Journal of Microbiology and Modern Techniques
Volume 1 | Issue 1

Research Article Open Access

Antifungal Potential in Crude Extracts of Five Selected Brown Seaweeds Collected from the Western Libya Coast

Khallil AM*[1], Daghman IM[2] and FadyAA[2]

[1]Department of Botany and Microbiology, Faculty of Science, Assiut University, Egypt
[2]Department of Microbiology, Faculty of Science, Misurata University, Libya

***Corresponding author:** Khallil AM, Department of Botany and Microbiology, Faculty of Science, Assiut University, Egypt, E-mail: raouf42@yahoo.com

Citation: Khallil AM, Daghman IM, FadyAA (2015) Antifungal Potential in Crude Extracts of Five Selected Brown Seaweeds Collected from the Western Libya Coast. J Micro Creat 1(1): 103

Received Date: May 05, 2015 **Accepted Date:** June 23, 2015 **Published Date:** June 27, 2015

Abstract

In vitro antifungal screening of six organic extracts of five seaweeds belong to Phaeophyta (*Sargassum vulgare, Cystoseira barbata, Dictyopteris membranacea, Dictyota dichotoma, and Colpomenia sinuosa*) against eight fungal species (*Alternaria alternata, Cladosporium cladosporioides, Fusarium oxysporum, Epicoccum nigrum, Aspergillus niger, Aspergillus ochraceus, Aspergillus flavus, and Penicillium citrinum*). Cyclohexanic extracts were almost the most active exhibiting a broad spectrum inhibitory action irrespective to the experimented algal extract or fungal species whereas both acetone and ethyl acetate extracts exhibited the lowest antifungal activity. Some algal extracts did not show recognizable inhibitory actions, and some others enhanced some fungal species. The experimented fungal species exhibited variable responses to the tested algal extracts depending upon the experimented fungal and algal species as well as the applied extract. Interestingly, some algal extracts exerted higher antifungal potential in comparable with the patented antifungal medicine (Nystatin and Clotrimazole). Generally, *Alternaria alternata* was relatively more resistant to most of the tested seaweed extracts where as *Fusarium oxysporum* was more sensitive. The present study confirms the potential use of seaweed extracts as a source of antifungal compound and may constitute a basis for promising future applied research that could investigate the use of seaweeds. We conclude that the Libyan coast is a source of bioactive compounds with potential applications in controlling undesired microorganisms in the fields of medicine, pharmacy and agriculture, as well as food additives and food preservation. This may encourage the use of natural products for substituting chemical preservations in food systems.

Keywords: Seaweeds; Organic extracts; Fungi

Introduction

Seaweeds are one of these marine organisms that has been considered as a source of major metabolites that possess bioactive effects [1]. There are numerous reports of compounds derived from macroalgae with a broad range of biological activities, such as antifungal and antibacterial [2,3]. Investigations of the antibiotic properties of marine algae have focused on the effects of algal extracts on bacteria, with relatively little attention being paid to their potential antifungal properties [4]. M. Kausalya* and G.M. Narasimha Rao (2015) indicated that the presence of active constituents with antimicrobial activity in the extractions of marine algae such as *Sargassum polycystum* and *Sargassum tenerrimum* which can be exploited for the production of innovation drugs for the benefit of the humanity [5]. Several investigations have been carried out worldwide. In this respect, in USA, Martin, (1995) tested extracts from macroalgae by spraying on plants and reported a pronounced reduction in the disease incidence of *Botrytis cinerea* on strawberries, *Erysiphe polygonion* turnips, and reported that macroalgae, produce various, biologically active compounds [6]. In France, Hellio, *et al.* (2000) tested antimicrobial potentiality of many seaweed extracts and observed a conspicuous decrease in the development of the fungi tested [7]. In China, Yi, *et al.* (2001) used ethanol, acetone and methanol-toluene to extract antibiotics from 23 species of marine algae belonging to the Chlorophyta, Phaeophyta and Rhodophyta and revealed that the strongest antifungal activities were exhibited by the ethanol extract but the least were by the methanol-toluene extract [8]. In Pakistan, Khanzada, *et al.* (2007) screened various fractions of ethanolic extract of *Solieria robusta* (Rhodophyta) for antifungal activity against five fruit spoiling fungi isolated from fruits and reported that all fractions were able to inhibit fungal growth [9]. Aqueous fraction showed maximum inhibition ratios followed by methanol, ethyl acetate, chloroform and ethanol. In Korea, Kim and Kim, (2008) explored the inhibitory effect of Nostoc commune against *Fusarium oxysporum f. sp. Lycopersici* [10]. Lee, *et al.* (2010) investigated the antifungal activities of Dieckol isolated from the marine brown alga *Ecklonia cava* against *Trichophyton rubrum* and reported the minimum inhibitory concentration of dieckol was 200μM [11]. In Algeria, Saidani, *et al.* (2012) explored antifungal activity of four species of marine algae of Bejaia coast and reported that all the experimented extracts exhibited antifungal activity, the highest inhibiting effect was noted for *Rhodomela confervoides* (red algae) and *Padin apavonica*

