Al-Kitab Journal for Pure Sciences (2023); 7(1):100-113.

https://doi.org/10.32441/kjps.07.01.p9

Al-Kitab Journal for Pure Sciences
ISSN: 2617-1260 (print), 2617-8141(online)
www.kjps.isnra.org

Active and phenolic compounds in Spirogyra sp. PDNA1 is an antibiotic for some bacteria and fungi

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Citation: Mohammed D. H., Al-Katib M. A. Active and phenolic compounds in Spirogyra sp. PDNA1 is an antibiotic for some bacteria and fungi. *Al-Kitab Journal for Pure Sciences* (2023); 7(1): 100-113. DOI: https://doi.org/10.32441/kjps.07.01.p9.

Keywords: Algal Antibiotics, Spirogyra sp. Phenols, Algal extract active compounds.

Article History

Received	02 May	2023
Accepted	15 July	2023
Available online	25 Aug.	2023

Abstract:

Green algae are a biological source rich in phenolic compounds and potentially inhibit the growth of microorganisms. Spirogyra sp. PDNA1 is one of the most types of green algae found in freshwater. Because of the increasing resistance of most bacteria and fungi to available antibiotics, a continuous search is required for the most effective, economical, and environmentally friendly alternatives. There are 30 compounds were identified, including alkaloids, phenols, and esters, and the highest percentage was oleic acid, with a retention time of 21.949 min and a concentration of 32.89%. The highest percentage of inhibition showed on the bacteria for the methanolic algal extract was against Salmonella typhi (22.5 mm), while the lowest percentage of it was against Bacillus cereus (10 mm). The hexane extract had the highest inhibition percentage against Salmonella typhi (19.5 mm) and the lowest inhibition percentage against Klebsiella pneumoniae (11 mm). It was also noted that the effect of the methanolic extract was highest against Trichoderma asperallum (22 mm) and the lowest percentage of inhibition against Candida albicans (7 mm), while the hexane extract recorded the highest percentage of inhibition against Candida albicans (15 mm) and the lowest percentage of inhibition was against the fungus Aspergillus Niger with inhibition diameter (8 mm). Phenols

were identified by HPLC technology. The phenolic compounds included Rutin, Gallic acid, Tannic acid, Quercetin, and Kaempferol, where the highest percentage of Rutin was in the phenolic methanolic extract (240.99) ppm, Kaempferol (7.2124) ppm, while the phenolic hexane extract had the highest percentage of Rutin (19.606) ppm, Kaempferol (10.997) ppm. The phenols showed the highest inhibition rate of the phenolic-methanolic extract against (Klebsiella pneumoniae) (30) mm and the least inhibition against (Escherichia. coli) (11) mm while the phenolic hexane extract has the highest inhibition to (Salmonella typhi) (27) mm and the lowest effect was against (Escherichia coli) (10) mm. The antifungal effect of the phenolic methanolic extract recorded the highest percentage against (Candida albicans) (30) mm and had the lowest effect on Mucor racemosus (18) mm, while the phenolic hexane extract had the highest effect with Candida albicans (22.5) mm, and the least inhibition percentage was in Mucor racemosus with (11) mm. Therefore, the study aimed to isolate and identify the effective compounds of the methanolic and hexanoic extract of this algae, and active phenolic compounds were detected using GC-MS and HPLC technology.

Keywords: Algal Antibiotics, Spirogyra sp. Phenols, Algal extract active compound.

المركبات الفعالة والفينولية في طحلب *Spirogyra* sp. PDNA1 كمضاد حيوي لبعض البكتريا والفطريات

