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The Biosynthesis of Silver Nanoparticles by the Extract of the Algae Cladophora crispata and Testing their Antibacterial Activity.

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Abstract

The present research aimed to investigate the potential of the ethanolic extract of Cladophora crispata algae for synthesizing AgNPs. The GC-mass technique was used in the identification of active compounds present in the algal ethanolic extract. The extract's capability to synthesize silver nanoparticles was examined, as well as the formation of these nanoparticles was deduced by the color transformation of the mixture from light green to dark brown. Some of the techniques used to characterize the synthesized nanoparticles include an Ultraviolet-Visible spectrophotometer (UV-vis), Fourier Transform Infrared (FTIR) spectroscopy, a Scanning Electron Microscope (SEM), and Energy-Dispersive X-ray spectroscopy (EDX). They were also characterized by X-ray Diffraction (XRD). The antibacterial effectiveness was tested through the Agar well diffusion method. The GC-mass results indicated that the ethanolic extract of the algae contains different active compounds with different percentages of each, such as Oleic acid (11.7%), Neophytadiene (12.0%), and n-Hexadecanoic acid (283.1%). UV-vis absorption spectroscopy was used to confirm the formation of the silver nanoparticles, which appeared at a wavelength of 417nm. The XRD analysis confirmed the crystalline nature of the resulting silver nanoparticles, while SEM revealed that the AgNPs were spherical with particle sizes between 29-99 nm. EDX analysis revealed that the synthesized silver nanoparticles were composed of 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.

Keywords: Biosynthesis , Silver Nanoparticles , Extract , Cladophora crispata , Antibacterial activity

1- Introduction

Nanotechnology is a science that deals with the use and studying of the molecule's and particle's applications at the nanoscale, dimensions do not exceed 100 nanometers, as nano is a Greek word derived from the word nano and means dwarf, and this unit represents a part of a billion.

Therefore, the nanometer is equivalent to 10^{-9} a meter, equivalent to three carbon atoms lined up next to each other (Madkhour, 2019).

One of the newest sciences is nanotechnology, which focuses on creating particles with nanoscale dimensions that differ from the metals they are made of in terms of their properties (Rao and Gan, 2017).

Nanoscience has become one of the most interesting sub-disciplines of life sciences research (Bayda et al., 2019). Many studies have revealed the enormous potential of the science of nanotechnology in the field of biomedicine and this is due to the discovery and treatment of numerous ailments in human beings (Kinnear et al., 2017).

There are many types of diagnosis, delivery and molecular imaging research, all these research are very effective and many new nanomedical products like nano pharmaceuticals, nanoparticles to carry and deliver drug and antibacterial nanoparticles have been invented (Weissing et al., 2014).

Nanotechnology is an emerging interdisciplinary field of study that focuses on manipulating materials and structures at the nanoscale. these tiny particles called nanoparticles are made from algae, this is because algae has some qualities that make it suitable for this purpose. For example, they have a high capacity of adsorbing metals as well as decreasing their ions. They are also relatively cheap to use and can yield massive quantities of nanoparticles. Also, algae are easy to cultivate and handle, especially in a laboratory. They are more adapted to withstand the environmental effects than other organisms; they have short generation times and are not known to produce toxic compounds to the environment (Negi and Singh, 2018).

Nanoscience, which focuses on the production of dried and dry biomass can also be utilized in the biosynthesis of nanoparticles, therefore referred to as biofactories (Omar et al., 2017).

Nanoparticle production through biomass is a very simple process which does not require any extra steps; instead of cultivating the live algae, nanoparticles are produced in two ways: one is intracellular way, where particles are formed inside the algae body, and the other one is extracellular way in which active compounds are secreting outside the body of algae and hence the reduction of silver ions (Rahman et al., 2020).

Thus, an environmentally friendly formula was developed for its commercial availability to address diseases affecting fish. They examined the efficiency of the manufactured nanoparticles against the fish pathogens: vibrio alginolyticus, vibrio anauillarum, vibrio harveyi, and vibrio parahaemolyticus. It was also observed that Vibrio harveyii and Vibrio parahaemolyticus had the highest activity of silver nanoparticles (Fatima et al., 2020).

Another study involved the generation of silver nanoparticles by green algae chlorella vulgaris to evaluate the antibacterial properties against gram-positive S. aureus, and the inhibition rate was recorded as 98%. Dasilva et al., (2017) investigated the efficiency of AgNPs on Klebsiella pneumonia that belong to Gram negative bacteria and found that the cell survival rate can be determined by the concentration used in the experiment, which noted the increase of inhibition is positively related to higher concentration. The study of Bhuyar et al., (2020) demonstrated

that pandina sp. (green algae) may considered a potential source for the production of AgNPs, which can serve as an alternative antibiotic, and proved that AgNPs have effective efficiency against some infectious bacteria like; Staphylococcus aureus and pseudomonas aeruginosa, depending on these result AgNPs production by pandina sp can have substantial effect on antibiotic-resistant bacteria. In another study, the antibacterial effectiveness of prepared AgNPs by using the aqueous extract of green algae Caulerpa serrulate was found to have a significant effect against some bacterial species like E.coli, Pseudomonas aeruginase, Salmonella sp., and Staphylococcus aureus (Aboelfetoh et al., 2017).

The antimicrobial activity of Padina tetrastomatica nanoparticles was also tested, and the results proved the efficiency against different bacterial strains such as Klebsiella planticola, Pseudomonas aeruginosa, and Bacillus subtilis (Rajeshkumar et al. 2012).

2-Methodology

2.1 Collection of Algal Samples

Algal mass was directly collected from the different water area of Kutiban-Shatt Al-arab in Basrah Governorate in southern Iraq. Net was used for collection, and the algal sample placed in sterile plastic containers, after which they were brought to the laboratory. Algal sample was washed with tap water to remove impurities. The sample washed several time with distilled water to ensure cleanliness. The sample was examined under light microscope to determine the type of isolated alga, then morphological identified based on taxonomic references.

2.2 Identification and purification of Algal Isolates

Cladophora crispata was identified phenotypically by preparing temperorary glass slides and examined under light microscope to determined its morphological characteristics and identification based on taxonomic references (Prescott, 1975; Bourrely,1980). sonicator apparatous used to isolate diffierent microorganism (Bacteria and fungi) attached to the alga and thus obtain a pure isolated alga (Axenic calture).

