ISSN:2523 - 0522
Volume 8, Issue 2, October 2025
ISRAA UNIVERSITY JOURNAL OF APPLIED SCIENCE (IUJAS)

ORIGINAL ARTICLE

Evaluation of the Antimicrobial Activity of Selected Algal Extracts Against Pathogenic Bacteria and *Candida* Species In Vitro study.

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Received:06/07/2025 ***Accepted:08/09/2025*** ***Published:01/10/2025***

OPEN ACCESS

Doi:
https://doi.org/10.52865/krbz4160

Competing Interests:
The authors declare that this manuscript was approved by all authors in its form and that no competing interest exists.

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ABSTRACT:

Background: *The worldwide increase in microbial resistance to antibiotics necessitates immediate research on sustainable and advantageous natural treatment alternatives.*

Method: *Algae isolated from the aquatic environment of Baqubah city (Khreesan River and Othmania River) and subsequently identified using taxonomic references. The study utilized hexane and ethanol solvents to extract by maceration and shaking. After that, analyze the active compounds present in these algae species. After extraction, the compounds were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) to fully characterize their chemistry and identify potential bioactive substances with therapeutic properties. To determine the antimicrobial properties of the ethanol and hexane extracts, in vitro experiments were conducted on three strains of Klebsiella, two strains of Pseudomonas, two strains of Acinetobacter, and , two strains of Candida albicans. They have also undergone testing for antibiotic resistance.*

Results: *Cladophora glomerata and Mougeotia floridana are two species of algae isolated from the environment. The GC-MS analysis determined Several organic compounds, including phenolic compounds, nitrogen compounds, hydroxylamines, aldehydes, fatty acid methyl esters, diterpenoids, long-chain primary fatty alcohols, long-chain fatty acids, aromatic solvents, phenylpropanes, and alkanes. The findings revealed that the crude ethanolic extract of Cladophora glomerata exhibited the highest activity at a concentration of 100 mm/L, which was consistent with the performance of its crude hexane extract at the same concentration. Conversely, the crude ethanolic and hexane extracts of Mougeotia floridana demonstrated superior activity only at a higher concentration of 500 mm/L. When comparing the results of the algal extracts with the antibiotic susceptibility test according to the CLSI system, the algal extracts showed a remarkable superiority. This is attributed to the fact that the tested microorganisms exhibited a high level of resistance to conventional antibiotics.*

Conclusion: *Since antibiotic resistance is on the rise worldwide, these algae have shown promise as potential alternative medicine projects.*

KEYWORDS: Cladophora glomerata, Mougeotia floridana, GC-MS, Antibacterial, Antifungal.

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INTRODUCTION:

Throughout human history, infectious diseases have had a tremendous impact on society's structure and the development of medical knowledge. Many diseases have had a horrific, deadly impact throughout human history, such as the Spanish flu of 1918, which claimed the lives of more than 50 million people, and the Black Death, which killed more than a third of Europe in the fourteenth century (Lusk, *et a*l.,2016; Chrystal, 2021). These catastrophes demonstrated how vulnerable humans are to infections, which resulted in important developments in therapeutic medicine (Theodosiou *et al*., 2023). The field of medicine underwent an enormous transformation with the discovery of antibiotics. Penicillin was discovered in 1928, which opened the door to a great deal of success in getting rid of infections (Elshobary *et al*., 2025). However, a new worldwide epidemic known as antibiotic resistance arose as decades went by and antibiotics were overused. It had to be managed using natural alternatives, the most significant of which is algae (Maji *et al*., 2025). Recently, one of the most important sources of natural products has been algae. Since algae can survive and develop in fresh, salty, and brackish water, their ease of cultivation, quick growth, short life cycle, and lack of competition for arable land are important, especially when compared to terrestrial plants (Edward and David, 2010; Levasseur *et al*., 2020; Nova *et al*., 2020). Algae are a fundamental and significant material for humans on an industrial, environmental, and therapeutic level (Rani *et al*., 2018; Jung *et al*., 2019). It is abundant in natural compounds that might have application in the medicinal and cosmetics sectors (Sathasivam *et al*., 2019). According to AAh (2016), algal biomass accumulates bioactive substances as lipids, proteins, fats, phenols, carbohydrates, enzymes, flavonoids, toxins, amino acids, fatty acids, growth regulators, and pigments. Numerous properties, including antioxidant, antidiabetic, antibacterial, antiviral, anti-parasitic, and cytotoxic activity, are exhibited by biologically active substances (Michalak, and Messyasz, 2021). *Cladophora* spp. are a possible source of basic materials for cosmetic, dietary, and medicinal uses, and believed that Cladophora extracts' antifungal and antibacterial qualities may also result from the presence of thymol, a chemical that frequently appears in cosmetics (Horincar, *et al*., 2014). This study aimed to collect algae from Baqubah city, extract them, and analyze them using GC-MS. The sensitivity of these extracts to a range of pathogenic microbes was then tested, and the efficacy of these extracts was compared with a range of commercial antibiotics.

Materials and Methods

Description of the study area

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Samples of macroalgae were collected from two freshwater river sites in Baqubah city (Khreesan River and Othmania River) during October and November / 2023, as illustrated in Figures 1 and 2. These sites are subjected to pollution from agricultural fertilizers and wastewater discharge, which, according to Al-Abboodi (2018), increases the probability of algal presence. Furthermore, environmental parameters such as water temperature, pH, and electrical conductivity were measured, as presented in Table 1.

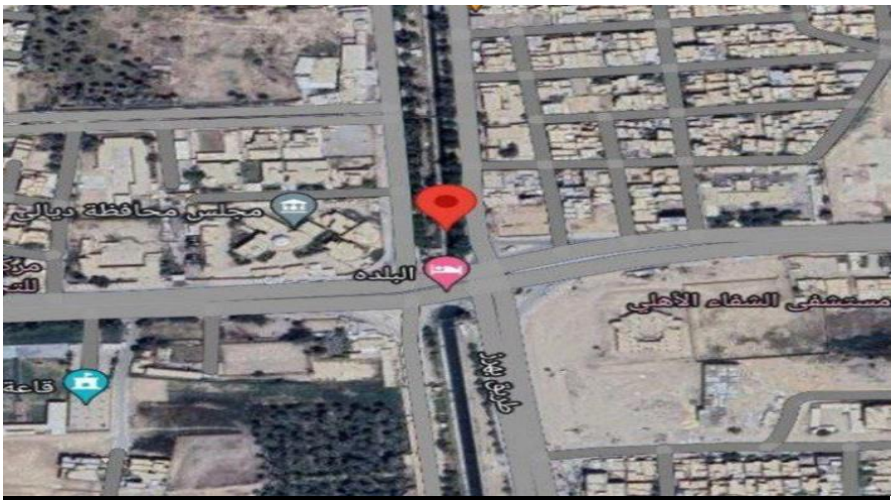

Figure 1: Location of the khreesan according to Google Maps08 GPS (33.745841,44.6468)

Figure 2: Location of the Othmania according to Google Maps GPS (33.717908,44.612806)

Sample collection

In the current study. Large quantities of algal mats were taken and placed in sterile containers. They were then transported to the laboratory and thoroughly cleaned with running tap water for more than half an hour, with light rubbing to remove any suspended impurities and other microorganisms, so that we could diagnose them (Milledge, *et al.,* 2018).

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Table (1): Measurements of the studied environment within months

The Month	Temperature (C°)	pH	Electrical conductivity (EC) (ppm)
Khreesan			
October	**20**	**7.8**	**258**
Othmania			
November	**26.5**	**7.8**	**268**

Algal identification

In order to be able to identify and confirm the genus and species of the algae isolated from the water, several filaments were randomly selected from each sample and examined under a light microscope at various magnifications. The presence of branching, the location of branching (if present), the presence of a sporangium, the distribution of plastids within the cells, and whether the algal filament was divided. By referring to classical taxonomic references, the species was confirmed (Desikachary, 1959; Prescott, 1982; Mahdi and Al-Hussieny, 2022).

Preparation of algal extract

The following modified method was used to produce crude extracts of algae (Elnabris *et al.*, 2013; Al-Tmimi *et al.,* 2018; Hashimi *et al.*, 2022): Five grams of powdered algae were macerated and shaken for seven days in 250 ml of 95% hexane and ethanol.

Analysis of Gas Chromatography–Mass Spectrometry (GC-Ms)

These experiments were carried out at the Basra Oil Company at Basra Governorate / Iraq to identify the active compounds in microalgae extracts using GC-Ms (Agilent). And identified through matching mass spectra to library spectra (Salman, 2018) as shown in table 2.

Antimicrobial susceptibility test

The method outlined below has been used to carry out the sensitivity test (CLSI, 2022). Five to six colonies with a diameter of about 1 mm were chosen over a period of a day from a nutrient Agar culture plate. The suspension was then injected into five milliliters of sterile saline, and its turbidity was visually adjusted according to 0.5 McFarland standards. A swab made of sterile

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cotton wool was moistened with the modified inoculum suspension. On the inside surface of the tube, the swab was rolled above the fluid surface to remove any remaining excess fluid. Stripping was done on the Muller-Hinton Agar (MHA) surface to create a lawn out of the isolate. The following antibacterial discs (CondalabTM) were used on Mueller-Hinton Agar: Trimethoprim – sulphathiazole, Chloramphenicol, Trimethoprim, Nitrofurantoin, Tobramycin, Tetracycline, Ampicillin, Imipenem, Clarithromycin, Azithromycin, Penicillin, Meropenem for *Klebsiella pneumoniae*. Piperacillin, Piperacillin – tazobactam, Ceftazidime, Cefepime, Imipenem, Trimethoprim – sulphamethoxazole, Gentamicin, Tetracycline, Amikacin, Ciprofloxacin, Levofloxacin for *Acinetobacter baumannii,* and *Pseudomonas aeruginosa.* And fluconazole disk (25 µg) and Amphotericin B (20 µg) antifungal discs (CondalabTM).

Table 2: GC/MS conditions. (Satar, and Abdul-Jalil, 2022).

Instrument	Agilent 5977A GC Mass Spectrometer
Analytical Column	Agilent HP-5MSUI 30 m × 0.25 mm, 0.25 µm column
Injection volume	1µl
Pressure	11.933 psi
Temperature	Transfer line temperature 100–350 °C Ion source temperature 150–350 °C Quadrupole temperature 106 -200 °C
Carrier Gas	He 99.99%
Injector Temperature	250 ˚C Scan Range: m/z 25-1000
Injection Type	Split less Injection

Evaluation of the activities of crude algal extract against pathogenic microbes by the Agar well diffusion method

The approach (Nesrullah, *et al.,* 2023) was used to investigate the sensitivity of pathogenic microorganisms to algal extracts.

To produce a suspension, five to six colonies were selected from a 24-hour-old culture of the pathogenic microbe on a nutritional agar plate for bacteria and an SDA culture plate for *Candida*, each with a diameter of approximately 1 mm. The following colonies were placed in 5 mL of sterile saline; turbidity adjusted to 0.5 McFarland standards. The sterile cotton swab was moistened with the modified inoculum suspension. The swab was then rolled along the inside surface of the tube above the fluid surface to remove any excess fluid. The Muller-Hinton agar (MHA) surface was streaked in order to create a lawn out of the isolates. A sterile cork borer was used to make holes in the culture media that were 5 mm in diameter. 100 µl of the algal extract

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should be added to each hole using a micropipette (Two concentrations: 100mg/ml and 500mg/ml). The control is water. Following that, incubate the dishes for 24 hours at 37 °C.

Statistical Analysis

Statistical analysis of the experiments, the curves were drawn, and the ratios were calculated using the Excel program, while the significant differences were determined using One-way analysis of variance ANOVA were used to test group variance significance. Significance was defined as $p<0.01$.

Results and Discussion

In the current study, two species of macro-algae were collected as follows: *Mougeotia floridana, Cladophora glomerata*. Figures 3,4 show the isolated and identified algal species photographed under a microscope in the Botany Laboratory in the Biology Department, College of Science, University of Diyala.

