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Original article

Biological activities of the red algae *Galaxaura rugosa* and *Liagora hawaiiana* butters

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ABSTRACT

The biological activities; antimicrobial, antioxidant and anticancer, of the red algae *Galaxaura rugosa* and *Liagora hawaiiana* were determined. The total ethanol, lipoidal matters, chloroform, n-butanol, aqueous extracts and powder of both algae showed and bacterial and antifungal activities. However, the chloroform extract of *Galaxaura rugosa* showed antibacterial activity against *Klebsiella pneumoniae* (24 mm, 0.15 mg/ml) higher than gentamycin (23 mm, 0.49 mg/ml). Moreover, the total ethanol, lipoidal matter and chloroform extracts showed antifungal activity (21, 22 and 25 mm, 1.25, 0.312 and 0.156 mg/ml) similar to the antibiotic Ketoconazole activity (23, 24 and 27 mm, 1.25, 0.312 and 0.156 mg/ml) against *Aspergillus fumigatus*, *A. niger* and *Candida trobicalis*, respectively. A good antioxidant activity (80.96%, IC_{50} = 27.8 μg/ml) was provided by *Galaxaura rugosa*. The anticancer activity results revealed that the lipoidal matters of *Galaxaura rugosa* and *Liagora hawaiiana* possessed antitumor activity (IC_{50} = 15 ± 1.7 and 21.2 ± 1.6, respectively) against lung carcinoma (A-549) better than vinblastine sulfate (IC_{50} = 24.6 ± 0.7). Although, the lipoidal matters of *Galaxaura rugosa* and *Liagora hawaiiana* antitumor activity against cervical carcinoma (HeLa) and intestinal carcinoma (CACO-2) (IC_{50} = 10.2 ± 0.6 and 12.2 ± 0.6, respectively) preferable than vinblastine sulfate (IC_{50} = 59.7 ± 2.1 and 30.3 ± 1.4, respectively).

1. Introduction

The interest in ancient herbal remedies has been significantly increased in the last few decades. In the worldwide, all the natural resources including medicinal plants, fungi and algae are screened for their biological activities (Awaad et al., 2013, Zain et al., 2012, Amornlerdpison et al., 2007). Accordingly, the therapeutic values and pharmaceutical usage of numerous herbal medicines have already been validated. The herbal medicines which obtained from natural sources are considered as safe for human beings. However, they would have some antagonistic effects due to presence of other active ingredients (Izzo and Ernst, 2009).

Algae are found everywhere: in the sea, rivers, lakes, soil, walls, and as symbiont in animal and plants. Algae include four main divisions; namely, Red algae (Rhodophya), Brown Algae (Phycophyta), Green Algae (Chlorophyta) and Diatoms. Although, Seaweeds which are macroscopic, multicellular, and marine algae, are divided into three categories; red, green and brown organisms comprises about 30000 species. In most of Asian countries, seaweeds are traditionally traded as food items including sushi wrappings, seasonings, condiments, and vegetables (El Gamal, 2010; Mark et al., 2016).

Antioxidants have attracted the most interest among the many biologically-active compounds found in algae. Antioxidants are important compounds in the treatment and recovery from various diseases including cancer, chronic inflammation, atherosclerosis, cardiovascular disorders, and aging process (Kohen and Nyska, 2002). Although, the search for anticancer drugs has similar attention as marine compounds revealed promising results at different stages of cancer progress (Mayer and Gustafson, 2006). On the other hand, in developed and developing countries, the most people died following infectious bacterial and/or fungal diseases. The bacterial Gram-positive and Gram-negative organisms including

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different species of *Bacillus*, *Proteus*, *Klebsiella*, *Staphylococcus*, *Salmonella* and *Pseudomonas* are the main source of severe infections in animals including humans (Nathan, 2004).

Among seaweeds, numerous macroalgae have potent cytotoxic activities (Mayer and Gustafson, 2006, Smit, 2004) and algal consumption has been suggested as a chemo-preventive agent against several cancers (Yuan and Walsh, 2006). Recently, due to their exceptional richness in bioactive compounds (e.g., antimicrobial, anti-inflammatory, and antitumoral activities), the seaweeds has significantly expanded into the pharmaceutical and parapharmaceutical industry (Kornprobst, 2005; Smit, 2004). The current study aimed to assess the biological activity including antioxidant, antimicrobial, and anticancer of different extracts of the red algae *Galaxaura rugosa* and *Liagora hawaiiana*.

2. Material and methods

2.1. Algal samples collection, extraction and screening

2.1.1. Algal species collections

The algal species used in this study; namely, *Galaxaura rugose* and *Liagora hawaiiana* Butters were collected from Alharra, Umluj, Red Seashore, Kingdom of Saudi Arabia. Algal species were identified according to Aleem (1993) 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.