2.3 Preparation of the Ethanol Extract

3 grams of the freeze-dried algal material were mixed with 100 ml 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 evaporated at room temperature, and the extract was stored in the refrigerator at -20 °C until use.

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2.5 Biosynthesis of Silver Nanoparticles

The biosynthesis of silver nanoparticles was performed according to the method of Kumar et al. (2012) by adding 10 ml of the extract to 90 ml of 1 mM 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 6000 rpm 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.

2.6 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 Identified 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 1 ml/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 44-750 m/z. The data was confirmed through the INST 2014,2020 database library as an additional tool to confirm the identities of the compounds.

2.7 Characterization of Silver Nanoparticles

The synthesized silver nanoparticles, created using the ethanolic extract of C. crispata, were characterized through several techniques. UV-vis spectrophotometry was employed using a Shimadzu spectrophotometer, with the absorbance range recorded between 300-700 nm. The dried AgNPs were analyzed using a Jasco FTIR 4200 machine (Japan) in the region of 500-4000 cm^{-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.

2.8 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, Klebssiella 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 6 mm were punched into the agar, and 70 microliters of the nanoparticle solutions at concentrations of (62.5, 125, 250, 500, and 1000) µg/ml were applied to the agar. The plates were incubated at 37C° 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 millimetres (Naser et al. 2023).

3-Results and Discussion

3.1 Morphological identification of alga

After examining the alga sample with a light microscope, its determined based on their morphology, that it belong to the green alga Cladophora crispata (Figure 1).

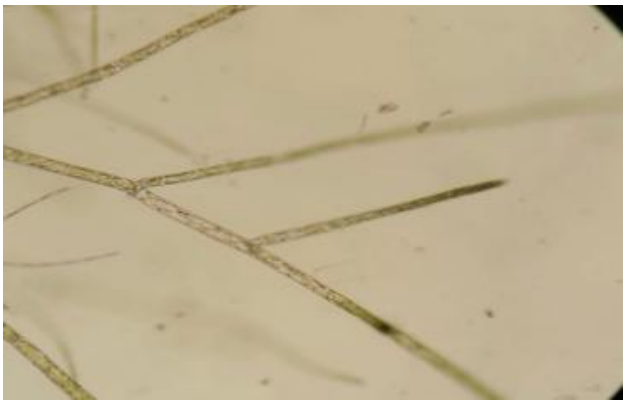

Figure 1: Green alga Cladophora crispata

Division: Chlorophyta

Class: Ulvophyceae

Order: Cladophorales

Family: Cladophoraceae

Genus: Cladophora

Species: Cladophora crispata (Roth) Kutzing 1843

3.2 Isolation and Diagnosis of Compounds from the C. crispata Algal Extract Using Gas Chromatography-Mass Spectrometry (GC-MS)

The components of the C. crispata 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 28 compounds (Figure 1, Table 1).

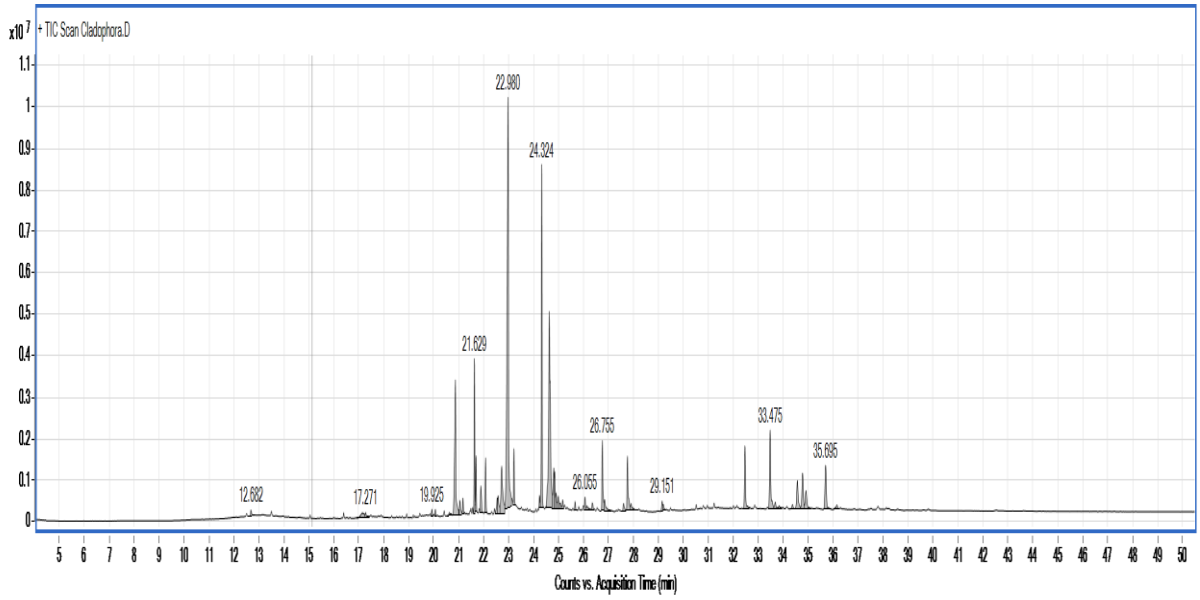

Figure (2): The Spectrum of the test results for the ethanolic extract of the alga C. crispata using Gas Chromatography (G.C.) technology.

Table (1) illustrates the compounds isolated from the extract of the alga C. crispata.

