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# Research Article
# Antioxidant and Antifungal Activity of Some Moroccan Seaweeds Against Three Postharvest Fungal Pathogens

[1]Lahbib Fayzi, [2]Latifa Askarne, [3]El Houssine Boufous, [4]Ouafa Cherifi and [1]Khalil Cherifi

[1]Laboratory of Biotechnologies and Valorization of Natural Resources, Faculty of Sciences, Ibn Zohr University, Agadir, Morocco
[2]Laboratory of Microbial Biotechnology and Plant Protection, Faculty of Sciences, Ibn Zohr University, Agadir, Morocco
[3]Department of Biochemistry and Microbiology, Laval University, Quebec City (Quebec), Canada
[4]Laboratory of Hydrobiology, Ecotoxicology, Sanitation and Global Changes, Cadi Ayyad University, Marrakech, Morocco

## Abstract

**Background and Objective:** Seaweeds are the most abundant marine resource, including novel bioactive molecules that exhibit a wide range of biological activities. The use of these resources to develop eco-friendly antifungal products is a suitable alternative to chemical fungicides in controlling postharvest decays. **Materials and Methods:** In this study, aqueous extracts of four seaweeds were collected from the Moroccan Atlantic Coastline (Agadir) *Bifurcaria bifurcata*, *Corallina officinalis*, *Codium tomentosum* and *Ulva fasciata* were phytochemically investigated and evaluated regarding their antioxidant and antifungal properties. The total phenolic and flavonoid contents were measured using the Folin-Ciocalteu and aluminium chloride methods, respectively. The antifungal activity was assessed *in vitro* against 3 postharvest phytopathogenic fungi *Penicillium digitatum*, *Penicillium expansum* and *Penicillium italicum*. Furthermore, the antioxidant activity was determined by DPPH assays. **Results:** The studied algal extracts showed significant variation in antioxidant activity, whereas, in most of the cases, the analysis revealed remarkable antioxidant capacity and high total phenolic and flavonoid contents. Specifically, *B. bifurcata* followed by *U. fasciata* contained the highest levels of TPC, TFC and exhibited higher antioxidant activity ($IC_{50}$ values of 0.18 and 0.23 mg $mL^{-1}$, respectively). These extracts also exhibited strong antifungal activity, particularly that of *B. bifurcata*, which proved to be more effective in reducing the mycelial growth of *P. digitatum*, *P. italicum* and *P. expansum*. **Conclusion:** The present findings suggest that local seaweeds, especially *B. bifurcata* and *U. fasciata* are potential sources of bioactive compounds and should be investigated for natural fungicides to control or delay fruit postharvest decay.

Key words: Seaweeds, postharvest pathogen, DPPH, phytochemicals, bioactive compounds, *Penicillium*, biofungicides

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Corresponding Author: Khalil Cherifi, Laboratory of Biotechnologies and Valorization of Natural Resources, Faculty of Sciences of Agadir, Ibn Zohr University, BP 8106, 8000 Agadir, Morocco

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Fresh fruits are often exposed to many postharvest diseases, mainly due to pathogenic fungi associated with decay. During storage and transportation, postharvest fungi affect significantly the shelf life of fresh agro-commodities leading to a loss of quality, reduction of nutrients, mycotoxin production and thereby reduced market value. The use of synthetic fungicides remains the primary means of controlling postharvest diseases. Nevertheless, the global trend appears to be shifting towards limiting their use and substituting them with alternative methods like biofungicides[1,2]. In this regard, transition to more sustainable agriculture requires less use of harmful chemical pesticides to preserve the environment and human health[3]. Furthermore, chemical control generally provides effective protection against pathogen diseases of crops, but their effectiveness is compromised because of the rapid adaptation of the pathogenic populations over time[4]. Hence, this serious worldwide problem has prompted researchers to explore new sources of bioactive compounds to control fungal pathogens, with low side effects and no toxicity. The marine environment and its organisms constitute an important reservoir of original active natural products. However, these metabolites are still under explored for their various structures and functional properties[5]. Among marine organisms, seaweeds, which are macroscopic marine algae that are adapted to harsh conditions. They can develop chemical defences to prevent themselves from environmental stresses and predation, including defence against microbial pathogens[6]. Compared with terrestrial plants, their secondary metabolites have original and unique structures and are synthesized by different biochemical pathways owing to their complex living environment. Furthermore, they can produce high biomass yield and do not depend on cultivable land and freshwater[7].

