RASĀYAN *J. Chem.*
Vol. 18 | No. 4 |2134-2143| October-December | 2025
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

# ANTIMICROBIAL ACTIVITY OF RED SEAWEEDS HARVESTED FROM THE ATLANTIC COAST OF SIDIBOUZID, MOROCCO

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**ABSTRACT**

The screening of antibacterial and antifungal activity of forty extracts from eight red algae harvested on the Atlantic coast of Sidi Bouzid, El Jadida, was performed against six pathogenic bacteria strains: *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeroginosa,* and against two fungi: *Candidas tropicalis, Aspergilus niger*. The best antimicrobial activity was assessed using the standard protocol of the disc diffusion method. Methanol-water was the best solution for extracting the effective antibacterial materials from the algae species used in this experiment, and for fungi strains, dichloromethane was the most effective extraction solution. The highest antibacterial activity was shown by the methanol water extract of *Gelidium pulchellum* against *Escherichia coli* with a diameter of inhibition of 19 mm and MIC value (5µg/ml). Antifungal activity was shown by the methanol extract of *Caulacanthus ustulatus* against *A.niger*(Φ= 20mm) with an MIC value of 3.5µg/ml. The result of this study revealed that the red seaweeds appear to have higher potential as a source of antibacterial and antifungal compounds; they can be used in treating diseases caused by these organisms.

**Keywords:** Antimicrobial Activity, Antibacterial Activity, Antifungal Activity, Pathogenic Bacteria, Seaweeds.
RASĀYAN *J. Chem.*, Vol. 18, No.4, 2025

## INTRODUCTION

Nowadays, Germs are a real health problem, causing a range of infectious diseases that can be fatal due to their virulence. Many antibiotics are developed to treat them; however, their misuse is causing the appearance of bacterial multidrug resistance.[1] Increasing the resistance of bacteria to antimicrobial agents requires finding solutions for the treatment of infections resistant to conventional antibiotics and to prevent the emergence of new resistant strains. The fight against bacterial infections is complicated by the fact that many bacteria are resistant to most antibiotics, which represents a major challenge for global health.[2] Despite the advantages of using synthetic molecules as antioxidants or antibacterial agents, their drawbacks are a major concern. It is necessary to find a natural alternative that is safe for human health. As a source of active biomolecules, algae present a promising avenue for advancing conventional medication research. Several studies have been published on the research of antibacterial[3,4,5], antioxidant[6,7], antifungal[8,9], antiviral[10], and anti-inflammatory activities[11] of algae harvested along the Atlantic and Mediterranean coasts of Morocco. Seaweeds produce various bioactive compounds, including sterols, terpenoid carotenoids, vitamins, fatty acids, amino acids, antioxidants like polyphenols, alkaloids, polysaccharides like agar agar, carrageenan, proteoglycans, and alginate[13], which can inhibit the growth of certain Gram-positive and Gram-negative pathogenic bacteria.[12] Red seaweeds (Rhodophyta) are well known as producer of phycocolloids such agar, agarose, carragenan and great variety of secondary metabolites Red seaweeds (Rhodophyta) are well known as producer of phycocolloids such agar, agarose, carragenan and great variety of secondary metabolites Red seaweeds (Rhodophyta) are well known as producer of phycocolloids such agar, agarose, carragenan and great variety of secondary metabolites Various secondary metabolites are derived from red algae, the best known of which are phycocolloids such as carrageenan, agar, and agarose. Several chemical compounds, including terpenoids, steroids, acetogenins, and halogens, are found in red algae.[14,15] This study aimed to analyse the antibacterial and antifungal properties of extracts from eight species of red algae harvested from the Atlantic coast of Sidi Bouzid, El Jadida in Morocco, against bacterial strains (Gram+ and Gram-) and


http://doi.org/10.31788/RJC.2025.1848315
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fungal strains. Also, the efficacy of different solvents used with different polarities, to obtain the most effective crude extract.

## EXPERIMENTAL

### Algal Materials

Along Sidi Bouzid El Jadida's coastline in April 2015, eight red algae species (*Corralina officinalis*, *Corralina elongata*, *Laurencia pinnatifida*, *Boergeseniella thuyoides*, *Gracilaria multipartita*, *Caulcanthus ustulatus*, *Bornetia secundiflora*, and *Gelidium pulchellum*) were manually collected during the low water spring tides. After washing the samples three times with tap water to remove any possible salt or epiphytes, they were carefully washed with new distilled water before extraction, let to air dry, crushed into a powder, and stored.

