



Original article

Antimicrobial, antioxidant and anticancer activities of *Laurencia catarinensis*, *Laurencia majuscula* and *Padina pavonica* extractsNouf M. Al-Enazi^a, Amani S. Awaad^{b,*}, Mohamed E. Zain^c, Saleh I. Alqasoumi^d^a Biology Department, College of Science and Humanity Studies, Prince Sattam Bin Abdulaziz University, Alkharj, Saudi Arabia^b Pharmacognosy Department, College of Pharmacy, Prince Sattam bin Abdulaziz University, Al-Kharj, Saudi Arabia^c Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt^d Pharmacognosy Department, College of Pharmacy, King Saud University, Saudi Arabia

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ABSTRACT

The antimicrobial, antioxidant, and anticancer activities of ethanolic extract of *Laurencia catarinensis*, *L. majuscula* and *Padina pavonica* were determined. The highest antibacterial activity; 23.40 ± 0.58 mm ($00.98 \mu\text{g/ml}$) and 22.60 ± 2.10 mm ($03.90 \mu\text{g/ml}$) were obtained against *Klebsiella pneumonia* by *Laurencia catarinensis* and *Padina pavonica*, respectively. However, *Padina pavonica* showed excellent antibacterial activity against *Bacillus subtilis* (21.7 ± 1.5 mm; $1.95 \mu\text{g/ml}$), *Staphylococcus aureus* (21.7 ± 0.58 mm; $1.95 \mu\text{g/ml}$), *Streptococcus pyogenes* (20.7 ± 1.2 mm; $1.95 \mu\text{g/ml}$) and *Acinetobacter baumannii* (20.1 ± 1.2 mm; $3.9 \mu\text{g/ml}$). Moreover, the highest antifungal activity; 24.7 ± 2.0 mm ($0.98 \mu\text{g/ml}$), 23.7 ± 1.5 mm ($0.98 \mu\text{g/ml}$), 23.6 ± 1.5 mm ($0.98 \mu\text{g/ml}$) was obtained by *Padina pavonica* against *Candida tropicalis*, *C. albicans* and *Aspergillus fumigatus*, respectively. The algal extracts showed DPPH radical scavenging activity in a concentration-dependent manner with maximum scavenging activity (77.6%, $\text{IC}_{50} = 5.59 \mu\text{g/ml}$ and 77.07%, $\text{IC}_{50} = 14.3 \mu\text{g/ml}$) was provided by *Padina pavonica* and *Laurencia majuscula*, respectively. The *in vitro* antitumor activity revealed that the IC_{50} values of *Padina pavonica* were 58.9, 115.0, 54.5, 59.0, 101.0, 101.0, and $97.6 \mu\text{g/ml}$; *Laurencia catarinensis* were 55.2, 96.8, 104.0, 78.7, 117.0, 217.0, $169.0 \mu\text{g/ml}$; and *Laurencia. majuscula* were 115.0, 221.0, 225.0, 200.0, 338.0, 242.0, and $189.0 \mu\text{g/ml}$; respectively against A-549 (Lung carcinoma), Caco-2 (Intestinal carcinoma), HCT-116 (Colon carcinoma), Hela (Cervical carcinoma), HEP-2 (Larynx carcinoma), HepG-2 (Hepatocellular carcinoma), and MCF-7 (Breast carcinoma) cell lines.

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0. Introduction

Marine algae have long been used as food and medicine in many Asian countries including Japan, China, Thailand and Korea. Natural products of marine algae are in great demand due to their prolific biological activities that might represent useful leads in the discovery of novel bioactive compounds and new pharmaceutical agents (Blunden, 2001; Iwamoto et al., 2001). Consumption of the marine algae is thought to ameliorate some inflammatory disorders, breast

cancer and high cholesterol level (Fitton and Helen, 2003). Numerous novel compounds have been isolated, during the last few decades, from marine organisms and many of these substances have been proved to possess remarkable biological activities (El Gamal, 2010; Proksch et al., 2002; Faulkner, 2002, 2001).

Different compounds isolated from marine algae have shown antimicrobial activities and are used in pharmaceutical industries (Rajasulochana et al., 2009; El-Fatemy, 2008; Venkateswarlu et al., 2007; Tüney et al., 2006; Ely et al., 2004; Lima-Filho et al., 2002). Antioxidant activity is important in various pharmacological activities such as anti-aging, anti-inflammatory, and anti-cancer activities (Lee et al., 2004; Middleton et al., 2000). Antioxidant activity is claimed to be present in most of the nutraceuticals and cosmeceuticals. However, numerous synthetic antioxidants are produced, but are quite unsafe and their toxicity is of concern (Madhavi et al., 1995). On the other hand, Natural products with antioxidant activity are used for human consumption because of their safety. Different compounds with cytostatic, antiviral,

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antihelmintic, antioxidant, antifungal and antibacterial activities have been detected in green, brown and red algae (Newman et al., 2003; Lindequest and Schweder, 2001).

One of the most life-threatening in developed and developing countries is cancer. Natural anticancer compounds are able to control the growth of cancer cells with no or minor side effects. Accordingly, identification of new effective cancer chemopreventive agents has become an important worldwide strategy in cancer prevention. Different compounds isolated from marine algae were found to have antiproliferative activity in cancer cell lines *in vitro*, as well as inhibitory activity of tumor growth in mice (Yang et al., 2008; Ye et al., 2008; Rocha de Souza et al., 2007; Kwon and Nam, 2006). The current study was carried out to determine the antimicrobial, antioxidant and anticancer activities of *Laurencia catarinensis*, *L. majuscula* and *Padina pavonica*.

