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USE OF CLADOPHORA GLOMERATA EXTRACT AGAINST MULTIDRUG RESISTANT BACTERIAL PATHOGENS

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ABSTRACT

The antibacterial effect of the crude hot Chloroform extracts and purified fractions of *Cladophoraglomerata* (Linnaeus) Kützing (Cladophoraceae) against multidrug resistant human pathogen were isolated from burn and bound. The test bacterial strains were *Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris* and *Escherichia coli*. Hot Chloroform extracts (128 and 256 mg/mL) of *Cladophora glomerata* inhibited growth of all the test organisms. Primary detection of active compounds showed that macroalgae (*Cladophoraglomerata*) containing flavonoids, alkaloids, phenols, saponins, Glycosides and tannins. Gas Chromatography-Mass Spectrometry was used to know the compounds which responsible of antibacterial activity and they were Salicylic acid was found to be a major compound (16.09%) followed by 6-Octadecenoic acid, (14.22%) and 10- Heptadecenoic acid (7.68%) and Tetradecanoic acid (7.29%) in addition to Dodecanoic acid (3.18%).These findings suggest the possibility of using the *Cladophoraglomerata* as a novel source of natural antimicrobial agents in pharmaceutical industries.

KEYWORDS: Cladophoraglomerata, antimicrobial, active compound, extracts.

1. INTRODUCTION

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents [1]. Marine algae are found to be important sources of useful bioactive substances since two decades [2]. More than 150,000 macroalgae or seaweeds species are found in the oceans of the globe but only a few of them were identified [3]. Algae are a large

and diverse group of simple, typically autotrophic organisms, ranging from unicellular to multicellular forms and fall under the category macro-algae. Algae are extremely fast growing in marine and fresh water plants that can grow to considerable size up to 60m in length. Micro-algae are, as the name suggests, microscopic photosynthetic organisms. Like macro algae, these organisms grow very rapidly, and are found in both marine and fresh water environments [4].

Algae are useful in numerous therapeutic applications. Algae have been used for centuries in Asian countries, as remedy to cure or prevent various physical ailments. Researchers found that algae contain remarkable amount of components valuable for human health. Amongst algae, those have been reported to inhibit growth of microorganism's are mostly planktonic and benthic fresh and marine water [5]. Secondary or primary metabolites obtained from these organisms maybe potential bioactive compounds of interest for the pharmacological industry [6]. Many substances obtained from marine algae such as alginate, carragenean and agar as phycocolliods have been used for decades in medicine and pharmacy [7]. Marine secondary metabolites are organic compounds produced by microbes, sponges, seaweeds and other marine organisms [8]. There are numerous reports of macroalgae derived compounds that have a broad range of biological activities, such as antibiotic, antiviral, anti-neoplastic, antifouling, anti-inflammatory, cytotoxic and antimitotic [9, 10]. In this investigation, antibacterial effects of macroalgae (Cladophoraglomerata) belonging to Chlorophyta, was studied againstmultidrug resistant bacterial pathogens (Staphylococcus aureus, Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa and Streptococcus faecalis).

2. MATERIAL AND METHODS

2.1 Collection and Preparation of Sample

Samplings were carried out from Baher Al Najaf region in south of Iraq, which located on longitude 44°7'11.81"E and latitude 33°45'13.6"N, 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 [11, 12]. 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.

2.2 Preparation of Alcoholic Extract

The alcoholic extract was prepared by soxhelet extraction according to Davis [13]. In this process the dried powders form of plant material extracted by using (100%) Chloroform. The concentrated active constituents from macroalgae were kept in sterilized test tubes stored in refrigerator till further use. The traces of methanol were removed by keeping the tubes at 50°C for 1 h.

2.3 Bacterial Strains

Bacterial strains used in this study were obtained from the Department of Microbiology, Collage of Science University of Mustansiryah, they were *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa and Proteus vulgaris* all these strains were isolated from burns and wounds.

2.4 Antibacterial Assay

Antibacterial tests of algal extracts were performed *in vitro* using the disc diffusion method [14], in Petri dishes. Sterile disks of 6 mm in diameter were impregnated with 25 µL of seaweeds extract, deposited on the surface of agar medium (Mueller-Hinton Agar, pH 7.4 ± 0.2 at 25 °C) previously inoculated with bacteria strains and incubated at 37 °C for 24 h [15]. The results are expressed by measuring the diameters in millimeter of the inhibition halos of bacterial growth around the disk. Chloroforme (100%) without seaweed extract was used as negative control. All tests were performed in triplicate, and clear halos greater than 10 mm were considered as positive results [16], experimental in comparison data represent mean ± SD of each sample.

2.5 Qualitative Estimation of Active Compounds

The presence of active compounds in the studied macro algae were determined by adopting standard protocols [17, 18].

2. 6 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 (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.

3. RESULTS AND DISCUSSION

3.1 Morphological Structure of CladophoraGlomerata

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. 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.

3.2 Bacterial Strain

Sensitivity to antibiotics for all bacteria were tested Table 1 results were measured in millimeters.

3.3 Evaluation of Antibacterial Activity

The antibacterial activity of *Cladophora glomerata* crude chloroform extracts of which isolated from Baher Al-Najaf region in south of iraq are shown in the Table 2.The antibacterial activity was ranged between (10-18 mm) the highest was in *S. aureus* at concentration 256 mg/ml and the lowest was in *E. coli* at concentration 128 mg/ml .

