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Microbiological Studies On antimicrobial activity of some seaweeds from Egypt

Eman H. Abdelaal[1], Ahmed A. Hamed[2], Mohamed G. Battah[1], Mohamed E. El awady[3], Sohier S. Abdel Salam[1] and Mervat G. Hassan[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: emanhamed11435@gmail.com

Abstract

This study investigates the antibacterial activity of crude extracts from isolated marine environments that contain the algae *Ulva lactuca* and *Hormophysa*. The algal strains' morphological characteristics provide information about their taxonomic identity. Using solvents such as methanol, ethanol, and ethyl acetate, crude extracts were made, and their antibacterial properties were assessed against a variety of bacteria and fungi. While the methanol fractions showed broad-spectrum antibacterial activities, the ethanol fractions had substantial antibacterial activity against Gram-positive bacteria, specifically *Bacillus* and *Staphylococcus aureus*. The ethyl acetate fraction of *Ulva lactuca* showed the best efficacy against *Candida albicans*, whereas the methanol fractions showed significant antifungal activity against *Aspergillus fumigatus*. These findings highlight the possibility of *Ulva lactuca* and *Hormophysa* algae extracts as viable sources of substitute antimicrobial agents, with various solvents exhibiting varying degrees of efficacy against various bacteria.

Key words: *Hormophysa*, *Uvla*, bioactive natural products, antibacterial and antifungal.

1.Introduction

Antibiotic resistance has emerged as a significant global health threat, with the World Health Organization [1]warning of the diminishing effectiveness of commonly used antibiotics against bacterial infections. This poses serious challenges in the treatment of various medical conditions, particularly during critical procedures such as cancer treatments, cesarean sections, and organ transplants. Consequently, there is an urgent need to explore alternative strategies to combat this escalating problem [2].

Natural products have emerged as promising alternatives in the search for novel antimicrobial agents. They offer a rich source of bioactive compounds that possess therapeutic potential in preventing and treating diseases. Among these natural sources, *Hormophysa cuneiformis*, an abundant brown marine alga found in the coral reefs of the Red Sea, holds significant promise. Despite its prevalence, this alga remains underexplored in terms of its bioactivity and chemical composition [3], [4].

in recent years, the exploration of natural compounds from marine sources has gained increasing attention due to their vast biodiversity and potential therapeutic applications. Marine algae, in particular, have emerged as valuable reservoirs of bioactive compounds with diverse pharmacological properties. *H. cuneiformis*, with its prolific presence in the Red Sea's coral reefs, represents a compelling target for bioactivity screening. Its unique ecological niche and resilience in harsh marine environments suggest the presence of specialized metabolites that may confer antimicrobial activity [5].

Furthermore, the identification of novel antimicrobial agents from marine sources holds particular significance in the context of the current antibiotic resistance crisis. With many conventional antibiotics losing effectiveness against pathogenic bacteria, there is a critical need to discover alternative therapeutic options. Natural products derived from marine organisms offer a promising avenue for drug discovery, providing structurally diverse compounds with unique modes of action against microbial pathogens [6], [7], [8].

This study seeks to investigate the antimicrobial properties of mthanol extract derived from *H. cuneiformis*, shedding light on its potential as a source of novel antimicrobial agents. By delving into the unique attributes of *Hormophysa* algae, including their nutritional richness and potential medicinal applications, this research aims to expand our understanding of their biological properties. Furthermore, elucidating the antibacterial of these algae holds promise for their utilization in various industrial and healthcare settings

Through a comprehensive exploration of the bioactivity and chemical profile of *Hormophysa* algae, this study contributes to the advancement of knowledge in the field of natural products research. By uncovering the therapeutic potential of these algae, this research paves the way for the development of novel antimicrobial agents and underscores the importance of marine organisms in addressing contemporary healthcare challenges

2.Materials and Methods

Obtaining of algae

Hormophysa were collected at a depth of 3 m from Ras Sudr, which is east of the Suez Gulf. In the field, algae samples were cleaned with seawater, and in the lab, they were cleaned using tap water. Dryness was maintained at ambient temperature and out of direct sunlight to prevent active chemical breakdown.