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تعد الطحالب الخضراء مصدرًا بيولوجيًا غنيًا بالمركبات الفينولية ولها دور محتمل في تثبيط نمو الكائنات الحية الدقيقة مثل البكتيريا والفطريات المسببة للأمراض للإنسان. أن طحلب Spirogyra sp. PDNA1 هو أحد أكثر أنواع الطحالب الخضراء الموجودة في المياه العذبة، بسبب المقاومة المتزايدة لمعظم البكتيريا والفطريات للمضادات الحيوية المتاحة والشائعة، يتوجب البحث المستمر عن البدائل الأكثر فعالية واقتصادية وصديقة للبيئة. تم تحديد ٣٠ مركبًا ، بما في ذلك القلويدات والفينولات والإسترات في مستخلصات الطحلب، وكانت أعلى نسبة هي لحامض الأوليك ، مع زمن احتباس ٢١,٩٤٩ دقيقة وبتركيز ٣٢,٨٩٪. كانت أعلى نسبة تثبيط لمستخلص الطحالب الميثانولي ضد Salmonella typhi بقطر (٢٢,٥ ملم) ، بينما وكانت أقل نسبة له ضد بكتيريا Bacillus cereus (١٠ ملم) أما بالنسبة لمستخلص الهكسان فقد كان له أعلى نسبة تثبيط ضد Salmonella typhi بقطر تثبيط (١٩,٥ ملم) وأقل نسبة تثبيط ضد Klebsiella pneumoniae بقطر تثبيط (١١ ملم). كما لوحظ أن تأثير المستخلص الميثانولي ضد الفطريات كان أعلى ضد فطر Trichoderma asperallum بقطر (٢٢ ملم) وأقل نسبة تثبيط ضد Candida albicans بقطر (٧ ملم). بينما سجل المستخلص الهكساني أعلى نسبة تثبيط لفطر Candida albicans بقطر تثبيط (١٥ ملم) وأقل نسبة تثبيط كانت ضد فطر **A.** Niger بقطر تثبيط

(٨ ملم). علاوة على ذلك ، تم عزل الفينولات وتشخيصها بواسطة تقنية HPLC. اشتملت المركبات الفينولية على Rutin, Gallic acid, Tannic acid, Quercetin, Kaempferol ، حيث كانت أعلى نسبة Rutin في مستخلص الفينول الميثانولي (٢٤٠,٩٩ ppm) Kaempferol (٧,٢١٢٤ ppm)، بينما احتل مستخلص الفينول الهكسان أعلى نسبة من Kaempferol (١٠,٩٩٧ ppm) بالإضافة إلى ذلك ، أظهرت الفينولات أعلى معدل تثبيط Rutin (19.606) ppm للمستخلص الفينول الميثانولي كان ضد (Klebsiella pneumoniae) بقطر تثبيط (٣٠) ملم وأقل تثبيط ضد (Escherichia coli) مع قطر تثبيط (١١) ملم بينما أظهر مستخلص الهكسان الفينولي أعلى نسبة تثبيط لـ (Salmonella typhi) بقطر (٢٧) ملم وأقل تأثير كان ضد (Escherichia coli) بقطر (١٠) ملم. سجل تأثير المستخلص الفينولي الميثانولي أعلى نسبة ضد (Candida albicans) بقطر ٣٠ملم وأقل تأثير عـــــلى 18 ((Mucor racemosus ملم ، بينما كان لمستخلص الفينول الهكسان أعلى تأثير مع (Candida albicans) بقطر ٢٢,٥ ملم واقل نسبة تثبيط كانت في Mucor racemosus بقطر (١١) ملم. هدفت الدراسة إلى عزل وتحديد المركبات الفعالة للمستخلص الميثانولي والهكساني لهذا الطحلب والمركبات الفينولية التي تم الكشف عنها باستخدام تقنيتي GC-MS وHPLC.

الكلمات المفتاحية: المضادات الحيوية الطحلبية ، فينولات.*Spirogyra* sp ، المركبات الفعالة للمستخلص الطحلبي.

1. Introduction:

Algae are living, autotrophic organisms, and they represent a wide range of organisms that can carry out the process of photosynthesis, and they are widespread on the surface of the globe as they live in land and water areas [1], and they have recently found popularity in scientific research and various industrial fields because of their direct and indirect relationship in different areas of human life, as they are a source of many vital active compounds [2].

Algae produce various compounds such as alkaloids, carbohydrates, fatty acids, vitamins, enzymes, and proteins [3]. It is also a useful source of phenolic compounds. Although these compounds are usually associated with plants, algae are also a rich source of these compounds [4]. Phenols are used in various fields such as the agricultural, food, industrial, and pharmaceutical fields, they are anti-inflammatory and effective against the human immunodeficiency virus (HIV) [5].

Phenols are a class of organic chemical compounds that are structurally composed of a hydroxyl functional bond directly to an aromatic hydrocarbon. The name phenols are attributed to the simplest of these compounds, which is phenol C_6H_5OH. Phenols can be simple, or they can be multiple according to the number of phenol groups in the molecule. Phenols exist in nature in the form of several compounds, and they can also be obtained industrially [6].

