Moroccan Journal of Chemistry
ISSN: 2351-812X
http://revues.imist.ma/?journal=morjchem&page=login
Bahammou & al. / Mor. J. Chem. 9 N°4 (2021) 639-648
DOI: https://doi.org/10.48317/IMIST.PRSM/morjchem-v9i3.25910

Assessment of the Antifungal and Antioxidant Activities of the Seaweeds Collected from the Coast of Atlantic Ocean, Morocco

N. Bahammou[(a,b,c)*], R. Raja[(d)], I. S. Carvalho[(d)], K. Cherifi[(e)], H. Bouamama[(c)], O. Cherifi[(a,b)]

[(a)] Laboratory of Water Biodiversity and Climat Changes, Cadi Ayyad University, Marrakech, Morocco

[(b)] National Center for Studies and Research on Water and Energy, Cadi Ayyad University, Marrakech, Morocco

[(c)] Laboratory of Sustainable Development and Health Research Cadi Ayyad University, Marrakech, Morocco

[(d)] MED-Mediterranean Institute for Agriculture, Environment and Development, Food Science Laboratory, FCT, Ed. 8, University of Algarve, Gambelas, 8005-139 Faro, Portugal

[(e)] Laboratory of Biotechnology and Valorization of Natural Resources, Faculty of Sciences, Ibn Zohr University, Agadir, Morocco

** Corresponding author:*
nadia.bahammou@edu.uca.ac.ma

Received 10 April 2021,
Revised 03 Dec 2021,
Accepted 05 Dec 2021

Abstract

Totally seventeen seaweeds (nine Phaeophyceae, six Rhodophyceae and two Ulvophyceae members) were collected from the Atlantic coast of Morocco in order to assess their antifungal and antioxidant activities. The crude methanolic extracts of 30 samples were screened for in vitro antifungal activities against phytopathogenic fungi *Botrytis cinerea* and *Penicillium digitatum* that infect fruits and vegetables. The methanolic extracts of *Carpodesmia tamariscifolia*, *Cystoseira humilis*, *Carpodesmia mediterranea*, *Sacchoriza polyschides*, *Bifurcaria bifurcata*, *Hypnea musciformis* and *Caulacanthus ustulatus* were considerably active against all fungi depending on seasonal variations in the levels of inhibitory activity. *B. cinerea* was highly sensitive to most algal extracts than *P. digitatum*. Phytochemical screening revealed that the most active extracts contain alkaloids, polyphenols and tannins. The antioxidant activity of the algae that possessed high antifungal activity was studied. Methanolic extract of *C. tamariscifolia* contains high phenolic content while the highest content of flavonoids was found in the *C. humilis* extract. The organic farming in the context of sustainable agriculture may use such natural products for the management of fungal diseases. With respect to such alternative in an integrated crop disease management program, further studies on these seaweeds are needed.

Keywords: *Biofungicide, Chlorophyceae, Methanolic extract, Phaeophyceae, Rhodophyceae.*

1. Introduction

For many years, synthetic fungicides were used to control and eliminate the plant pathogenic fungi. However, the extensive use of these fungicides leads to the development of fungal resistance [1,2,3]. In order to overcome this problem, the use of alternative natural fungicides is explored to control pests and diseases in agriculture. Seaweeds represent an inexhaustible source of highly bioactive secondary metabolites that are used in food industries, pharmaceutical, cosmetics and agriculture [1,2,4,5,6]. Seaweeds have secured interest as a potential source of antioxidants. Many researches have shown that the marine macroalgae are rich in various natural antioxidants including polyphenols which plays an important role and inhibit lipid peroxidation. A lot of polyphenolic compounds such as catechins, flavonols as well as flavonol glycosides were identified in the methanol extracts of red and brown algae [5,7]. In Morocco, although there are some available reports on antimicrobial activities of algae there is relatively little care on their potential antifungal and antioxidant properties against crop diseases [8,9,10]. The present investigation was undertaken on the evaluation of the antifungal activities of methanolic extracts of seventeen common seaweeds harvested from the Atlantic coast of Morocco, against economically important fungi *Botrytis cinerea* and *Penicillium digitatum*. In addition, antioxidant activities of some species identified with strong phytopathogenic activities have been screened.

