Saudi Pharmaceutical Journal 26 (2018) 44–52

Contents lists available at ScienceDirect

Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Original article

Antimicrobial, antioxidant and anticancer activities of *Laurencia catarinensis*, *Laurencia majuscula* and *Padina pavonica* extracts

Nouf 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

ARTICLE INFO

Article history:
Received 29 September 2017
Accepted 5 November 2017
Available online 10 November 2017

Keywords:
HCT-116 (Colon carcinoma)
Hela (Cervical carcinoma)
HEp-2 (Larynx carcinoma)
HepG-2 (Hepatocellular carcinoma)
MCF-7 (Breast carcinoma)
Acinetobacter baumannii
Bacillus subtilis

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 μg/ml) and 22.60 ± 2.10 mm (03.90 μg/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 μg/ml), *Staphylococcus aureus* (21.7 ± 0.58 mm; 1.95 μg/ml), *Streptococcus pyogenes* (20.7 ± 1.2 mm; 1.95 μg/ml) and *Acinetobacter baumannii* (20.1 ± 1.2 mm; 3.9 μg/ml). Moreover, the highest antifungal activity; 24.7 ± 2.0 mm (0.98 μg/ml), 23.7 ± 1.5 mm (0.98 μg/ml), 23.6 ± 1.5 mm (0.98 μg/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%, IC_{50} = 5.59 μg/ml and 77.07%, IC_{50} = 14.3 μg/ml) was provided by *Padina pavonica* and *Laurenica 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 μg/ml; *Laurencia catarinensis* were 55.2, 96.8, 104.0, 78.7, 117.0, 217.0, 169.0 μg/ml; and *Laurencia. majuscula* were 115.0, 221.0, 225.0, 200.0, 338.0, 242.0, and 189.0 μg/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.

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,

* Corresponding author at: College of Pharmacy, Prince Sattam bin Abdulaziz University, Al-Kharj, P.O. Box 173, Riyadh 11942, Saudi Arabia.
E-mail address: amaniawaad@hotmail.com (A.S. Awaad).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jsps.2017.11.001
1319-0164/

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, cardinolides 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 pneumonia* (RCMB 0010093), *Proteous mirablilis* (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), *Geotricum candidum* (RCMB 05097), *Microsporum 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 antitumor activity of *Laurencia catarinensis*, *L. majuscula* and *Padina pavonica*. The tumor cell lines were suspended in medium at

concentration 5×10^4 cell/well in Corning® 96-well tissue culture plates and then incubated for 24 hr. The tested algal extracts were then added into 96-well plates (six replicates) to achieve seven concentrations for each extract. Six vehicle controls with media or 0.5% DMSO were run for each 96 well plate as a control. After incubation for 24 h, the numbers of viable cells were determined by the MTT assay method.

Briefly, the media was removed from the 96 well plate and replaced with 100 μl of fresh culture RPMI 1640 medium without phenol red, then 10 μl of the 12 mM MTT (Sigma) stock solution (5 mg of MTT in 1 mL of PBS) was added to each well including the untreated controls. The 96 well plates were then incubated at 37 °C and 5% CO_2 for 4 hours. An 85 μl aliquot of the media was removed from the wells, and 50 μl of DMSO was added to each well and mixed thoroughly with the pipette and incubated at 37 °C for 10 min. Then, the optical density was measured at 590 nm with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells.

The percentage of viability was calculated as:

$$1 - (ODt/ODc) \times 100\%$$

where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells.

The relation between surviving cells and extract concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified extract. The 50% inhibitory concentration (IC50), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each concentration using Graphpad Prism software (San Diego, CA. USA) (Kameyama et al., 2005).

1.5. Statistical analysis

All values were expressed as mean ± S.D. Comparisons between means were carried out using a one-way ANOVA test followed by the Tukey HSD test using SPSS, version 14 (SPSS, Chicago, IL). Differences at p50.05 were considered statistically significant.

2. Results and discussion

2.1. Preliminary phytochemical screening

Preliminary phytochemical screening of the three alga under investigations (*Laurencia catarinensis*, L. *majuscula and Padina pavonica methanolic*) contains; unsaturated sterols and/or triterpenoides, Flavonoids, Carbohydrates or glycosides, Proteins and/or amino acids, Tannins and Coumarin.

2.2. Antimicrobial activity

The antibacterial and antifungal activities of *Laurencia catarinensis*, *L. majuscula* and *Padina pavonica* methanolic extracts were determined using well-diffusion method. All the investigated algal extracts showed antibacterial and antifungal activities (Tables 1 and 2).

The antibacterial activity of *Laurencia catarinensis*, *L. majuscula* and *Padina pavonica* revealed that the highest activities; 23.40 ± 0.58 mm (00.98 μg/ml) and 22.60 ± 2.10 mm (03.90 μg/ml) were obtained against *Klebsiella pneumonia* by *Laurencia catarinensis* and *Padina pavonica*, respectively (Table 1). The extract of *Padina pavonica* revealed significant antibacterial activity against *Bacillus subtilis* (21.7 ± 1.5 mm; 1.95 μg/ml), *Staphylococcus aureus* (21.7 ± 0.58 mm; 1.95 μg/ml), *Streptococcus pyogenes* (20.7 ± 1.2 mm; 1.95 μg/ml) and *Acinetobacter baumannii* (20.1 ± 1.2 mm; 3.9 μg/ml). The antibacterial activity of *Laurencia catarinensis* was obtained against *Pseudomonas aeruginosa* (21.3 ± 0.63 mm; 1.95 μg/ml), *Escherichia coli* (21.2 ± 2.1 mm; 1.95 μg/ml), *Acinetobacter baumannii* (20.7 ± 1.5 mm; 3.9 μg/ml) and *Staphylococcus aureus* (20.49 ± 1.2 mm; 3.9 μg/ml). The highest antibacterial activity obtained by *L. majuscule* was 20.5 ± 1.2 mm (1.95 μg/ml) against *Klebsiella*, 18.30 ± 2.10 (7.81 μg/ml) against *Acinetobacter baumannii* and 17.7 ± 0.58 (15.63 μg/ml) against *Streptococcus pyogenes* (Table 1).

