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Extraction and Identification of Certain Bioactive Compounds With Antibacterial Activity From The Green Algae *Cladophora glomerate*

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ABSTRACT

Cladophora glomerata algae extract was used to inhibit the growth of five bacterial species: *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis*, Citrobacter, and *Klebsiella pneumoniae*. Algae samples were collected from the river Euphrates in Al-Fadhiliya/ Thi-Qar governorate and extracted into alcohol. The concentrations of the extracts were compared with five antibiotics. The 200% concentration showed the highest effectiveness against bacteria, with an inhibition zone of 28mm. The antibiotics Imipenem and Meropenem had an inhibition zone of 28mm, while Amikacin, Norfloxacin, and Piperacillin had an inhibition area of 12-21mm. The 20% concentration was least effective against *Escherichia coli*, with an inhibition diameter of 2mm. The alcoholic extract of *C. glomerata* alga had the highest inhibition region of 28 against *Klebsiella pneumoniae* and 13mm against *Proteus mirabilis*. The chemical algal contents were identified using mass GC technology, revealing compounds like 10-Undecyn-1-ol, n-hexadecanoic acid, 4,4-dimethyl-1-hex, and isoamyl nitrite, which were found to have the major function of inhibiting bacterial growth.

Keywords: Antibiotics, *Cladophora glomerata*, Pathogenic Bacteria

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INTRODUCTION

Algae are a group of thallophytes, or plants without stems, roots, leaves, fruits, or flowers. Because they contain auxiliary representative pigments like carotenoids, xanthophylls, and phycobiloproteins, as well as different types of chlorophyll pigments, algae are autotrophic organisms that can produce oxygen during the process of photosynthesis Winnacker (2015).

Algae can live in the ocean's dark, deep waters. The majority of them are separated into red, brown, yellow, green, and other types of algae based on the pigment components found in them. Not only are they useful in pharmacology, medicine, and the environment, but they are renewable living resources that are utilized as fertilizer and food in many parts of the world Nithya and Dhanalakshmi (2016).

Antibiotics have revolutionized therapeutics globally, but their uncontrolled use has led to antibacterial resistance, a major public health threat in the 21st century. Over 40 countries have shared reports on antimicrobial resistance surveillance with the World Health Organization, indicating its criticality. As a result, alternatives are needed to address this challenging situation and protect public health. Bhowmick et al., (2020).

Many alternatives, including algae, have been studied for their potent antibacterial qualities. Their capacity to endure harsh conditions and competitive settings enables them to produce a diverse array of bioactive substances through intricate metabolic processes that differ significantly from those of terrestrial animals. Algae metabolites are interesting sources of antibacterial chemicals because they have strong antibacterial action against a variety of bacteria, including ones that are resistant to drugs Surendhiran et al., (2021); Gao et al., (2023).

C. glomerata, a group of over 183 species of green algae. These algae are rich in antimicrobial, antioxidant, antidiabetic, and antitoxic cell activities, making them valuable for human health. They are classified as a healthy food by the World Health Organization. However, some antibiotics produced by microorganisms have become useless due to severe antimicrobial symptoms, high costs, and side effects like hypersensitivity. This has led to the development of new antibiotics in diverse environments, highlighting the importance of algae as active substances. Mohammed et al., (2021).

C. glomerata can be found in both fresh and saltwater habitats. It is well-known for its intense, recurring blooms. Saturated and unsaturated fatty acids with antibacterial properties are abundant in C. glomerata. Additionally, phenolic compounds-which are well-known for their antioxidant qualities-are present in it. In a rat study, its ethanolic extract also shown analgesic, anti-inflammatory, and anti-ulcer properties Petchsomrit et al., (2023).

The current study aims to extract the active substances from algae *C. glomerata* using the organic solvent of ethanol, and test its effect against the bacterial isolated from pathogenic samples.

METHOD

Collecting of algae

In January 2024, during the winter, C. glomerata was collected in plastic bags in the form of biomass algae from the Euphrates River in the city of Al-Fadhiliya/ Thi-Qar governorate. After that, the algae was brought into the lab and cleaned three times using distilled water after first being cleaned with regular water to get rid of any remaining dust, dirt, and living organisms. The algae mass was exposed to sunlight for five days, grinding into a powder, and then canning it. After that, it was kept at 0–4°C until used Peller et al., (2007); Weidman et al., (1984).