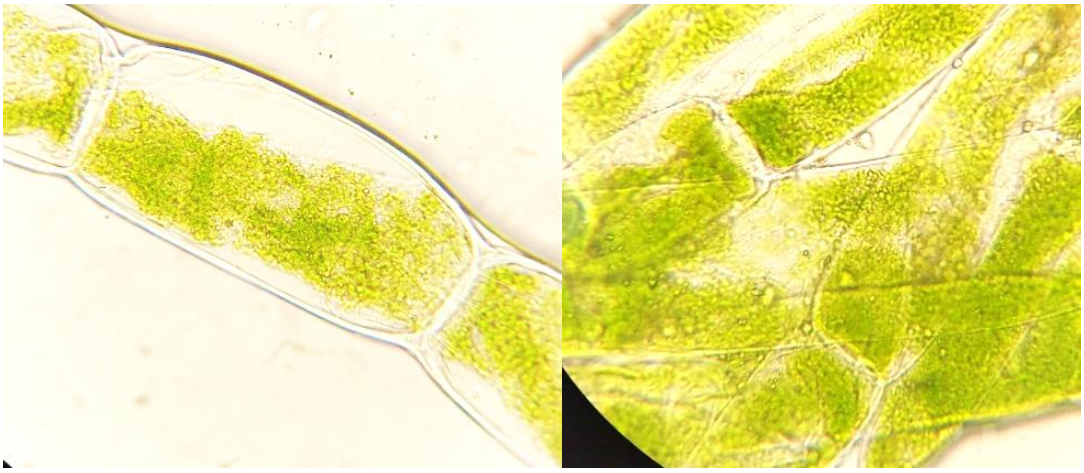

Figure3: *Mougeotia floridana* with 400X magnification

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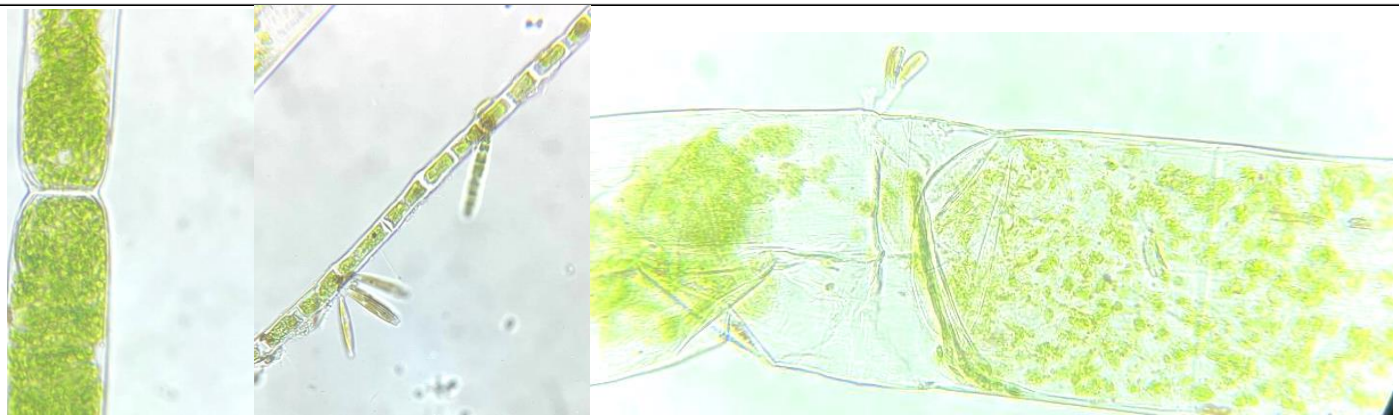

Figure4: *Cladophora glomerata* with 400X magnification

The studied geographical area lacks studies on the presence of algae and the biological diversity of this aquatic environment. However, in other regions of Iraq, the presence of these two algae was widespread, as this study agrees with the study (Aldaraji, 2015), which confirmed the presence of *Cladophora* in five different areas in the Beat Zwana River in Diyala. The study (Al-Moula and Dwaish, 2017) isolated Cladophora algae from water channels at the University of Baghdad. As well as the study (Toma and Aziz, 2022), which found, through a biological survey, *Mougeotia* algae in the city of Shaqlawa/Erbil, northern Iraq. In addition to environmental factors, it should be noted that the large biodiversity of algae is the significant rise in contaminants in freshwater, particularly phosphates and nitrates, which are the main drivers behind the growth and reproduction of all algae. As algae are a more valuable environmental indicator than other physical and chemical indicators, the vast number of different species of algae is a potent bioindicator that demonstrates a significant rise in pollutants (O'Neill and Rowan, 2022; Stoyneva-Gärtner *et al*., 2023).

The Gas chromatography–mass spectrometry

This method is crucial for identifying various chemical and inorganic substances.

The (GC–MS) of ethanol *Mougeotia floridana* as figure (5) and Table (3)

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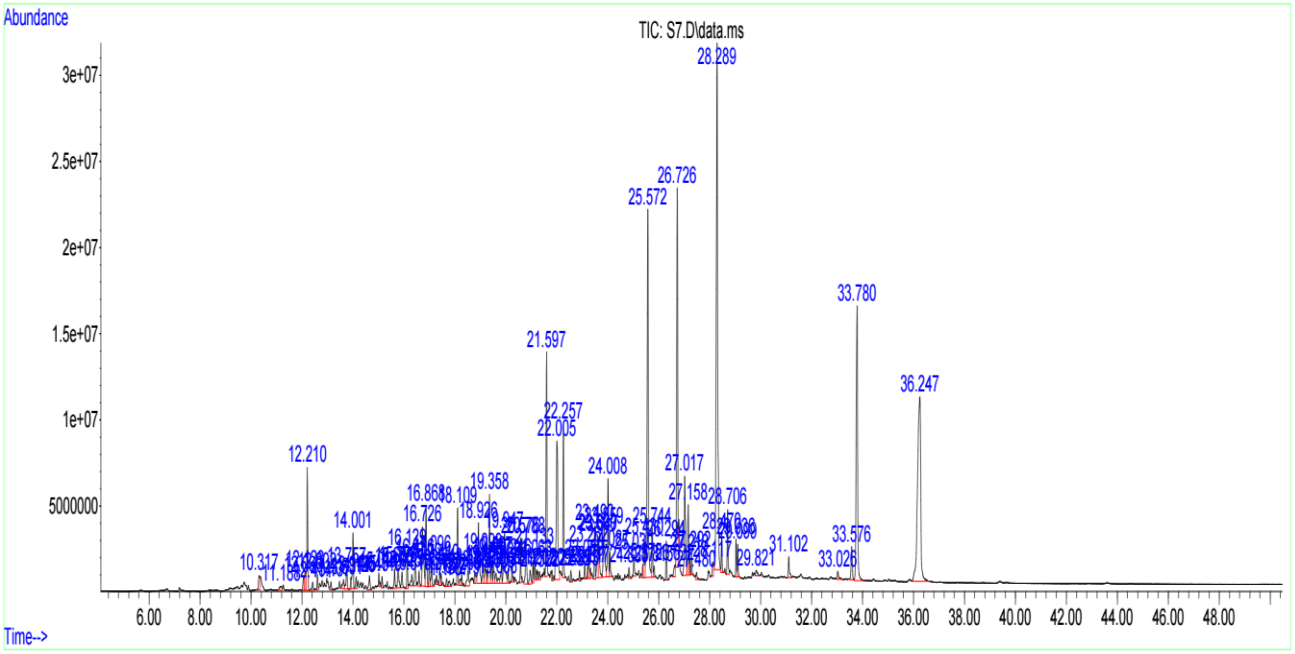

Figure 5: The GC-Ms Analysis of ethanol *Mougeotia floridana*

Table (3): The GC-Ms Analytical of *Mougeotia floridana*

NO	Chemical Class	Compounds	Molecular formula	Area Pct	Biological Activity	Ref
1	Fatty acid	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	3.6	Antibacterial	Shaaban *et al.*, 2021
2	Fatty acid	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	4.1	Antibacterial Antioxidant	Ganesan *et al.*, 2024
3	Organo silicon	Silane, cyclohexyldimethoxy methyl-	$C_9H_{20}OSi$	1.8	Anti-inflammatory	Chetehouna, *et al.*, 2024
4	Polycyclic Alkane	Tetracyclo[6.1.0.0(2,4).0(5,7)]nonane,3,3,6,6,9,9-hexamethyl-(1.alpha.,2.alpha.,4.alpha.,5.beta.,7.beta.,8.alpha.)-	$C_{15}H_{24}$	1.5	-	-----
5	alkanes	Hexadecane	$C_{16}H_{34}$	1.4	Lipase inducer	Miranda *et al*, 2024
6	Phthalate Ester	Diethyl Phthalate	$C_{12}H_{14}O_4$	1.1	Anti-Cancer	Flocchetti *et al.*, 2021
7	Ketone	aR-Turmerone	$C_{15}H_{20}O$	1.3	Cyto productive activity	Megumi et al.,2017
8	alkanes	Octadecane	$C_{18}H_{38}$	1.5	Antibacterial	Rouis-Soussi *et al.*, 2014
9	Fatty acid	Tetradecanoic acid	$C_{14}H_{28}O_2$	1.4	Anti-Virulence Properties	Juárez-Rodríguez *et* al., 2021

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10	Fatty acid	Hexadecanoic acid, ethyl ester	$C_{17}H_{34}O_2$	2.0	Anti-cancer Antimicrobial	Nisa *et al.*, 2022 Musa *et al.*, 2015
11	Fatty acid	Octadecanoic acid	$C_{18}H_{36}O_2$	1.3	Antiviral	Entigu *et al.*, 2013
12	Fatty amide	Hexadecanamide	$C_{16}H_{33}NO$	1.6	Anti-inflammatory	Bao *et al.*, 2023
13	Fatty amide	9-Octadecenamide, (Z)-	$C_{18}H_{55}NO$	8.1	Hypolipidemic	Cheng *et al.*, 2010
14	Fatty acid ester	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	7.1	Anti-bacterial Anti-inflammatory	Tyagi, and agarwal, 2017
15	Phthalate ester	Bis(2-ethylhexyl)phthalate	$C_{24}H_{38}O_4$	2.2	Antibacterial and Larvicidal	Javed *et al* ., 2022
16	N-Oxide	Methadone N-oxide	$C_{21}H_{27}NO_2$	1.1	--	---
17	Fatty acid monoester	Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$	13.2	Anti-biofilm Anti-oxidant Anti-bacterial	Canli *et al.*, 2023
18	Phosphite ester	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	$C_{42}H_{63}O_3P$	7.1	Anti-Phytopathogenic Fungi	Ren *et al.*, 2018
19	Aryl Phosphate	Tris(2,4-di-tert-butylphenyl) phosphate	$C_{42}H_{63}O_3P$	10.6	Toxic	Kang *et al.*, 2025

The (GC–MS) Analysis of hexane *Mougeotia floridana* as figure (6) and Table (4)

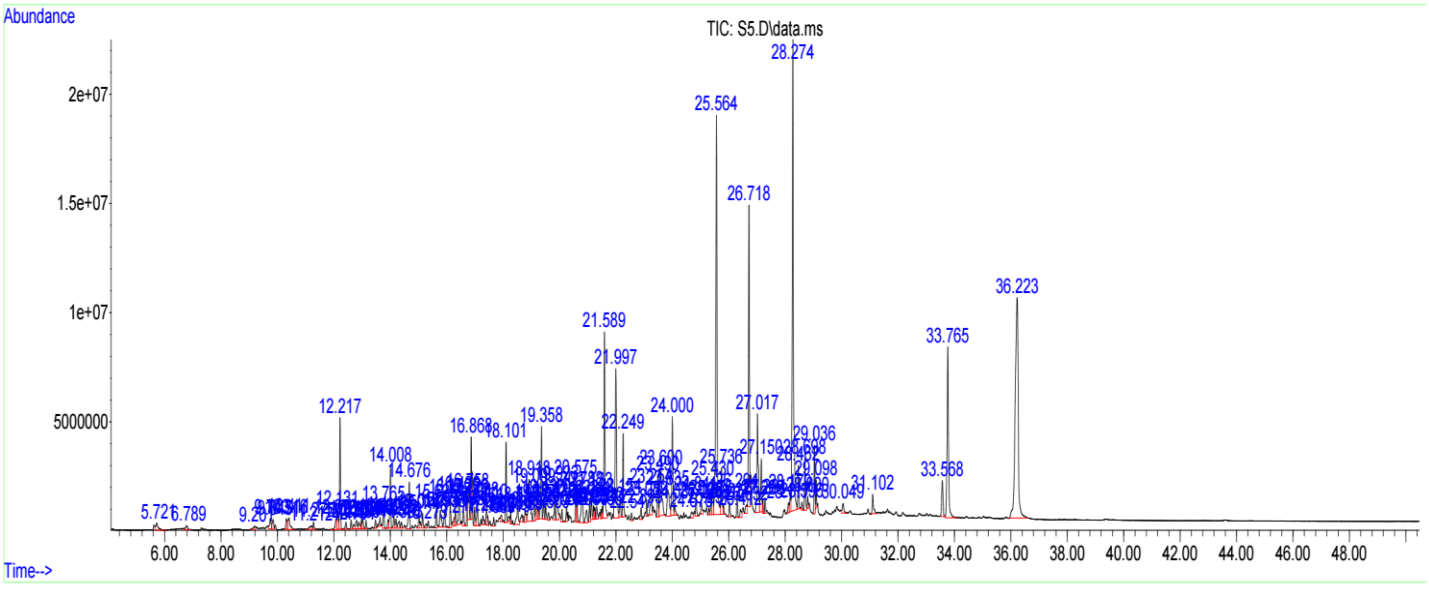

Figure 6: The GC-Ms Analysis of hexane *Mougeotia floridana*

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Table :(4) The GC-Ms Analytical of *Mougeotia floridana*

