Anti –phytopathogenic Activities of *Cladophora glomerata* extract against plant fungi

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Abstract :

The antiphytopathogenic effects of the crude methanol extract of *Cladophora glomerata* (Lin.) Kützing (Cladophoraceae) which isolated from Al Rashidiya region at north of Baghdad was investigated against two type of plant fungi *(Pythium altimum* and *Rhizoctonia solani*) which causes damping off disease where isolated from covered cucumber field in Al-Alyosifia region.

Hot methanol extract showed antifungal activity against the two species of fungi in different concentrations(10,25,50 mg/ml) of extract to *Cladophora glomerata* as percentage inhibition (51.63 ,72.8 ,83.71) and (56.18 ,77.41 ,100)comparing with *Pythium altimum* and *Rhizoctonia solani* respectively .primary detection of active compounds showed that macroalgae (*Cladophora glomerata*) containing flavonoids,alkaloids, phenols and tannins. Our findings suggest the possibility of using the *Cladophora glomerata* as a novel source of natural antimicrobial agents for pharmaceutical industries.

Key words: phytopathogenic, Activities, Macro alage, extract, fungi.

Introduction:

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 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 environment[1].

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 remakeable amount of components valuable for human health. Amongst algae, those have been reported to inhibit growth of micro organisms are mostly planktonic and benthic fresh and marine water [2]. Many marine macro algae produce a variety of secondary metabolites [3].

These metabolites are mainly terpenes, alkaloids and polyphenolics, many of these compounds being halogenated [4].

Cladophora glomerata is a filamentous green alga widely distributed throughout lentic and lotic freshwaters of the world [5, 6, 7]. In general, *C. glomerata* grows attached to hard substrates in nutrient rich alkaline freshwaters, although high morphological plasticity makes classification to species level difficult[8].

Materials and Methods:

Collection and preparation of sample:

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Samplings were carried out from Al Rashidiya region in north of Baghdad city ,Iraq. Which located on longitude 44°20'15.62"E and latitude 33°36'12.29"N, during autumn 2012. Samples of *C. glomerata* were collected manually from the rock of irrigation drainage which discharge their water directly to Tigris river . The harvested macro algae were stored in plastic bags for transported to the laboratory. Voucher specimen of species were pressed and stored in 5% formalin for identification according to [9] and [10]. 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 by the blender.

Preparation of alcoholic extract:

The alcoholic extract was prepared by soxhelet extraction according to[11]. In this process the dried powder form of plant material extracted by using ethyl alcohol. 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 hr.

Isolation of pathogenic fungi:

Soil samples were collected from covered cucumber field in Al-Alyosifia region , The samples were taken to the laboratory for isolation within 7 days. The particles of the soil samples were put on Petri dishes with potato dextrose agar (PDA) and were incubated at 25°C for 2–3 days. When mycelia growth was observed, purification was carried out by cutting a small piece of media with mycelia at the edge of a colony and then transplanted onto new medium plates

Antifungal Activity Assay:

In this experiment the crude extracts of the macro algae were mixed with Potato Dextrose Agar (PDA) medium to get different concentrations (50 ,25,10) mg/ml and the fungal mycelia were inoculated to grow. Data on the radial growth were recorded.

Potato Dextrose agar plates were inoculated with each fungus by placing a 5 mm diameter disc from an actively growing culture in the centre of each plate. Six replicates were used per treatment. Fungi were also grown on non-ameliorated PDA as a control. All fungi were incubated at 25°C for seven days in the dark. Fungal growth (colony diameter) was measured and percentage of inhibition calculated according to the formula:

Percentage inhibition = (C-T) x 100/C

Where,

C = Colony diameter (mm) of the control.

T = Colony diameter (mm) of the test plate.

Percentage inhibition and analysis of variance for the different treatments were calculated.

Qualitative estimation of active compounds :

The presence of active compounds of studied macro algae were determined by adopting standard protocols [12,13].

Results and Discussion:

Morphological Structure of

***Cladophora glomerata* :**

Cladophora glomerata 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, arbuscular, 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 (Fig. 1&2) .

Fig. (1): *Cladophora glomerata* after harvested from water.

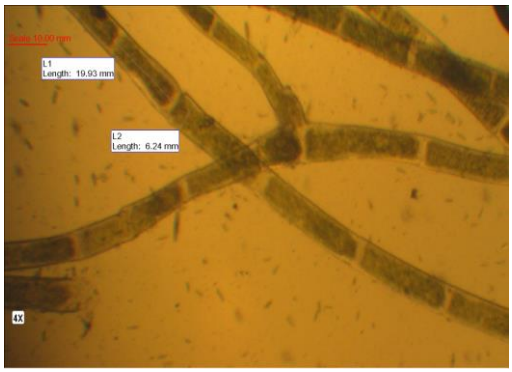

Fig. (2): *Cladophora glomerata* under microscope 4X .

Two species of plant pathogenic fungi were isolated from covered cucumber field in Al-Alyosifia region ,they were *(Pythium ultimum* and *Rhizoctonia solani*) fungi were identified on the basis of morphological characteristics suggested by [14,15].