Peak	R.T.	Chemical formula	Molecular weight	Area%	Chemical compound
1	20.869	$C_{14}H_{28}O_2$	228.37	8.3967	Tetradecanoic acid
2	21.052	$C_{10}H_{14}O$	150.21	0.6573	(Z)-2,6-Dimethylocta-2,5,7-trien-4-one
3	21.168	$C_{13}H_{26}O_2$	214.34	0.4969	Undecanoic acid, ethyl ester
4	21.629	$C_{20}H_{38}$	278.5	4.7745	Neophytadiene
5	21.69	$C_{18}H_{36}O$	268.47	1.907	2-Pentadecanone, 6,10,14-trimethyl-
6	21.887	$C_9H_{18}O$	142.23	1.3049	Cyclohexanol, 2-(1-methylethyl)-
7	22.077	$C_{20}H_{40}O$	296.5	1.9129	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
8	22.539	$C_{16}H_{32}O_2$	256.42	0.6324	Pentadecanoic acid, 14-methyl-, methyl ester
9	22.722	$C_{16}H_{30}O_2$	254.41	2.4696	Palmitoleic acid
10	22.98	$C_{16}H_{32}O_2$	256.42	28.1011	n-Hexadecanoic acid
11	23.211	$C_{18}H_{36}O_2$	284.47	1.9526	Hexadecanoic acid, ethyl ester
12	24.236	$C_{19}H_{36}O_2$	296.48	0.5457	11-Octadecenoic acid, methyl ester
13	24.324	$C_{20}H_{38}$	278.5	12.0734	Neophytadiene
14	24.63	$C_{18}H_{34}O_2$	282.5	11.722	Oleic Acid
15	24.806	$C_{18}H_{36}O_2$	284.4	0.8959	Octadecanoic acid
16	25.662	$C_{11}H_{22}O_2$	218.36	0.3107	4-n-Hexylthiane, S,S-dioxide
17	26.055	$C_{13}H_{24}O_2$	212.33	0.3872	Oxacyclotetradecan-2-one
18	26.354	$C_{20}H_{36}O_2$	308.5	0.3404	Linoleic acid ethyl ester
19	26.755	$C_{22}H_{42}O_4$	370.56	3.0598	Hexanedioic acid, dioctyl ester
20	27.603	$C_{18}H_{34}O_2$	282.5	0.3514	cis-13-Octadecenoic acid
21	27.759	$C_{19}H_{38}O_4$	330.5	2.6617	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
22	29.151	$C_{10}H_{14}O$	150.22	0.3011	2-tert-Butylphenol, tert-butyldimethylsilyl ether
23	32.464	$C_{27}H_{44}$	368.6	2.8598	Cholesta-3,5-diene

24	33.475	$C_{28}H_{46}O$	398.7	3.9738	Ergost-5,8(14)-dien-3-ol
25	34.568	$C_{29}H_{48}$	396.7	1.7645	Stigmasta-3,5-diene
26	34.772	$C_{23}H_{20}$	296.41	2.1448	9-Isopropyl-10-phenylanthracene
27	34.908	$C_{13}H_{22}OSi$	250.48	1.2798	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-
28	35.695	$C_{30}H_{50}O$	426.72	2.7222	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-

3.1 he Compound n-Hexadecanoic acid

Figure 4 shows the mass spectrum of the compound n-Hexadecanoic acid, separated with a retention time of 22.98 minutes. Upon matching it with the database electronically, the type of the compound was identified, and its chemical formula was determined to be $C_{16}H_{32}O_2$, with a molecular weight of 256.42 Daltons. The area it occupied was 28.10% of the total area of the isolated compounds.

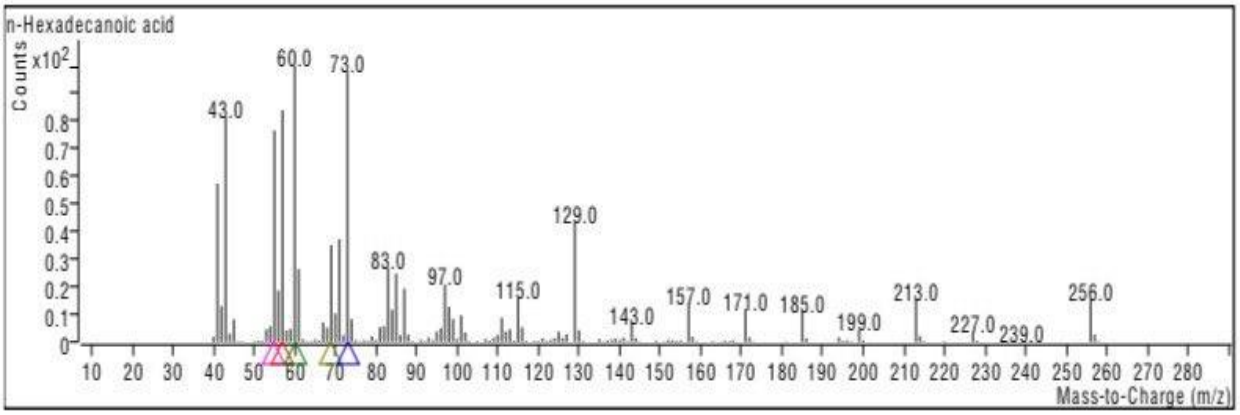

Figure 3: Mass Spectrum of the Compound n-Hexadecanoic acid isolated from the Alga C. crispata

3.2 The Compound Neophytadiene

Figure 3 illustrates the mass spectrum of the compound Neophytadiene, which was separated with a retention time of 24.32 minutes. Upon matching it with the database electronically, 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 12.07% of the total area of the isolated compounds.

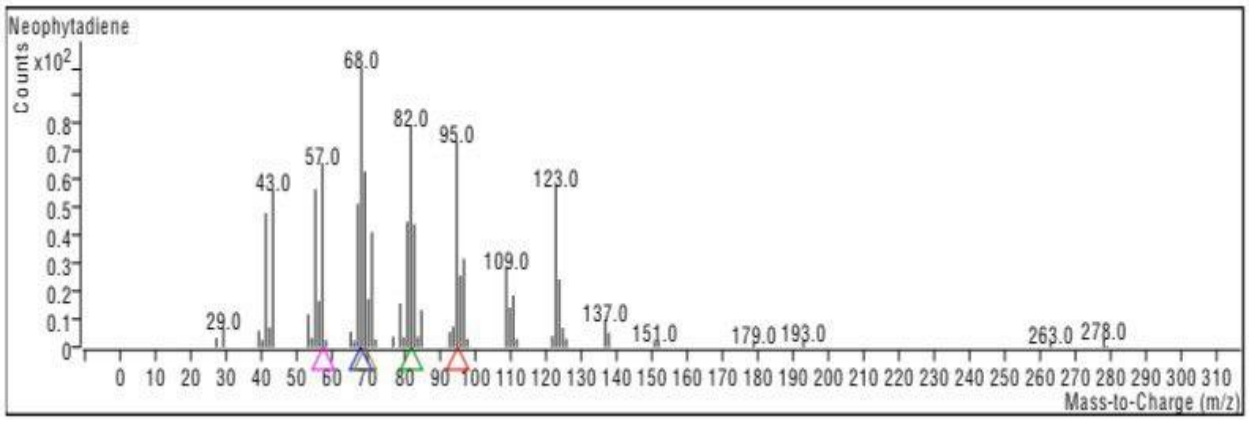

Figure 4: Mass Spectrum of the compound Neophytadiene isolated from the Alga C. crispata

3.3 The Compound Oleic Acid

Figure 5 illustrates the mass spectrum of the compound Oleic Acid, which was separated with a retention time of 24.63 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 282.5 Daltons. The area it occupied was 11.72% of the total area of the isolated compounds.