### Algal Extract Preparations

We used five distinct solvents (methanol, methanol/water (40/60), dichloromethane, and the dichloromethane/methanol mixture (50/50), and ethyl acetate), to extract the different algae studied, using a proportion of 1 g per 5 ml, according to the method of Caccamese *et al*., 1979.[16] Extracts are then filtered and evaporated. At 4°C, the dried extracts are kept for further testing; only the methanol/water extract is stored after lyophilization.

### Antimicrobial Bioassay

### Microbial Strains

The strains utilized in these tests were from the American Type Culture Collection (ATCC), the Collection of the Institute Pasteur of Paris (CIP), and Mohamed V Hospital in El Jadida, Morocco. Bacterial strains used for assay were as follows: Gram-negative strains were *Pseudomonas aeruginosa* (ATCC 9027), *Klebsiella pneumonia*, and *Escherichia coli* (ATCC10536), and Gram-positive strains: *Staphylococcus epidermidis*, *Staphylococcus aureus*(ATCC 25295), and *Streptococcus pyogenes,* while *Aspergillus niger* (CIP 1275) and *Candida tropicalis* (ATCC 127581) were the fungal strains.

### Antimicrobial Assay

The test for agar disk-diffusion was used for antibacterial assays.[17] Using a wire loop, three colonies of each bacteria were taken out of the original plate of the culture and placed within a test tube with five milliliters of broth. After a night of cultivation, A suspension of $10^6$ bacteria/ml was obtained, which was used to inoculate Petri dishes with 12ml Mueller-Hinton agar. A half hour was spent drying the plates before inoculation. Following a temperature equalization at 4°C, Paper disks having a diameter of 6 mm were impregnated with 150µg of each organic extract for testing purposes.. After that, the plates were incubated for the whole night at 37 °C. Next, the inhibitory zone diameters were measured. The susceptibility test discs for streptomycin (100µg) and ofloxacine (50µg) represented the positive control. Zones of inhibition for fungicidal activity were measured following a 24-hour incubation period at 27°C. In the antifungal activity test, 100µg of conventional antibiotics, such as Amphotericin B, were impregnated on discs, which served as the reference. Furthermore, control disks containing all of the solvents utilized were made. Every test had at least three duplicates.

### Minimum Inhibitory Concentration

The method using microdilution plates was utilized to estimate the resazurin's minimum inhibitory concentration (MIC).[18] A 96-well microplate was created by adding 100 µL of either the fungal strain PDA or the bacterial strain Mueller-Hinton broth to each well. The first row of the plate was covered with 100 µL of the 40 mg/ml stock solution of the examined extract. Next, A multichannel pipette was used to transfer 100 µl of solution from one row to the next in order to perform two-fold serial dilutions. The range of concentrations that were achieved was 20 to 0.0003 mg/ml. Each $10^6$ CFU/ml bacterial solution was applied in 10 µl increments to the wells. Lastly, resazurin solution (10 µL) was added. An oxidation-reduction indicator used to assess microbial growth is called resazurin. It is a blue, non-fluorescent dye that becomes pink and brilliant when oxidoreductases in live cells convert it to resorufin. For 24 hours, the plates inoculated were incubated at 37°C for bacterial strains, and 48 hours at 22°C for fungal strains. The minimum inhibitory concentration (MIC) of the examined algae extracts was measured by preventing the color from changing from blue to pink. Amphotericin B was employed for fungal strains, while

antibiotic streptomycin and ofloxacin, dissolved in Mueller-Hinton broth, was used as a positive control for bacterial strains. A solvent control test using 10% DMSO was conducted. It was shown that 10% DMSO did not affect bacterial growth inhibition. Every assay consisted of a growth verification and a sterility verification. All tests were carried out in duplicate, and the MICs were consistent.

## RESULTS AND DISCUSSION

Seaweeds extracts have been tested in vitro to evaluate their antibacterial and antifungal activity. The antimicrobial activity against microorganisms analyzed in this study was evaluated, the diameter of the zone and compared to streptomycin, ofloxacine, and amphotericin B used as antibiotic drugs and antifungal reference (Table-1 and 2).

### Antibacterial Assay

Table-1 presents the antibacterial effectiveness of various seaweed extracts using the agar disc diffusion method. The findings demonstrated that, at different concentrations, the algal extracts exhibited strong antibacterial activity.

