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Research Article

Antibacterial Activity and Qualitative Phytochemical Analysis of *Cladophora glomerate*

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ABSTRACT

Filamentous specie of algae (*Cladophora glomerata*) collected from Baghdad University –Iraq were tested against bacterial species. Extracts of *Cladophora glomerata* species were prepared in acetone, hot and cold aqueous extracts. four different concentrations (w/vol.) 12.5 ,25 ,50 and 100 mg/ml were made in each of the above mentioned extracts. Extracts were loaded on agar plates, containing test bacteria, *staphylococcus aureus, staphylococcus epidermidis, Bacillus substilis, Escherichia coli, Klebseilla sp. and pseudomonas aeruginosa*. Hot and cold aqueous extracts were inefficiency in all bacterial species, while the hot acetone extract was efficiency for making extract that showed good zone of inhibition in bacterial species maximum up to 18 mm than the lower value was 7.5 mm. Chemical analyses showed that the active chemical compounds for hot acetone extract alga (*Cladophora glomerata*) extract was contains alkaloids, phenols, Tannins, Flavones, Resins and tannins. The acetone extract was further chemically characterized by using GC–MS in order to be tentative identify the compounds responsible for such activities. The main compositions were Methadone, Benzonitrile, bromobutyloxychalcone, Benzeneethanamine and Cyclodecasiloxane compounds which had antimicrobial activity. These results indicate that the acetone extract of *C. glomerata* exhibited appreciable antimicrobial activity and could be a source of valuable bioactive materials for health products.

Keywords: *Cladophora glomerata* ,antibacterial and active compounds.

INTRODUCTION

Macroalgae are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. Compounds with antioxidant, antiviral, antifungal and antimicrobial activities have been detected in brown, red and green algae[1,2]. The use of algal species extracts for medicine has been well known and their analysis begun from 1950 in medical industry. The antimicrobial action was an indication that the algae have potential to synthesize vital bioactive secondary metabolites[3]. Macroalgae have been known to produce antimicrobial action against gram positive and gram negative bacteria[4]. Algae have been reported to synthesize compounds like antibiotics which are effective against fish and human pathogen bacteria[5].

The problem of getting treatment against resistant pathogenic bacteria is becoming increasingly difficult[6]. Since pathogens gaining resistance to antibiotic, is common due to indiscriminate use of antibiotics, much attention is needed to kill or control the pathogens using bioactive substances. In this study, our aim was to study the chemical composition of extracts from fresh water green algae *C. glomerata*, and to determine their antibacterial activity in order to find a potential natural source of bioactive compounds, food supplements and biomedical uses.

MATERIALS AND METHODS

Collection and Preparation of Sample

Samplings were carried out from Baghdad University – Iraq, which located on longitude (33°16'09.5"N) and latitude (44°20'19.41" E), during autumn 2014. Samples of *C. glomerata* were collected manually from the rock. The harvested macro algae were stored in plastic bags and transported to the laboratory. Voucher specimen of species were pressed and stored in 5% formalin for identification according to Davis and Burrows[7,8]. Biomass was rinsed with fresh water to eliminate other materials such as sand, shells, etc. The macroalgae were stored in the laboratories and dried at 50°C under ventilation in an oven and then grounded to powder form by the blender.

Preparation of algae extracts

Algae samples were washed with distilled water to remove the adhering particles. They were dried in the shaded place. The dried algae were powdered, weighed and stored in clean containers all algae extraction were done according to[9]. There are two types of water extraction method used

Aqueous Extraction

Powdered material of algae (5gm) is taken with 150 ml of distilled water for 1 hr on rotary shaker. The extract is filtered by using muslin cloth and Whatman no.1 filter paper and concentrated by evaporation on water bath.

Cold Water extract

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Figure 1: Filaments of *Cladophora glomerata* showing the branching (40X) .

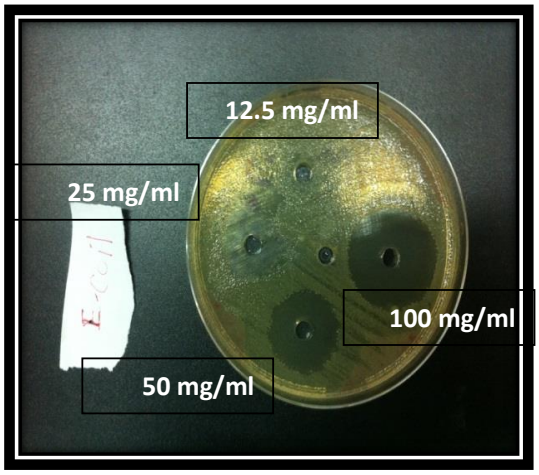

Figure 2: antibacterial activity of Crude extract *Cladophora glomerata* against *E.coli* at different concentrations.

