RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2023.081

ScienceAsia 49 (2023): **791–796**

Variations in total phenolics in *Sargassum plagiophyllum* and their cytotoxic activities

Raphatphorn Navakanitworakul[a], **Dailamee Wanso**[a], **Sinjai Phetcharat**[b], **Parinuch Chumkaew**[c], **Jaruwan Mayakun**[b,*]

[a] Department of Biomedical Sciences and Biomedical Engineering, Faculty of Medicine, Prince of Songkla University, Songkhla 90110 Thailand
[b] Department of Biological Science, Faculty of Science, Prince of Songkla University, Songkhla 90110 Thailand
[c] Faculty of Science and Industrial Technology, Prince of Songkla University, Surat Thani Campus, Surat Thani 84000 Thailand

*Corresponding author, e-mail: jaruwan.may@psu.ac.th

Received 27 Nov 2022, Accepted 2 Aug 2023
Available online 22 Nov 2023

ABSTRACT: The brown alga *Sargassum plagiophyllum* is one of the most abundant species in the Andaman Sea. It is rich in phenolic compounds that show variations possibly influenced by biotic and abiotic factors. In the present study, variations of total phenolic compounds within-thallus and in different life stages were investigated, and the cytotoxicity of the phenolic compounds were evaluated against cancer cell proliferation. The results showed that total phenolic compounds in *S. plagiophyllum* significantly differed within-thallus with the highest concentration being found in the reproductive cells. There was significant difference in concentrations among life stages, with the lowest concentration found in juvenile plants. Total phenolic compounds revealed anticancer activity against colon (HCT116 and PMF-k014) and cervical cancer cells (HeLa and SiHa). The cytotoxic activity of the extracted phenolics produced half-maximal inhibitory concentrations (IC_{50} values) of >80 µg/ml against the cell lines HCT116 and PMF-k014, and of 44 ± 11.4 and 41.9 ± 6.1 µg/ml against HeLa and SiHa, respectively. Overall, this work found that total phenolic compounds from *S. plagiophyllum* have potential health benefits. In addition, the results of the study imply that to collect and use total phenolic compounds of *S. plagiophyllum* efficiently and sustainably, parts of the thallus and life stages should be carefully considered.

KEYWORDS: cytotoxicity, phenolic compounds, within-seaweed variation, *Sargassum*

INTRODUCTION

Phenolic compounds found in brown algae displays multifunctional ecological roles [1]. The primary function of these metabolites is structural, as components that strengthen cell walls, and the secondary function is chemical, as a deterrent to herbivores, as a protection against UV radiation, and as antifouling, antioxidant, and antibacterial agents [1–5]. In recent years, phenolic compounds from algae have been extensively and widely used commercially in cosmetics, medicine, food, health supplements, and industry [3, 6, 7]. They also have beneficial effects for human on reducing cardiovascular diseases and involving in antioxidant and antitumor activities [8].

In algae, phenolic concentrations vary within and between different species [9]. Within species, variable concentrations within different parts of the same individual and between individuals in the same population depend on developmental stage, algal age, thallus size, maturity, and location [2, 10–12]. Many studies have indicated that environmental factors such as temperature, light intensity, nutrient supply, and season, together with biotic factors such as grazing and epiphyte attachment, can strongly influence phenolic concentrations [1, 2, 12–14]. Faced with biotic or abiotic changes, seaweed will allocate resources to produce and translocate chemical compounds to younger tissue, to reproductive parts, and to vulnerable parts in order to maximize fitness. These responses support the Optimal Defense Theory (ODT) [15], which predicts that plants will allocate resources to tissues that have the highest fitness value or the most at risk of attack by grazers [9, 16]. In seaweed, these tissues correspond to attachment structures, which are valuable and at high risk to benthic grazers, and to meristematic and reproductive tissues with a high fitness value. These tissues, therefore, should have higher chemical concentrations than non-meristematic vegetative tissues [10, 15].