Table-1: Antibacterial Activity of Red Seaweeds Against Pathogens Bacteria Using Different Solvents

| | Diameter of inhibition Φ (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Gram$^+$ | | | Gram$^-$ | | |
| Seaweed species | Solvent extraction | *S.P* | *S. A* | *S.E* | *K.P* | *P.A* | *E.C* |
| *C. elongata* | M | 7±0.461 | 8±0.816 | - | 11±0.927 | - | 12±0.734 |
| | DC | - | - | - | 16±0.763 | - | 9±0.881 |
| | M/DC | 9±0.440 | - | - | - | - | - |
| | EA | 10±0.959 | 10±0.85 | 10±0.577 | 10±0.548 | 10±0.866 | - |
| | M/W | 10±1.424 | 9±0.577 | - | 10±0.721 | 11±1.00 | 10±0.850 |
| *B.thuyoides* | M | 7±0.726 | 8±0.493 | - | 9±1.154 | 8±0.666 | 10±1.386 |
| | DC | - | - | - | 9±0.577 | - | - |
| | M/DC | 8±1.201 | - | 8±0.503 | - | 7±0.333 | - |
| | EA | 10±1.732 | - | - | 11±1.000 | 9±1.527 | - |
| | M/W | 8±0.666 | 9±0.986 | - | 11±0.763 | 9±1.527 | 10±0.000 |
| *L. pinnatifida* | M | 7±0.00 | 7±0.392 | - | 7±0.416 | 7±1.047 | 8±0.503 |
| | DC | - | - | - | - | - | - |
| | M/DC | - | - | - | - | - | - |
| | EA | 10±0.416 | - | 10±0.721 | 9±0.986 | 10±0.416 | - |
| | M/W | - | 8±0.666 | - | 10±0.959 | 12±0.734 | 10±0.416 |
| *B. secundiflora* | M | - | - | - | 7±0.00 | 7±0.392 | 7±0.577 |
| | DC | 8±0.666 | - | 9±0.982 | 7±1.00 | - | - |
| | M/DC | 7±1.00 | - | 8±1.000 | 7±0.392 | - | - |
| | EA | - | - | 8±0.577 | 8±0.493 | 10±0.000 | - |
| | M/W | - | 8±1.201 | - | 10±1.424 | 12±0.763 | 10±0.959 |
| *C. ustulatus* | M | 7±0.333 | - | 9±0.881 | 7±0.881 | 7±0.333 | 7±0.000 |
| | DC | - | - | - | 7±1.000 | - | 7±0.881 |
| | M/DC | 9±0.577 | 9±0.440 | - | - | - | - |
| | EA | - | - | - | 8±1.047 | 10±0.959 | - |
| | M/W | 10±1.527 | 9±1.201 | 19±0.635 | - | 12±0.907 | 15±0.433 |
| *C. officinalis* | M | 13±0.712 | 11±0.763 | 9±0.577 | 7±0.577 | 10±1.424 | 7±0.577 |
| | DC | 8±0.493 | - | - | 16±0.00 | 9±0.881 | 7±0.440 |
| | M/DC | 10±1.386 | 7±0.333 | - | - | 7±0.33 | - |
| | EA | - | - | - | 8±1.201 | - | - |
| | M/W | 9±0.881 | 8±1.33 | - | 9±0.881 | 11±1.000 | 11±0.927 |
| *G. pulchellum* | M | - | 7±0.666 | - | - | - | 7±0.392 |
| | DC | - | 8±1.047 | 10±0.959 | 7±0.440 | - | - |
| | M/DC | 8±1.000 | 7±0.392 | 10±1.201 | 16±0.895 | 12±0.763 | - |
| | EA | - | - | - | - | - | 10±1.424 |
| | M/W | 9±0.577 | 7±0.726 | - | 9±1.154 | 11±0.927 | 19±0.635 |
| *G.multipartita* | M | 8±0.493 | 10±0.392 | 9±1.224 | 10±0.721 | 10±0.721 | 9±1.201 |
| | DC | 7±0.577 | - | - | 10±0.548 | 12±1.000 | - |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | M/DC | 7±0.00 | - | 10±0.959 | 8±0.493 | 9±0.440 | - |
| | EA | - | - | - | - | 15±0.881 | 8±1.047 |
| | M/W | 8±0.666 | 8±0.577 | - | 9±0.986 | 13±0.712 | 16±0.5 |
| Streptomycin | 100µg | 20±0.577 | 29±0.976 | 23±0.768 | 27±0.606 | 10±0.577 | 11±1.000 |
| Ofloxacine | 50µg | 21±0.577 | 30±0.907 | 30±1.013 | 28±0.953 | 25±0.577 | 30±0.866 |

