International Journal of Ecology and Environmental Sciences 50: 623-629, 2024
ISSN: 2320-5199 (Online); https://doi.org/10.55863/ijees.2024.0205

Phyto-chemical Profiling, Antimicrobial and Antioxidant Activity of Marine Brown Algae, *Sargassum polycystum*

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ABSTRACT
Marine algae contain diverse bioactive compounds with pharmaceutical and nutraceutical importance. The present study was aimed at exploring one such alga, *Sargassum polycystum,* belonging to the phaeophyceae family. The seaweed was extracted with two different solvent systems and subjected to phytochemical analysis, antimicrobial and antioxidant studies. The extract showed wide variety of phytocomposition and was found to possess significant antimicrobial activity against bacterial and fungal pathogens. For antioxidant activities, DPPH assay, Hydroxyl radical scavenging assays, Phosphomolybdenum reduction and Fe^{3+} reducing power assays were carried out. The extract exhibited remarkable antioxidant activity in all the assays, especially in Fe^{3+} reducing power assay and hydroxyl radical scavenging, a maximum inhibition of 96.27 and 84.14% was observed, respectively. Also, the GC-MS analysis further confirmed the presence of isoflavone, a phenolic compound that signifies the role in antioxidant activity.

Key words: *Sargassum polycystum*, seaweed, phytochemistry, antioxidant, antibacterial, antifungal.

INTRODUCTION

Marine environment is a rich source of biological and chemical diversity. The diversity has been a unique source of chemical compounds of potential for pharmaceuticals, cosmetics, dietary supplements and agrochemicals (Chau et al. 2005). It also represents a great challenge that requires inputs from various scientific areas to bring the marine chemical diversity up to its therapeutic potential. In folk medicine, seaweeds have been used for a variety of remedial purposes, such as in eczema, gallstone, renal trouble, scabies, Psoriasis, asthma, arteriosclerosis, heart disease, ulcers and cancer (Raghavendran et al. 2006). Seaweeds are considered as a rich source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities (Rajasulochana et al. 2009). Brown seaweeds are rich sources of bioactive secondary metabolites. Among the marine flora and fauna marine algae are rich sources of diverse bioactive compounds with various biological activities. Recently, their importance as a source of novel bioactive substances is growing rapidly and researchers have revealed that marine algal originated compounds exhibit various biological activities (Wijesekara and Kim 2010).

For nearly 2000 years *Sargassum* spp., brown seaweed, has been used in Traditional Chinese Medicine (TCM) to treat a variety of diseases including thyroid disease (eg. goitre). *Sargassum* has been used traditionally for treating scrofula, goiter, tumor, edema, testicular pain and swelling (Kandale et al. 2011). The therapeutic effects of *Sargassum* spp. are scientifically plausible and may be explained partially by key in vivo and in vitro pharmacological activities of *Sargassum*, such as anticancer, anti-inflammatory, antifouling, antibacterial (Mansuya et al. 2010), antiviral (Yangthong et al. 2009, and Liu et al. 2012), hepatoprotective (Raghavendran et al. 2006), larvicidal (Lee and Kang 2015) and neuroprotective (Subramaniam et al. 2014).

Sargassum species are found throughout tropical and subtropical areas of the world and are reported to produce metabolites of structural classes such as terpenoids, polysaccharides, polyphenols, sargaquinoic acids, sargachromenol, plastoquinones, steroids, glycerides etc., which possesses several therapeutic activities (Chennubhotla et al. 1981). Phyto chemical properties, anti-microbial potential and anti-oxidant activities of several seaweed species of Tamil Nadu coastlines are still unexplored and unidentified. Though there have been a number of

studies on ecology, anatomy, taxonomy and distribution from the coast of Bay of Bengal but a little attention was paid on the phytochemical properties, antimicrobial activities and anti-oxidant nature of these seaweeds. Hence, the present study is focused on the seaweed *Sargassum polycystum* from the coast of Mandapam, Ramanathapuram District, Tamil Nadu.