Extraction of bioactive metabolites from isolated algae

Once the algae had sufficiently dried, 10 grams of carefully measured material were weighed in order to extract the bioactive components from the algae. During the extraction process, these three organic solvents were used: methanol, ethanol,dichloromethane, and ethyl acetate. The procedure of choosing the right solvent sought to extract from the algae a variety of beneficial elements while taking into account its unique qualities.

The dried algae were submerged in each solvent throughout the extraction procedure, enabling the bioactive ingredient ds to dissolve into the solvent medium. After the algae had sufficiently dried, 5 grams of carefully measured material were weighed in order to extract the bioactive components of the algae. During the extraction process, these three organic solvents were used: methanol, ethanol, dichloromethane, and ethyl acetate. The procedure of choosing the right solvent sought to extract from the algae a variety of beneficial elements while taking into account its unique qualities.

The dried algae were submerged in each solvent throughout the extraction procedure, allowing the bioactive ingredient ds to dissolve into the solvent medium.

[9].

Evaporation of crude extract

After the extraction procedure, the liquid crude extract was poured into a flask with a circular bottom. Next, the rotary evaporator apparatus was connected to the flask containing the crude extract.

A water bath, a revolving flask, a condenser, and a vacuum pump make up the rotary evaporator. The temperature and vacuum level of the rotary evaporator were adjusted to be optimal for the particular solvent employed in the extraction process [10]. In order to promote evaporation without subjecting the bioactive chemicals to thermal degradation, the water bath was heated to a temperature that was just below the solvent's boiling point [11].As a result of the vacuum pump's decreased pressure, the solvent began to evaporate from the crude extract in the flask with a circular bottom. The efficient evaporation of the crude extract was made possible by the spinning flask, which exposed a greater surface area of the extract to the vacuum conditions. Consequently, the concentrated bioactive substances stayed in the flask with a circular bottom, while the volatile solvent evaporated and condensed in the condenser. In order to avoid overheating and maintain the integrity and activity of the bioactive metabolites, the evaporation process was closely supervised [12].The concentrated crude extract containing the bioactive components was taken out of the round-bottomed flask and subjected to additional examination and characterization once the solvent had fully evaporated.

Antimicrobial screening

Examined against a variety of test microorganisms, including those resistant to penicillin, the antibacterial activity of the algae crude extracts was assessed. The microplate dilution method was applied to the experiment. In summary, 10 μL of different quantities of fungal extract were combined with 180 μL of appropriate culture conditions, which included potato-dextrose sauce for fungi and lysogeny sauce for bacteria. Follow the logarithmic growth period with the addition of 10 L of a bacterial or fungal solution. The microplates were incubated at 37 °C for the full night prior to being measured for absorbance at OD600 using a Spectrostar Nano Microplate Reader [13]

3.Results

sample collection

To ascertain their taxonomic identity and possible uses, two strains of algae were separated and given morphological analysis. Samples of *Hormophysia* were taken in April from Ras Sudr (east of Suez Gulf) at a depth of three meters, while *Ulva lactuca* was taken in February from intertidal waters in Suez Bay (north of Suez Gulf). The algae samples were collected, and after being cleaned in the field with seawater, they were cleaned in the lab with tap freshwater. The samples were dried at room temperature in the absence of direct sunlight in order to maintain the integrity of the active ingredients.

Each strain of algae has unique characteristics, according to morphological examination. Brown coloring, thallus habit, and pseudo-dichotomously articulated branches with dentate margins were all distinctive characteristics of *Hormophysia* [14].On the other hand, *Ulva lactuca* was seen to be a thin, flat green algae that grew from discoid holdfasts. Its highest dimensions were 18 cm for length and 30 cm for width [15]. Interestingly, *Ulva lactuca* is made out of a delicate, translucent membrane that is two cells thick and devoid of a stipe (Table 1).

These thorough anatomical examinations offer insightful information about the variety of algae strains present in the biotech unit. They also open up new avenues for further investigation into the biotechnological potential of these algae strains, from the generation of biofuel to the use in pharmaceuticals.