(brown algae), respectively against *Candida albicans* and *Mucor ramaniannus* for the first one and *Candida albicans* for the second one [12]. *Aspergillus niger* showed resistance against majority of methanolic extracts. In Brazil, Peres, *et al.* (2013) reported that ten seaweed extracts significantly inhibited the *Colletotrichum lagenarium* growth, but not inhibited significantly the *Aspergillus flavus* growth [13]. In Ireland, Rajauria and Abu-Ghannam (2013) screened antimicrobial activity and bioactive compounds of *Himanthalia elongate* (Brown Seaweed) [14]. In India, Indira, *et al.* (2013) displayed the antifungal property of seaweed *Halimeda tuna* against nine fungal strains (*Aspergillus niger, Aspergillus flavus, Alternaria alternaria, Candida albicans, Epidermophyton floccossum,Trichophyton mentagrophytes, Trichophyton rubrum, Pencillium sp.* and *Rhizopus sp.)* [15]. It is recorded that methanolic extracts exhibited a broad spectrum of antifungal activity compared to the ethanolic and chloroform extracts. Puthamohan Vinayaga Moorthi and Chelliah Balasubramanian (2015) explored the antimicrobial properties of marine seaweed, *Sargassum muticum* against human pathogens [16]. In Thialand, Rattaya, *et al.* (2015) explored the antioxidative, and antimicrobial activities of brown seaweed extracts, *Turbinaria ornate* and *Sargassum polycystum* [17]. In Morracco, El Wahidi, *et al.* (2015) screened of antimicrobial activity of macroalgae extracts from the Moroccan Atlantic coast [18]. Amudha, *et al.* (2015) tested the antimicrobial activity of three seaweeds (*Gelidiella acerosa, Turbinaria conoides* and *Sargassum wightii*) from gulf of Mannar, South-east cost of India [19].

According to the available literatures, the Libyan marine algae have been neglected with respect to assessments of their antifungal activity. Thus, the current investigation represents an attempt to bridge this gap and was designed to evaluate the antifungal potential of different extracts of the five commonest seaweeds which were collected from the western Libyan coast against eight economically important fungi.

Materials and Methods

Study area and algal samples collection

During the period from April to May, 2013, five of the commonest seaweeds were collected in clean polyethylene bags by hand picking from the Libyan western coast (Misurata– Elkhoms regions), immediately transferred to the laboratory for further processing, identified to species level using the standard literature and taxonomic keys and then described [20-26].

Preparation of extracts

The shaded dry seaweeds were cut into small pieces and powdered in a mixer grinder. Every sample was preserved in a freezer until compound extraction. Twenty grams of the powder was charged in the thimble and extracted successively with 400 ml of different solvent (Ethanol, Acetone, Chloroform, Ethyl acetate and Cyclohexane) using a Soxhlet apparatus for 8h. The extracts were filtered through bacterial filters (Millipore, 0.45µm). The crude extracts were stored at -20 °C in airtight brown bottle for further study.

The experimented fungal species

The experimented fungal species were isolated from outdoor and indoor air of some hospitals at Misurata and identified as *Alternaria alternata* (Fr.) Keissl, *Aspergillus niger* Tiegh, *Aspergillus ochraceus* Wilh, *Aspergillus flavus* Link, *Fusarium oxysporum* Schltdl., *Cladosporium cladosporioides* (Fresen.) G.A. de Vries, *Epicoccum nigrum* Link and *Penicillium citrinum* Thom depending upon the macroscopic and microscopic characteristic features. Reliable references were used for identification. Potato dextrose agar (PDA), and Sabouraud dextrose agar (SDA) media were used [27,28].

Antifungal activity assay

Antifungal potentiality of the algal extracts is performed using the disk diffusion technique [29]. Two patented medicine namely Clotrimazole and Nystatin as a reagent-grade powder were used for comparison as positive controls. The experimented fungi were separately and uniformly swabbed across a culture plate [30]. A filter-paper disk of 6 mm in diameter was prepared from Whatman No. 1, and impregnated with the extract to be tested, then placed on the surface of the agar. The plates were incubated at 28±2 °C. The results are expressed as:-

(0): No antifungal activity.
(≤ 10 mm inhibition zone): Weak antifungal activity.
(10 –15 mm inhibition zone): Moderate or mild antifungal activity.
(≥15 mm inhibition zone): High or strong antifungal activity.

Statistical analyses

Activities of the extracts were evaluated as described in Experimental Section. All values represent the mean of triplicate determinations. Data were statistically analyzed by using one way analysis of variance (ANOVA) according to SPSS (1999) [31]. The least significant difference is abbreviated as LSD and measured at $P \leq 0.05$.

Results

Macro-algae (Marine Seaweeds)

Five species belonging to Phaeophyta were isolated and identified as *Sargassum vulgare* C. Agardh, *Cystoseira barbata* (Stackhouse) C. Agardh, *Dictyopteris membranacea* (Stackhouse) Batters, *Dictyota dichotoma* (Hudson) J. V. Lamouroux and *Colpomenia sinuosa* (Mertens ex Roth) Derbès & Solier.

Antifungal activity

The obtained results showed that antifungal activities of tested seaweeds varied depending upon the experimented fungal species, seaweed species and the employed solvent.

