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Research Article

Investigate the Antimicrobial Activity of Methanolic Extract of *Cladophora glomerata*

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A B S T R A C T

Background: Macroalgae have a wide range of natural compounds which have natural antioxidants.

Method: In this study, four concentrations of the methanolic extract of the Chlorophyta-related macroalgae-isolate *Cladophora glomerata* were tested (concentrations of 12.5, 25, 50, and 100 mg/ml) *in vitro* to evaluate their effect on the growth inhibition of pathogenic fungal isolate (*Candida albicans*) and pathogenic bacterial isolates (*Bacillus cereus, Micrococcus spp., Pseudomonas aeruginosa,* and *Proteus mirabilis*).

Result: Methanolic extracts had an inhibitory effect on *Candida albicans* and all bacterial isolates tested. Microbiological fungal and pathogenic bacterial isolates are inhibited by a diameter of the inhibition zone (in millimeters). The maximum biological inhibitory action was observed at dosages of 100 mg/ml. *Micrococcus* spp. exhibited the highest susceptibility to the treatment. While the smallest diameter of inhibition zones was observed at a concentration of 12.5 mg/ml against microorganisms, the observed diameters ranged between 0 mm (*Proteus mirabilis*) and 7 mm (*Micrococcus* spp). Alkaloids, tannins, flavones, resins, saponins, terpenes, and steroids were among the active chemicals found in the methanol extract of *Cladophora glomerata.* To tentatively identify the compounds responsible for these activities, Gas Chromatography-Mass Spectrometry GC–MS was used to chemically characterise the methanol extract. There were six main components in the extract: tridecyne, hexadecanoic acid, octadecadienoic acid, octadecadienoic acid, octadecadien-1-ol, and tetradecenal.

Conclusion: The methanolic extract of C. glomerata showed significant antimicrobial activity, which implies that it might be useful as a source of bioactive compounds and could be potentially used as an antimicrobial agent.

Keywords: *C. glomerata,* Fungi, Active Compounds

Introduction

Algae are common and widespread in most water bodies all over the world. In addition to being branching, attached, benthic, and filamentous, *Cladophora*, a green alga found in both fresh and marine water, has hair-like filaments with cross walls that separate segments of the macroalga. Each segment has multiple nuclei. Germination and growth of *Cladophora* usually require hard substrates like rocks. They do not produce hepatotoxins.[1,2] Algal extracts have been found to be highly active against *Candida albicans*, and similar results have been observed against other fungal strains and bacteria.[3,4] In a study published by Yi et al., it was found that methanol extracts possess the highest antibacterial and antifungal activity.[5]

The *Rhodophyta* genus includes four species with broad-spectrum antimicrobial activity. Extracts from seaweeds had a greater impact in inhibiting bacterial growth than fungal growth; *Rhodophyta* species had the highest antibacterial and antifungal activity.[6] Green algae were shown to be the most effective antimicrobial agents.[7] Bhagavathy et al. investigated the presence of bioactive phytochemicals in green algae and the antimicrobial activity of these compounds using a variety of organic solvents and found that the extracts had potent effects against pathogenic fungus and bacteria.[8] Al-Asady conducted a study which clearly demonstrated the presence of antibacterial activity in the crude methanol extract of *S. platensis* at dosages of 12.5, 25, 50, and 100 mg/ml. Among the tested microorganisms, doses of 100 mg/ml demonstrated the highest biological inhibitory activity.[9] In another study, five different types of seaweed were extracted, and all extracts showed antimicrobial activity.[10] When compared to extracts made using different solvents, the acetone extract of *Ulva lactuca* exhibited the widest range of antibacterial activity. Fareed and Khair looked into the antifungal properties of four different marine algae species and found that all the tested extracts showed antifungal and antibacterial activity.[11] Thirteen researchers conducted a study to test the effectiveness of extracts from marine macroalgae, *Rhodophyceae, Chlorophyceae,* and *Phaeophyceae* against various microorganisms.[12] Numerous types of bacteria are capable of causing disease in a wide variety of organisms, including humans. Some of them are found in the plants that are found in the wild. Even while some of the bacteria are beneficial to the environment, others are not.[13] This study aimed to investigate the antimicrobial activity of methanolic extracts of *C. glomerata* collected in Mosul, Iraq.

Materials and Methods

Cladophora glomerata Collection

From May to June 2023, *C. glomerata* (macroalgae) were collected by hand from the Tigris River in Mosul, Iraq. The sample was transported directly to the laboratory for further analysis.[14] It was cleaned, washed with water, and stored.

