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FULL LENGTH ARTICLE

Antimicrobial activity of some seaweeds species from Red sea, against multidrug resistant bacteria

Shimaa M. El Shafay, Samh S. Ali, Mostafa M. El-Sheekh *

Botany Department, Faculty of Science, Tanta University, 31527 Tanta, Egypt

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Abstract This study evaluates the antibacterial activity of diethyl ether, methanol, ethanol and chloroform extracts of red algae *Ceramium rubrum* (Rhodophyta), *Sargassum vulgare*, *Sargassum fusiforme* and *Padina pavonia* (Phaeophyta) collected from Red sea, Egypt. The algal extracts were tested for their antibacterial activity against ten multidrug resistant clinical isolates of Gram positive and Gram negative bacteria. The highest inhibition activity among all extracts was obtained with 100 µl diethyl ether extract *S. fusiforme* against *Staphylococcus aureus* 2 and 50 µl ethanol extract of *S. vulgare* against *Klebsiella pneumoniae*. The algal extract of *S. fusiforme* and *S. vulgare* was characterized by Gas chromatography–mass spectrometry (GC–MS). The compounds with antimicrobial activity were identified, such as phenols, terpenes, acetogenins, indoles, fatty acids and volatile halogenated hydrocarbons. Transmission electron microscopy was applied for determining the morphological changes in *S. aureus* 2 and *K. pneumonia* treated with 100 µl diethyl ether extract of *S. fusiforme* and 50 µl ethanol extract of *S. vulgare*, respectively. Perforation of cell wall, leakage of cytoplasmic contents, severe distortion of outer cell shape, inner chromatin mild scattered cytoplasmic vacuolation, rupture of cell wall, and decreased cell size for both bacterial isolates treated with 100 µl diethyl ether of *S. fusiforme* extract and 50 µl *S. vulgare* ethanolic extract were recorded.

Introduction

Various natural antimicrobial compounds have been recorded in marine environment more than those in the terrestrial one (Ireland et al., 1988). Marine organisms such as marine algae are source material for structurally unique natural products with pharmacological and biological activities (Schwartsmann et al., 2001). Among the marine organisms, the macroalgae (seaweeds) occupy a special site as a source of biomedical compounds (Manilal et al., 2010). Seaweeds have been recognized as potential sources of the antibiotic substances. Synthesis of different metabolites from seaweeds is an indicator of the presence of antimicrobial active compounds (Chiheb et al., 2009). A wide range of bioactive compounds were derived from macro algae such as antibacterial active compounds (Lustigman and Brown, 1991). Seaweeds contain many different secondary metabolites which

* Corresponding author. Fax: +20 40 3350804.
E-mail address: mostafaelsheikh@science.tanta.edu.eg (M.M. El-Sheekh).

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have a wide spectrum of biological activities. It was observed, the presence of cytostatic, antiviral, anthelminthic, antifungal and antibacterial activities compounds in green, brown and red algae with cytostatic, antiviral, anthelminthic, antifungal and antibacterial activities (Lindequist and Schweder, 2001; Newman et al., 2003 and Chakraborthy et al., 2010). Seaweeds are considered to be the main source of bioactive compounds with a wide range of biological activities, such as antibiotics, antioxidant and anti-inflammatory (Tuney et al., 2006; Patra et al., 2008). Some macroalgae have bio-active components which affected the germination of some pathogenic bacteria (Kolanjinathan et al., 2009). Hornsey and Hide (1985) found that many species of marine algal crude extracts have inhibition activity against pathogenic bacteria. Seaweeds contain different substances which incorporated medicine and pharmacotherapy, whereas some of the isolated substances have bacteriostatic and bactericidal properties (Gorban et al., 2003). Different diseases were treated with antibiotics, extracted from terrestrial sources that were used as therapeutic agents; new compounds were present in oceans and have commercial value (Smit, 2004).

Clinical and public health problem due to antibiotic resistance and multi-resistant bacteria are difficult and sometimes impossible to treat (Levy, 2002). Using antibiotics in different medicines has a significant role in the emergence of bacterial strains resistant to antibiotics (Bacon et al., 2000). Recently, new mechanisms of resistance have resulted in the simultaneous development of resistance to several antibiotic classes creating very dangerous multidrug resistant (MDR) bacterial strains, some also known as "superbugs" (Sande-Bruinsma et al., 2008). The required number of new antimicrobial agents is higher than ever due to the rapid presence of new infections, emergence of multidrug resistance in common pathogens, and the potential for use of multidrug-resistant agents in bioweapons (Peters et al., 2008). Organisms resistant to more than one class of antimicrobial agents are identified as multidrug resistant organisms (MDROs) (Sameera et al., 2010).

