Research article

The antagonistic effect of *Anabaena circinalis* on some dermatophytes

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ABSTRACT

Introduction and Aim: *Anabaena circinalis*, a photosynthetic cyanobacterium belonging to the Gram-negative group, is widely distributed in freshwater ecosystems across the world. The scientific focus on *A. circinalis* primarily stems from its ability to produce numerous cyanotoxins, which have the potential to be hazardous. The objective of this study was to assess the antifungal efficacy of *A. circinalis* against the dermatophytes *Trichophyton rubrum* and *Trichophyton mentagrophytes*, with the aim of discovering a new antifungal agent.

Methodology*:* The identification of *Anabaena circinalis* in this study was conducted through the observation of its morphological characteristics. Molecular identification and confirmation of the algae was done using the polymerase chain reaction (PCR) method, employing a specific set of primers targeting the PCβ and PCα genes. Gas chromatography-mass spectrometry was employed in identifying *A. circinalis* antifungal compounds. The ability of 70% ethanolic and methanolic extracts produced from *A. circinalis* to inhibit the dermatophyte fungi *Trichophyton rubrum* and *Trichophyton mentagrophytes* was tested by *in vitro* tests.

Results: The findings indicate that the hot ethanolic extract of *Anabaena circinalis* exhibited a significant inhibitory effect (100% inhibition) on the growth of dermatophyte fungi tested. Gas chromatography-mass spectrometry studies identified several antifungal compounds in *A. circinalis* extracts, which included Hexadecen-1-ol, Phthalic acid, Octadecenoic acid, Octadecynoic acid, Hexadecanoic acid, Pentadecadien, Tetradecenal, and Octadecadien-1-ol.

Conclusion: Overall finding suggests hot ethanolic or methanolic extracts of *Anabaena circinalis* contain phyto components, which could be used as an antifungal agent in treating dermatophyte fungi-related infections.

Keywords: *Anabaena circinalis*; ethanolic extract; dermatophytes; GC mass spectrophotometry.

INTRODUCTION

Algae encompass a diverse array of photosynthetic organisms primarily found in aquatic environments. They exist in either unicellular or multicellular forms. Algae play a significant role as a valuable reservoir of bioactive compounds, encompassing proteins, carbohydrates, fats, phenols, flavonoids, toxins, antibiotics, lipids, vitamins, enzymes, pigments, growth regulators, and free amino acids. Algae have been identified as potent sources of antibiotics that exhibit antimicrobial activity against pathogenic bacteria and fungi, thereby contributing to the treatment of human illnesses (1, 2).

Fungal organisms are responsible for the development of superficial mycoses as well as chronic infections that affect both humans and animals on a widespread scale. Dermatophytes and Candida species are frequently recognized fungi that impact the subcutaneous layer of the skin and underlying tissues in the elderly and individuals with compromised immune systems. The incidence of opportunistic infections caused by drug-resistant fungal pathogens is increasing (3,4), prompting the exploration of alternative options such as plant-derived antimicrobials and pharmaceuticals (5). Academic researchers are currently placing significant emphasis on the examination of secondary metabolites derived from algae, as well as the exploration of naturally occurring antimicrobial compounds found within marine algae. Marine algae are recognized for their substantial content of fatty acids (both unsaturated and saturated), sterols, sugars, and terpenes, which possess significant pharmacological significance. In contrast, there is a scarcity of available information pertaining to freshwater algae. Limited research has been conducted on the lipid, terpene, phlorotannin, phenol, steroid, and antifungal and antibacterial compound content of freshwater algae (6, 7). Therefore, the objective of this study was to assess the antifungal efficacy of *Anabaena circinalis* against *Trichophyton rubrum* and *Trichophyton mentagrophytes*, with the aim of discovering a new antifungal agent.

MATERIALS AND METHODS

Sample collection

The specimens were obtained from a freshwater reservoir located in the Tigris River at Mosul, situated within the administrative region of Nineveh Governorate. The geographical coordinates of this station are situated at a latitude of (36°20'46"N) and longitude (43°08'46"E). The algae that were collected were streaked onto plates and subsequently purified in accordance with the procedure outlined previously (8). The obtained pure species of Cyanobacteria were transferred to a conical flask containing a CHU-10 solution with a volume ranging from 100 to 250 ml.

The flask was then incubated for a period of 14 to 21 days in order to facilitate optimal growth (9).

Identification of *Anabaena circinalis*

A. circinalis was initially identified through the application of the classical algal classification method, which relies on the examination of morphological characteristics (10). The molecular verification of the isolate was conducted through the utilization of the polymerase chain reaction (PCR) methodology, employing a specific pair of primers (PCβF: 5' GGCTGCTTGTTTACGCGACA 3' and PCαF: 5' CCAGTACCACCAGCAACTAA 6'). The set of primers employed facilitated the amplification *cpcBIGC*-*cpcA* region of a gene segment responsible for encoding the phycocyanin pigment. In the PCR studies conducted, a cyanobacterial species was employed as a negative control (11). The PCR procedures were for an initial denaturation for 2 min at 95°C, 35 cycles of denaturation for 90 sec at 95°C, annealing for 30 sec at 52°C (PCβ-PCα primer set), extension for 1 min at 72°C, and a final extension for 8 min at 72°C. To establish the precise size of the amplified products, 10 l of PCR product was separated in 1.5% agarose gel electrophoresis and stained with ethidium bromide before being seen on a UV transilluminator.

