ARTICLE / INVESTIGACIÓN

Antifungal Activity and Qualitative Phytochemical Analysis of Green alga Ulothrix sp.

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Abstract. The antifungal activity of the ethanolic hot extract of the green filamentous species Chlorophyta was evaluated in vitro at various doses (25, 50, and 100 mg/ml) and shown to be effective. Antifungal activity was performed by evaluating the percentage inhibition growth method against some fungus were obtained from the postgraduate laboratories in the Department of Biology - Faculty of Science / University of Mustansiriya (*Aspergillus niger, Fusarium oxysporum, Penicillium sp. and Rhizoctonia solani*). As a result of the research, it was discovered that the hot ethanol extract of Ulothrix sp had the most significant effect (91.8 %) on *Rhizoctonia solani* growth inhibition at a concentration of 100 mg/ml and the minor effect (22.4 %) at a concentration of 25 mg/ml against *Aspergillus niger* growth inhibition. It was discovered by primary chemical analysis of active substances that alkaloids, Terpenes, Saponines, phenols, Flavones, Resins, Steroids, and tannins were present in hot ethanolic alga extract. Finally, the GC-mass analysis performed on Ulothrix sp extracts revealed a large number of antibacterial activity-producing substances. Because the current research shows that algae have antifungal activity, it has the potential to be developed as a new source of active chemicals for human and plant consumption in a variety of applications shortly.

Keywords: Antifungal activity, Ulothrix, Active compounds,

Introduction

The green alga Ulothrix sp., which belongs to the family Ulothricaceae, includes about 30 species and is generally found in fresh and marine water.Thallus has the advantage which are long, filamentous, un-branched, multicellular, having a single line of cells (uniseriate) and attached to the substratum by a holdfast[1,2]. Antibiotics are used in an indiscriminate and illogical manner, and this is one of the causes that contribute to the formation of antibiotic-resistant strains of bacteria. The search for new and natural sources that have the efficacy of antimicrobial resistance. Algae consider being a potentially active source of antimicrobial and antioxidant compounds [3,4]. Because algae is an alternative source of many of diseases of cancer and other infectious diseases because of their chemical and biological diversity [5,6].

Materials and methods

Sample Collection and Preparation

Ulothrix specimens were collected. The sample was collected from aspiring of water in Tarjella village with Al-Hamdaniya District within Nineveh governorate, This station is located on longitude 43°28'33"E and latitude 36°19'49"N during spring 2021 figure (1). For the transfer to the laboratory, samples were placed in plastic bags. The samples are cleaned from dirt and substance by washing carefully with tap water and then drying for three days in the sun [7].

Soxhelet proses

According to [8,9] convert the dried powder of macroalgae ethyl alcohol to get hot alcoholic extract by Soxhelet extraction for six hours. When the extraction process is complete, evaporation increases concentration for an hour at 50˚C, which is then stored in sanitized test tubes until future usage.

Antifungal susceptibility

The antifungal activity of the macro-algae was investigated first by mixing different concentrations of the crude extracts of the macro-algae with Potato Dextrose Agar (PDA) medium [10, 11] to obtain different concentrations (25, 50, and 100) mg/ml and inoculating each plate with a block disk of fungal mycelia (1cm in diameter) and allowing it to grow at 2± 28°C for seven days in the dark. It was discovered that there was radial growth. For each treatment, three duplicate plates were utilized in total. According to the formula, the percentage of fungal inhibition was determined by testing.

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Figure 1. Collection region of the sample. At 36°19'49"N and 43°28'33"E 7Km

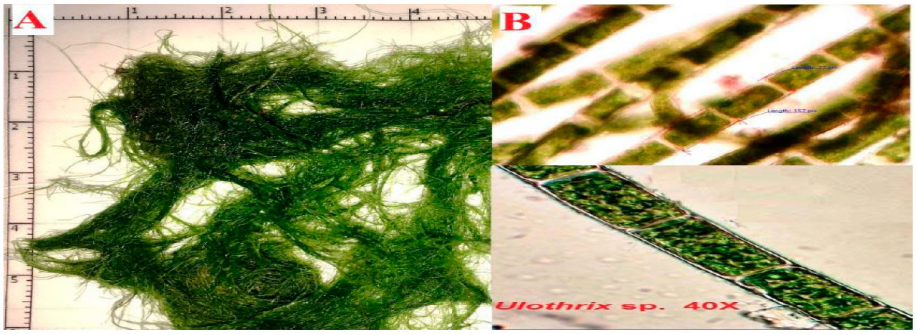

Figure 2. Filaments of Ulothrix specimens. (A) Length of filament in nature (B) unbranching under the microscope at 40X.

