



## Original article

Biological activities of the red algae *Galaxaura rugosa* and *Liagora hawaiiiana* buttersNouf M. Al-Enazi<sup>a</sup>, Amani S. Awaad<sup>b,\*</sup>, Saleh I. Alqasoumi<sup>c</sup>, Metab F. Alwethairi<sup>d</sup><sup>a</sup> Biology Department, College of Science and Humanity Studies, Prince Sattam Bin Abdulaziz University, Al-Kharj, Saudi Arabia<sup>b</sup> Pharmacognosy Department, College of Pharmacy, Prince Sattam bin Abdulaziz University, Al-Kharj, Saudi Arabia<sup>c</sup> Pharmacognosy Department, College of Pharmacy, King Saud University, Saudi Arabia<sup>d</sup> Clinical Pharmacist, King Abdulaziz Medicinal City, Saudi Arabia

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## ABSTRACT

The biological activities; antimicrobial, antioxidant and anticancer, of the red algae *Galaxaura rugosa* and *Liagora hawaiiiana* were determined. The total ethanol, lipoidal matters, chloroform, n-butanol, aqueous extracts and powder of both algae showed antibacterial 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 tropicalis*, respectively. A good antioxidant activity (80.96%, IC<sub>50</sub> = 27.8 µg/ml) was provided by *Galaxaura rugosa*. The anticancer activity results revealed that the lipoidal matters of *Galaxaura rugosa* and *Liagora hawaiiiana* possessed antitumor activity (IC<sub>50</sub> = 15 ± 1.7 and 21.2 ± 1.6, respectively) against lung carcinoma (A-549) better than vinblastine sulfate (IC<sub>50</sub> = 24.6 ± 0.7). Although, the lipoidal matters of *Galaxaura rugosa* and *Liagora hawaiiiana* antitumor activity against cervical carcinoma (HeLa) and intestinal carcinoma (CACO-2) (IC<sub>50</sub> = 10.2 ± 0.6 and 12.2 ± 0.6, respectively) preferable than vinblastine sulfate (IC<sub>50</sub> = 59.7 ± 2.1 and 30.3 ± 1.4, respectively).

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## 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 (Rhodophyta), Brown Algae (Phyco-phyta), Green Algae (Chlorophyta) and Diatoms. Although, Sea-weeds 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, sea-weeds are traditionally traded as food items including sushi wrap-pings, 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 pharmaceutical 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 hawaiiiana*.

## 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 rugosa* and *Liagora hawaiiiana* 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 rugosa* and *Liagora hawaiiiana*; 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 rugosa* and *Liagora hawaiiiana*, 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 rugosa* and *Liagora hawaiiiana*, 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 rugosa* and *Liagora hawaiiiana*, 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 rugosa* and *Liagora hawaiiiana*) 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 subtilis* (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), *Proteus vulgaris* (RCMB 01004) *Staphylococcus aureus* (RCMB 010010), *Staphylococcus epidermidis* (RCMB 010009), *Streptococcus byogenes* (RCMB 0100174-2), *Streptococcus 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 hawaiiiana* 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  $\mu$ l 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 hawaiiiana* 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 hawaiiiana* 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<sub>50</sub> 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 hawaiiiana* 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  $\pm$  S.D. Comparisons between means were carried out using a one-way ANOVA test followed by

the Tukey HSD test using SPSS, version 14 (SPSS, Chicago, IL). Differences at p50.05 were considered statistically significant.

### 3. Results and discussion

#### 3.1. Preliminary phytochemical screening

The preliminary phytochemical analyses of *Galaxaura rugosa* and *Liagora hawaiiiana* revealed the presence of different primary and secondary metabolites, they contains unsaturated sterols and/or triterpenoids, flavonoids, carbohydrates or glycosides, proteins and/or amino acids, tannins and coumarin, no saponins or alkaloids were detected. This variety of active metabolites give these algae high potentials to be used as source of medication specially the presence of flavonoids (Kosanić et al., 2015).

#### 3.2. Antimicrobial activity

The antimicrobial activity of total ethanol, lipoidal matters, chloroform, n-butanol, aqueous extracts, and powder of *Galaxaura rugosa* and *Liagora hawaiiiana* were determined against Gram-negative, Gram-positive bacteria and fungi (Tables 1 and 2). The results revealed that all the extracts of *Galaxaura rugosa* showed antibacterial and antifungal activities. On the other hand, only lipoidal matters, chloroform, n-butanol and aqueous extracts of *Liagora hawaiiiana* showed antibacterial and antifungal activity, in addition to the powder which has only antifungal activity (Tables 1 and 2).