Recent reports demonstrated that marine macroalgae are rich sources of several natural bioactive products with a wide variety of pharmacological properties, such as antibacterial, antiviral, antifungal, anticancer and antioxidant activities[8,9]. They have received increasing attention both in crop treatments and in postharvest diseases management because of their powerful antifungal activity, non-phytotoxicity, systemic effect and biodegradability[10]. Several studies have been focused on screening their extracts to develop new antifungal compounds for the control of postharvest diseases as a potential alternative or complements to synthetic fungicides[11-13]. Thus, extracts from edible seaweed *Osmundea pinnatifida* have been shown antifungal activity against *Alternaria infectoria* and *Aspergillus fumigatus* inhibiting growth and conidiation[14]. In *in vitro* experiments, polysaccharides from the three seaweeds *Anabaena* sp., *Ecklonia* sp. and *Jania* sp. reduced both the strawberry fruit-infected area and the pathogen sporulation in the preharvest treatment[15]. On the same fruit, *Ascophyllum nodosum* seaweed extract was able to reduce the soft rot incidence caused by the infection of *Rhizopus stolonifer* in the postharvest stage[16]. Extracts from the two brown macroalgae *Undaria pinnatifida* and *Laminaria digitata* and from the red one *Porphyra umbilicalis* strongly reduced brown rot disease on peaches, grey mould growing on strawberries and green mould on lemons at a dose of 30 g $L^{-1}$ through *in vivo* experiments[12]. Also, the effectiveness of 2 brown seaweed extracts in postharvest control of grey mould of tomato has been reported against the mycelial growth of *Botrytis cinerea* by Bahammou *et al.*[8]. In the same way, Omar[17] investigated the use of seaweed extracts to control postharvest decay on orange fruit and suggested them as a suitable means for improving fruit storability and quality of Navel orange fruit when compared with synthetic chemicals. Strong fungus-inhibitory effects of some Brazilian seaweed extracts were observed against anthracnose in papaya and banana caused by *Colletotrichum musae* during storage[18].

Generally, limited research is available on the activity of seaweed extracts against plant pathogenic fungi. The potentiality of using marine macroalgal biomass and its components in biological control constitutes a promising issue. Indeed, Moroccan coastlines are endowed with an important biodiversity reserve of seaweeds with more than 500 species that have not been completely explored[19]. Interestingly, this algal richness represents an important source of bioactive natural substances with multifunctional properties. However, these resources having high economic value are poorly exploited and need advanced investigations, especially, for novel natural compounds that have innovative applications. Investment in this area requires the inventory of Moroccan algal diversity on both its Atlantic and Mediterranean coasts as well as the development of new strategies to manage and valorize these marine natural resources, which represent a source of useful products[7,20].

In this context, this work aimed to evaluate *in vitro* antifungal activity of aqueous extracts, prepared from four locally abundant seaweeds *Bifurcaria bifurcata*, *Corallina officinalis*, *Codium tomentosum* and *Ulva fasciata* collected from the Moroccan Atlantic Seashore (North of Agadir City) against three postharvest phytopathogenic fungi *Penicillium digitatum*, *Penicillium expansum* and *Penicillium italicum*. The antioxidant activity of the extracts was also determined by DPPH assays and the total phenolic and flavonoid content was

evaluated spectrophotometrically using the Folin-Ciocalteu and aluminium chloride methods, respectively.

## MATERIALS AND METHODS

**Study area:** The study was carried out at the Laboratory of Biotechnologies and Valorization of Natural Resources (LBVRN), Faculty of Sciences, Agadir, Morocco from May, 2017-October, 2019. The fungal strains were obtained from the culture collection of the Microbial Biotechnology and Plant Protection Laboratory at the same institution.

**Seaweeds collection and preparation:** Four intertidal seaweeds *Corallina officinalis* (Rhodophyceae), *Ulva fasciata* and *Codium tomentosum* (Chlorophyceae) and *Bifurcaria bifurcata* (Phaeophyceae) were harvested from the Moroccan Atlantic Shoreline at Tamri National Park area, northern Agadir during low tide in May, 2017. Soon after they arrived in the laboratory, the algae were cleaned, carefully rinsed with distilled water to remove salt, epiphytes and sand particles and thereafter were air-dried in the shade at room temperature. The dried algae were reduced into a fine powder, sealed in an airtight container and preserved at 4°C until they were analyzed.

**Extract preparation:** The extraction with water, which can dissolve so many organic compounds due to its polar nature, was carried out using the cold maceration method as described by Sridharan and Dhamotharan[21] with modifications. In comparison to heat extraction, this approach was chosen since it is less harmful to most chemical compounds. A weighed amount of 20 g of each seaweed powder was macerated in 200 mL of distilled water for 48 hrs with continuous agitation at room temperature. After that, the macerate was sonicated for 30 min in an ultrasonic bath and then filtered. To remove the water, the filtrates were vacuum evaporated in a rotary evaporator at 40°C. The resultant extracts were then stored in sealed brown glass vials at -4°C until their use for analysis.