2. Materials and methods

2.1. Seaweed collection

Table 1. ***Dominant algal species harvested during the study period (X: harvest).***

	2017-2018			
Species	**Autumn**	**Winter**	**Spring**	**Summer**
Phaeophyceae				
Cystoseira humilis Schousboe ex Kützing	x			x
Carpodesmia tamariscifolia (Hudson) Orellana & Sansón	x			x
Carpodesmia mediterranea (Sauvageau) Orellana & Sansón		x		
Treptacantha baccata (S.G.Gmelin) Orellana & Sansón	x			
Bifurcaria bifurcata R. Ross	x	x	x	x
Sargassum vulgare C. Agardh, nom. Illeg			x	
Saccorhiza polyschides (Lightfoot) Batters	x	x	x	x
Fucus spiralis Linnaeus		x		
Laminaria digitata (Hudson) J.V. Lamouroux	x			
Rhodophyceae				
Halopithys incurva (Hudson) Batters		x		
Osmundea pinnatifida (Hudson) Stackhouse			x	
Hypnea musciformis (Wulfen) J.V. Lamouroux	x	x	x	
Caulacanthus ustulatus (Mertens ex Turner) Kützing			x	
Gelidium corneum (Hudson) J.V. Lamouroux	x	x	x	X
Corallina officinalis Linnaeus			x	
Ulvophyceae				
Ulva lactuca Linnaeus			x	
Ulva clathrata (Roth) C. Agardh	X			

In The seaweed species were collected at different seasons from October 2017 to July 2018 depending up on their abundance from the Atlantic Moroccan Coast of Sidi Bouzid located near El Jadida city (33°13′52″N, 8°32′51″W).

Nine Phaeophyceae, six Rhodophyceae and two Ulvophyceae members were collected during their dominant period as shown (Table 1). The collected seaweeds were rinsed with seawater at the sampling area and transferred to the laboratory in plastic bags. The seaweeds were then washed with freshwater to take off salts, epiphytes and sand particles.

2.2. Samples preparation and extraction

The dried and ground samples (20 g) of algae were dissolved in 500 mL of methanol by maceration at room temperature with continuous stirring for four days. The material was filtered using filters (Millipore, 0.45 µm) and concentrated by evaporation under reduced pressure to yield the final crude extract. The stocks of crude seaweed extracts were weighed, and stored at -4°C in the dark till further use.

2.3. Mold species

The strain of Botrytis cinerea was isolated from infected tomato whereas, *Penicillium digitatum* from *Citrus sinensis*. The strains were transferred aseptically to potato dextrose agar (PDA) and then incubated at 25±1°C for 7 days. The fungus was purified by spores isolation and maintained on PDA in distilled water and incubated at 25±1°C for 7 days. The phytopathogenic fungi were identified based on their morphological aspect.

2.4. Antifungal activity

The fungus suspensions ($3.8x10^5$ spores mL^{-1}) were spread on PDA in petriplates. Afterwards, disk of Whatman paper containing 10 µL of methanolic extract at 2 mg mL^{-1} was placed. Methanol was considered as negative control and nystatin and Amphotericin B as positive control. All tests were performed in triplicates. Growth of fungal isolates was scored after 3 days of incubation at 25±1°C. The area of inhibition was measured as diameter around the disk (in mm). All readings were taken in three different fixed directions.

2.5. Phytochemical assay

Among the seaweeds that showed strong antifungal activity, seven species were subjected to phytochemical analysis and antioxidant activity: *C. humilis* (summer), *B.bifurcata* (autumn), *S. polyschides* (autumn), *C. tamariscifolia* (summer), *H. musciformis* (winter), *C. ustulatus* (spring) and *C. mediterranea* (winter). The phytochemical algal screening (alkaloids, flavonoids, phenols, saponins, steroids, tannins and terpenoids) was performed according to Békro et al. [11] and Houta et al. [12].

2.6. Determination of total phenolic compound

The phenolic contents (TPC) were determined using a Folin-Ciocalteau method [13]. 1 mL of each seaweed extracts, 1 mL of 95% EtOH, 5 mL of distilled water, and 0.5 mL of 50% Folin-Ciocalteau reagent (Sigma Chemical, St. Louis, MO) were mixed. The mixtures were allowed to react 5 min and 1 mL of 5% Na_2CO_3 was added and placed in the dark for 1 hr. The absorbance was measured at 725 nm and gallic acid standard curve was obtained for the calibration of phenolic content.

