Naser *et al.*, 2023)**.

RESULTS

Morphological identification of alga

After examining the alga sample with a light microscope, it was determined based on their morphology that the sample belongs to the green alga *Enteromorpha intestinalis* (Fig. 1).

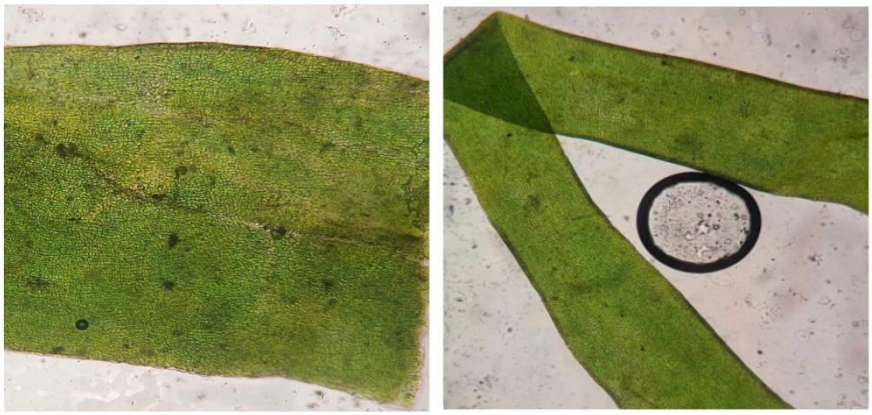


Fig. 1. Green alga *Enteromorpha intestinalis*

Division: Chlorophyta

Class: Ulvophyceae

Order: Ulvales

Family: Ulvaceae

Genus: Enteromorpha

Species: Enteromorpha intestinalis **(Linnaeus) Nees, 1820**

Isolation and diagnosis of compounds from the *E. intestinalis* algal extract using gas chromatography-mass spectrometry (GC-MS)

The components of the *E. intestinalis* algal extract were separated and identified using the gas chromatography-mass spectrometry (GC-MS) technique. Distinct bands representing the separated compounds of the algal extract appeared and were identified by the mass spectrometer, totalling 21 compounds (Fig. 2 & Table 1).

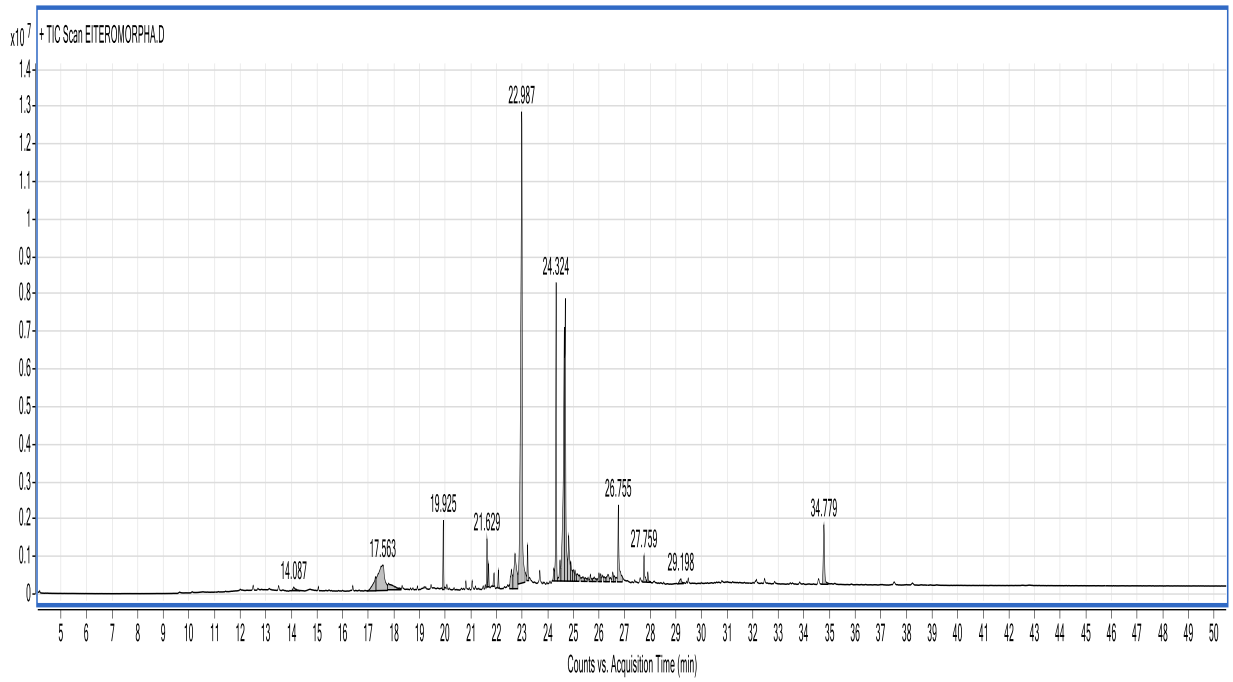


Fig. 2. The spectrum of the test results for the ethanolic extract of *E.intestinalis* using gas chromatography (G.C.) technology