*S. pyogenes: Streptococcus pyogenes*, *S. aureus: Staphylococcus aureus, S. epidermidis: Staphylococcus epidermidis, K. pneumoniae : Klebsciellapneumoniae, P. aeroginosa : Pseudomonas aeroginosa, E. coli: Escherichia coli,* MeOH: methanol, DC: dichloromethane, DC/MeOH: dichloromethanemethanol mixture(50/50), EA: ethylacetate, MW: methanol water (60/40). (-: no/very hazy inhibition zone).

**For Gram-Positive Bacteria**

Only the methanol water extract of *C. ustulatus* showed an inhibition with a diameter superior to 15 mm against *S.epidermidis*, fifteen seaweeds extracts exhibited antibacterial inhibition ranging between 10 mm to 15 mm. All seaweeds tested are active against at least one of the Gram-positive bacteria tested.

**For Negative Bacteria**

Among eight seaweeds, higher inhibition (diameter greater than 15 mm) was observed in the methanol water extract of *G.pulchellum*, *G.multipartita,* and *C.ustulatus* against *E.coli.* The dichloromethane extract of *C.officinalis and C.elongata* and the dichloromethane-methanol extract of *G.pulchellum* exhibited a higher activity against *K.pneumoniae.* Similar activity was obtained by the ethyl acetate extract of *G.multipartita.* A diameter of inhibition ranging between 10 and 15 mm against all Gram-negative bacteria tested was observed in the methanol water extract of *C.elongata, L.pinnatifida, and B.secundiflora.* A weak activity was observed in the extract of *B.thuyoides.* The majority of algal extracts demonstrated antibacterial activity against the tested bacterial species. *E.coli* and *K. pneumoniae* were the most susceptible bacteria to almost all of the seaweed extracts tested. The methanol water extract of *G.pulchellum* demonstrated the highest antibacterial activity (Φ=19 mm) against *E. coli.* The methanol water extract was found to be the most effective among the five solvent extracts tested.

**Antifungal Assay**

The result of the antifungal activity of different extracts using the agar disc diffusion method was summarized in Table-2. Among the five solvents tested, dichloromethane extracts exhibited maximum inhibition on the growth of the tested fungi strains.

Table-2: Antifungal Activity of Seaweeds Tested against Fungal Pathogens Microorganism

| | Diameter of inhibition Φ (mm) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Seaweed Species | M | | DC | | DC/M | | EA | | M/W | | Amphotericin B (100µg) | |
| | *C.t* | *A.n* | *C.t* | *A.n* | *C.t* | *A.n* | *C.t* | *A.n* | *C.t* | *A.n* | *C.t* | *A.n* |
| *C.elongata* | - | - | - | 16±0.76 | - | - | - | - | 10±1.52 | - | | |
| *B.thuyoides* | - | - | 10±0.00 | 15±0.43 | - | - | - | - | - | 10±0.41 | | |
| *L.pinnatifida* | - | - | - | 18±0.86 | - | - | - | - | 9±0.57 | - | | |
| *B.secundiflora* | 7±0.00 | 12±0.33 | - | - | - | - | - | - | - | - | | |
| *C.ustulatus* | 7±1.00 | 20±0.88 | 10±1.52 | - | 10±0.41 | - | - | - | - | - | 23±1.00 | 15±0.57 |
| *C.officinalis* | 9±0.53 | - | - | 14±0.50 | - | - | - | - | - | 12±0.90 | | |
| *G.pulchellum* | 8±1.15 | - | 10±0.85 | 12±1.73 | 10±0.54 | - | - | - | - | - | | |
| *G.multipartita* | 10±0.54 | - | 11±0.76 | - | 10±1.72 | - | - | - | 9±0.60 | - | | |

*A.n :Aspergilusniger, C.t: Candida tropicalis,* MeOH: methanol, DC: dichloromethane, DC/MeOH: dichloromethane methanol mixture(50/50), EA: ethyl acetate, MW: methanol water (60/40). Inactive (-: no/very hazy inhibition zone).