Spirogyra alga

This alga classification is Division: Chlorophyta, Class: Charophyceae, Order: Zygnematales, Family: Zygnemataceae, Genus: *Spirogyra* [7, 8].

It is a genus of filamentous green algae consisting of thin, unbranched chains of cylindrical cells whose width ranges between (10-100) micrometers and whose length is greater than its width and may reach several centimeters. The cell wall is made of an inner layer of cellulose and an outer layer of pectin, which is responsible for the sticky texture of algae, so it is called water silk and can produce masses that float near the surface of streams and ponds supported by oxygen bubbles emitted in the process of photosynthesis **[9,10].**

2. Materials and Methods

2.1 Preparation of crud alga extract

These samples (*Spirogyra* sp.) of algae were obtained from environments in Nineveh governorate in Bartella sub-district / Shaquli village (East of Mosul city). They were obtained from a narrow stream estimated at (1.0 m) coming from the connection of a small spring of water (freshwater) to a spring of water smaller than Ein al-Awwal (sulfuric water) that pass through the village of Shaquli toward the village of Karmelos in the Hamdaniya district, where it is located near the mountain of Ain al-Safra. To the southwest, it is bounded by the Tigris River, and the Bartella district represents a raining area of 425 mm/year (The algae samples were collected then washed and dried in Oven at 40 C° to obtain algae dry weight (DW). The dry mass of the alga under study was used and crushed using a small ceramic mortar and an electric grinder. The dried alga powder was placed in a Whatman type 1 filter paper and arranged in such a way as to be a cellulose thimble (Soxhlet) **[11]**. Then, the transferring of the extract was done to the round flask (after emptying the separation column) of the rotary evaporator to reduce the volume of the solvent and reuse it again and transfer the resulting extract to a small airtight container for use in measurements and determination of the active compounds in it **[12]**. The methanolic algae extract was prepared in the same way with the change of the solvent (hexane) using methanol. The chemical constituents of *Spirogyra elongate*, successively extracted with petroleum ether, methylene chloride, chloroform, acetone, and methanol were determined by GC–MS. The extract percentage varied greatly between different solvents, with the highest one (4.83%) recorded for methanol **[13,14]**As well as, the highest yield of total phenolic compounds for *Spirogyra* was found when sonicated at 45 kHz with methanol **[15]**.

2.2 Identification of biologically active compounds using Gas Chromatography-Mass Spectrometry(GC-MS)

To identify the active compounds using GC-MS, the volume of the methanolic extract was reduced and the solvent was removed by a rotary evaporator device. The analysis of the

components of the methanolic extract was carried out in the central chromatography laboratory / College of Agriculture and Forestry / Food Research and Protection Laboratories Consumer - University of Basrah using gas chromatography connected to the mass spectrometer. The GC-MS type (QP 2010) supplied by a Japanese company, Shimadzu, contains a capillary separation column with dimensions (diameter 0.32, thickness of the static phase 0.25 micrometers, length 30 meters) in which high-purity helium gas was used as a directed gas with a flow rate of (1.69 ml/min) and the initial temperature was programmed in it from 50 to 280 ˚m. Samples were injected into it by direct injection at the top of the separation column through a plug The compounds were identified by comparing them with materials with known structure by comparing them with the database of known compounds in the GC-MS library and relying on the evidence of retention for each compound. Whereas the hexane extract was detected previously for the same sample of this alga in the study of **[16]**.

2.3 Acidolysis and isolation of phenols from crude hexane and methanolic extracts

Since phenols do not exist in a free form, but are linked by a glycosidic bond with sugar, so they are in the form of glycosides inside the plants, and to purify and characterize the phenols, the process of acid hydrolysis is carried out to break the glycosidic bonds and liberate the phenols from the sugar, depending on the method of the researcher Harborne (1973) and using 200 ml of hydrochloric acid (HCL) with a concentration of 2 molars as a solvent for 4 ml of the alcoholic extract of the algae under study as they were placed In a glass beaker, the mixture was heated in a rocking water bath at a temperature of 100-90 °C for half an hour, then it was left to cool at the laboratory temperature, then it was placed in a separating funnel and 200 ml of ethyl acetate was added to it in two stages. Ethyl for all algae species under study is placed on the bottom layer and shaken well in the separation funnel. Two layers are also formed and concentrated using a rotary vacuum evaporator to obtain the ethyl acetate extract and then kept in the refrigerator until use. The phenolic extract is separated from the hexane algae extract in the same way **[17].**