Table 1. The compounds isolated from the extract of *E. intestinalis*

Peak	R.T.	Chemical formula	Molecular weight	Area%	Library/ID
1	19.925	$C_{18}H_{36}$	252.47	2.3077	3-Octadecene, (E)-
2	21.052	$C_{10}H_{14}O$	150.21	0.4187	(Z)-2,6-Dimethylocta-2,5,7-trien-4-one
3	21.629	$C_{20}H_{38}$	278.5	1.7103	Neophytadiene
4	21.69	$C_{18}H_{34}O_3$	298.5	0.8807	Carbonic acid, prop-1-en-2-yl tetradecyl ester
5	21.907	$C_{16}H_{22}O_4$	278.34	0.9052	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
6	22.077	$C_{20}H_{40}O$	296.5	0.7415	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
7	22.586	$C_{22}H_{32}O_2$	328.5	1.4995	Doconexent
8	22.722	$C_{16}H_{30}O_2$	254.41	1.5729	(E)-Hexadec-9-enoic acid
9	22.987	$C_{16}H_{32}O_2$	256.42	37.4024	n-Hexadecanoic acid
10	23.211	$C_{18}H_{36}O_2$	284.47	1.2569	Hexadecanoic acid, ethyl ester
11	23.693	$C_{16}H_{30}O_2$	254.41	0.6453	Palmitoleic acid
12	24.236	$C_{11}H_{24}$	156.31	0.5548	Undecane
13	24.324	$C_{20}H_{38}$	278.5	11.0838	Neophytadiene
14	24.48	$C_{16}H_{26}O$	234.38	0.9133	cis,cis,cis-7,10,13-Hexadecatrienal
15	24.65	$C_{18}H_{30}O_2$	278.42	17.3286	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-

16	24.684	$C_{18}H_{34}O_2$	284.47	10.2834	Oleic Acid
17	24.82	$C_{18}H_{36}O_2$	284.47	1.4246	Octadecanoic acid
18	26.755	$C_{22}H_{42}O_4$	370.56	3.6273	Hexanedioic acid, dioctyl ester
19	27.759	$C_{18}H_{36}$	252.47	0.9898	E-7-Octadecene
20	27.909	$C_{20}H_{40}$	280.53	0.3928	5-Eicosene, (E)-
21	34.779	$C_{12}H_{17}NO_2$	207.27	4.0601	Phenol, 6-methyl-2-[(4-morpholinyl)methyl]-

1- Mass spectrum of the compound n-hexadecanoic acid

Fig. (3) displays the mass spectrum of the compound n-hexadecanoic acid, which was separated at a retention time of 22.98 minutes. Upon computationally matching it with the database, the type of compound was identified. Its chemical formula was $C_{16}H_{32}O_2$, with a molecular weight of 256.42 Dalton. The area it occupied was 37.40% of the total area of the isolated compounds.

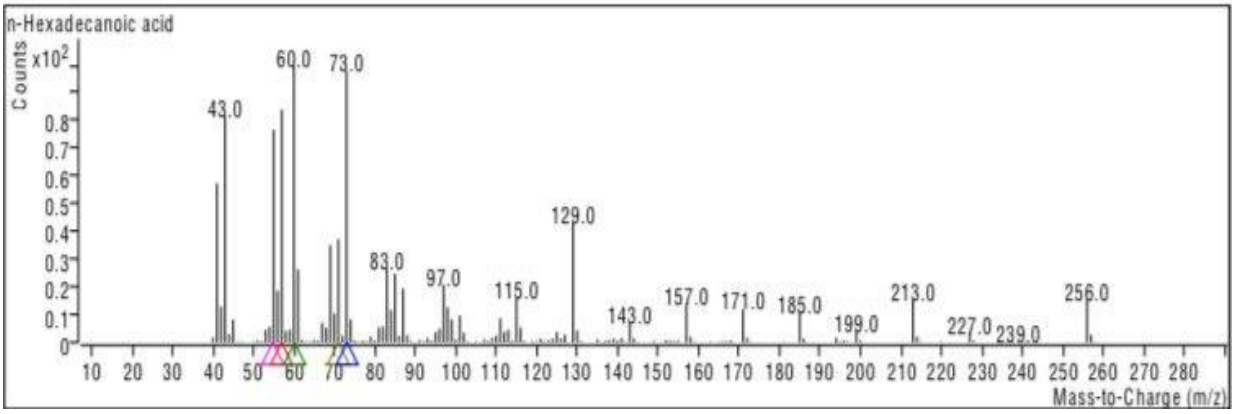


Fig. 3. Mass spectrum of the compound n-hexadecanoic acid isolated from *E.intestinalis*

2- The compound neophytadiene

Fig. (4) illustrates the mass spectrum of the compound neophytadiene, which was separated with a retention time of 24.32 minutes. Upon electronically matching it with the database, the type of the compound was identified, and its chemical formula was determined to be $C_{20}H_{38}$, with a molecular weight of 278.5 Daltons. The area it occupied was 11.08% of the total area of the isolated compounds.