2.7. Determination of Total Flavonoid Content

Total flavonoid content (TFC) was estimated spectrophotometrically by the aluminum chloride method based on the formation of complex flavonoid aluminum [14]. 0.5 mL of methanolic extract at a concentration of 1 mg/mL was mixed to 2 mL of distilled water and 0.15 mL of $NaNO_2$ solution (15%) for 6 min at room temperature followed by the

addition of 0.15 mL of $AlCl_3$ (10%). All samples were incubated for 6 min then mixed with 2 mL of NaOH (4%) and incubated for 15 min. The absorbance was read at 510 nm. The amount of TFC was estimated from the standard calibration curve of 0.2-1 mg mL^{-1} quercetin.

2.8. Total antioxidant activity

The total antioxidant activity (TAA) was determined by Prieto et al. [15]. In brief, 0.3 mL of methanolic extract at a concentration of 1 mg/mL was mixed with 3 mL reagent solution (0.6 M H_2SO_4, 28 mM sodium phosphate and 4 mM ammonium molybdate) and incubated at 95°C for 90 min in a water bath. The absorbance was measured at 695 nm using ascorbic acid as standard.

2.9. DPPH free radical scavenging activity

Free radical scavenging activity of the algal extracts were measured using a stable free radical DPPH [16]. DPPH solution was prepared in dimethyl sulfoxide (DMSO) at the concentration of $4x10^{-4}$ M. 100 µL of seaweed extract and 100 µL of freshly prepared DPPH solution were mixed. Incubation of the mixture was done in dark at room temperature for 1 hr. The absorbance was then recorded at 517 nm by ELISA reader (ELX tek Instrument Inc). Inhibition percentage was calculated as below:

$$[1 - (Ai - Aj)/Ac] \times 100$$

Ai: absorbance of extract mixed with DPPH solution; Aj: absorbance of the same extract mixed with 100 µL DMSO; Ac: absorbance of control with solvent (without seaweed extract).

2.10. FRAP (Ferric reducing ability plasma) assay

The FRAP test was performed according to Benzie and Strain [17] and Thaipong et al. [18]. It depends on the ability of the sample to reduce the ferric tripyridyltriazine (Fe(III)-TPTZ) complex to ferrous tripyridyltriazine (Fe(II)-TPTZ) at low pH. Fe (II)-TPTZ has an intensive blue color which can be read at 593 nm. 1.5 mL of fresh FRAP reagent (25 mL of 300 mM L-1 of acetate buffer pH 3.6, 2.5 mL of 10 mM L-1 2,4,6 tripyridyl-S-triazine (TPTZ) in 40 mM L-1 of HCl and 20 mM L-1 of ferric chloride solution) was mixed with 50 µL of algal extract (100 µg mL-1) in 150 µL of distilled water. The absorbance was monitored for 4 min (every 10 secs) at 593 nm. The absorbance is proportional to the combined ferric reducing or antioxidant power (FRAP value) of the antioxidants in the sample. The results are expressed as mMol of FRAP L-1 and were estimated using aqueous FeSO47H2O (200-1000 mM) as standard for calibration. The relative activity of the sample was compared with standard ascorbic acid (2-10 µgmL-1).

2.11. Statistical analysis

All analysises were done in triplicates. The data were statistically analyzed and the values are expressed as mean±SD. The results fit with single factor test and the ANOVA analysis.

3. Results

3.1. Antifungal activity

The present study is an attempt towards screening antifungal activities of crude extracts of seventeen marine macroalgal species that belongs to red, brown and green groups. The results of their biological activities are summarized in table 2. All methanolic extracts exhibited high antifungal activity than the positive control against both *B. cinerea* and *P. digitatum* with the first one being highly sensitive. The degree of seaweed activity differs on the basis of the seasons when the specimens were collected. The expansion of strongest inhibition of fungal mycelium was

observed in the nine algal species collected during all seasons; C. *humilis* (16.7 mm and 9.1 mm against *B. cinerea* and *P. digitatum* respectively in summer), *S. polyschides* (16.1 mm and 7.8 mm against *B. cinerea* and *P. digitatum* respectively in autumn), *H. musciformis* (19.2 mm and 6.4 mm against *B. cinerea* and *P. digitatum* respectively in winter). Also, *C. ustulatus*, *C. officinalis*, *G. corneum*, *C. tamariscifolia*, *C. mediterranea* and *B. bifurcata* possessed antifungal activities. However, remaining algae presented lower activity. Among the algae studied, only seven were selected for the rest of the experiments.