In Thailand, *Sargassum* species grow dominantly and abundantly on wave-exposed rocky shores and are widely distributed along coastal areas [17]. The brown alga, *S. plagiophyllum*, was chosen for the present study because it has a high density and biomass on the Andaman Coast. Additionally, it is a promising and potential natural source of raw materials and chemical compounds, such as sulfated fucoidans, phenolic compounds, antioxidant compounds, and other polysaccharides, that are of use in pharmaceutical, medical and other applications [18–20]. However, the variations in the concentration of phenolic compounds within different parts, among different life stages, and the cytotoxic activities of these concentrations remain

unexplored. The objectives of this study were: (1) to determine total phenolic compound variation within-thallus and among different life stages of *S. plagiophyllum*, and (2) to investigate the effect of total phenolic compounds on proliferation of cervical and colorectal cancer cells. The findings will help identify what part of the plant to collect and at what life stage in order to keep the resource's sustainability, develop high yield culture methods and elucidate cytotoxic activities of phenolic compounds on cancer cell lines. Additionally, the present data will confirm if this brown alga is a potential source of phenolic compounds for pharmaceutical, medical, and other uses.

MATERIALS AND METHODS

The whole thallus of *S. plagiophyllum* was collected from the coast of Ko Lanta, Krabi Province in the Andaman Sea, Southern Thailand. An ethanol extract of *S. plagiophyllum* was prepared for phytochemical screening. Chemical tests were performed using standard procedures [21–25] to identify the presence of alkaloids, steroids, terpenoids, saponin, anthraquinones, phenolic compounds, tannins, flavonoids, and glycosides. Thin layer chromatography (TLC) was applied for phytochemical screening. Chromatograms were developed and dried on silica gel TLC plates. The developed dried plate was sprayed with vanillin-sulphuric acid, and displayed bands were visualised under a UV lamp.

Juvenile, adult, and fertile individuals of *S. plagiophyllum* were collected for the study of intraspecific variation in phenolic concentration in different life stages. Blade, holdfast, and reproductive cells (or receptacles) were separated to determine within-thallus variation in phenolic concentration. Samples were collected in the dry (March 2021) and the rainy (October 2021) seasons. All collected samples were placed in Zip-loc bags and immediately transferred to the laboratory under dark and cold conditions. Samples were sorted, cleaned of sediment and epiphytes and frozen at −85 °C until used. Samples of each life stage and part were chopped into very fine pieces using a food cutter and, then, extracted in 80% aqueous ethanol in a 500-ml flask placed on an ultrasonic shaker for 1 h. The ethanol extract was filtered and dried under reduced pressure using a rotary evaporator. Phenolic concentrations were measured using a modified Folin-Ciocalteu assay [26]. Briefly, 500 µl of Folin-Ciocalteu's phenol reagent (Sigma-Aldrich, Germany) were added to the extract sample, and the mixture was incubated at room temperature for 3 min. Then, 100 µl of saturated sodium carbonate solution (Na_2CO_3) were added, and the reaction continued in the dark at room temperature for 1 h. The absorbance of the solution was measured at 725 nm with gallic acid used as standard. The analysis of extracts was performed in triplicate, and total phenolic compounds were expressed as GAE (gallic acid equivalent).

The cytotoxicity of extracted phenolic compounds from the whole thallus of *S. plagiophyllum* was determined against colorectal cancer cell lines (HCT116 and PMF-k014), cervical cancer cell lines (HeLa and SiHa), and normal embryonic kidney cell line (HEK293) by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. These cell lines were seeded at a density of 5×10^3 cells in 96-well plates containing 150 µl of completed medium per well. Cells were permitted to adhere for 24 h, and then treated with the crude extracts diluted to various concentrations of 5–80 µg/ml in medium. In addition, DMSO alone was added to another set of cells as a negative control. Doxorubicin (0.06–6 µg/ml) was used as the positive control. The cells were incubated for 72 h, washed with phosphate buffered saline (PBS), and then 100 µl of 0.5 mg/ml MTT solution were added into each well. The formazan crystals were dissolved with 150 µl of DMSO before absorbance was measured at 570 nm and 650 nm on a SpectraMax M5 multi-mode microplate reader (Molecular Device Corporation, USA). The whole experiment was independently replicated three times. The determination of cell viability and the fitting of response curves followed a previously described method [27]. The 50% inhibitory concentration (IC_{50}) of the crude extracts was calculated from the fitted response curves and determined according to the method of the US National Center Institute and Geran et al [28]. IC_{50} values were categorized as follows: $IC_{50} \leqslant 20$ µg/ml = highly active; IC_{50} 21–200 µg/ml = moderately active; IC_{50} 201–500 µg/ml = weakly active, and $IC_{50} > 500$ µg/ml = inactive.