No	Chemical class	Compounds	Molecular formula	Area Pct	Biological Activity	Ref
1	Organo silicon	Silane, cyclohexyldimethoxymethyl-	$C_9H_{20}OSi$	1.9	-	-
٢	Alkane	Pentacosane	$C_{55}H_{52}$	1.1	Anticancer	Mishra *et al.*, 2019
3	Alkane	Hexadecane	$C_{16}H_{34}$	1.2	Lipase production	Boekema *et al.*, 2007
4	Phthalate Ester	Diethyl Phthalate	$C_{12}H_{14}O_4$	1.2	Adipogenisis inhibitor	Mondal *et al.*, 2024
5	Ketone	aR-Turmerone	$C_{15}H_{20}O$	1.4	Antimicrobial	Lee, 2006
6	Haloalkane	2-Bromo dodecane	$C_{12}H_{25Br}$	1.6	Fatty acid procusor	Hamilton *et al.*, 1995
7	saturated fatty acid methyl ester (FAME	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	3.7	anticancer, antioxidant, antimicrobial, anti-androgenic, anti-inflammatory and hepatoprotective properties.	Momodu, *et al,.* 2022
8	Fatty acid	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	3.6	Anti inflammatory	Aparna *et al.,* 2012
9	Fatty acid ester	Hexadecanoic acid, ethyl ester	$C_{19}H_{38}O_4$	1.3	Anti-viral	Sagna *et al.*, 2023
10	Alkane	Heptadecane	$C_{17}H_{36}$	1.7	Antibacterial Cytotoxic	Abdel-Hady *et al.*, 2016
11	Fatty acid	Octadecanoic acid	$C_{18}H_{36}O_2$	1.1	Antiviral	Entigu *et al.*, 2013
12	Fatty amide	9-Octadecenamide, (Z)-	$C_{18}H_{55}NO$	9	Hypolipidemic	Cheng *et al.*, 2010
13	Fatty acid	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	5.5	Anti-microbial Cytotoxic	Manaswini *et al.*, 2025
14	Phthalate Ester	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	2.3	Antibacterial and Larvicidal	Javed *et al* .,2022

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15	Indole derivative	1H-Indole, 5-methyl-2-phenyl-	$C_{15}H_{13}N$	1.1	Antibacterial Antioxidant	Modi and Jain, 2019
16	Fatty acid monoester	Octadecanoicacid,2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$	10.7	Anti-biofilm Anti-oxidant Anti-bacterial	Canli *et al.*,2023
17	Treterpenes	Squalene	$C_{30}H_{50}$	1.1	Antioxidant	Cheng *et al.*, 2024
18	Phosphite ester	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	$C_{42}H_{63}O_3P$	4.6	Antifungal	Devi *et al.*, 2021
19	Aryl Phosphate	Tris(2,4-di-tert-butylphenyl)phosphate	$C_{42}H_{63}O_3P$	11.8	Toxic	Kang *et al.*, 2025

The (GC–MS) Analysis of ethanol *Cladophora glomerata* as figure (7) and Table (5)

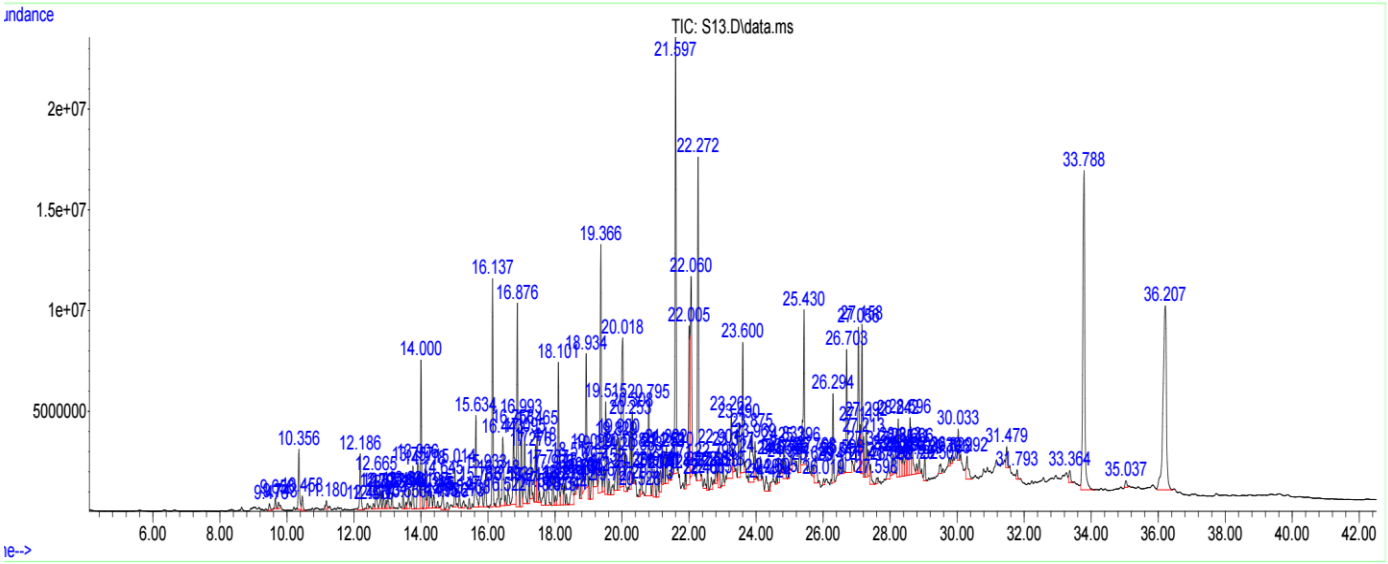

Figure 7: The GC-Ms Analysis of ethanol *Cladophora glomerata*

Table (5) The GC-Ms Analytical of *Cladophora glomerata*

No	Chemical class	Compounds	Molecular formula	Area Pct	Biological Activity	Ref
1	Organo silicon	Silane, cyclohexyldimethoxymethyl-	$C_9H_{20}OSi$	1.9	-	-
2	alkanes	Pentacosane	$C_{25}H_{52}$	1.2	Pheromone	Sun *et al.*, 2017
3	alkanes	Hexadecane	$C_{16}H_{34}$	1.8	Alkane metabolism substrate	Nie *et al.*, 2017
4	Phthalate Ester	Diethyl Phthalate	$C_{12}H_{14}O_4$	1.8	Anti-Cancer	Flocchetti *et al.*, 2021

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5	bromoalkanes	-٢Bromo dodecane	$C_{12}H_{25}Br$	1.7	Biodegradation	Shah *et al.*,2024
6	saturated fatty acid methyl ester (FAME)	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	3.8	anticancer, antioxidant, antimicrobial, anti-androgenic, anti-inflammatory and hepatoprotective properties.	Momodu, *et al.,* 2022
7	fatty acid	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	3.7	Anti-biofilm Quorum sensing inhibitor	Senthil *et al.*,2025
8	Fatty acid Ester	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_7$	1.5	Anti-cancer Antimicrobial	Nisa *et al.*, 2022 Musa *et al.*, 2015
9	Alkane	Heneicosane	$C_{21}H_{44}$	1.6	Micro biocidal Anti-biofilm	Vanitha *et al* ., 2020
10	Fatty amide	Octadecenamide, (Z) ٩-	$C_{18}H_{55}NO$	9.3	Hypolipidemic	Cheng *et al.*, 2010
11	Monoacylglycerols	Hexadecanoic acid,2-hydroxy-1hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	5	Anti microbial Cytotoxic	Manaswini *et al.*,2025
12	Phthalate Ester	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	2.8	Antibacterial and Larvicidal	Javed *et al* ., 2022
13	Amine-N-Oxided	Methadone N-oxide	$C_{21}H_{27}NO_2$	1.4	antioxidant and neuroprotective effects	Ekeanyanwu, *et al.,* 2024
14	Monoglyceride	Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$	9.6	Anti-biofilm Anti-oxidant Anti-bacterial	Canli *et al.*, 2023
15	Triterpene hydrocarbone	Squalene	$C_{30}H_{50}$	1.1	Antioxidant Antioxidant	Kim *et al.*, 2012
16	Phosphite ester	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite(٣:١)	$C_{42}H_{63}O_3P$	5.6	Antifungal	Devi *et al.*, 2021
17	Phosphite ester	Tris(2,4-di-tert-butylphenyl) phosphate	$C_{42}H_{63}O_3P$	12.8	Antioxidant and stabilizer in food	Shi *et al.*, 2020

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The (GC–MS) of hexane *Cladophora glomerata* as figure (8) and Table (6)

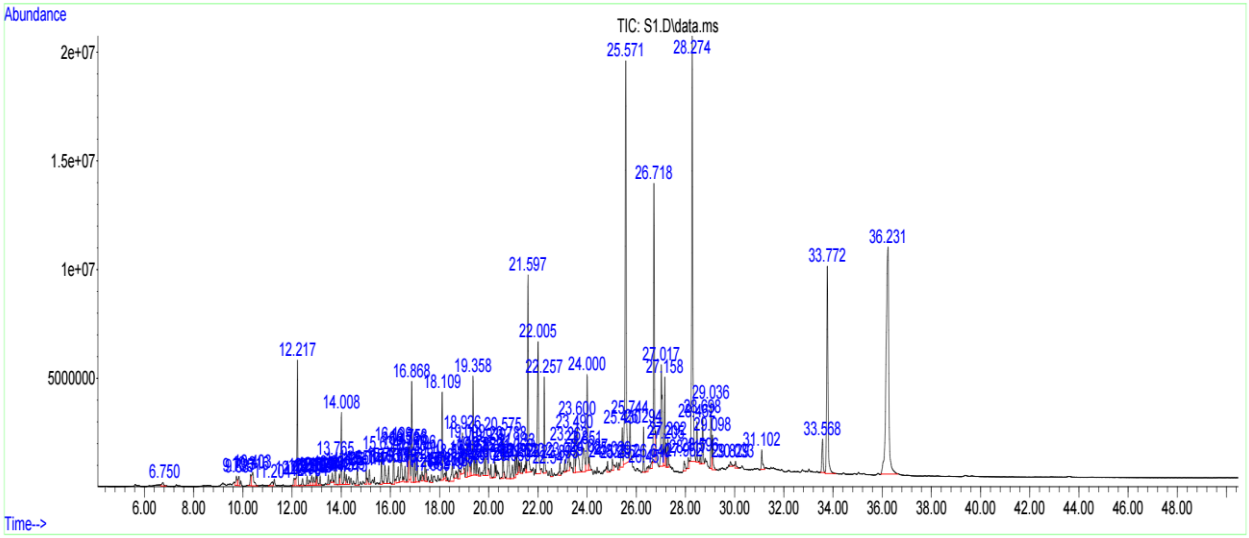

Figure 8: The GC-Ms Analysis of hexane *Cladophora glomerata*

Table (6): The GC-Ms Analysis of hexane *Cladophora glomerata*

No	Chemical class	Compounds	Molecular formula	Area Pct	Biological Activity	Ref
1.	alkanes	Pentacosane	$C_{25}H_{52}$	1.4	Antibacterial	Marrufo *et al.*, 2013
2.	Alkane	Tetradecane	$C_{14}H_{30}$	1.1	Antibacterial	Nasr *et al.*, 2022
3.	Terpenoid ester	-١Methyl-4-(1-acetoxy-1-methylethyl)-cyclohex-2-enol	$C_{12}H_{20}O_3$	2.3	-	-
4.	Alkanes	Hexadecane	$C_{16}H_{34}$	2.5	Support microbial degradation	Perera *et al.*, 2019
5.	Terpenoid aromatic compound	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	$C_{15}H_{22}$	1.3	antibacterial activity	Ohaegbu, *et al.*, 2022
6.	Alkanes	Heptadecane	$C_{17}H_{36}$	2.4	Antimicrobial	AlZahrani, 2025
7.	Alkane	aR-Turmerone	$C_{15}H_{22}O$	1.4	Anti-caner	Lee, 2009
8.	Alkane	Heneicosane	$C_{21}H_{44}$	2.8	Microbicidal	Vanitha *et al.*, 2020
9.	Fatty acid	Tetradecanoic acid	$C_{14}H_{28}O_2$	3.1	Anti - virulence properties	Juárez-Rodríguez *et al.*, 2021
10	Aliphatic ketone	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	5.3	Antibiofilm	Alkuwayti, 2023

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11	Fatty acid Methyl ester	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	4.8	Anti bacterial	Shaaban et al., 2021
12	Fatty acid	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	5.1	Anti-fungal Anti biofilm	Hrichi *et al.*,2022
13	Alkane	Heneicosane	$C_{21}H_{44}$	1.4	Anti-inflammatory	Khan and Sharma, 2020
14	Alkane	Eicosane	$C_{20}H_{42}$	1.7	Anti-biofilm Anti adhesion	Beema Shafreen *et al.*, 2022
15	Branched Alkane	Octadecane, 2,6,10,14-tetramethyl-	$C_{22}H_{46}$	1.4	anti-inflammatory antioxidant	Frahtia, and, Niemann, 2024
16	Branched Alkane	Dodecane, 4-methyl-	$C_{13}H_{28}$	2.2	antibacterial potential	Ajijolakewu, *et al.*, 2024
17	Long chain fatty acid	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	2	Ant Alcer	Syed *et al.*, 2024
18	Halogenated Alkane	1-Iodo-2-methylundecane	$C_{12}H_{25}I$	2.3	Estrogen dependent pheromone	Achiraman *et al.*, 2010
19	N-oxide	Methadone N-oxide	$C_{21}H_{27}NO_2$	1.6	Antioxidant	Ekeanyanwu *et al.*, 2024
20	Phosphate ester	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	$C_{42}H_{63}O_3P$	6.4	Anti-enterococcal Anti-oxidant	Tyagi *et al.*, 2021
21	Organa Phosphate ester	Tris(2,4-di-tert-butylphenyl) phosphate	$C_{42}H_{63}O_3P$	6.2	Autophagy induction	Chen *et al.*, 2025

The species of Pathogenic Microbes

Pathogens used: *Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Candida albicans* isolates were obtained from the postgraduate laboratory in the Biology Department, College of Science, University of Diyala. The diagnosis was confirmed through differential media and biochemical tests.