2.4 Identification of active phenolic compounds using high-performance liquid chromatography (HPLC)

The separation device used is High-Performance Liquid Chromatography (HPLC), which relies on the capillary and polar property, to separate the phenolic compounds separated from plants, as most of these compounds are characterized by being weakly acidic, that is ionizes under basic conditions and dissolves in polar solvents easily **[18,19]**. As the diagnostic process for these compounds was carried out in the HPLC Lab/Mosul the decomposition process for them using a high-performance liquid chromatography (HPLC) type. Shimadzu, Japan) where

the carrier phase was used: methanol: distilled water (75: 25) and the separation column was (C18 - ODS) with dimensions (25 cm * 4.6 mm) to separate phenols and the use of an ultraviolet detector: UV 280 nm, where the flow rate of the carrier phase was 1ml/min. The concentration of the unknown sample (ml/µg) was calculated = area of the sample \ area of the standard x concentration of the standard solution x volume of extract (ml) \ weight of the unknown sample (g) **[20]**.

2.5 Antibacterial activity of green algae extracts

The sensitivity method (diffusion in agar well) was used according to the method of **[21]** and to test the inhibitory effectiveness of the extracts used under study. Young colonies of pathogenic bacteria were transferred to the nutrient broth medium and incubated at a temperature of 37 °C for 24 hours. The diluted bacterial suspension was spread on the nutrient agar medium in a homogeneous manner using a sterile cotton swab. The Agar Wells Diffusion Method was adopted to study the effect of the extracts on the bacteria, as holes were made in the nutrient agar using a stainless still pourer with a diameter of 6 mm and the extracts were added at a volume of 50 microliters to each hole.

2.6 Antifungal activity of green algae extracts

The Well Diffusion Method **[22]** was prepared. Potato dextrose agar (PDA) fungus development medium was prepared under sterile conditions, poured into Petri dishes, then inoculated with pathogenic fungi with a diameter of 6 mm, and extracts were added at a volume of 50 microliters to each well.

3. Results and Discussion

The results of the GC-MS technique for the methanolic extract of *Spirogyra* alga showed the active compounds, where 30 compounds were identified including alkaloids, phenols, and esters, and the highest percentage was for the compound Oleic acid for a retention time of 21.494 minutes and a concentration of 32.89%, as in Table (1) and Figure (1,2). One of the reasons for inhibition with algae extracts is that they contain steroidal fatty and protein compounds that have an inhibitory effect on bacteria. Also, pigments and their derivatives such as chlorophyll and carotene have activity against bacteria, and fatty acids have antibacterial activity against positive and negative bacteria. The fatty acids alone or in combination have either a Bacteriostatic or a Bactericidal effect **[23]**. The terpenes, Neophytadiene and Phytol, were present in methanolic extracts of the (*Spirogyra longata*). Both phytocompounds were detected in several plants and some microalgae **[24]**.The phytocompounds constituents of two species of Rhodophyta, *Liagora divaricata* and *Trematocarpus flabellatus* methanol solvent.

GC-MS analysis allowed the identification of 42 phytocompounds from methanolic extracts of the red seaweed *L. divaricate* and *T. flabellatus*. Diverse groups of secondary metabolites were found within the phytocompounds, such as sterols (Cholesterol and Desmosterol), fatty acids (Hexadecanoic acid methyl ester and n-Hexadecanoic acid), and terpenes (Neophytadiene and Phytol). **[13]**.Neophytadiene was identified as strong bactericidal, antifungal, antipyretic, analgesic, antioxidant, and vermifugic **[24]**.

Table 1: the active compounds identified in the *Spirogyra* methanolic extract by the GC-MS technique.