Sargassum vulgare: Cyclohexane, chloroform and ethanol extracts of *S. vulgare* displayed higher antifungal activity than acetone and ethyl acetate extracts. The highest inhibitory actions of cyclohexanic extract was recorded against, *E. nigrum, F. oxysporum, C. cladosporioides and A. ochraceous.* A moderate inhibitory action was recorded against *P. citrinum, A. niger, A. alternata* and *A. flavus.* Both *A. flavus* and *A. niger* were not affected by the chloroform extract of *S. vulgare* whereas other experimented fungal species were slightly or moderately affected. Acetone extract did not display inhibitory action against all tested fungi except *E. nigrum* which were slightly affected. Nearly similar inhibitory action was recorded for ethyl acetate extract against *F. oxysporum* and a moderate inhibitory action was recorded against *E. nigrum. A. alternata, A. flavus, E. nigrum* and *C. cladosporioides* did not show responses against the ethanol extract of *S. vulgare* but the other tested fungi were slightly affected (Table 1).

Fungal species	N	Cl	*Sargassum vulgare* (IZ)				
			A	B	C	D	E
Alternaria alternata	12	22	*11	7	0	0	0
Penicillium citrinum	11	19	*14	*7	0	0	*8
Aspergillus flavus	9	22	*10	0	0	0	0
Aspergillus niger	14	10	12	0	0	0	*7
Aspergillus ochraceus	8	20	*15	*8	0	0	*8
Epicoccum nigrum	25	16	*21	11	*10	14	0
Fusarium oxysporum	10	10	*20	*8	0	*7	*8
Cladosporium cladosporioides	7	16	*19	*7	0	0	0

*Significant differences ($P \leq 0.05$) from control fungal cultures
Solvents: A: Cyclohexane B: Chloroform C: Acetone D: Ethyl acetate E: Ethanol

Table 1: Antifungal activities of different organic extracts of *Sargassum vulgare* in comparable with Clotrimazole (Cl) and Nystatin (N) against 8 fungal species using disk diffusion technique and diameter of the inhibition zone (IZ; mm)

Cystoseira barbata: The obtained results from Table 2 revealed that antifungal activity varied according to the employed organic solvent and test fungal species. It was observed that cyclohexane was the best organic solvent for extracting the effective antifungal material from the applied algal species and exhibited the highest antifungal potential particularly against *F. oxysporum* followed by, *A. ochraceous* and *E. nigrum*. The weakest inhibitory action of cyclohexanic extract was recorded against *A. alternata* and *A. flavus*. A moderate inhibitory action was recorded against P.*citrinum, A. niger,* and *C. cladosporiodes.*

Chloroform and ethanol extracts followed cyclohexanic extract as antifungal activity exhibiting low potentiality against most of tested fungi. There was no activity recorded in chloroform extract against *A. alternata, A. niger* and *E. nigrum.* A slight antifungal activity of chloroform extract was recorded against *F. oxysporum* followed by *A. flavus, A. ochraceus, P. citrinum,* and *C. cladosporiodes.* Five of the experimented fungi did not negatively affected by ethanol extract whereas the other three fungal species were slightly inhibited. Both acetone and ethyl acetate extracts displayed the lowest antifungal activity since the former did not display antifungal activity against all experimented fungi. Similarly, all experimented fungi except *F. oxysporum* resisted ethyl acetate extract (Table 2).

Dictyopteris memberanacea: The highest antifungal activity was recorded for cycloxanic extract followed by chloroform and ethanol extracts. The maximum inhibitory action of cyclohexanic extract was recorded against, *F. oxysporun*, and a moderate inhibitory action was recorded against *P. citrinum, A. ochraceus,* and *E. nigrum*. A slight inhibitory action was recorded for *A. alternata, C. cladosporiodes* and *A. niger.* Chloroform extract did not display inhibitory action against *A. alternata* and *E. nigrum* whereas the remaining fungal species were slightly inhibited. Both acetone and ethyl acetate extracts presented the weakest inhibitory action since all the experimented fungi do not inhibited except *F. oxysporum*. A similar slight inhibitory action was recorded for *F. oxysporum* when treated with ethyl acetate extract. No inhibitory action was recorded for ethanol extract against *A. alternata, A. flavus, A. niger, E. nigrum,* and *C. cladosporiodes* whereas a slight inhibitory action was recorded against the remaining fungal species (Table 3).

Fungal species	N	Cl	*C. barbata* (IZ)				
			A	B	C	D	E
Alternaria alternata	12	22	*9	0	0	0	0
Penicillium citrinum	11	19	*13	*7	0	0	*8
Aspergillus flavus	9	22	*10	*8	0	0	0
Aspergillus niger	14	10	*15	0	0	0	0
Aspergillus ochraceus	8	20	16	*8	0	0	*8
Epicoccum nigrum	25	16	16	0	0	0	0
Fusarium oxysporum	10	10	*23	9	0	*7	8
Cladosporium cladosporioides	7	16	15	*7	0	0	0

*Significant differences ($P \leq 0.05$) from control fungal cultures
Solvents: A: Cyclohexane B: Chloroform C: Acetone D: Ethyl acetate E: Ethanol

Table 2: Antifungal activities of different organic extracts of *Cystoseira barbata* in comparable with Clotrimazole (Cl) and Nystatin (N) against 8 fungal species using disk diffusion technique and diameter of the inhibition zone (IZ; mm)

