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Evaluation of biological activities of some seaweed and seagrass species in the coastal area of Vietnam

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Abstract
Although seaweeds and seagrasses have been used for food and traditional medicine for centuries, merely a small amount of them is exploited and used. Positive biological activities of seaweed and seagrass products on humans, animals and plants have also been recorded for a long time. Vietnam is a tropical country with 3,260 km long coastline and about 350 species of seaweeds, including 60 widely used species. In this study, 57 seaweed and seagrass samples were extracted using $CHCl_3$/MeOH solvent systems and their crude extracts were tested for selected biological actives, including antimicrobial, antioxidant activities and cytotoxicity. The results revealed that 13 out of 57 extracts (accounting for 24.07%) were cytotoxic to one of the two tested cancer cell lines (Hepatocellular carcinoma cell line Hep-G2 and human lung adenocarcinoma cell line LU-1), and 4 extracts (accounting for 7.4%) were cytotoxic to both cancer cell lines. In antimicrobial activity assay, 18 of all 57 extracts (accounting for 37.5%) were capable of inhibiting 1 to 2 test microorganisms and 16 extracts (accounting for 33.33%) inhibited at least 3 test microorganisms. There were solely 1 extract (accounting for 1.85%) of the 57 extracts performing antioxidant activity in DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay.

Keywords: Antioxidant, antimicrobial, cytotoxicity, seagrass, seaweed, Vietnam Sea.

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INTRODUCTION

The ocean accounts for 70% of the earth's surface, which is the living environment for organisms belonging to 34 of the 36 biological branches on the earth, in which about 20 branches are completely non-terrestrial. In the marine environment, organisms compete fiercely for shelter, food and enemies, so they are theoretically thought to either produce chemical compounds that are toxic to competitive species or have mutual relationship with symbionts that are capable of synthesizing inhibitory compounds against competitive species. As the chemical compounds from marine organisms and its biological activities are diverse, they have become a source for exploiting and using to fulfill human needs.

Vietnam has a huge potential of seaweeds (macroalgae) with about 350 species, and many of them were known to have industrial, agricultural and medicinal importance [1]. Seaweeds are considered a source of valuable metabolites, including pigments, such as chlorophyll and carotenoids, biliprotein and polysaccharides, such as alginic acid, agar, carrageenan, fucoidan, glucan and mannitol, macro- and micro-elements such as proteins, vitamins and polyphenols, polyunsaturated fatty acids (PUFAs) such as omega-3,... [2]. Pal et al., [2] reported biological activities of seaweed products, such as antiviral activity of carrageenan and fucoidan, antimicrobial activity of phenolic, aldehyde-based, hydroquinone-based and ketone-based compounds, anti-inflammatory activity of unsaturated fatty acids such as eicosapentaenoic and docosahexaenoic, anti-coagulating effect of fucoidan, anti-obesity and cholesterol-lowering effects such as sesquiterpene and plastoquinones,... Fucoxanthin, a secondary metabolite from brown algae *Sargassum siliquastrum*, *Hizikia fusiformis* and *Undaria pinnatifida*, was observed to possess antioxidant, antimicrobial and anticancer activities [3]. Currently, about 60 species of seaweeds are cultivated in Vietnam, in which more than 30 are being used as food, 20 are serving as pharmaceutical materials or in traditional medicine [1]. The genera *Sargassum* (brown seaweed), *Fucus* (brown seaweed), *Gracilaria* (red seaweed), *Kappaphycus* (red seaweed) and *Porphyra* (red seaweed) are amongst the most popularly cultivated and exploited ones in Vietnam [1].

In addition to seaweeds, seagrasses were known to contain diverse bioactive and pharmaceutically potential metabolites such as aquaporins, phenol, polyphenol, sulfated polysaccharide, dimethylsulfoniopropionate (DMSP) [4]. Species *Zostera japonica* comprises fatty acids with anti-inflammatory activity [5]. Seagrasses *Halodule pinifolia* and *Cymodocea rotundata* have antimicrobial activity against human pathogenic bacteria [6]. The crude extract from *Enhalus acoroides* showed antimicrobial and cytotoxic effects on human pathogens and cancer cells [7]. Compound zosterin produced by seagrass *Zostera asiatica* showed ability of purging heavy metals from human organisms [4]. L-chiro-inositol, a high proportion (up to 2.5% of dry weight) in seagrass *Syringodium flotsam*, presented anti-diabetes activity. It is estimated that there are about 14 species of seagrasses in Vietnam, belonging to 4 families, occupying an area of about 17,000 ha [8].