Peak#	R. Time	Area	Area%	Name
1	3.771	58514	0.41	L-(-)-Fucose, tetrakis(trifluoroacetate), benzyl oxime (isomer 2)
2	4.099	601515	4.23	o-Xylene
3	18.566	64050	0.45	Phthalic acid, isobutyl 2-(2-methoxyethyl)hexyl ester
4	18.648	625070	4.39	Neophytadiene
5	18.902	112434	0.79	Neophytadiene
6	19.103	148093	1.04	1-Octadecyne
7	19.452	953219	6.70	7,10,13-Hexadecatrienoic acid, (Z,Z,Z)-
8	19.506	371645	2.61	E-11-Tetradecenoic acid
9	19.764	3395961	23.86	n-Hexadecenoic acid
10	20.741	152538	1.07	Arachidonic acid
11	21.075	95758	0.67	8-Tetradecyn-1-ol acetate
12	21.114	106892	0.75	trans-1,4-Cyclohexanedimethanol, bis(heptafluorobutyrate)
13	21.162	126900	0.89	Hexadecane, 1,1-bis(dodecyloxy)-
14	21.347	568436	3.99	Phytol
15	21.494	4681401	32.89	Oleic Acid
16	21.731	321098	2.26	Octadecanoic acid
17	22.000	101307	0.71	Hexacontanoic acid
18	22.214	90455	0.64	Pentadecafluorooctanoic acid, tetradecyl ester
19	22.456	88952	0.63	Tributyl acetylcitrate
20	22.866	126024	0.89	1-Hexadecanaminium, N,N,N-trimethyl-, octadecanoate
21	23.275	149753	1.05	Glycerol 1-palmitate
22	23.387	228539	1.61	9-Amino-1-methyl-3,6-diazahomoadamantane
23	23.550	326246	2.29	9-octadecenoic acid, 2,2,2-trifluoroethyl ester
24	23.617	144082	1.01	Tetratriacontyl pentafluoropropionate
25	23.933	105062	0.74	Diethyl docosanedioate
26	24.414	48568	0.34	Hexacontanoic acid, propyl ester
27	24.642	79442	0.56	Octaethylene glycol monododecyl ether
28	25.483	149768	1.05	Octatriacontyl pentafluoropropionate
29	25.617	44575	0.31	Hexadecane, 1,1-bis(dodecyloxy)-
30	25.704	165224	1.16	
31		14231521	100.00	

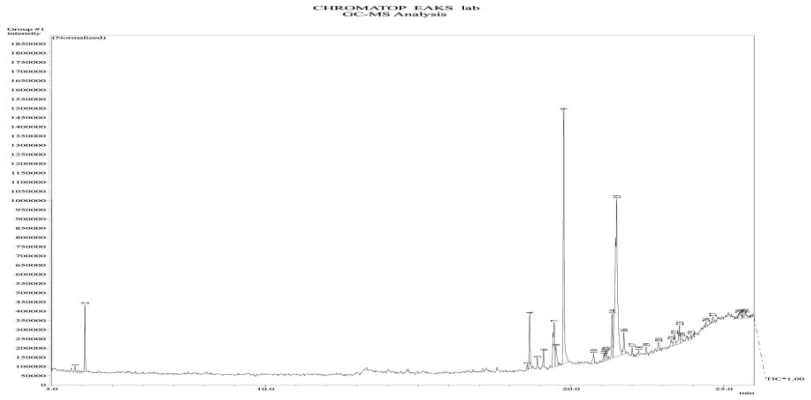
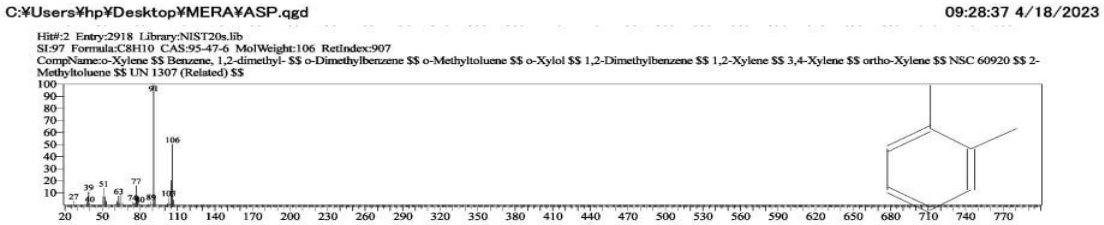
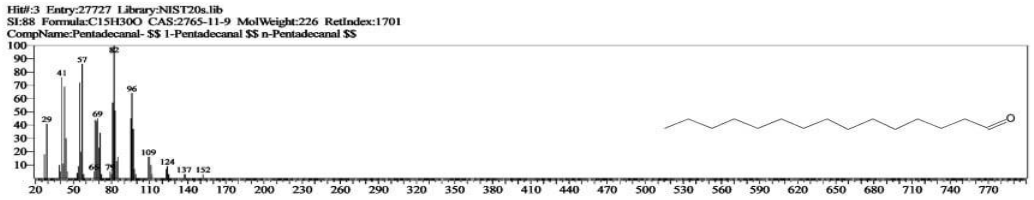


