Isolation and Identification of Active Substances from Brown Algae and Study of their Biological Activity against Pathogenic Fungi

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Abstract

The present study included the isolation and characterization of chemical compounds from the two alga Sargassum sp. and Laminaria sp. and the qualitative detection of the active compounds, the extracts of algae (hot water and ethanolic at a concentration of 80%) were banned using the Soxhlet device.
The primary detection showed the presence of secondary metabolites represented by phenols, flavonoids, tannins, glycosides, steroids and amino acids. The results of the gas chromatography-mass spectrometry detection revealed the presence of many active compounds, and the highest percentage among the active substances was for the compounds (n-Hexadecanoic acid and Oleic acid and Fucosterol) in both algae. The biological activity of the two algae extracts against fungi , Aspergillus niger , Candida albicans and Candida krusei was studied.
The hot aqueous extract of the two algae did not show any activity against the studied fungi, while the alcoholic extract of Sargassum algae showed biological activity as the inhibition diameters were (21.76, 22.00 and 20.76) against A.niger, C.albicans and C.krusei respectively, while The diameters of inhibition of the alcoholic extract of Laminaria moss were (20.90, 21.53, and 20.00) against A.niger, C.albicans , and C.krusei respectively.
Keywords: *Antifungal, GC-mass, Laminaria, Sargassum, bioactive compounds.*

INTRODUCTION

Fungi are eukaryotic organisms, as there are approximately (50,000) types of fungi in nature, as about (80) types of molds and yeasts have the ability to cause various diseases for humans and animals alike, and diseases caused by fungi are known as (Mycosis) The infection is usually chronic because the fungus grows slowly and includes superficial, systemic and opportunistic infections that usually affect people who are immunocompromised (1) . The genus Candida is one of the natural flora of the body, as it is found naturally in the oral cavity, in addition to its presence in the vagina and respiratory tract (2). However, when an imbalance occurs in the microbial balance due to a change in conditions, the candida grows in excess of its normal limit, which causes human diseases known as Candiaiasis, and this term is applied to a fungal infection of any kind of the genus Candida (3)

Aspergillus fungus is one of the pathogens of opportunistic fungal diseases as a result of its spread in the air, soil and various surfaces, as humans inhale the spores of this genus continuously, and the pathogenicity of the fungus depends on the amount of spores entering the body, as the infections range from mild to causing acute and chronic pulmonary diseases, in addition to The immune status of a person, and the chances of infection increase in people who suffer from weak immunity and

chronic diseases, and who take immunosuppressive drugs, and A. flavus , and A. nigar are among the most pathogenic types of this genus (4) . One of the main reasons for using algae extracts as antimicrobials is their natural origin, as they have minimal harmful side effects on humans and animals and have less environmental risks compared to synthetic alternatives (5) . The increase in microbial resistance to common antibiotics that occurred as a result of excessive and indiscriminate use of them forced researchers to study new resources for antibiotics from different sources, as marine organisms, including algae, are a rich source of bioactive compounds, as studies showed the isolation of more than (15000) natural products Navy in the time period (1965-2005) (6) .

Aim of study :

The aim of the study was to isolate and identify the active substances from the two algae and to know their biological activity against pathogenic fungi.

Materials and Methods:

-Samples Collection

The algae sample was obtained in the form of a powder from the American Amazon company. The solvents (ethanol at a concentration of 80% and hot water 100%) were used ,Diagnosed pathogenic fungal isolates were obtained from Al-Ameen Center for Research and Advanced Biotechnology.

-prepevation of brown alga extracts

The hot extraction was carried out using a Soxhlet extractor at a temperature of (70) °C for the ethanolic extract. And a temperature of (100) C ^{o} for the aqueous extract for a period of (24) hours.

-Gas chromatograph mass spectrometry

Identification of some chemical compounds in ethanolic extracts of Sargassum sp. By (Gc-mass) of the Basra Oil Company / Department of Laboratory and Quality Control / Nahran Omar site.