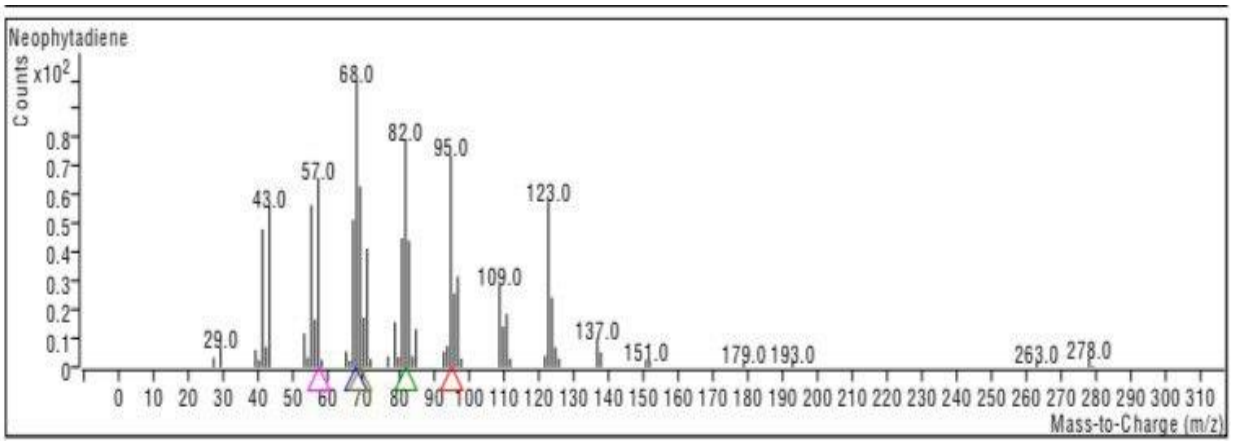


Fig. 4. Mass spectrum of the compound neophytadiene isolated from *E.intestinalis*

3- The compound 9, 12, 15-octadecatrienoic acid, -(Z, Z, Z)

Fig. (5) shows the mass spectrum of the compound 9, 12, 15-octadecatrienoic acid - (Z, Z, Z), separated with a retention time of 24.65 minutes. Upon matching it electronically with the database, the type of the compound was identified, and its chemical formula was determined to be $C_{18}H_{30}O_2$, with a molecular weight of 278.42 Daltons. The area it occupied was 17.32% of the total area of the isolated compounds.

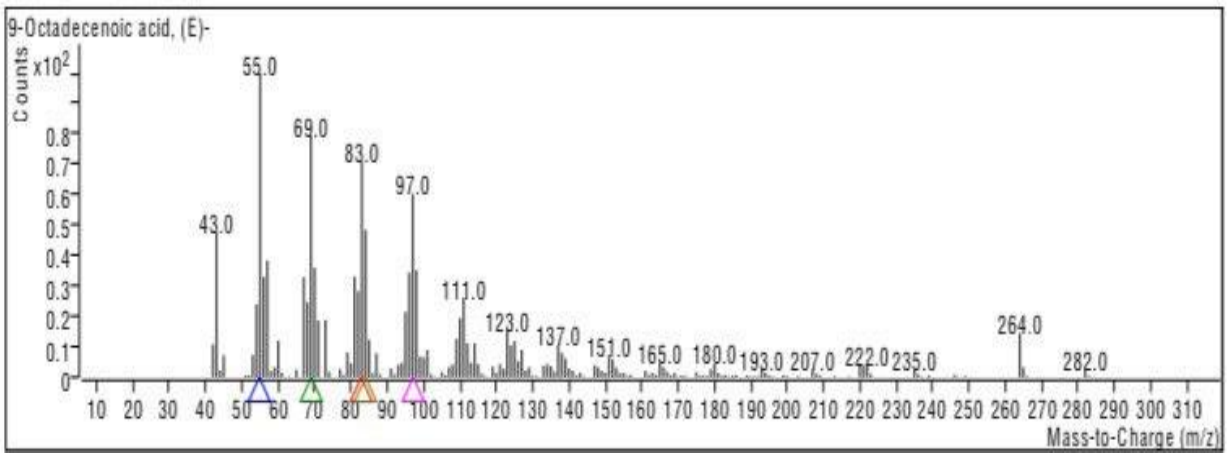


Fig. 5. Mass spectrum of the compound 9, 12, 15-octadecatrienoic acid, (Z, Z, Z)- isolated from *E.intestinalis*

4- The compound oleic acid

Fig. (6) signifies the mass spectrum of the compound oleic acid, which was separated with a retention time of 24.68 minutes. Upon matching it with the database electronically, the type of the compound was identified, and its chemical formula was determined to be $C_{18}H_{34}O_2$, with a molecular weight of 282.47 Daltons. The area it occupied was 10.28% of the total area of the isolated compounds.

