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Antibactrial and antifungal activity of some seaweeds species from Egypt

Israa S. Esmail[1], Mervat G. Hassan[1], Ahmed A. Hamed[2], Mohamed E. El awady[3] and Mohamed G. Battah[1]
[1]Botany and Microbiology Department, Faculty of Science, Benha University, Benha, Egypt
[2]Microbial Chemistry Department, National Research Centre, El-Buhouth Street, Dokki, Cairo, Egypt.
[3]Microbial Biotechnology Department, National Research Centre, El- Buhouth Street, Dokki, Cairo, Egypt.
E-Mail: israasamir2020@gmail.com

Abstract

Algae are rich in bioactive compounds that have potential use in the pharmaceutical, nutraceutical, and functional food industries. In this study, the antibacterial properties of crude extracts from isolated marine environments containing *Ulva lactuca* and *Cystosera myrica* algae were investigated. The physical traits of the algal strains reveal details about their taxonomic affiliation. Crude extracts were prepared using solvents such methanol, ethanol, and ethyl acetate, and their antibacterial qualities were evaluated against a range of bacteria and fungi. The ethanol fractions exhibited strong antibacterial activity against Gram-positive bacteria, particularly *Klebsiella* and *Staphylococcus aureus*, whereas the methanol fractions shown broad-spectrum antibacterial activity. *Ulva lactuca*'s ethyl acetate fraction was most effective against *Candida albicans*, whereas the methanol fractions demonstrated notable antifungal activity against *Aspergillus fumigatus*. The results show that *Ulva lactuca* and Cystosera myrica algae extract can be used as effective alternatives to traditional antibacterial treatments, with different solvents showing different levels of effectiveness against different bacteria.

Key words: *Cystosera myrica*, *Ulva*, bioactive natural products, antibacterial and antifungal

Introduction

Since ordinary antibiotics are becoming less efficient at treating bacterial illnesses, antibiotic resistance has become a serious threat to world health, according to the World Health Organization [1]. The treatment of many medical problems is made extremely difficult by this, especially when doing crucial surgeries like organ transplants, cesarean sections, and cancer therapies.

Thus, it is imperative to investigate alternate tactics in order to counteract this growing issue[2].

As innovative antimicrobial agents are sought after, natural compounds have surfaced as viable substitutes. They provide an abundance of bioactive chemicals with potential applications in illness prevention and treatment.

The abundant brown marine algae *Cystosera myrica*, which is found in the coral reefs of the Red Sea, is one of these natural sources that shows great potential. The bioactivity and chemical makeup of this alga are still poorly understood despite its widespread use[3], [4].

The tremendous variety and possible medicinal applications of natural chemicals derived from marine sources have led to an increased focus on their research in recent years. A valuable source of bioactive chemicals with a variety of pharmacological effects has been found in marine algae, namely. *C. myrica* is a highly attractive candidate for bioactivity screening due to its widespread distribution in the coral reefs of the Red Sea. Its distinct ecological niche and ability to withstand severe maritime settings point to the possibility of the presence of specific metabolites with potential antibacterial activity[5].

Moreover, given the present antibiotic resistance dilemma, the discovery of new antimicrobial drugs from marine sources is very important. Finding alternative therapeutic options is vital because many conventional antibiotics are no longer effective against harmful germs. Marine-derived natural products provide structurally varied molecules with distinct mechanisms of action against microbial pathogens, making them a prospective pathway for drug discovery[6], [7], and [8].

The objective of this research is to examine the antibacterial characteristics of the mthanol extract obtained from *C. myrica*, thereby illuminating its potential as a source of innovative antimicrobial agents. This research attempts to deepen our understanding of the biological characteristics of *Cystosera myrica* algae by exploring their distinctive qualities, such as their nutritional richness and possible medical uses.

Clarifying these algae's antibacterial properties also offers potential for their application in a range of commercial and medical contexts. This work advances our understanding of natural products research by providing a thorough investigation of the bioactivity and chemical profile of *Cystosera myrica* algae. This research highlights the significance of marine creatures in solving modern healthcare concerns and lays the road for the creation of novel antimicrobial medicines by revealing the therapeutic potential of these algae.

2. Materials and Methods

Obtaining of algae

In April, *Cystosera myrica* was taken from Ras Sudr, east of the Suez Gulf, at a depth of 3 m. Algal samples were cleaned using tap water in the lab and seawater in the field. To stop active

chemical decomposition, the dryness was kept at room temperature and out of direct sunshine.