The Effect of Antifungal agents

This study investigated the effects of two antifungal agents: fluconazole disk (25 g) and amphotericin B (20 g). Figures below (9) and Table (7) show the results of antibiotic therapy for each isolate.

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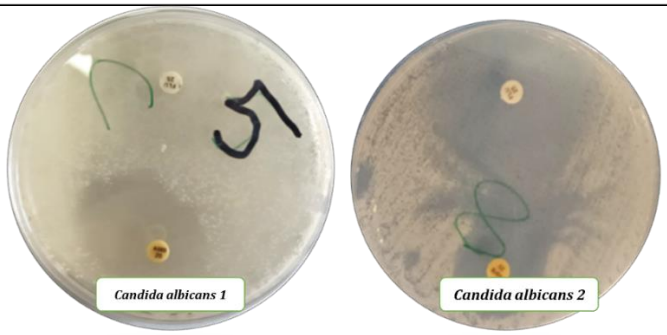

Figure 9: The Antifungal agent effect of two kinds (Amphotericin B (20 mg) and Fluconazole disk (25 mg)) on the isolate of *C. ablicans*.

Table (7) The Antifungal agent effect of two kinds (Amphotericin B (20 mg) and Fluconazole disk (25 mg)) on two isolates of *C. ablicans*.

Name of Sample	Fluconazole	Amphotericin B
C. albicans 1	40	26
C. albicans 2	0	40

In hospitals, fungal infections have increased alongside bacterial infections due to the development of antifungal resistance. (Brown et al., 2012; Roy et al., 2023). *Candida* spp. is the most significant fungal infection known to exist worldwide. because practically every organ in the body is susceptible to its spread. As a result, *Candida* species infections provide a serious threat to public health. According to Kaur and Nobile (2023), one of the species of *Candida* that is most common in the human microbiome is *Candida* albicans. It asymptomatically colonizes the mouth, skin, genitourinary system, and gastrointestinal tract, among other parts of the body. The connection between *Candida* infections and biofilms is one of the primary causes of their severity. They are resistant to higher doses of antifungal drugs, making biofilm infections challenging to treat. because their complex three-dimensional Biofilms offer their individuals the perfect environment. Additionally, only a few types of antifungal medications are available for treating patients with invasive fungal infections. (Bohner et al., 2022; Kaur and Nobile, 2023).

Antibiotic susceptibility of isolated bacteria (Disc diffusion)

The antibiotic sensitivity of three different pathogenic bacterial species—*Klebsiella pneumoniae, Acinetobacter baumannii,* and *Pseudomonas aeruginosa*—was investigated. To determine an isolate's susceptibility to or resistance to the antibiotics currently used in healthcare settings, sensitivity tests were conducted against 11 groups of antibiotics for *Acinetobacter baumannii* & *Pseudomonas aeruginosa*, and 14 for *Klebsiella pneumoniae*. Figure 10: Isolated bacteria's susceptibility to

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antibiotics (Disc diffusion)

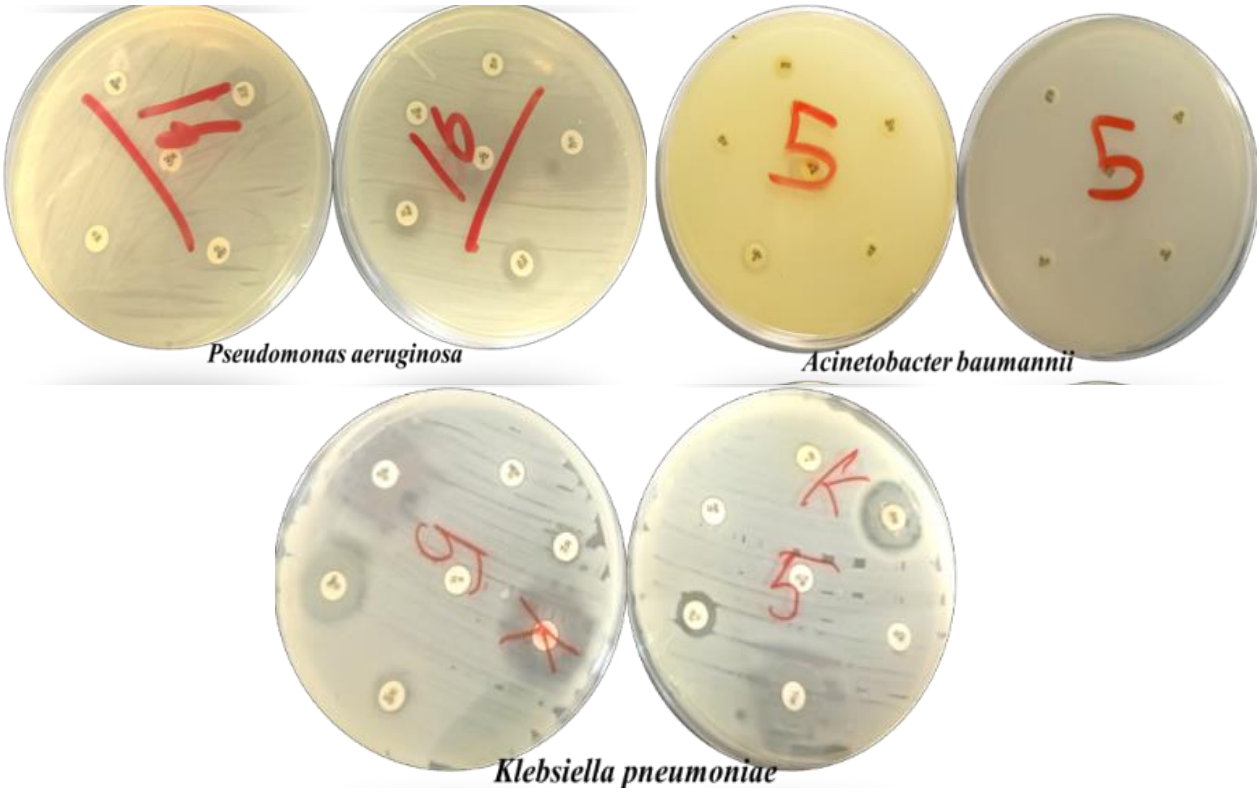

Figure 10: Antibiotic susceptibility of bacteria

P. aeruginosa & *A. baumannii*, based on previous studies, are widely produced beta-lactamase enzymes, biofilms, and efflux pumps, among other mechanisms, which contribute to their resistance to antibiotics. Additionally, it carries its R-resistance plasmids, which are packed with genes that resist antibiotics (Pang, *et al*., 2019; Pachori, *et al*., 2019). Additionally, the concentration of antibiotics inside the cell can be decreased by constricting the channels in the outer membrane (Zahn et al., 2016; Kyriakidis *et al*., 2021). Moreover, *Klebsiella pneumoniae*, which belongs to the Enterobacteriaceae family, developed resistance to antibiotics through several mechanisms. It involves using the beta-lactamase enzyme to analyze the antibiotics and reduce their permeability into the cell. and reducing the enzyme's affinity for them, Penicillin Binding Proteins (Manolitsis *et al*., 2023; Su *et al*., 2023).

Antimicrobial activity of extracts (ethanol and hexane) of *Mougeotia floridana* & *Cladophora glomerata*

In the current study, all isolates (*P. aeruginosa, A. baumannii, and K. pneumoniae* and *Candida spp*.) were inhibited by the crude extracts of two algae. The highest value of inhibition zone is 90mm in (*P. aeruginosa, A. baumannii, and Candida Spp*), while the lowest value of inhibition zone is 20 mm with

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(*Candida Spp*). Table 8 shows the inhibition zone of all isolates. Figures (11, 12, 13, 14, 15) below describe the effect of the hexane and ethanol extract. of *Mougeotia floridana* and *Cladophora glomerata* on all isolates.

**According to the analysis of variance, Statistical analysis revealed a highly significant variation among the tested groups (p = 0.01).

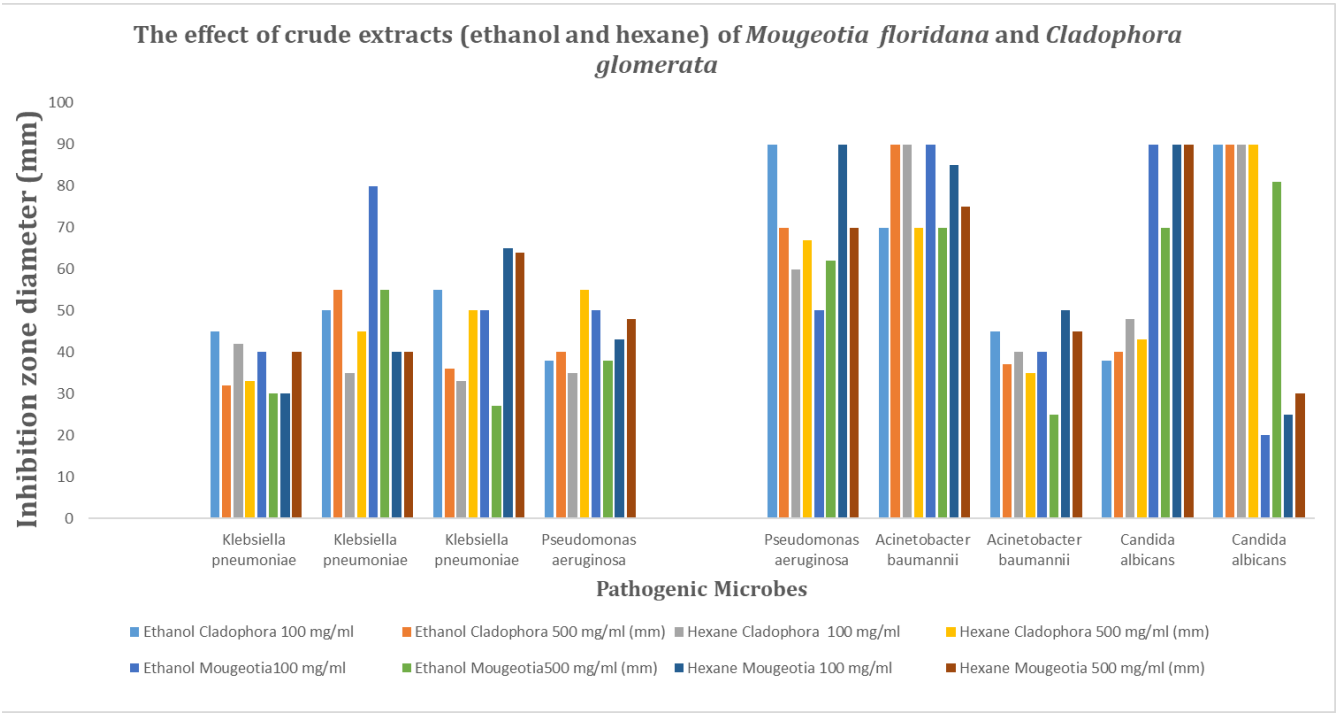

Figure 11: The effect of crude extracts (ethanol and hexane with Two concentrations) of *Mougeotia floridana* and *Cladophora glomerata* on all isolates of microbes

Table 8: The effect of crude extracts (ethanol and hexane with Two concentrations) of *Mougeotia floridana* and *Cladophora glomerata* on all isolates of microbes

Name of sample	Ethanol *Cladophora* 100 mg/ml (mm)	Ethanol *Cladophora* 500 mg/ml (mm)	Hexane *Cladophora* 100 mg/ml (mm)	Hexane *Cladophora* 500 mg/ml (mm)	Ethanol *Mougeotia* 100 mg/ml (mm)	Ethanol *Mougeotia* 500 mg/ml (mm)	Hexane *Mougeotia* 100 mg/ml (mm)	Hexane *Mougeotia* 500 mg/ml (mm)
Klebsiella pneumoniae	45	32	42	33	40	30	30	40
Klebsiella pneumoniae	50	55	35	45	80	55	40	40

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Klebsiella pneumoniae	55	36	33	50	50	27	65	64
Pseudomonas aeruginosa	38	40	35	55	50	38	43	48
Pseudomonas aeruginosa	90	70	60	67	50	62	90	70
Acinetobacter baumannii	70	90	90	70	90	70	85	75
Acinetobacter baumannii	45	37	40	35	40	25	50	45
Candida albicans 1	38	40	48	43	90	70	90	90
Candida albicans 2	90	90	90	90	20	81	25	30