Among the extracts of *Galaxaura rugosa*, chloroform, n-butanol, and aqueous extracts inhibited the growth of nine, out of ten, bacterial test organism. While total ethanol extract and lipoidal matters showed antifungal activity against 8, out of ten, fungal test strains. Interestingly, the chloroform extract of *Galaxaura rugosa* exhibited antibacterial activity against *Klebsiella pneumoniae* (24 mm, 0.15 mg/ml) higher than the standard antibiotic 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 tropicalis*, respectively (Tables 1 and 3). The chloroform extract of *Liagora hawaiiiana* showed the best antibacterial and antifungal activities. With the exception of *Microcanis canis* and *Trichophyton mentagrophytes*, it inhibited the growth of all tested fungal strains in addition to all the bacterial strains. Furthermore, the potency of chloroform extract against *Candida tropicalis* (27 mm, 0.078 mg/ml) was similar to that of the standard antibiotic, Ketoconazole (27 mm, 0.98 mg/ml) (Tables 2 and 4).

From the previous studied it was concluded that researchers have isolated different compounds from algae including terpenoids, phlorotannins, polyphenols, phenolic acids, anthocyanins, hydroxycinnamic acid derivatives, and flavonoids (Bhat and Madyastha, 2000, 2001; Benedetti, 2004). Nevertheless, the antibacterial, antifungal and antiviral activities of algal extracts are extensively published (El-Fatemy and Said, 2011; Manilal et al., 2009; Rajasulochana et al., 2009; Ely et al., 2004). Although, the obtained results of the current study revealed the antimicrobial activity of extracts of *Galaxaura rugosa* and *Liagora hawaiiiana* using different solvents which indicates the multiplicity and diversity of the compounds present in algae

#### 3.3. Antioxidant activity

The antioxidant activity of *Galaxaura rugosa*, and *Liagora hawaiiiana* were screened using DPPH assay. It is the most commonly used assay because it can run many samples in short time and detect the active components at low concentration (Piao et al., 2004). The current results exhibited that the total ethanol extract of *Galaxaura rugosa*, and *Liagora hawaiiiana* have DPPH radical scavenging activity in a concentration-dependent manner (Table 5).

**Table 1**  
Antimicrobial activity of different extracts of *Galaxaura rugosa*.

Extract Test organism	Mean diameter of inhibition zone (mm)						
	Total (Ethanol)	Lipoidal matter	Chloroform	n-Butanol	Aqueous	Powder	Standard antibiotic Gentamycin
<b>Bacteria</b>							
<i>Gram-negative</i>							
<i>Escherichia coli</i>	15	18	22	16	22	15	36
<i>Klebsiella pneumoniae</i>	14	19	24	15	16	14	23
<i>Proteus vulgaris</i>	18	22	20	19	15	16	31
<i>Pseudomonas aeruginosa</i>	00	00	15	00	00	00	25
<i>Salmonella typhimrium</i>	00	14	21	15	15	00	27
<i>Gram-positive</i>							
<i>Bacillus subtilis</i>	14	18	17	16	16	15	32
<i>Staphylococcus aureus</i>	19	14	22	21	18	20	30
<i>Staphylococcus epidermidis</i>	20	00	19	21	17	14	34
<i>Streptococcus mutans</i>	00	00	14	15	20	00	26
<i>Streptococcus pyogenes</i>	00	00	00	14	18	00	28
<b>Fungi</b>							
<i>Ketoconazole</i>							
<i>Aspergillus fumigatus</i>	21	16	00	00	00	00	23
<i>Aspergillus niger</i>	15	22	00	00	00	00	24
<i>Candida albicans</i>	18	16	20	18	15	18	26
<i>Candida tropicalis</i>	17	19	25	19	16	23	27
<i>Cryptococcus neoformans</i>	20	15	27	22	18	15	31
<i>Geotricum candidum</i>	15	17	22	20	15	22	30
<i>Penicillium expansum</i>	14	23	00	00	00	00	28
<i>Syncephalastrum racemosum</i>	14	14	00	00	00	00	24
<i>Amphotericin B</i>							
<i>Dermaphrytes</i>	00	00	00	00	00	00	30
<i>Trichophyton mentagrophytes</i>	00	00	00	00	00	00	29

Values are expressed as mean ± SEM of 3 determinants.

**Table 2**  
Antimicrobial activity of different extracts of *Liagora hawaiiiana*.

Extract Test organism	Mean diameter of inhibition zone (mm)						
	Total(Ethanol)	Lipoidal matter	Chloroform	n-Butanol	Aqueous	Powder	Standard Antibiotic
<b>Bacteria</b>	<i>Gentamycin</i>						
<i>Gram-negative</i>							
<i>Escherichia coli</i>	00	21	21	15	23	00	36
<i>Klebsiella pneumoniae</i>	00	22	20	16	16	00	23
<i>Proteus vulgaris</i>	00	15	18	21	15	00	31
<i>Pseudomonas aeruginosa</i>	00	14	15	00	00	00	25
<i>Salmonella typhimrium</i>	00	19	16	14	00	00	27
<i>Gram-positive</i>							
<i>Bacillus subtilis</i>	00	15	17	00	00	00	32
<i>Staphylococcus aureus</i>	00	17	18	00	00	00	30
<i>Staphylococcus epidermidis</i>	00	14	14	00	00	00	34
<i>Streptococcus mutans</i>	00	20	24	00	00	00	26
<i>Streptococcus pyogenes</i>	00	19	19	00	00	00	28
<b>Fungi</b>	<i>Ketoconazole</i>						
<i>Aspergillus fumigatus</i>	00	00	16	00	00	00	23
<i>Aspergillus niger</i>	00	00	16	00	00	00	24
<i>Candida albicans</i>	00	21	22	14	16	14	26
<i>Candida tropicalis</i>	00	22	27	15	18	15	27
<i>Cryptococcus neoformans</i>	00	24	25	19	21	17	31
<i>Geotricum candidum</i>	00	21	21	14	15	14	30
<i>Penicillium expansum</i>	00	00	20	00	00	00	28
<i>Syncephalastrum racemosum</i>	00	00	15	00	00	00	24
<b>Dermatophytes</b>	<i>Amphotericin B</i>						
<i>Microsporium canis</i>	00	00	00	00	00	00	30
<i>Trichophyton mentagrophytes</i>	00	00	00	00	00	00	29