Preparation of Extracts

The shade-dried *C. glomerata* was ground. After filling the thimble with 30 g of powder, 6 hours were spent using a Soxhlet device to extract the substance from 500 ml of methanol solvent for later analysis.

Identification of Microbial Isolates

For the purpose of this experiment, potato dextrose agar (PDA) was produced in accordance with the instructions provided by the manufacturer. As a means of determining the degree to which antifungal medications are effective against *Candida albicans*, five different drugs were tested (amphotericin B, caspofungin, ketoconazole, fluconazole, and metroconazol). The bacterial isolates were examined using the Vitek-2 compact system at Al-Jumhury Hospital, Iraq. Gram-positive and gram-negative bacterial isolates were confirmed with the automated Vitek-2 compact system using GPB-592 Kit (ID) and GNB-419 Kit (ID) cards. The cards had bar codes that contained information on product type, lot number and expiration date.[15]

Table 1.Microbial Isolates and Source of Isolation

Microbial Isolates	Isolation Source
Micrococcus spp.	Skin
Bacillus cereus	Stool
Pseudomonas aeruginosa	Wound
Proteus mirabilis	Stool
Candida albicans	Stool

Antimicrobial Activity Assay

The agar well diffusion method was utilised in order to evaluate the effectiveness of the *C. glomerata* extract as an antibacterial agent in vitro.[16] It was necessary to quantify the diameter of the inhibitory rings that surrounded the well in millimetres so that the findings of this investigation could be utilised. It was determined that halos with a diameter of more than 10 millimetres were regarded to be positive. All of the tests were carried out in triplicate. The control solution that was utilised was DMSO.

Identification of Active Compounds in *C. glomerata* Extract

The presence of active chemicals in algae was determined by using established methods.[17]

Gas Chromatography-Mass Spectrometry

The gas analysis was conducted on a high-temperature column provided by Agilent Technologies by the method

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of Gas Chromatography-Mass Spectrometry (GC-MS), with the initial temperature of the column set at 100 °C. A 5 L sample volume was injected, and oven temperatures were raised to 225 °C and 300 °C. Mass spectra were recorded and analysed using Agilent GC-Mass Solution and postrun software. As part of the identification process, mass of the molecules was compared to the NIST [ADR1] database and to the genuine standards provided by the National Institute of Standards and Technology (NIST).[18]

Results and Discussion

Morphology of *Cladophora glomerata*

There are many species of *C. glomerata*, including the branching, attached, and filamentous macroalgae, which are widespread in both marine and freshwater habitats. The filaments are often long and hair-like, and cross walls separate each segment; each segment contains more than one nucleus. For *Cladophora* to germinate and grow, hard substrates such as rocks are usually needed. Water motion is also crucial to *Cladophora* growth.[16, 19] Experienced biologists can identify *Cladophora* species easily, and sampling is easy.[20]

Figure 1.Naturally Occurring C. glomerata

Antimicrobial Activity

The antimicrobial activity of *C. glomerata* was analysed against bacterial (*Bacillus cereus, Micrococcus spp., Pseudomonas aeruginosa,* and *Proteus mirabilis*), and fungi (*Candida albicans*) isolates. The effects of *C. glomerata* extract (methanol extract) were observed at four concentrations (100, 50, 25 and 12.5 mg/ml). The extract was found to have a strong and widespread inhibitory impact on all microbial isolates (Table 2). *Micrococcus* spp. exhibited the highest level of biological inhibitory action among the studied microorganisms, at values of 100 mg/ml. *Bacillus cereus, Candida albicans, Proteus mirabilis,* and *Pseudomonas aeruginosa* have the most significant inhibitory zones, measuring 18 mm in diameter, indicating that they are the most susceptible to treatment. The inhibitory zone diameters of *Bacillus cereus, Bacillus mirabilis, Proteus mirabilis,* and *Pseudomonas aeruginosa* are 16, 15, 13, and 11 mm, respectively. The inhibitory zones with the smallest widths were observed at a concentration of 12.5 mg/ml. At this concentration, *Proteus mirabilis* and Micrococci species were observed to have diameters ranging from 0 to 7 mm.