Fungal species	N	Cl	*D. memberanacea*				
			A	B	C	D	E
Alternaria alternata	12	22	*9	0	0	0	0
Penicillium citrinum	11	19	14	*7	0	0	*8
Aspergillus flavus	9	22	*10	*8	0	0	0
Aspergillus niger	14	10	8	6	0	0	0
Aspergillus ochraceus	8	20	14	*9	0	0	*9
Epicoccum nigrum	25	16	*14	0	0	0	0
Fusarium oxysporum	10	10	*16	9	*7	*7	*8
Cladosporium cladosporioides	7	16	*9	*7	0	0	0

*Significant differences ($P \leq 0.05$) from control fungal cultures
Solvents: A: Cyclohexane B: Chloroform C: Acetone D: Ethyl acetate E: Ethanol

Table 3: Antifungal activities of different organic extracts of *Dictyopteris memberanacea* in comparable with Clotrimazole (Cl) and Nystatin (N) against 8 fungal species using disk diffusion technique and diameter of the inhibition zone (IZ; mm)

Dictyota dichotoma: In general, extracts of *D. dichotoma* displayed low antifungal potential in comparable with the other applied seaweeds. Cyclohexanic and ethanolic extracts of *D. dichotoma* exhibited higher antifungal activity than the other three applied extracts. Cyclohexanic extract was more effective than both employed patented medicine reagents. The tested fungi varied in response to cyclohexanic extract since *A. alternata* and *A. niger* were not retarded. *A. flavus, A. ochraceus* and *C. cladosporiodes* were slightly or moderately inhibited but *P. citrinum, E. nigrum*, and *F. oxysporum* were strongly inhibited.

Chloroform extract did not display inhibitory against both *A. alternata* and *A. niger* whereas the remaining of experimented fungi were slightly inhibited. All tested fungi did not affected by acetone extract of *D. dichotoma*, except *F. oxysporum* which was slightly inhibited. Similarly, no inhibitory action was recorded against all tested fungi resulting from ethyl acetate application. Ethanol extract showed different results ranging from no inhibitory action against *A. alternata, A. flavus, E. nigrum,* and *C. cladosporiodes* to slight inhibitory action against the remaining three fungal species (Table 4).

Colpomenia sinuosa: Cyclohexanic extract exhibited the highest antifungal activity in comparable with the other applied extracts. The strongest inhibitory action was recorded against *F. oxysporum,* followed by *C. cladosporiodes*. The weakest inhibitory action was recorded against *A. flavus, A. niger, A. ochraceus,* and *E. nigrum*. A moderate inhibitory action was monitored against *P. cirinum.*

Chloroform extract did not inhibit both *A. alternata* and *A. niger* but displayed a slight inhibitory action against the remaining tested fungi. Acetone extract exhibited no inhibitory action against all tested fungi except for *C. cladosporiodes* which was strongly inhibited and *E. nigrum,* which were slightly inhibited. Ethyl acetate extract exerted the weakest inhibitory action since all the tested fungi did not negatively affected by its application except for *F. oxysporum* which was slightly inhibited. Ethanol extract showed a slight inhibitory action against all experimented fungi except for *A. alternata, A. flavus* and *A. niger* which exhibited no inhibitory action (Table 5).

Fungal species	N	Cl	*D. dichotoma*				
			A	B	C	D	E
Alternaria alternata	12	22	0	0	0	0	0
Penicillium citrinum	11	19	*15	*7	0	0	*7
Aspergillus flavus	9	22	*10	7	0	0	0
Aspergillus niger	14	10	0	0	0	0	*7
Aspergillus ochraceus	8	20	*10	*8	0	0	*7
Epicoccum nigrum	25	16	*15	*9	0	0	0
Fusarium oxysporum	10	10	15	*8	*7	0	9
Cladosporium cladosporioides	7	16	*7	*7	0	0	0

*Significant differences ($P \leq 0.05$) from control fungal cultures
Solvents: A: Cyclohexane B: Chloroform C: Acetone D: Ethyl acetate E: Ethanol

Table (4): Antifungal activities of different organic extracts of ***D. dichotoma*** in comparable with Clotrimazole (Cl) and Nystatin (N) against 8 fungal species using disk diffusion technique and diameter of the inhibition zone (IZ; mm)

Fungal species	N	Cl	*C. sinuosa (IZ)*				
			A	B	C	D	E
Alternaria alternata	12	22	*9	0	0	0	0
Penicillium citrinum	11	19	*15	*7	0	0	*6
Aspergillus flavus	9	22	*10	*9	0	0	0
Aspergillus niger	14	10	12	0	0	0	0
Aspergillus ochraceus	8	20	*12	*7	0	0	*7
Epicoccum nigrum	25	16	*12	9	7	0	*9
Fusarium oxysporum	10	10	*23	*7	*0	7	7
Cladosporium cladosporioides	7	16	16	*7	16	0	*7

*Significant differences ($P \leq 0.05$) from control fungal cultures
Solvents: A: Cyclohexane B: Chloroform C: Acetone D: Ethyl acetate E: Ethanol

Table 5: Antifungal activities of different organic extracts of *Colpomenia sinuosa* in comparable with Clotrimazole (Cl) and Nystatin (N) against 8 fungal species using disk diffusion technique and diameter of the inhibition zone (IZ; mm)