Extraction of bioactive metabolites from isolated algae

The bioactive ingredients of the algae were extracted by weighing ten grams of precisely measured material after it had adequately dried. Methanol, ethanol, dichloromethane, and ethyl acetate were the three organic solvents employed in the extraction process. Selecting the appropriate solvent for the algae involved considering its distinct characteristics and trying to extract as many useful ingredients as possible.

During the extraction process, the dried algae were immersed in each solvent, allowing the bioactive element, , to dissolve into the solvent medium. To extract the bioactive components of the algae, 5 grams of precisely measured material were weighed after the algae had dried sufficiently. These three organic solvents—methanol, ethanol, dichloromethane, and ethyl acetate—were employed in the extraction process.

The process of selecting an appropriate solvent aimed to extract various useful components from the algae while considering its distinct characteristics. During the extraction process, the dried algae were immersed in each solvent, enabling the bioactive ingredient ds to dissolve into the solvent medium. [9].

Evaporation of crude extract

Following the extraction process, a flask with a round bottom was filled with the liquid crude extract. The flask holding the crude extract was then attached to the rotating evaporator device. The rotary evaporator is composed of a water bath, a spinning flask, a condenser, and a vacuum pump. The rotary evaporator's temperature and vacuum level were tuned to be ideal for the specific solvent used in the extraction procedure [10].

To encourage evaporation while preventing thermal destruction of the bioactive compounds, the water bath was heated to a temperature just below the boiling point of the solvent [11].The solvent in the crude extract in the flask with a circular bottom started to evaporate due to the vacuum pump's dropping pressure. The rotating flask, which exposed a larger surface area of the extract to the vacuum conditions, allowed for the effective evaporation of the crude extract. The volatile solvent consequently evaporated and condensed in the condenser, while the concentrated bioactive compounds remained in the flask with a circular bottom.

Close supervision was used during the evaporation process to prevent overheating and preserve the integrity and activity of the bioactive metabolites [12].After the solvent had completely evaporated, the concentrated crude extract with the bioactive ingredients was removed from the round-bottomed flask and then further analysis and characterization was performed.

Antimicrobial screening

The antibacterial effectiveness of the crude extracts from algae was evaluated by testing them against a range of test microorganisms, including some that were resistant to penicillin. The experiment was conducted using the microplate dilution method. To summarize, 180 µL of suitable culture conditions—potato-dextrose sauce for fungus and lysogeny sauce for bacteria—were mixed with 10 µL of various concentrations of fungal extract. Add 10 L of a bacterial or fungal solution after the logarithmic growth period. The microplates were tested for absorbance at OD600 using a Spectrostar Nano Microplate Reader after being incubated at 37 °C for the entire night [13].

3.Results

sample collection

Two strains of algae were separated and subjected to morphological study in order to determine their taxonomic identity and potential applications. While samples of *Ulva lactuca* were taken in February from intertidal waters in Suez Bay (north of Suez Gulf), samples of *Cystosera myrica* were taken in April from Ras Sudr (east of Suez Gulf) at a depth of three meters. Following their collection and field cleaning with saltwater, the algae samples were cleaned in the lab using fresh tap water. To preserve the active ingredient integrity, the samples were dried at ambient temperature without exposure to direct sunlight.

Morphological analysis indicates that every strain of algae has distinct features. *Cystosera myrica* was distinguished by its caespitose habit, smooth primary branches with square borders, and thickened walls [14]. It was also brown in color.*Ulva lactuca*, in contrast, was observed to be a thin, flat green algae that developed from discoid holdfasts. Its greatest measurements were 30 cm in width and 18 cm in length [15]. It's interesting to note that *Ulva lactuca* is composed of a thin, translucent membrane without a stipe that is two cells thick (Table 1).

These detailed anatomical analyses provide valuable insights into the range of strains of algae that are housed in the biotech unit. They also provide fresh directions for researching the biotechnological potential of these algae strains, including their potential for use in medications and the production of biofuel.

