مجلة الدراسات التربوية والعلمية - كلية التربية - الجامعة العراقية
العدد الثاني والعشرون - المجلد الأول - علوم الحياة - أيلول 2023 م
doi.org/10.52866/esj.2023.01.22.15

Investigate the anti-*Candida albicans* activity of an ethanolic extract of the macroalgae *Spirogyra aequinoctialis*.

Thaeer H.Khleel [1] & Ahmed S.Dwaish [2]

1&2 Department of Biology, College of Science, University of Mustansiriyah, Baghdad-Iraq

Abstract:

Macroalgae have a wide range of natural compounds which have natural antioxidants. In this study, four concentrations of the ethanolic extract of the chlorophyta-related macroalga Spirogyra aequinoctialis were tested in vitro to evaluate their ability on the growth inhibition of Candida species. Results showed that the ethanolic extract has a strong and widespread inhibitory impact on Candida albican. The diameter of the inhibition zone (in millimeters) against Candida albican was ranged between (32-10) mm also It's interesting to note that some concentrations had greater antifungal activity when compared to the trademarked antifungal drugs Nystatin and Clotrimazole. Chemical analyses showed that the active chemical compounds for hot ethanol extract alga (Spirogyra aequinoctialis) extract was contains alkaloids, phenols , Tannins , Flavones, Resins , Saponines and Flavones. The ethanol extract was further chemically characterized by using GC–MS in order to be tentative identify the compounds responsible for such activities. The main compositions were Decenoic acid, Pyrimidine, Tetradecanoic acid, Heptane, and Boranamine compounds which had antimicrobial activity. Acetone extract of Spirogyra aequinoctialis exhibited appreciable antimicrobial activity. This mean that could be a source of valuable bioactive materials for health products. The current study confirmed that macroalgae (Spirogyra aequinoctialis) ethanolic extract could be potentially used as antimicrobial agent.

KEY WORDS: ALGAE,FUNGI,ACTIVE COMPOUNDS .

التحري في النشاط المضاد للمبيضات البيض للمستخلص إلايثانولي للطحالب الكبير *Spirogyra aequinoctialis*.

ثائر حميد خليل و أحمد ساهي دويش
قسم علوم الحياة - كلية العلوم - الجامعة المستنصرية -بغداد - العراق

مستخلص:

تحتوي الطحالب الكبيرة على مجموعة واسعة من المركبات الطبيعية التي تحتوي على مضادات الأكسدة الطبيعية. في هذه الدراسة ، تم اختبار أربعة تراكيز من المستخلص الإيثانولي من الطحالب الكبيرة العائدة الى شعبة الطحالب الخضراء Spirogyra aequinoctialis في المختبر لتقييم قدرتها على تثبيط نمو أنواع المبيضات. أظهرت النتائج أن المستخلص الإيثانولي له تأثير مثبط قوي وواسع الانتشار على المبيضات البيضاء. تراوح قطر منطقة التثبيط (بالمليمترات) ضد المبيضات البيضاء بين (10–32) ملم ، ومن المثير للاهتمام أيضًا ملاحظة أن بعض التركيزات كان لها نشاط مضاد للفطريات أكبر عند مقارنتها بالعقاقير المضادة للفطريات Nystatin و Clotrimazole. أظهرت التحليلات الكيميائية أن المركبات الكيميائية الفعالة لمستخلص الايثانولي الحار لطحلب (Spirogyra aequinoctialis) تحتوي على قلويدات ، فينولات ، تانين ، فلافون ، راتنجات ، صابونين وفلافون. تم وصف مستخلص الإيثانول كيميائيًا باستخدام GC-MS من أجل تحديد مؤقت للمركبات المسؤولة عن مثل هذه الأنشطة. التركيبات الرئيسية هي حمض ديسينويك ، بيريميدين ، حمض تترادیكانويك ، هيبتان ، ومركبات البورانامين التي لها نشاط مضاد للميكروبات. تشير هذه النتائج إلى أن مستخلص الأسيتون من Spirogyra aequinoctialis أظهر نشاطًا مضادًا للميكروبات ملحوظًا ويمكن أن يكون مصدرًا للمواد الحيوية النشطة للمنتجات الصحية. أكدت الدراسة الحالية أن المستخلص الإيثانولي للطحالب الكبيرة (Spirogyra aequinoctialis) يمكن استخدامه كعامل مضاد للميكروبات.