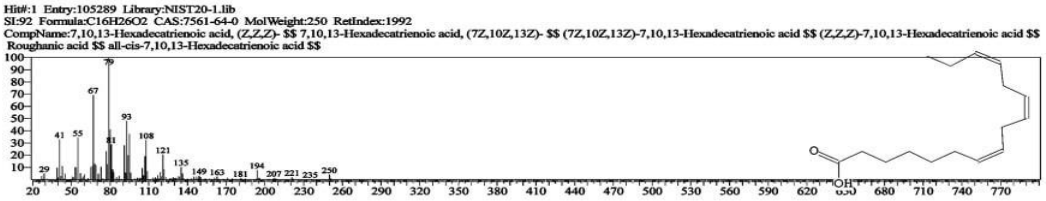
Figure 1: Active compound bands identified in the *Spirogyra* methanolic extract.



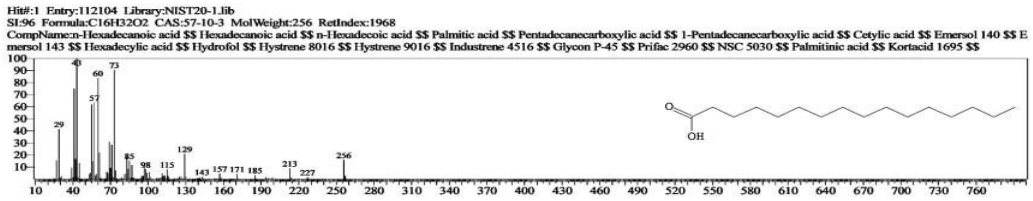
o-Xylene



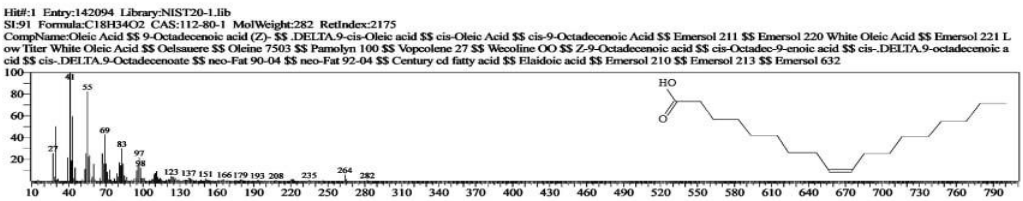
Neophytadiene



7,10,13-Hexadecatrienoic acid, (Z,Z,Z)-



n-Hexadecanoic acid



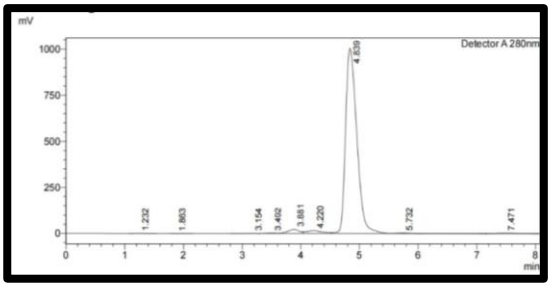
Oleic Acid

Figure 2: Structural formulas and bands of the active compounds identified in the methanolic extract.

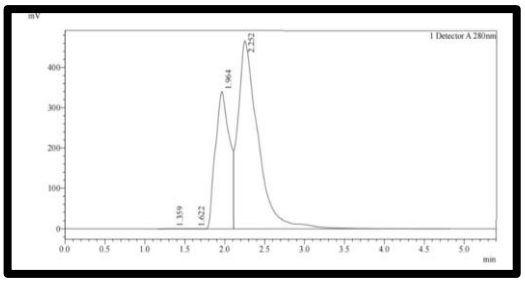
An HPLC analysis was conducted for the phenolic extracts after they were isolated from the crude extracts of alga, where the results showed the presence of phenolic compounds: Rutin, Gallic acid, Tannic acid, Quercetin, Kaempferol, where the highest percentage of Rutin was in the phenolic methanolic extract (240.99ppm), Kaempferol (7.2124ppm), while the phenolic hexane extract had the highest percentage of Rutin (19.606ppm), Kaempferol (10.997ppm) Table (2) and **Figure 3**.