1. Material and methods

1.1. Algal samples collection, extraction and screening

1.1.1. Algal species collections

The algal species used in this study; namely, *Laurencia catarinensis*, *Laurencia majuscula* and *Padina pavonica* were collected from Alharra, Umluj, Red Seashore, Kingdom of Saudi Arabia. Algal species were identified according to Aleem (1978, 1993), Bold and Wynne (1978) and Coppejans et al. (2009). Samples collected were air-dried in shade, reduced to fine powder, packed in tightly closed containers and stored for phytochemical and biological studies.

1.1.2. Algal extraction

Dry powder of each alga under investigation were separately (600 g) was extracted by percolation in 95% ethanol (Awaad et al., in press) at room temperature for two days. The ethanol extracts were separately filtered and the residues were re-percolated for five times for each alga. The total ethanol extracts were separately concentrated under reduced pressure at a temperature not exceeding 35 °C

1.1.3. Phytochemical screening

Powdered samples from the of the investigated alga were subjected to phytochemical screening for their different constituents such as; carbohydrates and/or glycosides, flavonoides, tannins, sterols and/or triterpenes, proteins and/or amino acids, alkaloids and/or nitrogenous bases, saponins, anthraquinones, cardenolides and oxidase enzyme (Khan et al., 2011).

1.2. Antimicrobial activity

1.2.1. Test organisms

Different clinically isolated microorganisms including 10 bacterial strains; Gram-negative bacteria, *Acinetobacter baumannii* (RCMB 0100282-9), *Escherichia coli* (RCMB 010056), *Klebsiella pneumoniae* (RCMB 0010093), *Proteus mirabilis* (RCMB 0100254-2) and *Pseudomonas aeruginosa* (RCMB 0100243-5), Gram-positive bacteria, *Bacillus subtilis* (RCMB 0100169-3), *Staphylococcus aureus*, *Staphylococcus epidermidis* (RCMB 010027), *Streptococcus pyogenes* (RCMB 0100174-2) and *Streptococcus sanguinis* (RCMB 0100171-3); and 10 fungal strains including *Aspergillus fumigatus* (RCMB 02568), *Aspergillus niger* (RCMB 02724), *Candida albicans* (RCMB 05036), *C. tropicalis* (RCMB 05239), *Cryptococcus neoformans* (RCMB 05642), *Geotrichum candidum* (RCMB 05097), *Microsporium canis* (RCMB 0834), *Penicillium expansum* (RCMB 01924), *Syncephalastrum racemosum* (RCMB 05922) and *Trichophyton mentagrophytes* (RCMB 0925) were identified by in the Microbiology

Laboratory, Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt and used as test organisms.

1.2.2. Antimicrobial assay

The antibacterial and antifungal activities of ethanolic extract of *Laurencia catarinensis*, *L. majuscula* and *Padina pavonica* were determined using the well diffusion method (Zain et al., 2012). Petri plates containing 20 ml of, nutrient (for bacteria) or malt extract (for fungi), agar medium were seeded with 1–3 day cultures of microbial inoculums. Wells (6 mm in diameter) were cut off from agar and 50 µl of algal extracts were tested in a concentration of 100 mg/ml and incubated at 37 °C for 24–48 h (bacterial strains) and for 3–5 days (fungal strains). The antibacterial and antifungal activities were determined by measurement of the diameter of the inhibition zone around the well.

1.2.3. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was determined by micro-dilution method using serially diluted (2 folds) algal extracts (Zain et al., 2012). The MIC of *Laurencia catarinensis*, *L. majuscula* and *Padina pavonica* extracts were determined by dilution of concentrations from 0.0 to 100 mg/ml. Equal volume of each extract and nutrient broth were mixed in a test tube. Specifically 0.1 ml of standardized inoculum ($1-2 \times 10^7$ cfu/ml) was added in each tube. The tubes were incubated at 37 °C for 24–48 h and/or 3–5 days. Two control tubes, containing the growth medium, saline and the inoculum were maintained for each test batch. The lowest concentration (highest dilution) of the algal extract that produced no visible microbial growth (no turbidity) when compared with the control tubes were regarded as MIC.

1.3. Antioxidant activity (DPPH (1-diphenyl-2-picrylhydrazyl) radical-scavenging assay)

The antioxidant activity of *Laurencia catarinensis*, *L. majuscula* and *Padina pavonica* extract was determined using the DPPH free radical scavenging assay according to the method described by Yen and Duh (1994). The assay was carried out in triplicate and the mean value was recorded.

Freshly prepared (0.004%w/v) methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was prepared and stored at 10 °C in the dark. A methanol solution of the test compound was prepared. A 40 µL aliquot of the methanol solution was added to 3 ml of DPPH solution, under light protection. Absorbance measurements were recorded immediately with a UV–visible spectrophotometer (Milton Roy, Spectronic 1201). The decrease in absorbance at 515 nm was determined continuously, with data being recorded at 1 min intervals until the absorbance stabilized (16 min). The absorbance of the DPPH radical without antioxidant (control) and the reference compound ascorbic acid were also measured. The percentage inhibition (PI) (scavenging activity) of the DPPH radical was calculated according to the formula (Yen and Duh, 1994):

$$PI = (AC - AT) / AC \times 100$$

where AC = Absorbance of the control at t = 0 min and AT = absorbance of the sample + DPPH at t = 16 min.

1.4. Antitumor activity

The cell lines A-549 (Lung carcinoma), Caco-2 (Colorectal carcinoma), HCT-116 (Colon carcinoma), Hela (Cervical carcinoma), HEP-2 (Larynx carcinoma), HepG-2 (Hepatocellular carcinoma), and MCF-7 (Breast carcinoma) were used for determination of anti-tumor activity of *Laurencia catarinensis*, *L. majuscula* and *Padina pavonica*. The tumor cell lines were suspended in medium at

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