Table 1: antibacterial activity of *Cladophora glomerata hot acetone extract.* (inhibition zone was measured to the nearest millimeter).

Bacteria	Concentrations mg/ml				LSD value
	100	50	25	12.5	
S. aureus	18±1	13±2	12±0.5	9±1	3.266 *
S. epidermdis	17±2	15±1	12±1	8.5±1	3.089 *
E. coli	13±1	11±0.5	10.5±0.5	-	2.341 *
Klebseilla sp.	11.5±1	9±1	7.5±0.5	-	2.056 *
P. euroginosa	11±1	9±1	8.5±1	-	2.226 *
Bacillus substilis	13.5±0.5	11±2	10.5±1	8.5±1	2.762 *
LSD value	2.975 *	2.355 *	3.026 *	1.94 NS	---

* (P<0.05), NS: Non-Significant.

Table 2: Presence or absence of active compounds in *Cladophora glomerata* extract.

Chemicals Compound	Hot Acetone Extract
Glycosides	+
Phenols	+
Alkaloids	+
Resins	+
Saponines	-
Tannins	+
Flavones	+
Coumarines	-

5g of dried algae powder was added to 150ml of distilled water (15°C) and was mixed thoroughly and kept it on rotary shaker for 1 h on 100 r.p.m. It was then filtered through muslin cloth or whatman no.1 filter paper. Filtrate was taken and concentrated through evaporation on water bath at 70-80 c.

Hot Water extract

5g of dried algae powder was added to 150ml of distilled water (70°C) and kept on rotary shaker for 1 hour. It was then filtered through muslin cloth or whatman no.1 filter paper. Filtrate was taken and concentrated through evaporation on water bath at 100 °c.

Soxhlet extraction

5g of dried algae powder was extracted for 4-5 hrs with (150ml) organic solvent (acetone.) by hot continous perlocation method in Soxhlet apparatus. After the effective extraction solvent was concentrated using rotary evaporator.

Preparation of different algaE extracts concentrations

Stock solution was prepared for each extract, 0.5 g were dissolved with 2.5 ml of the appropriate DMSO, and then the volume was made up to 5 ml that equal 100 mg /ml.

Antibacterial Assay

Antibacterial tests of algal extracts were performed *in vitro* using the well diffusion method[10], in Petri dishes. The results are expressed by measuring the diameters in millimeter of the inhibition halos of bacterial growth around the well. Acetone (100%) and water without macroalgae extracts were used as negative control. All tests were performed in triplicate, and clear halos greater than 10 mm were considered as positive results, experimental in comparison data represent mean ± SD of each sample.

Indicators of Active Compound in Extracts

The presence of active compounds in the studied algae were determined by adopting standard protocols[11,12].

Gas Chromatography-Mass Spectrometry

For GC-MS analysis, a high-temperature column (Inert cap 1MS; 30 m × 0.25 mm id × 0.25µm film thickness) was purchased from Agilent Technologies

Table 3: GC-MS Analysis of Major Compounds in acetone extract of *Cladophora glomerata.*

Number	Rt.	Area%	Compounds
1.	26.374	1.36	hexadecamethyl
2.	26.597	1.56	hexadecamethyl
3.	30.474	1.57	octadecamethyl
4.	30.541	1.80	octadecamethyl
5.	30.812	4.40	heptane
6.	33.210	2.09	Tetradecanoic acid
7.	33.954	0.83	Dibutyl phthalate
8.	34.612	3.17	n-Hexadecanoic acid
9.	39.388	3.76	octadecamethyl-
10.	39.649	0.95	Oleic Acid
11.	39.727	1.45	Heptadecane
12.	41.477	7.34	Hexanedioic acid
13.	41.776	3.70	octadecamethyl-
14.	42.985	1.42	Pentacosane
15.	44.010	4.28	hexadecamethyl-
16.	49.946	1.58	octadecamethyl

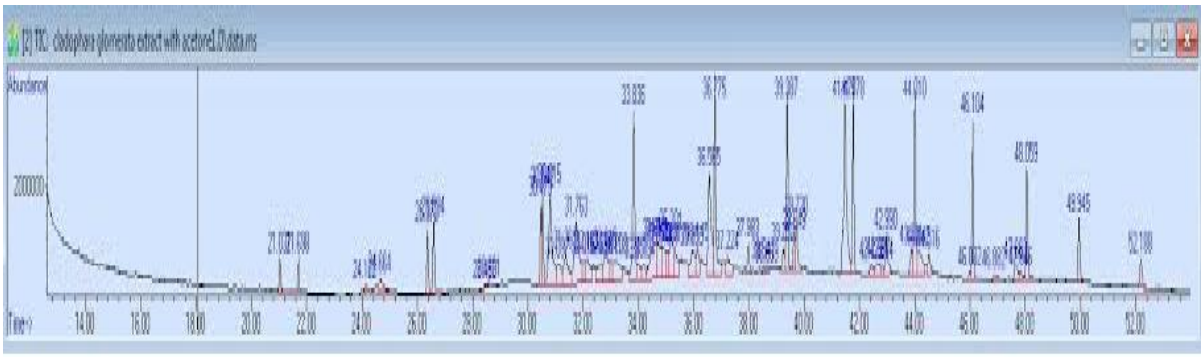

Figure 3: The chromatogram of GC-Mass spectrophotometery showed in acetone extract of *Cladophora glomerata*.