Values are expressed as mean  $\pm$  SEM of 3 determinants

**Table 3**  
The minimum inhibitory concentration (MIC) of different extracts of *Galaxaura rugosa*.

Extract Test organism	Minimum Inhibitory Concentration (mg/ml)						
	Total (Ethanol)	Lipoidal matter	Chloroform	n-Butanol	Aqueous	Powder	Standard Antibiotic
<b>Bacteria</b>	<i>Gentamycin</i>						
<i>Gram-negative</i>							
<i>Escherichia coli</i>	5.000	2.500	0.312	5.000	0.625	5.000	03.90
<i>Klebsiella pneumoniae</i>	10.00	2.500	0.156	5.000	5.000	10.00	00.49
<i>Proteus vulgaris</i>	2.500	0.625	1.250	2.500	5.000	5.000	01.95
<i>Pseudomonas aeruginosa</i>	ND	ND	5.000	ND	ND	ND	01.95
<i>Salmonella typhimrium</i>	ND	10.00	0.625	5.000	5.000	ND	01.95
<i>Gram-positive</i>							
<i>Bacillus subtilis</i>	10.00	1.250	2.500	5.000	5.000	5.000	01.95
<i>Staphylococcus aureus</i>	ND	10.00	0.625	0.625	2.500	1.250	01.95
<i>Staphylococcus epidermidis</i>	ND	ND	1.250	0.625	2.500	10.000	00.98
<i>Streptococcus mutans</i>	ND	ND	10.00	5.000	1.250	ND	01.95
<i>Streptococcus pyogenes</i>	ND	ND	ND	10.00	2.500	ND	00.98
<b>Fungi</b>	<i>Ketoconazole</i>						
<i>Aspergillus fumigatus</i>	1.250	5.000	ND	ND	ND	ND	00.49
<i>Aspergillus niger</i>	5.000	0.312	ND	ND	ND	ND	03.90
<i>Candida albicans</i>	2.500	5.000	1.250	1.250	5.000	1.250	01.95
<i>Candida tropicalis</i>	2.500	1.250	0.156	2.500	5.000	0.312	00.98
<i>Cryptococcus neoformans</i>	1.250	5.000	0.078	0.625	2.500	5.000	01.95
<i>Geotricum candidum</i>	5.000	2.500	0.312	1.250	5.000	0.625	03.90
<i>Penicillium expansum</i>	10.00	0.312	ND	ND	ND	ND	01.95
<i>Syncephalastrum racemosum</i>	10.00	10.00	ND	ND	ND	ND	00.98

ND, not determined. Values are expressed as mean  $\pm$  SEM of 3 determinants.

The maximum scavenging activity (80.96%,  $IC_{50}$  = 27.8  $\mu$ g/ml) was provided by *Galaxaura rugosa*. However, the scavenging activity of *Liagora hawaiiiana* was 66.87% ( $IC_{50}$  = 57.2  $\mu$ g/ml) (Table 5).

The free radicals are involved in several diseases including cancer, AIDS and neurodegenerative diseases. The scavenging activity of antioxidants is very useful for the control of those diseases (Suresh et al., 2008; Koleva et al., 2002). Interestingly, the antioxidant activity of *Galaxaura rugosa* was very good ( $27.8 \pm 1.22$ ) and almost similar to the antioxidant activity of ascorbic acid (86.36%,  $IC_{50}$  = 11.2  $\mu$ g/ml) (Table 5), this can be due to the

presence of flavonoids in both algae (Farasat et al., 2014; Yen & Duh, 1994).