Fungal pathogen	Algal concentrations (mg/ml)			Control
	100	50	25	
Fusarium oxysporum	86.4	72.3	27.6	00.00
Aspergillus niger	88.6	65.3	22.4	00.00
Penicillium sp.	85.4	60.3	30.2	00.00
Rhizoctonia solani	91.8	80.2	66.2	00.00

Table 1. Demographical Characteristics and Homogeneity Between Experimental and Control Groups

Active compounds	alkaloids	phenols	Terpenes	Steroids	Flavones	Resins	Saponines	Tannins
Presence or absence	+	+	+	+	+	+	+	+

(+) Presence of active compounds. (-) Absence of active compounds.

Table 2. Active components of Ulothrix sp ethanolic hot extract

% Inhibition = (Cd-Td) x 100/Cd

Where,

Cd = The diameter of the control colony (in millimeters).

Td = Test plate colony diameter measured in millimeters (mm).

The percentage of inhibition was computed, as well as an analysis of variance for each treatment option.

Qualitative estimation of active compounds

The standard protocols determined of presence and absence of active compounds from Ulothrix specimens[12].

C/MS It is the method of choice for separating smaller and more volatile compounds in a sample, but it is also the most expensive because of its complexity. The characteristics of an Agilent Technologies (SHIMADZU / Japan) high-temperature column (Inert cap 1MS; 30 m x 0.25 mm id x 0.25 mm film thickness) were investigated. Using a high-temperature column, we were able to remove the necessity for the derivatization of each sample. 280°C was selected as the temperature for the injector and detector, while 100°C was set as the temperature for the starting column in the experiment. It was decided to run the column in split (1:10) mode with a 5 µl sample volume injected into it. A ramp rate of 12.5°C/min was used to increase the oven temperature to 225°C after one minute (hold time four minutes), and then a ramp rate of 7.5°C/min was used to raise the oven temperature to 300°C after five minutes (hold time five minutes). GC-Mass Solution and Postrun software, both of which can be downloaded from the Agilent website, were used to record and analyze the mass spectra and keep the helium carrier gas flowing at a constant rate of 17.5 ml/min. It was determined which chemicals were involved by comparing their mass to those found in the NIST library and legitimate standards [11].

Results

The green algae Ulothrix sp. isolate from Tarjella village with Al-Hamdaniya District within Nineveh governorate, cells consist of unbranched, cylindrical, uniseriate filaments figure (2). These findings agreed with [1,2].

The antifungal activity of the ethanolic hot extract was evaluated based on the validity of experimental data of percent inhibition obtained against fungi, which were used to determine the extract's efficacy (Ulothrix sp). Table (1) and Figure (3) shows the inhibitory of ethanolic hot extract (Ulothrix sp) against fungi. It shows more high antifungal activity due to fungi and concentration of algal extract. The percentage of inhibition ranged at 100mg/ml concentration between (85.4-91.8) %. The highest value (91.8)% in Rhizoctonia solani. While the lower value (85.4)% at Penicillium sp. The percentage of inhibition ranged at 50 mg/ml concentration between (60.3-80.2) %. The highest value (80.2) % was in Rhizoctonia solani. But, the lower value (60.3)% at Penicillium sp. Finally, The percentage of inhibition ranged at 25 mg/ml concentration between (22.4-66.2)%. The highest value (66.2)% was in *Rhizoctonia solani.* On the other hand, the lower value (22.4) % was for Aspergillus niger. The findings revealed a flexible relationship between algal extract concentrations and the percentage inhibition of fungi; nevertheless, when the concentration of algal extract is high, the percentage of fungal inhibition is also high.

Evaluation of Phyto-active compounds

The findings indicated that the hot alcohol extract of Ulothrix sp. included a significant amount of saponins, tanins, alkaloids, phenols, and flavonoids. Additional metabolites, such as glycosides and terpenoids, were not detected in the extracted sample; the findings are summarized in Table (2).

Evaluations of Gas Chromatography-mass Spectrometry:

The GC-MS study of the Ulothrix sp. extract revealed various compounds, some of which were ignored because they did not include eight compounds, and of these only three significant components accounted for 85.6 % of the total mass (Fig. 4). Due to its scarcity, the remaining 14.4 % composition could not be determined. Table (3) lists the principal discovered chemicals ethanolic hot extract of Ulothrix sp. Alkane hydrocarbons Nonadecane (16.2%) and Pentadecane (39.5%) were identified in Ulothrix sp hot ethanolic crud.