Table (1) algae sample collection

No	Algae name	Location	Depth (m)	Morphology
1	*Ulva lactuca*	Suez Bay (North of Suez Gulf)	Intertidal waters	thin, flat green algae growing from discoid holdfasts, with a maximum length and width of 18 cm and 30 cm
2	*Cystosera Myrica*	Ras Sudr (East of Suez Gulf)	3m	brown coloration, with caespitose habit and smooth primary branches featuring dentate edges

Table (2) Summary of Solvent Extraction Parameters

Solvent	Sample Amount (g)	SolventVolume (mL)	Extraction Time	Filtration	Centrifugation Speed (rpm)	Centrifugation Time (min)
Methanol	5	50	Overnight	Yes	6,000	15
Ethanol	5	50	Overnight	Yes	6,000	15
Ethyl Acetate	5	50	Overnight	Yes	6,000	15

Extraction of crude extract from different samples

At room temperature, three distinct solvents were made from powdered materials: ethyl acetate, methane, and ethanol. Five grams of the sample (powder) were dissolved in fifty milliliters of solvent to create each extract, which was then allowed to stand at room temperature on a rotary shaker for the whole night. After filtration, the mixture was centrifuged for 15 minutes at 6,000 rpm (Table 2).

Antimicrobial screening for the obtained extracts

Antibacterial activity

The antibacterial properties of the ethanol, methanol, and ethyl acetate fractions derived from Ulva lactuca and Cystosera myrica algae were assessed in relation to a range of microorganisms, such as Escherichia coli (E. coli), Klebsiella pneumoniae (Klebsiella), Proteus vulgaris (Proteus), and Staphylococcus aureus (Staph). To evaluate the efficacy of the algae fractions with conventional antibacterial treatments, a control group was also included.

The ethanol fraction of Ulva lactuca had the greatest antibacterial activity against E. coli (47.92%) and Klebsiella (69.55%), whereas Staph (58.77%) and Proteus (24.22%) showed only moderate activity. Comparatively, the Cystosera myrica ethanol fraction showed reduced activity against Klebsiella (22.01%) and Proteus (30.66%), but greater activity against E. coli (51.11%) and Staph (72.95%). According to these findings, both algae species' ethanol fractions have significant antibacterial qualities, especially against Gram-positive bacteria.

In relation to the methanol fractions, Ulva lactuca exhibited moderate to strong antibacterial activity against every microbe examined, with the greatest activity shown against E. coli (72.2%) and Klebsiella (70.61%). On the other hand, Cystosera myrica showed variable degrees of activity; there was limited or no action against Klebsiella and Proteus, but considerable inhibition against Staph (76.46%) and E. coli (73.64%) was noted. This suggests that the methanol fractions from both species of algae have broad-spectrum antibacterial activity, with Cystosera myrica showing particularly strong antimicrobial efficacy against Escherichia coli. With inhibitory percentages of 72.64% and 72.2%, respectively, the ethyl acetate fractions of Ulva lactuca and Cystosera myrica also showed noteworthy antibacterial activity, especially against E. coli. However, in comparison to the ethanol and methanol fractions, both fractions showed less activity against other tested bacteria. When compared to the control group, which consisted of standard antibacterial medications, the effectiveness of the algal fractions differed. While certain fractions exhibited lower antibacterial activity, others showed activity comparable to or greater than the control.

All things considered, our findings indicate that Ulva lactuca and Cystosera myrica algae fractions have potential as alternative antibacterial agents, particularly against Gram-positive and Gram-negative bacteria. The ethanol and methanol fractions exhibit the most promising results (Fig.1).

