

Exploration of Antimicrobial Potential in Oualidia Algae – Unveiling *Cystoseira Humilis* as a Promising Source of Bioactive Molecules

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ABSTRACT

The potential of seventeen marine seaweed extracts (five Chlorophyceae, six Pheophyceae, six Rhodophyceae) was investigated to determine their antibacterial activity, aiming to evaluate their viability in pharmaceutical applications. The assessment of their bioactivity involved utilizing crude extracts from dried samples against five Gram-positive bacteria, one Gram-negative bacteria, yeast, and fungus using the disk diffusion technique. These samples were collected from Oualidia, situated along Morocco's Atlantic coast. Out of the diverse macroalgae studied, 36% of the extracts exhibited activity against at least one of the tested microorganisms. This outcome strongly supported the notion of leveraging algae extracts as a promising source of antibacterial compounds. In particular, extracts from *Cystoseira humilis*, *Bifurcaria bifurcata* and *Asparagopsis armata* showed zones of inhibition greater than 17 mm. Purification of the compounds responsible for the inhibitory activity against several types of *C. humilis* microorganisms was performed using chromatography and thin layer chromatography.

Keywords: algae, antibacterial activity, antifungal activity, lagoon, Moroccan Atlantic coast.

INTRODUCTION

Several natural molecules used by man for pharmacological purposes have a marine origin, demonstrating the potential wealth of molecules with original structures and promising biological activities [Maggio et al., 2022; Manino, 2014; Mannino et al., 2016; Martín-Martín et al., 2022].

The Moroccan beaches and lagoons are a veritable reservoir of plant and animal species because of the immense diversity of marine flora and their unique richness. This presents Morocco with significant economic potential. In recent years, a great deal of research has been carried out into active substances of marine origin in the algae of the Atlantic coast of El Jadida. This work has led to the selection of the most active algal species and the isolation of numerous molecules with significant pharmacological activity [Fayzi et al., 2022; Khaya et al., 2022; Rhimou, 2013].

In this study, algal screening was performed to investigate the antibacterial activity of crude extracts (dichloromethane/ethanol) of seventeen species of marine algae, five green algae, six brown algae, and six red algae collected from the Oualidia lagoon on the Atlantic coast of Morocco. *C. humilis* is one of the species that showed a high inhibition zone (19mm), and the active molecule was purified.

MATERIALS AND METHODS

Collection of algal material and preparation of extracts

Seaweed harvesting occurs in March and April in the Oualidia lagoon. After harvesting, the algae are rinsed several times with distilled water to remove any foreign matter that might interfere with the analysis of biological activities. The algae are then sorted and identified.

For each species, the prepared algal powder was extracted in a mixture of organic solvents methanol/ethanol (50:50) at a rate of 5 g algal powder/ml solvent, following the extraction protocol described by Caccamese [Caccamese & Azzolina, 1979]. The extracts are then filtered through Whatman paper and evaporated in a rotary evaporator. Extracts dried in this way are stored at 4 °C until they are used for biological testing.

Search for biological activities

Antimicrobial activity

Gram-positive bacterial species tested: *Bacillus cereus* CIP 783, *Bacillus subtilis* CIP 783, *Bacillus thuringiensis* (ATCC 10792), *Staphylococcus aureus* ssp. ATCC 6538, and *Escherichia coli* ATCC 10536 as Gram-negative bacteria and *Cryptococcus coliforme* and *Candida tropicalis* (IP 201773) as fungi.

Measuring antimicrobial activity

The antimicrobial activity was quantified using cellulose disks for organic extracts [Bauer et al., 1966]. Quantities of 100 µg to 500 µg of the extract to be analyzed were deposited on cellulose paper disks, and after evaporation of the solvent, these disks were applied directly to a Petri dish previously inoculated with the test strain.

After 16 h of incubation at 37 °C, the antibacterial activity was measured as the diameter (in mm) of the zone of inhibition that appeared around the pellets. Similarly, antifungal activity was assessed after 24 h of incubation at 27 °C. Discs impregnated with standard antibiotics such as chloramphenicol, streptomycin, or tetracycline are used (at 50 or 100 µg/ml) as controls in antibacterial activity tests.

Methods used to separate active compounds

Column chromatography

This method relies on the phenomena of adsorption. A column of varying length and cross-section is filled with solid-phase silica. The extract is deposited at the top of the column, and the components separate because the eluent flows continuously through the column either under low pressure or by gravity. Compound displacement is accelerated by progressively increasing the polarity of the eluent employed.