***In vitro* antifungal test:** Three pathogenic fungi, *Penicillium digitatum*, *Penicillium expansum* and *Penicillium italicum*, were obtained from the culture collection of the Microbial Biotechnology and Plant Protection Laboratory of Faculty of Sciences, Ibn Zohr University. The agar plates method was used to screen for antifungal activity of the four seaweed extracts according to the protocol described by Karim *et al.*[2] with slight modifications. The different seaweed aqueous extracts (at 5 mg $mL^{-1}$) were added to the Potato Dextrose Agar (PDA) medium and then autoclaved for 15 min. Agar plates were inoculated with one of the three fungal pathogens using 5 mm diameter agar discs, taken from the margins of actively growing fungal colonies (one-week-old cultures). To maximize contact, the plug was positioned on the agar surface with its mycelial surface facing down. The agar plates were then incubated at 25°C for 6 days. The control consisted of an unamended PDA medium. The average radial growth was determined by measuring colony diameters along with 2 perpendicular directions. The antifungal activity was expressed in terms of the percentage of mycelial growth inhibition and calculated according to the formula suggested by[22] as follows:

$$\text{MGI (\%)} = \frac{C - T}{C} \times 100$$

Where, MGI (%) is the percentage of mycelial growth inhibition, C and T represent mean mycelial growth diameter in control and in treated Petri dishes, respectively.

All tests were performed in triplicates under a completely random design and each experiment was repeated twice.

**Antioxidant activity:** The DPPH (2,2-diphenyl-1-picrylhydrazyl) reactivity test is one of the simplest and most reliable methods for estimating *in vitro* antioxidant activity. Therefore, this accurate analysis is recommended as a simple and rapid way to evaluate antioxidants. The hydroxyl radical scavenging activity of the different seaweed extracts was assayed according to the method described by Ganesan *et al.*[23] with slight modifications. Initially, 0.1 mM DPPH solution was prepared freshly in methanol and kept protected from light. Two millilitres of this solution was added to the test tube containing a 2.0 mL aliquot of samples. The mixture was vortexed for 1 min and kept at room temperature for 30 min in the dark. The sample solutions were tested by measuring of bleaching purple coloured methanol solution of DPPH radical spectrophotometrically at 517 nm. BHT (butylated hydroxytoluene) was used as positive control and distilled water as blank control. The ability to scavenge DPPH radical (%) was evaluated by applying the formula proposed by Duan *et al.*[24]:

$$\text{Scavenging effect (\%)} = 1 - \frac{A_{\text{sample}} - A_{\text{sample blank}}}{A_{\text{control}}} \times 100$$

where, the $A_{sample}$ is the absorbance of the test sample (DPPH solution plus test sample), the $A_{sample\ blank}$ is the absorbance of the sample only (sample without DPPH solution) and the

$A_{control}$ is the absorbance of the control (DPPH solution without samples). The scavenging capacity of the various extracts was compared using their corresponding $IC_{50}$. This parameter was used to express the concentration of the selected antioxidant sample extracts needed to scavenge 50% of the DPPH radical and it was calculated by plotting the inhibition percentages against the extract concentrations. The higher $IC_{50}$ value reflects the lower antioxidant activity of the examined samples. The test was carried out in triplicate and $IC_{50}$ values were reported as Means±SD.

**Total phenolic content:** Phenolic contents of the four seaweed extracts were estimated by Folin-Ciocalteu reagent method[25,26]. A volume of 125 µL of each extract was mixed with 2.0 mL of 2% $Na_2CO_3$. The mixture was allowed to stand for two minutes at room temperature. After that, 125 µL of 50% Folin-Ciocalteu phenol reagent was added and mixed thoroughly and then the solution was incubated at 28°C for 30 min in the dark. The absorbance was measured at a wavelength of 765 nm against a blank sample and compared with a standard gallic acid calibration curve. The phenolic contents were expressed as Milligram (mg) gallic acid equivalents per gram of dry weight (mg GAE/g DW).

**Total flavonoid content:** Flavonoids were quantified by the colorimetric method using aluminium chloride ($AlCl_3$) described by Brighente *et al.*[27] with minor adjustments. Briefly, 1 mL of sample solution, dissolved in methanol, was added to 1 mL of the solution of $AlCl_3$ (2% in methanol). The mixture was vigorously stirred, then incubated in the dark at room temperature for 30 min. The absorbance was measured spectrophotometrically at 415 nm and the flavonoid content of the seaweed extracts were obtained by referring to a calibration curve for rutin. The total flavonoid contents were expressed as mg of rutin equivalents per g of the dry weight of seaweed extracts. All samples were analyzed in triplicate.