كلمات مفتاحية : طحالب – فطريات – مركبات فعالة .

Introduction :

Algae are common and widespread in the water bodies of various countries. It could be possible to categorize macro-algae through their nutrients and chemicals contain. Some marine organisms, including seaweeds, have been examined upon as potential sources of pharmacological main metabolites. (1). Algal extracts were found to be highly active against *Candida albicans*, and the same results were achieved against other fungal strains (2 , 3). (4) Using paper disc agar diffusion methods, tested the antimicrobial and antifungal effects of ethanolic crude extracts from seven marine algal species (*Cladophora glomerata*, *Enteromorpha linza, Ulva rigida*), *Cystoseira barbadensis*, *Padina pavonica*, and Rhodophyceae (*Corallina officinalis*, *Ceramium ciliatum*) found along the coast of Vona. When (5) obtained antimicrobial agents from 23 species of marine algae belonging to the Chlorophyta, Phaeophyta, and Rhodophyta using three different solvents (ethanol, acetone, and methanol-toluene), they found that the ethanol extract had the highest antibacterial and antifungal activities and the methanol-toluene extract had the lowest. The Rhodophyta genus includes four species with broad-spectrum antimicrobial activity: *Laurencia okamurai*, *Dasya scoparia*, *Grateloupia filicina*, and *Plocamium telfairiae*. Extracts from seaweeds had a greater impact in inhibiting bacterial growth than fungal growth, while Rhodophyta species had the highest antimicrobial and antifungal activity. Some green, red, and brown seaweeds were identified as possible sources of antibiotic compounds, according to a report from (6). Green algae were shown to be the most effective antibacterial agents (7). (8) investigated the presence of bioactive phytochemicals in green algae (*Chlorococcum humicola*) and the antimicrobial activity of these compounds using a variety of organic solvents, and they found that organic extracts of *C. humicola* had potent effects against the pathogenic fungal strains of *C. albicans* and *A. flavus.*

Five different types of seaweed were extracted using petroleum ether, benzene, chloroform, ethanol, ethyl acetate, and water, and all of the extracts showed antifungal activity. Eleven human infections and five fish

pathogens were tested for resistance to nine different solvents of algal extracts (10). When compared to extracts made using different solvents, the acetone extract of *Ulva lactuca* exhibited the widest range of antibacterial activity. Researchers from Bejaia, Algeria, looked into the antifungal properties of four different marine algae species and found that all of the extracts tested showed antifungal activity; however, the red algae *Rhodomela confervoides* and the brown algae *Padina pavonica* had the greatest inhibiting effect against *Candida albicans* (inhibition zone: 24 mm) and *Mucor ramanianus* (inhibition zone: 26 mm), respectively. *Ulva lactuca* (Chlorophyceae) was shown to be more effective as an antibacterial agent than Jania rubens (Rhodophyceae), according to a study (12).Thirteen researchers tested the effectiveness of extracts from nine marine macroalgae (three Rhodophyceae species, four Chlorophyceae species, and two Phaeophyceae species) found in Abu-Qir bay (Alexandria) against various microorganisms. The variety of algae seen in Iraq is remarkable. According to the existing literatures, analyses of antifungal properties of Iraqi algae have been ignored. Therefore, the purpose of this research is to investigate the potential therapeutic value of biologically active substances produced by extract of macroalgal species collected in Baghdad, Iraq.

Materials and Methods:

Study area and algal samples collection:

From September to October of 2022, macro-algae were collected by hand from the Tigris River in the Rashididia regni of Baghdad, Iraq. All samples were placed polyethylene bags and transported directly to the laboratory for diagnosis and description using standard reference and taxonomy keys (14). After that algae was , cleaned of epiphytes, unwanted waste, and decaying tissue in the field, then washed completely with water, and stored in the shade for 7 days.

Preparation of extracts:

The shade-dried macro algae was ground and kept frozen. After filling the thimble with 20g of powder, 8 hours were spent using a Soxhlet device to extract the substance from 400ml of Ethanol solvent. For later analysis, the crude extracts were kept in an airtight

reagent bottle.