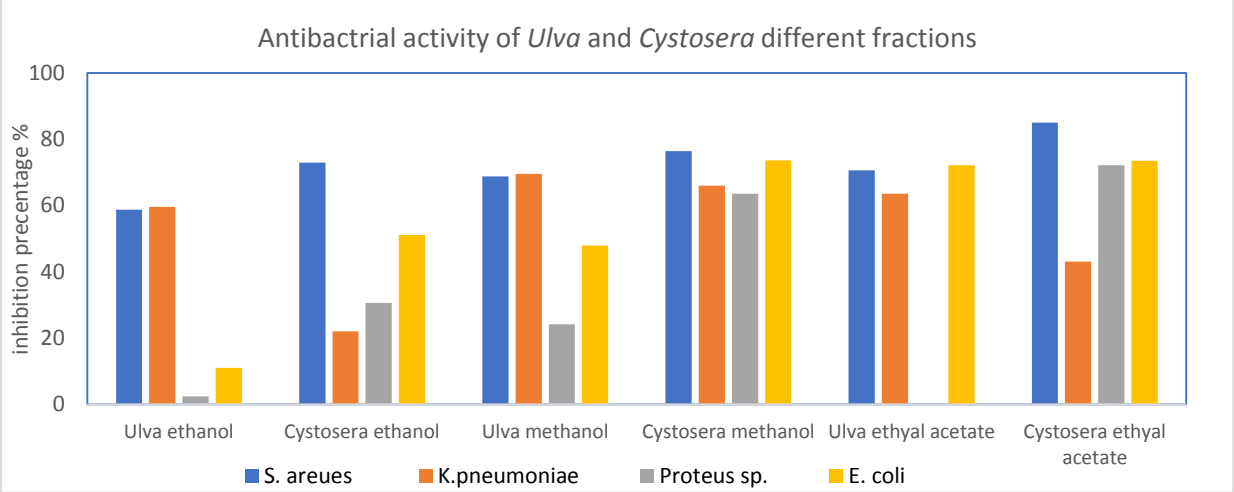

Fig. (1) Antibacterial activity of *Ulva* and *Cystosera* different fractions.

Antifungal activity

Aspergillus fumigatus (Aspergillus) and *Candida albicans (Candida)* are two common fungal infections that were investigated for the antifungal activity of ethanol, methanol, and ethyl acetate fractions from *Ulva lactuca* and *Cystosera myrica* algae. A control group was also included to evaluate the effectiveness of the algal fractions against popular antifungal medicines.

The ethanol fraction of *Ulva lactuca* exhibited noteworthy antifungal activity against *Aspergillus* (45.06%) and *Candida* (66.07%), suggesting that it may have broad-spectrum antifungal properties. Comparably, the *Cystosera myrica* ethanol fraction had much greater activity against *Aspergillus* (69.45%) and *Candida* (79.40%), indicating its potent antifungal characteristics.

In the methanol fractions, *Ulva lactuca* and *Cystosera myrica* both exhibited potent antifungal activity against *Candida* and *Aspergillus. Ulva lactuca* displayed inhibition percentages of 79.4% and 69.45% against *Candida* and *Aspergillus*, respectively, while *Cystosera myrica* displayed inhibition percentages of 85.09% and 78.16% against the same fungus. These results imply that the methanol portions of both algae species have promising antifungal properties.

Comparing the results with the control group showed Out of all the fractions, the ethyl acetate fraction of *Ulva lactuca* was shown to have the highest antifungal activity, with inhibitory percentages of 86.16% against *Candida* and -0.49% against *Aspergillus*. The ethyl acetate fraction of *Cystosera myrica,* however, demonstrated reduced activity (48.83%) against *Candida* and more activity (73.17%) against *Aspergillus*.

While some algal fractions showed less antifungal activity than traditional antifungal medications, other fractions exhibited antifungal activity that was comparable to or greater than that of the pharmaceuticals. Overall, these findings show the potential of *Ulva lactuca* and *Cystosera myrica* algae fractions as alternative antifungal medications; the ethyl acetate fractions show particularly promising effects against *Candida* and *Aspergillus* (Fig 2)

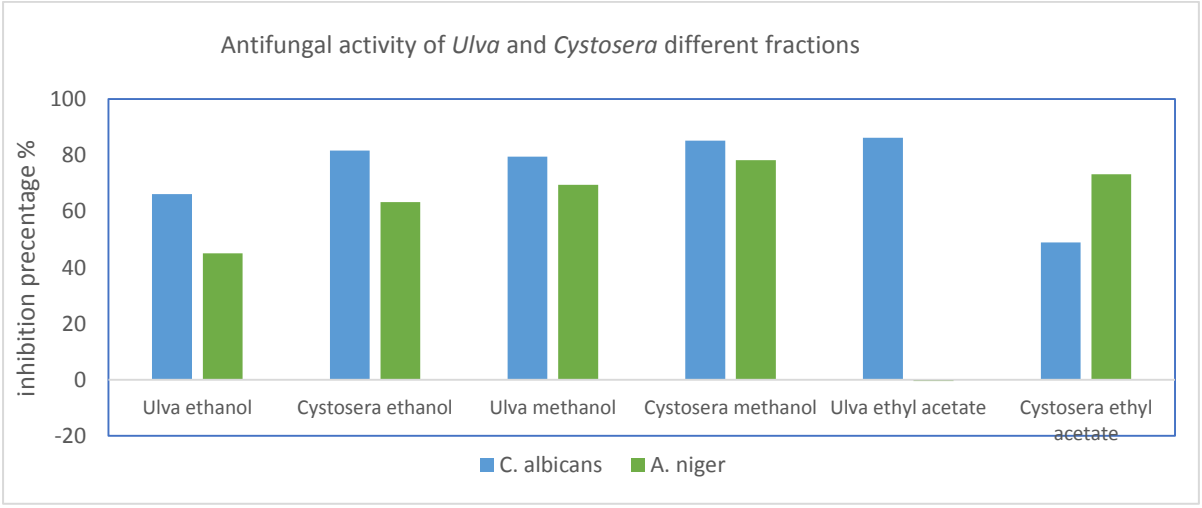

Fig. (2) Antifungal activity of *Ulva* and *Cystosera* different fractions.