*P-value = 0.997664. According to the analysis of variance, at a significant 0.01 **.*

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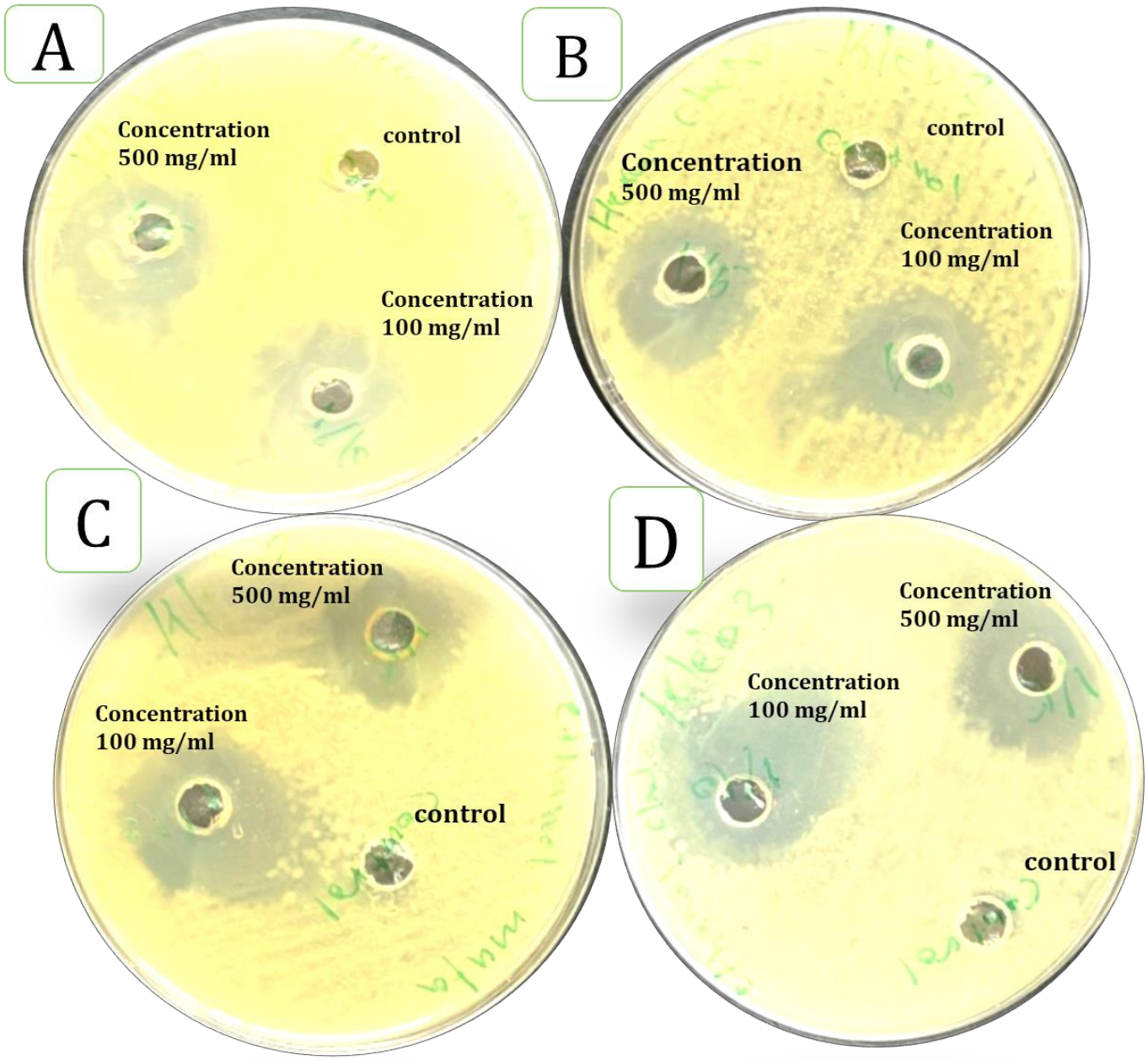

Figure 12: The effect of crude extracts (A. Hexane extract of *Mougeotia floridana, B.* Hexane extract of *Cladophora glomerata*, c. Ethanol extract of *Mougeotia floridana,* D. Ethanol extract of *Cladophora glomerata*) on all isolates of *Klebsiella pneumoniae*

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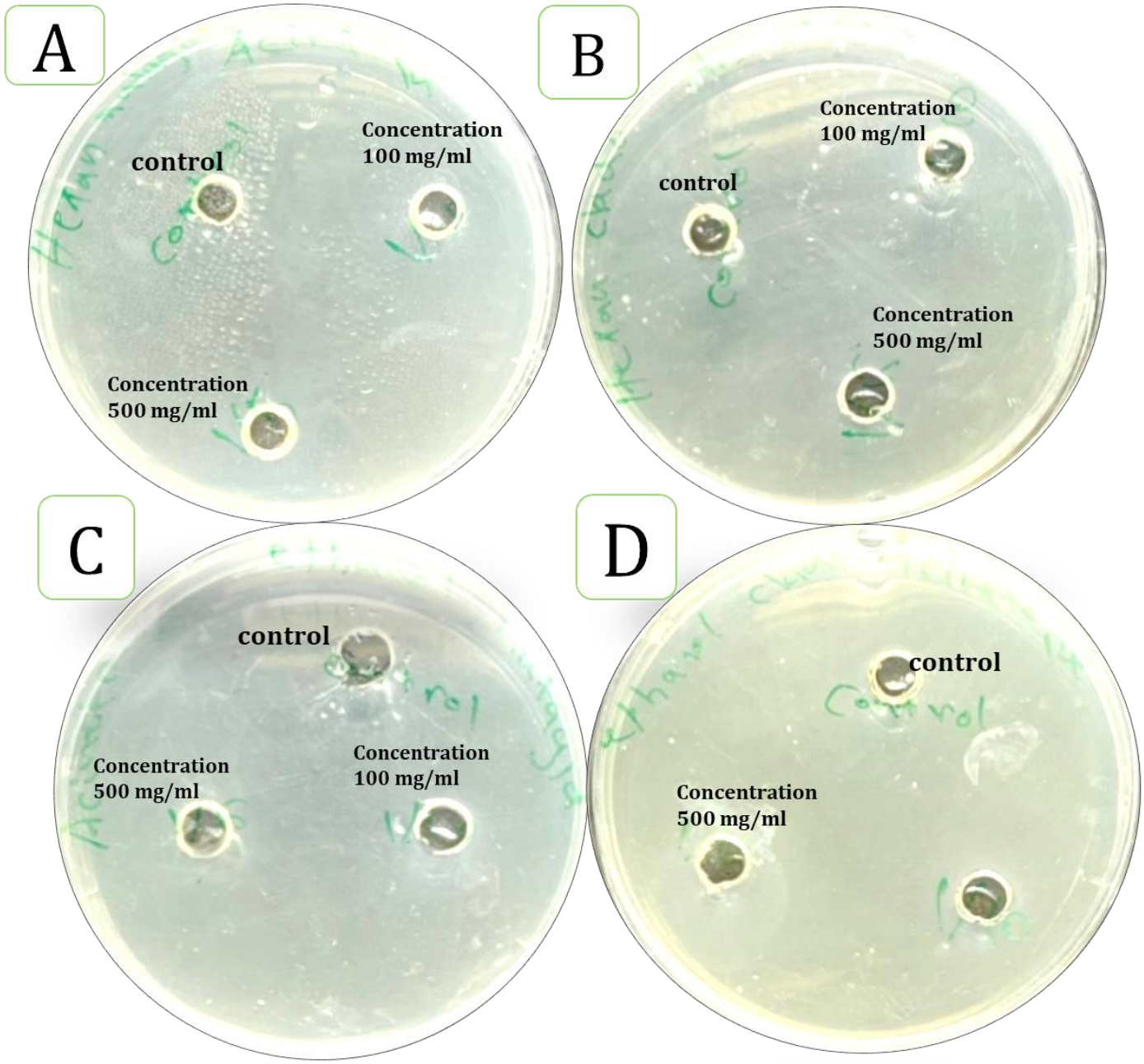

Figure 13: The effect of crude extracts (A. Hexane extract of *Mougeotia floridana, B.* Hexane extract of *Cladophora glomerata*, c. Ethanol extract of *Mougeotia floridana,* D. Ethanol extract of *Cladophora glomerata*) on all isolates of *Acinetobacter baumannii*

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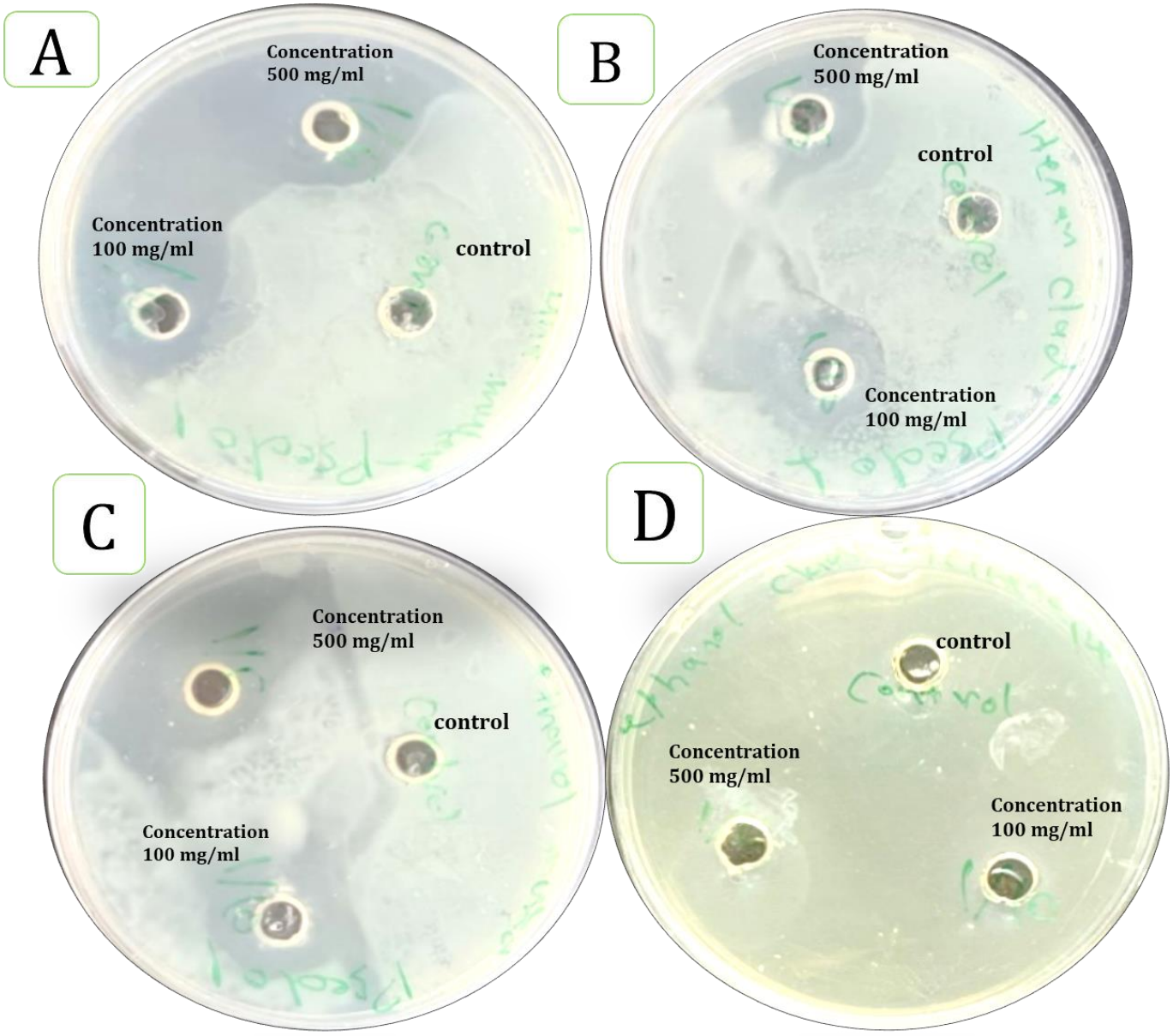

Figure 14: The effect of crude extracts (A. Hexane extract of *Mougeotia floridana, B.* Hexane extract of *Cladophora glomerata*, c. Ethanol extract of *Mougeotia floridana,* D. Ethanol extract of *Cladophora glomerata*) on all isolates of *Pseudomonas aeruginosa*