Discussion

The present study is an endeavor towards the screening antifungal activities through crude extracts of five marine macroalgal species belonging Phaeophyta (*S. vulgare, C. barbata, D. membranacea, D. dichotoma, C. sinuosa*) against eight economically fungal species (*A.alternata, C. cladosporioides, F. oxysporum, E.nigrum, A. niger, A. ochraceus, A. flavus, P. citrinum*). The main reason for this is that the marine seaweeds which abundantly occur are not adequately exploited as a source for bioactive compounds of potential medicinal applications. The selected fungal species are deteriorating, opportunistic, pathogenic, or antagonistic fungi. This surely helps in aiding to understand whether the application of marine algae derived compounds can be of any assistance in treating food deterioration or preservations well as plant and human fungal diseases, both of topical and systemic in nature. The marine seaweeds were extracted with five different organic solvents (cyclohexane, chloroform, acetone, ethyl acetate and ethanol).

The present screening revealed that cyclohexanic extracts were almost the most effective and exhibited a broad spectrum inhibitory action irrespective to the experimented algal extract or fungal species. On the contrary, some algal extracts did not show recognizable inhibitory actions, but some others enhanced fungal growth of some species. Some of tested extracts which did not alter the experimented fungal species suggesting that either that they are not able to diffuse across the agar or that the extracting condition does not allow the isolation of active compounds with properties to alter fungal growth under these conditions. Similar finding was reported by Jiménez, *et al.* (2011) [32]. Both chloroform and ethanol extracts followed cyclohexanic extracts as antifungal potential whereas both acetone and ethyl acetate extracts exhibited the lowest antifungal activity. Inconsistently Yi, *et al.* (2001) reported that the ethanol extract of 23 species of marine algae (belonging to Chlorophyta, Phaeophyta and Rhodophyta) was the strongest antifungal activity in comparable with acetone and methanol-toluene extracts which exhibited the lowest antimicrobial activity [8]. Variable findings were obtained by several authors concerning the best solvent for extraction the bioactive compounds. In this respect, Manivannan, *et al.* (2011) revealed that the strongest antimicrobial activity was exhibited by the methanol extract and the least by chloroform and Petroleum ether [33]. Salem, *et al.* (2011) reported that ethyl acetate was to be the best solvent for isolation of antimicrobial compounds from the tested marine algae followed by methanol [34].

The present study revealed that the experimented fungi exhibited variable responses for the tested seaweed extracts depending upon the applied solvent, tested algal and fungal species. In this respect, *Alternaria alternata* exhibited the highest resistance for all five tested algal extracts except in case of cyclohexanic extracts of most seaweeds and chloroform extract of *S. vulgare* which displayed slight inhibitory action. Inconsistently, Galal, *et al*. (2011) reported that crude ethyl acetate extract of *Padina gymnospora* and methanolic extract of *Codium fragile* exhibited strong activity against most of the tested fungi including *Fusarium oxysporum, Alternaria alternata*, and *Alternaria brassicicola* [35]. Cosoveanu, *et al*. (2010) reported that *Alternaria alternata* was to be the most sensitive isolate to *Alaria esculenta* extracts [36].

Fusarium oxysporum was on the contrast to *Alternaria alternata*, where it was the most sensitive for the majority of the tested algae and the strong inhibitory action was recorded particularly for cyclohexanic extracts. Cyclohexanic extract of *Cystoseira barbata, Colpomenia sinuosa,* and *Sargassum vulgare* displayed the highest inhibitory actions against *Fusarium oxysporum.* No inhibitory action was recorded against *Fusarium oxysporum* with ethyl acetate extract of *D. dichotoma*, acetone extract of *S. vulgare, C. barbata, Colpomenia sinuosa* and *H. musciformis*. The other extracts of the experimented Phaeophyta seaweeds exhibited slight inhibitory actions against *Fusarium oxysporum.* Similarly, Kim and Kim (2008) reported that extract of *Nostoc commune* displayed significant inhibitory action against *Fusarium oxysporum f. sp. lycopersici.* Asnad and Tanver (2014) recorded antifungal activity of the water and ethanol extracts of eight seaweed species against *Fusarium solani* and *F. oxysporum* isolated from deteriorated fruits and vegetables [37]. Galal, *et al.* (2011) reported that crude ethyl acetate extract of *Padina gymnospora* and methanolic extract of *Codium fragile* exhibited strong activity against *Fusarium oxysporum* [35]. Lavanya and Veerappan (2012) reported the minimum inhibitory action of *Sargassum wightii* was recorded in methanol water extracts against *Fusarium udum. Penicillium citrinum* was strongly impeded with cyclohexanic extracts of *D. dichotoma, C. sinuosa, H. musciformis* and *L. papillosa* whereas cyclohexanic extract of *S. vulgare, C. barbata,* and *D. membranacea* displayed moderate inhibitory action [38]. The other extracts of the tested seaweeds showed slight or no inhibitory action against *Penicillium citrinum*. Yi, *et al.* (2001) reported that out of the 23 screened seaweeds, 17 showed antifungal activity against *Penicillium citrinum* [8]. Cosoveanu, *et al*. (2010) reported Penicillium expansum was the most sensitive isolates to *Alaria esculenta* extracts [36]. Kumar, *et al*. (2014) noted the highest inhibiting effect in chloroform and ethanolic extracts of *Sargassum tenerrimum* and *Turbinaria ornate* (brown algae), against *Penicillium janthinellum* [39]. *Aspergillus flavus* was moderately and slightly inhibited with cyclohexanic and chloroform extracts, respectively of all experimented brown seaweeds. However, the other applied extracts did not display inhibitory action against *Aspergillus flavus.* On the contrary, our results are entirely different from the finding of Manivannan, *et al.* (2011) who showed that extract of the *Sargassum tenerrimum* was highly effective against *Aspergillus flavus* [33].