On the other hand, the antifungal activity of the three algal extracts showed that the highest activity; 24.7 ± 2.0 mm (0.98 μg/ml), 23.7 ± 1.5 mm (0.98 μg/ml), 23.6 ± 1.5 mm (0.98 μg/ml) was

Table 1
Antibacterial activity of *L. catarinensis, L. majuscula and Padina pavonica* against clinically isolated bacteria.

Bacteria	Sample							
	L. catarinensis		*L. majuscule*		*Padina pavonica*		Standard antibiotic	
	Inhibition zone (mm)	MIC (μg/ml)	Inhibition zone (mm)	MIC (μg/ml)	Inhibition zone (mm)	MIC (μg/ml)	Inhibition zone (mm)	MIC (μg/ml)
Gram negative							**Gentamycin**	
Acinetobacter baumannii (RCMB 0100282-9)	20.70 ± 1.50	03.90	18.30 ± 2.10	07.81	20.10 ± 1.20	03.90	23.40 ± 1.20	00.98
Escherichia coli (RCMB 010056)	21.20 ± 2.10	01.95	16.30 ± 2.10	31.25	18.20 ± 0.63	07.81	20.30 ± 0.85	03.90
Klebsiella pneumoniae (RCMB 0010093)	23.40 ± 0.58	00.98	20.50 ± 1.20	01.95	22.60 ± 2.10	03.90	27.20 ± 2.10	00.49
Proteous mirablilis (RCMB 0100254-2)	00.00	ND	00.00	ND	00.00	ND	21.20 ± 1.20	01.95
Pseudomonas aeruginosa (RCMB 0100243-5)	21.30 ± 0.63	01.95	17.20 ± 1.50	15.63	19.60 ± 0.63	03.90	20.60 ± 1.50	01.95
Gram positive							**Ampicillin**	
Bacillus substilis (RCMB 0100169-3)	17.39 ± 2.10	15.57	14.70 ± 1.50	62.50	21.70 ± 1.50	01.95	22.30 ± 0.63	01.95
Staphylococcus aureus (RCMB 010027)	20.49 ± 1.20	03.90	17.10 ± 1.00	15.63	21.70 ± 0.58	01.95	22.00 ± 1.00	01.95
Staphylococcus epidermidis (RCMB 010024)	15.70 ± 0.58	>1000	14.30 ± 1.50	62.50	18.30 ± 0.58	07.81	23.00 ± 1.20	00.98
Streptococcus pyogenes (RCMB 0100174-2)	16.20 ± 1.50	5000	17.70 ± 0.58	15.63	20.70 ± 1.20	01.95	22.70 ± 0.58	00.98
Streptococcus sanguis (RCMB 0100171-3)	00.00	ND	00.00	ND	00.00	ND	21.70 ± 1.50	01.95

ND, not determined. These are the mean of three determinations.

Table 2
Antifungal activity of *L. catarinensis, L. majuscula and Padina pavonica* against clinically isolated fungi.

Sample								
Fungi	*L. catarinensis*		*L. majuscule*		*Padina pavonica*		Standard Antibiotic (Amphotericin B)	
	Inhibition zone (mm)	MIC (μg/ml)	Inhibition zone (mm)	MIC (μg/ml)	Inhibition zone (mm)	MIC (μg/ml)	Inhibition zone (mm)	MIC (μg/ml)
Aspergillus fumigatus (RCMB 02568)	19.50 ± 1.20	03.90	22.40 ± 1.00	01.95	23.6 ± 1.50	00.98	25.70 ± 1.50	0.49
Aspergillus niger (RCMB 02724)	16.30 ± 0.58	31.25	18.60 ± 1.50	03.90	20.3 ± 0.58	03.90	20.44 ± 0.36	03.90
Candida albicans (RCMB 05036)	15.20 ± 0.51	62.50	21.30 ± 1.50	03.90	23.7 ± 1.50	00.98	21.30 ± 1.50	01.95
Candida trobicalis (RCMBA 05239)	19.10 ± 0.32	03.90	23.10 ± 1.30	00.98	24.2 ± 2.00	00.98	23.70 ± 2.00	00.98
Cryptococcus neoformans (RCMB 05642)	00.00	ND	00.00	ND	00.00	ND	21.00 ± 1.44	01.95
Geotricum candidum (RCMB 05097)	20.10 ± 0.58	03.90	20.30 ± 1.50	03.90	21.30 ± 1.50	01.95	20.31 ± 1.50	03.90
Microsporum canis (RCMB 0834)	00.00	ND	00.00	ND	00.00	ND	23.30 ± 1.50	00.98
Penicillium expansum (RCMB 01924)	14.60 ± 1.50	>1000	16.10 ± 1.70	31.25	21.70 ± 2.00	01.95	21.70 ± 2.00	01.95
Syncephalastrum racemosum (RCMB 05922)	00.00	ND	00.00	ND	00.00	ND	24.30 ± 1.20	0.98
Trichophyton mentagrophytes (RCMB 0925)	11.80 ± 1.21	>1000	00.00	ND	00.00	ND	21.30 ± 1.50	01.95

ND, not determined. These are the mean of three determinations.

