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ANTIFUNGAL ACTIVITY OF ALGAL *SPIROGYRA* SP. AGAINST FUNGAL *FUSARIUM OXYSPORUM*

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ABSTRACT

In the present study the antifungal activities of hot and cold alcoholic extracts of fresh water green macro-algae *Spirogyra* sp. was investigated against plant fungi (*Fusarium oxysporum*). Hot and cold alcoholic extract showed antifungal activity against the specie of fungi in different concentrations (25,50,75mg/ml)as percentage inhibition (87.11, 90.33,100) and (67.18,81.21,89.43) respectively. Also the results showed the ability of algal extract to protect tomato seed and promoted seed growth when soaked in extracts for less than 48 hours. Primary detection of active compounds showed that macro-algae (*Spirogyra* sp.) containing Flavonoids, Alkaloids, Phenols and Saponins. In this study the bioactive compound have been evaluated by using GC-Mass spectrophotometer.

KEYWORDS: Flavonoids, Alkaloids, Phenols.

INTRODUCTION

Spirogyra sp. is a genus of filamentous un branched green algae that forms free-floating mats in shallow waters. It widely occurs in stagnant waters, such as ponds and canals, in shaded littoral zones of lakes, and in slow streams.[1,2] Which belong to class (Chlorophyta) and order Zygnematales, named for the helical or spiral arrangement of the chloroplasts that is diagnostic of the genus .Currently, *Spirogyra* is gaining interest and considered as an ingredient or supplement for cosmetics, antioxidants or in foods, as well as in pharmaceutical products.[3, 4]

Fusarium oxysporum is genus of filamentous fungi that contains many agronomical important plant pathogens.[5,6] Many approaches were applied to control this fungal pathogen.

[7,8,9] Unfortunately, most these approach have negative effect due to the development of resistant strains of pathogens against various chemical fungicides.[10]

Many marine macro-algae produce a variety of secondary metabolites.[11] These metabolites are mainly Terpenes, Alkaloids and Polyphenolics, with many of these compounds being halogenated.[12]

MATERIALS AND METHODS

Collection and Preparation of Sample

Spirogyra specimens was collected from Tigris river in Baghdad city ,Iraq. Which located on longitude33°36'01.94"N and latitude44°20'19.41"E, during summer 2014. Samples of *Spirogyra* sp. was collected manually .The harvested macro-algae were stored in plastic bags for transportation to the laboratory. *Spirogyra* samples washed with water to clean the dirt, then dried for three days in the sun.

Preparation of alcoholic extract

The hot alcoholic extract was prepared by Soxhelet extraction according to.[14] In this process the dried powder form of algae material extracted by using ethyl alcohol. After completion the process the concentrated active constituents from macro-algae were kept in sterilized test tubes stored in refrigerator till further use. The traces of ethanol were removed by keeping the tubes at 50˚c for 1 hr. while cold alcoholic extract was prepared by take 10 gram powder of the macro-algae material and mixed in a beaker in 90ml ethanol and kept for 24 hours (stirred in between thrice). The mixture was filtered with the help of Whatman filter paper No-1.

Isolation of pathogenic fungi

Samples of infected tomato were collected from local markets , the samples were taken to the laboratory for isolation within 7 days. The piece of the infect tomato tissue were put on Petri dishes with potato dextrose agar (PDA) after sterilized and were incubated at 2 ± 28°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. The fungus *(Fusarium oxysporum*) was identified under the microscope for comparison of fungal morphology with descriptions given by.[8,15,16]

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 (75,50 ,25) mg/ml and the fungal mycelia were inoculated to grow. Data on the radial growth were recorded. Potato Dextrose agar plates were inoculated with fungus by placing a 6cm diameter disc from an actively growing culture in the centre of each plate. Six replicate plates were used per treatment. Fungus was also grown on non-ameliorated PDA as a control. Then incubated at 2 ± 28°C for seven days in the dark. Fungal growth (colony diameter) was measured and percentage 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 was calculated and an analysis of variance for the different treatments.

Qualitative estimation of active compounds from the *Spirogyra* specimens

The presence of active compounds from macro-algae of the studied were determined by adopting standard protocols.[14]

Seed treatment

Seeds of tomato(*Solanum lycopersicum*) were soaking in (25,50,75 mg/ml) concentrations of hot and cold alcoholic macro-algae extracts for 24 ,48 and 72 hours in test tubes, after seeds surface disinfestations with 1% sodium hypochlorite. Treated seeds were dried on clean sheets of paper under room conditions. Seeds were also soaked in distilled water for the same periods and dried under the same conditions to be served as a control.