#### 3.4. Antitumor activity

The cancer, cells growing out of control, causes are diverse, complex and not fully understood. The cancer diseases are classified according to the type of cell that the tumor cells resemble and are presumed to be the origin of the tumor. Herbal medicines are used worldwide for cancer prevention and treatment. The

**Table 4**  
The minimum inhibitory concentration (MIC) of different extracts of *Liagora hawaiiiana*.

Extract Testorganism	Minimum Inhibitory Concentration (mg/ml)						
	Total (Ethanol)	Lipoidal matter	Chloroform	n-Butanol	Aqueous	Powder	Standard antibiotic
<b>Bacteria</b>	<i>Gentamycin</i>						
<i>Gram-negative</i>							
<i>Escherichia coli</i>	ND	0.625	0.625	5.000	0.625	ND	03.90
<i>Klebsiella pneumoniae</i>	ND	0.312	1.250	5.000	5.000	ND	00.49
<i>Proteus vulgaris</i>	ND	10.00	2.500	0.625	10.00	ND	01.95
<i>Pseudomonas aeruginosa</i>	ND	10.00	5.000	ND	ND	ND	01.95
<i>Salmonella typhimrium</i>	ND	1.250	5.000	10.00	ND	ND	01.95
<i>Gram-positive</i>							
<i>Bacillus subtilis</i>	ND	5.000	2.500	ND	ND	ND	01.95
<i>Staphylococcus aureus</i>	ND	2.500	1.250	ND	ND	ND	01.95
<i>Staphylococcus epidermidis</i>	ND	10.00	10.00	ND	ND	ND	00.98
<i>Streptococcus mutans</i>	ND	1.250	0.312	ND	ND	ND	01.95
<i>Streptococcus pyogenes</i>	ND	2.500	1.250	ND	ND	ND	00.98
<b>Fungi</b>	<i>Ketocona-zole</i>						
<i>Aspergillus fumigatus</i>	ND	ND	5.000	ND	ND	ND	00.49
<i>Aspergillus niger</i>	ND	ND	10.00	ND	ND	ND	03.90
<i>Candida albicans</i>	ND	0.625	0.625	10.00	5.000	10.00	01.95
<i>Candida tropicalis</i>	ND	0.312	0.078	5.000	2.500	5.000	00.98
<i>Cryptococcus neoformans</i>	ND	0.156	0.312	1.250	0.625	5.000	01.95
<i>Geotricum candidum</i>	ND	0.625	0.625	10.00	5.000	10.00	03.90
<i>Penicillium expansum</i>	ND	ND	1.250	ND	ND	ND	01.95
<i>Syncephalastrum racemosum</i>	ND	ND	5.000	ND	ND	ND	00.98

ND, not determined. Values are expressed as mean  $\pm$  SEM of 3 determinants.

**Table 5**  
The scavenging activity of DPPH radicals of *Galaxaura rugosa* and *Liagora hawaiiiana*.

Concentration ( $\mu$ g/ml)	DPPH scavenging (%)		
	<i>Galaxaura rugosa</i>	<i>Liagora hawaiiiana</i>	Ascorbic acid
000	00.00	00.00	00.00
001	10.87 $\pm$ 1.50	4.96 $\pm$ 1.32	12.98 $\pm$ 1.41
002	12.35 $\pm$ 1.11	9.83 $\pm$ 1.21	16.38 $\pm$ 1.44
004	21.39 $\pm$ 1.71	16.61 $\pm$ 1.54	62.98 $\pm$ 1.62
008	28.09 $\pm$ 1.32	22.35 $\pm$ 1.33	76.81 $\pm$ 1.57
016	34.35 $\pm$ 1.91	27.91 $\pm$ 1.38	78.72 $\pm$ 1.75
032	55.48 $\pm$ 1.22	35.65 $\pm$ 1.30	78.94 $\pm$ 1.51
064	66.00 $\pm$ 1.58	53.83 $\pm$ 1.27	80.21 $\pm$ 1.14
128	80.96 $\pm$ 1.30	66.87 $\pm$ 1.12	86.36 $\pm$ 1.09
<b>IC<sub>50</sub></b>	<b>27.8 <math>\pm</math> 1.22</b>	<b>57.2 <math>\pm</math> 1.35</b>	<b>11.2 <math>\pm</math> 1.55</b>

Values are expressed as mean  $\pm$  SEM of 3 replicates.

**Table 6**  
The antitumor activity of extracts of *Galaxaura rugosa* against different cell lines.

Cell line	Concentration ( $\mu$ g/ml)	Cell viability (%)						
		Total (Ethanol)	Lipoidal matters	Chloro-form	n-butanol	Aqueous	Powder	Vinblastine sulfate
<b>A-549</b> <i>Lung carcinoma</i>	000.00	100	100	100	100	100	100	100
	001.00	100	98.1	100	100	100	100	98.2
	002.00	98.1	92.3	100	100	100	100	94.7
	003.90	94.0	84.9	98.6	100	100	98.4	81.4
	007.80	87.3	67.2	93.7	100	98.1	91.7	73.8
	015.60	76.9	48.6	85.1	99.2	92.8	85.0	62.5
	031.25	63.1	40.9	70.4	95.0	84.0	72.3	40.7
	062.50	41.9	32.8	54.8	89.4	69.5	59.1	32.9
	125.00	30.6	21.9	38.7	68.1	42.7	38.6	25.2
	250.00	22.8	12.8	23.8	40.7	29.4	23.1	15.3
500.00	10.7	06.3	12.9	27.8	14.5	10.9	06.8	
<b>CACO-2</b> <i>Intestinal carcinoma</i>	<b>IC<sub>50</sub> (<math>\mu</math>g/ml)</b>	<b>50.7 <math>\pm</math> 3.5</b>	<b>15 <math>\pm</math> 1.7</b>	<b>81.4 <math>\pm</math> 4.5</b>	<b>208 <math>\pm</math> 17.2</b>	<b>108 <math>\pm</math> 9.2</b>	<b>90.4 <math>\pm</math> 7.8</b>	<b>24.6 <math>\pm</math> 0.7</b>
	000.00	100	100	100	100	100	100	100
	001.00	100	100	100	100	100	100	99.2
	002.00	100	98.7	100	100	100	100	93.8
	003.90	100	95.4	100	100	97.8	100	86.2
	007.80	99.4	89.2	99.4	97.4	92.4	99.4	79.4