Table 2. ***Antifungal activity of methanolic extracts of dominant seaweeds of Sidi Bouzid Coast against B. cinerea and P. digitatum (Inhibition diameter in mm).***

		Inhibition diameter (mm)			
Seaweeds species	**Tested fungi**	**Autumn**	**Winter**	**Spring**	**Summer**
Phaeophyceae					
S. polyschides	*B. cinerea*	16.1 ± 0.11	12.9 ± 0.15	4.8 ± 0.02	14.9 ± 0.01
	P. digitatum	7.8 ± 0.04	6.9 ± 0.01	8.8 ± 0.02	2.8 ± 0.12
B. bifurcata	*B. cinerea*	12.4 ± 0.11	5.2 ± 0.07	1.5 ± 0.12	8.1 ± 0.15
	P. digitatum	13.3 ± 0.12	5.1 ± 0.01	8.6 ± 0.05	13.4 ± 0.11
C. humilis	*B. cinerea*	3.4 ± 0.10	-	-	16.7 ± 0.18
	P. digitatum	6.3 ± 0.09	-	-	9.1 ± 0.13
C. tamariscifolia	*B. cinerea*	7.1 ± 0.10	-	-	18.9 ± 0.08
	P. digitatum	5.6 ± 0.06	-	-	5.3 ± 0.09
C. mediterranea	*B. cinerea*	-	17.6 ± 0.13	-	-
	P. digitatum	-	9.5 ± 0.04	-	-
F. spiralis	*B. cinerea*	-	9.6 ± 0.01	-	-
	P. digitatum	-	11.2 ± 0.07	-	-
S. vulgare	*B. cinerea*	-	-	15.5 ± 0.15	-
	P. digitatum	-	-	6.7 ± 0.07	-
T. baccata	*B. cinerea*	2.2 ± 0.27	-	-	-
	P. digitatum	8.8 ± 0.09	-	-	-
L. digitata	*B. cinerea*	17.6 ± 0.15	-	-	-
	P. digitatum	13 ± 0.11	-	-	-
Rhodophyceae					
G. corneum	*B. cinerea*	14.4 ± 0.16	3.8 ± 0.08	14.4 ± 0.14	15.8 ± 0.08
	P. digitatum	5.5 ± 0.13	2.9 ± 0.08	6.9 ± 0.05	8.5 ± 0.09
H. musciformis	*B. cinerea*	11.8 ± 0.07	19.2 ± 0.19	16.4 ± 0.17	-
	P. digitatum	11.3 ± 0.01	6.4 ± 0.09	7.6 ± 0.07	-
H. incurvis	*B. cinerea*	-	0.2 ± 0.17	-	-
	P. digitatum	-	4.9 ± 0.04	-	-
C. officinalis	*B. cinerea*	-	-	16.8 ± 0.07	-
	P. digitatum	-	-	16.2 ± 0.11	-
C. ustulatus	*B. cinerea*	-	-	13.9 ± 0.15	-

	P. digitatum	-	-	11.1 ± 0.13	-
O. pinnatifida	*B. cinerea*	-	-	12.7 ± 0.13	-
	P. digitatum	-	-	5.2 ± 0.02	-
Ulvophyceae					
U. lactuca	*B. cinerea*	-	-	9.5 ± 0.07	-
	P. digitatum	-	-	10.1 ± 0.13	-
U. clathrata	*B. cinerea*	3.4 ± 0.11	-	-	-
	P. digitatum	7.3 ± 0.09	-	-	-
Nystatin	*B. cinerea*	2.67 ± 0.10	2.68 ± 0.00	2.58 ± 0.11	2.75 ± 0.10
	P. digitatum	2.13 ± 0.0	2.35 ± 0.00	2.16 ± 0.07	2 ± 0.00
Amphotericin B	*B. cinerea*	0.92 ± 0.09	1.02 ± 0.06	0.97 ± 0.05	0.99 ± 0.11
	P. digitatum	0.6 ± 0.00	0.7 ± 0.00	0.6 ± 0.00	0.9 ± 0.00

(Average [n=3] ± standard deviation)

3.2. Phytochemical test

All seven seaweeds, especially the brown ones are rich on alkaloids, highly present in *B. bifurcata* and *C. tamariscifolia* and moderately present in *S. polyschides* and *C. mediterranea.* Whereas, polyphenols and tannins are highly present in *C. humilis.* In contrast, red algae, *H. musciformis* and *C. ustulatus* are rich on saponins than the other phytochemical products (Table 3).