MATERIALS AND METHODS

Collection of samples

Fresh algae were collected in polythene bags containing sea water from the coastal line of Mandapam (9°16'37.34"N; 79°7'30.78"E), Ramanathapuram district, India and transported to the laboratory. Then the algal materials were thoroughly washed in running tap water to remove the attached epiphytes and other marine debris. Finally, it was washed with distilled water and allowed to dry under shade for 5-7 days, until the moisture was completely removed. The dried material was ground to a coarse powder and stored in air tight containers at room temperature.

Preparation of crude extract and analysis

Finely ground algal material was extracted with chloroform and aqueous in the ratio of 1:10 (w/v) in a conical flask for 72 hrs. The mixture was filtered using Whatman No.1 filter paper in a separate container. The above process was repeated for two times with the same residue using fresh solvent. All the supernatants were collected together and then the solvent was removed by rotor evaporator. The filtrate thus obtained was allowed to concentrate and stored for further studies. All parameters were performed on the crude extracts of seaweeds to explore the phytoconstituents present in them by adapting the methods of Harborne (1998) and Kokate (2001).

RESULTS AND DISCUSSION

Phytochemical analysis

The phytochemical constituents of the selected brown seaweed *Sargassum polycystum* are summarized in Table 1. The chloroform extract showed the presence of alkaloids, phenol, sugar, foam, flavonoids, steroids and terpenoids while the aqueous extract showed the presence of alkaloids, foam, flavonoids, tannins, steroids and terpenoids. The results of the present study confirmed rich sources of phytoconstituents which can be isolated and further screened for various biological activities. In agreement to the above results, many research studies has reported the phytoconstituents of the genus *Sargassum* with structurally unique secondary metabolites such as alkaloids, anthraquinones, cardiac glycosides, flavonoids, reducing sugars, saponins and terpenoids (Arsianti et al. 2020). In a similar study conducted by Mehdinezhad et al. (2016), three different *Sargassum* species namely, *Sargassum angustifolium*, *Sargassum oligocystum* and *Sargassum boveanum* also showed the presence of alkaloids, saponins, flavonoids, tannins, steroids, anthraquinones and terpenoids.

Table 1. Phytochemical screening of *Sargassum polycystum*

Test	Chloroform extract	Aqueous extract
Alkaloids	+	+
Terpenoids	+	+
Steroid	+	+
Phenol	+	+
Flavonoid	+	+
Tannin	-	+
Sugar	+	-
Saponin	-	+

'+' Present, '-' Absent

Antimicrobial activity

Seaweed produces metabolites aiding in the protection against different environmental stresses. These compounds show antiviral, antiprotozoal, antifungal, and antibacterial properties (Perez et al. 2016). Antimicrobial activity of crude extracts and solvent fractions from *Sargassum* has been reported as effective against bacterial and fungal pathogens (Kim et al. 2007). The antibacterial activity of *S. polycystum* against Gram-negative bacteria and Gram-positive bacteria was reported to show bacteriostatic as well as inhibitory activity (Chiao-Wei et al. 2013). *Accordingly,* in the present study, the chloroform extract was found to be the most

Table 2. Antibacterial activity (expressed as zone of inhibition (cm)) of *Sargassum polycystum*

Microorganism	Chloroform extract				Antibiotic	Aqueous extract				Antibiotic
	25 µg	50 µg	75 µg	100 µg	(25 µg /ml)	25 µg	50 µg	75 µg	100 µg	(25 µg /ml)
Stapylococcus aureus	1.2	1.3	2	2.3	2.4	1	1.2	1.3	1.4	**1.8**
Bacillus subtilis	1.2	1.4	2	2.2	3.1	1.3	1.7	1.8	1.9	-
Klebsiella pneumoniae	-	1.3	1.3	1.5	2.6	-	_-	-	1.5	2.8
Pseudomonas aeruginosa	1.1	1.5	1.7	1.8	2	1.2	1.3	1.4	1.9	2.6