**Statistical analysis:** All assays were carried out in triplicates and values, expressed as a percentage, were arcsine square-root transformed before performing statistical analysis to normalize the data and improve the homogeneity of variance[28]. The data were subjected to statistical analysis of variance (ANOVA), followed by the Student-Newman-Keuls post hoc test. A difference was considered to be statistically significant when $p<0.05$. Principal Component Analysis (PCA) was also performed to investigate the correlation between the determined parameters and simplify the interpretation of the results. All statistical analysis was performed using Xlstat software for windows version 2016[29,30].

## RESULTS

**Total phenolic (TPC):** Total phenol content was estimated by the Folin-Ciocalteu colorimetric method in comparison with standard gallic acid and the results were expressed in terms of mg GAE/g dry extract. The obtained gallic acid calibration curves were used for the calculation of the total phenolic amount in the four seaweed extracts. According to these results, the aqueous extracts of seaweeds studied had an important charge of phenols and their values varied greatly from one species to another. It ranged from 0.42±0.33-14.26±0.41 mg gallic acid equivalents per g of dry weight (mg GAE/g) (Table 1). The amounts of phenolic compounds in the brown macroalgae *Bifurcaria bifurcata* extract showed the highest TPC and the lowest amount was revealed in the *Codium tomentosum* extract.

**Total flavonoid contents (TFC):** As in the case of total phenolic content, the concentration of flavonoids in the studied extracts varied significantly among the seaweed species. Their values, ranging from 1.20±0.15 to 9.16±0.18 quercetin equivalents of the dry weight of the seaweed extract (mg QE/g), were in the descending order *Bifurcaria bifurcata* > *Ulva fasciata* > *Corallina officinalis* > *Codium tomentosum* (Table 1).

**Antioxidant activity:** The antioxidant activities were determined using a DPPH assay which is simple, sensitive and widely used for investigating the free radical scavenging activities of extracts prepared using various solvents. The radical-scavenging capacities of the studied seaweeds as well as the standard BHT, were determined by using the $IC_{50}$. This parameter represented the concentration of extract required to scavenge 50% of the free radicals and a lower $IC_{50}$ value reflects better protective action. As shown in Table 2, antioxidant activity was detected in all of the tested aqueous seaweed extracts with some variation. Higher DPPH scavenging activities were observed in the extract of the brown seaweed *Bifurcaria bifurcata* and the green seaweed *Ulva fasciata* with $IC_{50}$ values of 0.18 and 0.23 mg $mL^{-1}$, respectively. In contrast, *Corallina officinalis* and *Codium*

Table 1: Total phenolic content (TPC) and total flavonoid content (TFC) of the four aqueous seaweed extracts

| Seaweeds | TPC (mg GAE/g) | TFC (mg QE/g) |
|---|---|---|
| *Bifurcaria bifurcata* | 14.26±0.41[a] | 9.16±0.18[a] |
| *Corallina officinalis* | 1.07±0.40[c] | 2.21±0.12[c] |
| *Ulva fasciata* | 10.11±0.17[b] | 6.16±0.71[b] |
| *Codium tomentosum* | 0.42±0.33[d] | 1.20±0.15[d] |

Values are given as Mean±SD (n = 3). Means in each column not followed by the same letter are significantly different ($p<0.05$)

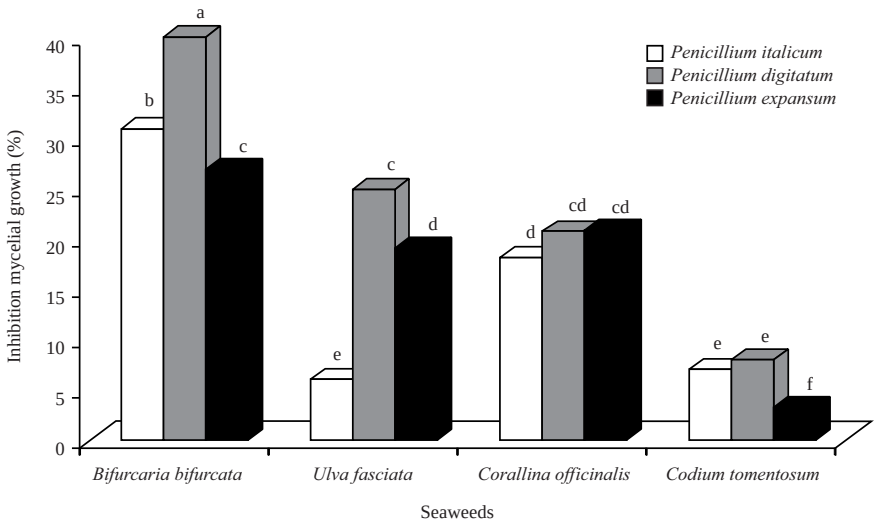


Fig. 1: *In vitro* effects of the four aqueous seaweed extracts on mycelial growth of the tested pathogenic fungi
Values (means of three measurements) having the same letter are not significantly different (p<0.05) (Newman-Keuls test)