Table 3
The scavenging activity of DPPH radicals of *L. catarinensis*, *L. majuscula* and *Padina pavonica*.

Concentration (μg/ml)	DPPH scavenging (%)		
	L. catarinensis	*L. majuscula*	*Padina pavonica*
000	00.00	00.00	00.00
001	12.17 ± 1.50	06.67 ± 1.32	14.13 ± 1.41
002	13.65 ± 1.11	09.20 ± 1.21	26.53 ± 1.44
004	17.83 ± 1.71	18.93 ± 1.54	44.80 ± 1.62
008	24.09 ± 1.32	30.93 ± 1.33	57.87 ± 1.57
016	30.26 ± 1.91	55.07 ± 1.38	73.60 ± 1.75
032	38.00 ± 1.22	70.00 ± 1.30	75.73 ± 1.51
064	55.57 ± 1.58	74.80 ± 1.27	76.67 ± 1.14
128	67.65 ± 1.30	77.07 ± 1.12	77.60 ± 1.09
IC_{50}	53.80 ± 1.22	14.30 ± 1.35	05.59 ± 1.55

These are the mean of three determinations.

obtained by *Padina pavonica* against *Candida tropicalis*, *C. albicans* and *Aspergillus fumigatus*, respectively (Table 2).

2.2.1. Antioxidant activity

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. The DPPH assay is most commonly used method for screening antioxidant activity and it is a sensitive method to determine the antioxidant activity of different plant, fungal, or algal extracts (Suresh et al., 2008; Koleva et al., 2002).

The ethanolic extract of *Laurencia catarinensis*, *L. majuscula* and *Padina mavonica* showed DPPH radical scavenging activity in a concentration–dependent manner (Table 3, Fig. 1). The maximum

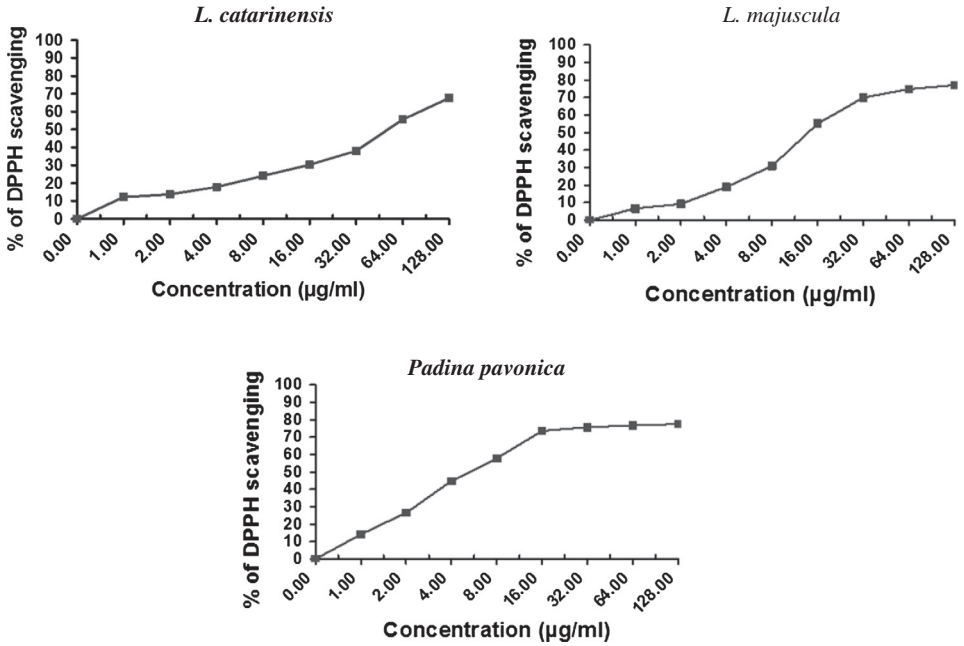

Fig. 1. The scavenging activity of DPPH radicals of *Laurencia catarinensis*, *L. majuscula and Padina pavonica*.

scavenging activity (77.6%, IC_{50} = 5.59 μg/ml and 77.07%, IC_{50} = 14.3 μg/ml) was provided by *Padina pavonica* and *Laurenica majuscula*, respectively (Table 3). However, the scavenging activity of *Laurencia catarinensis* was 67.65% (IC_{50} = 53.8 μg/ml).

2.2.2. Antitumor activity

Marine Algae when uttered strikes about its healing property (Dziwornu et al., 2017) due its unique bioactive compounds present in it. The compounds present in it paves way for the synthesis of new drug molecules in treating various diseases (Alves et al., 2016).