Table 1Sensitivity test for all bacteria were used in millimeter (Inhibition zone was measured to the nearest millimeter).

Test organism	AM	CE
S. aureus	12	-
P. vulgaris	14	21
E. coli	17	18
P. aeruginosa	18	22

AM: ampicillin 10 µg; CE:cefazolin 30 µg; -: no inhibition.

Table 2. Crude extract antibacterial activity of *Cladophora glomerata* (inhibition zone was measured to the nearest millimeter).

Test organism	Crude extract (128 mg/mL)	Crude extract (256 mg/mL)	Negative control chloroform
S. aureus	16 ±0.8	18±0.4	0.00±0.00
P.vulgaris	11±0.4	13±0.1	0.00±0.00
E. coli	10±0.00	13±0.2	0.00±0.00
P. aeruginosa	15±0.4	17±0.2	0.00±0.00

Antibacterial activity of crude extract with concentration (128 and 256 mg/mL) has a great potential for the discovery of lead compounds that could be used against infectious diseases and parasites [19]. Among crude chloroform extract, the green seaweed *Cladophoraglomerata* showed high inhibiting activity against *S. aureus*. Similar observation was made in a methanol extract of green seaweed *Ulvalactuca* (500 µg/mL) which showed high inhibiting activity against *Staphylococcus aureus* [20]. In the present study, the minimum inhibitory of chloroform extract of *C. glomerata* was found against Gram-negative pathogens, *E. coli and P. vulgaris*. Earlier studies showed that methanol extracts of seaweeds *Enteromorpha intestinalis* and *Gracilaria corticata* were active against Gram positive bacteria [21]. In the present study, we observed that chloroform extracts of green seaweed *C. glomerata* was active against Gram-positive bacteria. Many species of seaweeds were screened and found that members of red algae exhibited high antibacterial activity [22, 23]. The variation of antibacterial activity of our extract might be due to the presence of antibacterial substances, which varied from species to species [24]. Previous reports showed that Gram-positive bacteria were more effectively controlled by the extracts of algae used in their study in comparison to Gram-negative bacteria [25, 26]. This may be probably due to the presence of more complex cell wall structure in Gram negative bacteria [27, 28]. In addition to that, the resistance displayed by the pathogens might be due to masking of antibacterial activity by the presence of some inhibitory compounds or factors in the extract [29]. Interestingly, in the present study, it was observed that seaweed which was used in this work exhibited good antibacterial activity to all Gram-positive pathogens tested except a few. Conflicting reports were observed on the presence of bioactive compounds in the seaweeds related to the seasonal variation, as well as the method of extraction and organic solvents used for bioactive compounds extraction and differences in assay methods.

3.4 Qualitative Estimation of Active Compounds from the Macroalgae

The results showed the presence of active compounds in chloroform extract of Cladophora

glomerata. The results showed that chloroform extract of Cladophora glomeratahad Alkaloids, Tannins, phenols, Flavonoids, glycosides and saponins, while terpenoids, were absent. This results agreed with many studies such as [30, 31] 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.

3.5 GC-MS Analysis

Chloroform extracts of green seaweed Cladophora glomeratawere dissolved in hexane and subjected to GCMS to analyze the chemical constituents. Characteristic Gas Chromatograph-Mass Spectrometry analysis of hydrocarbons has been summarized. In the active fraction, were Salicylic acid was found to be a major compound (16.09%) followed by 6-Octadecenoic acid, (14.22%) and 10- Heptadecenoic acid (7.68%) and Tetradecanoic acid (7.29%) in addition to Dodecanoic acid (3.18%). These results are in accordance with the reported investigations [32, 33]. Straight chain paraffins (n-alkanes), branched chain paraffins (alkyl-alkanes) and unsaturated hydrocarbons (alkenes) were already reported from many marine algae [34, 35]. Hydrocarbon distribution pattern in C. glomeratais closely similar to prokaryotic Anacystis. montaneand Btryococcusbraunii belonging to Cyanophycophyta and Chrysophytarespectively. Similar group of hydrocarbons heptadecane and hexadecane have been reported as common major volatile components in many other algae [36].

Table 3. Presence or absence of active compounds in *Cladophoraglomerata* extract.

Active compounds	Presence(+) orabsence(-)
Alkaloids	+
Glycosides	+
Tannins	+
Terpenoid	-
Flavonoids	+
Phenols	+
Saponins	+

Table 4. GC-MS Analysis of Major Compounds of *Cladophoraglomerata.*

Rt (min)	Compound	Area (%)
14.19	Salicylic acid	16.09
16.88	6-Octadecenoic acid	14.22
13.86	10- Heptadecenoic acid	7.68
11.78	Tetradecanoic acid	7.29
27.34	Dodecanoic acid	3.18

The presence of methyl and hexyl groups could be a result of alkylation of hydrocarbons with methanol and hexane which was used in extraction and purification process in the present study, also Salicylic acid is used as a preservative in food, as a chemical raw material for the synthesis, and salicylates derivatives (aspirin), as an antiseptic and antimicrobial by topical application in medicine. Use of antibiotics to control pathogens culture is banned. There is a need for alternative methods or substances for controlling microbial pathogens diseases. The results of this study prove that methanol fraction of green seaweed *C. glomerata*can control some microbial pathogens. 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.*

4. CONCLUSIONS

The present study provides data to show the appreciable antibacterial activity of seaweed *Cladophoraglomerata* crude extract and purified fractions against Gram-negative and positive human pathogens.

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