MATERIALS AND METHODS

Seaweed and seagrass samples

Fifty-two seaweed and five seagrass samples were collected in coastal regions of Vietnam, including Hai Phong, Nam Dinh, Hue, Thai Binh, Quang Ninh and Ninh Binh. The samples were morphologically identified and preserved under standard conditions in Institute of Marine Environment and Resources, Vietnam Academy of Science and Technology. Samples were dried (55^{o}C) immediately after being collected, followed by crushing and storing at -20^{o}C for extraction purpose. Table 1 listed sampling data and taxonomy profiles of seaweed and seagrass samples in this study.

Table 1. List of collected seaweed and seagrass samples

No.	Sample name	Sample type	Taxonomy	Place and time of sampling
1	LP4	Seagrass	*Ruppia maritime*	
2	LP5	Seagrass	*Halodule pinifolia* (Miki) Den Hartog	Co To, Quang Ninh 04.2014
3	LP6	Seagrass	*Halophila ovalis* (R. Br.) Hooker	Co To, Quang Ninh 04.2014
4	LP7	Seaweed	*Gracilaria bainilae* Chang et Xia	Tien Hai, Thai Binh 05.2014
5	LP 9	Seaweed	*Gracilaria salicornia* (C. Ag.) Daws.	Tien Hai, Thai Binh 05.2014
6	LP10	Seaweed	*Gracilaria gigas* Harv	Tien Hai, Thai Binh 05.2014
7	LP11	Seaweed	*Gracilaria tenuispititata* Zhang et Xia	Tien Hai, Thai Binh 05.2014
8	LP12	Seaweed	*Hydropuntio eucheumoides* Gyrgel et Fred.	Con Thoi, Ninh Binh 06.2014
9	LP15	Seagrass	*Halodule pinifolia* (Miki) Den Hartog	Tien Yen, Quang Ninh 04.2015
10	LP16	Seaweed	*Gracilaria salicornia* (C.Ag) Daws	Cat Ba, Hai Phong 03.2015
11	LP17	Seaweed	*Polycavernosa fastigiata* Zhang et Xia	Ha Long, Quang Ninh 07.2014
12	LP18	Seaweed	*Gracilaria tenuistipitata* Zhang et Xia	Xuan Thuy, Nam Dinh 03.2015
13	LP19	Seaweed	*Acanthophora muscoides* (L.) Bory	Cat Ba, Hai Phong 03.2015
14	LP20	Seaweed	*Gracilaria tenuistipitata* Zhang et Xia	Tra Co, Quang Ninh 04.2015
15	LP21	Seaweed	*Pterocladia pinnata* (Huds.) Papenf	Tien Yen, Quang Ninh 04.2015
16	LP22	Seaweed	*Gracilaria tenuistipitata* Zhang et Xia	Ha Long, Quang Ninh 04.2015
17	LP23	Seaweed	*Gracilaria tenuistipitata*	Quang Yen, Quang Ninh 03.2015
18	LP24	Seaweed	*Enteromorpha-Clathrata*	Kim Son, Ninh Binh 03.2015
19	LP25	Seaweed	*Chaetomorpha linum* (Muell.) Kuetzing	Cat Ba, Hai Phong 03.2015
20	LP26	Seaweed	*Gracilaria tenuistipitata* Zhang et Xia	Tien Hai, Thai Binh 04.2015
21	LP27	Seaweed	*Gracilaria tenuistipitata* Zhang et Xia	Dinh Vu, Hai Phong 01.2015
22	LP28	Seaweed	*Enteromorpha linum* (Muell.) Kuetzing	Ninh Binh 03.2015
23	LP29	Seagrass	*Halophila ovalis*	Tien Yen, Quang Ninh 04.2015
24	LP30	Seaweed	*Caulerpa verticillata* J.Ag.	Tien Yen, Quang Ninh 04.2015
25	LP31	Seaweed	*Gracilaria tenuistipitata* Zhang et Xia	Cat Hai, Hai Phong 02.2015
26	LP32	Seaweed	*Gracilaria blodgettii* Korr	Quang Yen, Quang Ninh 04.2015
27	LP33	Seaweed	*Enteromorpha clathrata* (Roth.) Grev.	Cat Ba, Hai Phong 03.2015
28	LP34	Seaweed	*Gracilaria tenuistipitata* Zhang et Xia	Do Son, Hai Phong 01.2015
29	LP35	Seaweed	*Gracilaria tenuistipitata* Zhang et Xia	Tien Yen, Quang Ninh 04.2015
30	LP36	Seaweed	*Gracilaria tenuistipitata* Zhang et Xia	Cau Hai, Hue 05.2014
31	LP37	Seaweed	*Gracilaria tenuistipitata* Zhang et Xia	Cat Hai, Hai Phong 03.2015
32	LP38	Seaweed	*Gracilaria tenuistipitata* Zhang et Xia	Trang Cat, Hai Phong 01.2015
33	LP39	Seaweed	*Gracilaria tenuistipitata* Zhang et Xia	Con Thoi, Ninh Binh 04.2013
34	LP40	Seaweed	*Gracilaria tenuistipitata* Zhang et Xia	Thinh Hung, Nam Dinh 04.2013
35	LP41	Seaweed	*Gracilaria tenuistipitata* Zhang et Xia	Thinh Hung, Nam Dinh 04.2013
36	LP42	Seaweed	*Gracilaria tenuistipitata* Zhang et Xia	Giao Xuan, Nam Dinh 04.2013
37	LP43	Seaweed	*Gracilaria tenuistipitata* Zhang et Xia	Giao Xuan, Nam Dinh 04.2013
38	LP44	Seaweed	*Gracilaria tenuistipitata* Zhang et Xia	Xuan Thuy, Nam Dinh 04.2013
39	LP45	Seaweed	*Gracilaria tenuistipitata* Zhang et Xia	Thai Thuy, Thai Binh 04.2013
40	LP46	Seaweed	*Gracilaria tenuistipitata* Zhang et Xia	Tien Hai, Thai Binh 04.2013
41	LP47	Seaweed	*Gracilaria tenuistipitata* Zhang et Xia	Tien Hai, Thai Binh 04.2013
42	LP48	Seaweed	*Gracilaria tenuistipitata* Zhang et Xia	Tien Lang, Hai Phong 04.2013
43	LP49	Seaweed	*Gracilaria tenuistipitata* Zhang et Xia	Do Son, Hai Phong 04.2013
44	LP50	Seaweed	*Gracilaria tenuistipitata* Zhang et Xia	Thai Thuy, Hai Phong 04.2013
45	LP51	Seaweed	*Gracilaria tenuistipitata* Zhang et Xia	Do Son, Hai Phong 04.2013