(continued on next page)

effect of natural products as anti-cancer was widely studied because their nature, low toxicity and side effects (Manglani et al., 2014, Mulla & Swamy, 2012, Jani & Jain, 2011).

In the present study, the *in vitro* antitumor activity of *Galaxaura rugosa* and *Liagora hawaiiiana* extracts was determined against different cell lines including A-549 (Lung carcinoma), CACO (Intestinal carcinoma), HCT-116 (Colon carcinoma), Hela (Cervical carcinoma), HEp-2 (Larynx carcinoma), HepG-2 (Hepatocellular carcinoma), and MCF-7 (Breast carcinoma). Because it is reliable to assess the *in vitro* cytotoxicity of the anticancer compounds, MTT assay method (Allely et al., 1998) was used.

The obtained results revealed that the extracts of *Galaxaura rugosa* and *Liagora hawaiiiana* have a remarkable antitumor activity against different types of tumor cells (Tables 6 and 7). Interestingly, the lipoidal matters of *Galaxaura rugosa* and *Liagora hawaiiiana* possessed antitumor activity (IC<sub>50</sub> = 15 $\pm$ 1.7 and 21.2 $\pm$ 1.6,

Table 6 (continued)

Cell line	Concentration ( $\mu\text{g/ml}$ )	Cell viability (%)						
		Total (Ethanol)	Lipoidal matters	Chloro-form	n-butanol	Aqueous	Powder	Vinblastine sulfate
<b>HCT-116</b> <i>Colon carcinoma</i>	015.60	96.1	72.3	95.2	90.6	85.2	96.2	67.5
	031.25	89.2	49.2	84.1	81.4	78.1	89.7	48.9
	062.50	72.5	38.4	70.6	68.0	65.7	70.8	31.4
	125.00	43.8	27.1	53.4	47.1	42.9	42.9	20.3
	250.00	31.7	14.2	34.9	36.2	28.7	28.8	08.9
	500.00	16.4	06.4	23.6	21.4	12.5	15.2	04.0
	<b>IC<sub>50</sub> (<math>\mu\text{g/ml}</math>)</b>	<b>112 <math>\pm</math> 10.4</b>	<b>30.7 <math>\pm</math> 4.1</b>	<b>149 <math>\pm</math> 12.2</b>	<b>117 <math>\pm</math> 9.1</b>	<b>106 <math>\pm</math> 8.4</b>	<b>109 <math>\pm</math> 11.4</b>	<b>30.3 <math>\pm</math> 1.4</b>
	000.00	100	100	100	100	100	100	100
	001.00	100	79.4	100	100	100	100	66.4
	002.00	100	72.9	100	100	100	100	58.1
	003.90	98.7	60.7	100	100	100	98.7	47.3
	007.80	93.2	45.9	99.1	98.1	100	95.1	39.8
	015.60	86.9	38.2	93.7	91.8	99.7	89.5	28.7
031.25	69.1	30.6	86.0	85.2	92.4	73.1	18.9	
062.50	43.5	22.8	68.1	68.0	69.5	49.5	15.5	
125.00	30.7	16.4	45.2	49.8	42.8	36.9	12.1	
250.00	18.6	08.7	31.7	35.4	24.9	23.8	06.7	
500.00	06.	03.9	18.6	23.8	08.7	09.2	04.0	
<b>IC<sub>50</sub> (<math>\mu\text{g/ml}</math>)</b>	<b>54.7 <math>\pm</math> 1.2</b>	<b>6.7 <math>\pm</math> 0.2</b>	<b>112 <math>\pm</math> 7.2</b>	<b>125 <math>\pm</math> 5.3</b>	<b>108 <math>\pm</math> 3.9</b>	<b>61.9 <math>\pm</math> 3.1</b>	<b>3.5 <math>\pm</math> 0.2</b>	
<b>HeLa</b> <i>Cervical carcinoma</i>	000.00	100	100	100	100	100	100	100
	001.00	100	92.5	100	100	100	100	100
	002.00	99.5	81.4	100	100	100	100	98.1
	003.90	94.6	69.0	99.4	100	100	98.4	95.4
	007.80	89.7	52.7	96.0	100	97.8	92.3	90.6
	015.60	80.9	43.9	88.9	100	91.3	81.4	82.7