Table 3. ***Qualitative phytochemical screening of tested algae***

Seaweed species	Alkaloids	Polyphenols	Tannins	Saponins
Phaeophyceae				
B. bifurcata	+++	++	+	-
C. tamariscifolia	+++	+++	-	+
C. humilis	+	++	+++	+
C. mediterranea	++	+++	+	+
S. polyschides	++	+	-	+
Rhodophyceae				
C. ustulatus	+	+	-	+++
H. musciformis	+	++	+	+++

+++: Highly present; ++: Moderately present; +: Present;--: Absent

3.3. Total phenolic and flavonoid contents

The methanolic extracts of *C. mediterranea* and *C. tamariscifolia* contain highest phenolic content with 24.71 ± 0.82 and 36.92 ± 1.68 GAE g^{-1} dry wt, respectively (Table 4). In contrast, that of *S. polyschides* possesses low phenolic content with 2.07 ± 0.12 GAE g^{-1} dry wt. Significant differences were found in TFC among different seaweed species, ranging from 26.64 ± 1.73 to 7.24 ± 0.7 mg quercetin g^{-1} dry wt (Table 4). Flavonoid content of *C.humilis* and *B. bifurcata* are higher than *C.mediterranea* that possessed the lowest 7.24 ± 0.7 mg quercetin g^{-1} dry wt. Tukey test shows a significant difference in TFC between *C. humilis* and all algal species (p<<0.05).

3.4. Total antioxidant activity (TAA)

The methanolic extracts were used to find out their total antioxidant capacities by subsequent formation of phosphomolybdenum complexes. This method is based on the reduction of Mo(VI) to Mo(V) and the formation of a green Mo(V) complex by the antioxidant compounds with a maximal absorption at 695 nm at acidic pH. The different seaweed extracts showed various degrees of antioxidant capacity (Table 4). Except *S. polyschides* and *C. ustulatus*, other algae have shown a relatively good antioxidant activity. The highest TAA was attributed to *B.bifurcata* with 6.22 mg AAE g^{-1} dry wt followed by *C.tamariscifolia* with a value around 3.01 mg AAE g^{-1} dry wt.

3.5. FRAP test

FRAP assay is a method for assessing antioxidant power, whose the ferric to ferrous ion reduction revealed by the formation of a colored ferrous-tripyridyltriazine complex at low pH. The FRAP values are obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing ferrous ions in known concentration. The methanolic extracts of *B.bifurcata* showed higher FRAP value of 57.38 mg TE g^{-1} dry wt, followed by *C. humilis* (Table 4). Tukey test shows a significant difference in ferric reducing ability plasma between *B. bifurcata* and all algal species ($p<<0.05$).

3.6. DPPH test

The DPPH method is based on the reduction of alcoholic DPPH solution to the non-radical form DPPH–H in the presence of a hydrogen-donating antioxidant [19]. The results clearly showed a significant reduction of the free radical DPPH by the extract *S. polyschides* with EC_{50} around 0.045 mg mL^{-1} following by *C. tamariscifolia*, *C.humilis* and then *C.mediterranea* which have a moderate value (Table 4). Tukey test shows a highly significant difference in DPPH between the majority of algal species ($p<<0.05$).

4. Discussion

Most of the tested algal species have proved their antifungal activity against two tested fungal species with a significant difference between seasons. Pearson's correlation showed significant positive relationship between antifungal activity against *B. cinerea* and TPC (R2 = 0.54) and negative relationship with, DPPH, TAA and FRAP (R2 = -0.51; -0.32 and -0.51, respectively). The antifungal activity against *P. digitatum* is positively correlated to TFC, DPPH, TAA and FRAP (R2 =0.17; 0.57; 0.36 and 0.53, respectively). Therefore, high antifungal activity in seaweed extracts is dependent on the phenolic contents flavonoids and antioxidant activity (Table 5). The results obtained were in line with many authors as they also showed the antimicrobial activity of macroalgae [2,3,20,21,22,23]. The higher antifungal and antioxidant activity of some algae may be due to their higher content on phenolic compounds and flavonoids. The roles of phenolic compounds as the main contributors to the antimicrobial and antioxidant activities of various marine algae have been found [24,25,26,27]. Some authors reported that the antimicrobial activity of algae may be influenced by some factors specifically the habitat, the season of collection, different growth stages of the algae and experimental methods, which may explain the variations in antimicrobial activity in their findings [27,28,29]. Despite various solvents have been employed in screening algae for their antimicrobial activity, it is as yet uncertain to determine which solvent is the most effective and suitable for algal extraction procedures [29,30,31].