Among the fungi tested, *A.niger* was the most sensitive to almost all of the seaweed extracts, and almost all of the algal extracts exhibited antifungal activity against *A.niger*. *C.tropicalis* was the most resistant strain. The highest antifungal activity (diameter superior to 15 mm) was shown by the methanol extract of *C.ustulatus (Φ=20mm)* and the dichloromethane extract *of L.pinnatifida(Φ=18 mm)*. This activity is higher than that given by amphotericin B (Φ=15mm) against *A.niger.* An important activity was observed in the dichloromethane extract of *C.elongata* showed inhibition (Φ=16 mm) against *A.niger*, followed by the extract of *B.secundiflora* (Φ=15 mm) and *C.officinalis (Φ=14mm)* prepared by the dichloromethane. However, no inhibition was observed in the ethyl acetate extract of seaweeds tested against both fungi strains. Fungi are eukaryotic cells with a single nucleus, which prevents the action of antimicrobial drugs, which explains their low antifungal activity.[19] Among the five solvent extracts tested, the dichloromethane extract was found to be the most effective.

**Minimum Inhibitory Concentration (MIC)**

Evaluation of algal antimicrobial effect has also been performed based on MIC values. Under normal circumstances, the MIC is the minimum inhibitory concentration of an active ingredient that prevents observable growth. In this regard, estimated MIC values ranged between 5–10000 µg/ml for bacteria (Table-3). Whereas, it varied between 3.5 and 5000 µg/ml for fungal isolates(Table-4).

Table-3: Algal Minimum Inhibitory Concentration Values Against Bacterial Strains

| Algal minimum inhibitory concentration | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Gram$^+$ | | | Gram$^-$ | | |
| Seaweed | Solvent extraction | *S.pyogenes* | *S. aureus* | *S.epidermidis* | *K.pneumoniae* | *P.aeroginosa* | *E. Coli* |
| *C. elongata* | M | 117 | 117 | 625 | 58 | 7500 | 58 |
| | DC | 312 | 5000 | 2500 | 20 | 2500 | 312 |
| | M/DC | 156 | 312 | 1250 | 1250 | 1250 | 625 |
| | EA | 78 | 78 | 39 | 78 | 78 | 1250 |
| | M/W | 78 | 78 | 312 | 78 | 39 | 78 |
| *B.thuyoides* | M | 117 | 117 | 1875 | 58 | 117 | 58 |
| | DC | 2500 | 1250 | 1250 | 156 | 2500 | 625 |
| | M/DC | 156 | 312 | 156 | 625 | 156 | 1250 |
| | EA | 78 | 1250 | 312 | 156 | 78 | 1250 |
| | M/W | 156 | 156 | 2500 | 78 | 156 | 78 |
| *L. pinnatifida* | M | 234 | 117 | 937 | 117 | 234 | 117 |
| | DC | 156 | 1250 | 5000 | 625 | 5000 | 312 |
| | M/DC | 625 | 10000 | 2500 | 625 | 625 | 2500 |
| | EA | 39 | 2500 | 78 | 156 | 78 | 1250 |
| | M/W | 1250 | 156 | 1250 | 78 | 39 | 78 |
| *B. secundiflora* | M | 468 | 1870 | 3750 | 234 | 117 | 234 |
| | DC | 156 | 625 | 312 | 156 | 2500 | 1250 |
| | M/DC | 156 | 625 | 156 | 156 | 312 | 5000 |
| | EA | 625 | 625 | 156 | 156 | 78 | 625 |
| | M/W | 2500 | 78 | 1250 | 78 | 39 | 156 |
| *C. ustulatus* | M | 117 | 234 | 58 | 117 | 117 | 117 |
| | DC | 2500 | 1250 | 5000 | 156 | 5000 | 156 |
| | M/DC | 78 | 156 | 2500 | 1250 | 2500 | 10000 |
| | EA | 5000 | 312 | 1250 | 156 | 78 | 312 |
| | M/W | 78 | 156 | 10 | 2500 | 39 | 20 |
| *C. officinalis* | M | 29 | 58 | 117 | 117 | 58 | 234 |
| | DC | 156 | 2500 | 625 | 39 | 156 | 312 |
| | M/DC | 78 | 156 | 5000 | 2500 | 312 | 5000 |
| | EA | 312 | 1250 | 2500 | 156 | 5000 | 312 |
| | M/W | 78 | 156 | 1250 | 312 | 625 | 156 |
| *G. pulchellum* | M | 468 | 234 | 234 | 469 | 469 | 117 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | DC | 312 | 156 | 78 | 156 | 2500 | 625 |
| | M/DC | 156 | 312 | 78 | 20 | 78 | 2500 |
| | EA | 625 | 2500 | 1250 | 312 | 312 | 78 |
| | M/W | 156 | 312 | 5000 | 156 | 78 | 5 |
| *G.multipartita* | M | 117 | 58 | 117 | 117 | 78 | 117 |
| | DC | 156 | 312 | 5000 | 156 | 78 | 312 |
| | M/DC | 156 | 312 | 78 | 156 | 156 | 1250 |
| | EA | 2500 | 625 | 2500 | 312 | 39 | 156 |
| | M/W | 156 | 156 | 5000 | 78 | 39 | 19 |
| Ofloxacine | 50µg | 0.97 | 0.06 | 0.06 | 0.24 | 0.97 | 0.12 |
| Streptomycin | 100µg | 1.9 | 0.12 | 0.48 | 0.78 | 2.4 | 1.9 |