The *in vitro* antitumor activity of algal species like *Laurencia catarinensis*, *L. majuscula* and *padina pavonica* extract was evaluated on 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

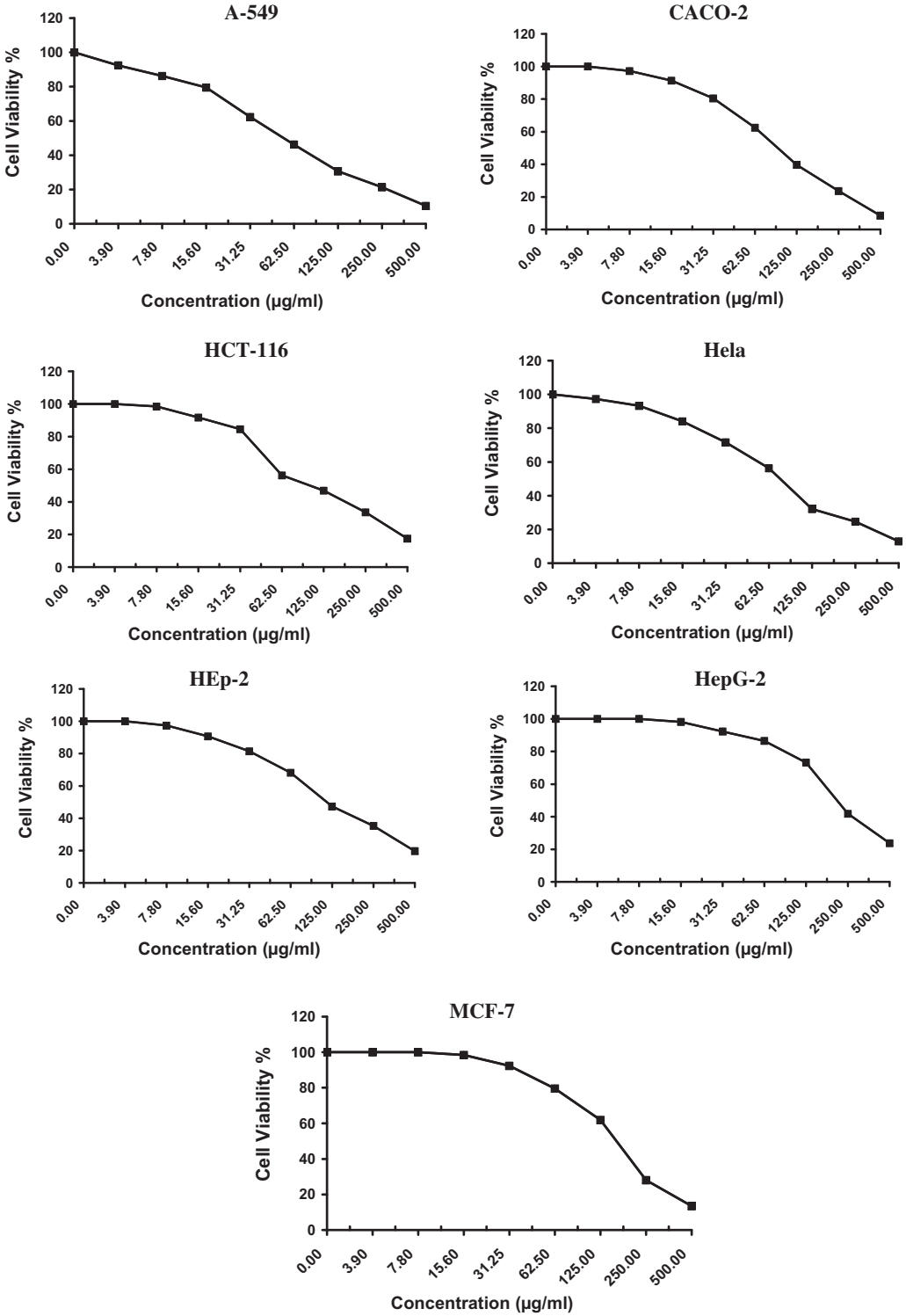

Fig. 2. The cytotoxic effect of *L. catarinensis* on A-549, CACO, HCT-116, Hela, HepG-2, and MCF-7 cell lines.

(Breast carcinoma) cell lines by using MTT assay method which is reliable to assess the *in vitro* cytotoxicity of the anticancer compounds (Allely et al., 1998). The obtained results exhibited direct cytotoxic effect of the investigated algal extracts on the cell lines in a concentration dependent manner (Figs. 2–4). The results indicated that the extract of *Padina pavonica* has the lowest percentage of viability and shows significant antitumor activity followed by *Laurencia catarinensis* and *L. majuscula* (Table 1).

The IC_{50} values of *Padina pavonica* were 58.9, 115.0, 54.5, 59.0, 101.0, 101.0, and 97.6 μg/ml; *Laurencia catarinensis* were 55.2, 96.8, 104.0, 78.7, 117.0, 217.0, 169.0 μg/ml; and *L. majuscula* were 115.0, 221.0, 225.0, 200.0, 338.0, 242.0, and 189.0 μg/ml; respectively against 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) (Table 4). Standard reference Vin-