(SHIMADZU—Japan), by employing a high-temperature column. Derivatization of each sample was eliminated. The injector and detector temperatures were set at 280°C while the initial column temperature was set at 100°C. A 5 µL sample volume was injected into the column and ran using split (1:10) mode After 1 min, the oven temperature was raised to 225°C at a ramp rate of 12.5°C/min(hold time 4 min). The oven temperature was then raised to 300°C at a ramp rate of 7.5°C/min (hold time 5 min). The helium carrier gas was programmed to maintain a constant flow rate of 17.5 mL/min and the mass spectra were acquired and processed using both Agilent GC-Mass. Solution (SHIMADZU—Japan) and postrun software. The compounds were identified by comparison of their mass with NIST library search and authentic standards[10].

RESULTS AND DISCUSSION

Morphological Structure of *Cladophora glomerata*

Cladophoraglomerata is green or light green, filamentous in form, attached on rock or cobble in the bed of shallow rivers. Microscopically, thalli are composed of joined cylindrical cells, with lengths of 6-20 µm and widths of 4-10 µm and with dichotomously branching filaments figure 1. Branches are tufted, arising singly, the branches becoming irregular in old algae. Branches are narrowed towards tips, cell walls are thick and usually lamellate. The chloroplast is in a parietal network with numerous pyrenoids. Usually it tends to stay on one spot, which makes it easy to remove, this findings agreed with[13,14].

Antibacterial activity of algae extracts

Antibacterial activities of crude extracts of algae (*Cladophora glomerata)* were carried out from Baghdad University –Iraq determined by well diffusion assay. Aqueous forms of the extracts of the algae exhibited no action of antibacterial activities against the test organisms. While the hot acetone form of the extract of algae exhibited varying degree of antibacterial activities against the test organisms in different concentrations and the results are summarized in Table 1 and figure 2.

The antibiotics are synthetic chemicals and may have side effects are now time to replace with natural antibiotic source[15]. It is necessary to make natural extracts that are equally effective as the artificially prepared antibiotics. There are chances that bacteria become resistant against the used antibiotic which is serious threat in biological treatments. According to table 1, *S. aureus* showed maximum zone of inhibition. it was clear that extract in acetone at concentration 100mg/ml of algae with 18 mm of zone of inhibition were closer than others. According to table 1, *Klebseilla sp.*showed minimum zone of inhibition. it was clear that extract in acetone at concentration 12.5mg/ml of algae with 7.5 mm of zone of inhibition.

Algae had proven a good source for bacterial resistance[16] proved that methanolic extracts of *Spirulina platensis*, *Chlorella pyrenoidosa* and *Nostoc muscorum* were good against the human pathogenic bacteria and fungi.

Phytochemical evaluation

The results showed the presence of active compounds in acetone extract of *Cladophora glomerata*. The results showed that extract of *Cladophora glomerata* had alkaloids, phenols, Tannins, Flavones, Resins and tannins. While Coumarines and Saponines, were absent as shown in table 2. This results agreed with many studies such as[10] they screened the most active compounds in macroalgae.Biochemical analysis were being undertaken to determine the structure and nature of compounds responsible of the bio-activity of the extract with high antibacterial potency.

At present in table 3 and figure 3, analysis by Gas chromatography–mass spectrometry (GC-MS) is essential for the identification of natural organic compounds. *C. glomerata* extract chemically characterized in order to determine the compounds responsible for the biological activity observed using GC–MS techniques. Different natural antimicrobial compounds have been described in algae belonging to a wide range of chemical classes, including indoles, terpenes, acetogenins, phenols, fatty acids and volatile halogenated hydrocarbons[17]. Thus, GC–MS methods were used to analyze both, fatty acids and volatile compounds, in separated fraction of hexane and ethyl acetate extracts that showed antibacterial activity from the studied algae. The result presumes that the long chain hydrocarbons may act as potential bioactive substance and can be exploited in pharmaceutical preparations. The cultivable nature of seaweeds is an added advantage for mass production of potential antibacterial products. Further study is in progress to find out the mechanism of inhibition of pathogens by the purified compounds and to study the antioxidant in addition to anti-inflammatory properties of *C. glomerata.* Our results are in accordance with the reported investigations[18,19].

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