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FULL LENGTH ARTICLE

GC/MS spectroscopic approach and antifungal potential of bioactive extracts produced by marine macroalgae

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Abstract The antifungal activity and the chemical constituents of selected macroalgae collected from the Egyptian Mediterranean coast of Alexandria have been investigated. Agar well diffusion assay was used to determine the antifungal potential of the extractable matter against *Fusarium solani*, *Fusarium oxysporum*, *Tricoderma hamatum*, *Aspergillus flavipes* and *Candida albicans*. The ethyl acetate and methanolic extracts (ULE2 and ULM5) of *Ulva lactuca* obtained from Al Selsela exhibited the highest activity with (AI) = 1.05 ± 0.053 and 1.03 ± 0.052, respectively, compared with fluconazole. However, the methanolic extract of *U. lactuca* (ULM1) from Abu Qir Bay showed (AI) = 0.73 ± 0.037. This followed by methanolic extracts of *Pterocladia capillacea* (PCM1: AI = 0.70 ± 0.035 and *Ulva fasciata* (UFM1: AI = 0.69 ± 0.035). GC/MS analysis of ULM1 and ULM5 indicated the existence of different constituents revealing ecological impacts. The methanolic extract (UFM1) contains six major components including palmitic acid, methylester, trichloromethyloxirane, linolenic acid, ethylester, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, 11-octadecenoic acid, methylester and 12,15-octadecadienoic acid, methylester. High percentages of palmitic acid, n-heptacosane, 2-methylhexadecan-1-ol, methoxy acetic acid, 2-tridecylester and myristic acid are found in the methanolic extract of *P. capillacea* (PCM1). Most of the identified components have been reported to possess antimicrobial activity that could be responsible for the antifungal potential reported in the present study.

Introduction

Marine environment is a good source of bioactive natural products exhibiting structural/chemical features that are not found in terrestrial natural compounds (Carter, 1996). Several marine organisms produce bioactive metabolites in response to

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ecological pressures (Ireland et al., 2000). They develop a chemical strategy for defense against other organisms in their environment and to ensure their survival (König et al., 1994). Numerous novel compounds have been isolated from marine organisms (Jha and Zi-rong, 2004; Arunkumar et al., 2010; D'Orazio et al., 2012; Mayer et al., 2013; Balakrishnan et al., 2014; Pal et al., 2014). Blunt et al. (2016) reported that approximately 25 700 new marine natural products (MNPs) have been discovered over the period 1965–2014 from 22 oceanic regions or countries. There have been extensive collections with 2358 compounds in the Mediterranean, Arabian Peninsula and Black sea regions. This high diversity has been the source of unique natural compounds used for various industrial developments such as pharmaceuticals, cosmetics and nutritional supplements (Suleria et al., 2015). Marine macroalgae have been widely recognized as producers of a broad range of biogenic compounds including polyunsaturated fatty acids, flavonoids, terpenoids, alkaloids, quinones, sterols, polyketides, phlorotannins, polysaccharide, glycerols, peptides and lipids (Al-Saif et al., 2014; El-Din and El-Ahwany, 2015) that have antimicrobial (Zbakh et al., 2012), antifouling (Bhadury and Wright, 2004), anti-inflammatory (Jaswir and Monsur, 2011), antiviral (Bouhlal et al., 2011), antioxidant (Devi et al., 2011), anticancer (Kim et al., 2011), anti-allergic (Na et al., 2005) and anticoagulant activities (Dayong et al., 2008; Kolanjinathan et al., 2014; Smit, 2004). For example, the green alga *Enteromorpha compressa* is rich in C_{18} PUFAs ± α-linolenic (18:3n-3) and octadecatetraenoic (18:4n-3) acids where α-linolenic acid is the main polyunsaturated acid (Khotimchenko et al., 2002) and the flavonoids content is 33.39 mg/g as reported by Sarojini et al. (2012).