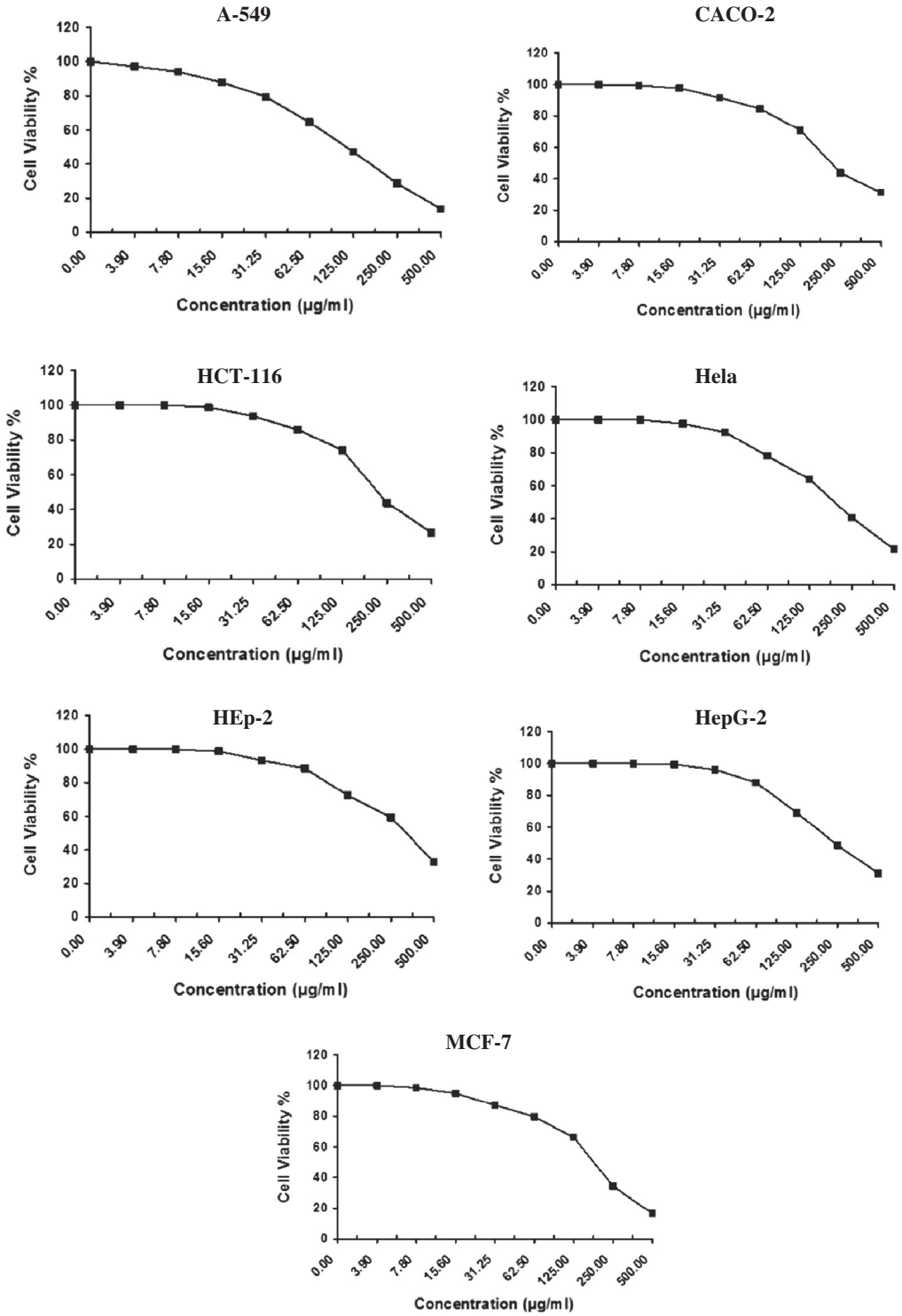

Fig. 3. The cytotoxic effect of *L. majuscula* on A-549, CACO, HCT-116, Hela, HepG-2, and MCF-7 cell lines.