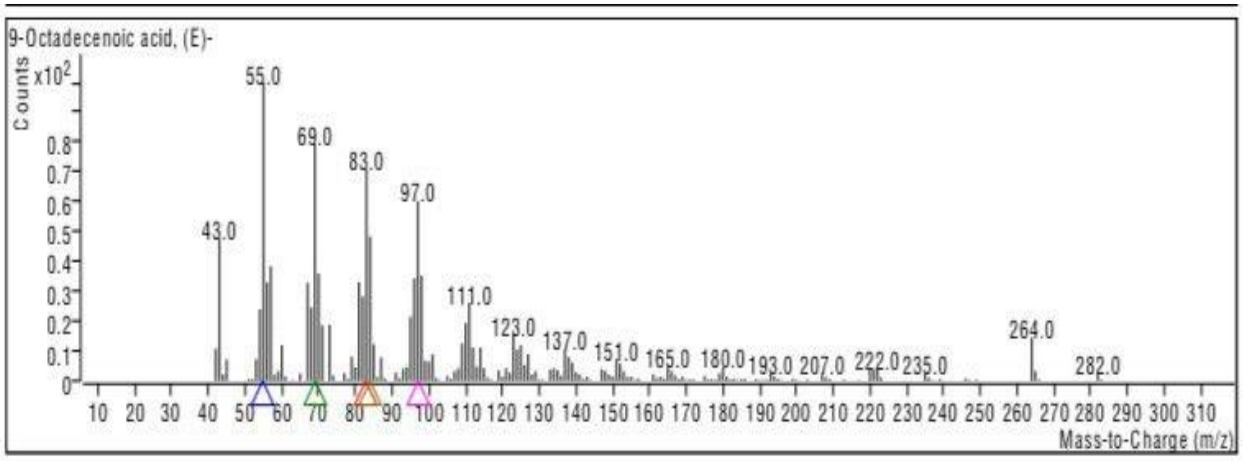


Fig. 6. Mass spectrum of the compound oleic acid isolated from *E.intestinalis*

Biosynthesis of silver nanoparticles from algal isolates under study

The results indicated that the extract from *E. intestinalis* can synthesize silver nanoparticles, as evidenced by the initial identification of a color change in the mixture of the extract with silver nitrate solution, which turned dark brown. This color change was an initial indicator of the algae's ability to fabricate silver nanoparticles, as shown in Fig. (7).

Fig. 7. Stages of color change

1- Ultraviolet-visible spectrophotometer analysis

The nanoparticles synthesized from the *E. intestinalis* algal extract were characterized using ultraviolet-visible spectrophotometry in the 300- 800nm wavelength range. The results indicated that the synthesized silver nanoparticles exhibited their highest peak at 435 nanometers, as shown in Fig. (8).

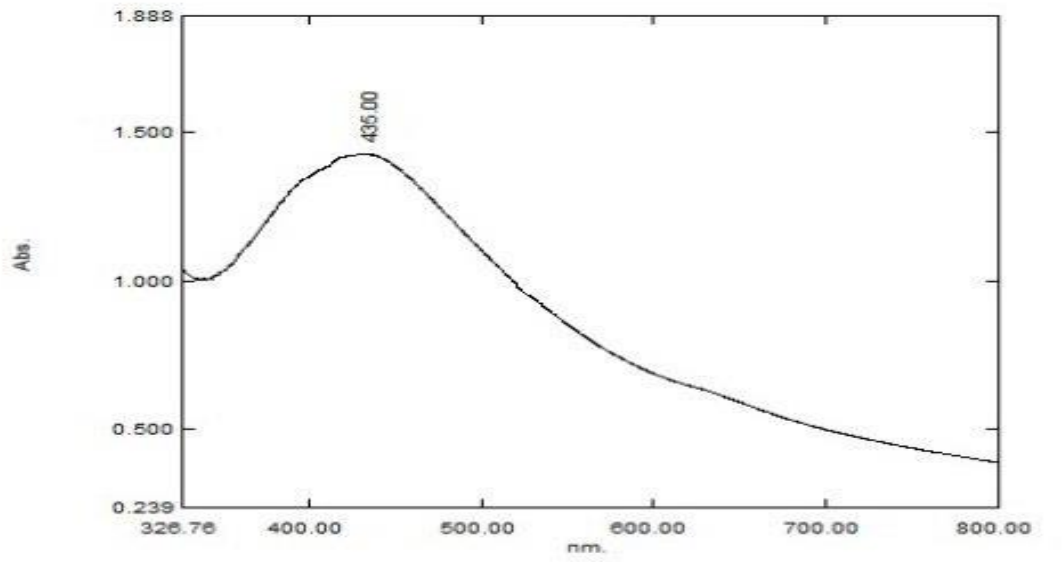


Fig. 8. Visible and ultraviolet absorption spectrum of *E. intestinalis*

2. Infrared spectroscopy using Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) analysis revealed the nature of the functional groups acting as reducing agents and capping agents for the silver nanoparticles. The findings are recorded in Table (2) and Fig. (9). The analysis revealed bands, in the *E. Intestinalis* extract, which appeared at frequencies of 3803 and 3734 cm^{-1}. These frequencies indicate the presence of amine N-H stretch, suggesting the existence of amine groups **(Nithya *et al.*, 2019)**. A band at 3259cm^{-1} indicated the presence of O-H stretch, which signifies the occurrence of hydroxyl groups found in alcohols or phenols **(Ibrahim *et al.*, 2014)**. Another band at 2922cm^{-1} indicated the presence of C-H stretch confirming the existence of alkanes **(Rahmi *et al.*, 2021)**. Moreover, a band at 2362cm^{-1} suggested the presence of C=O stretch implying carboxyl group in acids **(Basha *et al.*, 2008)**. Additionally, a band at 1631cm^{-1} pointed to C=O stretch due to amides represented by alkenes **(Grace *et al.*, 2020)**. Lastly, a band at 1424cm^{-1} emerged due to C-H stretch caused by the occurrence of alkanes **(Yu *et al.*, 2021)**. Moreover the presence of the C-N stretch is indicated by a band at 1043cm^{-1}, suggesting the existence of amines **(Wei *et al.*, 2021)**. Additionally, the presence of amine groups is indicated by a band at 842cm^{-1}, pointing to the existence of the amine N-H stretch **(Theivandran *et al.*, 2015)**.

