Eco. Env. & Cons. 28 (2) : 2022; pp. (1055-1060)
Copyright@ EM International
ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2022.v28i02.074

Study on biochemical analysis, antioxidant assay and GCMS analysis of *Saccharina japonica*

Reshmi Mohamed Ali[1], O.S. Aysha[2] and S. Shamini[3]

[1]Department of Microbiology, Bhaktavatsalam Memorial College for Women, Korattur, Chennai, Tamilnadu, India
[2]PG and Research Department of Microbiology, Mohamed Sathak College of Arts and Science, Sholinganallur, Chennai, Tamilnadu, India
[3]Organogenik Biotech Pvt. Limited, Chennai, Tamilnadu, India

(Received 19 January, 2022; Accepted 26b February, 2022)

ABSTRACT

Marine algae are one of the largest sources for biogenic compounds. They produce a wide variety of chemically active metabolites. They are found to possess significant antioxidant, and phytochemical activities. Macroalgae provide a great variety of metabolites and natural bioactive compounds with antimicrobial activity, such as polysaccharides, polyunsaturated fatty acids, phlorotannins other phenolic compounds and carotenoids. In the present study the phytochemical, α–amylase activity, GCMS and Antioxidant assays such as ABTS assay and DPPH assays were carried out for *Saccharina japonica* using two different solvents, petroleum ether and ethyl acetate. For antioxidant activity, DPPH radical assay and ABTS radical cation scavenging assay were carried out.

Key words: *Saccharina japonica, Phytochemical screening, ABTS, DPPH, GCMS.*

Introduction

Marine algae having a numerous commercial applications in various fields. Marine algae produce sixty trace elements in a concentration much higher than terrestrial plants. Macroscopic marine algae also contain protein, iodine, bromine, vitamins and substances of stimulatory and antibiotic nature.

Seaweeds are microscopic and primarily macroscopic, multicellular, polyphyletic and photosynthetic marine algae and usually grow on the seabed between the coastal region's high tide and low tide zones. Marine algae or seaweeds mostly grow in the rocky part of the littoral zone of the ocean, where usually 0.01% light penetration can assist the photosynthesis of the seaweeds. The maximum significant application of seaweed is found in food industries, cosmetic industries, an industry of phycocolloid or hydrocolloid, biofuel production, wastewater treatment, pharmaceutical industry and in fertilizers. In general, seaweed contains different secondary metabolites, for example, tannins, saponins, phenols, and flavonoids in varying concentration. In addition, seaweeds have a wide range of bioactive compounds that have antibacterial, anti-Alzheimer, anti-inflammatory, antifungal, anti-hyperglycemic, anti-aging activities, and preferable antioxidant properties.

Saccharina japonica is one of the most important marine brown seaweed having lot of pharmacological applications. ***Saccharina japonica*** is a commercially important marine brown algae which grows

1. Assistant Professor, 2. Vice Principal and Head, 3. Director
Corresponding author's email: reshtas@gmail.com

as a single blade (reaching 10 meters in length) with a short stipe. *S. japonica* contains many bioactive substances that are valuable for cosmetics, foods and health.

Materials and Methods

Collection and Processing of *Saccharina japonica*

Algal samples were collected from the red gate end of the Hare Island, Tuticorin, Tamil Nadu during the early morning low tide period. The Contaminated free samples were cut into small fragments and shade dried. The dried samples were granulated or powdered using a blender and sieved to get uniform particles by using Sieve. No.60. The final uniform powder was used for the extraction of active constituents of the algal sample.

Preparation of *Saccharina japonica* extract

The coarse powder (100 g) of *Saccharina japonica* was extracted consecutively with 250 ml of acetate and petroleum ether in soxhlet apparatus for 24 hours.

Phytochemical screening of Algal Sample

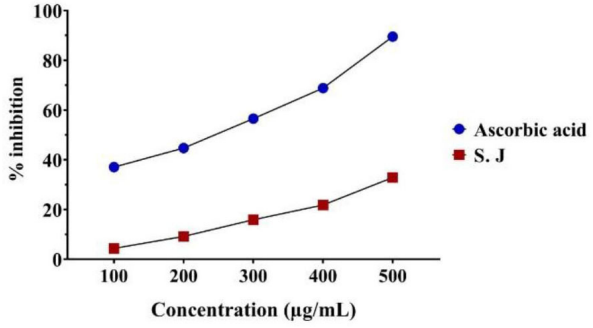
The Petroleum ether and ethyl acetate extract of *Saccharina japonica* was subjected to preliminary phytochemical screening using standard methods .Both the extracts were screened for different classes of phyto-constituents such as alkaloids, steroids, terpenoids, flavonoids and phenolic compounds using specific standard reagents.