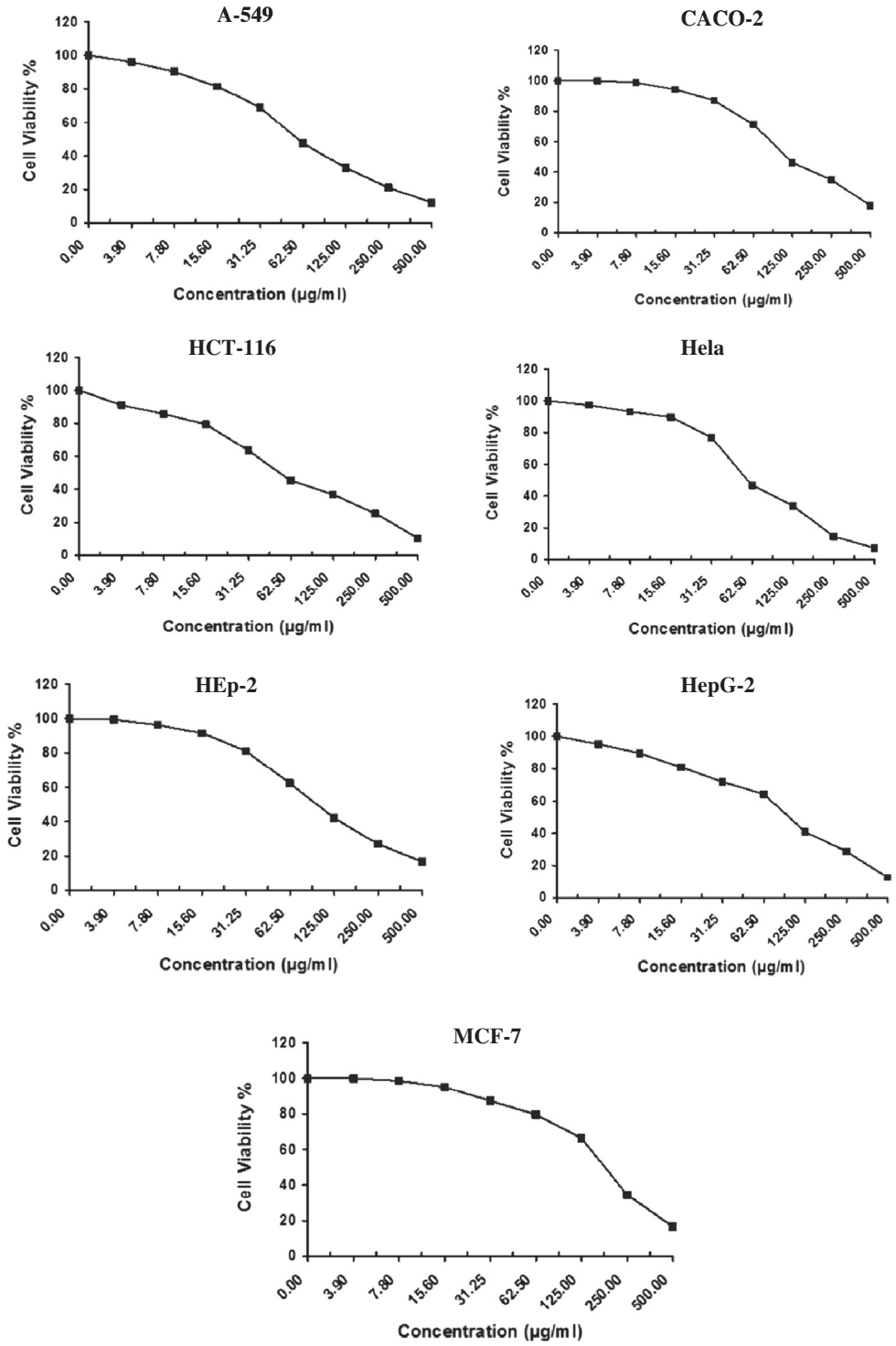

Fig. 4. The cytotoxic effect of *Padina pavonica* on A-549, CACO, HCT-116, Hela, HepG-2, and MCF-7 cell lines.

blastine Sulphate showed various effect on the same sell lines (Table 4 and Fig. 5).

L. catarinensis effect (55.2 ± 0.7 µg/ml) on A-549 (Lung carcinoma) showed activity the closest to Vinblastine Sulphate (24.6 ± 0.7 µg/ml) followed by *Padina pavonica* (58.9 ± 0.1 µg/ml), While *Padina pavonica* effect (59.0 ± 0.1 µg/ml) was equal to the standard (59.0 ± 0.7 µg/ml) when it tested on HCT-116 (Colon carcinoma).

In general *Padina pavonica* reported to have the best anticancer activities on the 7 tested cell lines *followed* by *L. catarinensis* and *L. catarinensis* respectively (Table 4 & Figs. 2–5)

Table 4
The IC_{50} values of *L. catarinensis*, *L. majuscula and Padina pavonica* extracts on cell lines.

Algal extract				
Cell line	IC_{50} (μg/ml)			
	L. catarinensis	*L. majuscula*	*Padina pavonica*	Vinblastine Sulphate
A-549 (Lung carcinoma)	055.2 ± 0.7	115.0 ± 0.4	058.9 ± 0.1	24.6 ± 0.7
CACO-2 (Colorectal carcinoma)	096.8 ± 0.3	221.0 ± 0.6	115.0 ± 0.9	30.3 ± 1.4
HCT-116 (Colon carcinoma)	104.0 ± 0.4	225.0 ± 0.2	054.5 ± 0.3	3.5 ± 0.2
Hela (Cervical carcinoma)	078.7 ± 0.5	200.0 ± 0.3	059.0 ± 0.1	59.7 ± 2.1
HEp-2 (Larynx carcinoma)	117.0 ± 0.2	338.0 ± 0.5	101.0 ± 0.2	21.2 ± 0.9
HepG-2 (Hepatocellular carcinoma)	217.0 ± 0.3	242.0 ± 0.2	101.0 ± 0.4	2.93 ± 0.3
MCF-7 (Breast carcinoma)	169.0 ± 0.1	189.0 ± 0.1	097.6 ± 0.3	5.9 ± 0.4

These are the mean of three determinations.