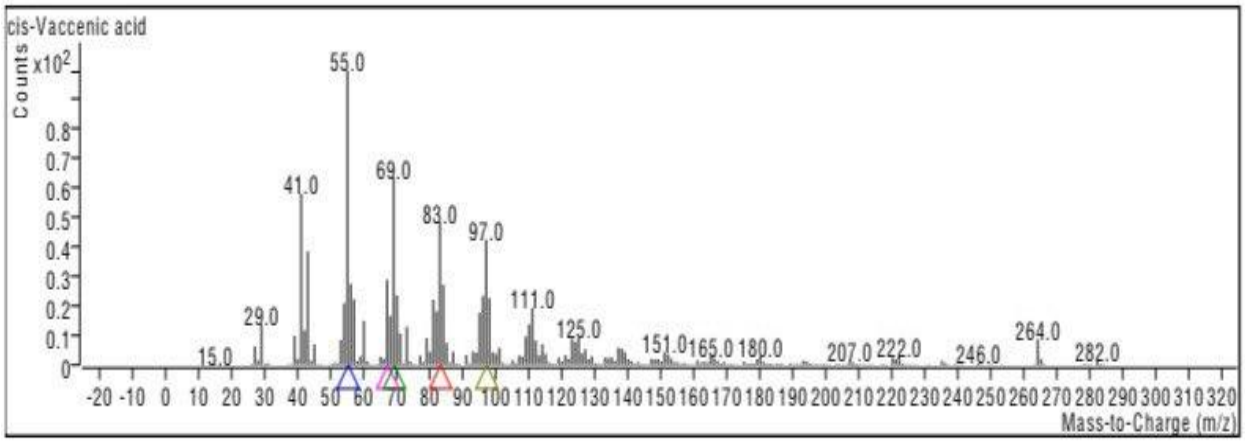

Figure 5: Mass Spectrum of the compound Oleic Acid isolated from the Alga C. crispata

A study using gas chromatography mass spectrometry identified 28 compounds. The abundant ones were Hexadecanoic acid, Neophytadiene and Oleic acid. The compound n-Hexadecanoic acid was extracted from Ipomoea eriocarpas extract has shown effectiveness against both Gram positive and Gram negative bacteria. Neophytadiene was isolated from Eupatorium odoratum has exhibited antibiotic properties, against bacteria and fungi while also possessing antioxidant properties (Venkata et al., 2012). A study by Yoon et al., 2018, demonstrated that Oleic Acid shows antibacterial activity against Gram-positive bacteria by damaging their cell membrane, explaining the algal extract's ability to inhibit the bacterial species under study.

3.4 Biosynthesis of Silver Nanoparticles from Algal Isolates:

The results indicated that the extract from C. crispata can synthesise silver nanoparticles, as evidenced by the initial Identified of a colour 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 Figure 6.

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 (Javed et al., 2020; Ocsoy et al., 2018). 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 and Singh 2018).

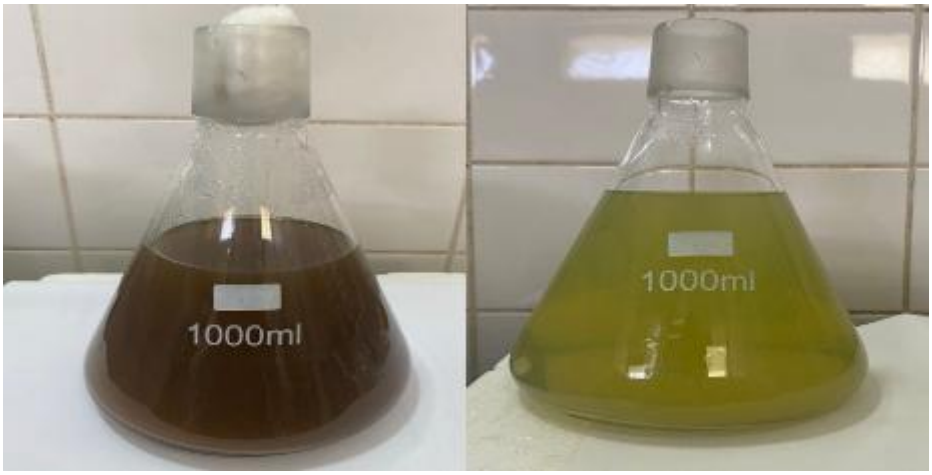

Figure 6: Color Change.

1- Ultraviolet-Visible Spectrophotometer Analysis

The nanoparticles synthesized from the C. crispata algae extract were characterized using ultraviolet-visible spectrophotometry in the (300-800) nm wavelength range. The results indicated that the synthesized silver nanoparticles exhibited their highest peak at 417 nanometers, as shown in Figure 7.

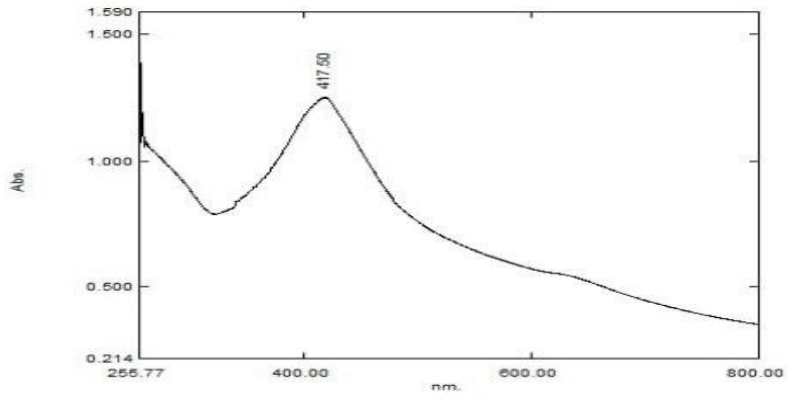

Figure 7: Visible and Ultraviolet Absorption Spectrum of the Alga C. crispata

UV-vis spectroscopy within the 400-500 nm range confirmed the excitation of the surface plasmon resonance of AgNPs (Salari et al. 2016; Ibrahim, 2016).