Table 2. Effect of Methanolic *C. glomerata* extract at four different concentrations on selected microbial isolates

Microbial Isolates	Concentration (mg/ml)			
	100	50	25	12.5
Micrococcus spp. (mm)	18 ± 1	14 ± 1	8 ± 2	7 ± 1
Bacillus cereus (mm)	15 ± 2	13 ± 1	7 ± 1	5 ± 2
Pseudomonas aeruginosa (mm)	11 ± 1	9 ± 2	6 ± 1	4 ± 1
Proteus mirabilias (mm)	13 ± 2	10 ± 2	6 ± 2	0 ± 0
Candida albicans (mm)	16 ± 1	13 ± 1	9 ± 2	6 ± 1

In contrast to the results obtained from the sensitivity test for the isolates of *C. glomerata*, which indicated that most of them were resistant to antifungal drugs, As a result of the methanolic extract, the colony diameter of the pathogenic fungi that were investigated was dramatically reduced. In agreement with the findings of Dwaish, which found that algae extracts have greater antifungal properties than common drugs against C. albicans, the current findings are consistent with the findings of Dwaish.[21] In a study by Manivannan et al., methanol-based seaweed extracts showed to be most effective in killing microbes.[22] However, methanol extracts of algae have a moderate antimicrobial activity. Acetone, methanol, and chloroform extracts of green algae have no effect on microorganisms.[8] There are many alternatives to antibiotics that act as antimicrobials against pathogenic microbes that might have caused the variations in the mentioned results. These alternatives may have occurred due to differences in the season, location, and isolate solvents used for the algae.[23–27]

Phytochemical Evaluation

According to the findings, the methanolic extract of *C. glomerata* contains active chemical compounds. Alkaloids, tannins, flavones, resins, saponins, terpenes and steroids were found in the extract. The researchers examined the most chemically active compounds in macroalgae, and their findings matched those of a number of other studies.[15]

GC-Mass Spectrometry Analysis

It was determined that the extracts with high antimicrobial activity had biochemical properties that could be identified through the identification of the chemical structures and identities that contributed to their antimicrobial activity.

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For this identification of natural organic substances, GC-MS is used. An investigation was conducted to determine the active components responsible for the bioactivity of the extract of *C. glomerata*, the extract was chemically analysed using GC-MS. Consequently, tridecyne, hexadecanoic acid, octadecadienoic acid, octadecynoic acid, pentadecadien-1-ol, and tetradecenal were discovered to be components. Several antimicrobial compounds have been detected in the algae, including indoles, terpenes, phenols, and fatty acids. [28] As GC-MS techniques indicated medication action, active compounds in algae acetate extracts were analyzed. Seaweeds are believed to possess antimicrobial properties, and their ability to be cultivated is an advantage in increasing their production. It is currently being investigated whether *C. glomerata* has antioxidant and anti-inflammatory activity, and the mechanism by which the natural components of this plant reduce infections is being investigated. Several studies have previously reported similar findings to those of this study.[29,30]

Conclusion

A recent study confirmed that methanolic extract of *Cladophora glomerata* could potentially be used as an antimicrobial agent.