Table 4. ***Total phenols (TPC), flavonoids (TFC) contents and antioxidant activities of methanolic extract of Bifurcaria bifurcata, Carpodesmia tamariscifolia, Cystoseira humilis, Carpodesmia mediterranea, Saccorhiza polyschides, Caulacanthus ustulatus and Hypnea musciformis.***

Seaweed species	TPC	TFC	DPPH	TAA	FRAP
Phaeophyceae					
B. bifurcata	5.36 ± 0.52^{b}	12.57 ± 0.72^{c}	44.32 ± 1.53^{d}	6.22 ± 2.74^{b}	57.38 ± 2.72^{d}
C. tamariscifolia	36.92 ± 1.68^{e}	$8.89 \pm 0.35^{a,b}$	0.471 ± 0.29^{a}	$3.01 \pm 1.9^{a,b}$	$26.14 \pm 4.07^{b,c}$
C. humilis	5.52 ± 0.62^{b}	26.64 ± 1.73^{d}	0.556 ± 0.47^{a}	$2.15 \pm 1.5^{a,b}$	31.06 ± 0.48^{c}
C. mediterranea	24.71 ± 0.82^{d}	7.24 ± 0.7^{a}	0.593 ± 0.36^{a}	$0.96 \pm 1.1^{a,b}$	$21.08 \pm 1.67^{a,b}$
S. polyschides	2.07 ± 0.12^{a}	$10.45 \pm 0.6^{b,c}$	0.045 ± 0.83^{a}	0.35 ± 1.9^{a}	$25.6 \pm 1.39^{b,c}$
Rhodophyceae					
C. ustulatus	$4.17 \pm 0.02^{a,b}$	$10.38 \pm 0.35^{b,c}$	5.74 ± 0.71^{b}	0.35 ± 1.2^{a}	18.49 ± 1.11^{a}
H. musciformis	7.83 ± 0.07^{c}	$9.28 \pm 0.00^{a,b}$	19.84 ± 0.65^{c}	1.41 ± 1^{a}	28.52 ± 1.82^{c}
Ascorbic acid	-	-	0.03 ± 0.00^{a}	0.530 ± 0.14^{a}	89.80 ± 1.00^{e}

Content is represented as follows TPC as mg GAE g-1 DW; TFC as mg Quercetin g-1 DW; TAA as mg AAE g-1 DW; RP and FRAP as mg TE g-1 DW; DPPH as EC_{50} mg/ml. (Average [n=3] ± standard deviation).

Some authors found that methanolic extracts of the seaweeds give higher antimicrobial activities [32,33,34]. For others, the antimicrobial activity of brown seaweeds was yielded with methanol; whereas acetone was better solvent for green algae [35]. This may be due to the variety of antifungal components that are present in the seaweed extracts. For instance, phenolic compounds were isolated from the brown and red seaweeds; among them *C.tamariscifolia* and *B. bifurcate* [22,36,37] and terpenic and alkaloids compounds, isolated from brown and red algae were more active [21,22]. Other researchers suggest that the antifungal activity could be due to a direct inhibition of fatty acids found at the highest concentrations in *Laminaria digitata*, *Undaria pinnatifida*, *Porphyra umbilicalis* and other species instead of phenolic and phlorotannin compounds [2,16,21]. Biochemical analysis are currently undertaken in order to determine the structure and nature of the responsible antifungal compounds.

Some pigments, like carotenoids, astaxanthin, fucoxanthin and polyphenols as phenolic acids, flavonoids and tannins were identified as antioxidant compounds that have a wide distribution in algae. They exhibit higher anti-oxidative activities as reported through various methods of reactive oxygen species scavenging activity and the inhibition of lipid peroxidation [16,28]. Phlorotannins, phenolic compounds, were detected in various brown seaweed families especially Alariaceae, Sargassaceae and Fucaceae [38,39]. In addition, many studies showed that this group is the only phenolic group identified in brown seaweeds [40,41]. It may explain the high antifungal and antioxidant activities found in the most studied brown seaweeds. The presence of phenolic compounds (Flavonoids, Coumarins ...), Alkaloids and Terpenoids would probably the origin of the antioxidant activity of the species. Flavonoids, recognized as an excellent anti-oxidants [42,43, 44].