This work aims to evaluate the antimicrobial activity of some seaweeds extracts from Red sea coast against some collected clinical multidrug resistant bacterial isolates in order to find alternative drugs and promising source of pharmaceutical agents.

Material and methods

Algal collection and preparation

Four seaweeds species, *Ceramium rubrum* (Rhodophyta), *Sargassum vulgare*, *Sargassum fusiforme* and *Padina pavonia* (Phaeophyta) were collected from Hurghada coastal along the Red sea, Egypt, and identified according to Aleem (1993). Different species of collected algae were cleaned with seawater to remove impurities. The seaweeds were transported to the laboratory in sterile polythene bags. In the laboratory, samples were rinsed with tap water and were shade dried, cut into small pieces and powdered in a mixer grinder.

Preparation of organic algae extracts

Different organic solvents (ethanol, methanol, chloroform and diethyl ether) were used for extraction. Five grams of each powdered sample were soaked in 40 ml of the solvent for three days. Remain extracts were filtered and concentrated in a rotatory evaporator at 35 °C. The residual water was removed with a vacuum pump. The weighted crude extracts were suspended in the dimethyl sulfoxide (DEMSO) to a final concentration of 50 mg/ml and stored in a refrigerator (Mohanta et al., 2007; Patra et al., 2008).

Collection of bacteria

Two isolates of *Pseudomonas aeruginosa* (PA1 and PA2) were recovered and identified by Khalil et al. (2015). Four isolates of *Staphylococcus aureus* (SA1, SA2, SA3 and SA4), *Shigella flexneri*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Corynebacterium* sp. Bacterial species were brought from microbiology collection, Faculty of Pharmacy, Tanta University, Egypt. The morphological and biochemical tests were carried out continuously to ensure purity (Collee et al., 1996).

Antibiotic susceptibility testing

The antimicrobial susceptibility of the collected bacteria was assessed using the modified Kirby-Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI, 2012). The following concentrations of antibiotics were tested: Ampicillin (AMP, 10 μg), Amoxicillin (AX, 25 μg), Amoxycillin/Clavulanic acid (AMC, 20/10 μg), Oxacillin (OX, 1 μg), Piperacillin-tazobactam (TPZ, 100/10 μg), Ceftazidime (CAZ, 30), Cefepime (FEP, 30 μg), Ceftriaxone (CRO, 30 μg), Imipenem (IPM, 10 μg), Meropenem (MEM, 10 μg), Cefoperazone/sulbactam (CES, 75/30 μg), Aztreonam (ATM, 30 μg), Gentamicin (CN, 10 μg), Amikacin (AK, 30 μg), Neomycin (N, 30 μg), Streptomycin (S, 10 μg), Tobramycin (TOB, 10 μg), Kanamycin (K, 30 μg), Chloramphenicol (C, 30 μg), Colistin Sulfate (CT, 10 μg), Nalidixic acid (NA, 30 μg), Ciprofloxacin (CIP, 5 μg), Co-trimoxazole (SXT, 25 μg), Tetracycline (TE, 30 μg), Vancomycin (VA, 30 μg). The antibiotic disks were then applied to the prepared plates and incubated at 37 °C for 18 h then, the diameter of the growth inhibition zones was measured. The multiple antibiotic resistances (MAR) index was calculated for each isolate by dividing the number of antibiotics to which the isolate is resistant by the total number of antibiotics tested (Krumpernam, 1983; Olayinka et al., 2009; Jayaraman et al., 2012).

Ultra-structure of multiple drug resistant (MDR) bacterial isolates

The changes in ultra-structure of selected MDR, *S. aureus* 2 and *K. pneumoniae*, due to algal extract treatment were investigated by transmission electron microscope (JEOL-JEM-100SX, Japan). The samples were incubated by shaking at 37 °C for 18 h followed by centrifugation, and washing using saline solution (Richards and Cavill, 1976).