46	LP52	Seaweed	*Gracilaria tenuistipitata* Zhang et Xia	Cong Trang, Hai Phong 04.2013
47	LP53	Seaweed	*Gracilaria tenuistipitata* Zhang et Xia	Cat Hai, Hai Phong 04.2013
48	LP54	Seaweed	*Gracilaria tenuistipitata* Zhang et Xia	Cat Hai, Hai Phong 04.2013
49	LP55	Seaweed	*Gracilaria gigas* Harv.	Thai Thuy, Thai Binh 04.2013
50	LP56	Seaweed	*Gracilaria gigas* Harv.	Thai Thuy, Thai Binh 04.2013
51	LP57	Seaweed	*Gracilaria gigas* Harv.	Do Son, Hai Phong 04.2013
52	LP58	Seaweed	*Gracilaria gigas* Harv.	Thuy Hai, Thai Thuy 04.2013
53	LP59	Seaweed	*Gracilaria gigas* Harv.	Cat Hai, Hai Phong 26.4.2013
54	LP60	Seaweed	*Gracilaria busas-pastoris* (Gmel.) Silva	Giao Xuan, Nam Dinh 04.2013
55	LP61	Seaweed	*Gracilaria gigas* Harv.	Yen Hung, Quang Ninh 03.2012
56	LP62	Seaweed	*Gracilaria busas-pastoris* (Gmel.) Silva	Cat Hai, Hai Phong 07.2014
57	LP63	Seaweed	*Gracilaria gigas* Harv.	Cat Hai, Hai Phong 07.2014

Microbial strains and cell lines

Eight test microbial strains were supplied by Department of Experimental Biology - Institute of Natural Products Chemistry, including *Bacillus subtilis* ATCC 27212, *Staphylococcus aureus* ATCC 12222, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 25923, *Saccharomyces cerevisiae* ATCC 7754, *Candida albicans* SH 20, *Aspergillus niger* 439 and *Fusarium oxysporum* M42.