	031.25	65.2	35.1	80.7	100	80.6	69.8	71.9
	062.50	48.1	26.7	68.9	100	62.9	45.1	47.8
	125.00	31.4	18.4	47.5	100	43.0	30.6	34.5
	250.00	14.7	09.6	31.7	97.1	29.4	21.8	22.8
	500.00	08.9	05.7	17.2	89.2	15.8	09.2	09.1
	<b>IC<sub>50</sub> (<math>\mu\text{g/ml}</math>)</b>	<b>59.1 <math>\pm</math> 3.2</b>	<b>10.2 <math>\pm</math> 0.6</b>	<b>118 <math>\pm</math> 5.1</b>	<b>&gt; 500</b>	<b>103 <math>\pm</math> 4.8</b>	<b>56.3 <math>\pm</math> 3.4</b>	<b>59.7 <math>\pm</math> 2.1</b>
	<b>HepG-2</b> <i>Hepatocellular carcinoma</i>	000.00	100	100	100	100	100	100
001.00		100	90.6	100	100	100	100	60.9
002.00		98.2	83.1	100	100	100	98.7	54.2
003.90		91.7	70.4	99.3	100	98.6	94.0	45.0
007.80		80.1	49.2	94.1	100	91.7	88.7	34.1
015.60		69.4	37.0	86.2	100	82.0	72.1	26.8
031.25		48.1	28.5	72.6	100	67.4	48.5	19.2
062.50		34.5	20.6	51.3	100	53.9	36.4	14.3
125.00		23.7	11.3	34.9	99.0	37.1	24.9	10.9
250.00		11.9	06.9	26.4	93.7	21.3	15.3	05.8
500.00		05.6	03.5	13.8	81.4	09.4	06.1	03.2
<b>IC<sub>50</sub> (<math>\mu\text{g/ml}</math>)</b>		<b>29.9 <math>\pm</math> 2.3</b>	<b>7.6 <math>\pm</math> 0.5</b>	<b>67.6 <math>\pm</math> 4.2</b>	<b>&gt; 500</b>	<b>77.2 <math>\pm</math> 5.9</b>	<b>30.3 <math>\pm</math> 2.6</b>	<b>2.9 <math>\pm</math> 0.3</b>
<b>MCF-7</b> <i>Breast carcinoma</i>		000.00	100	100	100	100	100	100
	001.00	100	94.1	100	100	100	100	67.1
	002.00	100	89.2	100	100	100	100	58.7
	003.90	99.4	78.3	100	100	100	99.4	52.9
	007.80	96.2	63.1	100	100	100	96.2	47.2
	015.60	89.4	42.5	98.1	100	98.7	89.5	40.5
	031.25	72.3	31.7	89.7	100	92.4	70.8	31.9
	062.50	40.9	23.8	74.0	100	78.1	41.7	23.8
	125.00	26.4	14.7	48.7	98.7	45.2	28.5	15.1
	250.00	13.8	07.5	32.8	91.4	30.9	18.7	07.8
	500.00	06.7	03.8	19.4	76.8	13.7	08.9	05.4
	<b>IC<sub>50</sub> (<math>\mu\text{g/ml}</math>)</b>	<b>53.5 <math>\pm</math> 2.3</b>	<b>12.8 <math>\pm</math> 1.4</b>	<b>122 <math>\pm</math> 9.3</b>	<b>&gt;500</b>	<b>116 <math>\pm</math> 8.2</b>	<b>53.6 <math>\pm</math> 4.6</b>	<b>5.9 <math>\pm</math> 0.4</b>
	<b>PC-3</b> <i>Prostate carcinoma</i>	000.00	100	100	100	100	100	100
001.00		100	100	100	100	100	100	93.0
002.00		100	100	100	100	100	100	88.2
003.90		98.0	100	100	100	100	100	74.8
007.80		91.7	97.8	100	100	98.7	100	68.9
015.60		86.9	90.6	100	100	93.8	100	56.7
031.25		71.4	79.5	97.6	100	84.1	100	37.8
062.50		43.5	64.0	93.8	99.5	67.2	99.4	24.9
125.00		29.4	38.1	85.1	92.4	43.9	96.2	13.7
250.00		17.2	27.8	69.4	83.9	28.6	88.4	09.5
500.00		08.2	15.8	46.2	70.2	15.8	74.5	05.3
<b>IC<sub>50</sub> (<math>\mu\text{g/ml}</math>)</b>		<b>55.3 <math>\pm</math> 5.4</b>	<b>96.4 <math>\pm</math> 8.3</b>	<b>459 <math>\pm</math> 24.8</b>	<b>&gt; 500</b>	<b>109 <math>\pm</math> 7.8</b>	<b>&gt; 500</b>	<b>21.2 <math>\pm</math> 0.9</b>

Values are expressed as mean  $\pm$  SEM of 3 determinants.

Table 7

The antitumor activity of extracts of *Liagora hawaiiiana* against different cell lines.