2- Infrared Spectroscopy using Fourier Transform Infrared Spectroscopy (FTIR)

The diagnostic results obtained from Fourier transform infrared spectroscopy have revealed the nature of active compounds, which act as reducing and capping agents for silver nanoparticles.

The recorded results are presented in the table (2) and figures (8).

A beam showed up at a frequency of 3744 cm^{-1} in the study of C. crispate algae extract, showing that N-H stretch amine aggregates were present in the extract (Nithya et al., 2019).

A beam also appeared at 3666 cm^{-1}, 3593 cm^{-1},3505 cm^{-1}, and 3419 cm^{-1} indicating O-H stretching and hydroxyl groups in alcohols and phenols (Ibrahim et al., 2014).

a package appeared at the frequency 3317 cm^{-1}, which indicated the presence of N-H stretch, which indicates the presence of amine groups; a package appeared at the frequency 3180 cm^{-1}, which indicates the presence of O-H which indicates the presence of hydroxyl group in alcohols; a package appeared at the frequency 2918 cm^{-1} , which indicated the presence of C-H stretch, which indicates the presence of alkanes, as well as the appearance of a beam at the frequency 2334 cm^{-1}, which indicates while the aromatic bond C = C showed that alkenes were present, the appearance of a bundle at 1642 cm^{-1} showed that the amino bond N- H stretch was present, which meant that a amines were present.

FTIR measurements were performed to verify silver nanoparticles, as the results of the analysis of silver nanoparticles manufactured from C. crispata algae extract table (2) and figure (9) showed the appearance beams at frequencies 3870 cm^{-1} and 3773 cm^{-1}, which indicated the presence of amino bond N-H stretching, indicating the presence of amine group.The beam at 3320 cm^{-1} indicated the presence of amine groups and the N-H stretch amino bond.We observed a beam at the beam at 3049 cm^{-1} and 2921 cm^{-1}, indicating the presence of the asymmetry C- H stretch and alkane. A beam appeared at the frequency 2368 cm^{-1}, which indicated the presence of aromatic bond C = Which indicates the presence of alkynes; a package appeared at the frequency 1695 cm^{-1} which indicated the presence of aromatic compounds; a package appeared at 1641 cm^{-1}, which indicated the presence of N-H stretch, which indicates the presence of amines; and a package appeared at 1542 cm^{-1}, which indicated the presence of N-O stretch, which indicates the presence of organic Nitro compounds.

Table 2: Illustrates the Functional Groups of the C. crispata Algae Extract and the Nanoparticles Derived from It.

C. crispata extact			**C. crispata (AgNPs)**		
Wave number (cm^{-1})	**Group**	**Compound class**	**Wave number (cm^{-1})**	**Group**	**Compound class**
3882	NH2	Primary amine	3870	NH2	Primary amine
3744	NH2	Primary amine	3773	NH2	Primary amine
3666	O-H	Alcohol	3673	O-H	Alcohol
3593	O-H	Alcohol	3558	O-H	Alcohol
3505	O-H	Alcohol	3478	N-H	Primary amine
3419	O-H	Secondary amine	3320	N-H	Secondary amine
3317	N-H	Alcohol	3049	C-H	Alkene
3180	O-H	Alkane	2921	C-H	Alkane

2918	C-H	Alkane	2368	C≡C	Alkyne
2334	C≡C	Alkyne	1695	C-H	Aromatic compound
1642	N-H	Amine	1641	N-H	Amine
			1542	N-O	Nitro compound