M: methanol, DC: dichloromethane, M/DC: methanol-dichloromethane mixture (50/50), EA: ethyl acetate, M/W: methanol-water (60/40).

The best MIC (5µg/ml) value was recorded for the methanol water extract of *G.pulchellum* against *E.coli,* followed by the dichloromethane extract of *C.ustulatus* (10µg/ml) against *S.epidermidis*.

Table-4: Algal Minimum Inhibitory Concentration Values against Fungal Strains

| Seaweed species | Algal minimum inhibitory concentration (MIC) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | | DC | | DC/M | | EA | | M/W | | Amphotericin B (100µg/mL) | |
| | *C.t* | *A.n* | *C.t* | *A.n* | *C.t* | *A.n* | *C.t* | *A.n* | *C.t* | *A.n* | *C.t* | *A.n* |
| *C. elongata* | 469 | 234 | 1250 | 20 | 312 | 2500 | 625 | 1250 | 78 | 625 | 0.61 | 0.48 |
| *B.thuyoides* | 468 | 1870 | 78 | 39 | 625 | 312 | 1250 | 1250 | 5000 | 156 | | |
| *L. pinnatifida* | 234 | 234 | 312 | 5 | 625 | 312 | 5000 | 2500 | 156 | 312 | | |
| *B. secundifloira* | 117 | 58 | 2500 | 2500 | 1250 | 5000 | 5000 | 2500 | 2500 | 1250 | | |
| *C. ustulatus* | 117 | 3.5 | 78 | 5000 | 78 | 312 | 312 | 625 | 312 | 625 | | |
| *C. officinalis* | 58 | 234 | 2500 | 39 | 625 | 1250 | 1250 | 625 | 1500 | 39 | | |
| *G. pulchellum* | 117 | 468 | 78 | 39 | 156 | 325 | 625 | 1250 | 156 | 2500 | | |
| *G. multipartita* | 58 | 234 | 78 | 625 | 78 | 312 | 312 | 625 | 625 | 312 | | |

*A.n: Aspergilus niger, C.t: Candida tropicalis*, MeOH: methanol, DC: dichloromethane, DC/MeOH: dichloromethane methanol mixture(50/50), EA: ethyl acetate, MW: methanol water (60/40)

The best MIC was obtained against *A. niger,* (3.5 µg/ml) value was recorded for the methanol extractof *C.ustulatus,* followed by the dichloromethane extract of *L.pinnatifida* (5 µg/ml).

In the present study, eight seaweed species were tested for their antibacterial activity. Extracts of different species inhibited bacteria with diverse degree. This difference in activity can be explained by several factors, the most important of which are: the method and solvent used for extraction, bacteria used for evaluation of antibacterial activity. Effects of habitat and harvested season are also important.[18] A few researchers tested the antibacterial activity of seaweeds using various solvents and compared the results. Filho *et al*., 2002 stated that hexane was a suitable solvent for extracting the antibiotic principle.[20] Dichloromethane proved to be the most effective solvent among several others in a different investigation that extracted antibiotic compounds[21]. Good antibacterial activity was also demonstrated by some additional investigations that used ethyl acetate and chloroform to extract seaweeds.[18] According to a study, tests using seaweed methanol extracts revealed broad-spectrum antibacterial efficacy against human pathogenic microorganisms.[11,22,23] In contrast, the current study found that the most effective organic solution for removing the potent antibacterial substance from the nearly examined algal species was methanol-water. Three species of the eight algae studied had a favorable action against the bacteria *E.coli.* A significant activity with a diameter of inhibition greater than 15 mm was found in the methanol-water extracts of *G.pulchellum, G.multipartita, and C.ustulatus* against *E.coli*. This result is in agreement with those obtained by Dayuti *et al.,* 2018, who observed that among all the extracts of *Gracilaria verrucosa*, the highest inhibition against *E coli* was obtained with a methanol-water ratio of 75:25 and for 72 hours of extraction.[24] A moderate activity was observed in the methanol water extract of *G.multipartita,* which inhibed *P.aeroginosa* with a diameter of inhibition of 13 mm. Similar activity was obtained by