effective showing the largest inhibition zone particularly with *Staphylococcus aureus* (2.3 cm), followed by *Bacillus subtilis* (2.2 cm), *Pseudomonas aeruginosa* (1.8 cm) and *Klebsiella pneumonia* (1.5 cm) at 100 µg/ml concentration. The zone of inhibition increased as the concentration increased (Table 2). Also, the aqueous extract showed similar trend in inhibition against the tested pathogens. Of the two extracts, chloroform was comparatively significant than the aqueous extract. Overall, both the extracts were sensitive to all the tested pathogens.

Also, seaweeds are known to possess remarkable antifungal potential. Sargassum has more polysaccharides compounds compared with other algae, this polysaccharides have the ability to invade inside of the pathogens and damages the nucleus effectively (Rajivgandhi et al. 2021). The antifungal activity of *Sargassum* sp. against *Aspergillus niger* and *Candida albicans* has been reported to significantly high (Abdi et al. 2022). The results in the present study revealed the antifungal potential of *S. polycystum* extract against the tested fungal isolates. The highest activities were recorded in the chloroform extract against *Penicillium notatum* (2.8 cm) and *Microsporangium gypsium* (2 cm) followed by *Candida* and *Rhizopus* (Table 3). Similarly, the aqueous extract also presented notable inhibition against *Penicillium notatum* (1.3 cm), *Microsporangium gypsium* (1.7 cm), *Candida* (1 cm) and *Rhizopus* (1 cm). However, both the extracts were ineffective to *Aspergillus flavus.*

Antioxidant activity

Sargassum polycystum is well known as macroalgae that contain active compounds with great function as antioxidants. The antioxidants content of the seaweed has links closely to phenolic compounds (Sumandiarsa et al. 2022). Several studies have demonstrated a highly significant correlation between the phenolic content and the antioxidant activity in seaweed extracts (Siriwardhana et al. 2003). It has been reported that the antioxidant activity of *Sargassum polycystum* is well correlated with the content of their phenolic compounds (Zhang et al. 2007). Thus, in the present investigation, the maximum DPPH radical scavenging activity of *S. polycystum* was 63.56% at 600 µg/mL and minimum 16.58% at 100 µg/ml concentration (Fig. 1). The hydroxyl radical scavenging assay demonstrated a maximum antioxidant activity of 84.14% in 600 µg/ml concentration and minimum of 56.2% in 100 µg/ml concentration (Fig. 2). In Phosphomolybdenum assay and Fe^{3+} reducing power assay, the seaweed showed significant antioxidant potential of 75.57 and 96.27% in 60 µg/ml concentration, respectively (Figs. 3, 4). Overall, the algal extract exhibited remarkable antioxidant activity. In agreement to the above findings, study reports on the antioxidant

Table 3. **A**ntifungal activity (expressed as Zone of inhibition (cm)) of *Sargassum polycystum*

Microorganism	Chloroform extract (100 µg /ml)	Antibiotic (25 µg /ml)	Aqueous extract (100 µg /ml)	Antibiotic (25 µg /ml)
Penicillium notatum	2.3	2.4	1.3	3.3
Microsporangium gypsium	2.2	3.1	1.7	3.5
Candida albicans	1.5	2.6	1	0
Rhizopus sp	1.8	2.0	1	4
Aspergillus flavus	-	-	-	-