Data analysis

A 3-way analysis of variance and Tukey's post-hoc test were used to examine the effects of: (1) life stage, (2) within-thallus, and (3) sampling time on total phenolic concentrations of *S. plagiophyllum*. Cochran's C-test was used to determine homogeneity of variance. All statistical analyses were performed using the computer program SPSS for Windows 13.0 (SPSS, Chicago, IL, USA).

RESULTS AND DISCUSSION

Phytochemical screening of the ethanol extract of *S. plagiophyllum* showed distinct patterns of chemical constituents of terpenoids, steroids, saponins, phenolic compounds, flavonoids, and coumarins (Table 1).

Phenolic compounds are frequently found in brown algae. Their multiple functions and biological activities were reported to include herbivore deterrence, antifouling with epiphytes, UV protection, and antioxidant, antibacterial, anticoagulant, anti-inflammatory, and anticancer activities [17, 29, 30]. It was also reported that phenolic compounds and

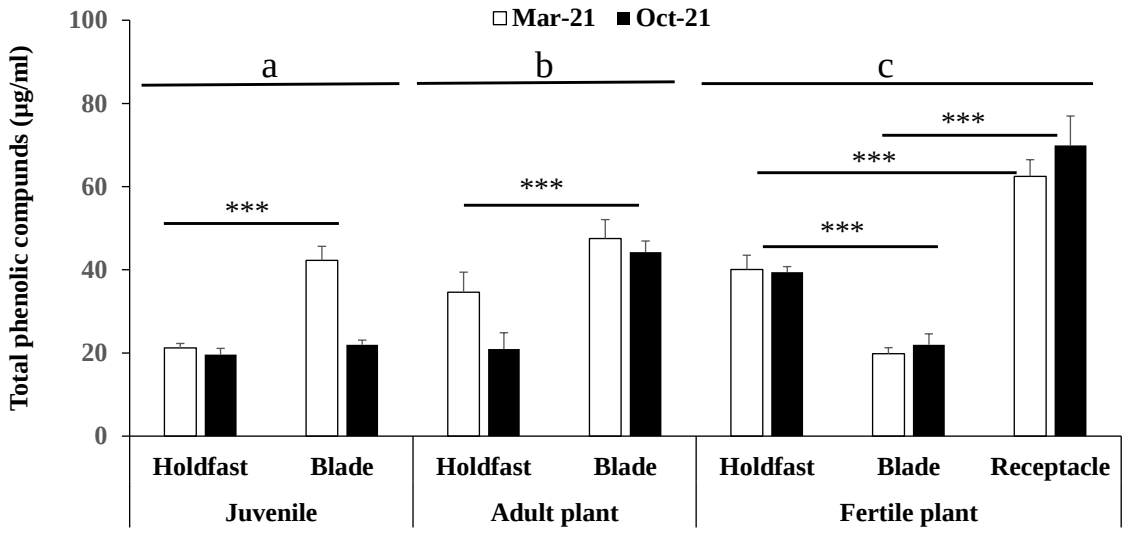

Fig. 1 Total phenolic compounds in different thallus parts of each life stage of *S. plagiophyllum* from Ko Lanta, Krabi Province, collected in March (dry season) and October (rainy season) 2021. Data are means ± SE. Different letters and *** indicate statistically significant differences at $p < 0.001$.

Table 1 Phytochemical screening of *S. plagiophyllum*.

Phytochemical screening	*S. plagiophyllum*
Terpenoids	+
Steroids	+
Saponins	+
Phenolic compounds	+
Flavonoids	+
Coumarins	+

+ = present, − = absent.

Table 2 The effects of sampling time, life stage, and thallus part on total phenolic concentrations of *S. plagiophyllum*.