Table (1) algae sample collection

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

Extraction of crude extract from different samples

Three different solvents—methanol, ethanol, and ethyl acetate—were prepared from powdered materials at room temperature. Each extract was made by dissolving 5 grams of the sample (powder) in 50 milliliters of solvent and letting it stand at room temperature on a rotary shaker for the whole night. Following filtering, the mixture was centrifuged at 6,000 rpm for 15 minutes Table (2).

Table (2) Summary of Solvent Extraction Parameters.

Solvent	Sample Amount (g)	Solvent Volume (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

Antimicrobial screening for the obtained extracts

Antibacterial activity

The antibacterial activity of ethanol, methanol, and ethyl acetate fractions obtained from *Ulva lactuca* and *Hormophysa* algae was evaluated against various microorganisms, including *Staphylococcus aureus* (*Staph*), *Bacillus subtilis* (*Bacillus*), *Proteus vulgaris* (*Proteus*), and *Escherichia coli* (*E. coli*). Additionally, a control group was included to compare the effectiveness of the algae fractions against standard antibacterial agents.

For *Ulva lactuca* ethanol fraction, the highest antibacterial activity was observed against *Bacillus* (69.55%) and *E. coli* (47.92%), while moderate activity was recorded against *Staph* (58.77%) and *Proteus* (24.22%). In comparison, the *Hormophysa* ethanol fraction exhibited superior activity against *E. coli* (63.41%) and *Staph* (63.32%), with lower activity against *Bacillus* (58.19%) and *Proteus* (37.33%). These results suggest that the ethanol fractions of both algae species possess notable antibacterial properties, particularly against Gram-positive bacteria.

Regarding the methanol fractions, *Ulva lactuca* showed moderate to high antibacterial activity against all tested microorganisms, with the highest activity against *Bacillus* (70.61%) and *E. coli* (72.2%). Conversely, *Hormophysa* displayed varying levels of activity, with significant inhibition observed against *Staph* (51.1%) and *E. coli* (80.99%), but minimal or no activity against *Bacillus* and *Proteus*. This indicates that the methanol fractions of both algae species possess broad-spectrum antibacterial activity, with *Hormophysa* exhibiting potent activity against *E. coli*.

The ethyl acetate fractions of *Ulva lactuca* and *Hormophysa* also demonstrated notable antibacterial activity, particularly against *E. coli*, with inhibition percentages of 72.2% and 80.99%, respectively. However, both fractions exhibited lower activity against other tested microorganisms compared to the ethanol and methanol fractions.

The efficacy of the algal fractions varied when compared to the control group, which was made up of common antibacterial drugs. Certain fractions had antibacterial activity that was on par with or even higher than the control, whereas other fractions had reduced activity. All things considered, our results demonstrate the potential of *Ulva lactuca* and *Hormophysa* algae fractions as substitute antibacterial agents, especially against Gram-positive and Gram-negative bacteria, with the most promising outcomes being shown by the ethanol and methanol fractions (Fig 1).

