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

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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 algal 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 Clotrimazol). Generally, Alternaria alternata was relatively more resistant to most of the tested seaweed extracts whereas 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 tenerirmum 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 (Rhophyta) 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.

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 ºC. 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≤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).

<table>
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*Significant differences (P ≤ 0.05) from control fungal cultures

Table 1: Antifungal activities of different organic extracts of Sargassum vulgare in comparable with Clotrimazole (C) 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. cladosporioides.

Chloroform and ethanol extracts followed cyclohexan 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. ochraceous, P. citrinum, and C. cladosporioides. 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 membranacea: The highest antifungal activity was recorded for cycloxyan extract followed by chloroform and ethanol extracts. The maximum inhibitory action of cyclohexanic extract was recorded against, F. oxysporum, and a moderate inhibitory action was recorded against P. citrinum, A. ochraceous, and E. nigrum. A slight inhibitory action was recorded for A. alternata, C. cladosporioides 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. cladosporioides whereas a slight inhibitory action was recorded against the remaining fungal species (Table 3).

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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. cladosporioides* 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. cladosporioides* 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. cladosporioides*. The weakest inhibitory action was recorded against *A. flavus*, *A. niger*, *A. ochraceus*, and *E. nigrum*. A moderate inhibitory action was monitored against *P. citrinum*.

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. cladosporioides* 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).
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. cladспорioides, 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 as 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 antifungal activity in comparable with acetone and methanol-toluene extracts which exhibited the lowest antimicrobial activity 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].

### Discussion

<table>
<thead>
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<th>Fungal species</th>
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<td>Cladosphorium cladспорioides</td>
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*Significant differences (P ≤ 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)

<table>
<thead>
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<th>D. dichotoma</th>
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<td>Cladosphorium cladスポリoides</td>
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*Significant differences (P ≤ 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)
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. vulgaris which displayed slight inhibitory action. Inconsistently, Galal, et al. (2011) reported that crude ethyl acetate extract of Padina gymnospora and methanol 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. vulgaris, 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]. Lavana 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. vulgaris, 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 isolate to Alaria esculenta extracts [36]. Kumar, et al. (2014) noted the highest inhibiting effect in chloroform and ethanolic extracts of Sargassum tenerrimum and Turbinaria ornata (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, Lavana 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. vulgaris 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 ornata (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. vulgaris 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 ornata (brown algae), against Aspergillus niger and Penicillium janthinellum in chloroform extracts and ethanolic extracts which are causing opportunistic infection of HIV-infected person, lung disease, aspergillosis, and otomycosis (fungal ear infections) [39].

Cladosporium cladosporioides was strongly inhibited with Cyclohexanic extracts of S. vulgaris, 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 sphaerosperrum [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 spateine, diterpene, tetrac, 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 the 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.

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