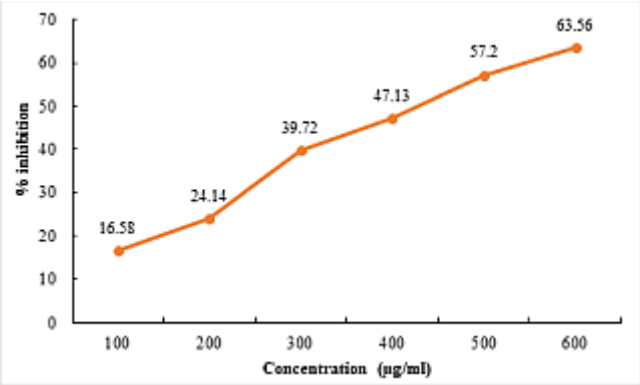

Figure 1. DPPH assay

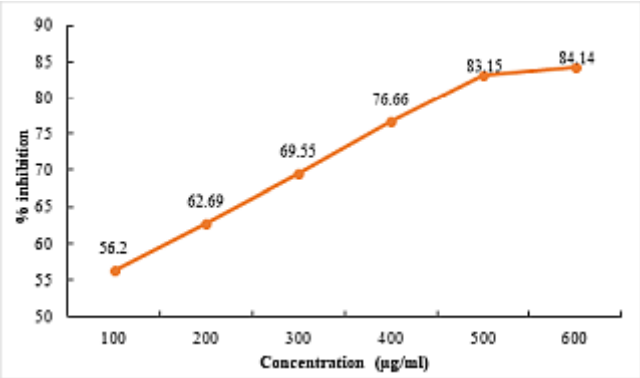

Figure 2. Hydroxyl radical scavenging assay

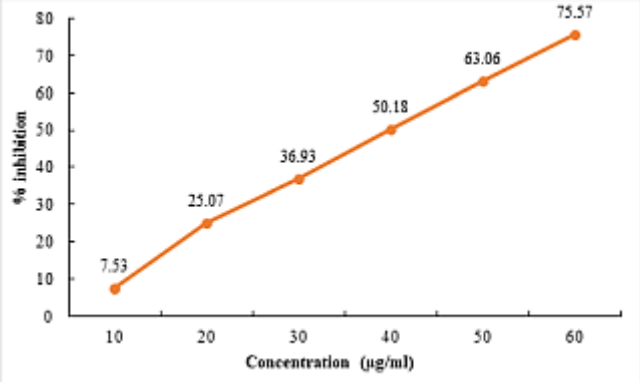

Figure 3. Phosphomolybdenum reduction assay

activity of brown seaweed, *Sargassum olygocystum* has shown similar trends in antioxidant potential establishing the significant of the genus (Sanger et al. 2022). Also, the antioxidant activity of the hydroethanolic extract of *S. polycystum* investigated using DPPH and Fe^{3+} reducing power assay has been reported to possess significantly high antioxidant levels (Woonnoi et al. 2023).