Preparation of alcoholic algal extracts

In a 500 cm3 volumetric flask, 10g of the dry weight of *C. glomerata* was combined with 250 ml of 70% ethyl alcohol. A Soxhelt device was used to extract the material for 12 hours at 78°C. After that, the mixture was allowed to cool and filtered through Whattman No. 1 filter paper. Finally, the mixture was concentrated using a rotary evaporator set at 50°C. The procedure was carried out multiple times to produce a sticky substance, which was then stored in the refrigerator until used Al-Saeed (2002). A stock solution of 200% was created by dissolving 2 g of the C. glomerata extract in 10 ml of distilled water. This solution was then diluted several times.

GC-Mass device analysis of the extract

This instrument is unique as a detector for volatile organic compounds and gaseous mixtures of different inorganic compounds due to the sophisticated technology used to link two gas chromatography and mass spectrometer devices. A sample of the substance's solution can be injected into the gas chromatography apparatus, which separates the mixture's constituent parts, in order to identify any pure unknown substance or mixture. After the materials have been separated, they are each fed into a mass spectrometer, which uses their molecular weights and the information library that is attached to it to identify the types of materials. This process does not require the use of a standard substance and produces results that can be compared to a database that has been stored, which numbers more than 400,000 compounds Kazem and Kraidy (2017).

Testing the biological effectiveness of the extracts

The effectiveness of the alcoholic extracts at concentrations (20, 40, 100, 200%) was tested against five pure pathogenic bacterial isolates (Escherichia coli, Staphylococcus aureus, Proteus mirabilis, Citrobacter, and Klebsiella pneumoniae). Bacteria cultured on Petri dishes containing Mueller-Hinton medium were inoculated using the spreading method. A holes were made using a cork porer with a diameter of 6mm, and the equivalent of 40 micrograms of extract was added to the holes and left to dry under sterile conditions.

The bacteria were then incubated at a temperature of 37°C for 24 hours, and the corona formed around the holes was measured with a ruler, which is an indication of growth inhibition Jeremiah et al., (2007).

RESULT AND DISCUSSION

The control group had a considerably greater mean RBCs, Hb, platelet count, and total cellular volume, whereas the hypothyroid group had a significantly lower mean RBC count, MCV, and MCH. When comparing MCV and MCH, the hyperthyroid group could not identify any significant differences. There were no statistically significant MCHC results in either the hypothyroid or hyperthyroid groups compared to the control group. In terms of total leukocyte count and platelet count, neither the control group nor the hypothyroid group, nor the hyperthyroid group differed significantly from the other two (Table 1).

C. glomerata extracts

TABLE 1. Displays the percentages of alcoholic and aqueous extracts produced from C. glomerata

Substance	Alcoholic extract
Wight of Powder	60g
Wight of extract	3g
Percentage	0.05

Identification the C. glomerata extracts by GC-mass spectrum

The absorption spectra of the ethanolic extract looked to be composed of seven components. There are four compounds that have a high percentage among the other compounds and are thought to be the key reason for the extract's potency against germs. The compounds that occupy these high proportion is:

The mass spectrum of the chemical separated by gas chromatography - the mass spectrum with a detention duration of 21,580 minutes - and by matching this component with the computer information base in The gadget demonstrated that it is the molecule 10-Undecyn-1-ol, with the chemical formula C11H20O and a molecular weight of 168 Daltons, occupying 51.99% of the total area of the separated compounds as in (Figure 1).

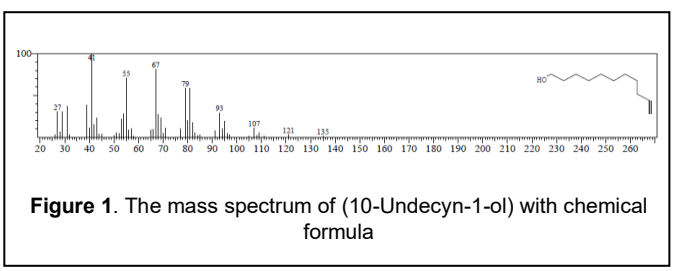

Figure 1. The mass spectrum of (10-Undecyn-1-ol) with chemical formula

The mass spectrum of the compound that was separated using the gas chromatography technique is depicted in Figure 2. The compound was found to be compound C16H3202, with a molecular weight of 256 Dalton and an area that accounted for 16.31% of the total area of isolated compounds, after being matched with the information base computerised in its chemical formula (Palmitic acid) n-Hexadecanoic acid.