Source	Df	MS	*F*
Time	1	348.693	109.594***
Life stage	2	340.347	106.970***
Within thallus	1	119.465	37.548***
Time × life stage	2	114.603	36.019***
Time × within thallus	1	7.691	2.417^{ns}
Life stage × within thallus	2	1164.598	366.030***
Time × life stage × within thallus	2	170.673	53.642***
Error	24	3.182	
Total	36		

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, not significant; Df, degrees of freedom in the source; MS, mean sum of squares due to the source; and *F*, *F*-statistic.

Table 3 Inhibitory effects of total phenolic compounds from the whole thallus of *S. plagiophyllum* on the cervical cancer HeLa and SiHa cell lines, and the colorectal cancer HCT116 and PMF-k014 cell lines.

Cell type	Cell line	IC_{50} (µg/ml)	
		Compounds	Doxorubicin
Cervical cancer	HeLa	44.0 ± 11.4	0.57 ± 0.01
	SiHa	41.9 ± 6.1	0.55 ± 0.00
Colorectal cancer	HCT116	>80	0.55 ± 0.02
	PMF-k014	>80	0.55 ± 0.01
Normal Kidney	HEK293	>80	<0.06

flavonoids were among the most effective antioxidant agents [31]. Steroids have an antibacterial potential, and saponins have shown anti-inflammatory and antimicrobial properties [32]. However, phytochemical variations between species could be generated by life cycle and environmental factors such as temperature, light intensity, salinity, nutrient availability, and grazing pressure [1, 2, 4, 5, 33, 34]. Phytochemical investigations of *S. oligocystum*, *S. crassifolium* and *S. tenerrimum* showed that phenolic compounds and flavonoids were the major metabolites in these species [29, 30].

Table 2 presents the result from a 3-way analysis of variance examining the effects of within-thallus, life stage, and sampling time on total phenolic concentrations of *S. plagiophyllum*. There were significant differences in total phenolic concentrations within-thallus (blade, stipe, and receptacles), in different life stages (juvenile, adult, and fertile plants), and sampling times (March and October) ($p < 0.001$, Table 2). For the within-thallus variation, the receptacles had the highest phenolic concentration, whereas the holdfast had the lowest value. For the life stage, the lowest

and the highest phenolic concentrations were found in the juvenile and the fertile plants, respectively (Fig. 1). For juvenile and adult plants, the concentration was higher in the blade than in the holdfast in both March and October. For fertile plants, the concentration varied between holdfast, blade, and receptacle. The receptacles of fertile plants exhibited the highest within-thallus concentrations in both March and October at 62.47 ± 4.01 and 69.92 ± 7.05 µg/ml, respectively (Fig. 1). Concentrations fluctuated significantly through time; however, *S. plagiophyllum* in March had a greater phenolic concentration than that in October ($p < 0.001$, Table 2).

Adult plants exhibited the highest concentration of phenolic compounds in blade, while fertile plants exhibited the highest concentration of the compounds in receptacles. These findings supported the prediction of the ODT that tissues with a higher fitness value and risk to be attacked by grazers would have higher concentration of phenolic compounds. Thus, meristematic and reproductive tissues, which have high fitness values, should be better defended than non-meristematic vegetative tissues. Our results were consistent with the work of Van Alstyne et al [10], who found that reproductive cells of the kelp *Alaria marginata* contained higher concentrations of phlorotannins than vegetative tissues. It is stated that newly growing tissues of *Halimeda* contained higher chemical concentrations than older tissues [35]. *Halimeda*, thus, follows the prediction of the ODT. Similarly, Siphonous green algae have the structures (siphons) to transport chemical defences from the meristem to the actively growing parts [36]. It is also showed that the tips of *Neomeris annulata* had high concentrations of secondary metabolites, and adult life stages had more phenolic compounds than juvenile stages [37]. Many studies have concluded that concentrations fluctuated in juvenile plants because they were more susceptible to changing environmental factors than adult plants [38, 39], and seaweeds might allocate more resources to growth than to chemical defence.