Depending on their solubility in the eluent and affinity for the adsorbent, molecules are transported downhill at varying speeds. A series of colored cylindrical zones that split as they move downhill form the chromatogram. Various fractions were gathered on the basis of their polarity.

Every recovered fraction was examined for its potential to inhibit microorganisms or reduce inflammation.

Thin layer chromatography

Thin-layer chromatography (TLC) is mainly based on adsorption phenomena. The extract migrates through the solvent or solvent mixture. After being deposited on a 0.2 mm thick silica 60F254 plate (MERK), the substances migrate at a speed that depends on their polarity and that of the solvent. All recovered fractions were tested for antibacterial or anti-inflammatory activity.

RESULTS AND DISCUSSION

Screening for the antibacterial activity of algal extracts

The various dichloromethane/methanol extracts prepared from the five species of green algae (Chlorophyceae) harvested in the Oualidia region *C. tomentosum*, *U. lactuca*, *U. crispa* *E. linza*, and *E. intestinalis*, were tested against the following microbial strains: *S. aureus*, *B. cereus*, *B. thuringiensis*, *B. subtilis*, *C. sporogenes*, *E. coli*, *C. neoformans*, and *C. tropicalis* (Figure 1). Of the extracts tested, 30% showed an inhibitory effect on the strains tested. The extracts of *E. linza*, *E. intestinalis*, and *U. lactuca* showed an inhibition diameter greater than 10 mm for *S. aureus*. In contrast, algal extracts tested against *B. subtilis*, *C. sporogenes*, *E. coli* (gram-negative bacteria), and *C. tropicalis* showed no inhibitory effect. The same result was obtained by par [Anjali & al., 2019; Elkouri & al., 2004]. In contrast, extract of *C. tomentosum* showed the strong antimicrobial activity [Elkhateeb & al., 2021]. Ulvae and Entermorphe have antimicrobial effects on gram-positive and gram-negative bacteria [Anjali & al., 2019; EL-Sayed and al., 2023; Patra & al. 2015]. Ardita et al. [2019] described the potential of *U. lactuca* as a source of a methicillin-resistant *S. aureus* agent that could be used to treat common surgical site infections. whereas [Arun Kumar & Rengasamy, 2000; Caccamese & Azzolina,

1979; Ktari, 2000] have shown that antimicrobial activity is absent in algal extracts from ulvae.

Extracts of the pheophyceae: *B. bifurcata*, *C. humilis*, *C. tamariscifolia*, *F. spiralis*, *L. pinnatifida*, *S. bulbosa*, and *L. ochroleuca* were tested

against bacteria (gram-positive and gram-negative), yeast, and fungi. The results showed that 48% of the algae extracts showed activity (Figure 2). The *B. bifurcata* and *C. humilis* extract showed inhibition diameters of 18 and 19 mm against