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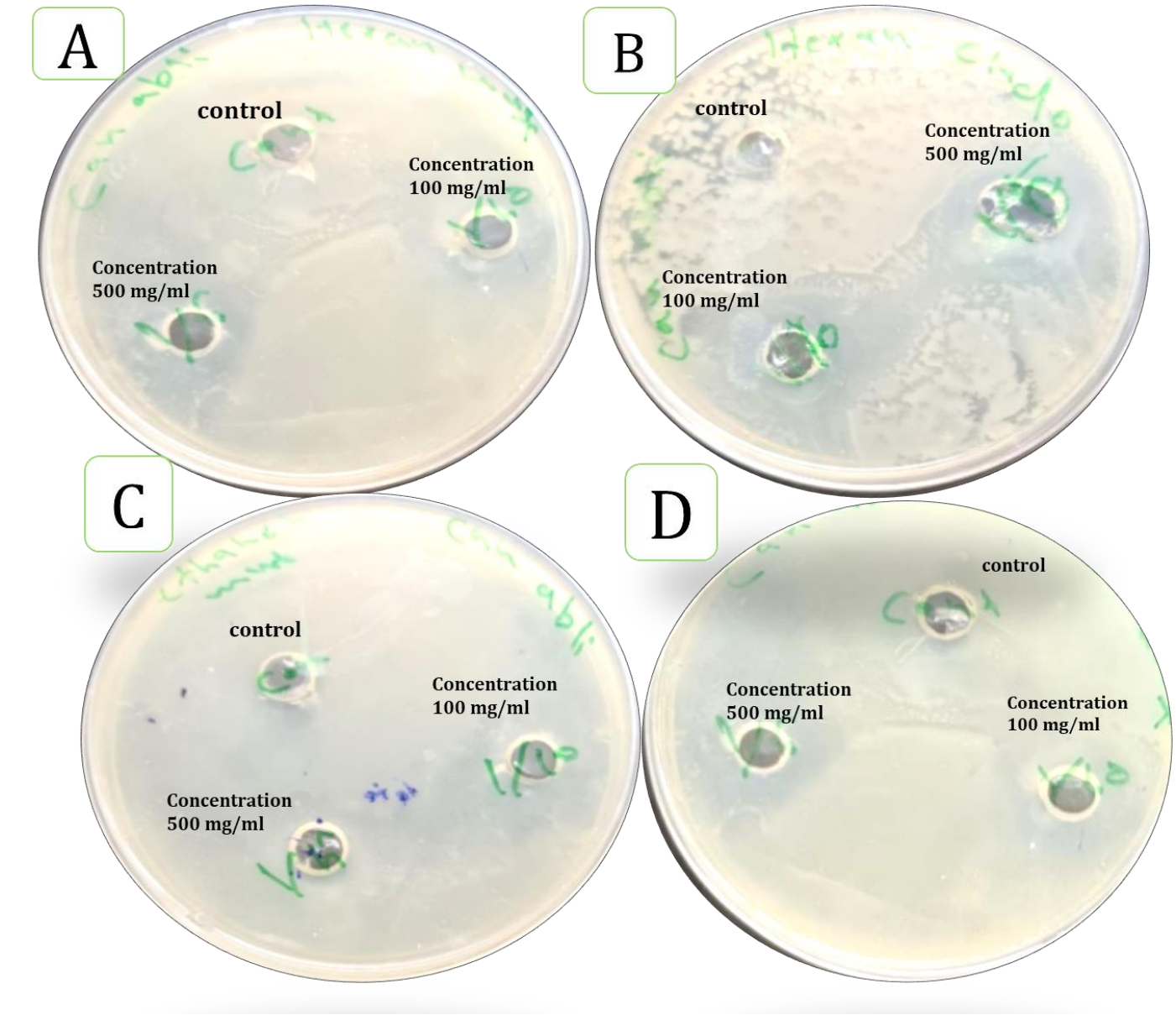

Figure 15: The effect of crude extracts (A. Hexane extract of *Mougeotia floridana,* B. Hexane extract of *Cladophora glomerata,* C. Ethanol extract of *Mougeotia floridana,* D. Ethanol extract of *Cladophora glomerata*)on all isolates of *Candida albicans*

Algae can produce several secondary metabolites that are physiologically active against a range of pathogenic bacterial and fungal strains, which may help explain their antibacterial capabilities. The research results showed that the algal extract affected the used microorganisms. In the current investigation, the crude extract of two algae inhibited every bacterial isolate that exhibited antibiotic resistance. Gram-negative bacteria's reaction is because the extract can penetrate their cell wall due to its few layers. Additionally, the protein enzyme phosphatase, which is essential for transferring chemicals into the organism's body, is blocked by the algae extract, which also inhibits the negative

bacteria (Gram stain) (Salman, 2024). The results of this study agree with previous studies conducted by Mohammed *et al*. (2024), which showed the antibacterial activity of *C. glomerata* alcohol extract against five distinct bacterial species. Additionally, research conducted in Thailand by Laungsuwon and Chulalaksananukul (2014) showed that hexane and ethyl acetate extract of *C. glomerata* was effective against *Vibrio parahaemolyticus* and *Bacillus* cereus. Many compounds are shown in Tables 2, 3, 4, and 5of the GC-MS analysis. Among these compounds are alkanes such as heneicosane and chain alkanes that have been shown to have antibacterial activity in a study by Vanitha et al. (2020). Since heneicosane is non-polar and can integrate into lipid bilayers, it can increase membrane fluidity, weaken structural integrity, and impair the functions of membrane-associated proteins. This mechanism of action is linked to the hydrophobic interaction between alkanes and microbial membranes (Thawabteh *et al*., 2024). These algae also contain a lot of fatty acids with antibacterial properties, like n-hexadecanoic acid. According to the theory, these compounds mostly affect the cell membrane. The permeability of the membrane increases as holes enlarge. Cell death results from the dissolution of the protein membrane. Fatty acids may increase oxidative stress by interfering with respiratory enzymes and electron transport (Wei *et al*.,2023). According to El-Adl *et al*. (2022), transmission electron microscopy demonstrated that fatty acids from *C. glomerata* and *C. vieillardii* induced structural damage to *K. pneumoniae*, including membrane disorder and cell deformation.

CONCLUSION

This study's findings indicate that two algae species, *Mougeotia floridana* and *Cladophora glomerata*, show strong antimicrobial properties against isolates obtained from the University of Diyala's College of Science. Since antibiotic resistance is on the rise worldwide, these algae have shown promise as a source for new drugs. According to a chemical investigation by (GC-MS), this is because of the biologically beneficial chemicals it contains

Disclosure statement

There is no declared competition.

The Future scope

Future research will concentrate on fully purifying polysaccharides and fatty acids from algae and using them biologically in pharmaceutical products. After this study demonstrated their effectiveness against pathogenic bacteria and fungi as antifungal and antibacterial agents.

Competing interests

No competing interests

Funding sections.

The Project was personally financed by the researcher.

REFERENCES:

1. Aah, E. (2016). Antimicrobial activity of microalgal extracts isolated from Baharia Oasis. *Journal of Microbiology, 5*(3), 33–41.
2. Abdel-Hady, H., Abdel-Wareth, M. T. A., El-Wakil, E. A., & Helmy, E. A. (2016). Identification and evaluation of antimicrobial and cytotoxic activities of *Penicillium islandicum* and *Aspergillus tamarii* ethyle acetate extracts. *World Journal of Pharmacy and Pharmaceutical Sciences, 5*(9), 2021–2039. https://doi.org/10.20959/wjppx20169-7674
3. Achiraman, S., Archunan, G., Ponmanickam, P., Rameshkumar, K., Kannan, S., & John, G. (2010). 1-Iodo-2-methylundecane [1I2MU]: An estrogen-dependent urinary sex pheromone of female mice. *Theriogenology, 74*(3), 345–353. https://doi.org/10.1016/j.theriogenology.2010.01.027
4. Ajijolakewu, K., Ajadi, A. E., Ayoola, S. A., Bale, S. I., Sorunke, T. A., Onifade, S. A., & Oyawoye, T. (2024). Phytochemical profiling, GC-MS analysis and antibacterial activity of *Sida acuta* leaf extracts against *Helicobacter pylori*. *UMYU Journal of Microbiology Research, 9*(2), 149–158.
5. Al Zahrani, N. A. (2025). Design and synthesis of heptadecane-linked 1,2,4-triazoles as antimicrobial agents. *Chemistry & Biodiversity.* Advance online publication. https://doi.org/10.1002/cbdv.202500089
6. Al-Abboodi, A. K. A. (2018). *Evaluation of Chlorella vulgaris extracts against bacterial and fungal biofilm* (Master's thesis, Mustansiriyah University, College of Science).
7. Aldaraji, I. K. A. H. (2015). *Use of phytoplankton as biological indicator to evaluate the anthropogenic impact on water quality of Beat–Zwana River–Diyala* (Doctoral dissertation, University of Baghdad).
8. Alkuwayti, M. A. (2023). Anti-*Staphylococcus aureus* activity of the aqueous ethanolic extract of *Solenostemma argel* aerial parts. *Journal of Pure & Applied Microbiology, 17*(4), 2445–2453. https://doi.org/10.22207/JPAM.17.4.47
9. Al-Moula, M. S., & Dwaish, A. S. (2017). Detection of some active compounds from algae *Cladophora glomerata* & *Chara vulgaris* isolated from water canal in Baghdad University. *Journal of the College of Basic Education, 23*(99), 51–62.
10. Al-Tmimi, G. S. L., Al-Rrubaai, G., & Zaki, N. H. (2018). Antibiofilm activity of intracellular extracts of *Westiellopsis prolifica* isolated from local environment in Baghdad. *Journal of Global Pharma Technology, 10*, 281–288.
11. Aparna, D., Dileep, K. V., Mandal, P. K., Karthe, P., Sadasivan, C., & Haridas, M. (2012). Anti-inflammatory property of n-hexadecanoic acid: Structural evidence and kinetic assessment. *Chemical and Biological Drug Design, 80*(3), 434–439. https://doi.org/10.1111/j.1747-0285.2012.01418.x
12. Bao, L., Sun, H., Zhao, Y., Feng, L., Wu, K., Shang, S., Wang, Y., Wang, C., & Liu, Y. (2023). Hexadecanamide alleviates *Staphylococcus aureus*–induced mastitis in mice by inhibiting inflammatory responses and restoring blood–milk barrier integrity. *PLOS Pathogens, 19*(11), e1011764. https://doi.org/10.1371/journal.ppat.1011764
13. Beema Shafreen, R. M., Seema, S., Alagu Lakshmi, S., Srivathsan, A., Tamilmuhilan, K., Shrestha, A., Balasubramanian, B., Dhandapani, R., Paramasivam, R., Al Obaid, S., Salmen, S. H., Mohd Amin, M. F., & Muthupandian, S. (2022). In vitro and in vivo antibiofilm potential of eicosane

against *Candida albicans*. *Applied Biochemistry and Biotechnology, 194*(10), 4800–4816. https://doi.org/10.1007/s12010-022-03984-8

14. Boekema, B. K., Beselin, A., Breuer, M., Hauer, B., Koster, M., Rosenau, F., Jaeger, K. E., & Tommassen, J. (2007). Hexadecane and Tween 80 stimulate lipase production in *Burkholderia glumae* by different mechanisms. *Applied and Environmental Microbiology, 73*(12), 3838–3844. https://doi.org/10.1128/AEM.00097-07
15. Bohner, F., Papp, C., & Gácser, A. (2022). The effect of antifungal resistance development on the virulence of *Candida* species. *FEMS Yeast Research, 22*(1), foac019. https://doi.org/10.1093/femsyr/foac019
16. Brown, G. D., Denning, D. W., Gow, N. A. R., Levitz, S. M., Netea, M. G., & White, T. C. (2012). Hidden killers: Human fungal infections. *Science Translational Medicine, 4*(165), 165rv13. https://doi.org/10.1126/scitranslmed.3004404
17. Canli, K., Yetgin, A., Benek, A., Bozari, S., & Yanmis, D. (2023). Biochemical and antioxidant properties as well as antimicrobial and antibiofilm activities of *Allium scorodoprasum* subsp. *jajlae* (Vved.) steam. *Current Issues in Molecular Biology, 45*(1), 1–15. https://doi.org/10.3390/cimb45010001
18. Chen, X., Feng, L., Xia, Y., Li, H., Liu, F., Li, Y., Xu, H., & Liu, Y. (2025). Exposure to tris(2,4-di-tert-butylphenyl) phosphate during adolescence induces testicular toxicity in mice: Autophagy-apoptosis interplay. *Ecotoxicology and Environmental Safety, 302,* 118598. https://doi.org/10.1016/j.ecoenv.2025.118598
19. Cheng, L., Ji, T., Zhang, M., & Fang, B. (2024). Recent advances in squalene: Biological activities, sources, extraction, and delivery system. *Trends in Food Science & Technology, 146,* 104392. https://doi.org/10.1016/j.tifs.2024.104392
20. Cheng, M. C., Ker, Y. B., Yu, T. H., Lin, L. Y., Peng, R. Y., & Peng, C. H. (2010). Chemical synthesis of 9(Z)-octadecenamide and its hypolipidemic effect: A bioactive agent found in the essential oil of mountain celery seeds. *Journal of Agricultural and Food Chemistry, 58*(3), 1502–1508. https://doi.org/10.1021/jf903573g
21. Chetehouna, S., Derouiche, S., Reggami, Y., Boulaares, I., & Frahtia, A. (2024). Gas chromatography analysis, mineral contents and anti-inflammatory activity of *Sonchus maritimus*. *Tropical Journal of Natural Product Research, 8*(4), 6787–6798.
22. Chrystal, P. (2021). *The history of the world in 100 pandemics, plagues and epidemics*. Pen & Sword History.
23. Desikachary, T. V. (1959). *Cyanophyta*. Indian Council of Agricultural Research.
24. Devi, T. S., Krishnan, R., & Kandasamy, S. (2021). Antifungal activity and molecular docking of phenol, 2,4-bis(1,1-dimethylethyl) produced by plant growth-promoting actinobacterium *Kutzneria* sp. strain TSII from mangrove sediments. *Archives of Microbiology, 203*(7), 4481–4492. https://doi.org/10.1007/s00203-021-02343-9
25. Edward, G. B., & David, C. S. (2010). *Freshwater algae: Identification and use as bioindicators*. John Wiley & Sons.
26. Ekeanyanwu, C. R., Ekeanyanwu, C. L., & Ugochukwu, K. N. (2024). Towards a natural treatment for mania: Red onion husk extract modulates neuronal resilience, redox signalling, and glial activation. *International Journal of Bipolar Disorders, 12*(1), 16. https://doi.org/10.1186/s40345-024-00338-7
27. El-Adl, M. F., Deyab, M. A., El-Shanawany, R. S., & Abu Ahmed, S. E. (2022). Fatty acids of *Cladophora glomerata* and *Chaetomorpha vieillardii* (Cladophoraceae) of different niches inhibit pathogenic microbial growth. *Aquatic Botany, 176,* 103461. https://doi.org/10.1016/j.aquabot.2021.103461