Two human cancer cell lines were provided by Department of Experimental Biology - Institute of Natural Products Chemistry, including Hep-G2 cell line (Hepatocellular carcinoma - liver cancer) and LU-1 (Human lung adenocarcinoma - lung cancer).

Antimicrobial assay

Antimicrobial activity of the extracts was tested on sterile 96-well plates according to the broth dilution method that was previously described by Vanden and Vlietlinck [9]. The antimicrobial testing method is currently applied in College of Pharmacy, University of Illinois at Chicago, USA.

Cytotoxicity assay

Cancer cell lines were cultured in vitro according to Skehan et al., [10]. The cytotoxicity on cancer cell lines was conducted by SRB method as described by Likhiwitayawuid et al. [11]. This method has been applied in Department of Experimental Biology - Institute of Natural Products Chemistry since 1996.

Antioxidant assay

Antioxidant activity of extracts was estimated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method described by Shela et al. [12]. In brief, a mixture containing 10 µL of sample in dimethyl sulfoxide (DMSO) and 190 µL of DPPH in ethanol was incubated in the dark for 30 min at 37°C. The absorbance of the reaction was recorded at 517 nm using a microplate reader (Tecan F150, Austria). DMSO and ascorbic acid were used as negative and positive controls, respectively. The antioxidant capacity of the tested samples was calculated using the following equation:

$$\%SC = \left[Ac - As\right]/Ac * 100\%$$

In which: Ac: Measured value of without sample; *As*: Measured value of the sample.

SC50 value is the sample concentration at which 50% of DPPH is scavenged.

Sample extraction

Total lipids were extracted using chloroform and methanol solvent system following the method described by Folch et al. [13]. Briefly, collected samples were ground to a size of 1-3 mm, then the lipids were extracted in $CHCl_3$/MeOH (2/1, v/v) (30 ml of solvent was used to extract 10 g of sample) (6 h, 4°C) (2×30 ml). After adding 35 ml of H_2O and 30 ml of $CHCl_3$, lipid retaining layer (lower layer) was separated. The lipids were then removed from water by adding anhydrous sodium sulfate

Na_2SO_4, then filtered to remove salt. Rotary evaporation was subsequently performed at 40°C under reduced pressure to obtain total lipid crude extracts. The total lipid fraction was dissolved in $CHCl_3$ and stored at -18°C.

RESULTS AND DISCUSSION

Cytotoxic activity

57 crude extracts of seaweed and seagrass samples were tested for cytotoxicity in two human cancer cell lines Hep-G2 and LU-1. The percentages of cell survival as well as IC_{50} values of cytotoxic samples were determined and recorded in table 2.

Table 2. Cytotoxicity of seaweed and seagrass extracts

No.	Sample name	Conc. (µg/ml)	Cell survival (CS, %)		IC_{50} (µg/ml)*	
			Hep-G2	LU-1	Hep-G2	LU-1
	DMSO		100.0 ± 0.0	100.0 ± 0.0		
	(+) control (ellipticine)	5	2.2 ± 1.5	3.4 ± 0.7		
1	LP5	40	16.09 ± 2.1	67.41 ± 2.1	30.03	-
2	LP6	40	18.47 ± 2.7	67.97 ± 1.8	30.52	-
3	LP7	40	20.21 ± 2.0	74.27 ± 2.7	33.98	-
4	LP 9	40	39.85 ± 2.1	87.13 ± 0.4	31.42	-
5	LP10	40	16.91 ± 1.3	77.20 ± 1.3	31.15	-
6	LP11	40	47.35 ± 1.2	83.03 ± 2.0	39.21	-
7	LP19	40	42.35 ± 2.7	72.31 ± 1.7	36.15	-
8	LP21	40	0	27.59 ± 2.0	28.91	25.79
9	LP23	40	46.37 ± 0.5	82.55 ± 1.5	38.76	-
10	LP29	40	0	43.21 ± 1.5	29.12	33.16
11	LP33	40	0	43.37 ± 2.1	19.19	37.95
12	LP37	40	24.73 ± 2.7	78.07 ± 2.4	30.53	-
13	LP38	40	2.09 ± 0.9	54.48 ± 2.2	38.15	-
14	LP41	40	46.14 ± 0.9	96.62 ± 1.2	38.53	-
15	LP42	40	45.05 ± 2.6	91.13 ± 1.7	37.21	-
16	LP45	40	0	0	4.36	6.04
17	LP54	40	32.94 ± 1.5	82.83 ± 0.8	31.58	-

Note: *IC_{50}: The concentration of extracts at which 50% of cell growth was inhibited.