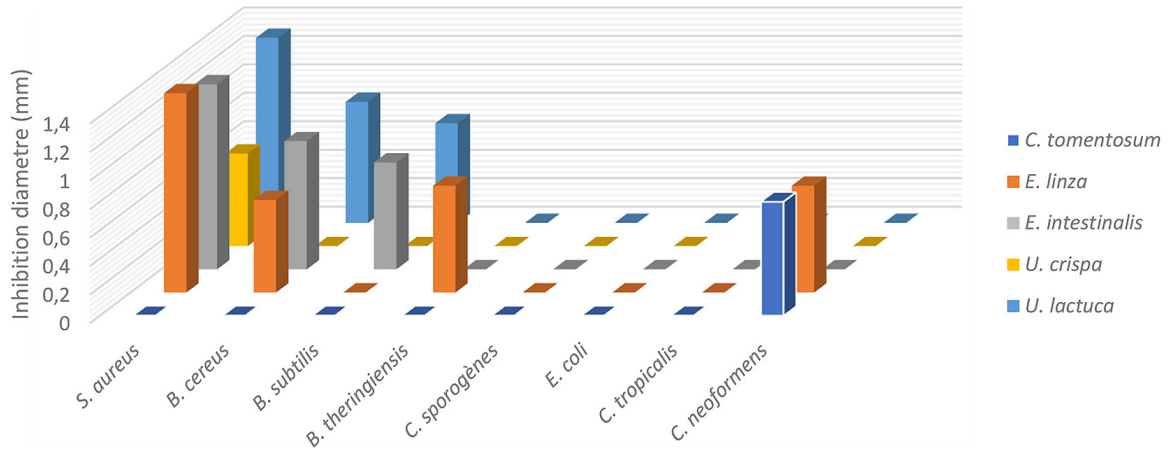


Figure 1. Antimicrobial activity of Chlorophyceae

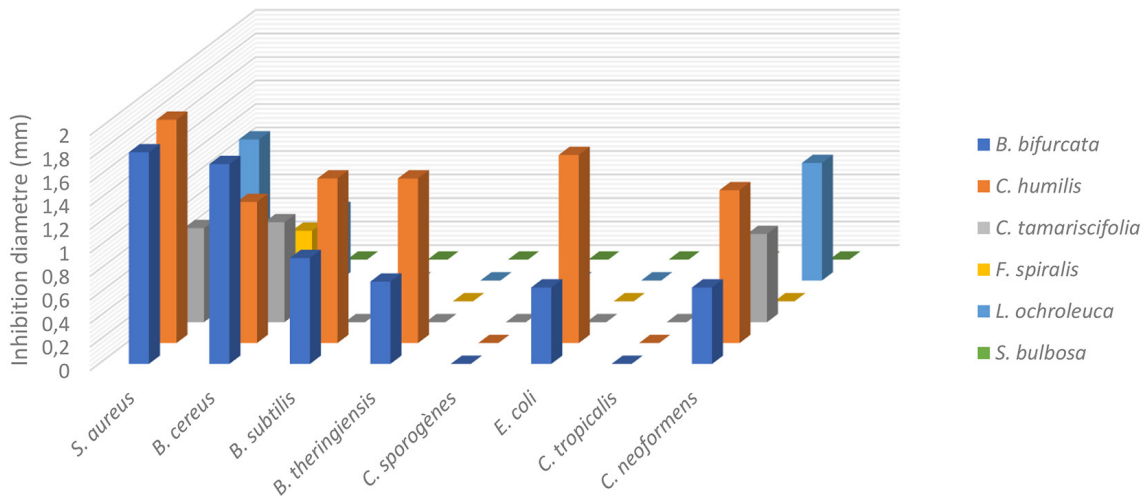


Figure 2. Antimicrobial activity of Phaeophyceae

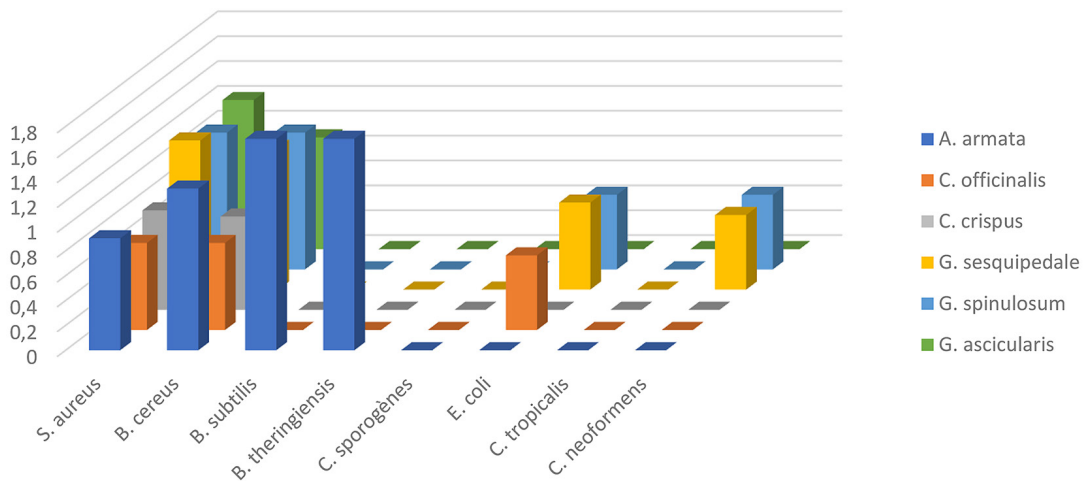


Figure 3. Antimicrobial activity of Rhodophyceae

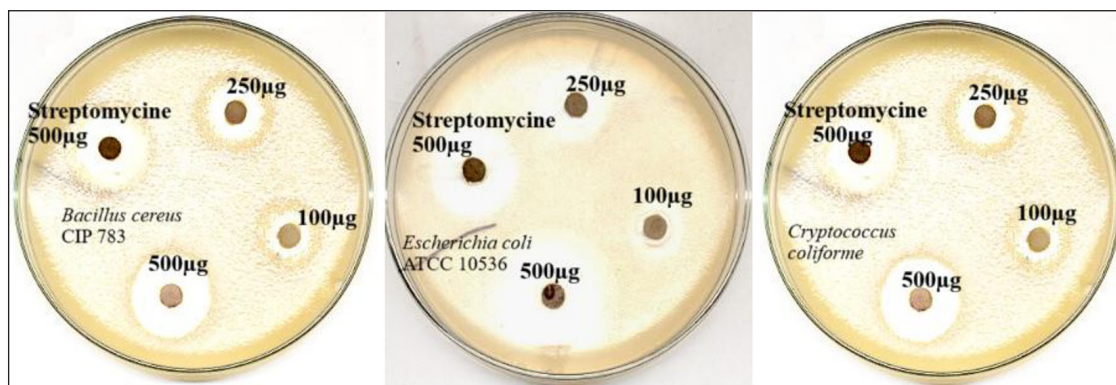


Figure 4. Three antibiograms (*B. cereus*, *E. coli*, and *C. coliforme*) against of *C. humilis* algal extract

S. aureus and *B. cereus*, respectively, and significant inhibitory activity against *B. thuringiensis* and *B. subtilis*. These results concur with those found by [Benhniya and al., 2022], who showed that *B. bifurcata* extract was collected from different locations.

Harvested from the Atlantic coast of El Jadida, had an inhibitory effect against the bacteria tested. The same result was found for *B. bifurcata* harvested in Rabat against *S. aureus* [Ibtissam et al., 2009]. The *C. humilis* extract harvested showed inhibitory activity against germs [Saidani et al., 2022; Ibtissam et al., 2009].

The other extracts showed only weak inhibitory activity against some of the microbial strains

tested. With the exception of *S. bulbosa*, no activity was observed. A similar result was obtained [Elkouri et al., 2004].

The results obtained for *F. spiralis* and *L. ochroleuca* extracts showed inhibitory activity against *S. aureus* and *B. cereus*. The same result was obtained by [Etahiri, 2002]. *F. spiralis* extract harvested showed significant inhibitory activity against *S. aureus* and *B. subtilis* [Ayrapetyan et al., 2021; Ibtissam et al., 2009].

Extracts of Rhodophyceae: *A. armata*, *C. officinalis*, *C. crispus*, *G. sesquipedale*, *G. spinulosum*, and *G. acicularis* were tested on the microorganisms studied, with only 40% of extracts active against germs (Figure 3). *A. armata* extract