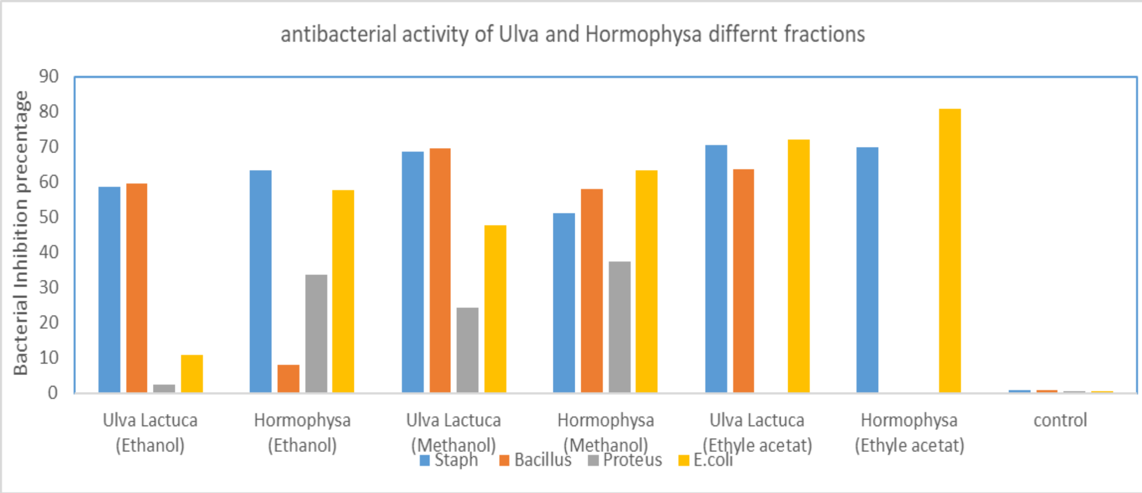

Fig. (1) Antibacterial activity of *Ulva* and *Hormophysa* different fractions

Antifungal activity

Ethanol, methanol, and ethyl acetate fractions from *Ulva lactuca* and *Hormophysa* *a*lgae were tested for their antifungal efficacy against two prevalent fungal pathogens: *Aspergillus fumigatus* (*Aspergillus*) and Candida albicans (Candida). To assess the efficacy of the algae fractions against common antifungal medications, a control group was also included.

For *Ulva lactuca* ethanol fraction, significant antifungal activity was observed against both *Candida* (66.07%) and *Aspergillus* (45.06%), indicating its potential as a broad-spectrum antifungal agent. Similarly, the *Hormophysa* ethanol fraction exhibited even higher activity against both *Candida* (81.55%) and *Aspergillus* (62.95%), suggesting its strong antifungal properties.

Ulva lactuca and *Hormophysa* both showed strong antifungal activity against *Candida* and *Aspergillus* in the methanol fractions. *Hormophysa* revealed inhibition percentages of 81.93% and 76.07% against the same fungi, whilst *Ulva lactuca* showed inhibition percentages of 79.4% and 69.45% against *Candida* and *Aspergillus*, respectively. These findings suggest that both algae species' methanol fractions have encouraging antifungal potential.

Comparison with the control group revealed With inhibitory percentages of 86.16% against *Candida* and -0.49% against *Aspergillus*, the ethyl acetate fraction of *Ulva lactuca* was shown to have the highest antifungal activity out of all the fractions. *Hormophysa*'s ethyl acetate fraction, on the other hand, had considerable activity against *Aspergillus* (76.07%) but less activity against *Candida* (7.58%).

found while some algae fractions had less antifungal activity than the conventional antifungal drugs, others had antifungal activity that was on par with or even higher. Overall, these results demonstrate the potential of *Ulva lactuca* and *Hormophysa* algae fractions as substitute antifungal drugs; against *Candida* and *Aspergillus*, the ethyl acetate fractions exhibit especially encouraging results (Fig 2)

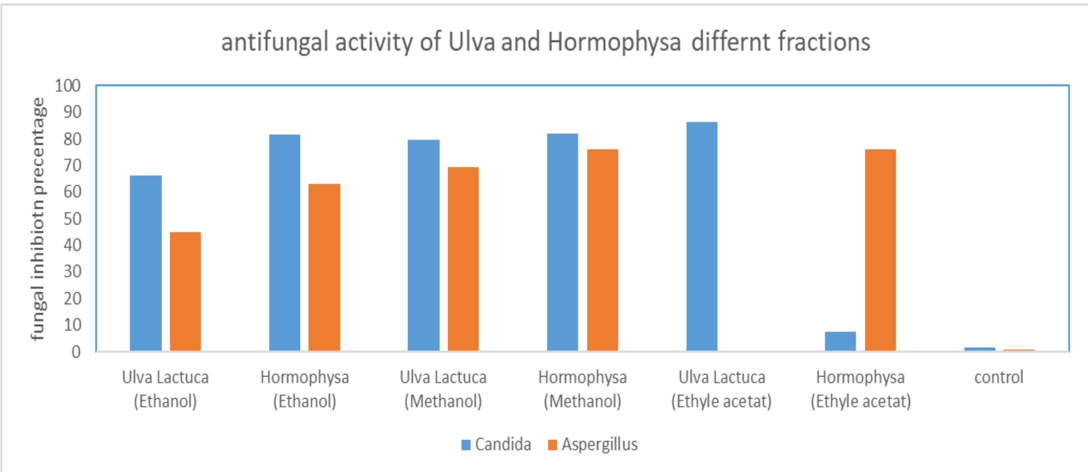

Fig. (2) Antifungal activity of *Ulva* and *Hormophysa* different fractions

4.Discussion

The study's findings cover a wide range of topics, such as sample collection, crude extract extraction from various substances, and antimicrobial screening of the extracted materials. The study also assessed the ethanol, methanol, and ethyl acetate fractions from *Ulva lactuca* and *Hormophysa* algae against a variety of microbes for their antibacterial and antifungal properties. We go into great detail about these outcomes here.