GC-MS analysis of *Sargassum polycystum*

The GC-MS analysis was carried out for the

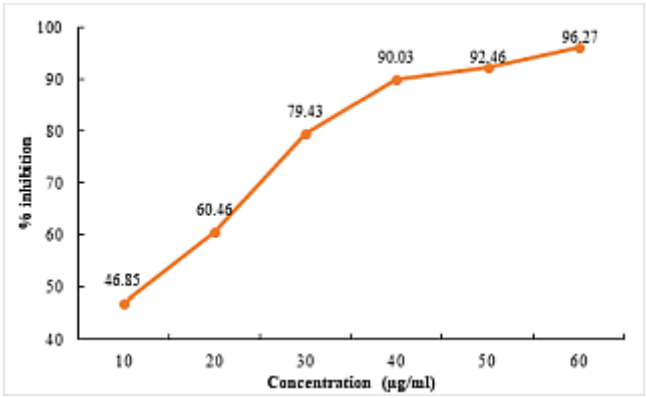

Figure4. Fe^{3+} reducing power assay

chloroform extract of *Sargassum polycystum* and the eluted compounds are presented in Figure 5. The extract showed the presence of many biologically active compounds (Table 4). A flavone compound Isoflavone was observed at a retention time (RT) of 16.03, which is a potential antioxidant compounds and could be the reason for the antioxidant property of the extract. Also, other compounds like Palmitic acid, Octadecanoic acid, Methyl isostearate, Methoxyacetic acid Octadecyl ester were found in the crude extract. A medically important compound a-Pinene was also observed at a RT of 13.08. Similarly, many previous studies have reported that the phenolic compounds produced by the marine algae significantly contribute to their antioxidant capacity. The presence of fatty acids, esters, flavonoids and other phenol derivatives are considered to possess pharmaceutical values that can be further explored (Nagai and Yukimoto 2003, Chabake and Chaubal 2020). Accordingly, the metabolites extracted from *S. polycystum* has been previously reported to comprise n-hexadecanoic acid, 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester, benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy- methyl ester, 1-dodecanol, 3,7,11-trimethyl (Nazarudin et al. 2021). Similarly, Cyclopropanepentanoic acid and oxohexanoic acid were found to be present in the methanolic extract of *S. tenerrimum* (Ponnuchamy et al. 2013).

CONCLUSIONS

The marine macroalgae *Sargassum polycystum* are a rich source of structurally novel and biologically

