Bio- Active Properties of Selected Brown, Green and Red Algae species Collected from Egypt

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Abstract:

Algae are an important source of secondary metabolites and several compounds that have a wide range of bioactivities. However, algae of the Red sea have not been adequately explored for their potential as a source of bioactive substances. In this study, *Caulerpa sp.*, *Chondria sp.*, *Galaxaura sp.*, *Turbinaria sp.* and *cystoseira sp.*, collected from Red sea, Egypt, were screened for their potential bioactivity. Algal extracts were prepared using Ethanol/Dichloromethane (1:1) solvent. It was found that the crude extract of *Caulerpa sp.* showed the highest antibacterial and anti-yeast activity among all tested extracts. It could be concluded that the Red Sea marine environment of Egypt has a potential to introduce pharmaceutically useful seaweeds as a great candidate for further investigations towards drug development, however further deep investigations are requested.

Keywords: Algae, Red Sea, Bioactivity, Antibacterial

Introduction

Algae are the oldest members of the plant kingdom, extending back to many millions of years. They are common in aquatic environment with a high variety in terms of morphological, biochemical, taxonomic and ecological aspects ***(Mareš et al., 2011 and Guiry, 2012)***. Algae can be classified and identified into two major groups; Microalgae, which float freely in water and form plankton, and Macroalgae, which exhibit complex degrees of organization of thalli ***(Bold and Wynne, 1985 and Garson, 1989).*** The biomass of algae delivers a vast spectrum of secondary metabolites that are synthesized in the reaction to ecological stress as quick changes of temperature, light force, salinity, drying up, osmotic pressure and nitrogen insufficiency ***(Gupta and Abu-Ghannam, 2011 and Shanab et al., 2012).*** These compounds have a wide range of bioactivities including; antiallergic ***(Na et al., 2005),*** antifungal ***(de Felício et al., 2010),*** antiviral ***(Kim and Karadeniz, 2011a),*** antimicrobial ***(Bouhlal et al., 2011),*** anticancer ***(Kim et al., 2011b),*** antioxidant and antifouling effects ***(Devi et al., 2011).***

This study aimed to investigate the potential anti-fungal, anti-yeast and antibacterial activities of selected brown, green and red algae, collected from Red Sea, Egypt.

Materials and Methods:

Algae:

Algal samples were collected from the Red Sea in Egypt at two different locations in a water depth of 2-4 m. On May, 2017, two algal species were collected from Sharm El-sheikh (Ras Mohamed protected area), Sinai. The six other species were collected from Red Sea at Marsa Alam, where three species were collected in November, 2017, and the other three were collected on February, 2018. All samples were washed thoroughly several times with seawater to remove the adhering sand particles and impurities then washed thoroughly with fresh water to remove salts ***(Kamaladhasan and Subramanian, 2009)***. Samples were iced in the freezer, until they were transferred to the laboratory for processing. The algal species were identified based on the schemes reported in the literature ***(Nasr and Aleem, 1949; Smith, 1944; Levring, 1946; Bouck, 1965; Scagel, 1966; Bold, 1978*** and ***Coppejans et al., 2009)*** and saved in the Lab of Aquaculture Biotechnology, Fish Farming and Technology Institute, Suez Canal University, Ismailia.

Preparation of algae extract:

Each sample of the marine algae was washed again with distilled water several times, followed by 5% ethanol to remove any epiphytes or any salt precipitates ***(Rhimou et al., 2010)***. Samples were spread on white sheets and left for air drying. They were cut into small pieces and powdered in a mixer grinder.

Extraction of grounded algal samples was done using Ethanol: Dichloromethane (1:1) and stored in a dark place. Remain extracts were filtered and concentrated in a rotatory evaporator at 35 ℃. The weighted crude extracts were suspended in the dimethyl sulfoxide (DMSO) to a final concentration of 20 mg/ml and stored in a refrigerator at 4ºC ***(Mohanta et al., 2007 and Patra et al., 2008)***.