Antioxidant assay -ABTS

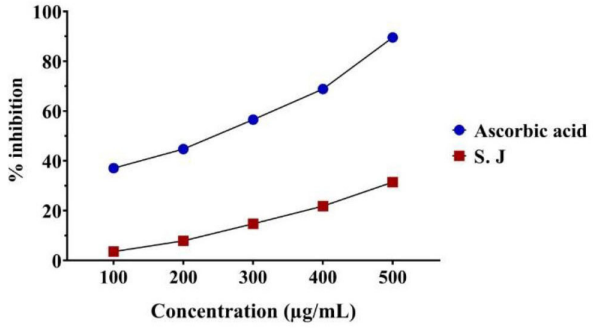
The ABTS (7 mM, 25 ml in deionized water) stock solution was prepared with potassium persulfate (K2S2O8) (140 mM, 440 µl). Different concentration of test samples and standard (Ascorbic acid) were mixed with the ABTS working solution (2.0 ml) and the reaction mixture was allowed to stand at room temperature for 20 min; then, the Abs was measured using an ultraviolet-visible spectrophotometer at 734 nm. The radical scavenging activity was given as ABTS radical scavenging effect was calculated by the equation:

ABTS radical scavenging effect (%) = [(A0 - A1)/ A0] ×100, respectively.

Fig. 1. ABTS of *Saccharina japonica* sample using different solvents



Graph 1. Petroleum ether



Graph 2. Ethyl acetate

The IC50 values of the given sample

Solvent	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml
Petroleum ether	364.51	282.92	323.93	267.80	419.07
Ethyl acetate	61.22	111.96	193.46	177.48	248.13

The standard drug (**Rutin**) was **4.87** µg/ml

Solvent	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml
Petroleum ether	156.71	162.41	237.75	119.402	165.09
Ethyl acetate	156.87	179.48	151.92	176.65	179.60

The standard drug (Ascorbic acid) was 56.83 µg/mL

Where, A0 is the control, A1 is the test

Antioxidant assay-DPPH radical scavenging assay

DPPH radical scavenging assay of *Saccharina japonica* was performed according to modified method described by Perumal *et al*., 2018. In brief, 0.135 mM DPPH was prepared in methanol. Different concentration of extract (100, 200, 300, 400 and 500 µg/ml) was mixed with 2.5 ml of DPPH solution. The reaction mixture was vortexed thoroughly and kept at room temperature for 30 min. The Absorbance of the mixture was measured at 517 nm. Rutin was used as the reference standard. The ability of algal extract to scavenge DPPH radical and control was calculated from the following formula: respectively.

% DPPH inhibition = [(OD of control - OD of test)/(OD of control)] × 100

GCMS Analysis

GC–MS analysis of *Saccharina japonica*, extract was carried out using different solvents- Petroleum ether and ethyl acetate, (GC/MS –series QP2010, Shimadzu, Tokya, Japan) using Thermal Desorption (TD) system. The contents of phytochemicals present in the test samples were identified based on comparison of their retention time (min), peak area, peak height and mass spectral patterns with those spectral database of authentic compounds stored in the National Institute of Standards and Technology (NIST) library.

Results and Discussion

Phytochemical Screening

The Phytochemical analysis of petroleum ether extracts of *Saccharina japonica* revealed the presence of Alkaloids, flavonoids, sugar, Tannins and amino acids and in the case of ethyl acetate extract showed the presence of alkaloids, flavonoids, steroids, terpenoids, phenols, sugars and glycosides. Phytochemical screening of extract sample – *Saccharina japonica* is shown in Table 1.