In recent years, a significant number of reviews reported numerous investigations that have been carried on crude and purified compounds obtained from marine algae to evaluate their bioactive potentials (Mhadhebi et al., 2012; Balamurugan et al., 2013; Barbosa et al., 2014; Torres et al., 2014; Kolanjinathan et al., 2014; El-Din and El-Ahwany, 2015; Hamed et al., 2015; Blunt et al., 2016). The first antimicrobial substances produced by algae have been reported by Harder (1917). The variation in the production of antimicrobial substances by the same species might be due to ecology, sexual maturity or the stage of active growth (Pesando, 1990; Burkholder et al., 1960). The extracts of an alga collected from the same location are more effective than the same species collected from another location as reported by Arunkumar et al. (2010). Lee et al. (2010) studied the antifungal activity of *Ecklonia cava* brown algae against *Trichophyton rubrum*. A study on the antifungal activities of five species of *Laurencia* red algae against three strains of *Candida* spp. was carried out (Stein et al., 2011). Crude extracts of green, brown and red algae have been screened for the antifungal potential against standard dermatophytes strains (Guedes et al., 2012). Recently, screening of the antifungal activity from marine algae as prominent natural antibiotic was presented by Chowdhury et al. (2015) where new terpenoid derivatives from the red alga *Laurencia obtusa* considered as effective antifungal-antitumor agents (Alarif et al., 2015). Different analysis methods such as GC/MS, LC/MS, HPLC/DAD and CE/DAD (diode array detection) have been developed for the identification of the bioactive constituents (Gong et al., 2001). Mass selective detector spectral information in addition to retention time, peak height and peak area information enhances the identification of the components.

Several studies have been reported on the antibacterial activity of marine algae however, available information on their antifungal activity is limited. Consequently, the present investigation aimed to evaluate the antifungal activity of six Mediterranean macroalgae extracts, collected from the coastline of Alexandria, against five pathogenic fungi in order to discover potential antifungal metabolites. Qualitative identification of the most potential antifungal extracts of *Ulva lactuca*, *Ulva fasciata* and *Pterocladia capillacea* was performed using retention times and mass spectra in the GC/MS analysis.

Materials and methods

Samples collection

Six fresh seaweeds; *U. lactuca*, *U. fasciata*, *E. compressa*, *P. capillacea*, *Hypnea musciformis* and *Padina pavonica* were harvested at various sites along the Mediterranean Egyptian coast of Alexandria (Abu Qir Bay, Al Selsela and El-Anfoushy). After collection, the samples were rinsed with fresh water to remove associated epiphytes and debris. The cleaned algal materials were then air dried to dryness in the shade and ground into fine powder using electric grinder mixer.

Preparation of the algal extracts

Extraction of the bioactive algal extracts has been carried out using ethyl acetate and methanol as follows; the finely powdered algal material (100 g) was macerated separately in ethyl acetate (1.5 L) followed by methanol (1.5 L) at room temperature for a period of one week with regular shaking. After filtration, organic solvents were evaporated under vacuum at 45 °C to furnish dry ethyl acetate and methanolic extracts. The extraction procedure with each solvent was repeated once again to furnish ethyl acetate extracts as green residues while methanolic extracts were separated as green residues (ULM1, ULM5, UFM1, ECM, PCM1, HM1, and PPM1) or white precipitates (ULM3, ULM4), crystals (ULM7) or pale yellow liquids (ULM2, ULM6, UFM2, PCM2, HM2, PPM2). The crude extractable materials were then stored at -20 °C until use (Choudhury et al., 2005; Wefky et al., 2009; Shobier et al., 2010).

Test organisms

The test organisms used in this study (*Fusarium solani* AUMC 6448, *Fusarium oxysporum* AUMC 6449, *Tricoderma hamatum* AUMC 382, *Aspergillus flavipes* AUMC 6450 and *Candida albicans* ATCC 10231) were kindly provided by Assiut University Mycological Center, Faculty of Science. These fungal strains were subcultured onto potato-dextrose agar (PDA) supplemented with Rose Bengal for 5–7 days at 28–33 °C.

Antifungal assay

The *in vitro* antifungal analysis of the studied algal extracts was recorded in terms of a well cut technique (Bodet et al., 1985). Fungal colonies were diluted in sterilized water to 0.5