Table 5. ***Correlation between antifungal, and antioxidant activities. Phenolic and flavonoid contents of the seaweed extracts.***

	TPC	TFC	DPPH	TAA	FRAP	*B. cinerea*	*P. digitatum*
TPC	1						
TFC	- 0,37	1					
DPPH	- 0,33	- 0,07	1				
TAA	0,10	0,17	0,68	1			
FRAP	- 0,24	0,23	0,87	0,84	1		
B. cinerea	0,55	- 0,15	- 0,51	- 0,32	- 0,51	1	
P. digitatum	- 0,50	0,17	0,57	0,36	0,53	- 0,91	1

5. Conclusion

Seaweeds collected from Sidi Bouzid Atlantic Coast showed a significant antifungal activity against *B. cinerea* and *P. digitatum* with a notable seasonal variation in the level of inhibitory activity for the same species. The highest inhibition was recorded for *C. tamariscifolia* (summer), *C. humilis* (summer), *C. mediterranea* (winter), *S. polyschides* (autumn), *H. musciformis* (spring) and *C. ustulatus* (spring). The methanolic extract of *C. tamariscifolia* was found to contain high phenolic content while the highest concentration of flavonoids are recorded in *C. humilis* extract. Hence, seaweeds can be considered as a potential source of bioactive compounds and it could be served as organic fungicides instead of harmful synthetic or chemical ones. Further, this kind of study should be undertaken in order to purify and identify the bioactive compounds involved in phytopathogenic and antioxidant activities.

Acknowledgement

The second author, Rathinam Raja is grateful to the University of Algarve, Faro, Portugal for funding the researcher under DL57/2016 rule.

References

[1] S. Ambika, K. Sujatha, *Sci. Res. Essays*, 10 (2015) 232-235.
[2] U. De Corato, R. Salimbeni, A. De Pretis, N. Avella, G. Patruno, *Postharvest Biol. Tec*., 131 (2017) 16-30.
[3] A. Leandro, L. Pereira, A. M. Gonçalves, *Mar. Drugs*, 18 (2020) 17.
[4] M. Kosanié, B. Rankovié, T. Stanojković, *Saudi J. Biol. Sci*., 22 (2014) 390-397.
[5] M. A. Abbassy, I. K. M. Gehan, M. H. R. Selwan, *Intern. J. of Plant and Soil Sci*., 3 (2014) 1366-1373.
[6] L. P. Machado, M. C. de Godoy Gasparoto, N. A. Santos Filho, R. Pavarini, CRC Press, 2019, pp. 100-127.
[7] L. Pereira, *Cosmetics*, 5 (2018) 68.
[8] A. Bennamara, A. Abourriche, M. Berrada, M. H. Charrouf, N. Chaib, M. Boudouma, F. X. Garneau, *Phytochemistry*, 52 (1999) 37-40.
[9] M. El Hattab, M. Ben Mesaaoud, M. Daoudi, A. Ortalo-Magné, G. Culioli, R. Valls, L. Piovetti, *Biochem. Syst. Ecol*., 36 (2008) 484-489.
[10] M. El Wahidi, B. El Amraoui, M. El Amraoui, T. Bamhaoud, *Ann. Pharm. Fr*., 73 (2015) 190-196.
[11] Y. A. Bekro, J. A. Mamyrbekova, B. B. Boua, F. T. Bi, E. E. Ehile, *Rev. Sci. Nat.*, 4 (2007) 217-225.
[12] O. Houta, H. Chouaeb, M. Nffati, H. Amri, *Journal de la Société Chimique de Tunisie*, 14 (2012) 77–82.
[13] Y. C. Huang, Y. H. Chang, Y.Y. Shao, *Food Chem.*, 98 (2006) 529-538.
[14] R. Socha, L. Juszczak, S. Pietrzyk, T. Fortuna, *Food Chem.*, 113 (2009) 568-5742.
[15] P. Prieto, M. Pineda, M. Aguilar, *Anal. Biochem*., 269 (1999) 337-341.