Culture media:

Potato dextrose agar (PDA) and Sabouraud dextrose agar (SDA) were used and prepared according the manufacturer details, Combine all ingredients in ~900 ml of deioinized water, adjust to pH 5.6 with hydrochloric acid and adjust final volume to 1 liter, heat to boiling to dissolve the medium completely,autoclave at 121ºC for 15 minutes. Cool to ~45 to 50°C and pour into petri dishes or tubes for slants.

Candida albicans :

Candida albicans were obtained from the postgraduate Bio-lab at the College of Science at Dialla University in Iraq to use in the following experiments. Five different antifungal drugs (table 1) were tested for their sensitivity to each of these pathogens**.**

Antifungal activity assay:

Efficacy antifung of algal extract was tested in vitro with use the well diffusion method (15). Fungal growth inhibition rings around the well are measured in millimeters to express the results. The solvent DMSO was employed as a placebo. Experiment-to-experiment comparison data are presented as the mean with standard error, and all tests were run in triplicate with halos larger than 10 mm deemed to be positive.

Indicators of Active Compound in Extract :

Algae were tested using established methods (16, 17) to identify the presence of active chemical substances.

samples	Sources.	Amphotricn B	Caspofangn	Ketoconazol	Fluconazol	Metroconazol	result
C. albicans1	**Urine**	**S**	**I**	**R**	**R**	**R**	
C. albicans2	Urine	**S**	**S**	**S**	**S**	**S**	
C. albicans3	Stool	**R**	**I**	**R**	**R**	**R**	MDR
C. albicans4	Stool	**S**	**R**	**R**	**R**	**R**	
C. albicans5	Urine	**R**	**R**	**R**	**R**	**R**	MDR
C. albicans6	Vaginal swap	**R**	**R**	**R**	**R**	**R**	MDR
C. albicans7	Burn	**S**	**S**	**S**	**R**	**R**	
C. albicans8	Stool	**S**	**S**	**R**	**R**	**R**	
C. albicans9	Urine	**S**	**R**	**R**	**R**	**R**	MDR
C. albicans10	Urine	**S**	**S**	**S**	**R**	**R**	

Gas Chromatography-Mass Spectrometry :

GC-MS analysis was performed using a high-temperature column from Agilent Technologies, with initial column temperature set at 100°C. A 5 L sample volume was injected, and oven temperatures were raised to 225°C and 300°C. Mass spectra were recorded and analyzed using Agilent GC-Mass Solution and postrun software. Chemicals were identified by comparing their masses to NIST database and genuine standards. (15).

Results and discussion:

Morphological Structure of algae:

Spirogyra aequinoctialis is a type of green algae that float freely in freshwater environments such as ponds, lakes, and other similar environments. In common usage, Spirogyra are referred to as "water silk" or "pond silk" (Figure 1). They have a vegetative structure that is unbranched and filamentous all throughout. There are around 400 distinct species of the genus Spirogyra. The distinctive spiral chloroplasts that are found inside the cells of algae (Figure 2) inspired the naming of the genus Spirogyra (18,19).

Spirogyra aequinoctialis species have rapid response times, easy identification by experienced biologists, and simple sampling. They exhibit tolerance or sensitivity to environmental changes, as noted by the US Environmental Protection Agency. (20).

Figure (1): *Spirogyra aequinoctialis* in nature after isolate.

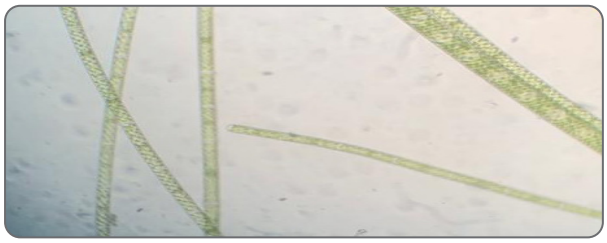

Figure (2): *Spirogyra aequinoctialis* under microscope (40X) .