Antimicrobial activity assay:

The in vitro antimicrobial activity testing was carried out using the standard well diffusion assay ***(Flemer et al., 2012)***, against standard pathogenic fungus *Aspergillus fumigatus* (RCMB 002008), yeast *Candida albicans* (RCMB 005003 (1) ATCC 10231) and *Cryptococcus neoformans* (RCMB 0049001), and bacteria *Staphylococcus aureus* (RCMB010010), *Bacillus subtilis* (RCMB 015 (1) NRRL B-543), *Escherichia coli* (RCMB 010052(1) ATCC 25955) and *Proteus vulgaris* (RCMB 004 (1) ATCC 13315). The standard pathogenic strains were cultured in nutrient broth at 37 ºC overnight. Antimicrobial activity was evaluated using the agar diffusion technique in Petri dishes. Briefly, sterile filter paper discs, 6 mm in diameter, were loaded with 100µl of the different extracts dissolved in DMSO in a concentration of 20 mg /ml and air dried. Discs containing standard concentrations of Gentamycin and Ketoconazole were used as controls. The discs were placed on Muller Hinton agar plates ***(Mueller and Hinton, 1941)*** inoculated with each of the previously mentioned microorganisms. Plates were incubated at 37℃ for 24 h for bacteria and 48 h for fungus and yeast, and the inhibition zones that formed around the

discs were measured (mm diameter). Each set was prepared in triplicate. The effect of DMSO against all the used pathogenic strains was also tested as a negative control.

Results:

In this study, eight samples of the collected algal species were used for the bioactive assay, which were; brown alga *Turbinaria sp.*, red alga *Chondrophycus sp.*, brown alga *Cystoseira sp.*, green alga *Caulerpa sp.* [(At); collected in Autumn and (W); collected in Winter], red alga *Chondria sp.* and red alga *Galaxaura sp.* [(HG) representing High Growth form and (LG) representing Low Growth form].

There was no anti-fungal activity of any of the crude extract of the tested algae on the fungus *A. fumigatus*. The results shown in Fig. (1) below represent a moderate inhibition of the crude extract of the green alga *caulerpa* Sp. [(At) and (W)] against yeast; C. albicans with an inhibition zone of 10 mm, while the rest of the algal extracts didn't exhibit any activity. All algal extracts exhibited bioactivity against the pathogenic yeast *C. neoformans*, where the green alga *Caulerpa sp.* (W) and *Caulerpa sp.* (At) had the highest bioactivity, with an inhibition zone of 25 and 22 mm respectively, where sample (W) was similar to that of Ketoconazole, followed by *Turbinaria sp.*, *Galaxaura sp.* (HG), *Chondrophycus sp.*, *Cystoseira sp.*, *Chondria sp.* and *Galaxaura sp.* (LG) with an inhibition zone of 18, 18, 16, 16, 16 and 16 mm respectively, as illustrated in Fig. (2).

Also, as shown in Fig. (3), all algae species exhibited moderate anti-bacterial activity against the pathogenic bacteria *S. aureus*, where the green alga *Caulerpa sp.* (W) and *Caulerpa sp.* (At) had the highest anti-bacterial activity, with an inhibition zone of 16 and 15 mm respectively, followed by *Turbinaria sp.*, *Chondria sp.*, *Galaxaura sp.* (HG & LG), *Chondrophycus sp.* and *Cystoseira sp.* with an inhibition zone of 12, 12, 12, 12, 10 and 10 mm respectively. Furthermore, all algal species exhibited moderate anti-bacterial activity against *B. subtills* except for *Chondrophycus sp.*, which didn't exhibit any activity. The green alga *Caulerpa sp.* (W) had the highest anti-bacterial activity with a 16 mm inhibition zone, followed by *Turbinaria sp.*, *Chondria sp.*, *caulerpa sp.* (At), *Cystoseira sp.*, *Galaxaura sp.* (HG), *Galaxaura sp.* (LG) with an inhibition zone of 14, 12, 11, 10, 10 and 8 mm respectively, as illustrated in Fig. (4). As shown in Fig. (5); all algal species exhibited moderate anti-bacterial activity against *E. coli*. The green alga *Caulerpa sp.* (W) had the highest anti-bacterial activity with a 14 mm inhibition zone, followed by *Turbinaria sp.*, *Chondria sp.*, *caulerpa sp.* (At), *Galaxaura sp.* [(HG) and (LG)], *Cystoseira sp.* and *Chondrophycus sp.* with an inhibition zone of 12, 12, 12, 10, 10, 9 and 9 mm respectively. On the other hand, only four algal extracts showed activity against the *P. vulgaris*. *Turbinaria sp.* and *Chondria sp.* were the highest with an inhibition zone of 13 mm, followed by *Caulerpa sp.* [sample (W), then (At)] with an inhibition zone of 12 and 10 mm respectively, as illustrated in Fig. (6).