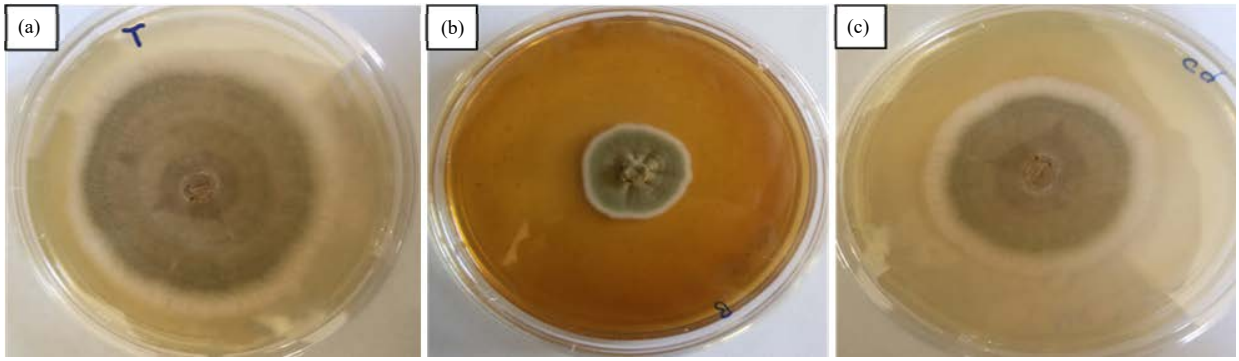


Fig. 2(a-c): Antifungal activity of aqueous extracts against *Penicillium digitatum*, (a) Control and (b) Extract of *Bifurcaria bifurcata* and (c) Extract of *Codium tomentosum*

Table 2: DPPH radical scavenging activity ($IC_{50}$) detected in the selected seaweed aqueous extracts

| Seaweeds | $IC_{50}$ (mg $mL^{-1}$) |
|---|---|
| *Bifurcaria bifurcata* | $0.18\pm0.02^{d}$ |
| *Corallina officinalis* | $0.72\pm0.15^{b}$ |
| *Ulva fasciata* | $0.23\pm0.27^{c}$ |
| *Codium tomentosum* | $0.87\pm0.17^{a}$ |
| Control BHT | $0.02\pm0.04^{e}$ |

Values are given as Mean±SD (N = 3). Means in each column not followed by the same letter are significantly different (p<0.05), $IC_{50}$: Concentration of extract which scavenged 50% of the DPPH free radical, BHT: Butylated hydroxytoluene

*tomentosum* showed significantly the lowest scavenging activities ($IC_{50}$ = 0.72 and $IC_{50}$ = 0.87 mg $mL^{-1}$, respectively).

**Antifungal test:** The evaluation of the 4 seaweed aqueous extracts (5 mg $mL^{-1}$) tested for their effects on mycelial growth revealed significant antifungal activity of all tested samples (Fig. 1). Indeed, the inhibitory impact and pathogen sensitivity of the different seaweed extracts varied considerably when compared with the control (Fig. 2a). Aqueous extract of *Bifurcaria bifurcata* revealed the best activity against most tested organisms, especially *P. digitatum* followed by *P. italicum* (Fig. 2b) and *P. expansum* with 40, 30 and 27% of mycelial growth inhibition respectively. Extract of *Corallina officinalis* also affected the growth of the three postharvest pathogens, but to a lesser degree, with inhibition percentages of 18% for *P. italicum* and nearly 21% in the case of both *P. digitatum* and *P. expansum*. However, the green macroalgae *U. fasciata* and *C. tomentosum* showed a significantly lower activity, chiefly towards *P. italicum* (less than 8%) (Fig. 2c). Interestingly, this last fungus was generally the less sensitive pathogen to the antifungal effect of all the seaweed extracts if compared to the other 2 fungi.