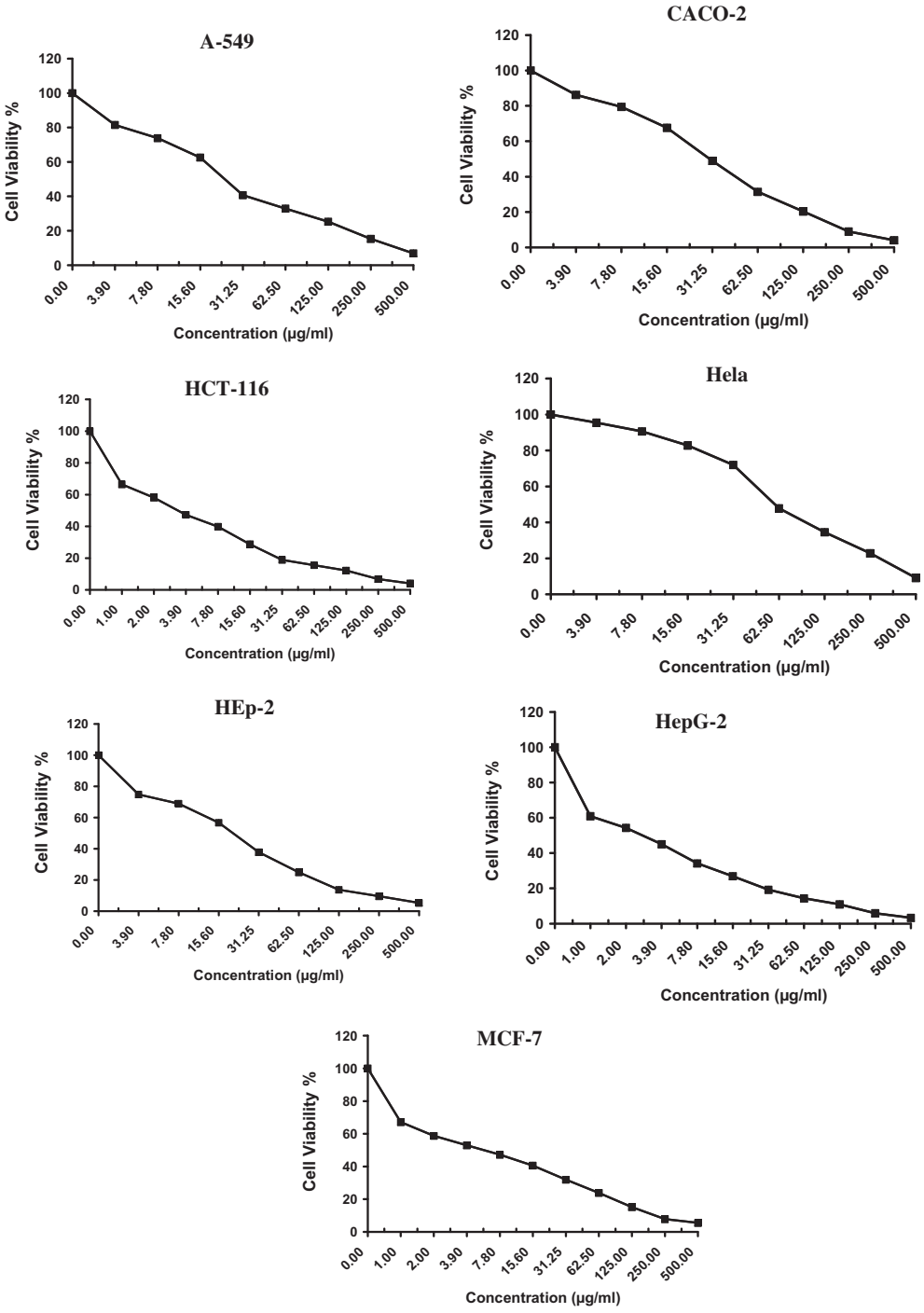

Fig. 5. The cytotoxic effect of Vinblastine Sulfate as Reference Standard on A-549, CACO, HCT-116, Hela, HepG-2, and MCF-7 cell lines.

References

Aleem, A.A., 1978. Contributions to the study of the marine algae of the Red Sea: III-Marine Algae from Obhor, in the vicinity of Jeddah, Saudi Arabia. Bull. Fac. Sci. K. A.U. Jeddah., 99–118

Aleem, A.A., 1993. The marine Algae of Alexandria, Egypt. In: Aleem (Ed.) Faculty of Science, University of Alexandria, Egypt.

Allely, M.C., Seudero, D.A., Monks, A., 1998. Feasibility of drug screening with panels of human tumor cell lines using micro culture tetrazolium assay. Can. Res. 58, 589–601.

Awaad, Amani S., AL-Mudhayyif, Hind A., Al-Othman, Monerah R., Zain, Mohamed E., El-Meligy, Reham M., 2017. Amhezole, a novel fungal secondary metabolite from Aspergillus terreus for treatment of microbial mouth infection. Phytother. Res. <https://doi.org/10.1002/ptr.5760> (in press).

Alves, C., Pinteus, S., Horta, A., Pedrosa, R., 2016. High cytotoxicity and anti-proliferative activity of algae extracts on an in vitro model of human hepatocellular carcinoma. Springerplus 5 (1), 1339.

Blunden, G., 2001. Biologically active compounds from marine organisms. Phytother. Res. 15, 89.

Bold, H.C., Wynne, M.J., 1978. Introduction to the Algae. Structure and Reproduction. Prentice-Hall, Englewood Cliffs, New Jersey. xiv+706 p.

Coppejans, E., Leliaert, F., Dargent, O., Gunasekara, R., Clerck, O., 2009. University of Ruhuna, Dept. of Botany; Matora, Srilanka. Srilanka Seaweeds Methodologies and Field Guide to the Dominant Species. pp. 1–265.

Dziwornu, G.A., Caira, M.R., Mare, J.A., Edkins, A.L., Bolton, J.J., Beukes, D.R., Sunassee, S.N., 2017. Isolation, characterization and antiproliferative activity of new metabolites from the South African Endemic Red Algal Species Laurencia alfredensis. Molecules 22 (4).

El Gamal, A.A., 2010. Biological importance of marine algae. Saudi Pharm. J. 18, 1–25.

El-Fatemy, A.S., 2008. Study of the effective of some brown algal species extractions (order: Dictyotales) against pathogenic fungi. M.Sc. Thesis, Botany Department, Faculty of Science, Garyounis University, Libya.