Preparation of ethanolic and methanolic extraction

In order to get a high concentration of cells, the identified isolates of *A. circinalis* were cultured in bioreactors with CHU-10 media after spending two weeks growing in aerated bioreactors. After being separated by centrifugation, the cells were collected and put to use in the extraction process. The fresh algal biomass was extracted through the use of Soxhlet with 70% ethanol and methanol in accordance with the procedure that had been described earlier (12).

Dermatophytes

The pathogenic fungi that were identified were acquired from the Laboratories of Al-Mosul Hospital in Mosul, Iraq.

Antifungal assessment

Anti–fungal activity of *A. circinalis* ethanolic and methanolic extracts was first evaluated by inoculating fungal inoculations onto Potato Dextrose agar medium after mixing the crude solution of alga with the extract concentrations (100 mg/ml). The percentage inhibition of fungal development was calculated using the formula (13);

% inhibition = 100×(A-B/) A, where,

A=mycelial biomass of fungi / dry weight in control.

B=mycelial biomass of fungi / dry weight in control in many test concentration

Analysis using GC-MS spectrophotometry

GC-MS spectrophotometry was carried out using the column provided by Agilent Technologies (Shimadzu, Japan). The temperature of the column was initially fixed at 110°C. To the column 5 mL of the test sample was introduced using the split (1:100.0) mode, following which the oven temperature was gradually ramped up to 225°C via a ramp ratio of 13.5°C per minute following a one-minute interval and then maintained at 225°C for a period of four minutes after the ramp ratio had been reached. A ramp rate of 9.5°C per minute was then applied to progressively raise the temperature of the oven to 320°C within five minutes, and this temperature was maintained for another five minutes. Helium served as the carrier gas, flowing at a rate of 18.5 mL per minute. Mass spectra analysis was carried out with the post-run software provided by the company (Agilent Technologies, Shimadzu, Japan). The identification of compounds was accomplished by comparing their masses with the NIST library using an authentic reference criterion (14-16).

RESULTS

Identification of *Anabaena circinalis*

A. circinalis is a multicellular organism that exhibits a filamentous structure composed of coccoid cells when observed under a microscope. Some filament cells have the ability to undergo a transition from a generic state to more specialized cell types. This phenomenon could potentially provide benefits to the organism under specific conditions (Fig. 1).

Fig. 1: The morphological appearance of *A. circinalis* under light microscope (40X)

Molecular identification of *A. circinalis*

In the gel electrophoresis analysis, a positive band was observed at 650 base pairs, indicating successful amplification of the cpcBA-IGC gene through PCR. The intergenic spacer (IGS) region was identified within the gene fragment of the phycocyanin operon derived from the alga *A. circinalis*. DNA extracts yielded a discernible amplicon pattern, which exhibited an anticipated size. However, DNA was extracted from a green alga. The species Cladophora does not exhibit the presence of the phycocyanin operon, as evidenced by the absence of a PCR product (Fig. 2).

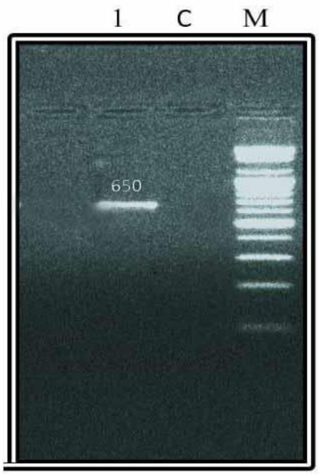

Fig. 2: PCR amplification of cpcBA-IGC gene (650bp) in *A. circinalis* isolates. M: 100 bp DNA ladder. Lane 1: *A. circinalis* Lane 2: *Cladophora* sp. (negative control).

Antifungal effect of *A. circinalis* extracts

The anti-fungal effectiveness of the ethanolic and methanolic extracts of *A. circinalis* in managing the biomass of the clinical fungal species *T. rubrum* and T. *mentegrephytes* was assessed. A statistical analysis was performed on the data presented in Table 1 to assess the validity of the experimental results regarding the inhibition percentage at a concentration of 100mg/ml. Statistically, clinical fungal species were significantly inhibited by alga A. *circinalis* extracts. As seen in the results, maximum inhibition was caused by *T. mentegrephytes*, in case of using ethanolic hot extracts of *A. circinalis* (100% inhibition growth) and minimum inhibition was affected by *T. mentegrephytes*, in case of methanolic hot extracts of *A. circinalis* (97.1%) at ten days of incubation at tested concentration, the extracts caused 100% and 85% inhibition rates, respectively.