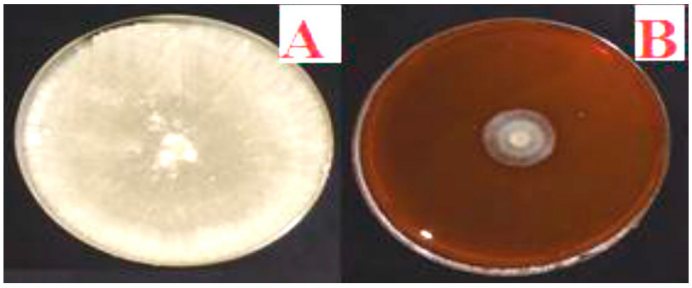

Figure 3. Growth inhibition of *Rhizoctonia solani* on PDAM plates by using ethanolic hot extract (*Ulothrix* sp) at 100 mg/ml concentration A: Control. B: Ethanolic hot extract (*Ulothrix sp.*) at 100 mg/ml.

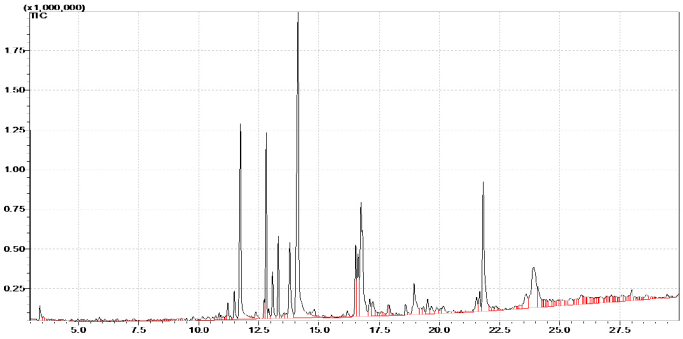

Figure 4. *Ulothrix sp*. extract chromatogram of GC-Mass spectrophotometery that combination of at least 8 chemicals

DISCUSSION

In different studies, the alga extract has been demonstrated to exhibit antibacterial activity both in vivo and in vitro; in the past three decades, there has been a significant surge in the discovery of algae-derived metabolites having biological activity[13]. In addition to their other features, these compounds exhibit various biological actions, including antibacterial, antiviral, and antifungal, insecticidal, and antiproliferative effects [14,15]. Interestingly, it was shown that hot ethanolic alga extract exhibited antifungal efficacy at all doses, which is consistent with previous findings [16]; it was discovered that macro-algae extracts were efficient against the majority of the tested fungus, including *Botryotrichum piluliferum, Fusarium oxysporium, and Alternaria brassicicola*, when extracted in methanol or ethyl acetate. There have been several research on the antibacterial activity of algal extracts that have been mentioned [5,1]. Results from these studies are difficult to compare because the antimicrobial activity of algae extracts can be influenced by many factors, including the algal species used in extraction, testing methodologies and the type of solvent used in extraction, the amount of time or period that samples were stored, and the thallus regions that were used [17,18]. Mammals and plants benefit from algae-derived bioactive chemicals, which have been demonstrated to protect them against biotic and abiotic stressors by enhancing their defense mechanisms. Mammals and plants benefit from the use of biologically active chemicals (antimicrobial activity, hunting of free radicals and host defense activity etc.); it has been discovered that the extract contains an alkane hydrocarbon known as Cn-H2n+2. The conclusions of this study are consistent with past studies on this issue [19]. It has previously been observed that a variety of marine algae include straight-chain paraffin (n-alkanes), divided-chain paraffin (alkyl-alkanes), and unsaturated hydrocarbons (alkenes) [19,20]. Octadecane, Tetradecane, and hexadecane were discovered as standard major volatile components in all algal extracts, and these results were verified when compared to other examined hydrocarbons [21]. A vast number of researches on the methodologies for production and the configuration of algal extracts have lately been published. Extract composition is highly dependent on both the source material (geographic location of macro-algae and algal species collected) and the extraction process used. From the algal biomass to the molten phase, polyphenols, polysaccharides, proteins, polyunsaturated fatty acids, minerals, pigments, plant growth hormones, and other physiologically active substances are transported. Humans, animals, and plants can all benefit from their well-predicted benefits, which include protection against environmental and internal stresses (such as antibacterial activity, free radical scavenging, and host defense), and they can be found in a variety of pharmaceuticals, feed additives, and dietary supplements [22,23].

Conclusions

Because they include a variety of active compounds that affect fungus development directly, algal Ulothrix extracts have shown dramatic suppression when used against several soil-borne pathogens, generally. Antifungal chemicals might be generated from this.

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Conflicts of Interest: Nil

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