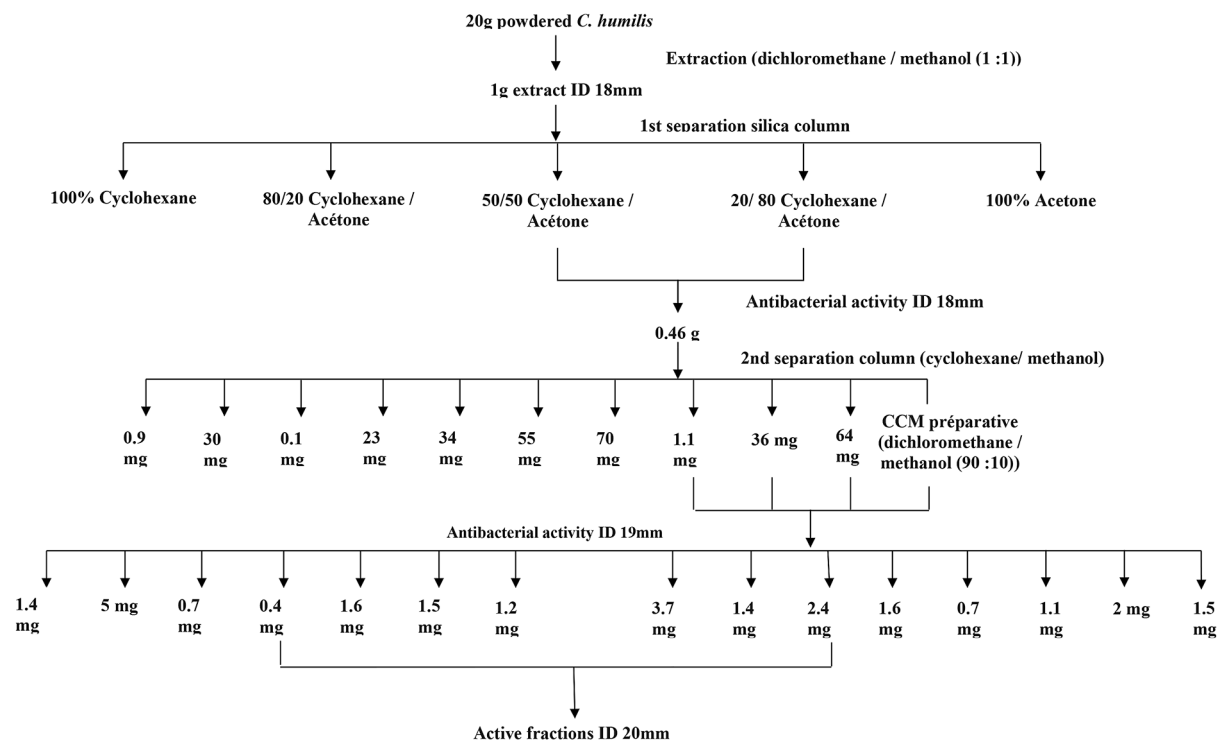


Figure 5. Schematic diagram for purification of the molecule responsible for antibacterial activity from *C. humilis*

showed a significant inhibition diameter of over 13 mm against *B. cereus*, *B. thuringiensis*, and *B. subtilis*. The antibacterial activity was obtained by the *A. armata* extract against *B. subtilis* and *S. aureus* harvested from the Peniche coast (Portugal) and Spanish coast [Pinteus and al., 2015].

Purification trial of the molecule responsible for antibacterial activity from *C. humilis*

Screening for antibacterial activity in algal extracts led to the selection of *C. humilis*, a brown alga with high antibacterial activity, which can live in calm or slow-moving waters in the eulitoral zone, attached to the substrate by a disk. It is characterized by several main axes with cylindrical primary and secondary branches.

Several species of brown algae in the Cystoseira genus (Cystoseiraceae, Pheophyta) are widespread in the Atlantic and Mediterranean, and some species have been reported to contain biologically active compounds [Valls et al., 1993].

The antibacterial effect induced by *C. humilis* extract, harvested on the Moroccan coast, has been reported by [Etahiri, 2002; Ibtissam et al., 2009], whereas the methanolic extract of *C. humilis*, harvested on the Canary Islands, shows no antimicrobial activity [Val et al., 2001].

In the screening performed, *C. humilis* extract showed an inhibition diameter of 18 mm against *B. cereus*.

To purify the molecule responsible for this activity, we adopted the protocol shown in Figure 5.

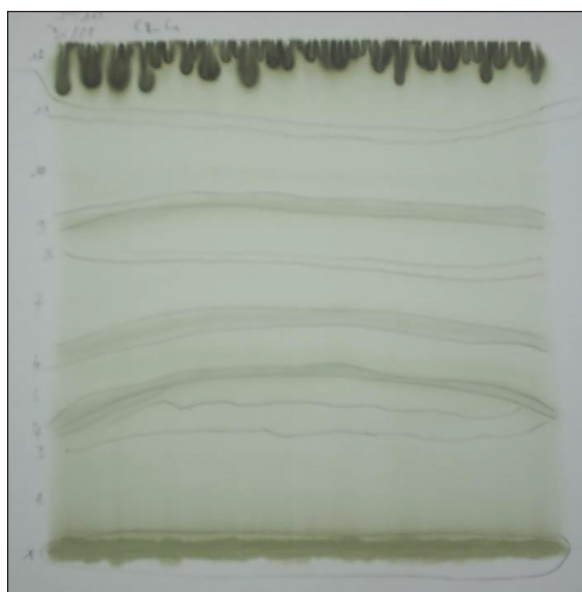


Figure 6. Separation of the active fraction using dichloromethane/methanol (90:10)

In this assay, 20g of dried powder was extracted in a dichloromethane/methanol (1:1) solvent mixture. After filtration and evaporation of the mixture, 1g of dry extract was obtained. The separation was performed on a normal-phase silica column, elution was performed with a mixture of solvents of increasing polarity, and a 3rd separation was performed on a TLC plate with a mixture of dichloromethane/methanol solvents (90:10). Fractions 4 and 7, 8, and 9 showing interesting antibacterial activity were collected (inhibition diameter greater than 20 mm against *S. aureus* and *B. cereus*) for purification by HPLC.

CONCLUSION

This study reveals the existence of algae with significant biological activity, which will undoubtedly become a valuable source of pharmaceutically interesting molecules in the future. In view of these results, we have the opportunity to pursue the purification of *C. humilis* extracts by HPLC to isolate the molecules responsible for its antimicrobial activity and to extend the purification to other selected antimicrobial active species.

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