The study of the cytotoxicity of *S. plagiophyllum* phenolic compounds against cancer cell proliferation revealed anticancer activity against all tested cell lines. The IC_{50} values were 44 ± 11.4 and 41.9 ± 6.1 µg/ml against HeLa and SiHa, respectively; and >80 µg/ml against HCT116 and PMF-k014 as well as normal kidney HEK293 cells (Table 3). Many studies have reported similar results, showing that phenolic compounds from *Sargassum* had anticancer effects [30, 39]. Extracts of polyphenols effectively inhibited cervical and breast cancer cells. Namvar et al [39] also reported that polyphenols inhibited approximately 50% of the growth of breast cancer cell lines MCF-7 and MDA-MB-231 at concentrations of 22 and 55 µg/ml, respectively. IC_{50} values of doxorubicin were 0.57 ± 0.01, 0.55 ± 0.00, 0.55 ± 0.02, and 0.55 ± 0.01 µg/ml against HeLa, SiHa, HCT116, and PMF-k014 cell lines, respectively. The present finding confirmed cytotoxic effect of doxorubicin on cancer cells. However, doxorubicin was sensitive to normal kidney HEK293 cells with IC_{50} of <0.06 µg/ml. Our finding was in accordance with a previous study which reported that HEK293 cell line had the higher growth rate compared with MCF-7, and doxorubicin was more effective on HEK293 cells [40]. Although, doxorubicin showed higher anti-proliferative activity than the compounds, it was more cytotoxic in the normal cells. This indicated that *S. plagiophyllum* phenolic compound was more effective in cancer cell lines and, hence, could be a potential candidate for cancer therapy.

This study confirmed the variation in total phenolic concentrations within the thallus and in different life stages of *S. plagiophyllum*. Additionally, the cytotoxicity of extracted phenolic compounds against cervical and colorectal cancer cell lines were evaluated. The results indicated that phenolic compounds of *S. plagiophyllum* had potential health benefits. According to the present study, the harvest of *Sargassum* for extraction of phenolic compounds should take into consideration the parts and the life stage of the plants. These considerations would improve the efficiency and the sustainability of the plant. However, more studies on the population dynamics of this seaweed, phenolic qualification, and clinical trials would also help define the harvesting time, the use of biomass, and the properties of phenolic compounds.

Acknowledgements: This work was funded by the Fundamental Fund (FF), Thailand Science Research and Innovation (TSRI), Contract No. SCI6405076S and the Biodiversity-based Economy Development Office (a public organization), Thailand, Contract No. BEDO-NRCT23/2561. We would like to thank the Seaweed and Seagrass Research Unit team, Prince of Songkla University, for their assistance with the field work. Thanks go to Mr. Thomas Duncan Coyne for assistance with the English text.

REFERENCES

1. Mannino AM, Vaglica V, Oddo E (2017) Interspecific variation in total phenolic content in temperate brown algae. *J Biol Res* **90**, 6578.
2. Kamiya M, Nishio T, Yokoyama A, Yatsuya K, Nishigaki T, Yoshikawa S, Ohki K (2010) Seasonal variation of phlorotannin in sargassacean species from the coast of the Sea of Japan. *Phycol Res* **58**, 53–61.
3. Tanniou A, Vandanjon L, Incera M, Serrano Leon E, Husa V, Le Grand J, Nicolas JL, Poupart N, et al (2014) Assessment of the spatial variability of phenolic contents and associated bioactivities in the invasive alga *Sargassum muticum* sampled along its European range from Norway to Portugal. *J Appl Phycol* **26**, 1215–1230.
4. Van Hees DH, Olsen YS, Wernberg T, Van Alstyne KL, Kendrick GA (2017) Phenolic concentrations of brown seaweeds and relationships to nearshore environmental gradients in Western Australia. *Mar Biol* **164**, 74.