Ely, R., Supriya, T., Naik, C.G., 2004. Antimicrobial activity of marine organisms collected off the coast of south East India. J. Exp. Biol. and Ecol. 309, 121–127.

Faulkner, D.J., 2001. Marine natural products. Nat. Prod. Rep. 18, 1–49.

Faulkner, D.J., 2002. Marine natural products. Nat. Prod. Rep. 19, 1–48.

Fitton, J., Helen, 2003. Brown marine algae: A survey of therapeutic potentials. Altern. Complement. Ther., 29–33

Khan, F.Al., Iqbal, H., Shahid, F., Majed, A., Muhammad, A., Inayat, U.R., 2011. Phytochemical screening of some Pakistanian Medicinal Plants. Middle-East J. Sci. Res. 8 (3), 575–578.

Iwamoto, C., Yamada, T., Ito, Y., Minoura, K., Numata, A., 2001. Cytotoxic cytochalasans from a *Penicillium* species separated from a marine alga. Tetrahedron 57, 2904–2997.

Kameyama, Y., Yamashita, K., Kobayashi, K., Hosokawa, M., Chiba, K., 2005. Functional characterization of SLCO1B1 (OATP-C) variants, SLCO1B1*5, SLCO1B1*15 and SLCO1B1*15+ C1007G, by using transient expression systems of HeLa and HEK293 cells. Pharmacogenet. Genom. 15, 513–522.

Koleva, I.I., Van Beek, T.A., Linseen, J.P.H., de Groot, A., Evstatieva, L.N., 2002. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem. Anal. 13, 8–17.

Kwon, M.J., Nam, T.J., 2006. Porphyran induces apoptosis related signal pathway in AGS gastric cancer cell lines. Life Sci. 79, 1956–1962.

Lee, J., Koo, N., Min, D.B., 2004. Reactive oxygen species, aging, and antioxidative nutraceuticals. Compr. Rev. Food Sci. Food Safety 3, 21–33.

Lima-Filho, J.V., Carvalho, A., Freitas, S.M., 2002. Antimicrobial activity of extracts of six macroalgae from the Northeastern Brazilian Coast. Braz. J. Microbiol. 33, 311–313.

Lindequest, U., Schweder, T., 2001. Marine biotechnology. In: Rehm, H.J., Reed, G. (Eds.), Biotechnology, vol. 10. Wliey-VHC, Weinheim, Germany, pp. 441–484.

Madhavi, D.L., Deshpande, S.S., Salunkhe, D.K., 1995. Food Antioxidants. Dekker, New York, p. 267.

Middleton, E., Kandaswamy, C., Theoharides, T.C., 2000. The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer. Pharmacol. Rev. 52, 673–751.

Newman, D.J., Cragg, G.M., Snader, K.M., 2003. Natural products as sources of new drugs over the period 1981–2002. J. Nat. Prod. 66, 1022–1037.

Proksch, P., Edrada, R.A., Ebel, R., 2002. Drugs from the seas-current status and microbiological implications. Appl. Microbiol. Biotechnol. 59, 125–134.

Rajasulochana, R., Dhamotharan, P., Krishnamoorthy, S., Murugesan, 2009. Antibacterial activity of the extracts of marine red and brown algae. J. Am. Sci. 5 (3), 20–25.

Rocha de Souza, M.C., Marques, C.T., Dore, C.M.G., Ferreira da Silva, F.R., Rocha, H.A. O., Leite, E.L., 2007. Antioxidant activities of sulphated polysaccharides from brown and red seaweeds. J. Appl. Phycol. 19, 153–160.

Suresh, P.K., Sucheta, S., Sudarshana, V.D., Selvamani, P., Latha, S., 2008. Antioxidant activity in some selected Indian medicinal plants. Afr. J. Biotech. 7, 1826–1828.

Tüney, I., Çadircl, B.H., Ünal, D., Sukatar, A., 2006. Antimicrobial activity of the extracts of marine algae from coast of Urla (Izmir, Turkey). Turk. J. Biol. 30, 171–175.

Venkateswarlu, S., Panchagnula, G.K., Gottumukkala, A.L., Subbaraju, G.V., 2007. Synthesis, structural revision, and biological activities of 4′-chloroaurone, a metabolite of marine brown alga Spatoglossum variabile. Tetrahedron 63 (29), 6909–6914.

Yang, C., Chung, D., Shin, I.S., Lee, H.Y., Kim, J.C., Lee, Y.J., et al., 2008. Effects of molecular weight and hydrolysis conditions on anticancer activity of fucoidans from sporophyll of *Undaria pinnatifida*. Int. J. Biol. Macromol. 43, 433–437.

Ye, H., Wang, K., Zhou, C., Liu, J., Zeng, X., 2008. Purification, antitumor and antioxidant activities *in vitro* of polysaccharides from the brown seaweed *Sargassum pallidum*. Food Chem. 111, 428–432.

Yen, G.C., Duh, P.D., 1994. Scavenging effect of methanolic extracts of peanut hulls on free radical and active oxygen species. J. Agric. Food Chem. 42, 629–632.

Zain, M.E., Awaad, A.S., Al-Outhman, M.R., El-Meligy, R.M., 2012. Antimicrobial activities of Saudi Arabian desert plants. Phytopharmacology 2 (1), 106–113.