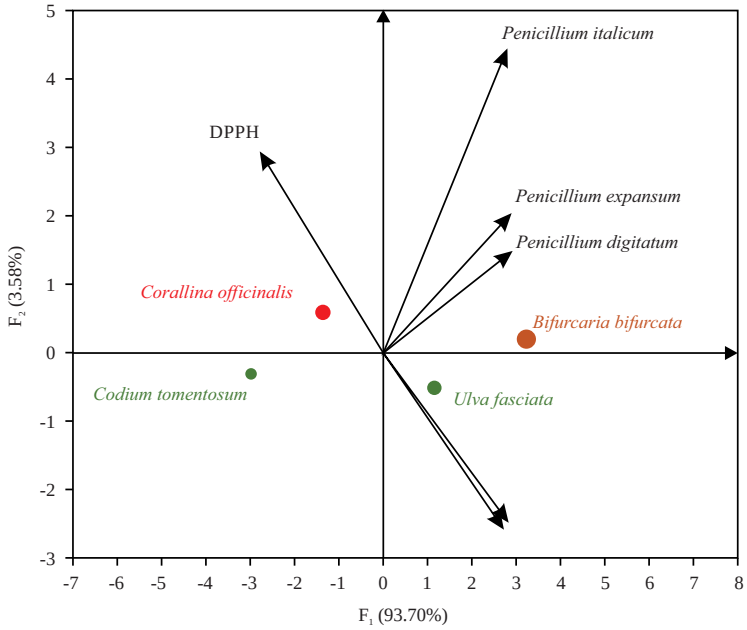


Fig. 3: Biplot of the principal component analysis based on phytochemical contents, antioxidant and antifungal activities of the four seaweed extracts
TPC: Total phenolic content, TFC: Total flavonoid content, DPPH: Antioxidant activity by DPPH (2,2-diphenyl-2-picrylhydrazyl) method

Table 3: Loadings, eigenvalues and percentage of variance for the first two principal components

| | $F_1$ | $F_2$ |
|---|---|---|
| TPC | 0.410 | -0.360 |
| TFC | 0.398 | -0.378 |
| DPPH | -0.404 | 0.427 |
| *P. italicum* | 0.402 | 0.641 |
| *P. digitatum* | 0.419 | 0.215 |
| *P. expansum* | 0.416 | 0.296 |
| Eigenvalues | 5.622 | 0.215 |
| Variance (%) | 93.699 | 3.575 |
| Cumulative % | 93.699 | 97.274 |

TPC: Total phenolic content, TFC: Total flavonoid content, DPPH: Antioxidant activity by DPPH (2,2-diphenyl-2-picrylhydrazyl) method

**Principal component analysis (PCA):** After examining each bioactivity assay and the amount of phenolic and flavonoid compounds separately, principal component analysis was carried out to explore the interactions between antifungal and antioxidant activity as well as TPC and TFC contents of the studied seaweed extracts. This multivariate analysis was applied to reduce the number of original variables and enable to obtain more information on the variables that mainly influence the seaweed extracts similarities and differences. The eigenvalues and contributions of the principal components obtained by the PCA analysis are shown in Table 3. It can be observed that the first 2 extracted factors are sufficient to explain 97.27% of the overall variation. The first principal component ($F_1$) explained 93.70% of the variation, while the second principal component ($F_2$) contributed to 3.57%.

The $F_1$ factor was positively correlated with TPC, TFC, anti-*P. digitatum* and anti-*P. expansum* and negatively with DPPH activity, while, $F_2$ was positively associated with anti-*P. italicum*. The PCA plot obtained with the first two scores is illustrated in Fig. 3. It indicated that through $F_1$ score analysis, a strong negative correlation between DPPH (on the left) and TPC and TFC (on the right), indicating that extracts with the highest DPPH activity (lower $IC_{50}$ values) displayed the highest TPC and TFC. Furthermore, this factor separated *B. bifurcata* extract from all others on the positive side of the plot, because this seaweed extract exhibited the highest positive loadings on $F_1$ and demonstrated a high content of TPC, TFC and high DPPH potential antioxidant and antifungal activities. Conversely, *C. tomentosum* had the highest negative loadings on $F_1$ and showed the lowest content of TPC and TFC as well as biological activities. Similarly, TFC and TPC, which fall on the positive side of both $F_1$ and negative loading on $F_2$ showed a significant correlation with each other. On the other hand, *B. bifurcata* extract was easily distinguished from *U. fasciata* mainly due to its DPPH values, whereas on the left side of PCA biplot, the red macroalga *C. officinalis* was characterized from *C. tomentosum* by its antifungal activity, chiefly against *P. italicum*.

## DISCUSSION

In this study, aqueous extracts of 4 seaweeds were phytochemically investigated and evaluated regarding their bioactive, antioxidant and antifungal properties. The TPC obtained in this study ranged from 0.42-14.26 mg GAE/g) (Table 1). These values were comparatively much higher than those measured by Farvin and Jacobsen[31] who reported TPC ranging from (0.11-6.10 mg GAE/g) of water extract in numerous marine macroalgae species. Nevertheless, Agregán *et al.*[32] reported lower levels of TPC content, although the results were expressed differently, ranging from (9.6-19.90 mg PGE/g) of aqueous extract for *Ascophyllum nodosum* and *Bifurcaria bifurcata*, respectively. As recorded by the same authors, this study also indicated that the brown seaweed *B. bifurcata* displayed the highest TPC with 14.26±0.41 mg GAE/g (Table 1). However, the TPC comparison between published results should be made carefully, since there are differences in extraction methods, local variations and the standards used for quantification.