Ten seeds which soaked in alcoholic extracts were plated on PDA media. These Petri dishes were inoculated at the centre with 0.5 cm disc of *Fusarium oxysporum*. They prior to incubated for 7 days at 2 ± 28°C. Seeds plated in Petri dish were examined for fungal growth and seed germination. Three replications were maintained for each treatment.

Different measurement of (*S. lycopersicum*) seedling were taken such as radical length (cm) plumule length (cm) ,number of secondary roots and percentage ratio of seedling.

RESULTS AND DISCUSSION

Evaluation of Antifungal Activity

The overall data presented gives us the indication that the antifungal activity is present in the isolated of *Spirogyra* sp. in table (1) and figure (1). alcoholic extracts of fresh water green macro-algae *Spirogyra* sp. was screened against fungal pathogen *F. oxysporum*. The antifungal activity in PDA plates ranged between (67.18 - 89.43) mm in cold alcoholic extracts and (100 –87.11) mm in hot alcoholic extracts. at different concentrations of extracts. These results confirm that the crud extracts antifungal activity as reported earlier.[13,17,18,19] While [20] which found that macro-algae extracts of *Spirogyra* sp. is act as stimulating factors to fungi and there is no antifungal activity. However, it is difficult to compare the result from this study because the antifungal activity of algal extracts may be influenced by a number of factors including environmental factors, algal species which can be associated with intra specific variability in product of secondary metabolic, season or time at which samples were collected and place of sample collection.[11,17,21,22]

Table (1): different concentrations of *Spirogyra* extracts.

Conc. of *Spirogyra* extract.				
Type of extracts	**25 mg/ml**	**50mg/ml**	**75mg/ml**	**Control**
Hot extracts	87.11	90.33	100	0.00
Cold extracts	67.18	81.21	89.43	0.00

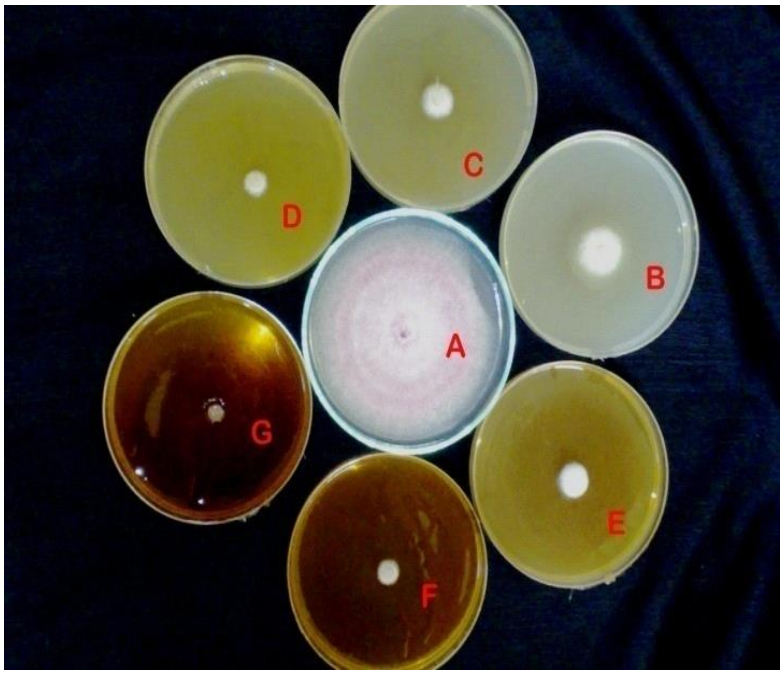

Figure (1): Growth inhibition of *F. oxysporum* on PDA plates by using extracts of *Spirogyra* sp. at different concentration.

A: Control.

B: Cold alcoholic extract at 25 mg /ml concentration.

C: Cold alcoholic extract at 50 mg /ml concentration.

D: Cold alcoholic extract at 75 mg /ml concentration.

E: Hot alcoholic extract at 25 mg /ml concentration.

F: Hot alcoholic extract at 50 mg /ml concentration.

G: Hot alcoholic extract at 75 mg /ml concentration.