Bala and Pushparaj, 2014, but in the methanol extract of *Gracilaria corticata* against *P.aeruginosa* (Φ=13±0.8 mm).[25] Ethyl acetate of *G.multipartita* exhibited a maximum inhibition against *P.aeroginosa* with a diameter of inhibition equal to 15 mm, while Abdellatif *et al.*, 2018 reported that ethyl acetate extract of the red algae *Grateloupia doryphora*[26] had a strong effect on *Pseudomonas aeroginosa* with an inhibition zone of 27 mm. Against the Gram-positive bacteria *S.epidermidis*, the methanol water extract of *C.ustulatus* showed the highest mean zone of inhibition, 19mm. Alghazeer *et al*., 2013 reported that *S. epidermidis* was more susceptible to the red algae *Gelidium latifolium, Hypnea musciformis* extracts, with a large high inhibition zone (Φ=15 mm)[27]. Similarly, dried ethyl acetate extract of *Gracilaria doryphora*[28] is beneficial in inhibiting the growth of *P. aeruginosa* according to Kanimozhi and Sridhar (2017). Among the algae tested, the presence of a best activity on *K. pneumoniae* was observed in dichloromethane extract of *C.elongata* and *C.officinalis*, with a diameter of inhibition of 16 mm, An inhibition was seen in the *G. pulchellum* dichloromethane/methanol extract for a diameter of 16 mm. this finding was consistent with the results of Rhimou *et al.*, 2010 which reported that species of red algae had an antibacterial potential on *K. pnemoniae.*[29] Magallanes *et al*., 2003, found that ethanolic extracts of Rhodophyceae[30] from *Grateloupia doryphora, Rhodymenia flabellifolia, Gracilariopsis lemaneiformis, Ahnfeltiopsis durveillaei, Porphyra columbina,* and *Cryptopleura sp*. inhibited the growth of the bacteria *E. coli ATCC 25922.* Only two out of the six pathogenic bacterial strains exhibited higher inhibition zones. However, these two strains *(K. pneumoniae and E. coli*) are all Gram-negative; these findings are according with those of Adaikalaraj G *et al.*, 2012[31], who stated that, as compared to Gram-positive bacteria (*B. subtilis and S. aureus*), red seaweed extracts were more efficient against Gram-negative pathogens (*P. aeruginosa and E. coli*). Hydrophilic substances like lipopolysaccharide and "porins" found in gram-negative bacteria's outer membrane act as barriers against hydrophobic chemicals. Antibacterial chemicals may be transported and enter bacterial cells more easily if to the study's findings, bacteria classified as Gram-positive or negative have a different cell wall composition and structure, making them more susceptible to the effects of algal extracts. Also in favor of this result were earlier studies employing a variety of algae species[32]. Furthermore, a lot of environmental chemicals, including antibiotics, target the lipids in Gram-negative bacteria's outer membrane, whereas Gram-positive bacteria's thick murine layer prevents inhibitor access[33]. Concerning the antifungal activity, the methanol extract of *C.ustulatus* inhibited *A. niger* with a diameter of inhibition equal to 20mm. These results are in agreement with the observations of Pandurngan *et al*., 2010, who reported that the extract of *Gracilaria cortica* and *Kappahycus alvarezii* prepared with methanol showed the best activity[34] (30 and 25 mm) against *A.niger,* respectively. Among eight seaweeds tested, the dichloromethane extract of three algae species exhibited a higher antifungal activity against *A.niger* with a diameter of inhibition higher than 15 mm. These results were consistent with those found by Moujahid *et al.*, 2004, who reported that some marine algae extracted by dichloromethane showed antifungal activity[35] against *A.niger*. These results showed that dichloromethane has a higher extraction capacity compared to that of the other solvents, and that red algae probably contained many substances having an antifungal effect. Wefqy *et al.*, 2008 reported that acetonic *Laurencia pinnatifida* and *Sargassum hystrix* extracts displayed the highest antifungal[36] effect against *A. niger* with a diameter of inhibition of 16 and 26 mm. The present investigation showed that the dichloromethane extract of *L.pinnatifida* exhibited the highest inhibitory activity against *A.niger* with a diameter of inhibition of 18mm. Saleh *et al*., 2018 reported that the acetone extract of *Corallina mediterranea* displayed a good antifungal effect against *A.niger* with a diameter of inhibition of 15 mm[37]. The present investigation showed a maximum activity of the dichloromethane extract of *C.elongata* against *A.niger* (Φ= 16 mm), followed by the dichloromethane extract of *B.thuydoies*(Φ=15 *mm).* The present study demonstrated that *A.niger* was the most sensitive to all the seaweed extracts except ethyl acetate and the dichloromethane-methanol mixture extract. This finding is consistent with the research of Naqvi *et al.* 1980, who demonstrated the ability of seaweed to inhibit the animal mould *Aspergillus niger.*[38] Therefore, the current study's results differ from previous studies in that all of the extracts showed strong antifungal efficacy against nearly all fungal pathogens. Depending on the solvent used and the fungal species, varying reactions of the investigated algal extracts to the fungi examined were observed. In this regard, *Candidas tropicalis* exhibited the highest resistance of all the extracts. Similar findings[39,40] by Sheikh *et al*., 2018, and Vimala *et al.*, 2017. Among all seaweeds extract tested, methanol extract and