FTIR measurements were conducted to verify the properties of silver nanoparticles, where the analysis of the nanoparticles produced from the *E. Intestinalis* extract referenced in Table (2) and Fig. (10) revealed bands at frequencies of 3479 and 3441cm^{-1}. These bands indicated the presence of the O-H stretch, suggesting the

existence of alcohols or phenols **(Moshfegh *et al.*, 2019)**. A band at 3311cm^{-1} indicated the amine N-H stretch's presence, pointing to the amine groups' presence **(Nithya *et al.*, 2019)**. A band at 3130cm^{-1} also suggested the presence of the O-H stretch, further indicating alcohols or phenols **(Abasi *et al.*, 2018)**. Furthermore, a band at 2918cm^{-1} indicated the presence of the asymmetric C-H stretch, which suggested the presence of alkenes **(Kathiraven *et al.*, 2015)**. A band at 2357cm^{-1} indicated the presence of the aromatic C=C stretch, suggesting the presence of alkenes **(Singh *et al.*, 2022)**. Moreover, a band at 1647cm^{-1} indicated the O-H stretch, pointing to the hydroxyl group **(Janadhanan *et al.*, 2009)**. The results also showed a band at 1038cm^{-1}, indicating the presence of the C-N stretch, which suggested the presence of aliphatic amines **(Wei *et al.*, 2021)**.

Table 2. The functional groups of the *E. intestinalis* extract and the nanoparticles derived from it

E. intestinalis extract			*E. intestinalis* (AgNPs)		
Wave number (cm-1)	Group	Compound class	Wave number (cm-1)	Group	Compound class
3803	NH2	Primary amine	3479	O-H	Alcohol
3734	NH2	Primary amine	3441	O-H	Alcohol
3259	O-H	Alcohol	3311	N-H	Primary amine
2922	C-H	Alkane	3130	O-H	Alcohol
2362	C-O	Carboxylic acid	2918	N-H	Alkane
1631	N-H	Primary amine	2357	C≡C	Alkyne
1424	C-H	Alkane (methyl group)			

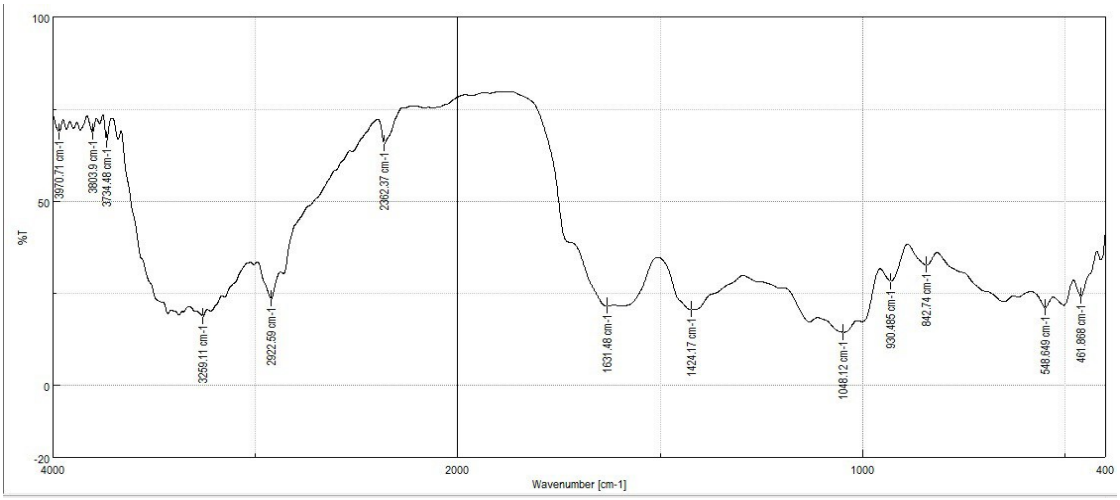


Fig. 9. Infrared spectrum of the extract from *E. intestinalis*

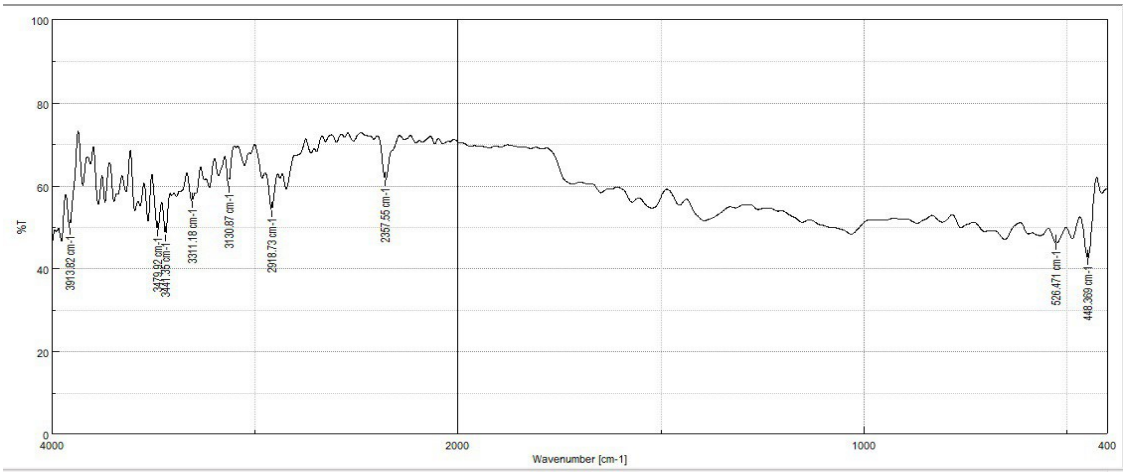


Fig. 10. Infrared spectrum of the nanoparticles fabricated from the extract *E. intestinalis*

3- Analysis using energy-dispersive X-ray spectroscopy (EDX)

Energy-dispersive X-ray spectroscopy analysis was conducted to identify the chemical elements composing the nanoparticles and their respective proportions. The spectrum of the nanoparticles derived from the extract of *E. intestinalis* revealed peaks corresponding to carbon, oxygen, chlorine, and silver, with proportions of 15.55, 4.41, 13.62, and 66.41%, respectively (Fig. 11).