Aspergillus flavus was moderately and slightly inhibited with cyclohexanic and chloroform extracts, respectively of all experimented brown seaweeds. However, the other applied extracts did not display inhibitory action against *Aspergillus flavus.* On the contrary, our results are entirely different from the finding of Manivannan, *et al*. (2011) who showed that extract of the *Sargassum tenerrimum* was highly effective against *Aspergillus flavus* [33].

Padmakumar, and Ayyakkannu (1997) [40] screened antifungal activities of 80 marine algal species and did not find a single algal extract was active against *Aspergillus flavus*. Similarly, Lavanya and Veerappan (2012) reported that dichloromethane and ethanol extracts of *Sargassum dentifolium, Laurencia papillosa* and *Janio corniculata* had no activity against *Aspergillus flavus* [38]. *Aspergillus niger* was highly inhibited with cyclohexanic extract of *Cystoseira barbata,* mildly inhibited with cyclohexanic extract of *S. vulgare* and *Colpomenia sinuosa,* but slightly inhibited with cyclohexanic extract of *D. membranacea*. The remaining extracts did not exert inhibitory action against *A. niger*. Erturk and Tas (2011) recorded highest antifungal activity of *Padina pavonica* (brown seaweed) against *Aspergillus niger* [41]. Kumar, *et al.* (2014) recorded the highest inhibitory action in chloroform and ethanolic extracts of *Sargassum tenerrimum* and *Turbinaria ornate* (brown algae), against *Aspergillus niger* which is causing opportunistic infection of HIV-infected person, lung disease, aspergillosis, and otomycosis (fungal ear infections) [39]. *Aspergillus ochraceus* was strongly inhibited with cyclohexanic extracts of *S. vulgare* and *Cystoseira barbata. Epicoccum nigrum* (the antagonistic fungus) was highly inhibited with cyclohexanic extracts of all tested brown seaweeds except for *D. membranacea* and *C. sinuosa* at which it was moderately inhibited. Karthick, *et al.* (2014) reported that antifungal assay of *Caulerpa scalpelliformis* showed the benzene extract rendered a maximum activity against *Aspergillus terreus* whereas a minimum activity obtained in chloroform extract against *Aspergillus flavus* [42].

Kumar, *et al.* (2014) explored the chemical composition of carbohydrate, protein, phenol, flavonoid, chlorophyll, and carotenoid and antifungal activity from various marine seaweeds, and noted the highest inhibiting effect was for *Sargassum tenerrimum* and *Turbinaria ornate* (brown algae), against *Aspergillus niger and Penicillium janthinellum* in chloroform extracts and ethanolic extract which are causing opportunistic infection of HIV-infected person, lung disease, aspergillosis, and otomycosis (fungal ear infections) [39].

Cladosporium cladosporiodes was strongly inhibited with Cyclohexanic extracts of *S. vulgare, Cystoseira barbata,* and *Colpomenia sinuosa*. Feleco, *et al.* (2010) presented high potentiality of marine red alga *Bostrychia tenella* against the phytopathogenic fungi *Cladosporium cladosporioides* and *Cladosporium sphaerospermum* [43]. Stirk, *et al.* (2007) reported that seaweeds provide a rich source of structurally diverse secondary metabolites [44]. These are mainly terpenes, acetogenins, alkaloids and polyphenolics, with many of these compounds being halogenated [45]. The functions of these secondary metabolites are defense against herbivores,

fouling organisms and pathogens; they also play a role in reproduction, protection from UV radiation and as allelopathic agents. Abou-Elwafa, *et al.* (2011) isolated some bioactive compounds such as spatane, diterpene, tetraol, fucosterol and linoleic acid from the brown alga *Sargassum subrepandum* [46]. Ambika and Sujatha (2015) recorded that ethanol extract of *Sargassum myricocystum* (brown alga) showed a significant antifungal activity against pathogen (*Colletotrichum falcatum*) followed by *Gracilaria edulis* (red alga) [47]. Rupapara, *et al.* (2015) recorded the presence of alkaloid, carbohydrates, protein, and phenolic compounds in methanol ethyl acetate and hexane extracts of *Sargassum johnstonii* which exhibited a considerable antimicrobial activity against pathogens [48]. Begum, *et al.* (2015) recorded that extract of seaweed (*Turbinaria conoides*) had shown antifungal activity against damping of pathogen *Pythium aphanidermatum* [49].