2.1.2. Algal extraction

Dry powder (830 and 795 g) of *Galaxaura rugose* and *Liagora hawaiiana*; respectively, were extracted by percolation in 95% ethanol (Awaad et al., 2017a) at room temperature for two days. The total ethanol extract was filtered and the residue was re-percolated by the same manor for five times. The ethanol extract was then concentrated, under reduced pressure at low temperature, and a yield of 81 and 77 g was obtained from *Galaxaura rugose* and *Liagora hawaiiana*, respectively.

The obtained extracts of each algae was separately suspended in water (300 ml) and filtered over a piece of cotton. The lipoidal matter, collected on top of the cotton piece (25 and 28 g. for *Galaxaura rugose* and *Liagora hawaiiana*, respectively) were obtained. The aqueous layer, which filtered off, was successively fractionated using chloroform and *n*-butanol. Each extract was dried over anhydrous sodium sulfate, concentrated and yielded 11 & 30 g and 14 and 26 g for chloroform and *n*-butanol of *Galaxaura rugos* and *Liagora* hawaiiana, respectively. However, after extraction with *n*-butanol some powder was precipitated from each algae and the filtration was carried out to separate it and. The leftover aqueous extract of each alga was dried using lyophilization (Awaad et al., 2017b) and kept for further investigation.

2.1.3. Phytochemical screening

Powdered sample of each investigated alga (*Galaxaura rugose* and *Liagora hawaiiana*) was subjected to phytochemical screening as published by Khan et al. (2011) to investigate their phytochemical constituents.

2.2. Antimicrobial activity

2.2.1. Test organisms

Different clinically isolated bacterial and fungal strains; namely, *Aspergillus fumigatus* (RCMB 02568), *Aspergillus niger* (RCMB 02724), *Bacillus substilis* (RCMB 010015), *Candida albicans* (RCMB 05003), *Candida. tropicalis* (RCMB 05004), *Cryptococcus neoformans* (RCMB 05642), *Escherichia coli* (RCMB 010052), *Geotricum candidum* (RCMB 05097), *Klebsiella pneumonia* (RCMB 0010093), *Microsporum canis* (RCMB 0834), *Penicillium expansum* (RCMB 01924), *Pseudomonas aeruginosa* (RCMB 0100243-5), *Proteous vlgaris* (RCMB 01004) *Staphylococcus aureus* (RCMB 010010), *Staphylococcus epidermidis* (RCMB 010009), *Streptococcus byogenes* (RCMB 0100174-2), *Stroptococcus mutans* (RCMB 0100017) *Salmonella typhimurium*, RCMB (RCMB 14028), *Syncephalastrum racemosum* (RCMB 05922) and *Trichophyton mentagrophytes* (RCMB 0925) were obtained from the Microbiology Laboratory, Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt and used as test organisms.

2.2.2. Antimicrobial assay

The antibacterial and antifungal activities of total ethanol, lipoidal matters, chloroform *n*-butanol, aqueous extracts and powder of *Galaxaura rugosa* and *Liagora hawaiiana* were determined using the well-diffusion method (Almalki, 2017). 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.

2.2.3. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of algal extract was determined by micro-dilution method using serially diluted (2 folds) algal extracts (Zain et al., 2012). The MIC of total ethanol, lipoidal matter, chloroform, *n*-butanol, aqueous extracts and powder of *Galaxaura rugosa* and *Liagora hawaiiana* were determined by dilution of concentrations from 0.0 to 100 mg/ml. Equal volumes 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.

2.3. Antioxidant assay

The antioxidant activity of *Galaxaura rugosa* and *Liagora hawaiiana* different extracts were determined using DPPH free radical scavenging assay as describe by Aksoy et al. (2013) in triplicate and average values were considered. The tested extracts were also compared using the IC_{50} value; i.e., the concentration leading to 50% inhibition which was estimated from graphical plots of DPPH Radical Scavenging% Vs concentrations.

2.4. Antitumor activity

The antitumor activity of total ethanol, lipoidal matters, chloroform, *n*-butanol, aqueous extracts and powder of *Galaxaura rugosa* and *Liagora hawaiiana* were determined using A-549 (Lung carcinoma), CACO (colorectal carcinoma), HCT-116 (Colon carcinoma), Hela (Cervical carcinoma), HEp-2 (Larynx carcinoma), HepG-2 (Hepatocellular carcinoma), and MCF-7 (Breast carcinoma) cell lines as described by Kameyama et al. (2005).

2.5. Statistical analysis

All values were expressed as mean ± S.D. Comparisons between means were carried out using a one-way ANOVA test followed by

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