In the case of TFC content, similar trend variations to that described for TPC was observed. Indeed, the multivariate biplot of Principal Component Analysis (PCA) revealed a strong positive correlation between these two phytochemical parameters (Fig. 3). However, the total flavonoid level varied from 1.20-9.16 mg QE/g. In this study, the highest flavonoid content was three times lower (26.89 mg GAE/g) than that reported by Haq *et al.*[33]. On the contrary, total flavonoid contents ranging only from 0.123-0.460 mg QE/g, were recorded in three seaweed extracts from Egypt[34]. Nevertheless, sunlight exposures at low tide, climate, location and extraction solvent always affect these chemicals content[35]. Because of their wide range of chemical and biological activities, flavonoids are the most important natural phenolics since they have been identified as antioxidants as well as possible antimicrobial agents for a wide range of diseases[36].

As regards the antioxidant activity, all seaweed extracts displayed significant DPPH radical scavenging capacity. Aqueous extract of *B. bifurcata* showed the highest antioxidant activity among all extracts, followed by *U. fasciata* ($IC_{50}$ values of 0.18 and 0.23 mg $mL^{-1}$, respectively) (Table 2). The inhibition level observed in this present study is higher than that discovered by Mhadhebi *et al.*[37] for aqueous extracts of three brown seaweeds of the genus Cystoseira. Divergence in DPPH radical scavenging activity might be attributed to the various extraction techniques and solvents employed in different experiments, which could alter antioxidant effects. Similar previous findings were also reported that brown algae, in general, have better DPPH radical scavenging than red ones[31]. In agreement with our results, several studies have shown a strong correlation between TPC and TFC with high antioxidant activity and many researchers found that phenolic compounds are among the most effective antioxidants in marine macroalgae[38,39]. Interestingly, *B. bifurcata* and *U. fasciata* samples were collected from the middle intertidal zone where the seaweeds are exposed to UV radiation for several hours per day. The other species tested in this experiment were from lower intertidal areas. Prolonged exposure of macroalgae to solar radiation may result in the production of bioactive compounds such as phenolics and flavonoids, which may explain higher antioxidant capacity and total phenolic contents of these seaweeds in comparison to the other tested species[39].

Several other studies have been conducted to investigate the biological activities of phenolic compounds, which are potent antioxidants and free-radical scavengers[31]. In general, polyphenol levels in brown macroalgae are found to be higher than in red and green seaweeds and the reported principal active compounds found in their extracts have been identified as phlorotannins[40]. Phlorotannins are unique marine polyphenolic compounds produced exclusively by brown seaweeds that can account for 10% of their dry weight. They are usually synthesized by the polymerization of phloroglucinol monomer units, which are characterized as 1, 3, 5-trihydroxy benzene monomer units and are biosynthesized via the acetate malonate pathway[41]. Because of the existence of up to eight linked phenol rings, polyphenols obtained from seaweeds may be more effective than equivalent polyphenols generated from terrestrial plant sources[42].

Recent reports have indicated that not just the phenolic compounds were implied in antioxidant activity, but there may be some effects involving other active chemical substances present in water extracts such as pigments, small molecular weight polysaccharides, proteins or peptides[32]. Thus, for the Ulva species, it was demonstrated that in addition to polyphenols, sulfated polysaccharides, known as ulvans, have been proven to exhibit strong antioxidant capability[43]. In this study, although the aqueous extracts had lower free radical-scavenging activity than the positive control BHT $IC_{50}$ = 0.023 mg $mL^{-1}$. This was not surprising because crude extracts, which are mixtures of many substances, frequently have lower activities than a purified single component.

Results concerning antifungal activity revealed that aqueous extract of *B. bifurcata* revealed the best activity against most tested phytopathogenic fungi, especially *P. digitatum* followed by *P. italicum* and *P. expansum* with

40, 31 and 27% of mycelial growth inhibition, respectively. The red macroalga *C. officinalis* also had an effect on these pathogens, but to a lesser degree, with inhibition percentages of 18% for *P. italicum* and nearly 21% in the case of both *P. digitatum* and *P. expansum* (Fig. 1). To our knowledge, only a few studies examined the antifungal activity of seaweed extracts against postharvest fungi used in this study, so it is quite difficult to compare. Nevertheless, several studies have revealed that seaweed extracts are active against a wide variety of fungal diseases[12,44]. The positive results obtained in the current study indicated that aqueous seaweed extracts contain active metabolites such as phenolic and flavonoid compounds that are, at least in part, involved in the observed antifungal activity. De Corato *et al*.[12] demonstrated that antifungal properties of 5 seaweeds analyzed against *Botrytis cinerea*, *Monilinia laxa* and *Penicillium digitatum* were mainly attributed to the toxicity of phenolic compounds, phlorotannins and fatty acids. Different organic extracts isolated from 23 seaweeds from the coast of Fujian, China also revealed significant antifungal activity against *Penicillium citrinum, Aspergillus niger, Fusarium oxysporum* and *Alternaria dianthi*[45].