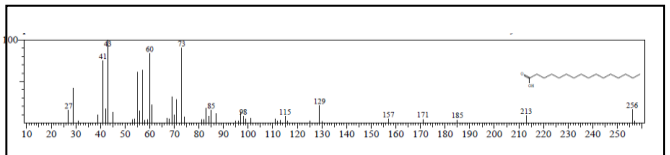

Figure 2. The mass spectrum of (n-Hexadecanoic acid) with chemical formula

The mass spectrum of the substance that was separated with a holding time of 21.103 minutes using the gas chromatography-mass spectrum technique is displayed in Figure 3. By comparing this component's weight of C8H16 and chemical formula, 4,4-Dimethyl-1-hexene, with the computerised information base, the gadget demonstrated that it is the molecular compound 112 Dalton and that it occupies 11.38% of the total area of separated compounds.

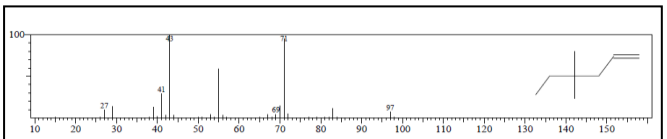

Figure 3.The mass spectrum of (4,4-Dimethyl-1-hexene) with chemical formula

The molecules that was separated using the gas chromatography technique is depicted in Figure 4 as its mass spectrum, which has a detention time of 17.571 minutes. It was established that this component is isoamyl nitrite by comparing it to the computer information base within the apparatus. Its molecular weight is 117 Daltons, its chemical formula is C5H11NO2, and its area of occupancy is 9.43%. of the entire space occupied by segregated automobiles.

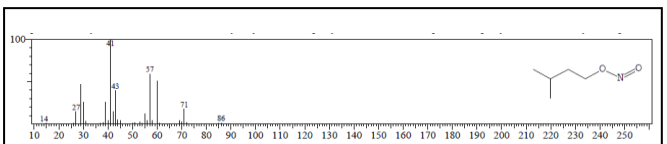

Figure 4. The mass spectrum of (isoamyl nitrite) with chemical formula

The effectiveness of the alcoholic extract

Table 2 shows the effectiveness of the size of the inhibition zone, estimated in (mm), as a result of the effectiveness of the alcoholic extract of C. glomerata algae, as it was shown from the results that the effectiveness of the concentration is 20% against the bacteria E. coli, Staph. aureus, Proteus mirbaili, Citrobacter, and K. pneumoniae, it was (4, 3, 4, 3, 4) mm, respectively. As for the 40% concentration, it was (7, 5, 7, 6, 8). As for the 100% concentration, its effectiveness was (10, 7, 10, 8, 14) mm. The concentration of 200% was equal to (12, 11, 13, 12, 28) mm, respectively.

TABLE 2. shows the effectiveness of the alcoholic extract of C. glomerata in increasing the size of the inhibition zone in millimeters.

Conc. Bacterial isolates	20%	40%	100%	200%
E. coli	4	7	10	12
Staph. aureus	3	5	7	11
P. mirbaili	4	7	10	13
Citrobacter	3	6	8	12
K. pneumoniae	4	8	14	28

Drug sensitivity to bacterial isolates

TABLE 3. The size of the inhabition zone, estimated in (mm), as a result of the effectiveness of antibiotics

Antibiotic Bacteria	Amikacin	Imipenem	Norfloxacin	Meropenem	Piperacillin
E. coli	18	25	15	30	20
Staph. aureus	13	25	0	15	3
P. mirbailis	18	30	20	32	0
Citrobacter	12	25	28	16	21
K. pneumonia	18	28	18	28	0

Table **3** shows the size of the corona formed, estimated in (mm), as a result of the effectiveness of antibiotics. From the table, the antibiotic Amikacin was effective against the pathogenic bacteria E coli K. pneumoni, Citrobacter, P. mirbailis, and Staph.aureus, and (18, 12, 13, 18, 18) mm, respectively. As for the antibiotic Imipenem, the results were (28, 25, 25, 30, 25) mm, respectively, and for the antibiotic Norfloxacin, the results were (28, 25, 25, 30, 25) mm, respectively. Its effectiveness against bacteria was (18,28,020,15) mm, respectively. As for the Meropenem, the results were (28,16,15,32,30) mm, respectively, and the Piperacillin was effective against bacteria (0,21,3,20) mm. Respectively.

The present research's results demonstrated that the green alga C. glomerata has a percentage of (0.05) for the alcoholic extract and a percentage of (0.2) for the aqueous extract, as indicated in Table **1**. The variation in the polar nature of the materials that are used in the extraction is what causes a differences in the amount and quality of the extracted materials. This can also be linked to variations in the culture media and extraction techniques that was used, as well as growth variables like pH, temperature, and the volume of culture used in batch culture to yield biologically active compounds Shareef and AL-Salami (2011).