dichloromethane extract of two marine algae showed a low antifungal activity against *C.tropicalis* with a diameter of inhibition of 10mm. The inhibition of mycelial growth of these fungi is probably due to interactions between the substances presented in the extracts. This interaction probably results in lipid peroxidation, resulting in morphological alterations of the thalli of the fungus.[41,42] The agar dilution method showed MIC values ranged from 5µg/ml to 10mg/ml for each of the bacteria strains that were inhibited. The ability of a substance to inhibit microbial growth at low concentrations is indicated by a low minimum inhibitory concentration (MIC) value[43]. The methanol water extract of *G.pulchellum* and of *C.ustulatus* was the most effective against *E.coli* and *S.epidermidis* with MIC 5 µg/ml. Balasankar *et al*., 2014 reported that the methanol extract[25] of *Gracilaria cortica* exhibited the maximum growth inhibition against *S.aureus*, and the MIC was 1.25µg/ml. The methanol extract of *C.ustulatus* was the most sensitive against A. *Aniger* with an MIC of 3.5µg/ml, followed by the dichloromethane extract of *L.pinnatifida,* which exhibited a higher inhibition against *A. niger* with an MIC of 5µg/ml. It was reported that *Laurencia dendroidea* extracts were found to have a fungistatic effect[44] on *Candida albicans*, with a minimum inhibitory concentration (MIC) of less than 31.25 µg/ml. Al-Enazi *et al.*, 2018 reported antifungal activity of tree seaweeds[45] against *C.tropicalis*. The previous investigation showed that the ethanol extract of *Laurencia majuscule* displayed a higher antifungal effect with an MIC of 0.95µg/ml.

## CONCLUSION

The present study showed that different species of algae studied have an antibacterial or antifungal potential. The obtained result suggests that extracts from the seaweed species employed in the current study demonstrated greater antibacterial activity against the pathogens tested. All of the red marine algae's methanol, water, and dichloromethane extracts generally show antibacterial action against gram-positive and gram-negative bacteria, with *G. pulchellum* and *C. ustulatus* showing particularly high levels of antibacterial activity. Dichloromethane extract of seaweeds showed a higher antifungal activity against *A. niger*. The observed inhibition of *E. coli* and *A.niger* indicates that the antimicrobial chemicals found in three of the studied algae may successfully restrict growth when extracted using dichloromethane and methanol-water as the solvents for the antifungal and antibacterial activities, respectively. Based on the present finding, Moroccan seaweeds are a possible source of novel substances that might be exploited in the medical industry.

## ACKNOWLEDGMENTS

The authors thank the Moroccan Ministry of Higher Education and Research for its support for this study.

## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

## AUTHOR CONTRIBUTIONS

The present work is related to *SDG-2: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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[RJC-8315/2025]