Cell line	Concentration ( $\mu\text{g/ml}$ )	Cell viability (%)						
		Total (Ethanol)	Lipoidal matters	Chloro-form	<i>n</i> -butanol	Aqueous	Powder	Vinblastine sulfate
<b>A-549</b> <i>Lung carcinoma</i>	000.00	100	100	100	100	100	100	100
	001.00	100	99.6	98.7	100	100	100	98.2
	002.00	99.8	94.8	95.2	100	100	100	94.7
	003.90	96.7	89.5	86.7	100	100	100	81.4
	007.80	90.3	70.4	79.5	100	98.1	100	73.8
	015.60	80.6	56.2	63.8	98.0	91.4	98.7	62.5
	031.25	68.5	38.7	40.8	86.9	79.5	94.3	40.7
	062.50	53.2	28.1	31.9	72.1	63.8	87.1	32.9
	125.00	34.9	22.4	23.5	50.6	45.9	69.2	25.2
	250.00	21.8	14.0	14.8	39.8	37.6	51.8	15.3
	500.00	09.79	06.1	06.9	26.5	24.9	32.7	06.8
<b>CACO-2</b> <i>Intestinal carcinoma</i>	<b>IC<sub>50</sub> (<math>\mu\text{g/ml}</math>)</b>	<b>73.6 <math>\pm</math> 5.8</b>	<b>21.2 <math>\pm</math> 1.6</b>	<b>25 <math>\pm</math> 3.1</b>	<b>132 <math>\pm</math> 11.4</b>	<b>111 <math>\pm</math> 8.9</b>	<b>274 <math>\pm</math> 26.2</b>	<b>24.6 <math>\pm</math> 0.7</b>
	000.00	100	100	100	100	100	100	100
	001.00	100	93.7	100	100	100	100	99.2
	002.00	100	89.4	99.4	98.1	100	100	93.8
	003.90	100	78.1	96.3	91.7	99.7	100	86.2
	007.80	98.0	65.3	87.2	86.4	93.8	100	79.4
	015.60	90.6	38.4	70.9	75.3	85.2	98.3	67.5
	031.25	78.1	28.9	37.4	60.8	69.1	92.5	48.9
	062.50	65.9	20.4	28.6	45.1	48.7	81.4	31.4
	125.00	47.8	13.2	19.4	36.2	39.4	65.1	20.3
	250.00	31.7	06.7	10.5	23.6	27.8	41.8	08.9
500.00	18.6	03.2	05.9	11.7	15.9	30.6	04.0	
	<b>IC<sub>50</sub> (<math>\mu\text{g/ml}</math>)</b>	<b>118 <math>\pm</math> 10.5</b>	<b>12.2 <math>\pm</math> 0.6</b>	<b>25.4 <math>\pm</math> 1.2</b>	<b>52.9 <math>\pm</math> 3.3</b>	<b>60.5 <math>\pm</math> 4.7</b>	<b>206 <math>\pm</math> 16.2</b>	<b>30.3 <math>\pm</math> 1.4</b>
<b>HCT-116</b> <i>Colon carcinoma</i>	000.00	100	100	100	100	100	100	100
	001.00	100	96.7	91.4	100	100	100	66.4
	002.00	98.1	90.6	86.2	100	100	100	58.1
	003.90	94.2	83.9	78.1	100	99.4	100	47.3
	007.80	86.1	70.8	63.9	98.2	95.2	100	39.8
	015.60	71.8	52.3	50.6	92.4	88.7	99.4	28.7
	031.25	59.4	42.9	41.0	86.9	79.4	95.2	18.9
	062.50	36.7	33.9	31.7	72.8	65.9	86.1	15.5
	125.00	21.8	20.6	22.4	45.1	41.8	70.9	12.1
	250.00	12.9	13.2	12.9	36.2	28.7	42.1	06.7
	500.00	05.6	06.3	05.2	20.4	16.2	28.6	04.0
	<b>IC<sub>50</sub> (<math>\mu\text{g/ml}</math>)</b>	<b>44.2 <math>\pm</math> 0.9</b>	<b>195 <math>\pm</math> 0.7</b>	<b>16.6 <math>\pm</math> 0.8</b>	<b>114 <math>\pm</math> 9.2</b>	<b>104 <math>\pm</math> 8.7</b>	<b>216 <math>\pm</math> 12.3</b>	<b>3.5 <math>\pm</math> 0.2</b>
<b>HeLa</b> <i>Cervical carcinoma</i>	000.00	100	100	100	100	100	100	100
	001.00	100	100	100	100	100	100	100
	002.00	100	99.7	98.6	100	100	100	98.1
	003.90	97.8	96.4	93.2	100	100	100	95.4
	007.80	90.3	90.6	85.4	100	100	100	90.6
	015.60	78.4	81.7	76.4	98.6	97.9	100	82.7
	031.25	63.1	69.4	62.1	91.7	90.6	100	71.9
	062.50	48.2	51.8	42.5	83.1	78.2	100	47.8
	125.00	31.5	36.7	30.4	70.8	65.1	99.7	34.5
	250.00	19.7	23.9	19.5	56.4	47.2	92.3	22.8
	500.00	08.3	14.5	08.7	38.6	31.5	81.6	09.1
	<b>IC<sub>50</sub> (<math>\mu\text{g/ml}</math>)</b>	<b>58.8 <math>\pm</math> 1.4</b>	<b>70.2 <math>\pm</math> 3.5</b>	<b>50.7 <math>\pm</math> 2.9</b>	<b>340 <math>\pm</math> 16.7</b>	<b>231 <math>\pm</math> 20.1</b>	<b>&gt; 500</b>	<b>59.7 <math>\pm</math> 2.1</b>
<b>HepG-2</b> <i>Hepatocellular carcinoma</i>	000.00	100	100	100	100	100	100	100
	001.00	100	100	99.8	100	100	100	60.9
	002.00	100	99.2	93.1	100	100	100	54.2
	003.90	98.5	93.1	84.0	100	98.7	100	45.0
	007.80	90.1	85.7	69.5	100	93.1	100	34.1
	015.60	78.0	76.9	46.2	99.2	86.2	100	26.8
	031.25	65.4	60.8	32.8	94.0	75.3	100	19.2
	062.50	40.6	43.1	25.6	88.6	61.4	98.1	14.3
	125.00	28.3	30.4	14.3	73.1	43.0	91.8	10.9
	250.00	14.6	18.7	06.9	46.2	31.7	80.7	05.8
	500.00	06.8	10.2	03.4	28.9	19.4	68.9	03.2
<b>MCF-7</b> <i>Breast carcinoma</i>	<b>IC<sub>50</sub> (<math>\mu\text{g/ml}</math>)</b>	<b>50.8 <math>\pm</math> 5.1</b>	<b>50.4 <math>\pm</math> 4.3</b>	<b>14.4 <math>\pm</math> 0.8</b>	<b>233 <math>\pm</math> 19.6</b>	<b>101 <math>\pm</math> 7.8</b>	<b>&gt; 500</b>	<b>2.9 <math>\pm</math> 0.3</b>
	000.00	100	100	100	100	100	100	100
	001.00	100	100	97.2	100	100	100	67.1
	002.00	100	100	91.7	100	100	100	58.7
	003.90	100	100	85.0	100	100	100	52.9
	007.80	99.5	97.0	76.9	100	99.3	100	47.2
	015.60	91.4	89.5	60.8	100	95.1	100	40.5
	031.25	79.8	71.3	39.6	99.4	89.5	100	31.9
	062.50	45.1	49.8	28.1	96.5	70.8	98.2	23.8
	125.00	32.7	36.2	19.4	81.4	47.2	94.5	15.1
	250.00	19.4	21.4	08.7	63.1	35.9	83.1	07.8