Table 2: The phenolic compounds identified in *Spirogyra* alga by the HPLC technique.

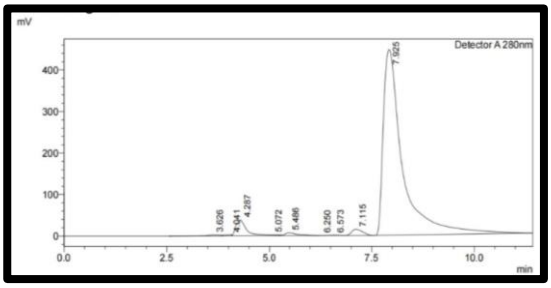
No.	Stan. Phenols	Rt. Time (min)	Methanol Extract Phenols Rt. Time (min)	Hexane Extract Phenols Rt. Time (min)
1	Quercetin	4.839	10.150	2.3317
2	L- Rutin	1.964	-	17.736
3	Rutin- R	2.252	240.99	19.606
4	Kaempferol	7.925	7.2124	10.997
5	Titanic acid_L	2.906	61.213	-
6	Tanic acid_R	3.601	-	-
7	Gallic acid	3.561	-	-



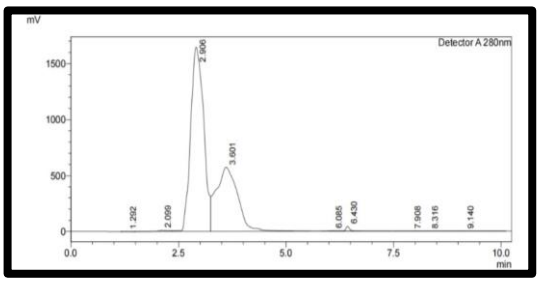
Quercetin



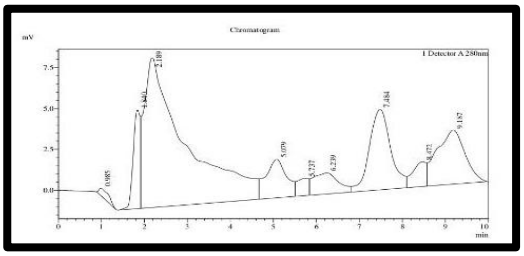
Rutin



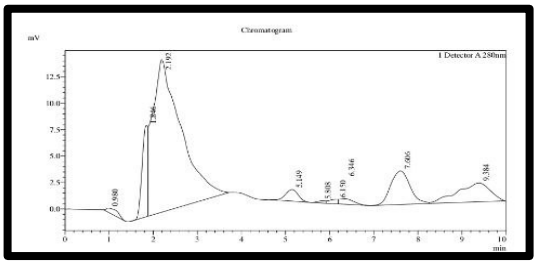
Kaempferol



Tanic acid



Phenol hexane *Spirogyra* extract



Phenol methanolic *Spirogyra* extract

Figure 3:The HPLC Data for alga phenolic compounds

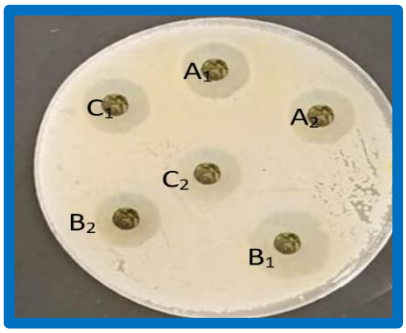
The effectiveness of the methanolic and hexane extracts of algae against bacteria was studied, where the highest percentage of inhibition on bacteria was for the methanolic extract of algae against *Salmonella typhi* with a diameter of inhibition (23 mm) and the lowest percentage of inhibition was against *Pseudomonas aeruginosa* with a diameter of inhibition (9 mm). The hexane extract had the highest percentage of inhibition against *Salmonella typhi* with a diameter of inhibition (20 mm), and the lowest percentage of inhibition against Pseudomonas aeruginosa with a diameter of inhibition (8 mm). As for the effect of methanolic extract against fungi, it had the highest inhibition against *Trichoderma asperallum* with a diameter of inhibition (20 mm) and the lowest percentage of inhibition against *Mucor racemosu* with a diameter of inhibition (10 mm). The effect of the hexane extract of moss against fungi showed the highest inhibition rate against the fungus *Trichoderma asperallum* with a diameter of inhibition (18 mm) and the lowest inhibition was against the fungus *Candida albicans* with a diameter of inhibition (10 mm), as shown in Figure (4) and (5) the effect of the phenolic and hexane extract on bacteria and fungi.

