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Study of Antifungi activity and bioactive Compounds of brown alga extract

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Abstract:
The objective of this study was to determine bioactive compounds and antifungi of extracts of *Laminaria sp*. against pathological fungi, which are *Aspergillusniger*, *Candida albicans* , and *Candida krusei*, as the effective compounds extracted from this algae solvents were used (hot water and ethanol alcohol) and the products of secondary metabolism from the aqueous extract were qualitatively detected, which indicates existence of chemical compounds such as flavonoids and phenols Glycosides, terpenes and carotenoids, Identified chemical compounds in the ethanolic extract of seaweed using gas chromatography-mass spectrometry (Gc-mass). These compounds were Oleic acid, n-Hexadecanoic acid , Fucosterol , Cyclohexane, 1,5-diethenyl-3-methyl-2-methylene-, (1.alpha.,3.alpha.,5.alpha.)- , Tetradecanoic acid and D-Mannitol. The aqueous extract of *Laminaria sp.* did not appear any biological activity versus all the studied fungal species. While the ethanolic extract was found to be more effective in its effect on pathogenic fungal species. The average zones of inhibition of the extract were (21.53, 21.46, 20.00) against *C.albicans* , *A.niger* and *C.krusei* , respectively.

Keywords: Bioactive compounds , *Laminaria* , antifungi , Gc-mass.

Introduction:

The misuse of antibiotics led to an increase in the resistance of fungi and bacteria to common drugs, The resistance of pathogens to antibiotics has forced researchers to develop new alternatives , This enabled algae to be used as natural alternatives to some antibiotics because it is a rich source of effective compounds (1). The nineteenth century witnessed a scientific trend aimed at studying many Clinical characteristics of secondary metabolites found at all Living organisms , especially algae, To take advantage of its natural products to treat many diseases, as the focus was on algae due to its diversity, vitality, and abundance in several places (2). Thus, interest in them has increased in the last four decades as a source of bioactive compounds, in addition to the increase in studies on their importance in the medical and pharmaceutical fields on the analogue of the use of medicinal plants rich in these compounds, as 35% of the compounds discovered from aquatic organisms during the period The period between(1977-1987) that has medical and pharmacological importance was from algae alone (3) .

Seaweed (Macro algae), including red and brown algae, is an important source of many biologically active compounds that are used in the manufacture of many medicines and drug development, as many of them have been used in the treatment of many diseases such as AIDS and bacterial and fungal infections (4). As the first use of algae in medicine was recorded to approximately (2700 BC), and it was used in the treatment of many malnutrition diseases and thyroid diseases, because it contains a high percentage of vitamins, proteins and other mineral elements, as well as its use in treating cough, fever and diarrhea (5). One of The main reason to use algae extracts as antimicrobials is their natural origin and therefore have fewer harmful side effects on humans and animals as pose fewer environmental risks than synthetic alternatives (6). 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 The Navy in the Time Period (1965-2005) (7)..

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 *Laminariasp*. By (Gc-mass) of the Basra Oil Company / Department of Laboratory and Quality Control / Nahran Omar site.

-Bioactivity test of extracts against pathogenic fungi

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 (8). 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 (Spssver 23) and the Least significant differences (L.S.D) test was used to compare the averages under the probability level $P \leq 0.05$

Results and discussion

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

Table [1]: Phytochemicals analysis of *Laminaria sp.* extract

Phytochemicals	Result
alkaloids	-
flavonoids	+
glycosides	+
phenols	+
terpenoids	+
saponins	-
carotenoids	+
tannins	-

+= Present ; - = Obsent

This result agreed with (9) Made on brown moss *Lmainaria sp. ,* The results presented in Table [2] showed that identified compounds through gas chromatography - mass spectrometry of the ethanolic extract of the seaweed *Laminariasp*. 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 (10). secondly the compound was n-Hexadecanoic acid, which took up an area (29636592) and this compound has antifungal activity according to the study of (11). 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 ofethalonic*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 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 (13). in his study on the brown alga *Laminaria sp*. *AgientsC.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 [3] 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. [3]: Antifungi activity of *Laminaria sp*. extract

Fungal species	**concentration mg/mL**			**Mean**
	100	200	300	
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
A.niger	16.70 ± 1.15	21.70 ± 0.57	26.00 ± 1.00	21.46
Mean	17.9	21.1	24.0	

L.S.D = 2.3 ($p \leq 0.05$)

These results are consistent with the findings of (15). in his study on the Tow type of brown alga *Gelidiumsesquipedale and Laminariaochroleuca*against pathogenic species *C.albicans , S.faecalis , S.oureus* . It also agreed with the study of (16) which was conducted on the brown alga *Laminariaochroleuca*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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