The present study confirms the potential use of seaweed extracts as a source of antimicrobial compound and may constitute a basis for promising future applied research that could investigate the use of seaweeds which are renewable prosperity against opportunistic, pathogenic and deteriorating fungi. Additional studies need to be performed to define and characterize, at the chemical and biochemical level, the preferential effect of algae extracts on microorganisms that have adverse effects. Finally we conclude that the Libyan coast is a source of bioactive compounds with potential applications in controlling undesired microorganisms in the fields of medicine, pharmacy and agriculture, as well as for a possible use in food preservation. This may encourage the use of natural products for substituting chemical preservations in food systems.

References

1. Yang RY, Li CY, Lin YC, Peng GT, She ZG, et al. (2006) Lactones from a brown alga endophytic fungus (No. ZZF36) from the South China Sea and their antimicrobial activities.Bioorg Med ChemLett 16: 4205-8.

2. Freile-Pelegrin Y, Morales JL (2004) Antibacterial activity in marine algae from the coast of Yucatan, Mexico. Bot Mar 47: 140-6.

3. Orandy MA, Verde MJ, Martinez-Lozano SJ, Waksman NH (2004) Active fractions from species of marine algae. Intern J Exp Bot 73: 165-70.

4. Usmanghani K, Shameel M, SuaJeh M, Khan KH, Mahmood ZA (1984) Antibacterial and antifungal activities of marine algae from Karachi seashore of Pakistan. Fitoterapia 55: 73-8.

5. Kausalya M, Narasimha GM (2015) Antimicrobial activity of marine algae. J Algal Biomass Utln 6: 78- 87.

6. Martin MK (1995) The potential for using cyanobacteria (blue-green algae) and algae inthe biological control of plant pathogenic bacteria and fungi. European J Plant Pathol 101: 585-99.

7. Hellio C, Bremer G, Pons AM, Gal YL, Bourgougnon N (2000) Inhibition of the development of microorganisms (bacteria and fungi) by extracts of marine algae from Brittany, France. Appl Microbiol Biotechnol 54: 543-9.

8. Yi Z, Yin-Shan C, Hai-Sheng L (2001) Screening for antibacterial and antifungalactivities in some marine algae from the Fujian coast of China with three different solvents. Chin J OceanolLimnol 19: 327-31.

9. Khanzada AKW, Shaikh TG, Kazi S, Kabir and Soofia S (2007) Antifungal activity, elemental analysis and determination of total protein of seaweed, Solieriarobusta (Greville) Kylin from the coast of Karachi. Pak J Bot 39: 931-7.

10. Kim J, Kim JD (2008) Inhibitory Effect of Algal Extracts on Mycelial Growth of the Tomato-Wilt Pathogen, Fusarium oxysporum, f. sp, Lycopersici. Mycobiology 36: 242-8.

11. Lee MH, Kyung BL, Sang MO, Bong HL, Hee YC (2010) Antifungal Activities of Dieckol Isolated from the Marine Brown Alga Ecklonia cava against Trichophyton rubrum. J Korean Soc Appl Biol Chem 53: 504-7.

12. Saidani BF, Benabdesselam F, Touati N (2012) Antifungal activity of methanolicextracts of four Algerian marine algae species. African J Biotechnol 11: 9496-500.

13. Peres JCF, De Carvalho LR, Gonçalez E, Berian LOS, Felicio JD (2012) Evaluation of antifungal activity of seaweed extracts. SciAgrotecnolo 36: 294-9.

14. Rajauria G, Abu-Ghannam N (2013) Isolation and Partial Characterization ofBioactive Fucoxanthin from HimanthaliaelongataBrown Seaweed: A TLC-Based Approach. Int J Anal Chem Article ID 802573: 1-6.

15. Indira KS, Balakrishnan M, Srinivasan S, Bragadeeswaran, Balasubramanian T (2013) Evaluation of in vitro antimicrobial property of seaweed (Halimeda tuna) from Tuticorin coast, Tamil Nadu, Southeast coast of India. African J Biotechnol 12: 284-9.

16. Moorthi PV, Balasubramanian C (2015) Antimicrobial properties of marineseaweed, Sargassummuticum against human pathogens. J Coastal Life Med 3: 122-5.

17. RattayaS, Benjakul S, Prodpran T (2015) Extraction, antioxidative, and antimicrobial activities of brown seaweed extracts, Turbinaria ornate and Sargassupolycystum, grown in Thailand. IntAquat Res 7: 1-16.

18. El Wahidi M, El Amraoui B, El Amraoui M, Bamhaoud T (2015) Screening of antimicrobial activity of macroalgae extracts from the Moroccan Atlantic coast. Ann Pharm Fr 73: 190-6.

19. Amudha, Prabuseenivasan, Harika N, Kumar V (2015) Bioactivity of selected seaweeds from gulf of Mannar, South-east cost of India. J Coastal Life Med 3: 52-5.

20. Gayral P (1966) Lea algues des cotes francaises (Manche et Atlantiquea). 632.

21. Nizamuddin M, West JA, Menez EG (1979) A list of marine algae from Libya. Bot Mar 3: 465-76.

22. Nizamuddin M (1981) Contribution to the marine algae of Libya Dictyotales. J Cramer 54: 1-122.

23. Nizamuddin M (1991) The green marine algae of Libya. ELGA 227.

24. Taylor WR (1960) Marine algae of the eastern tropical and subtropical coasts of Americas. University of Michigan press ANN Arbor: 870.