Antifungal activity:

Antifungal of Chlorophyta macroalga *Spirogyra aequinoctialis* was evaluated against ten different strains of *Candida albicans*. The efficacy of *Spirogyra aequinoctialis* differed build on the difference between isolates of C. albicans used in the studies. Effects

of *Spirogyra aequinoctialis* extract (ethanol extract) at four different concentrations that the ethanolic extract has a strong and widespread inhibitory impact on Candida *C. albican*(Table 2). According to the results of the current assessment, it appears that the ethanolic extract has a strong and widespread inhibitory impact on *Candida albican*. The diameter of the inhibition zone (in millimeters) against *Candida albican* was measured to evaluate the antifungal activity of *Spirogyra aequinoctialis* (Chlorophyta) in extracts of different treatments.(Table2). Notably, the colony diameter of the examined pathogenic fungi was significantly reduced by the ethanolic extract of *Spirogyra aequinoctialis*, which contrasts with the results of the sensitivity test for these isolates, most of those that were resistant to antifungal drugs. The current findings were consistent with those of (21) who found that extracts of some algae displayed more antifungal action than common drugs against *C. albicans*. Several authors found conflicting findings on the antifungal effects of algae extracts on various Candida species. This is significant since (22) found that seaweed extracts made with methanol were the most effective against *C. albicans*. In contrast, the anti-Candida activity of the ethanol extract of both algae is moderate. Extracts of green algae in acetone, methanol, and chloroform revealed no action against *C. albicans*, however (8) reported opposite results. All of the above mentioned may have occurred because of the season, location, and isolate solvent employed for the algae.

Phytochemical evaluation:

According to the findings, the *Spirogyra aequinoctialis* the extract of ethanol contains active chemical compounds. Alkaloids, phenols, flavonoids, resins, saponine, and tannins were found *in S. aequinoctialis* extract. Table 3 indicates that no coumarins nor glycosides were present. They examined the most chemically active compounds in macro algae, and their findings matched those of a number of other studies (15).

Table(2) : The effect (inhibition zone mm ± SD) of ethanolic *Spirogyra aequinoctialis* extract in four different concentration on different sample source of *Candida albicans* .

No.	Sample Source	Spirogyra algae extract in four different concentration			
		100mg/ml	50mg/ml	25mg/ml	12.5mg/ml
1	Urine	1±23	1±22	1±19	1±18
2	Urine	2±30	1±25	1±24	1±22
3	Stool	1±28	1±26	1±24	2±21
4	Stool	1±32	1±31	1±30	2±26
5	Urine	1±16	0.5±14	0.5±11	0.5±10
6	H.V.S	1±26	1±23	1±20	0.5±16
7	Burn	2±24	1±22	1±19	1±18
8	Stool	2±28	2±23	1±21	1±19
9	Urine	1±27	1±24	0.5±18	0.5±17
10	Urine	1±26	1±24	1±21	1±18

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

Chemicals Compound	ethanolic Extract
Glycosides	Absence
Phenols	Presence
Alkaloids	Presence
Resins	Presence
Saponines	Presence
Tannins	Presence
Flavones	Presence
Coumarines	Absence

GC-MASS analysis:

Extracts that had notable antimicrobial activity were investigated by biochemical research to identify the structures and identities of the chemicals responsible for their bio-activity. Table 4 and Figure 3 both show that identifying natural organic substances requires examination using Gas chromatography-mass spectrometry (GC-MS). Extract of *Spirogyra aequinoctialis* was chemically analyzed using GC-MS to identify the active components responsible for the extract's apparent biological activity.

Table(4): GC-MS Analysis of Major Compounds in ethanol extract of Spirogyra aequinoctialis.

Number	RT.	Area%	Compounds
1	4.704	2.09	Aziridine
2	6.833	1.27	3-Quinolinecarboxylic acid, 6,8-di
3	13.004	2.31	Decenoic acid
4	13.662	8.07	2-Decenoic acid
5	15.436	0.95	Pyrimidine, 4,6-dimethoxy-5-nitro-
6	16.631	1.14	2-Pyrrolidinethione
7	18.924	3.30	Tetradecanoic acid
8	19.192	2.40	Ethylcyclopent-1-ene-1-carboxyli
9	19.928	1.26	Bicyclo[3.1.1]heptane, 2,6,6-trime
10	20.006	6.81	2-Pentadecanone, 6,10,14-trimethyl
11	20.300	1.73	Heptane,
12	20.975	1.22	1,2-Cyclobutanedicarboxylic acid
13	21.798	53.12	n-Hexadecanoic acid
14	22.109	1.90	Heptadecyl heptafluorobutyrate
15	23.555	1.99	Pyrrolidin-2-one, 5-[2-butyrylethy
16	24.013	2.46	Boranamine