Among the investigated species, those belonging to the Phaeophyceae were the most active. The same result was obtained by several authors who reported, in more detail, the chemical composition of the macroalgae belonging to the genus of *Bifurcaria* and based on their observations, they suggested that antifungal activity is related to their high content of phenolic compounds, mainly phlorotannins[46-48]. These compounds are a special group of tannins found in brown seaweeds, since they are polar, highly reactive and have significant antioxidant activity. They are extremely hydrophilic polyphenolic secondary metabolites synthesized by polymerization of phloroglucinol-based phenolic compounds[49]. Up-to-date, the mechanical action of these compounds has not been completely elucidated but it is speculated that they exert their inhibitory effects through the disruption of the structural integrity and thus the function of fungal membrane[50-52]. Indeed, it has been reported that this family of compounds involves membrane destabilization as well as the destruction of fungal mitochondria[53].

The green marine macroalga *U. fasciata* also displayed good antifungal activity, especially against *P. digitatum* and *P. expansum*, although higher concentrations were needed to achieve better control on the tested fungi. This finding was consistent with earlier research indicating that the aqueous extract of this species is highly rich in bioactive compounds that can serve as antifungal agent[54]. Among these molecules, ulvans, which are water-soluble sulfated polysaccharides extracted from most the green seaweeds are endowed with significant biological activities including antifungal properties[55]. These last authors reported that Lebanese *Ulva lactuca* is particularly rich in ulvans and their fractions greatly inhibited germination of *P. digitatum* spores.

Sulfated polysaccharides derived from red seaweeds also possessed some bioactive properties that could explain the observed antifungal activity of *C. officinalis*, despite its low total phenolic and flavonoid contents. Recently, Ismail and Amer[56] revealed that sulfated polysaccharides, such as galactans, also known as carrageenan, extracted from *Corallina* sp. exhibited a strong antimicrobial activity. Furthermore, carrageenans extracted from the red macroalga *Chondracanthus teedei* var. *lusitanicus* have been proven to induce alterations on the *Aspergillus fumigatus* and *Aspergillus infectoria* cell walls, exhibiting a high level of antifungal activity[57].

Therefore, the findings of this study have clearly shown that the selected seaweeds are rich in polyphenol compounds that exhibit antioxidant and antifungal potential, especially against postharvest fungi that cause considerable economic losses. Moreover, this study represents an opportunity to valorise these local marine resources and their products. Unfortunately, till now there are only a few research reports available regarding the use of macroalgae as an eco-friendly antifungal product, particularly suitable to reduce the use of chemical fungicides for controlling postharvest decays.

## CONCLUSION

Based on the results of principal component analysis, *B. bifurcata* followed by *U. fasciata* contained the highest levels of TPC, TFC and exhibited higher antioxidant activity. In biocontrol assays, aqueous extract of *B. bifurcata* revealed the best activity against most tested postharvest fungal pathogens, especially *P. digitatum* followed by *P. italicum* and *P. expansum*. Therefore, the studied marine macroalgae, chiefly *B. bifurcata* followed by *U. fasciata* and *C. officinalis*, may be considered as a biological source of novel bioactive compounds and should be investigated as biological antioxidants and natural fungicides for controlling fruit postharvest decay. Further studies are required to confirm the effectiveness of this antifungal potential under *in vivo* and field conditions as well as to identify and quantify the predominant active chemicals involved in such biological proprieties.

## SIGNIFICANCE STATEMENT

This study discovered the potential of aqueous extracts from Moroccan seaweeds to be used as a natural antioxidant and antifungal agent for the control of postharvest diseases caused by *P. digitatum*, *P. italicum* and *P. expansum*. Thereby, the findings would be beneficial to farmers and producers to extend the shelf life of fresh fruits and vegetables. This study will help the researchers to uncover the antimicrobial abilities of other fungi or other microbes that many researchers were not able to explore. Thus, a new theory on the use of macroalgae as an eco-friendly antifungal product, particularly suitable to reduce the use of chemical fungicides, may be arrived at.

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