With the alcoholic extract of C. glomerata algae, E. coli bacteria colonies were inhibited within 24 hours; concentrations of 20%, 40%, 100%, and 200% mg/ml were used; the degree of inhibition reached 4, 7, 10, and 12 mm, respectively, which is close to what was obtained Al-Nasser (2011). These results were minimal in comparison to what Kamel et al. (2013) achieved with the alcoholic extract of Mougeati sp. Using the alcoholic extract of the moss Mougeotia sp., colonies of *E. coli* bacteria were inhibited within a period of 24 hours. The degree of inhibition reached 15 and 18 mm, respectively, at concentrations of 1 and 2 g/L.

The number of *S. aureus* bacteria colonies was inhibited within 24 hours to (3, 5, 7, 11) mm, respectively. These results are comparable to study of Danyal et al., (2013), which used Pithophora oedogonium's ethanolic extract to inhibit the pathogenic *S. aureus* bacteria. When contrasting the *C. glomerata* extract with the antibiotics listed above, as shown in Table **3**, which contain inhibition zones 13, 25, 0, 15, and 3mm. The antibiotics' inhibition results make it evident that the extract concentrations that is used were more effective to those of norfloxacin and piperacillin, with the exception of the concentration 20%, which was equivalent to the latter.

When *Proteus mirbaili* bacteria were exposed to antibiotics, all concentrations of algae extract performed better than Piperacillin at inhibiting colony counts to (4, 7, 10, 13) mm, respectively. The colony counts of Citrobacter bacteria were found to be reduced to 3, 6, 8, and 12 mm. This is consistent with research conducted by Leeds et al., (2014) which used an ethanolic extract of *Myagropsis myagroides* algae to combat Listeria monocytogenes, and by Cakmak et al., (2014) which used an ethanolic extract of Dunaliella salina algae to combat the same bacteria.

As for the bacterium Klebsiella pneumonia, its colonies were inhibited to 4, 8, 14, and 28 mm, where the concentration was 200% better than the alcoholic extract of the Mougeotia sp. used by Kamel et al. (2013) that inhibited *K. pneumoniae* bacteria colonies to 18 mm when using concentrations of 2 g/L, and the inhibition zone decreased with a concentration of 1 g/L, reaching 16 mm. All of the concentrations used outperformed the antibiotic Piperacillin in comparison to the other antibiotics, and the 200% concentration performed better than all other antibiotics except Meropenem, which was equal.

These substances are responsible for the inhibition's efficacy: Among the total chemical compounds identified from *C. glomerata* by the GC-Mass analysis were Undecyn-1-ol-10 with a ratio of 51.99 and a retention time of 21.580, n-Hexadecanoic acid with a ratio of 16.31 and a retention time of 19.733, 4,4-Dimethyl-1-hexene with a ratio of 11.38 and a retention time of 21.103, and isoamyl nitrite with a ratio of 9.43 and a retention time of 17.571.

The fact that algae extracts contain steroidal fatty and protein compounds that inhibit bacteria is one of the causes of their inhibition. Furthermore, pigments and their derivatives, like chlorophyll and carotene, have antibacterial properties. Additionally, fatty acids possess anti-gram-positive and anti-gram-negative bacterial activity. Whether used singly or in combination, fatty acids can have either a bacteriostatic or bacteriocidal effect Aubert (1979).

CONCLUSION

Cladophora glomerata algae extract was used to inhibit the growth of five bacterial species: *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis*, Citrobacter, and *Klebsiella pneumoniae*. The concentrations of the extracts

were compared with five antibiotics. The 200% concentration showed the highest effectiveness against bacteria, with an inhibition zone of 28mm. The antibiotics Imipenem and Meropenem had an inhibition zone of 28mm, while Amikacin, Norfloxacin, and Piperacillin had an inhibition area of 12-21mm. The 20% concentration was least effective against *E. coli*, with an inhibition diameter of 2mm. The alcoholic extract of *C. glomerata* alga had the highest inhibition region of 28 against *K. pneumoniae* and 13mm against *P. mirabilis*. The chemical algal contents were identified using mass GC technology, revealing compounds like 10-Undecyn-1-ol, n-hexadecanoic acid, 4,4-dimethyl-1-hex, and isoamyl nitrite, which were found to have the major function of inhibiting bacterial growth.

AUTHOR CONTRIBUTIONS

All authors played a role in the preparation of this article.

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