Ulva lactuca and *Hormophysa*, two strains of algae, were separated and given morphological descriptions. Samples of *Ulva lactuca* were taken from Suez Bay's intertidal waters, and samples of *Hormophysa* were taken from Ras Sudr at a depth of three meters. Each strain of algae has unique characteristics that the morphological study identified, offering important information about their taxonomic identity and their uses. *Ulva lactuca* had a thallus habit, pseudo-dichotomously articulated branches with dentate margins, and a distinctive brown coloring. On the other hand, *Hormophysa* appeared as a thin, flat green algae that grew from discoid holdfasts. It was made up of a soft, translucent membrane that was two cells thick and did not have a stipe [16].

Three solvents—methanol, ethanol, and ethyl acetate—were used to extract crude extracts from the collected algal samples. 5 grams of powdered materials were dissolved in 100 milliliters of solvent, and the mixture was then left to sit overnight on a rotary shaker. The mixture was filtered, and then centrifuged to extract the crude extracts. For every solvent, the extraction parameters—such as sample volume, solvent volume, extraction duration, filtration, centrifugation speed, and time—were meticulously tuned.

For antifungal activity, the ethanol, methanol, and ethyl acetate fractions of *Ulva lactuca* and *Hormophysa* exhibited significant inhibition The resulting extracts' antibacterial activity was assessed against a range of microorganisms, including fungus and bacteria. The ethanol, methanol, and ethyl acetate fractions of *Ulva lactuca* and *Hormophysa* shown notable suppression of antibacterial activity against *Escherichia coli*, *Bacillus subtilis*, *Proteus vulgaris*, and *Staphylococcus aureus* [17].Both *Ulva lactuca* and *Hormophysa*'s ethanol fraction shown significant antibacterial activity against Gram-positive bacteria; however, *Ulva lactuca*'s activity was greater against *Bacillus* and *Hormophysa*'s activity was greater against *Staphylococcus aureus*. The methanol fractions showed broad-spectrum antibacterial activity against all examined microbes, with *Ulva lactuca* showing moderate to high activity and *Hormophysa* showing considerable suppression against both *Escherichia coli* and *Staphylococcus aureus.* And against *Escherichia coli*, the ethyl acetate fractions had significant antibacterial activity.

against *Candida albicans* and *Aspergillus fumigatus.* The methanol fractions showed promising antifungal activity, with both *Ulva lactuca* and *Hormophysa* exhibiting potent inhibition against *Candida albicans* and *Aspergillus fumigatus*. Notably, the ethyl acetate fraction of *Ulva lactuca* displayed the highest antifungal activity among all fractions against *Candida albicans*.

5.Conclusion

To sum up, our work shows that crude extracts from *Ulva lactuca* and *Hormophysa* algae have antibacterial properties against a range of bacteria and fung All things considered, our results demonstrate the potential of Ulva lactuca and Hormophysa algae fractions as substitute antibacterial agents, with various solvents demonstrating differing degrees of efficacy against various pathogens.

i. The identification and possible application of the algal strains in different fields was made easier by the taxonomic insights obtained from their morphological characterisation [18].The ethanol and methanol fractions in particular exhibit strong antibacterial activity, demonstrating the extracts' effectiveness against both Gram-positive and Gram-negative bacteria. Furthermore, the methanol fractions' noteworthy antifungal activity against *Aspergillus fumigatus* and *Candida albicans* highlights their prospective medicinal uses. The different solvent fractions' differing levels of efficacy point to the significance of solvent selection in enhancing antibacterial characteristics. Overall, our results highlight the potential significance of *Hormophysa* and *Ulva lactuca* algae as sources of alternative antimicrobial agents, warranting further exploration and development in the field of natural product-based therapeutics.

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