The results from table 2 showed that 17 extracts were toxic to at least one cell line. Especially, three seaweed extracts (sample names LP21, LP33 and LP45) and one seagrass extract (LP29) performed cytotoxic activity in both cell lines. The cytotoxic activity of extracts from seagrasses, such as *Cymodocea serrulata* and *Halodule pinifolia*, was previously reported. Crude extract of *C. serrulata* inhibits cervical cancer cells (HeLa cell line) with IC_{50} value of 107.7 µg.ml^{-1} [14], *H. pinifolia* extract showed toxicity to human breast cancer cells (MCF7 cell line) with IC_{50} of 66.68 µg.ml^{-1} [15]. Seaweeds have been known with biological and pharmaceutical activities, for examples, *Gracilaria tenuistipitata* extracts exhibited cytotoxicity in throat cancer cells [16] and antiviral activity against Hepatitis virus C [17], *Gracilaria corticata* [18] and *G. verrucosa* [19] extracts were reported to be able to inhibit the replication of HeLa cancer cells. Seaweed species *Gracilaria tenuistipitata* is widely cultivated and populated in Vietnam, and in the present study, 20 out of 52 collected algal samples (from LP34 to LP54) were identified as *G. tenuistipitata*. It is noteworthy that among twenty *G. tenuistipitata* samples, only six (LP37, LP38, LP41, LP42, LP45 and LP54) were cytotoxic to at least one test cancer cell line. The result proposes a divergence in biological activities of samples belonging to

common taxonomical species. In our study, the crude extract of sample LP45 (seaweed *G. tenuistipitata*) exhibited the most potent cytotoxic activity in both tested cancer cell lines (Hep-G2 and LU-1) with IC_{50} values of 4.36 and 6.04 µg.mL^{-1}, respectively. The result indicates that the seaweed species (*G. tenuistipitata*) has a strong anticancer activity and potential to serve pharmaceutical purposes.

Antimicrobial activity

We have determined the antimicrobial activity of 57 crude extracts of seaweed and seagrass samples. Among them, 36 extracts showed antimicrobial activity to at least one test microbial strain. The minimal inhibitory concentrations (MIC) of them against 8 test strains in detail are presented at table 3.

Table 3. Antimicrobial activity of seaweed and seagrass extracts

No.	Sample name	MIC (µg/ml)							
		Gr (-) bacteria		Gr (+) bacteria		Filamentous fungi		Yeasts	
		E. coli	*P. aeruginosa*	*B. subtilis*	*S. aureus*	*A. niger*	*F. oxysporum*	*C. albicans*	*S. cerevisiae*
1	LP5	400	(-)	(-)	(-)	200	(-)	(-)	(-)
2	LP10	(-)	(-)	400	(-)	(-)	(-)	(-)	(-)
3	LP15	400	(-)	400	(-)	(-)	(-)	(-)	(-)
4	LP17	400	(-)	(-)	(-)	(-)	(-)	(-)	(-)
5	LP18	200	(-)	200	200	400	(-)	(-)	(-)
6	LP19	400	(-)	200	200	200	(-)	(-)	(-)
7	LP20	400	(-)	200	200	400	400	(-)	(-)
8	LP21	(-)	(-)	200	(-)	200	(-)	(-)	(-)
9	LP22	400	(-)	400	400	400	200	(-)	(-)
10	LP25	400	(-)	200	400	200	400	(-)	(-)
11	LP26	400	(-)	400	400	(-)	(-)	(-)	(-)
12	LP27	200	(-)	200	200	(-)	(-)	(-)	(-)
13	LP28	200	(-)	200	400	400	(-)	(-)	(-)
14	LP29	200	(-)	200	200	400	200	(-)	(-)
15	LP31	(-)	(-)	400	(-)	400	(-)	(-)	(-)
16	LP33	400	(-)	200	200	400	(-)	(-)	(-)
17	LP34	(-)	(-)	400	400	(-)	(-)	(-)	(-)
18	LP37	200	(-)	400	400	400	(-)	(-)	(-)
19	LP38	(-)	(-)	400	(-)	400	(-)	(-)	(-)
20	LP39	(-)	(-)	(-)	(-)	400	(-)	(-)	(-)
21	LP42	(-)	(-)	200	(-)	200	(-)	(-)	(-)
22	LP43	(-)	(-)	(-)	(-)	200	(-)	(-)	(-)
23	LP44	(-)	(-)	(-)	(-)	400	(-)	(-)	(-)
24	LP45	200	(-)	200	(-)	100	200	(-)	(-)
25	LP46	(-)	(-)	(-)	(-)	200	(-)	(-)	(-)
26	LP47	(-)	(-)	400	400	(-)	(-)	(-)	(-)
27	LP 49	(-)	(-)	400	(-)	(-)	(-)	(-)	(-)
28	LP51	400	(-)	(-)	(-)	(-)	400	(-)	(-)
29	LP52	(-)	(-)	400	(-)	(-)	(-)	(-)	(-)
30	LP 54	(-)	(-)	400	(-)	(-)	(-)	(-)	(-)
31	LP57	200	(-)	200	200	200	200	(-)	(-)
32	LP58	200	(-)	200	200	(-)	(-)	(-)	(-)
33	LP59	400	(-)	400	400	(-)	(-)	(-)	(-)
34	LP60	(-)	(-)	400	400	400	(-)	(-)	(-)
35	LP61	100	(-)	100	200	(-)	(-)	(-)	(-)
36	LP62	400	(-)	400	400	(-)	(-)	(-)	(-)