(continued on next page)

Table 7 (continued)

Cell line	Concentration (µg/ml)	Cell viability (%)						
		Total (Ethanol)	Lipoidal matters	Chloro-form	n-butanol	Aqueous	Powder	Vinblastine sulfate
PC-3 Prostate carcinoma	500.00	10.2	13.8	04.2	37.8	26.5	71.5	05.4
	IC <sub>50</sub> (µg/ml)	58.1 ± 3.7	62.2 ± 6.1	23.6 ± 3.4	380 ± 17.9	118 ± 82.3	> 500	5.9 ± 0.4
	000.00	100	100	100	100	100	100	100
	001.00	100	100	100	100	100	100	93.0
	002.00	100	98.6	98.0	100	100	100	88.2
	003.90	100	91.7	91.7	100	100	100	74.8
	007.80	100	84.3	84.1	100	98.7	100	68.9
	015.60	98.0	68.1	70.8	100	90.6	100	56.7
	031.25	90.6	47.2	41.5	99.5	82.1	98.4	37.8
	062.50	72.8	35.0	23.7	93.1	67.4	91.3	24.9
	125.00	47.8	23.6	19.5	86.4	46.2	80.1	13.7
	250.00	31.7	14.9	10.2	71.6	35.9	65.3	09.5
	500.00	18.9	06.3	06.3	46.8	21.3	41.9	05.3
IC <sub>50</sub> (µg/ml)	120 ± 9.3	29.2 ± 1.3	26.7 ± 1.4	469 ± 38.6	114 ± 10.5	414 ± 43.1	21.2 ± 0.9	

Values are expressed as mean ± SEM of 3 determinants.

respectively) against lung carcinoma (A-549) better than vinblastine sulfate (IC<sub>50</sub> = 24.6 ± 0.7). Although, the lipoidal matters of *Galaxaura rugosa* and *Liagora hawaiiiana* antitumor activity against cervical carcinoma (HeLa) and intestinal carcinoma (CACO-2) (IC<sub>50</sub> = 10.2 ± 0.6 and 12.2 ± 0.6, respectively) preferable than vinblastine sulfate (IC<sub>50</sub> = 59.7 ± 2.1 and 30.3 ± 1.4, respectively) (Tables 6 and 7). These results give new promising resource of anticancer drug discovery from marine this was clear from the variation of the anticancer effect of the algae extracts which due to their huge biodiversity and safety, as they have long been used in traditional Asian foods and folk medicine (Namvar et al., 2014)

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