The results showed in table (2) that when soaked seeds for 48 hours at 75 mg/ml concentration the highest value of the mean radical length, plumule length, No. Secondary roots and percentage ratio of *S. lycopersicum* seedling was 3.20, 1.5, 5.70 and 95 respectively of hot alcoholic extract Spirogyra sp. while the lowest value when soaked seeds for 24 hours was 1.44, 0.51, 3.60 and 66 respectively of hot alcoholic extract *Spirogyra* sp. The data showed that when soaked seeds for 48 hours .at 75 mg/ml concentration the highest value of the mean radical length, plumule length ,No. Secondary roots and percentage ratio of *S. lycopersicum* seedling was 2.75, 1.0, 4.3 and 74 respectively of cold alcoholic extract *Spirogyra* sp. while the lowest value when soaked seeds for 24 hours was 1.13, 0.23, 2.98 and 25 respectively.

Experimental results indicated that soaked tomato seeds less than 48 hours in 75 mg/ml concentrations of crude *Spirogyra* extract treatments have not only protect the infection by *F. oxysporum* but also promoted seeds germination and stimulated early seedling growth compared with negative control. This result is concordant to finding of other researchers[22,23] and that due to antifungal activity which found in macro-algae.[17,18,19]

Table (2): ***S. lycopersicum* seeds soaked in algal alcoholic extracts inoculums with *F. oxysporum*.**

Treatment Measures	Hot extracts (mg/ml)			Cold extracts (mg/ml)			control	LSD
	24hr	48hr.	72hr.	24hr	48h.	72hr		
Radical length (cm)	1.44	3.20	1.52	1.13	2.75	1.32	0.00	0.87*
Plumule length (cm)	0.51	1.5	0.66	0.23	1.0	0.43	0.00	0.17*
No. Secondary Roots	3.60	5.70	3.74	2.98	4.3	2.32	0.00	1.2*
Percentage ratio of seedling %	66	95	76	25	74	33	0.00	10.88 *
* (P<0.01)								

Qualitative estimation of active compounds from *Spirogyra* sp.

The results showed presence of active compounds in alcoholic extract of *Spirogyra sp*. in the table (3).

Table (3): Presence or absence of active compounds in *Spirogyra sp*. extract.

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

Results from current study show during analysis of the hot alcoholic extracts revealed that, Terpenoid, Flavonoids ,Phenols Saponins and Alkaloids are generally present in alcoholic extract of *Spirogyra sp*. other metabolites such as Glycosides and Tannins were absent in the extracts as show in table (3) .This result supports the findings of many authors[3,11,12] they screened the most active compounds in macro-algae , biochemical analysis are being undertaken to determine the structure and nature of compounds responsible of the bioactivity 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.[13,17,21,20,22]

Table (4): The major identified compounds of Hot crud alcoholic extract (*Spirogyra* sp.) by using GC-Mass spectrophotometer.

Rt	Compound	Area%
12.98	Pentadecane	10.2
13.78	Eicosane	19.2
14.11	Nonadecane	44.5
16.61	Tetradecane dihydroxyl	4.2
16.75	Octadecane	8.3
18.94	Hexadecane 2-hydroxyl	2.1
21.77	Hexadecane	1.3

The results reported in current study show in the table (4) that 6-major compounds were found in hot alcoholic crud extract of *Spirogyra sp*. ,these were: Nonadecane (44.5%) and Eicosane(19.2) are alkane hydrocarbon Alkanes .While, Pentadecane represented(10.2%) from the crud hot extract of *Spirogyra sp*. the alkane hydrocarbon the generic name for the

group of aliphatic hydrocarbons Cn-H2n+2 ,which represented reactive groups.[12,26] Materials in this group may be incompatible with strong oxidizing agents like nitric acid. Charring of the hydrocarbon may occur followed by ignition of un reacted hydrocarbon and other nearby combustibles. In other settings, aliphatic saturated hydrocarbons are mostly un reactive.

The present study provides data to show the appreciable antibacterial activity of macro-algae *Spirogyra sp*. crude extracts and purified fractions against phytopathogenic fungi. The result presumes that the long chain hydrocarbons may act as potential bioactive substance and can be exploited in pharmaceutical preparations.[26,27] The cultivable nature of seaweeds is an added advantage for mass production of potential antibacterial products, our finding agreed with[25] how reported the most similarly compound in macro-algae where isolated from green algae.[23,24] The main reasons for using algal extract as antifungal agents is their natural origin and low chance of pathogens developing resistance and less environmental hazards (eco-friend).[27,28]

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