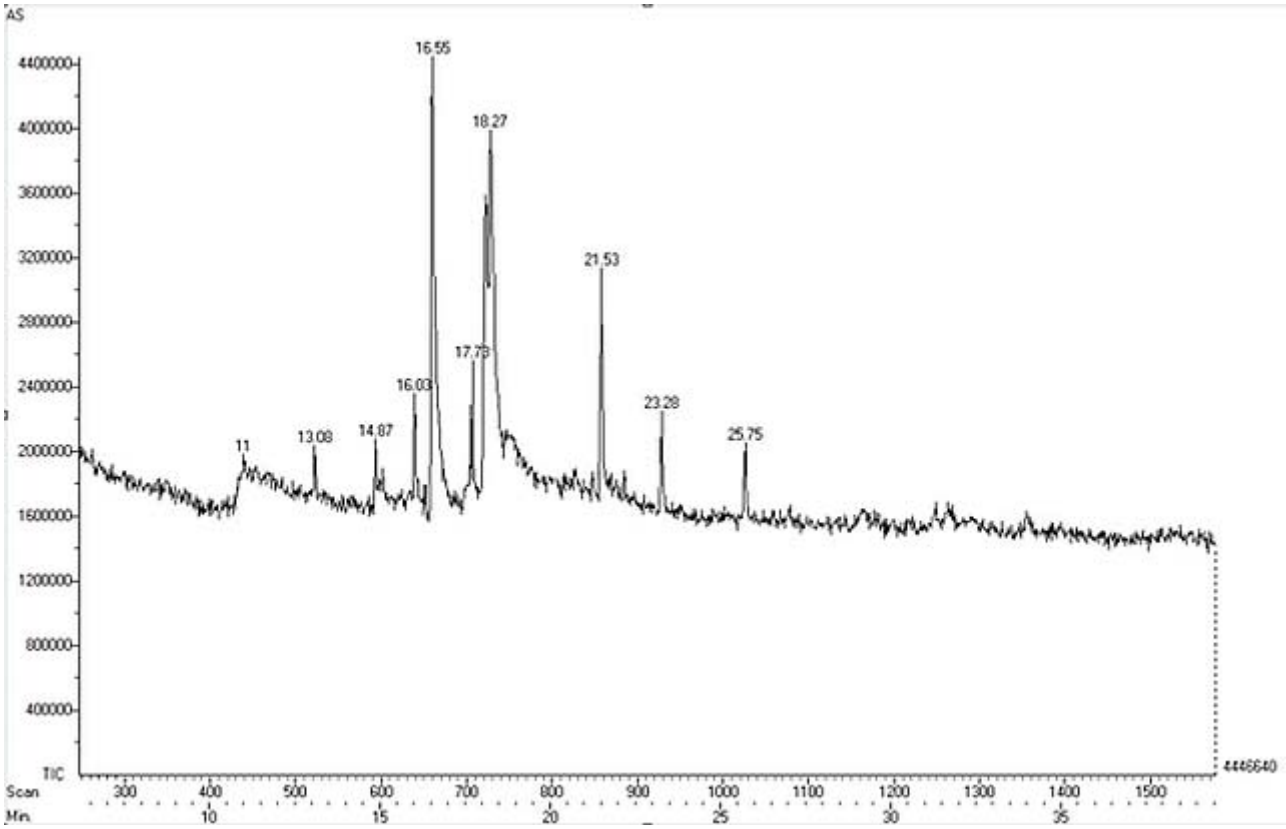

Figure 5. GC-MS chromatogram of S. polycystum crude extract

Table 4. Active compounds identified in S*argassum polycystum* by GC-MS analysis

S.No	RT	Name	Molecular weight (g/mol)	Molecular formula	IUPAC name
1.	11.37	Indenol	132.162	C_9H_8O	1H-Indenol
2.	14.87	1-Benzene	148.2447	$C_{11}H_{16}$	Benzene,(1-methylenebutyl)
3.	16.03	Isoflavone	222.243	$C_{15}H_{10}O_2$	Isoflavone
4.	16.55	Palmitic acid	256.43	$C_{16}H_{32}O_2$	Palmitic acid
5.	21.53	Benzene-dicarboxylic acid	278.3435	$C_{16}H_{22}O_4$	1,2-Benzenedicarboxylic acid, mono(2-ethyhexyl) ester
6.	23.28	Pyrimido	163.144	$C_5H_5N_7$	Pyrimido[5,4-E]1,2,4-triazine-5,6 (6H,8H)-dione, 6,8-dimethyl-3-(4-dimethylaminophenyl)
7.	18.27	Octadecanoic acid	284.4772	$C_{18}H_{36}O_2$	9-Octadecanoic acid
8.	17.73	Methyl isostearate	298.5038	$C_{19}H_{38}O_2$	Heptadecanoic acid, 16-methyl-, methyl ester
9.	25.75	Methoxyacetic acid, octadecyl ester	342.55638	$C_{21}H_{42}O_3$	Methoxyacetic acid, octadecyl ester
10.	13.08	á- Pinene	136.238	$C_{10}H_{16}$	a-Pinene

active metabolites. The present investigation presents significant data on the phytochemical constituents, antimicrobial and antioxidant potential of *S. polycystum* extracts with pharmaceutical importance. Given this antioxidant potential, the study will add value to the bioprospecting works on this seaweed which may be explored and developed for its potential utility as nutritional supplement.

ACKNOWLEDGEMENTS

Authors are thankful to IIT, Madras for assisting in performing GC-MS analysis.

Authors' contributions: All authors contributed equally.

Conflict of interest: Authors declare no conflict of interest.

REFERENCES

Abdi, G., Karande, V.C., Mohammed, A., Abbasi, T.M., Wen, G.K., Abdul, K.Z., Seong, W.L., Ahmad, M.R., Mohammadi, M., Ee, L.G. and Barwant, M.M. 2022. Pharmacological potential of *Sargassum* sp. of west coast of Maharashtra Kunkeshwar, India. Frontiers in Marine Science, 9, 1011218. https://doi.org/10.3389/fmars.2022.1011218

Arsianti, A., Bahtiar, A., Wangsaputra, V.K., Azizah, N.N., Fachri, W., Nadapdap, L.D., Fajrin, A.M., Tanimoto, H. and Kakiuchi, K. 2020. Phytochemical composition and evaluation of marine algal *Sargassum polycystum* for antioxidant activity and in vitro cytotoxicity on Hela cells. Pharmacognosy Journal, 12(1), 88-94. https://doi.org/10.5530/pj.2020.12.14

Chabake, V. and Chaubal, S. 2020. GC-MS profiling and characterization of *Sargassum prismaticum.* International Research Journal of Science & Engineering, A9, 161-165. https://irjse.in/ICETCL_2020/IRJSE_SPIA9_36.pdf

Chau, V.M., Phan, V.K. and Ngyen H.D. 2005. Marine natural products and their potential application in the future. Asean Journal on Science and Technology for Development, 22(4), 297-311. https://doi.org/10.3125/asean.v22i4.389