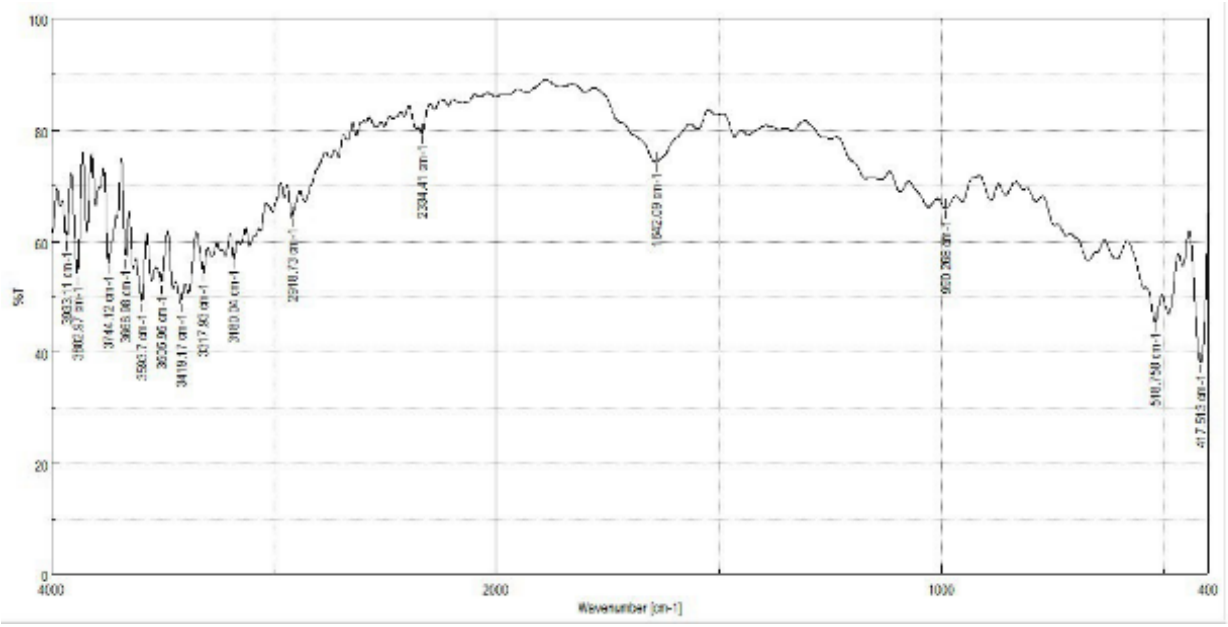

Figure 8: Infrared Spectrum of the Extract from the Alga C. crispata

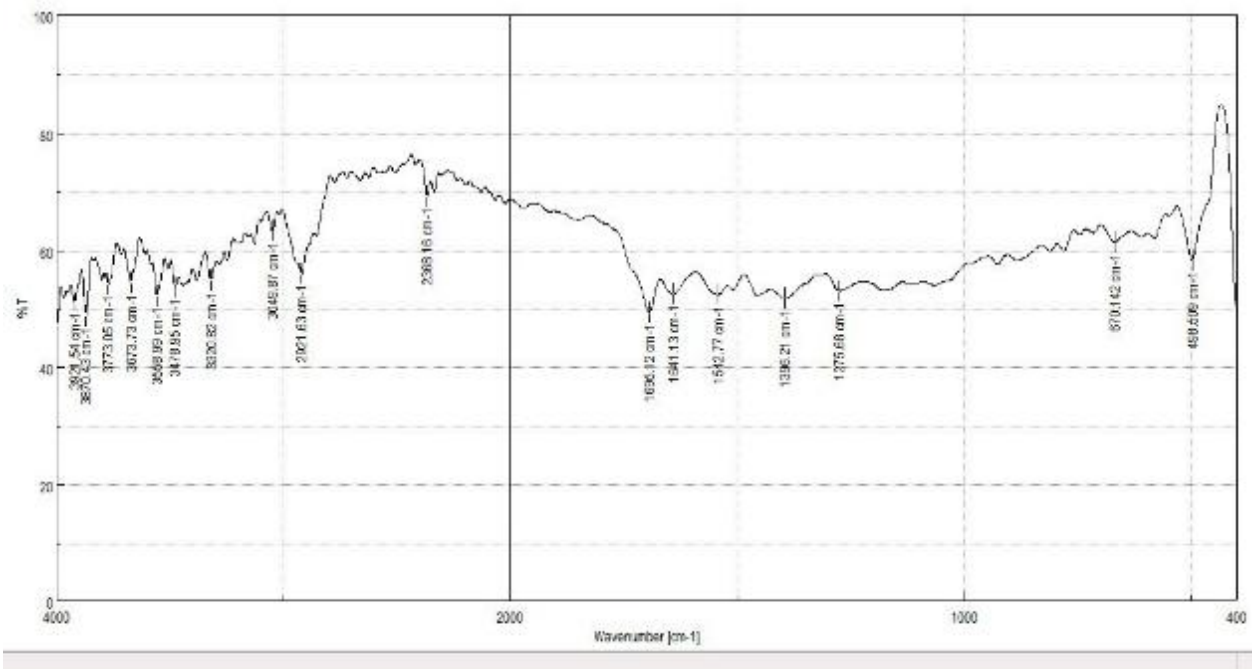

Figure 9: Infrared Spectrum of the Nanoparticles Fabricated from the Extract of the Alga C. crispata

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 and Iskefiyeli, 2023).

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 the algae C. crispata revealed peaks corresponding to carbon, oxygen, chlorine, and silver, with proportions of 10.95 %, 5.12%, 15.02%, and 68.90%, respectively (Figure 9).

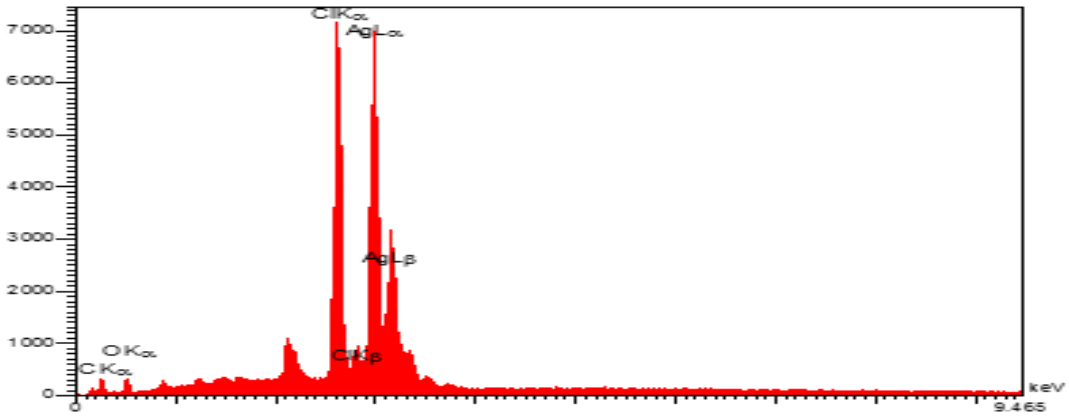

Figure 10: Energy-Dispersive X-ray Spectroscopy Analysis of the Synthesized Silver Nanoparticles from the Extract of the Alga C. crispata

EDX analysis indicated that the synthesized nanoparticles comprised 68% of silver, while other researchers using Anabaena variabilis for similar purposes revealed that the amount of silver in the synthesized nanoparticles was 66% (Ahmad et al., 2021).

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 the alga C. crispata ranged between (29-99)nm in size and had a semi-spherical shape (Figure 11).