[16] R. Raja, S. Hemaiswarya, K. Arunkumar, I. S. Carvalho, *Braz. J. Bot*., 39 (2016) 9-17.
[17] F. Benzie J. J. Strain, *Methods in enzymol.*, 299 (1999) 15-27.
[18] Thaipong, U. Boonprakob, K. Crosby, L. Cisneros-Zevallos, D. H. Byrne, *J. Food Comp. Anal*., 19 (2006) 669-675.
[19] M. Y. Shon, T. H. Kim, N. J. Sung, *Food chem*., 82 (2003) 593-597.
[20] A. Abourriche, M. Charrouf, M. Berrada, A. Bennamara, N. Chaib, C. Francisco, *Fitoterapia*, 70 (1999) 611-614.
[21] N. Kasanah, W. Amelia, A. Mukminin, A. Triyanto, A. Isnansetyo, *Nat. Prod. Res*., 33 (2019) 3303-3307.
[22] G. Arumugam, R. Rajendran, *Biocatal. Agric. Biotechnol*., 19 (2019) 101145.
[23] H. Zbakh, H. Chiheb, H. Bouziane, V. M. Sánchez, H. Riadi, *J. Microbiol. Biotechnol. Food Sci*., 9 (2020) 219-228.
[24] C. Reguant, A. Bordons, L. Arola, N. Rozes, *J. Appl. Phycol*., 88 (2000) 1065-1071.
[25] M. R. Alberto, M. E. Arena, M. C. M. de Nadra, *Food control*, 18 (2007) 898-903.
[26] F. S. Mohammed, H. Akgul, M. Sevindik, B. M. T. Khaled, *Fresenius Environ. Bull.*, 27 (2018) 5694-5702.
[27] I. Ak, G. Türker, *Turkish JAF Sci. Tech.*, 7 (2019) 154-159.
[28] S. J. Heo, S. U. Park, K. W. Lee, Y. J. Jeon, *Biores. Tech*., 96 (2005) 1613-1623.
[29] N. Salvador Soler, M. A. Gómez Garreta, L. Lavelli, M. A. Ribera Siguán, *Sci. Mar.*, 71 (2007) 101-113.
[30] S. Shanmughapriya, A. Manilal, S. Sujith, J. Selvin, G. S. Kiran, K. Natarajaseenivasan, *Ann. Microbiol*., 58 (2008) 535-541.
[31] E. O'Keeffe, H. Hughes, P. McLoughlin, S. P. Tan, N. McCarthy, *J. Appl. Phycol*., 31 (2019) 3759-3776.
[32] A. Manilal S. Sujith, G. S. Kiran, J. Selvin, C. Shakir, R. Gandhimathi, A. P. Lipton, *Ann. Microbiol*., 59 (2009) 207-219.
[33] G. S. Rangaiah, P. Lakshmi, K. Sruthikeerthi, *Drug Invent. Today*, 2 (2010) 311-314.
[34] Kolanjinathan, D. Stella, *Int. J. Pharm. Biol. Arch*., 2 (2011) 1722-1728.
[35] S. Cox, N. Abhu-Ghannam, S. Gupta, *Int. Food Res. J*., 17 (2010) 205-220.
[36] W. Glombitza, H. U. Rösener, D. Müller, *Phytochemistry*, 14 (1975) 1115-1116.
[37] W. Glombitza, H. U. Rösener, M. Koch, "*Phytochemistry*, 15 (1976) 1279-1281.
[38] S. Deal, M. E. Hay, D. Wilson, W. Fenical, *Oecologia*, 136 (2003) 107-114.
[39] S. H. Eom, Y. M. Kim, S. K. Kim, *Food Chem. Toxicol*., 50 (2012) 3251-3255.
[40] V. Jormalainen, T. Honkanen, *J. Evol. Biol*., 17 (2004) 807-820.
[41] R. Koivikko, J. Loponen, K. Pihlaja, V. Jormalainen, *Phytochem Anal*., 18 (2007) 326-332.
[42] A. Oussaid, M. Azzouzi, A. I. Mansour, M. Azouagh, M. Koudad, A. Oussaid, *Mor. J. Chem.*, 8 (2020) 8-3.
[43] I. Nounah, A. Hajib, A.Oubihi, H. Harhar, S. Gharby, B. Kartah, Z. Charrouf, K. Bougrin, *Mor. J. Chem.*, 7(2019) 7-1.
[44] S. Amalich, K. Fadili, M. Fahim, F. EL Hilali, T. Zaïr, *Mor. J. Chem*., 4(2016) 4-1