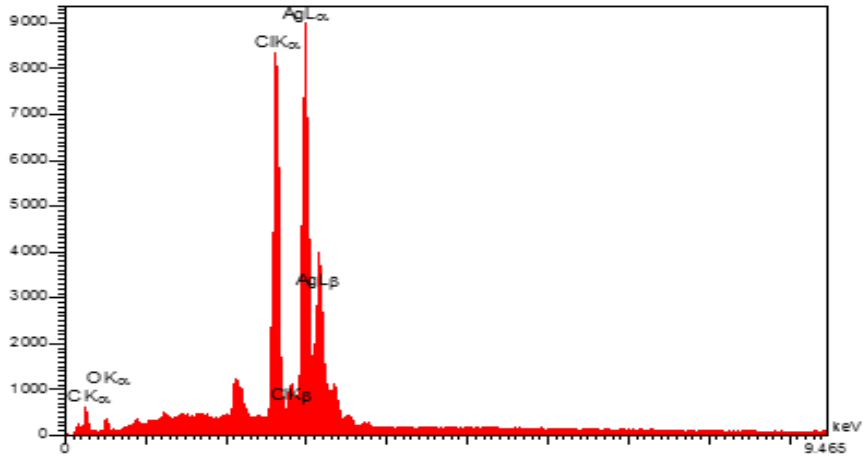


Fig. 11. Energy-dispersive X-ray spectroscopy analysis of the synthesized silver nanoparticles from the extract *E. intestinalis*

4- Scanning electron microscope (SEM) characterization

The scanning electron microscope (SEM) analysis results indicated that the nanoparticles fabricated using the alga extract were homogeneous in shape and densely packed. They featured pores or channels on their surface. The sizes of the nanoparticles were measured using the Image J software, revealing that the nanoparticles synthesized from the extract of *E. intestinalis* ranged between 47 & 78nm in size and had a semi-spherical shape (Fig. 12).

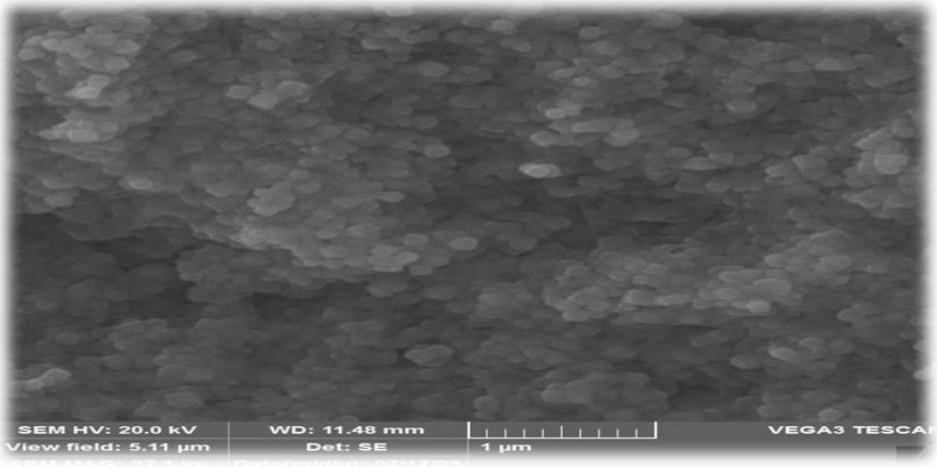


Fig. 12. Scanning electron microscope image of the nanoparticles fabricated from the extract of *E. intestinalis*

5- X-ray diffraction (XRD) characterization

The silver nanoparticles' crystalline size and physical structure were determined using X-ray diffraction (XRD) patterns. It was found that the silver nanoparticles synthesized from the extract of *E. Intestinalis* exhibited diffraction peaks at 2θ values of 27.82, 32.25, 46.30, 54.87, 57.53, 67.53, and 76.86, corresponding to the crystallographic planes of 111, 200, 220, 311, 222, 400, and 331, respectively (Fig. 13).

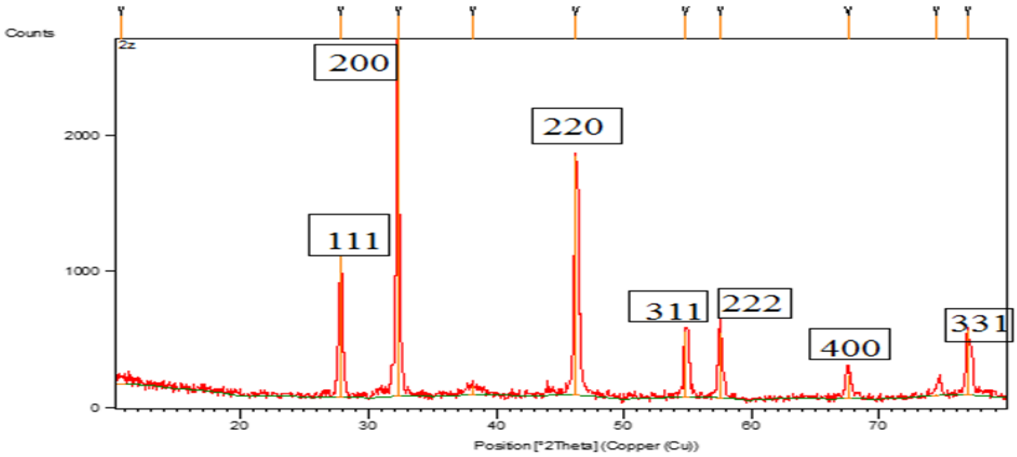


Fig. 13. X-ray diffraction of the nanoparticles fabricated from the extract of *E. intestinalis*