-Antifungal activity assay

A.niger , C.albicans , and C.krusei were used in the experiment. Culture media (potatoes and saproids) were used. As the concentrations of extracts were prepared (100 , 200 , 300) mg / ml. The antifungal test was carried out using the diffusion method by digging according to the method (7). Inhibition zones in all directions of the pits were calculated by ruler in millimeters. Experiments were repeated three times and the mean zones of inhibition were taken.

-Statistical analysis

The data were analyzed statistically according to the randomized complete block design (CRBD) with three replications using (Tow-Way-ANOVA) and using the statistical program (Spss ver 23) and the Least significant differences (L.S.D) test was used to compare the averages under the probability level $P \leqslant 0.05$

Results and discussion

The qualitative detection results recorded in Table [1] showed that the aqueous extract of Sargassum sp. and Laminaria sp. On secondary metabolites, flavonoids, phenols, saponins, terpenoids, carotenoids, and glycosides.

Table [1] Phytochemicals analysis of Sargassum sp. extract

Phytochemicals	Result	
	Sargassum sp.	***Laminaria sp.***
alkaloids	-	-
Flavonoids	+	+
Glycosides	+	+
Phenols	+	+
Terpenoids	+	+
Carotenoids	+	+
Saponins	+	-
Tannins	-	-

+ = Present ; - = Obsent

This result agreed with)8). and conducted on the brown moss Sargassum crassiflium and agreed with (9) Made on brown moss Lmainaria sp. The results presented in Table [2] revealed the identified compounds through gas chromatography - mass spectrometry of the ethanolic extract of Sargassum sp. The presence of a number of active chemical compounds . . The compounds occupied the largest area among the diagnosed compounds are, n-Hexadecanoic acid that took up space (125061416) It is a carboxylic acid and has antifungal activities, as indicated by a study (10). It is possible to attribute the counter activity to it as it occupies the largest part of the total area . secondly the compound was Oleic acid, which took up an area (44086904), which is an unsaturated fatty acid and has antioxidant and antifungal activity, according to the study of (11). As for the compound Fucosterol, which occupied an area of (31488409) , it is one of the steroid compounds and has antifungal activities, as indicated by(12). The results presented in Table [3] showed that identified compounds through gas chromatography - mass spectrometry of the ethanolic extract of the seaweed Laminaria sp. The presence of a number of active chemical compounds. The compounds occupied the largest area among the diagnosed compounds are oleic acid, which occupied an area (32340413) and is an unsaturated fatty acid and has an antifungal role as indicated by (11). secondly the compound was n-Hexadecanoic acid, which took up an area (29636592) and this compound has antifungal activity according to the study of (10). As for the compound Fucosterol, which is one of the steroidal compounds, as it occupies an area (17261997) and has an effective role in antifungal activity, according to the study of (12).

Table [2] Phytochemicals identified by the GC-mass of Sargassum sp. algae extract Biological activity

Compound name	**Molecular formula**	**M W g/mol**	**R T**	**Area**
Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37	17.996	22833127
Palmitoleic acid	$C_{16}H_{30}O_2$	254.41	19.765	10055719
n-Hexadecanoic acid	$C_{16}H_{32}0_2$	256.42	20.106	125061416
Pentane,2-(1-methylethyl)thio]	$C_8H_{18}S$	146.30	21.322	11893569
Oleic acid	$C_{18}H_{34}O_2$	282.468	21.619	44086904
Fucosterol	$C_{29}H_{48}O$	412.7	30.072	31488409
Cholesta-5,20,24-trien-3-ol, (3.beta.)-	$C_{27}H_{42}O$	382.6	31.424	25187468