Salmonella typhi | Bacillus cereus | Staphylococcus aureus

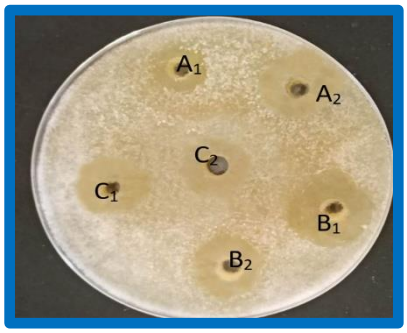
Escherichia coli | Klebsiella pneumoniae | Bacillus coagulase

A_1= Methanol *Chara* Extract(for thesis), B_1= Methanol *Spirogyra* Extract, C_1= Methanol *Cos Marium* Extract(for thesis)

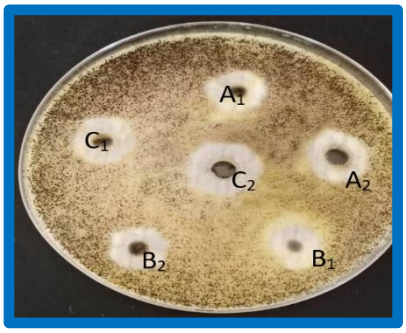
Figure (4) Effect of phenolic and hexane extract on bacteria



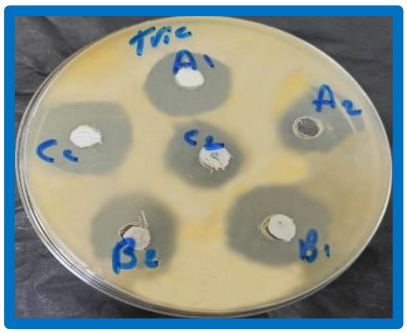
Candida albicans



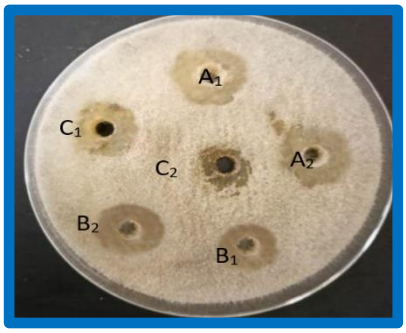
Trichoderma asperallum



Aspergillus Niger



Trichoderma harzianum



Mucor racemosus

$A_{1=}$ Hexane *Chara* Extract(for thesis), $B_{1=}$ Hexane *Spirogyra* Extract, $C_{1=}$ Hexane *Cosmarium* Extract(for thesis)

Figure 5: Effect of phenolic and hexane extract on fungi

Then, the effect of the phenolic-methanolic and hexane extracts on the bacteria and fungi used in the study, where the results showed that the highest inhibition rate of the phenolic-methanolic extract was against bacteria (*Klebsiella pneumoniae*) with a diameter of (21) mm., *Salmonella typhi* with a diameter of (14) mm, and the least inhibition percentage was against (*Pseudomonas aeruginosa*) with a diameter of (12) mm. The effect of phenol on the fungi used in the study was recorded by the phenolic methanolic extract, the highest percentage of inhibition against (*Candida albicans*) was with a diameter of (26) mm, and the least inhibition was against the fungus (*Aspergillus niger*) with a diameter of (20) mm, and the phenolic hexane extract had the highest inhibition against (*Candida albicans*) fungus, with a diameter of (26) mm, and the lowest inhibition rate was in (*Aspergillus niger*), with a diameter of (18) mm. The Inhibition diameters higher than the crude extract of algae indicated that it affected its pure isolated form better than it was mixed with the rest of the materials and components, which reduces its effect and concentration in the concentration used against bacteria, and this result was consistent with a study [25].

4. Conclusions

The quantitative and qualitative diagnosis of some different phenolic compounds and other biologically active compounds in the extracts of the crude methanolic *Spirogyra* sp. was carried out using the GC-MS technique. The study also succeeded in isolating phenols from the crude methanolic and hexane extracts of the alga. In addition to the identification of the isolated phenols by HPLC technique. Including fatty acids such as oleic acid in the crude methanolic extract, and phenols, the predominant of which is Rutin in the phenolic extracts.

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