6- Bioactivity of silver nanoparticles synthesized using the extract of *E. intestinalis*

The bioactivity of silver nanoparticles produced with the extract of *E. intestinalis* was assessed against bacterial strains, including *E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Staphylococcus aureus* at concentrations of 62.5, 125, 250, 500, and 1000µg/ ml. The evaluation revealed a statistically significant difference at the $P \leq 0.05$ level, indicating that all the concentrations utilized inhibited effectively the pathogenic bacterial species under study, though to varying extents. The greatest zone of inhibition was observed at 22mm against *P. aeruginosa* at a concentration of 1000µg/ ml, while the smallest was 11.33mm against *E. coli* at a concentration of 62.5µg/ ml (Table 3 & Fig. 14).

In addition, the algal extract was used as a positive control and was found to be effective, although to a lesser extent than the nanoparticles, with close inhibition zone diameters. The largest zone of inhibition for the algal extract was 12.33mm against the bacterium *P. aeruginosa* at a concentration of 1000µg/ ml, while the smallest was 9.33mm against the bacteria *K. pneumoniae* and *S. aureus* at a concentration of 62.5µg/ ml. DMSO was used as a negative control group, and it was observed that it had no inhibitory activity (Table 4 & Fig. 14).

Table 3. The inhibition zone diameters for AgNPs synthezied by algal extract of *E. intestinalis*

Bacteria	Concentration (µg\ml)				
	1000	500	250	125	62.5
E. coli	20	18	15.67	13.67	11.33
K. pneumoniae	17	15.67	14.67	13.33	12
P. areginosa	22	18.67	17	15.67	14.67
S. aureus	20.67	19	18.33	17.33	15

Table 4. The inhibition zone diameters for the extract of *E. intestinalis*

Bacteria	Concentration (µg\ml)				
	1000	500	250	125	62.5
E. coli	12	11.67	11.33	11	10.33
K. pneumoniae	11.33	11	10.33	10	9.33
P. aeruginosa	12.33	11.67	11.33	11	10.33
S. aureus	10.67	10.33	10	9.67	9.33

Furthermore, the results of the study, when comparing the nanoparticles with the extract, demonstrated a statistically significant difference at the $P \leq 0.05$ level, indicating that the nanoparticles were more effective than the algal extract in inhibiting pathogenic bacteria across all concentrations (62.5, 125, 250, 500, and 1000µg/ ml). The nanoparticles produced different inhibition zone diameters with respect to *E. coli*; they were 11.33, 13.67, 15.67, 18, 20mm, respectively. In contrast the inhibition zones for *K. pneumoniae* were 12, 13.33, 14.67, 15.67, 17mm, respectively. For *P. aeruginosa*, the inhibition zones were 14.67, 15.67, 17, 18.67, 22mm, respectively, whereas for *S. aureus*, the zones were 15, 17.33, 18.33, 19, 20.67mm, respectively.

In contrast, the extract of the algae *E. intestinalis* provided inhibitory diameters for the aforementioned concentrations as follows: for *E. coli*, the inhibition zones were 10.33, 11, 11.33, 11.67, 12mm, respectively; for *K. pneumoniae*, the zones were 9.33, 10,

10.33, 11, 11.33mm, respectively; for *P. aeruginosa*, the inhibition zones were 10.33, 11, 11.33, 11.67, 12.33mm, respectively; and for *S. aureus*, the zones were 9.33, 9.67, 10, 10.33, 10.67mm, respectively.

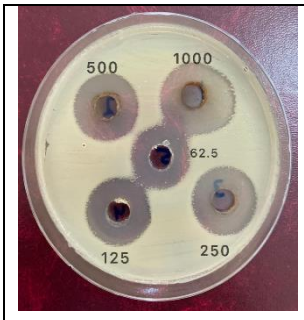
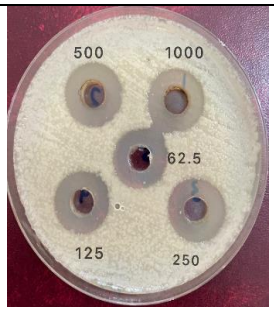
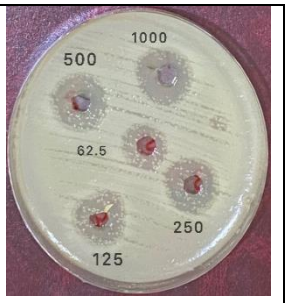
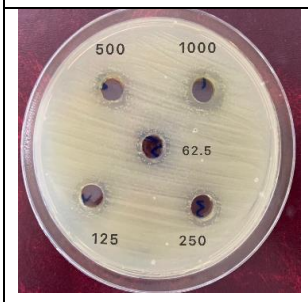
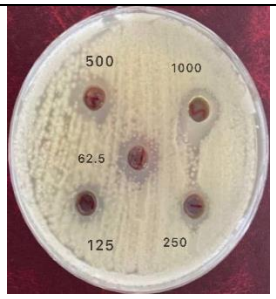
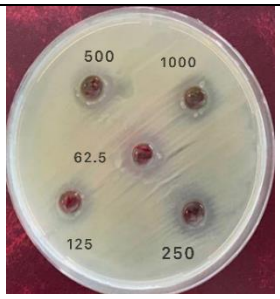
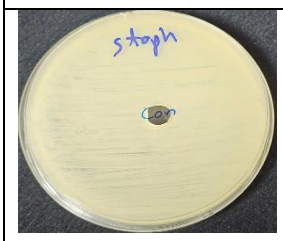
			