As being shown in table 3, 18 out of 57 crude extracts were antimicrobial active to 1–2 test microorganisms and 18 extracts (accounting for 31.57% of all tested extracts) exhibited inhibition effect against 3 or more test microorganisms. Especially, the number of extracts showing the activity on 4–5 test microorganisms was 9, equivalent to 15.8% of total extract number. These results indicate that seaweeds and seagrasses are a promising source of antibacterial and antifungal compounds. Most of extracts exhibiting activity on 5 test microorganisms in this study were originated from the genus *Gracilaria* (red seaweed). Ahneida et al., [20] investigated the activity of extracts of 160 seaweed species belonging to genus *Gracilaria* and found that there were 9 antibacterial active extracts (test organisms: *Vibrio, Staphylococus, Pseudomonas, Escherichia* and *Bacillus*) and 7 antifungal extracts (test organisms: *Candida, Fusarium, Aspergillus,...*). In addition to antimicrobial activity, many other biological activities were also investigated in the genus, such as antiviral, nematode, anti-diabetes, cardiovascular protection, intestinal, nervous system, anti-inflammatory, enzyme inhibitors... [20–22]. The extract fractions of seaweed *Gracilaria corticata* inhibited 8 human and animal pathogens (*Staphylococus aureus, Enterococcus faecalis, Salmonella typhi,...*), with MIC values of 1.25–20 µg/ml that were lower than ampicillin (MIC from 2.5–20 $\mu g.ml^{-1}$) [21]. The potential of antimicrobial against pathogens of seaweed extracts was also observed in five *Gracilaria* seaweed extracts [22], all of them were anti-bacterial, in which *G. verrucosa* extracts have highest activity. In another study, red seaweed *Gracilaria folifera* was antimicrobial active to 11 bacteria and 6 pathogenic fungi [23].

In seagrass samples, 3 (LP5, LP15 and LP29) of 5 extracts exhibited antimicrobial activity (table 3), in which extract LP29 (from *Halophila ovalis*) had a wide range of activity and effect against 6 out of 8 test microorganisms (both fungi and bacteria). Seagrass extracts have previously been studied for antimicrobial activity. Wisespongpand et al., [24] evaluated antimicrobial activity of extracts from 10 seagrass species and found that they were active to all tested bacterial and fungal pathogens and suggested that phenol and anthraglycoside were responsible to the activity. Three extracts of *H. stipulacea, H. pinifolia* and *Cymodocea serrulata* exhibited inhibiting effect to 7 human pathogenic bacteria, with MIC values ranging from 100 to 150 µg/ml (depending on the species of bacteria), equivalent to streptomycin with a MIC of 120–170 µg/ml [25].

Antioxidant activity

57 crude extracts of seaweeds and seagrasses were tested for antioxidant activity using DPPH radical scavenging assay. The results showed that almost all the samples were not antioxidant active. There was only one sample extracted from seagrass *Halophila ovalis* being observed with antioxidant activity, with SC50 value at 376.9 $\mu g.ml^{-1}$ (table 4).