Table [3] Phytochemicals identified by the Gc-mass of ethalonic Laminaria sp. algae extract

compound name	**molecular formula**	**M W g/mol**	**detention time**	**Area**
Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37	17.941	7970514
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	19.988	29636592
D-Mannitol	$C_6H_{14}O_6$	182.17	20.895	9403804
Oleic Acid	$C_{18}H_{34}O_2$	282.5	21.607	32340413
Cyclohexane, 1,5-diethenyl-3-methyl-2-methylene-, (1.alpha.,3.alpha.,5.alpha.)-	$C_{12}H_{18}$	162.27	23.029	11452453
Fucosterol	$C_{29}H_{48}O$	412.7	30.06	17261997

The results showed the hot aqueous extract of this alga did not show any activity against the studied fungi in the collection of the used concentrations. This result is consistent with the findings of (13) in his study on the brown alga Sargassum denticulatum. It is possible that the ineffectiveness of the aqueous extract against fungi is due to the fact that all the

identified substances are insoluble in water (14). The results recorded in Table [4] showed the difference in the effectiveness of the ethanolic extract of Sargassum sp. against the types of fungi used in the study. As the concentrations were used (100, 200, 300) mg / ml . There is a correlation between the used concentrations algae and the average inhibition diameters of the studied fungi. The higher the concentration, increased the inhibition zone.

Table [4] Antifungal activity of Sargassum sp. against the types of fungi used in the study

Fungal species	concentration mg/mL			Mean
	100	200	300	
A.niger	17.30 ± 0.57	22.00 ± 1.00	26.00 ± 0.57	21.76
C.albicans	19.00±1.00	22.00± 1.00	25.00 ± 1.00	22.00
C.krusei	19.00±1.00	21.00 ± 1.00	22.30 ± 1.00	20.76
Mean	18.40	21.66	24.43	

L.S.D = 2.3 ($P \leqslant 0.05$)

These results are consistent with the findings of (15) in his study on the brown alga Sargassum polycystum against pathogenic species of the genus Candida spp.. It also agreed with the study of (16) which was conducted on the brown alga Sargassum sp. against A. .niger . There are many factors that affect the results reached by the researcher in testing the activity of algae extracts against fungal growth. As there is a difference in the results reached by researchers in studying the same type of algae. This is due to the difference in regions, harvest time and preservation of the samples used in the test. In addition to the stage of algae growth, the method of extraction and the type of solvent used in the extraction.

The results showed the hot aqueous extract from this alga did not show any activity against the studied fungi in the collection of the used concentrations. This result is consistent with the findings of (17). in his study on the brown alga Laminaria sp. Agients C.albicans and A.niger. It is possible that the ineffectiveness of the aqueous extract against fungi is due to the fact that all the identified substances are insoluble in water (14). The results recorded in the table [5] showed the difference in the effectiveness of the ethanolic extract of Laminaria sp. against the types of fungi used in the study. As the concentrations were used (100, 200, 300) mg / ml. There is a correlation between the used concentrations algae and the average inhibition diameters of the studied fungi. The higher the concentration, increased the inhibition zone.

Table. [5] Antifungi activity of Laminaria sp. extract

Fungal species	concentration mg/mL			Mean
	100	200	300	
A.niger	16.70 ± 1.15	21.70 ± 0.57	24.30 ± 1.00	20.90
C.albicans	19.00±1.00	21.60 ± 0.57	24.00 ± 1.00	21.53
C.krusei	18.00±1.00	20.00 ± 1.00	22.00 ± 1.00	20.00
Mean	17.90	21.10	23.43	

L.S.D = 2.1 ($p \leqslant 0.05$)

These results are consistent with the findings of (18). in his study on the Tow type of brown alga Gelidium sesquipedale and Laminaria ochroleuca against pathogenic species C.albicans , S.faecalis , S.oureus . It also agreed with the study of (19) which was conducted on the brown alga Laminaria ochroleuca against C. albicans , A.flavus , Cryptococcus spp . There are many factors that affect the results reached by the researcher in testing the activity of algae extracts against fungal growth. As there is a difference in the results reached by researchers in studying the same type of algae. This is due to the difference in regions, harvest time and preservation of the samples used in the test. In addition to the stage of algae growth, the method of extraction and the type of solvent used in the extraction.

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