5. Ank G, Perez da Gama BAP, Pereira RC (2019) Latitudinal variation in phlorotannin contents from Southwestern Atlantic brown seaweeds. *PeerJ* **7**, e7379.
6. Okumura C, Miki O, Sakamoto Y, Fukami T (2018) Toxicological study for phenol using germling growth of the brown macroalga *Sargassum horneri*. *J Appl Phycol* **30**, 2083–2090.
7. Li Y, Fu X, Duan D, Xu J, Gao X (2018) Comparison study of bioactive substances and nutritional components of brown algae *Sargassum fusiforme* strains with different vesicle shapes. *J Appl Phycol* **30**, 3271–3283.
8. Vita JA (2005) Phenolic compounds and cardiovascular disease: effects on endothelial and platelet function. *Am J Clin Nutr* **81**(suppl), 292S–297S.
9. Fairhead VA, Amsler CD, McClintock JB, Baker BJ (2005) Within-thallus variation in chemical and physical defenses in two species of ecologically dominant brown macroalgae from the Antarctic Peninsula. *J Exp Mar Biol Ecol* **322**, 1–12.
10. Van Alstyne KL, McCarthy III JJ, Hustead CL, Kearns LJ (1999) Phlorotannin allocation among tissues of northeastern pacific kelps and rockweeds. *J Phycol* **35**, 483–492.
11. Pavia H, Toth GB (2000) Influence of light and nitrogen on the phlorotannin content of the brown seaweeds *Ascophyllum nodosum* and *Fucus vesiculosus*. *Hydrobiologia* **440**, 299–305.
12. Schiener P, Black KD, Stanley MS, Green DH (2015) The seasonal variation in the chemical composition of the kelp species *Laminaria digitata*, *Laminaria hyperborea*, *Saccharina latissima* and *Alaria esculenta*. *J Appl Phycol* **27**, 363–373.
13. Mayakun J, Kim JH, Lapointe BE, Prathep A (2013) Effects of nutrient enrichment and herbivory on morphology, reproduction, and chemical content of *Turbinaria conoides* (Phaeophyceae). *Phycol Res* **61**, 270–276.
14. Fellah F, Louaileche H, Dehbi-Zebboudj A, Touati N (2017) Seasonal variations in the phenolic compound content and antioxidant activities of three selected species of seaweeds from Tiskerth islet, Bejaia, *Algeria J Mater Environ Sci* **8**, 4451–4456.
15. Cronin G (2001) Resource allocation in seaweeds and marine invertebrates: Chemical defense patterns in relation to defense theories. In: McClintock JB, Baker BJ (eds) *Marine Chemical Ecology*, CRC Press, Boca Raton, Florida, USA, pp 325–352.
16. Toth GB, Langhamer O, Pavia H (2005) Inducible and constitutive defenses of valuable seaweed tissues: consequences for herbivore fitness. *Ecology* **86**, 612–618.
17. Praiboon J, Palakas S, Noiraksa T, Miyashita K (2018) Seasonal variation in nutritional composition and antiproliferative activity of brown seaweed, *Sargassum oligocystum*. *J Appl Phycol* **30**, 101–111.
18. Yangthong M, Hutadilok-Towatana N, Phromkunthong W (2009) Antioxidant activities of four edible seaweeds from the southern coast of Thailand. *Plant Foods Hum Nutr* **64**, 218–223.
19. Boonchum W, Peerapornpisal Y, Kanjapothi D, Pekkoh J, Amornlerdpison D, Pumas C, Sangpaiboon P, Vacharapiyasophon P (2011) Antimicrobial and anti-inflammatory properties of various seaweeds from the Gulf of Thailand. *Int J Agric Biol* **13**, 100–104.
20. Rattaya S, Benjakul S, Prodpran T (2015) Extraction, antioxidative, and antimicrobial activities of brown seaweed extracts, *Turbinaria ornata* and *Sargassum polycystum*, grown in Thailand. *Int Aquat Res* **7**, 1–16.
21. Kamba AS, Hassan LG (2010) Phytochemical screening and antimicrobial activities of *Euphorbia balsamifera* leaves, stems and root against some pathogenic microorganisms. *Afr J Pharm Pharmacol* **4**, 645–652.
22. Nisa S, Bibi Y, Waheed A, Zia M, Sarwar S, Ahmed S, Fayyaz Chaudhary M (2011) Evaluation of anticancer activity of *Debregeasia salicifolia* extract against estrogen receptor positive cell line. *Afr J Biotechnol* **10**, 990–995.
23. Anbuselvi S, Muthumani S (2014) Phytochemical and antinutritional constituents of sweet potato. *J Chem Pharm Res* **6**, 380–383.
24. Renjith Alex A, Ilango K, Viswanath BA, Shunmuga Sundaram R, Ganeshan S (2014) Phytochemical screening and antimicrobial activity of extracts of *Viburnum punctatum* Buch-Ham Ex D. Don against selected microbes. *J Chem Pharm Res* **6**, 1115–1120.
25. Yesufu HB, Khan IZ, Abdulrahman FI, Abatcha YZ (2014) A survey of the phytochemical and antioxidant potential of the fruit extracts of *Sarcocephalus latifolius* (Smith) bruce (Rubiaceae). *J Chem Pharm Res* **6**, 791–795.
26. Folin O, Ciocalteu V (1927) On tyrosine and tryptophane determinations in proteins. *J Biol Chem* **73**, 627–650.
27. Chotpirat A, Nittayaboon K, Kanokwiroon K, Srisawat T, Navakanitworakul R (2019) Anticancer potential of fruit extracts from *Vatica diospyroides* symington type SS and their effect on program cell death of cervical cancer cell lines. *Sci World J* **2019**, 5491904
28. Geran RI, Greenburg NH, Macdonald MM, Schumacher AM, Abbott BJ (1962) Protocols for screening chemical agents and natural products against animal tumors and other biological systems. *Cancer Chemother Rept* **25**, 1–29.
29. Baleta FN, Bolaños JM, Ruma OC, Baleta AN, Cairel JD (2017) Phytochemicals screening and antimicrobial properties of *Sargassum oligocystum* and *Sargassum crassifolium* extracts. *J Med Plants Stud* **5**, 382–397.
30. Mahendran S, Sankaralingam S, Sethupathi SM, Kathiresan D, Muthumani M, Kousalya L, Palpperumal S, Harinathan B (2022) Evaluation of antioxidant and cytotoxicity activities of polyphenol extracted from brown seaweed *Sargassum tenerrimum* biomass. *Biomass Conv Bioref*.
31. Sujatha R, Siva D, Nawas PMA (2019) Screening of phytochemical profile and antibacterial activity of various solvent extracts of marine algae *Sargassum swartzii*. *World Sci News* **115**, 27–40.
32. Mehdinezhad N, Ghannadi A, Yegdaneh A (2016) Phytochemical and biological evaluation of some *Sargassum* species from Persian Gulf. *Res Pharm Sci* **11**, 243–249.
33. Van Alstyne KL, Dethier MN, Duggins DO (2001) Spatial patterns in macroalgal chemical defenses. In: McClintock JB, Baker BJ (eds) *Marine Chemical Ecology*, Vol 3, CRC Press, Boca Raton, FL, USA, pp 301–324.
34. Jormalainen V, Honkanen T (2008) Macroalgal chemical defenses and their roles in structuring temperate marine communities. In: Amsler CD (ed) *Algal Chemical Ecology*, Springer, Berlin, Germany, pp 57–89.
35. Hay ME, Paul VJ, Lewis SM, Gustafson K, Tucker J, Trindell RN (1988) Can tropical seaweeds reduce her-