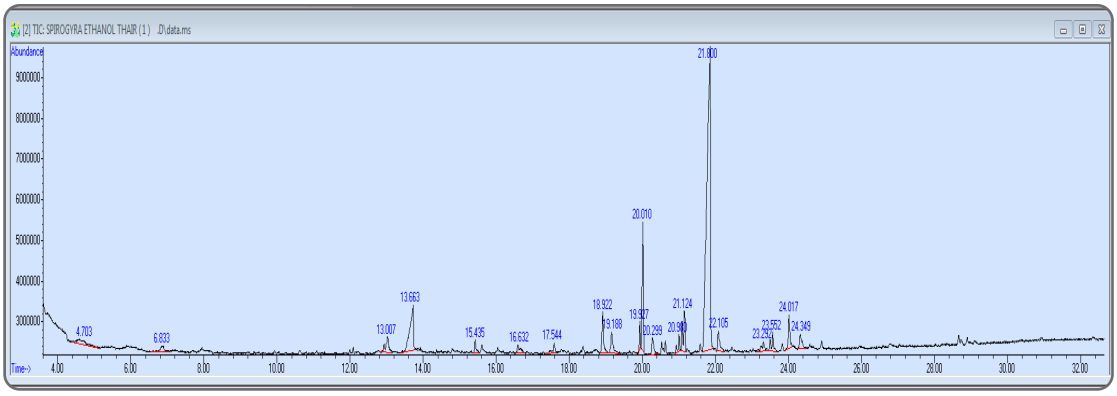

Figure (3): The chromatogram of GC-Mass spectrophotometry showed in ethanol extract of *Spirogyra aequinoctialis*.

A wide range of naturally produced antimicrobial compounds, including indoles, terpenes, acetogenins, phenols, fatty acids, and volatile halogenated hydrocarbons (23), have been detected in algae. Fatty acids and volatile compounds in the hexane and ethyl acetate extracts of the algae were therefore

analyzed using GC-MS techniques because they indicated medication action. This finding suggests that long-chain hydrocarbons might be a pharmacological substance that could be used in pharmaceuticals. Seaweeds' potential antimicrobial properties are promising, and the fact that they are cultivable is a boon to large the production process. The antioxidant and anti-inflammatory activities of *Spirogyra aequinoctialis* are now under investigation, and research into the mechanism by which its natural components reduce infections is occurring. Our findings are consistent with the findings of the previously published studies (24, 25).

References:

1. **Dwaish, A.S.(2019).** Antibacterial and wound healing activities of acetone cladophora glomerata extract. Plant Archives, 2019, 19, pp. 1394–1399.
2. **Gonzalez, Del Val, A., Platas, G., and Basilio, A. (2001).** Screening of antimicrobial activities in red, green and brown *macroalgae* from Gran Canaria (Canary Islands, Spain). Int. Microbiol. 4: 35-40.
3. **Fayyad, R.J., Dwaish, A.S., Sulman, I.M.A., Lefta, S.N.(2022)** Phytochemical profiling of hot and cold alcoholic extract from Spirulina platensis alga and Comparison between two extracts against multidrug-resistant bacteria Research Journal of Pharmacy and Technology, 2022, 15(1), pp. 399–404
4. **Erturk, O. and Tas, B. (2011).** Antibacterial and Antifungal Eff ects of Some Marine Algae. Kafkas Univ. Vet. Kaf Derg17 (Suppl A): S121-S124.
5. **Yi, Z., Yin-Shan., C., and Hai-Sheng, L. (2001).** Screening for antibacterial and antifungal activities in some marine algae from the Fujian coast of China with three different solvents. Chin J Oceanol Limnol, 19:327–331.
6. **Dwaish, A. S., Yousif, D. Y., & Lefta, S. N. (2016).** Use of Spirogyra sp. extract against multi drug resistant bacterial pathogens. International Journal of Advanced Research, 4(7), 575-579.
7. **Kandhasamy, M., and Arunachalam, K.D. (2008).** Evaluation of *in vitro* antibacterial property of seaweeds of southeast coast of India. Afr. J. Biotechnol., 7(12): 1958-

1961.