Anabaena circinalis GC-MS analysis

The results of analysis were screened by gas chromatography mass spectrometry (GC–MS/MS), it appeared eight different active compounds were the most abundant compounds in the hot alcoholic *A. circinalis* extract included : Hexadecen-1-ol (RT-9.55; 1.07 %), Phthalic acid (RT-13.2; 1.07 %), Octadecenoic acid (RT-18.79 ; 5.68 %), Octadecynoic acid (RT-18.94; 2.32 %), Hexadecanoic acid (RT-20.59; 0.81 %), Pentadecadien (RT-20,96; 1.26 %), Tetradecenal (RT-21.47; 1.72 %), and Octadecadien-1-ol (RT-22.82 ; 1.95 %) respectively (Fig. 3, Table 2). Most of these components possess various biological activities.

Table 1: Growth inhibition of dermatophytes *T. rubrum and T. mentegrephytes* by *A. circinalis* extract

Dermatophytes	***A. circinalis* Extracts Concentration (100mg/ml)**		**Control**	**LSD**
	Ethanolic	**Methanolic**		
T. rubrum	100 ± 0.1	85 ± 0.2	0.00 ± 0	**7.500 ***
T. mentegrephytes	100 ± 0.2	97.1 ± 0.05	0.00 ± 0	**7.428 ***

* (P<0.05).

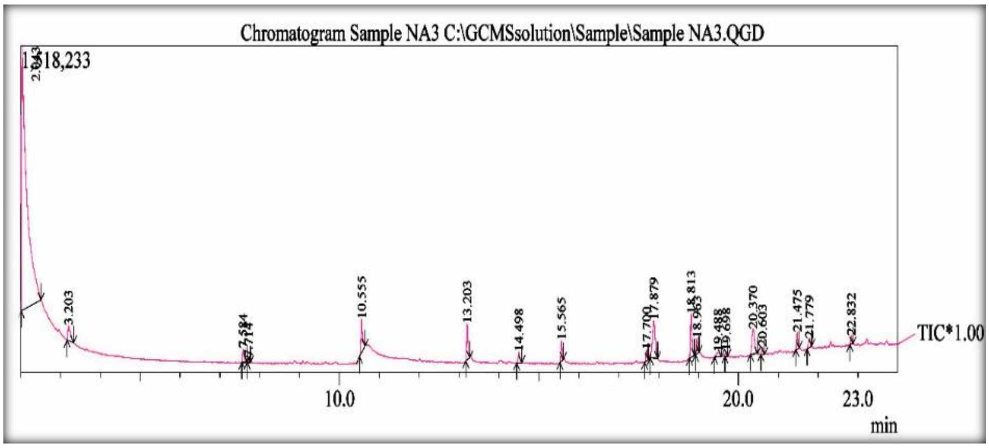

Fig. 3: GC-Mass spectrophotometry chromatogram of *A. circinalis* hot ethanolic extract

Table 2: Compounds identified in hot ethanolic extract *A. circinalis* using GC-Mass spectrophotometry

Rt.	**Area%**	**Compound name**
9.55	1.07	Hexadecen-1-ol
13.2	1.07	Phthalic acid
18.79	5.68	Octadecenoic acid
18.94	2.32	Octadecynoic acid
20.59	0.81	Hexadecanoic acid
20.96	1.26	Pentadecadien
21.47	1.72	Tetradecenal
22.82	1.95	Octadecadien-1-ol

DISCUSSION

The primary objective of this study was to assess the potential antifungal properties of *A. circinalis*, marking the first investigation of its kind conducted in Iraq. The findings demonstrated significant antifungal activity against *T. mentagrophytes*, particularly when compared to *T. rubrum,* as indicated by the larger zone of inhibition observed around colonies on the cultured media. The rationale goes back to the cell wall of fungus, which is composed of polysaccharides like hitchin and glucan, or because the membrane assembling mechanism offers or because of the permeability barrier that the cell wall provides (17). On the other hand, the linkages between the antibacterial component and the cell membrane can be the cause of this phenomenon. It is possible for the extract to return to the existence of a variety of compounds, some of which may contain flavonoids, triterpenoids, and phenolic. They could have an influence on fungal development that is either inhibiting or promoting, depending on their composition, composites, alkaloids, and amides, as well as their concentration (18, 19). A plethora of information is readily accessible pertaining to the substances synthesized by algae, which exhibit a wide array of biological effects. Several previous studies have indicated potential advantages in terms of antidiuretic, anticancer, and anti-inflammatory properties (20). Additionally, these studies have also highlighted the potential antimicrobial effects, including antibacterial and antifungal properties (21-24), as well as antioxidant effects (25). The analysis of microorganisms demonstrates that the inhibitory effects vary based on the microbe's ability to react to antimicrobial agents and extracts derived from algae. The observed variation in the extracted chemicals may be attributed to differences in the efficiency of the extraction process. The process of maceration is a relatively uncomplicated technique for extraction, although its effectiveness is comparatively lower when compared to the Soxhlet method (26).

CONCLUSION

This study suggests bioactive compounds that present in hot ethanolic and methanolic extracts of *A. circinalis* could be used to treat diseases caused by dermatophytic fungi *T.mentagrophytes* and *T. rubrum*.

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CONFLICT OF INTEREST

None.

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