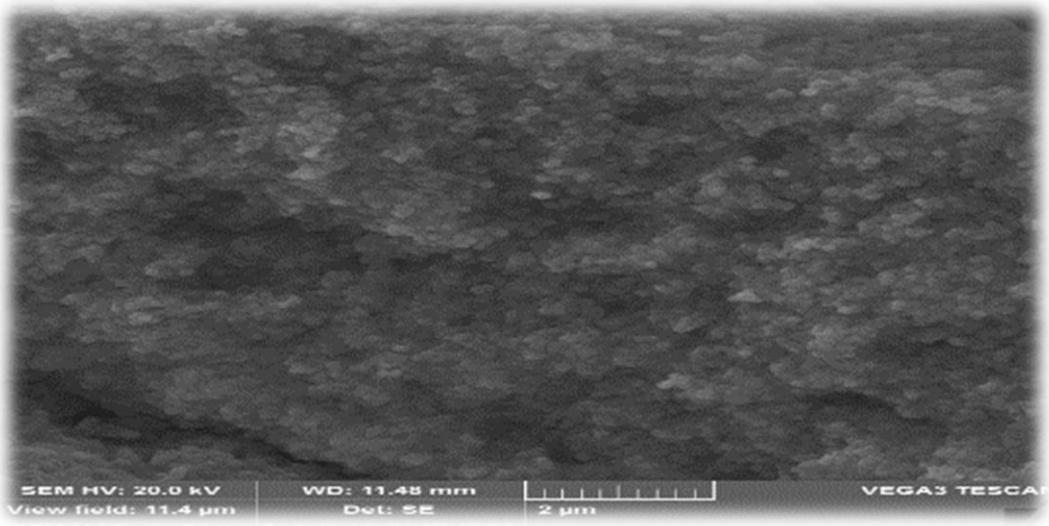

Figure 11: Scanning Electron Microscope Image of the Nanoparticles Fabricated from the Extract of the Alga C. crispata

SEM analysis supported the confirmation that the silver nanoparticles was semi-spherical and the size of the particles was between 29-99 nm, the nanoparticles synthesized from Zygnema carinthiacum extracts were spherical, and the size of the particles was between 19-52 nm (Alkinani et al., 2023).

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 the alga C. crispata exhibited diffraction peaks at 2θ values of (27.84, 32.27, 42.99, 46.38, 54.87,and 57.39), corresponding to the crystallographic planes (111), (200), (211), (220), (311), and (222) respectively (Figure 12).

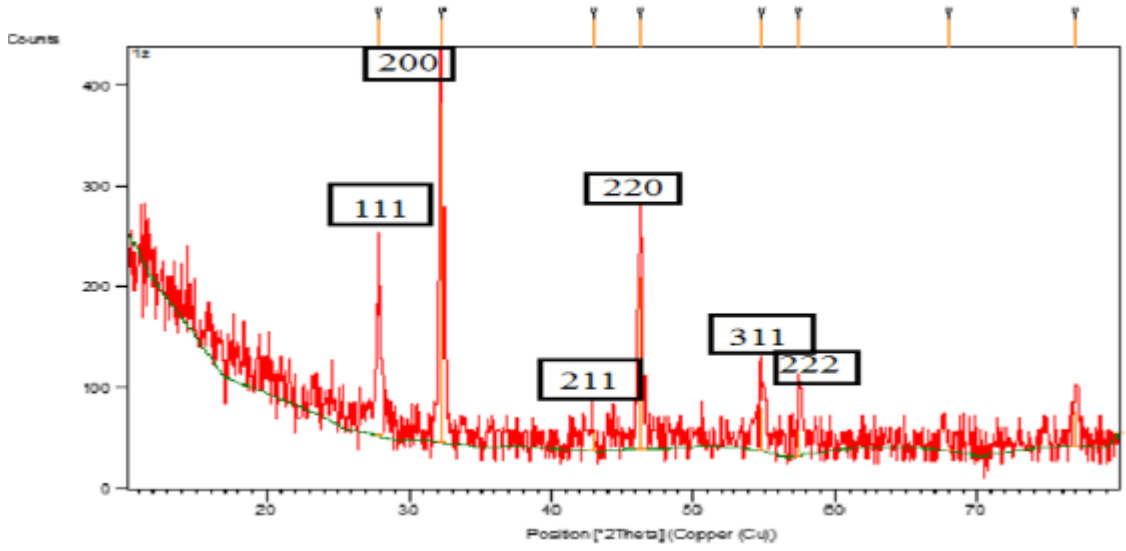

Figure 12: X-ray Diffraction of the Nanoparticles Fabricated from the Extract of the Alga C. crispata

In the XRD spectroscopy analysis, it was observed that the synthesized AgNPs were crystalline in nature with highest diffraction values at 2θ that corresponds to the crystal planes of silver and it affirmed that silver was the main element present in the AgNPs and the peaks with lower intensities were due to the biomolecules present in the extract used for the synthesis of AgNPs (Annadalakshmi et al., 2016).

6- Bioactivity of Silver Nanoparticles Synthesied Using the Extract of the Algae C. crispata

The bioefficacy of silver nanoparticles derived from C. crispate algae extract was evaluated against the following bacterial species E. coli, Klebssiella pneumoniae, Pseudomonas aeruginosa, and Staphylococcus aureus along with their respective concentrations (62.5, 125, 250, 500, and 1000), a significant difference was below the significance level of 0.05≥p, indicating that all the concentrations effectively inhibited the pathogenic bacterial species under study in different proportions the most significant inhibition diameter was 25.33mm on P. aeruginosa bacteria at a concentration of 1000 µg/ml, while the minor inhibition diameter was 12.67mm K. pneumoniae at 62.5 µg/ml.

No inhibitory activity when the DMSO solvent was used as a negative control group. the algal extract was also used as a positive control; it was similarly effective, but in lower proportions of nanoparticles than with close inhibition diameters, as the largest inhibition diameter was 11.1 mm on the bacteria P. aeruginosa the concentration of 1000 µg/ml and the minor inhibition diameter was 9mm for all bacterial species at a concentration of 62.5 µg/ml. When comparing nanoparticles with algae extract, it was found that the nanoparticles were significantly more effective in inhibiting pathological bacteria at all concentrations (62.5, 125, 250, 500, and 1000) µg/ml with a significance level of p≤0.05, it gave different inhibition diameters for E. coli (14, 15.67, 17, 19, 21) mm, respectively. While the inhibition diameters of K. pneumoniae (12.67, 14, 15.33, 18, 19) mm, respectively Table (3) Figure (13). On the other hand, the inhibition diameters of P. aeruginosa were (14.67, 17.33, 19.67, 23, and 25.33) mm, respectively. The inhibition diameters of the bacteria S. aureus were (13, 16.33, 18.67, 20.67, and 21.33) mm, respectively.