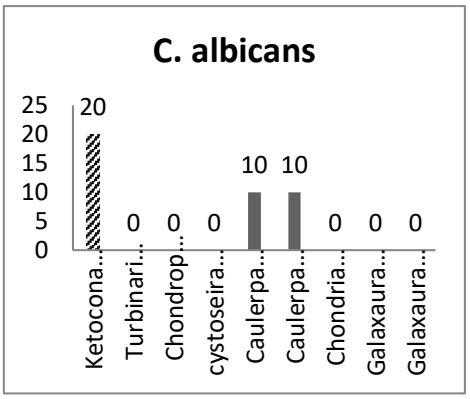

Fig (1): The anti-yeast activity of the crude extract of tested algal samples against *C. albicans*.

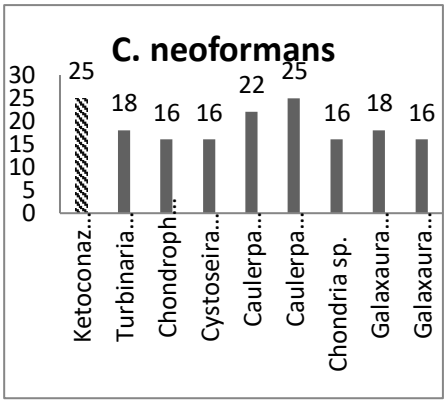

Fig (2): The anti-yeast activity of the crude extract of tested algal samples against *C. neoformans.*

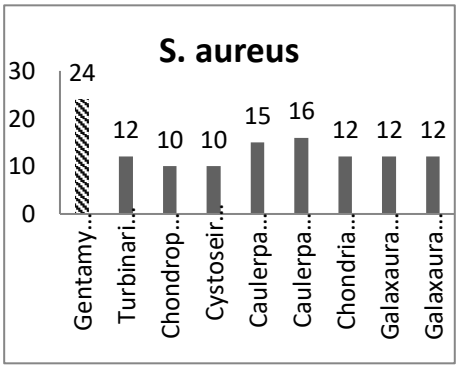

Fig (3): The anti-bacterial activity of the crude extract of tested algal samples against *S. aureus*.

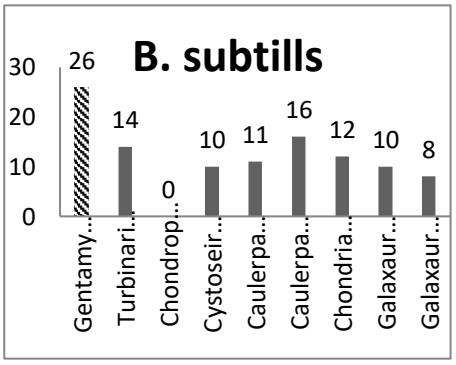

Fig (4): The anti- bacterial activity of the crude extract of tested algal samples against *B. subtills*.

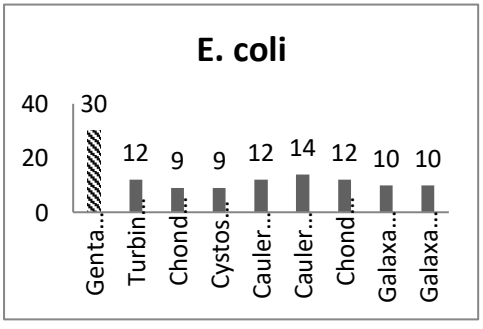

Fig (5): The anti-bacterial activity of the crude extract of tested algal samples against *E. coli.*

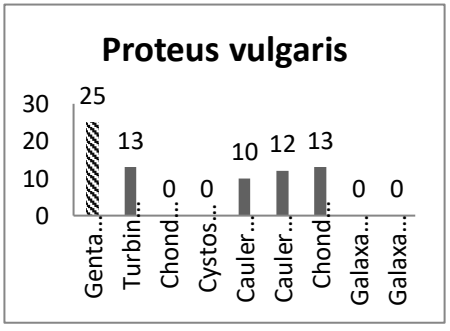

Fig (6): The anti-bacterial activity of the crude extract of tested algal samples against *P. vulgaris.*

Discussion:

In the present study, the antibacterial results indicated that the extract of *Caulerpa sp.* (collected in winter) was the most active among all tested algal extracts, exhibiting a high antibacterial activity against *S. aureus*, and a moderate inhibition to the rest of the pathogens tested. Similar results were reported by ***El-deen (2011)***, who mentioned in his study that *Caulerpa sp.* was found the one with the highest activity among other algal species. Also, ***Choudhury et al. (2005), Patra et al. (2008) and Rangaiah et al. (2010)*** indicated in their studies, the antimicrobial effect of the different extracts of marine algae *Caulerpa taxifolia* and the presence of active constituents in its extractions against bacterial and fungal pathogens.