25. Aleem AA (1981) Contributions to the study of the marine algae of the Red Sea. New or little known algae from the west coast of Saudi Arabia. Bull Fac Sci KAUJ 5: 1-49.

26. Abbott IA, Hollenberg GJ (1976) Marine algae of California. Stanford University Press [i]-xii: 1-827-701 figures.

27. Raper KB, Fennell DL (1965) The genusAspergillus.Williams and Wilkins.686.

28. Ainsworth GC (1971) Ainsworth and Bisby"s Dictionary of the fungi. Common wealth Mycological Institute. Kew Surrey England.

29. Hellio C, Broise DL, Dufosse L, Gal YL, Bourgougnon N (2001) Inhibition of marine bacteria by extracts of macroalgae: Potential use for environmentally friendly antifouling paints. Mar Environ Res 52: 231-47.

30. Thenmozhi K, Selvarani T, Karthikeya BP (2013) Estimation of antifungalactivity of aqueous extracts of brown Seaweed Sargassiumillicifolium. Adv Res Pharmaceut 3: 374 -7.

31. SPSS (1999) SPSS Base of 10.0 users Guide. SPSS Inc.

32. Jimenez E, Dorta F, Medina C, Ramirez A, Ramirez I, et al. (2011) Anti-Phytopathogenic Activities of Macro-Algae Extracts. Mar Drugs 9: 739-56.

33. Manivannan K, Karthikai DK, Anantharaman P, Balasubramanian T (2011) Antimicrobial potential of selected brown seaweeds from Vedalai coastal waters, Gulf of Mannar. Asian Pac J Trop Biomed 1: 114-20.

34. Galal HRM, SalemWM, Nasr El-Deen F (2011) Biological control of some pathogenic fungi using marine algae. Res J Microbiol 6: 645- 57.

35. Salem WM, Galal H, Nasr El-deen F (2011) Screening for antibacterial activities in some marine algae from the red sea (Hurghada, Egypt). African J Microbiol Res 5: 2160-7.

36. Cosoveanu A, Oana A, Iacomi B (2010) Antifungal activity of macroalgae extracts. Uasvm Bucharest, Series A LIII: 442-7.

37. Asnad A and Abbass T (2014) Screening of potential seaweeds against Fusarium species isolated from fruits and vegetables in Baluchistan, Pakistan. Int J Biosci 4: 131-8.

38. Lavanya R, Veerappan N (2012) Pharmaceutical Properties of Marine Macroalgal Communities from Gulf of Mannar against Human Fungal Pathogens. Asian Pac J Trop Dis 2: 5320-3.

39. Kumar N, JI, Barot M, Kumar RN (2014) Phytochemical analysis and antifungal activity of selected seaweeds from Okha Coast, Gujarat, India. Life sci leaflets 52: 57-70.

40. Padmakumar K, Ayyakkarmu K (1997) Seasonal Variation of Antibacterial and Antifungal Activities of the Extracts of Marine Algae from Scuthem Coasts of India. Bot. Mar. 40: 507-16.

41. Erturk O, Tas B (2011) Antibacterial and antifungal effects of some marine Algae. Kafkas Univ Vet Kaf Derg 17 (Suppl A): S121-4.

42. Karthick N, Fathimal MA, Ramesh K, Sridhar H, Natrajan M, et al. (2014) Screening of phytochemicals and antimicrobial activity of Caulerpa scalpelliformis collected from Manapad Coast, Tuticorin District, Tamilnadu, South India. J Coastal Life Med 2: 107-11.

43. Felicio R, Albuquerqueb S, Young MCM, Yokoyad NS, Debonsia HM (2010) Trypanocidal, leishmanicidal and antifungal potential from marine red alga Bostrychia tenella J. Agardh (Rhodomelaceae, Ceramiales). J Pharma Biomed Analysis 52: 763-9.

44. Stirk WA, Diana LR, Johannes S (2007) Seasonal variation in antifungal, antibacterial and acetylcholinesterase activity in seven South African seaweeds. J Appl Phycol 19: 271-6.

45. Watson SB, Cruz-Rivera E (2003) Algal chemical ecology: an introduction to the special issue. Phycologia 42: 319-23.

46. Abou-El-Wafaa GSE, Khaled A, Shaabanb M, El-Naggar EE, Shaabanb M (2011) Bioactive constituents and biochemical composition of the Egyptian brown algae Sargassium superandum (Forsk). Rev Latinoamer Quím 39: 62-74.

47. Ambika S, Sujatha A (2015) Antifungal activity of aqueous and ethanol extracts of seaweeds against sugarcane red rot pathogen (Colletotrichum falcatum). Acad J 10: 232-5.

48. Rupapara KV, Joshi NH, Vyas KG (2015) Evaluation of Antimicrobial Activityof Crude Extracts of Seaweed Sargassum johnstonii. Int J CurrMicrobiol App Sci 4: 300-4.

49. Begum AJ, Selvaraju P, Vijayakumar A (2015) Evaluation of antifungal activity of seaweed extract (Turbinaria conoides) against pythium aphanidermatum (edson) fitzp. Life Sci Leaflets 63: 162-8.