AgNPs *S. aureus*	AgNPs *K. pneumoniae*	AgNPs *P. aeruginosa*	AgNPs *E. coli*
			
Extrasct *S. aureus*	Extrasct *K. pneumoniae*	Extrasct *P. aeruginosa*	Extrasct *E. coli*
			
Control *S. aureus*	Control *K. pneumoniae*	Control *P. aeruginosa*	Control *E. coli*

Fig. 14. Diameters of inhibition zone

DISCUSSION

The green synthesis of silver nanoparticles is a biologically favored method, often involving biodegradable and biocompatible materials that act to reduce, encapsulate, and stabilize the nanoparticles, as postulated in the previous studies of **Singh *et al.* (2015)**, **Ocsoy *et al.* (2018)** and **Javed *et al.* (2020)**. Algae are widely recognized as sources for synthesis due to their high levels of proteins, peptides, sugars and pigments. This makes them appealing for use in bioreactors **(Negi & Singh 2018)**. A study using gas chromatography mass spectrometry identified 21 compounds. The abundant ones were n-hexadecanoic acid, neophytadiene, 9, 12, 15 octadecatrienoic acid -(Z, Z, Z)- and oleic acid. The compound n-hexadecanoic acid was extracted from *Ipomoea eriocarpas* extract and has shown effectiveness against both Gram-positive and Gram-negative bacteria **(Ganesan, *et al.*, 2022)**. Neophytadiene was isolated from *Eupatorium odoratum* and has exhibited antibiotic properties against bacteria and fungi, while also possessing antioxidant properties **(Venkata *et al.*, 2012)**. Another compound called 9, 12, 15 octadecatrienoic acid -(Z Z Z) was found in the leaves of *Jatropha curcas* and has been discovered to have beneficial properties **(Rahman *et al.*, 2014)**. A study by **Yoon *et al.* (2018)** demonstrated that oleic acid shows an antibacterial activity against Gram-positive bacteria by damaging their cell membrane, explaining the algal extract's ability to inhibit the bacterial species under study.

FTIR analysis was performed to detect the active groups that contributed to the reduction, encapsulation, and stabilization of the nanoparticles, finding that free electrons belonging to O.H., COOH, and NH2 groups played a role **(Basolgu & Iskefiyeli, 2023)**. UV-vis spectroscopy within the 400- 500nm range confirmed the excitation of the surface plasmon resonance of AgNPs **(Ibrahim, 2015; Salari, 2016)**. EDX analysis revealed a silver content of 66% in the synthesized nanoparticles, similar to the results obtained by other researchers using *Anabaena variabilis*, which also showed a 66% silver content **(Ahmad *et al.*, 2021)**. SEM analysis confirmed that the silver nanoparticles were semi-spherical and ranged in size from 47- 78nm, comparable to nanoparticles synthesized from *Zygnema carinthiacum* extracts, which were spherical and ranged in size from 19-52nm **(Alkinani *et al.*, 2023)**. XRD analysis confirmed the crystalline nature of the silver nanoparticles, showing the highest diffraction values at 2θ corresponding to the crystal planes of silver, indicating that silver was the main component in the production of AgNPs, while the lower peaks in XRD results were due to the presence of biomolecules in the extract used for synthesis **(Annadalakshmi *et al.*, 2016)**.

The study's findings revealed that silver nanoparticles have the ability to hinder the growth of both Gram-positive and Gram-negative bacterial species that were examined. This is achieved through the interaction of the nanoparticles with thiol groups on the surface of the bacterial cells. Consequently, the nanoparticles inhibit ATP

synthesis, disrupt the proton motive force, interfere with phosphate flow systems, detach the cell membrane from the cytoplasm, cause the release of intracellular content, induce DNA condensation, and prevent reproduction **(Marambio-Jones & Hoek, 2010)**. The results showed the highest inhibition zone diameter of 22mm against *P. aeruginosa* at a concentration of 1000µg/ ml, while the smallest was observed at 11.33mm against *E. coli* at a concentration of 62.5µg/ ml. These findings are similar to those of **Shareef *et al*. (2021)**, who reported the highest inhibition diameter of 22mm against *E.coli* at concentrations of 1000 and 500µg/ ml and the smallest diameter of 11mm against *E.coli, E. cloacae,* and *P. aeruginosa* at a concentration of 62.5µg/ ml.

CONCLUSION

The ethanolic extract of alga *Enteromorpha Intestinalis* proved to be an effective source for the biosynthesis of silver nanoparticles. These synthesized silver nanoparticles were characterized through a spectroscopic analysis (UV-vis, FTIR ,EDX, and XRD), and their antibacterial activity was tested against pathogenic bacterial species, such as *E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Staphylococcus aureus* at concentrations ranging from 62.5 to 1000µg/ ml. The silver nanoparticles synthesized from the *E. intestinalis* extract were highly effective against Gram-positive and Gram-negative bacteria. The results indicated that their antibacterial activity increased with higher concentrations of AgNPs, with *P. aeruginosa* being the most susceptible bacterial species.

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