8. **Bhagavathy, S. Sumathi, P. and Jancy Sherene Bell, I. (2011).** Green algae *Chlorococcum humicola*-a new source of bioactive compounds with antimicrobial activity. Asian Pacific Journal of Tropical Biomedicine (2011): S1-S7.
9. **Thinakaran, T., and K., Sivakumar. (2013).** Antifungal activity of certain seaweeds from puthumadam coas. IJRRPAS, 2013, June, 3(3)341-350.
10. **Saritha, K., Aswathi Elizabeth Mani, M., Priyalaxmi, and Jamila Patterson. (2013).** Antibacterial Activity and Biochemical Constit Global Journal of Pharmacology 7 (3): 276-282.
11. **Saidani, B.F., Benabdesselam, F. and Touati, N. (2012).** Antifungal activity of methanolic extracts of four Algerian marine algae species. African Journal of Biotechnology 11(39): 9496-9500.
12. **Fareed, M.F., and Khairy, H.M. (2008).** *In vitro* Antimicrobial Activities of Seaweeds Collected from Abu-Qir Bay Alexandria, Egypt. World Appl. Sci. J. 5(4): 389-396.
13. **Osman, M.E.H., A.M., Abushady, and M.E., Elshobary. (2010).** In *vitro* screening of antimicrobial activity of extracts of some macroalgae collected from Abu-Qir bay Alexandria, Egypt. African Journal of Biotechnology 9(12):7203-7208.
14. **Abbott, I.A. and Hollenberg, G. J. (1976)**. Marine algae of California. pp. [i]-xii, 1-827, 701 figs. Stanford, California: Stanford University Press.
15. **Siham. N and Ahmed. S. Dwaish (2014).** USE OF CLADOPHORA GLOMERATA EXTRACT AGAINST MULTIDRUG RESISTANT BACTERIAL PATHOGENS. W. J. of Pharm. Research. , Vol 3, Issue10.
16. **Harbone, J.B. (1973).** phytochemical methods. Chapman and Hall. London.
17. **Harbone, J. B. (1984).** Phytochemical Methods. A guide to Modern Techniques of Plant Analysis. 2nd. ed., Chapman and Hall. London. 288.
18. **Gibson, R.; Hextall, B. and Rogers, A. (2001).**Photographic Guide to the Sea and Shore Life of Brit-

ain and North-west Europe. Oxford University Press, Oxford.

19. **Fish, J.D. and Fish, S. (1989).** A student's guide to the Seashore. Unwin Hyman Ltd, London.
20. **USEPA (2005).** Draft Risk Assessment of the Potential Human Health Effects Associated with Exposure to Perfluorooctanoic Acid and Its Salts. Office of Pollution Prevention and Toxics. January 4, 2005.
21. **Dwaish, A.S.(2018).** Evaluation of antibacterial activity and qualitative phytochemical analysis of enteromorpha ralfsii. Indian Journal of Public Health Research and Development, 2018, 9(10), pp. 886–890.
22. **Manivannan, K., Karthikai, Devi, G., Anantharaman, P. and Balasubramanian, T. (2011).** Antimicrobial potential of selected brown seaweeds from Vedalai coastal waters, Gulf of Mannar. Asian Pacific Journal of Tropical Biomedicine , 114-120.
23. **AKarm I F , A. S. Dwaish, O. A. Dakhil (2021)** Investigation of Some Ecological Factors and Isolation Techniques for Some Local Algae in Iraq.
Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 1059 – 1068.
24. **Blumer, M., Guillard, R. R. L., & Chase, T. (1971).** Hydrocarbons of marine phytoplankton. Marine Biology, 8(3), 183-189.
25. **Youngblood, W. W., Blumer, M., Guillard, R. L., & Fiore, F. (1971).** Saturated and unsaturated hydrocarbons in marine benthic algae. Marine Biology, 8(3), 190-201.