Evaluation of Antifungal Activity:

The antifungal activity of *Cladophora glomerata* crude methanolic extracts of which isolated from Al Rashidiya region in north of Baghdad city are shown in the Table (1)and Figure (3,4).The antifungal activity is ranged between (51.63%-83.71%) and (56.18%-100%) to *Pythium ultimum* and *Rhizoctonia solani* respectively at different concentrations of extract.

Table (1): Mean Percentage inhibition of different fungi at different concentrations of *Cladophora glomerata* extract.

	Conc.of macroalgae extract.			
Name of Fungi	10 mg/ml	25mg/ml	50mg/ml	Control
Pythium ultimum	51.63	72.8	83.71	0.00
Rhizoctonia solani	56.18	77.41	100	0.00

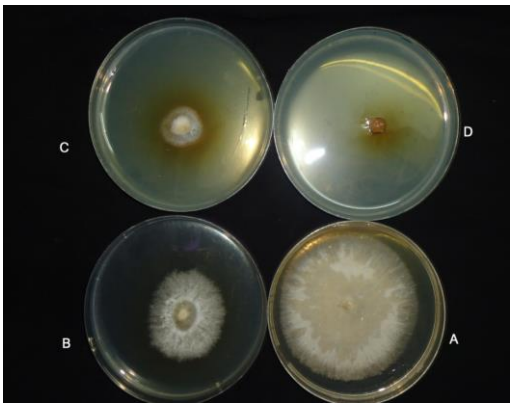

Fig. (3): percentage inhibition growth of *Rhizoctonia solani* in PDA plates by use methanol extract of *Cladophora glomerata*. A:control B:10 mg/ml C:25 mg/ml D:50 mg/ml.

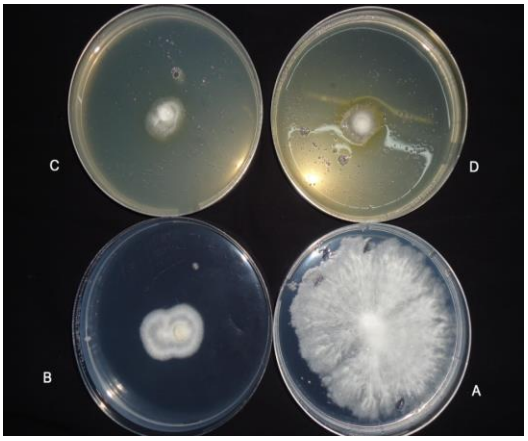

Fig. (4): percentage inhibition growth of *Pythium ultimum* in PDA plates by use methanol extract of *Cladophora glomerata*. A:control B:10 mg/ml C:25 mg/ml D:50 mg/ml.

The extracts and active constituents of various macroalgae have been shown to have antimicrobial activity *in vitro* [16]. Antimicrobial activity of marine algae from Brazil have been noticed. While [17] recorded antimicrobial activity of blue green and green algae .However and [18] studied seven algae for their antimicrobial activity against *Aspergillus flavus*, *Aspergillus niger* and *Alternaria brasica*.

Qualitative estimation of active compounds from the macroalgae:

The results showed presence of active compounds in methanol extract of *Cladophora glomerata* in the Table (2).

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

Active compounds	Presence OrAbsence
Alkaloids	+
Glycosides	-
Tannins	+
Terpenoid	-
Flavonoids	+
Phenols	+
Saponins	-

The results showed that methanolic extract of *Cladophora glomerata* had the Alkaloids, Tannins, phenols and Flavonoids while glycosides , terpenoids , and saponins ,were absent . This results agreed with many studies such as [19,20] they screened the most active compounds in macroalgae , biochemical analysis were being undertaken to determine the structure and nature of compounds responsible of the bioactivcompounds of the extracts with high antibacterial activity. Not only the presence of a particular compound which makes these organisms, interesting but also their huge diversity and the possibility of not only harvesting them but also of growing them at different conditions, leading to an enrichment of some bioactive compounds.

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الفعالية التضادية لمستخلص طحلب*Cladophora glomerata* ضد الفطريات الممرضة للنبات

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الخلاصة:

تـم اختبـار تـأثير الفعاليـة التضـادية لمسـتخلص الكـــــحولي الحـار الخـام للطحلـب *Cladophora glomerata* العائد للعائلة Cladophoraceae والمعزول من منطقة الراشدية شمال بغداد ، ضد نوعين من الفطريات النباتية *Pythium altimum* و *Rhizoctonia solani* المسببة لمرض تساقط البادرات والمعزولـه من احد حقول الخيار المغطاة في منطقة اليوسفية .

أظهر المستخلص الكحولي الحار لمستخلص الطحلب*Cladophora glomerata* فعالية تضادية ضد نوعين الفطريات بتراكيز مختلفة (10 ،25 ،50 ملغ/مل) حيث بلغت النسـبة المئوية للتثبيط (72.8,83.71 ,51.63) و (100, 77.41, 56.18) للفـطر *Pythium altimum* والفطر *Rhizoctonia solani* على التوالي. الكشف الاولي للمركبات الفعالة اظهر أن الطحلب الكبير(*Cladophora glomerata*) يحتـوي علـى الفلافونـات والقلويـدات و الفينـولات والتانينـات. وأقتراحنـا هنـا أمكانيـة اسـتخدام الطحلب(*Cladophora glomerata*) كمصدر طبيعي ضد المايكروبات في ا لصناعات الصيدلانية.