Table 4. Antioxidant activity of seaweed and seagrass extracts

Sample name	Sample conc. (µg/ml)	Scavenging capacity (SC, %)	SC_{50} (µg/ml)	Conclusion
Positive Control (+)	44	80.87 ± 0.13	20.7	Positive
Negative Control (-)	-	0.0 ± 0.0	-	Negative
LP29	400	54.98 ± 1.8	376.9	Positive

Seagrasses were known to possess remarkable bioactivities [26]. *Halophila ovalis* was claimed to have valuable bioactivities such as antibacterial ability with MIC values of 50–100 µg/ml; DPPH and superoxide free radical scavenging activity at 130 µg/ml and 650 µg/ml, respectively; anti-inflammatory activity with IC_{50} value at 78.72 µg/ml [27]. The main compositions of *H. ovalis* are fatty acids, carboxylic acids, phenols, saponins, flavonoids,

proteins, carbohydrates, alkaloids,... Other seagrass species such as *H. pinifolia*, *Syringodium isoetifolium* showed antioxidant activity in scavenging DPPH, hydrogen peroxide and nitrite oxide free radicals [28]. Even extracts of seagrass species such as *Halophila stipulacea*, *Halodule pinifolia*, *Thalassia hemprichii*, *Cymodocea serulata* exhibited more potent antioxidant activity than ascorbic acid, gallic acid [29].

In this study, extract of seaweed *Gracilaria tenuistipitata* was antioxidant inactive. However, antioxidant activity was observed in some seaweed species when tested at high concentration of sample such as *G. manilaensis* with SC50 = 0.51 $mg.ml^{-1}$, much lower than positive control (acid ascorbic) (SC50 = 12.4 µg/ml) [30], the crude extract of seaweed *G. gracilis* showed DPPH free radical scavenging activity with SC50 values ranging from 0.82 to 35.03 $mg.ml^{-1}$ [31], which was lower than SC50 values of seaweed *G. corticata* extract, with 90–100 $mg.ml^{-1}$ (depending on the solvent used). Extracts of seaweeds belonging to Gracilariaceae family were antioxidant active (in DPPH test) with the highest SC50 value of 24.22 $mg.ml^{-1}$ [32]. The water extracts of seaweed *Gracilaria tenuistipitata* were proved to contain bioactive compounds such as phenolic, flavonoid, and ascorbic acid, however their DPPH free radical scavenging activity was relatively weak, with 63.37% DPPH free radicals scavenged by 4 $mg.ml^{-1}$ extract [33].

From the test results of cytotoxic, antimicrobial and antioxidant activities of 57 seaweed and seagrass extracts in this study, it could be concluded that samples extracted from closely taxonomic species were not completely homogeneous in biological activities. The reason may result from the divergence in geographic distribution, ages of seaweed and seagrass samples, generating deviations in the bioactive compound synthesis. Data in tables 2–4 show that LP29 extract from seaweed *Halophila ovalis* that was collected in Tien Yen, Quang Ninh expressed all three investigated biological activities (cytotoxic to 2 cancer cell lines, antimicrobial active to 5 test microorganisms and antioxidant in DPPH free radical scavenging assay). This result suggests that seaweed *H. ovalis* is a promising candidate to serve in biological and pharmacological purposes. Yuvaraj et al., [27] agreed that the seaweed is a potential source owing to its potent antioxidant and anti-inflammatory activities. Therefore, it is necessary to conduct more studies on such research objects for a more effective and sustainable exploitation in future.

CONCLUSION

In conclusion, 57 crude extracts from 52 seaweed and 2 seagrass samples collected from Vietnam coast were evaluated for antimicrobial, cytotoxic, and antioxidant activities. The results show that among these 57 extracts:

13 extracts (accounting for 24.07%) were cytotoxic to one test human cancer cell line, and 4 extracts (accounting for 7.4%) showed cytotoxic activity to 2 cancer cell lines.

18 extracts (accounting for 31.57%) exhibited antimicrobial activity against 1–2 test microorganisms and 16 crude extracts (accounting for 33.33%) inhibited at least 3 test microbial strains.

1 extract (1.85%) originating from *H. ovalis* seaweed was antioxidant active in DPPH radical scavenging assay.

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