28. Elnabris, K. J., Elmanama, A. A., & Chihadeh, W. N. (2013). Antibacterial activity of four marine seaweeds collected from the coast of Gaza Strip, Palestine. *Mesopotamian Journal of Marine Science, 28*(1), 81–92.
29. Elshobary, M. E., Badawy, N. K., Ashraf, Y., Zatioun, A. A., Masriya, H. H., Ammar, M. M., … & Assy, A. M. (2025). Combating antibiotic resistance: Mechanisms, multidrug-resistant pathogens, and novel therapeutic approaches: An updated review. *Pharmaceuticals, 18*(3), 402. https://doi.org/10.3390/ph18030402
30. Entigu, R., Lihan, S., & Ahmad, I. (2013). The effect of combination of octadecanoic acid, methyl ester and ribavirin against measles virus. *International Journal of Scientific & Technology Research, 2*(10), 181–186.
31. Flocchetti, M., Bastari, G., Cipolletti, M., Leone, S., Acconcia, F., & Marino, M. (2021). The peculiar estrogenicity of diethyl phthalate: Modulation of estrogen receptor α activities in the proliferation of breast cancer cells. *Toxics, 9*(10), 237. https://doi.org/10.3390/toxics9100237
32. Frahtia, A., & Niemann, J. (2024). GC-MS analysis and quantification of some secondary metabolites of the Algerian *Phragmites australis* leaf extract and their biological activities. *Current Trends in Biotechnology and Pharmacy, 18*(4), 213–222.
33. Ganesan, T., Subban, M., & Seedevi, P. (2024). Structural characterization of n-hexadecanoic acid from the leaves of *Ipomoea eriocarpa* and its antioxidant and antibacterial activities. *Biomass Conversion and Biorefinery, 14,* 14547–14558. https://doi.org/10.1007/s13399-022-03686-1
34. Hamilton, J. T. G., McRoberts, W. C., Larkin, M. J., & Harper, D. B. (1995). Long-chain haloalkanes are incorporated into fatty acids by *Rhodococcus rhodochrous* NCIMB 13064. *Microbiology, 141*(10), 2611–2617. https://doi.org/10.1099/13500872-141-10-2611
35. Hashimi, A. A., Al-Rubaiee, G. H., & Zaki, N. H. (2022, October). Investigating the antimicrobial and anticancer action of spirulina hexan extract. In *AIP Conference Proceedings* (Vol. 2398, No. 1, p. 020001). AIP Publishing. https://doi.org/10.1063/5.0069876
36. Horincar, V. B., Parfene, G., Tyagi, A. K., Gottardi, D., Dinică, R., Guerzoni, M. E., & Bahrim, G. (2014). Extraction and characterization of volatile compounds and fatty acids from red and green macroalgae from the Romanian Black Sea in order to obtain valuable bioadditives and biopreservatives. *Journal of Applied Phycology, 26*(1), 551–559. https://doi.org/10.1007/s10811-013-0088-8
37. Hrichi, S., Chaâbane-Banaoues, R., Alibrando, F., Altemimi, A. B., Babba, O., Majdoub, Y. O. E., Nasri, H., Mondello, L., Babba, H., Mighri, Z., & Cacciola, F. (2022). Chemical composition, antifungal and anti-biofilm activities of volatile fractions of *Convolvulus althaeoides* L. roots from Tunisia. *Molecules, 27*(20), 6834. https://doi.org/10.3390/molecules27206834
38. Huang, Z.-R., Lin, Y.-K., & Fang, J.-Y. (2009). Biological and pharmacological activities of squalene and related compounds: Potential uses in cosmetic dermatology. *Molecules, 14*(1), 540–554. https://doi.org/10.3390/molecules14010540
39. Javed, M. R., Salman, M., Tariq, A., Tawab, A., Zahoor, M. K., Naheed, S., Shahid, M., Ijaz, A., & Ali, H. (2022). The antibacterial and larvicidal potential of bis-(2-ethylhexyl) phthalate from *Lactiplantibacillus plantarum*. *Molecules, 27*(21), 7220. https://doi.org/10.3390/molecules27217220
40. Juárez-Rodríguez, M. D., Chávez-Béjar, M. I., Balderas-Rentería, I., Rivera-Chavira, B. E., Nevárez-Moorillón, G. V., Morones-Ramírez, J. R., & Campos-García, J. (2021). Anti-virulence properties of saturated fatty acids in *Pseudomonas aeruginosa*: A new strategy to inhibit virulence factors. *Frontiers in Cellular and Infection Microbiology, 10,* 597517. https://doi.org/10.3389/fcimb.2020.597517

41. Jung, F., Krüger-Genge, A., Waldeck, P., & Küpper, J. H. (2019). Spirulina platensis, a super food? *Journal of Cellular Biotechnology, 5*(1), 43–54. https://doi.org/10.3233/JCB-189002
42. Kang, P., Huang, J., & Su, G. (2025). A critical review on sources, environmental occurrence, toxicity, and human exposure of an abundant organophosphate ester tris (2,4-di-tert-butylphenyl) phosphate. *Reviews of Environmental Contamination and Toxicology, 263*(1), 14. https://doi.org/10.1007/398_2024_333
43. Kaur, J., & Nobile, C. J. (2023). Antifungal drug-resistance mechanisms in *Candida* biofilms. *Current Opinion in Microbiology, 71,* 102237. https://doi.org/10.1016/j.mib.2022.102237
44. Khan, S., & Sharma, V. (2020). Evaluation of anti-inflammatory, analgesic, antipyretic effect of eicosane, pentadecane, octacosane, and heneicosane. *Asian Journal of Pharmaceutical and Clinical Research, 13*(4), 29–35. https://doi.org/10.22159/ajpcr.2020.v13i4.36414
45. Kim, S. K., & Karadeniz, F. (2012). Biological importance and applications of squalene and squalane. In J. Janick (Ed.), *Advances in food and nutrition research* (Vol. 65, pp. 223–233). Academic Press. https://doi.org/10.1016/B978-0-12-416003-3.00014-7
46. Kyriakidis, I., Vasileiou, E., Pana, Z. D., & Tragiannidis, A. (2021). *Acinetobacter baumannii* antibiotic resistance mechanisms. *Pathogens, 10*(3), 373. https://doi.org/10.3390/pathogens10030373
47. Laungsuwon, R., & Chulalaksananukul, W. (2014). Chemical composition and antibacterial activity of extracts of freshwater green algae, *Cladophora glomerata* Kützing and *Microspora floccosa* (Vaucher) Thuret. *Journal of BioScience and Biotechnology, 3*(3), 211–218. https://europub.co.uk/articles/-A-158251
48. Lee, H.-S. (2006). Antimicrobial properties of turmeric (*Curcuma longa* L.) rhizome-derived ar-turmerone and curcumin. *Food Science and Biotechnology, 15*(4), 559–563. https://doi.org/10.3746/jfn.2006.15.4.559
49. Lee, Y. (2009). Activation of apoptotic protein in U937 cells by a component of turmeric oil. *BMB Reports, 42*(2), 96–100. https://doi.org/10.5483/BMBRep.2009.42.2.096
50. Levasseur, W., Perré, P., & Pozzobon, V. (2020). A review of high value-added molecules production by microalgae in light of the classification. *Biotechnology Advances, 41,* 107545. https://doi.org/10.1016/j.biotechadv.2020.107545
51. Lusk, B., Keeling, A. W., & Lewenson, S. B. (2016). Using nursing history to inform decision-making: Infectious diseases at the turn of the 20th century. *Nursing Outlook, 64*(2), 170–178. https://doi.org/10.1016/j.outlook.2015.11.016
52. Mahdi, Z. M., & Al-Hussieny, A. A. (2022). Classification of some species algae in the Tigris River within the city of Baghdad-Iraq. *Journal of Bionatura, 7*(1), 72–79. https://doi.org/10.21931/RB/2022.07.01.9
53. Maji, R., Chowdhury, S., Kar, K., & Mazumder, K. (2025). Current strategies for addressing antibiotic resistance: A comprehensive review. *Journal of Bio-X Research, 8,* 0040. https://doi.org/10.1097/JBR.0000000000000040
54. Manaswini, S., Ashata, R., Bhoomika, V., Nandini, P., Ganapathy, K., & Deesha, K. P. (2025). Antimicrobial and cytotoxic potential of endophytic *Aspergillus versicolor* isolate from the medicinal plant *Plectranthus amboinicus*. *Current Microbiology, 82,* 84. https://doi.org/10.1007/s00284-024-04050-8
55. Manolitsis, I., Feretzakis, G., Katsimperis, S., Angelopoulos, P., Loupelis, E., Skarmoutsou, N., & Skolarikos, A. (2023). A 2-year audit on antibiotic resistance patterns from a urology department in Greece. *Journal of Clinical Medicine, 12*(9), 3180. https://doi.org/10.3390/jcm12093180
56. Marrufo, T., Nazzaro, F., Mancini, E., Fratianni, F., Coppola, R., De Martino, L., Agostinho, A. B., & De Feo, V. (2013). Chemical composition and biological activity of the essential oil from leaves

of *Moringa oleifera* Lam. cultivated in Mozambique. *Molecules, 18*(9), 10989–11000. https://doi.org/10.3390/molecules180910989