Our results indicated that *Turbinaria sp.* and *Chondria sp.* exhibited a moderate inhibition against tested pathogens except for *E. coli*. This result is in accordance with ***Vijayabaskar and Shiyamala (2011),*** who found that the methanolic extract of *Turbinaria ornata* exhibited a moderate activity against *S. aureus* and all other pathogens. Also, ***Oumaskour et al. (2013)*** mentioned in their work that, the methanol extract of *Chondria dasyphylla*, showed activity against *Bacillus sp.* and *Streptococcus faecalis*. The extract of the green alga *Caulerpa sp.* was the only extract exhibiting a moderate activity against the yeast, *C. albicans*. However the yeast, *C. neoformans*, was highly inhibited by the green alga *Caulerpa sp.*, and moderately inhibited by all other algal extracts. Our results were confirmed by ***Val et al. (2001)*** who reported that the extract of *Caulerpa prolifera* posed a reasonable activity against the yeast *C. albicans*. Also, ***Ibraheem et al. (2012)*** mentioned that the different extracts of *Caulerpa racemosa*, *Turbinaria ornata*, *Cystoseira myrica*, and *Cystoseira compressa*, had from strong to weak antifungal activity against *C. albicans*. In contrast with our results, ***Karabay-Yavasoglu et al. (2007)*** reported that the yeast *C. albicans* wasn't inhibited by any of the algal extracts tested.

In seaweeds, it was proved by many authors that species from different divisions exhibit varied antimicrobial activity. In the current study, the extract of Chlorophyta *(Caulerpa sp.)* exhibited higher antimicrobial activity than that of Rhodophyta and Phyophyta. Our results were in agreement with ***Reichelt and Borowitzka (1984)*** who reported that extracts of green algae were the most active. Also, ***Ibraheem et al. (2012)*** revealed that *Caulerpa racemose* and *Ulva lactuca* (Chlorophyceae) were the most effective against tested pathogens.

In this study, a slight difference in inhibition zone between the two samples of *Caulerpa sp.* collected in two different seasons (autumn and winter) was observed. This slight difference could be due to the close in the two times of samples' collection (November and February). ***Stirk et al. (2007) and Durmaz et al., (2008)*** revealed that habitat (geographical variations), maturity (life phase) and time of the year are from the factors that influence bioactivity of macroalgae's compounds.

Conclusion:

In conclusion the results of the present investigation on selected species of marine algae indicated scope for deriving biologically active compounds which are effective in inhibiting the growth of the pathogenic bacteria and yeast. Further the Red sea marine environment of Egypt has potential to return pharmaceutically useful seaweeds which can be a great scope for further investigations toward drug development.

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الخصائص الحيوية للانواع المختارة من الطحالب البنية والحمراء المجمعة من مصر

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تعد الطحالب مصدر هام للمستقبلات الثانوية و العديد من المركبات التي تتميز بالعديد من البشاطات الحيوية. ومع ذلك لم يتم الكشف بشكل كافي علي الطحالب الموجودة بالبحر الاحمر و دورها كمصدر للمواد ذات النشاط البيولوجي. في هذه الدراسة، تم تجميع *Caulerpa sp.* ، *Chondria sp.* ، *Galaxaura sp.* ، *Turbinaria sp.* و *cystoseira sp.* من البحر الاحمر في مصر و تم الكشف عن خصائصها الحيوية. تم تحضير مستخلصات الطحالب باستخدام المذيب Ethanol/Dichloromethane بنسبة ١:١. عند مقايسة مضادات الميكروبات، وجدنا ان مستخلص ال*Caulerpa sp.* اظهر أعلي نتيجة بالنسبة لمضادات البكتيريا و الخميرة. يمكن الاستنتاج ان البحر الاحمر في مصر يعد بيئة بحرية تحتوي علي العديد من الطحالب البحرية المهمة التي قد يكون لها دور كبير في تطوير الادوية، و مع ذلك يجب اجراء المزيد من الدراسات حول هذا الموضوع.

الكلمات الدالة: الطحالب، البحر الاحمر، النشاطات الحيوية، مضادات الميكروبات