Chennubhotla, V.S.K., Kaliaperumal, N. and Kalimuthu, S. 1981. Seaweed recipes and other practical uses of seaweeds. Sea Food Export Journal, 13, 9-16. https://core.ac.uk/download/pdf/33017588.pdf

Chiao-Wei, C., Siew Ling, H. and Wong, C. 2013. Antibacterial activity of *Sargassum polycystum* C. Agardh and *Padina australis* Hauck (Phaeophyceae). African Journal of Biotechnology, 10(64), 14125-14131. https://doi.org/10.5897/AJB11.966.

Harborne, A.J. 1998. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Springer, Netherlands. https://link.springer.com/book/9780412572609

Kandale, A., Meena, A.K., Rao, M.M., Panda, P., Mangal, A.K., Reddy, G. and Ramesh, B. 2011. Marine algae: An introduction, food value and medicinal uses. Journal of Pharmacology Research, 4(1), 219-21. https:// doi.org/10.5555/20113094129

Kim J-Y., Lee J-A., Kim K-N., Yoon W-J., Lee W. J. and Park S-Y. 2007. Antioxidative and antimicrobial activities of *Sargassum muticum* extracts. Journal of Korean Society for. Food Science and Nutrition, 36, 663-669. https://doi.org/10.3746/jkfn.2007.36.6.663

Kokate, C.K. 1991. Practical Pharmacognosy. 3rd ed. Nirali Prakashan, New Delhi. 120 pages.

Lee, S.G. and Kang, H. 2015. Neuroprotective effect of *Sargassum thunbergii* (Mertens ex Roth) kuntze in activated murine microglial cells. Tropical Journal of Pharmacology Research, 14(2), 235-240. https://doi.org/10.4314/tjpr.v14i2.7

Liu, L., Heinrich, M., Myres, S. and Dworjanyn, S.A. 2012. Towards a better understanding of medicinal uses of the brown seaweed *Sargassum* in traditional chinese medicine: a phytochemical and pharmacological review. Journal of Ethnopharmacology, 142(3), 595. https://doi.org/10.1016/j.jep.2012.05.046

Mansuya, P., Aruna, P., Sridhar, S., Suresh, K.J. and Babu, S. 2010. Antibacterial activity and qualitative phytochemical analysis of selected seaweeds from Gulf of Mannar Region. Journal of Experimental Science, 1(8), 23-26.

Mehdinezhad, N., Ghannadi, A. and Yegdaneh, A. 2016. Phytochemical and biological evaluation of some *Sargassum* species from Persian Gulf. Research in Pharmaceutical Sciences, 11(3), 243-249.

Nagai, T. and Yukimoto, T. 2003. Preparation and functional properties of beverages from sea algae. Food Chemistry, 81, 327-332. https://doi.org/10.1016/S0308-8146(02)00426-0

Nazarudin, M.F., Alias, N.H., Balakrishnan, S., Wan Hasnan, W.N.I., Noor Mazli, N.A.I., Ahmad, M.I., Md Yasin, I.S., Isha, A. and Aliyu-Paiko, M. 2021. Chemical, nutrient and physicochemical properties of brown seaweed, *Sargassum polycystum* C. Agardh (Phaeophyceae) collected from Port Dickson, Peninsular Malaysia. Molecules, 26(17), 5216. https://doi.org/10.3390/molecules26175216

Perez, M.J., Falque, E. and Domínguez, H. 2016. Antimicrobial action of compounds from marine seaweed. Marine Drugs, 14(3), 52. https://doi.org/10.3390/md14030052

Ponnuchamy, K., Senthamilselvi, S. and Munisamy, G. 2013. GC-MS profiling and antibacterial activity of *Sargassum tenerrimum*. Journal of Pharmacy Research, 6, 88-92. https://doi.org/10.1016/j.jopr.2012.11.019.