57. Megumi, C., Muroyama, K., Sasako, H., & Tsuge, N. (2017). Preventive activity of ar-turmerone and bisacurone isolated from turmeric extract against ethanol-induced hepatocyte injury. *Food Science and Technology Research, 23*(2), 275–281. https://doi.org/10.3136/fstr.23.275
58. Michalak, I., & Messyasz, B. (2021). Concise review of *Cladophora* spp.: Macroalgae of commercial interest. *Journal of Applied Phycology, 33*(1), 133–166. https://doi.org/10.1007/s10811-020-02175-w
59. Milledge, J. J., Nielsen, B. V., Sadek, M. S., & Harvey, P. J. (2018). Effect of freshwater washing pretreatment on *Sargassum muticum* as a feedstock for biogas production. *Energies, 11*(7), 1771. https://doi.org/10.3390/en11071771
60. Miranda, S. M., Lopes, M., & Belo, I. (2024). Exploring the use of hexadecane by *Yarrowia lipolytica*: Effect of dissolved oxygen and medium supplementation. *Journal of Biotechnology, 380,* 29–37. https://doi.org/10.1016/j.jbiotec.2023.12.006
61. Mishra, S., Verma, S. S., Rai, V., Awasthee, N., Arya, J. S., Maiti, K. K., & Gupta, S. C. (2019). *Curcuma raktakanda* induces apoptosis and suppresses migration in cancer cells: Role of reactive oxygen species. *Biomolecules, 9*(4), 159. https://doi.org/10.3390/biom9040159
62. Modi, A., & Jain, A. (2019). Green approach for the synthesis of 3-methyl-1-phenyl-4-((2-phenyl-1H-indol-3-yl)methylene)-1H-pyrazole-5(4H)-ones and their DNA cleavage, antioxidant, and antimicrobial activities. *Research on Chemical Intermediates, 45*(11), 5067–5083. https://doi.org/10.1007/s11164-019-03874-1
63. Mohammed, A. H., Lhwak, N. S., & Jabbar, A. A. (2024). Extraction and identification of certain bioactive compounds with antibacterial activity from the green algae *Cladophora glomerata*. *Medicra, 7*(1), 1–9. https://doi.org/10.21070/medicra.v7i1.1742
64. Momodu, I. B., Okungbowa, E. S., Agoreyo, B. O., & Maliki, M. M. (2022). Gas chromatography–mass spectrometry identification of bioactive compounds in methanol and aqueous seed extracts of *Azanza garckeana* fruits. *Nigerian Journal of Biotechnology, 38*(1), 25–38. https://doi.org/10.4314/njb.v38i1.3
65. Mondal, S., Basu, S., Ghosh, S., Guria, S., & Mukherjee, S. (2024). Diethyl phthalate, a plasticizer, induces adipocyte inflammation and apoptosis in mice after long-term dietary administration. *Journal of Biochemical and Molecular Toxicology, 38*(1), e23561. https://doi.org/10.1002/jbt.23561
66. Musa, A. M., Ibrahim, M. A., Aliyu, A. B., Abdullahi, M. S., Tajuddeen, N., Ibrahim, H., & Oyewale, A. O. (2015). Chemical composition and antimicrobial activity of hexane leaf extract of *Anisopus mannii* (Asclepiadaceae). *Journal of Intercultural Ethnopharmacology, 4*(2), 129–133. https://doi.org/10.5455/jice.20150106124652
67. Nasr, Z. S., El-Shershaby, H. M., Sallam, K. M., Nasr, N. E., Abed, I. E. A., Abd El-Ghany, I. Y., & Sidkey, N. M. (2022). Evaluation of antimicrobial potential of tetradecane extracted from *Pediococcus acidilactici* DSM 20284-CM isolated from curd milk. *Egyptian Journal of Chemistry, 65*(3), 705–713. https://doi.org/10.21608/ejchem.2021.70243.3547
68. Nesrullah, Z., Al-Rubaiee, G., & Zaki, N. (2023). Evaluation of the extract of microalgae and its fatty acids on *Candida* spp. isolated from renal impairment patients. *Israa University Journal for Applied Science (IUJAS), 7*(1), 252–271. https://doi.org/10.5281/zenodo.8406104
69. Nie, H., Nie, M., Xiao, T., Wang, Y., & Tian, J. (2017). Hexadecane degradation of *Pseudomonas aeruginosa* NY3 promoted by glutaric acid. *Science of the Total Environment, 575,* 1423–1428. https://doi.org/10.1016/j.scitotenv.2016.09.223
70. Nisa, S., Bibi, Y., Masood, S., Ali, A., Alam, S., Sabir, M., Qayyum, A., Ahmed, W., Alharthi, S., Santali, E. Y., Alharthy, S. A., Bawazir, W. M., & Almashjary, M. N. (2022). Isolation,

characterization and anticancer activity of two bioactive compounds from *Arisaema flavum* (Forssk.) Schott. *Molecules, 27*(22), 7932. https://doi.org/10.3390/molecules27227932

71. Nova, P., Martins, A. P., Teixeira, C., Abreu, H., Silva, J. G., Silva, A. M., … & Gomes, A. M. (2020). Foods with microalgae and seaweeds fostering consumers' health: A review on scientific and market innovations. *Journal of Applied Phycology, 32,* 1789–1802. https://doi.org/10.1007/s10811-019-02090-1
72. Ohaegbu, C. G., Ngene, A. C., & Alisigwe, C. V. (2022). GC-MS analysis, antibacterial and antibiofilm activities of extracts of *Zingiber officinalis* against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Pharmacology and Toxicology of Natural Medicines, 2*(1), 25–36.
73. O'Neill, E. A., & Rowan, N. J. (2022). Microalgae as a natural ecological bioindicator for the simple real-time monitoring of aquaculture wastewater quality including provision for assessing impact of extremes in climate variance: A comparative case study from the Republic of Ireland. *Science of the Total Environment, 802,* 149800. https://doi.org/10.1016/j.scitotenv.2021.149800
74. Pachori, P., Gothalwal, R., & Gandhi, P. (2019). Emergence of antibiotic resistance *Pseudomonas aeruginosa* in intensive care unit: A critical review. *Genes & Diseases, 6*(2), 109–119. https://doi.org/10.1016/j.gendis.2019.04.001
75. Pang, Z., Raudonis, R., Glick, B. R., Lin, T. J., & Cheng, Z. (2019). Antibiotic resistance in *Pseudomonas aeruginosa*: Mechanisms and alternative therapeutic strategies. *Biotechnology Advances, 37*(1), 177–192. https://doi.org/10.1016/j.biotechadv.2018.11.013
76. Perera, M., Wijayarathna, D., Wijesundera, S., … & Samarakoon, M. (2019). Biofilm-mediated synergistic degradation of hexadecane by a naturally formed community comprising *Aspergillus flavus* complex and *Bacillus cereus* group. *BMC Microbiology, 19,* 84. https://doi.org/10.1186/s12866-019-1460-4
77. Prathipkumar, S., Vijaykumar, S., & Kim, W. (2023). Biogenic-mediated silver nanoparticles using heneicosane and their enhanced antimicrobial, antiproliferative, sensing capability and photocatalytic potential. *Applied Nanoscience, 13,* 5839–5849. https://doi.org/10.1007/s13204-023-02867-5
78. Prescott, G. W. (1982). *Algae of the Western Great Lakes area* (16th ed.). W. C. Brown Co.
79. Rani, K., Sandal, N., & Sahoo, P. K. (2018). A comprehensive review on *Chlorella*: Its composition, health benefits, market and regulatory scenario. *Pharma Innovation Journal, 7*(7), 584–589.
80. Ren, J., Wang, J., Karthikeyan, S., Liu, H., & Cai, J. (2018). Natural anti-phytopathogenic fungi compound phenol, 2,4-bis(1,1-dimethylethyl) from *Pseudomonas fluorescens* TL-1. *Indian Journal of Biochemistry and Biophysics, 56*(2), 162–168. https://doi.org/10.56042/ijbb.v56i2.14227
81. Rouis-Soussi, L., Ayed, A., Skhiri, F., Chaabane, H., & Boussaid, M. (2014). Chemical composition and antibacterial activity of essential oils from the Tunisian *Allium nigrum* L. *EXCLI Journal, 13*, 526–535. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4463966
82. Roy, M., Karhana, S., Shamsuzzaman, M., & Khan, M. A. (2023). Recent drug development and treatments for fungal infections. *Brazilian Journal of Microbiology*, 1–22. https://doi.org/10.1007/s42770-023-01067-7
83. Sagar, P., Azeem, A., Banjara, S. K., & Veleri, S. (2023). The role of food chain in antimicrobial resistance spread and One Health approach to reduce risks. *International Journal of Food Microbiology, 394,* 110148. https://doi.org/10.1016/j.ijfoodmicro.2023.110148
84. Sagna, A., Nair, R. V. R., Hulyalkar, N., Rajasekharan, S., Nair, V. T. G., Sivakumar, K. C., Suja, S. R., Baby, S., & Sreekumar, E. (2023). Ethyl palmitate, an anti-chikungunya virus principle from

Sauropus androgynus, a medicinal plant used to alleviate fever in ethnomedicine. *Journal of Ethnopharmacology, 309,* 116366. https://doi.org/10.1016/j.jep.2023.116366

85. Salman, Z. N. (2018). *A comparative taxonomic study (anatomical and chemical) for fourteen wild species of Brassicaceae in Diyala Province* (Master's thesis). University of Diyala, College of Science.
86. Salman, Z. N. (2024). *Biological activity of some microalgae extracts on some pathogenic microbes and cancer cell lines* (Doctoral dissertation). Mustansiriyah University, College of Science.
87. Satar Al-Baaj, A., & Abdul-Jalil, T. Z. (2022). Phytochemical screening of petroleum ether fractions by GC/MS and isolation of lupeol from two different parts of Iraqi *Leucaena leucocephala*. *Iraqi Journal of Pharmaceutical Sciences, 31*(Suppl.), 62–74.
88. Sathasivam, R., Radhakrishnan, R., Hashem, A., & Abd_Allah, E. F. (2019). Microalgae metabolites: A rich source for food and medicine. *Saudi Journal of Biological Sciences, 26*(4), 709–722. https://doi.org/10.1016/j.sjbs.2017.11.003
89. Senthil, R., Archunan, G., Vithya, D., & Saravanan, K. M. (2025). Hexadecanoic acid analogs as potential CviR-mediated quorum sensing inhibitors in *Chromobacterium violaceum*: An in silico study. *Journal of Biomolecular Structure and Dynamics, 43*(7), 3635–3644. https://doi.org/10.1080/07391102.2023.2299945
90. Shaaban, M. T., Ghaly, M. F., & Fahmi, S. M. (2021). Antibacterial activities of hexadecanoic acid methyl ester and green-synthesized silver nanoparticles against multidrug-resistant bacteria. *Journal of Basic Microbiology, 61*(6), 557–568. https://doi.org/10.1002/jobm.202100061
91. Shah, S. B., Wang, Y., Anwar, N., Abbas, S. Z., Khan, K. A., Wang, S. M., & Ullah, M. W. (2024). Co-metabolic degradation and metabolite detection of hexabromocyclododecane by *Shewanella oneidensis* MR-1. *Applied Microbiology and Biotechnology, 108*(1), 25. https://doi.org/10.1007/s00253-023-12905-6
92. Shi, J., Xu, C., Xiang, L., Chen, J., & Cai, Z. (2020). Tris(2,4-di-tert-butylphenyl) phosphate: An unexpected abundant toxic pollutant found in PM2.5. *Environmental Science & Technology, 54*(17), 10570–10576. https://doi.org/10.1021/acs.est.0c03709
93. Stoyneva-Gärtner, M. P., Descy, J. P., Uzunov, B. A., Miladinov, P., Stefanova, K., Radkova, M., & Gärtner, G. (2023). Diversity of the summer phytoplankton of 43 waterbodies in Bulgaria and its potential for water quality assessment. *Diversity, 15*(4), 472. https://doi.org/10.3390/d15040472
94. Sun, X., Wang, M., Zhang, G., Ma, T., & Zhang, Y. (2017). *n*-Pentacosane acts as both contact and volatile pheromone in the tea weevil, *Myllocerinus aurolineatus*. *Journal of Chemical Ecology, 43*(6), 557–566. https://doi.org/10.1007/s10886-017-0856-6
95. Syed, R. U., Moni, S. S., Lila, A. S. A., Abdallah, M. H., Abouzied, A. S., Banu, H., Alreshidi, K. S. M., Alrashidi, B. M. W., Hadi, M. A., El-Horany, H., Abdelwahab, S. I., & Taha, M. M. E. (2022). Spectral analysis and antiulcer potential of *Lactuca sativa* through the amelioration of proinflammatory cytokines and apoptosis markers. *Life, 12*(10), 1641. https://doi.org/10.3390/life12101641
96. Thawabteh, A. M., Ghanem, A. W., AbuMadi, S., Thaher, D., Jaghama, W., Karaman, R., Scrano, L., & Bufo, S. A. (2024). Antibacterial activity and antifungal activity of monomeric alkaloids. *Toxins, 16*(11), 489. https://doi.org/10.3390/toxins16110489
97. Theodosiou, A. A., Jones, C. E., Read, R. C., & Bogaert, D. (2023). Microbiotoxicity: Antibiotic usage and its unintended harm to the microbiome. *Current Opinion in Infectious Diseases, 36*(5), 371–378. https://doi.org/10.1097/QCO.0000000000000945

98. Toma, J. J., & Aziz, F. H. (2022). Algal study in the springs and streams from Shaqlawa district, Erbil Province, Iraq II – Zygnematales. *Iraqi Journal of Science, 63*(3), 1438–1452. https://doi.org/10.24996/ijs.2022.63.3.25
99. Tyagi, S., Singh, R. K., & Tiwari, S. P. (2021). Anti-enterococcal and anti-oxidative potential of a thermophilic cyanobacterium, *Leptolyngbya* sp. HNBGU 003. *Saudi Journal of Biological Sciences, 28*(7), 4022–4028. https://doi.org/10.1016/j.sjbs.2021.04.003
100. Tyagi, T., & Agarwal, M. (2017). GC-MS analysis of invasive aquatic weed, *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) Solms. *International Journal of Current Pharmaceutical Research, 9*(3), 111–117. https://doi.org/10.22159/ijcpr.2017v9i3.19203
101. Vanitha, V., Vijayakumar, S., Nilavukkarasi, M., Punitha, V. N., Vidhya, E., & Praseetha, P. K. (2020). Heneicosane—A novel microbicidal bioactive alkane identified from *Plumbago zeylanica* L. *Industrial Crops and Products, 154,* 112748. https://doi.org/10.1016/j.indcrop.2020.112748
102. Wei, L., Ran, J., Li, Z., Zhang, Q., Guo, K., Mu, S., ... & Xiao, Y. (2023). Chemical composition, antibacterial activity and mechanism of action of fermentation products from *Aspergillus niger* xj. *Applied Biochemistry and Biotechnology,* 1–18. https://doi.org/10.1007/s12010-023-04788-5
103. Zahn, M., Bhamidimarri, S. P., Baslé, A., Winterhalter, M., & Van den Berg, B. (2016). Structural insights into outer membrane permeability of *Acinetobacter baumannii*. *Structure, 24*(2), 221–231. https://doi.org/10.1016/j.str.2015.12.009