4.Discussion

The study's conclusions address a wide range of subjects, including sample collection, the extraction of crude extract from diverse substances, and the extraction materials' antimicrobial screening. The antibacterial and antifungal qualities of the ethanol, methanol, and ethyl acetate fractions from *Ulva lactuca* and *Cystosera myrica* algae were also evaluated in this work against a range of microorganisms. Here, we discuss these outcomes in detail.

Morphological descriptions were provided for *Ulva lactuca* and *Cystosera myrica*, two strains of algae. Intertidal waters in Suez Bay were used to collect samples of *Ulva lactuca*, while three-meter-deep Ras Sudr was used to collect samples of *Cystosera myrica*. The morphological investigation revealed distinctive traits for every strain of algae, providing crucial details regarding their taxonomic identity and applications. *Ulva lactuca* was a thallus-forming plant with dentate edges, pseudo-dichotomously articulated branches, and a characteristic brown color. Contrarily, Cystosera myrica took the form of a thin, flat green algae that spread from discoid holdfasts.

It consisted of a two cell thick, translucent, soft membrane without a stipe [16]. Crude extracts were taken from the gathered algal samples using three different solvents: methanol, ethanol, and ethyl acetate. After dissolving 5 grams of powdered ingredients in 100 milliliters of solvent, the mixture was placed on a rotary shaker and allowed to settle overnight. The crude extracts were separated from the mixture by centrifuging it after filtering. The extraction parameters, which included sample volume, solvent volume, extraction time, filtration, centrifugation speed, and time, were carefully adjusted for each solvent.

The antifungal activity of *Ulva lactuca* and *Cystosera myrica* was significantly inhibited by the ethanol, methanol, and ethyl acetate fractions. The antibacterial activity of the resultant extracts was evaluated against a variety of microorganisms, including bacteria and fungi. The antibacterial activity of *Ulva lactuca* and *Cystosera myrica* against *Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, and Staphylococcus aureus* was significantly suppressed in the ethanol, methanol, and ethyl acetate fractions [17].

The ethanol fractions of *Ulva lactuca* and *Cystosera myrica* both exhibited strong antibacterial activity against Gram-positive bacteria; however, the activity of *Ulva lactuca* was higher against *Klebsiella* while the activity of *Cystosera myrica* was stronger against *Staphylococcus aureus.* With *Ulva lactuca* displaying moderate to high activity and *Cystosera myrica* demonstrating significant suppression against both *Escherichia coli* and *Staphylococcus aureus,* the methanol fractions demonstrated broad-spectrum antibacterial activity against all tested microorganisms. Furthermore, there was notable antibacterial activity of the ethyl acetate fractions against *Escherichia coli*.

against *Aspergillus fumigatus* and *Candida albicans*. *Aspergillus fumigatus* and *Candida albicans* were both significantly inhibited by *Ulva lactuca* and *Cystosera myrica* in the methanol fractions, which demonstrated encouraging antifungal efficacy. Among all the fractions of *Ulva lactuca*, the ethyl acetate fraction showed the strongest antifungal efficacy against *Candida albicans.*

5.Conclusion

In conclusion, our research demonstrates the antibacterial qualities of crude extracts from *Ulva lactuca* and *Cystosera* algae against a variety of bacteria and fungi. All things considered, our findings show that *Ulva lactuca* and *Cystosera* algae fractions have potential as alternative antibacterial agents, with different solvents showing varying levels of activity against different infections.

The taxonomic insights gleaned from the morphological characterization of the algal strains facilitated their identification and potential use in several domains [18].Strong antibacterial activity is shown by the ethanol and methanol fractions in particular, indicating the extracts' efficacy against both Gram-positive and Gram-negative bacteria. Moreover, the methanol fractions' potential medical applications are highlighted by their notable antifungal activity against *Candida albicans* and *Aspergillus fumigatus*. The varying degrees of efficiency of the various solvent fractions indicate how important solvent selection is for boosting antibacterial properties. Overall, our findings point to the potential importance of *Ulva lactuca* and *Cystosera myrica* algae as sources of substitute antimicrobial agents, which calls for more research and development in the area of treatments based on natural products.

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