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Conflict of Interest: None

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References

1. Dwaish AS. Antibacterial and wound healing activities of acetone Cladophora glomerata extract. Plant Arch. 2019;19(1):1394-9. [Google Scholar]
2. Al-Khafaji ZH, Dwaish AS. Molecular detection of toxogenic cyanobacteria isolated from Tigris river in Baghdad city–Iraq. Indian J Forensic Med Toxicol. 2020;14(2):446-50. [Google Scholar]
3. Fayyad RJ, Dwaish AS, Sulman IM, Lefta SN. Phytochemical profiling of hot and cold alcoholic extract from Spirulina platensis alga and comparison between two extracts against multidrug-resistant bacteria. Res J Pharm Technol. 2022;15(1):399-404. [Google Scholar]
4. Erturk O, Tas B. Antibacterial and antifungal effects of some marine algae. Kafkas Univ Vet Fak Derg. 2011;17(Suppl A):S121-4. [Google Scholar]
5. Yi Z, Yin-Shan C, Hai-Sheng L. Screening for antibacterial and antifungal activities in some marine algae from the Fujian coast of China with three different solvents. Chin J Oceanol Limnol. 2001;19:327-31. [Google Scholar]
6. Dwaish AS, Yousif DY, Lefta SN. Use of Spirogyra sp. extract against multi drug resistant bacterial pathogens. Int J Adv Res. 2016;4(7):575-9. [Google Scholar]
7. Al-Khafaji ZH. Antifungal activity and qualitative phytochemical analysis of green alga Ulothrix sp. Bionatura. 2022;7(3):1-5. [Google Scholar]
8. Bhagavathy S, Sumathi P, Bell IJ. Green algae Chlorococcum humicola-a new source of bioactive compounds with antimicrobial activity. Asian Pac J Trop Biomed. 2011;1(1):S1-7. [Google Scholar]
9. Al-Asady IN. Antibacterial activity of Spirulina platensis on some pathogenic bacteria. Biomedicine. 2023;43(5):1508-13. [Google Scholar]
10. Saritha K, Mani AE, Priyalaxmi M, Patterson J. Antibacterial activity and biochemical constituents of seaweed Ulva lactuca. Glob J Pharmacol. 2013;7(3):276-82.
11. Fareed MF, Khair HM. In vitro antimicrobial activities of seaweeds collected from Abu-Qir Bay Alexandria, Egypt. World Appl Sci J. 2008;5(4):389-96.
12. Osman ME, Abushady AM, Elshobaryme ME. In vitro screening of antimicrobial activity of extracts of some macroalgae collected from Abu-Qir Bay Alexandria, Egypt. Afr J Biotechnol. 2010;9(42):7203-8. [Google Scholar]
13. Al-Asady IN, Mohammed MA, Saeed YS, AL-Rubaii BA. Bioenergy production from bacteria (Methanogens). Bionatura. 2023;8(1):1-4. [Google Scholar]
14. Abbott IA, Hollenberg GJ. Marine algae of California. Stanford, California: Stanford University Press; 1992. p. i-xii,1-827,701. [Google Scholar]
15. Bagudo AI, Obande GA, Harun A, Singh KK. Advances in automated techniques to identify Acinetobacter calcoaceticus–Acinetobacter baumannii complex. Asian Biomed (Res Rev News). 2020;14(5):177. [PubMed] [Google Scholar]
16. Lefta SN, Dwaish AS. Use of Cladophora glomerata extract against multidrug resistant bacterial pathogens. World J Pharm Res. 2014;3(10):23-32. [Google Scholar]
17. Gibson R, Hextall B, Rogers A. Photographic guide to the sea and shore life of Britain and Northwest Europe. Oxford University Press, Oxford; 2001. 450 p. [Google Scholar]
18. Al-Khafaji ZH. The antagonistic effect of Anabaena circinalis on some dermatophytes. Biomedicine. 2023;43(4):1261-5. [Google Scholar]
19. Fish JD, Fish S. A student's guide to the seashore. London: Unwin Hyman Ltd; 1989.
20. United States Environmental Protection Agency. Draft risk assessment of the potential human health effects associated with exposure to perfluorooctanoic acid and its salts. US EPA Office of Pollution Prevention and Toxics; 2005.

21. Dwaish AS. Evaluation of antibacterial activity and qualitative phytochemical analysis of Enteromorpha ralfsii. Indian J Public Health Res Dev. 2018;9(10):886-90. [Google Scholar]
22. Manivannan K, Karthikai Devi G, Anantharaman P, Balasubramanian T. Antimicrobial potential of selected brown seaweeds from Vedalai coastal waters, Gulf of Mannar. Asian Pac J Trop Biomed. 2011;1(2):114-20. [PubMed] [Google Scholar]
23. Mohsin MR, AL-Rubaii BA. Bacterial growth and antibiotic sensitivity of Proteus mirabilis treated with anti-inflammatory and painkiller drugs. Biomedicine. 2023;43(2):728-34. [Google Scholar]
24. Jalil IS, Mohammad SQ, Mohsen AK, Al-Rubaii BA. Inhibitory activity of Mentha spicata oils on biofilms of Proteus mirabilis isolated from burns. Biomedicine. 2023; 43(2):748-52. [Google Scholar]
25. Saleh TH, Hashim ST, Malik SN, Al-Rubaii BA. The impact some of nutrients on swarming phenomenon and detection the responsible gene RsbA in clinical isolates of Proteus mirabilis. Int J Res Pharm Sci. 2020;11(1):437-44.
26. Husain AG, Alrubaii BA. Molecular detection and expression of virulence factor encoding genes of Pseudomonas aeruginosa isolated from clinical samples. Biomedicine. 2023;43(5):1514-9. [Google Scholar]
27. Al-Saadi HK, Awad HA, Saltan ZS, Hasoon BA, Abdulwahab AI, Al-Azawi KF, Al-Rubaii BA. Antioxidant and antibacterial activities of Allium sativum ethanol extract and silver nanoparticles. Trop J Nat Prod Res. 2023;7(6):3105-10. [Google Scholar]
28. Karm IF, Dwaish AS, Dakhil OA. Investigation of some ecological factors and isolation techniques for some local algae in Iraq. Ann RSCB. 2021;25(5):1059-68. [Google Scholar]
29. AL-Shahery YJ, Al-Asady IN. Molasses as a new nutrition medium for Scenedsmus quadricauda growth and production of some bio compounds. Bionatura. 2021;6(4): 2202-2208.
30. Youngblood WW, Blumer M, Guillard RL, Fiore F. Saturated and unsaturated hydrocarbons in marine benthic algae. Mar Biol. 1971;8(3):190-201. [Google Scholar]

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