As for the algae extract C. crispata, it gave inhibitory diameters for the concentrations mentioned above, represented by (9, 9.33, 10, 10.33, 10.67) mm, respectively, for the bacteria E. coli, while the bacteria K. pneumoniae had inhibition diameters (9, 9.67, 10, 10.33, 10.67) mm respectively.

The inhibition diameters of the bacteria were P. aeruginosa (9, 9.33, 9.67, 10.33, 11) mm, respectively, while the inhibition diameters of the bacteria S. aureus were (9, 9.33, 10, 10.33, 10.67) mm respectively Table (4) Figure(13).

Table (3): Demonstrates the Inhibition Zone Diameters for the Extract of the Alga C. crispata

Bacteria	Concentration				
	1000	500	250	125	62.5
E. coli	21.3	19	17	15.6	14
K. pneumoniae	19	18	15.3	14	12.6
P. aeruginosa	25.3	23	19.6	17.3	14.6
S. aureus	21.3	20.6	18.6	16.3	13

Table (4): Demonstrates the Inhibition Zone Diameters for the Extract of the Alga C. crispata

Bacteria	Concentration				
	1000	500	250	125	62.5
E.coli	10.6	10.3	10	9.3	9
K. pneumoniae	10.6	10.3	10	9.6	9

P. aeruginosa	11	10.3	9.6	9.3	9
S. aureus	10.6	10.3	10	9.3	9

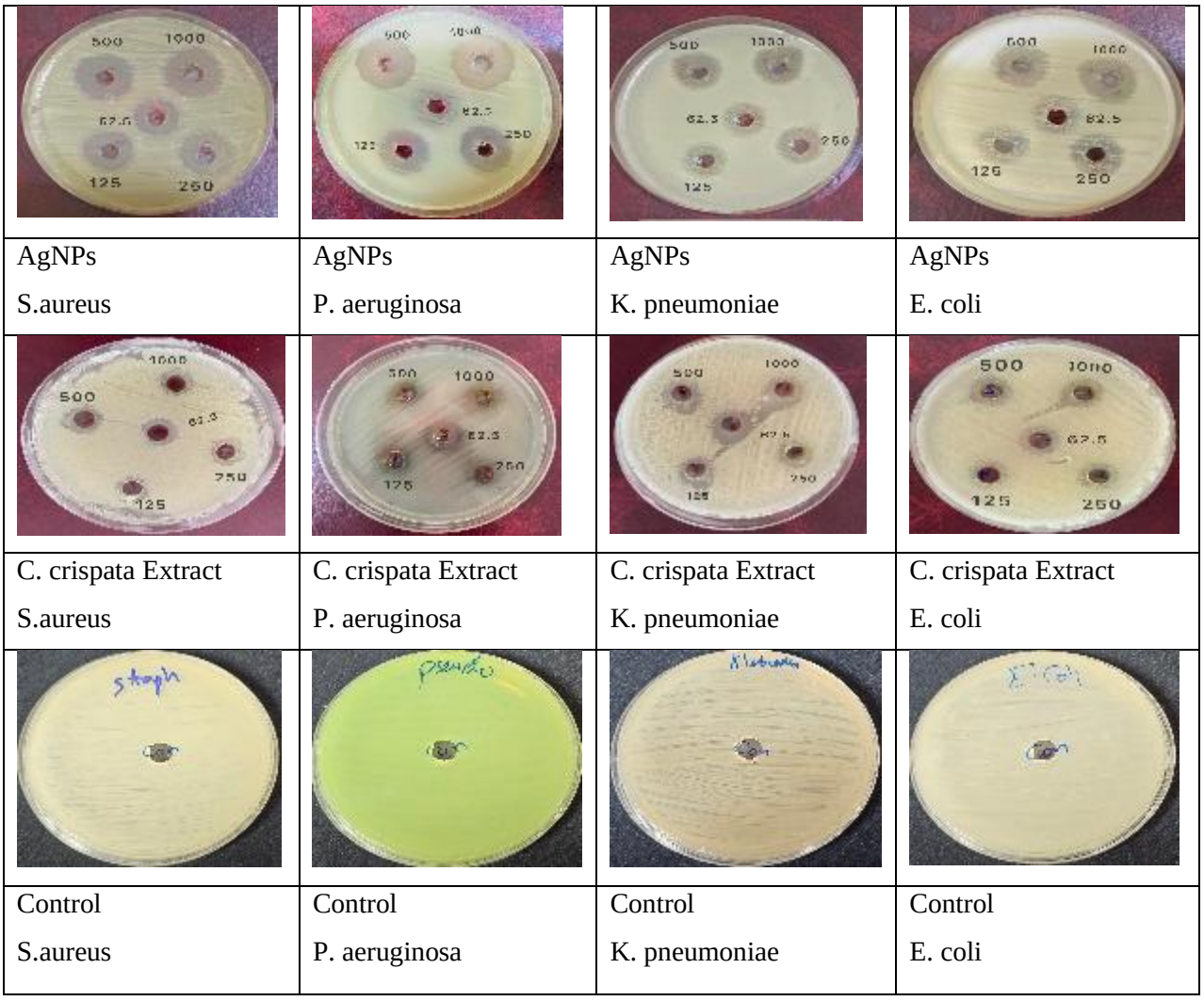

Figure 13: Diameters of Inhibition Zone

The current study concludes that AgNPs significantly inhibit the growth of both the G^+ and the G^- bacterial species that were considered in this research. This is made possible by employing nanoparticles that show a strong tendency to connect with the thiol groups present on the surface of bacteria cells. Therefore, the nanoparticles inhibit ATP production, modify the proton motive force, alter the phosphate flows, sever the link between the cell membrane and cytoplasm, induce the unwanted release of intracellular content, cause chromatin condensation, and interfere with replication (Marambio-Jones & Hoek, 2010). The results demonstrated that the maximum zone of inhibition was recorded 21 mm for E.coli and S. aureus at a concentration of 1000 µg/ml while the minimum zone of inhibition was 12. The average zone of inhibition was 67 mm against E. coli at a concentration of 62. 5 µg/ml. These findings were similar to those of (Shareef et al., 2021), which showed the highest inhibition diameter of 22 mm against E.coli at concentrations of 1000 and 500 µg/ml and the smallest diameter of 11 mm against E.coli, E. cloacae, and P. aeruginosa at a concentration of 62.5 µg/ml.

Conclusions

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

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