bivory by growing at night? Diel patterns of growth, nitrogen content, herbivory, and chemical versus morphological defenses. *Oecologia* **75**, 233–245.

36. Cronin G, Hay ME (1996) Within-plant variation in seaweed palatability and chemical defenses: optimal defense theory versus the growth-differentiation balance hypothesis. *Oecologia* **105**, 361–368.
37. Meyer KD, Paul VJ (1995) Variation in secondary metabolite and aragonite concentrations in the tropical green seaweed *Neomeris annulata*: effects on herbivory by fishes. *Mar Biol* **122**, 537–545.
38. Nielsen S, Nielsen H, Pedersen M (2014) Juvenile life stages of the brown alga *Fucus serratus* L. are more sensitive to combined stress from high copper concentration and temperature than adults. *Mar Biol* **161**, 1895–1904.
39. Namvar F, Mohamad R, Baharara J, Zafar-Balanejad S, Fargahi F, Rahman HS (2013) Antioxidant, antiproliferative, and antiangiogenesis effects of polyphenol-rich seaweed (*Sargassum muticum*). *BioMed res Int* **2013**, 604787
40. Akiyode O, George D, Getti G, Boateng J (2016) Systematic comparison of the functional physico-chemical characteristics and biocidal activity of microbial derived biosurfactants on blood-derived and breast cancer cells. *J Colloid Interface Sci 479*, 221–233.