Raghavendran, H.B., Sathivel, A. and Devaki, T. 2006. Defensive nature of *Sargassum polycystum* (Brown alga) against acetaminophen-induced toxic hepatitis in rats: role of drug metabolizing microsomal enzyme system, tumor necrosis factor-alpha and fate of liver cell structural

integrity. World Journal of Gastroenterology, 12(24), 3829-3834. https://doi.org/10.3748/wjg.v12.i24.3829

Rajasulochana, P., Dhamotharan, R., Krishnamoorthy, P. and Murugesan, S. 2009. Antibacterial activity of the extracts of marine red and brown algae. Journal of American Science, 5(3), 20-25.

Rajivgandhi, G.N., Kanisha, C.C., Ramachandran, G., Manoharan, N., Mothana, R.A., Siddiqui, N.A., Al-Rehaily, A.J., Ullah, R. and Almarfadi, O.M. 2021. Phytochemical screening and anti-oxidant activity of *Sargassum wightii* enhances the antibacterial activity against *Pseudomonas aeruginosa*. Saudi Journal of Biological Sciences, 28(3), 1763-1769. https://doi.org/10.1016/j.sjbs.2020.12.018

Sanger, G., Wonggo, D., Lita, Y. and Dotulong, V. 2022. Pigments constituents, phenolic content and antioxidant activity of brown seaweed *Sargassum* sp. IOP Conference Series: Earth and Environmental Science, 1033. 012057. https://doi.org/10.1088/1755-1315/1033/1/012057,

Siriwardhana, N., Lee, K.W., Kim, S.H., Ha, J.W. and Jeon, Y.J. 2003. Antioxidant activity of *Hizikia fusiformis* on reactive oxygen species scavenging and lipid peroxidation inhibition. Food Science and Technology International, 9(5), 339-346. https://doi.org/10.1177/1082013203039014

Subramaniam, D., Menon, T., Elizabeth, H.L. and Swaminathan, S. 2014. Anti-HIV-1 activity of *Sargassum swartzii* a marine brown alga. BMC Infectious Diseases, 14(3), E43. https://doi.org/10.1186/1471-2334-14-S3-E43

Sumandiarsa, I., Hamida, N., Santoso, J. and Tarman, K. 2022. Antioxidant activities from different parts of *Sargassum polycystum* thalli through ultrasound-assisted extraction (UAE) method. Omni-Akuatika - Journal of Fisheries and Marine Sciences, 18(2), 79-89. https://ojs.omniakuatika.net/index.php/joa/article/view/907/392

Wijesekara, I. and Kim, S.K. 2010. Angiotensin-I-converting enzyme (ACE) inhibitors from marine resources: Prospects in the pharmaceutical industry. Marine Drugs, 8, 1080-1093. https://doi.org/10.3390/md8041080

Woonnoi, W., Moolsap, F., Tanasawet, S., Khumpirapang, N., Aenglong, C. and Sukketsiri, W. 2023. In vitro antioxidant and wound healing activity of *Sargassum polycystum* hydroethanolic extract in fibroblasts and keratinocytes. Asian Pacific Journal of Tropical Biomedicine, 13(5), 222-232. https://doi.org/10.4103/2221-1691.377409

Yangthong, M., Towatana, N.H. and Phromkunthong, W. 2009. Antioxidant activities of four edible seaweeds from the southern coast of Thailand. Plant Foods for Human Nutrition, 64(3), 218-223. https://doi.org/10.1007/s11130-009-0127-y

Zhang, W.W., Duan, X.J., Huang, H.L., Zhang, Y. and Wang, B.G. 2007. Evaluation of 28 marine algae from the Qingdao coast for antioxidative capacity and determination of antioxidant efficiency and total phenolic content of fractions and sub fractions derived from *Symphocladia latiuscula*. Journal of Applied Phycology, 19(2), 976-108. https://doi.org/10.1007/s10811-006-9115-x

Received: 14th March 2024
Accepted: 12th May 2024