Table 1. Phytochemical screening of extract sample – *Saccharina japonica*

Solvent	Petroleum ether	Ethyl acetate
Alkaloids	+	+
Flavonoids	+	+
Steroids	-	+
Terpenoids	-	+
Sugar	+	+
Phenols	-	+
Tannins	+	+
Glycoside	-	+
Amino acid	+	-
Saponin	-	-

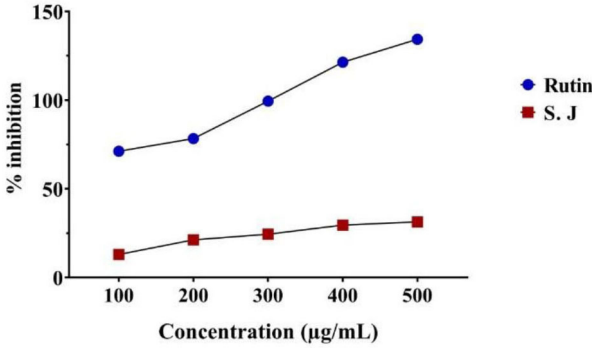
The GC-MS analysis report

GC-MS analysis of Petroleum ether and ethyl acetate extracts of *Saccharina japonica* showed the presence of 35 compounds each as shown in Table 4 and 5 and also represented in the chromatogram below.

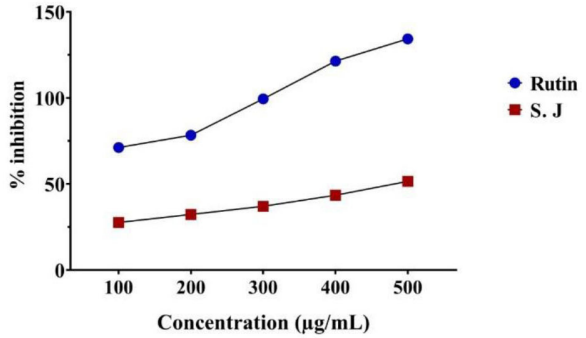
Acknowledgements

We would like to thank Mohamed Sathak College of Arts and Science, Shollinganallur, Chennai and Organogenick Pvt. Limited.

Fig. 2. ABTS of *Saccharina japonica* sample using different solvents



Graph 3. Petroleum ether



Graph 4. Ethyl acetate

Table 2. ABTS of *Saccharina japonica* sample using different solvents

Con. (ml)	Ascorbic acid	(Petroleum ether)	(Ethyl acetate)
100	37.07±1.45	4.40±1.34	3.58±0.05
200	44.76±0.05	9.20±2.78	7.92±0.23
300	56.58±2.34	15.92±1.23	14.74±2.02
400	68.87±0.23	21.85±0.56	21.84±0.19
500	89.54±0.65	32.89±0.24	31.43±0.38

Table 3. DPPH of *Saccharina japonica* sample using different solvent

Concen. (ml)	Rutin	(Petroleum ether)	(Ethyl acetate)
100	71.18±0.02	13.02±0.13	27.64±0.03
200	78.32±0.19	21.24±0.23	32.33±0.45
300	99.45±0.38	24.43±0.65	37.05±0.39
400	121.41±2.02	29.58±0.45	43.47±0.48
500	134.33±1.26	31.41±0.05	51.55±0.22

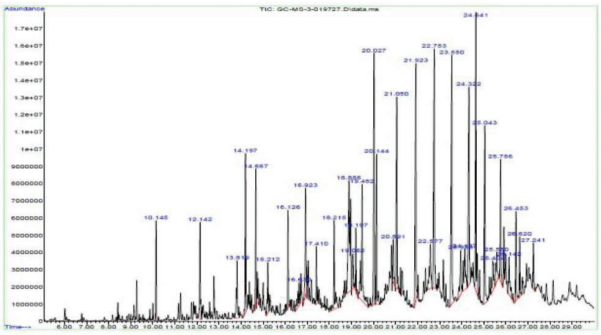
Table 4. The GC-MS of Petroleum ether extract of *Saccharina japonica*

S. No.	RT	Compound name	Molecular formula	Molecular weight	Peak area
1.	10.145	Benzene, 1,3-bis(1,1-dimethylethyl)-	$C_{14}H_{22}$	190.3245	1.60
2.	12.142	2-Bromo dodecane	$C_{12}H_{25}Br$	249.231	2.07
3.	13.819	Hexadecane	$C_{16}H_{34}$	226.4412	1.67
4.	14.197	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206.3239	3.01
5.	14.667	Eicosane	$C_{20}H_{42}$	282.5475	2.15
6.	15.212	Hexacosane	$C_{26}H_{54}$	366.7070	1.17
7.	16.126	Octadecane	$C_{18}H_{38}$	254.4943	1.59
8.	16.630	Octadecane	$C_{18}H_{38}$	254.4943	1.02
9.	16.923	2-Bromo dodecane	$C_{12}H_{25}Br$	249.231	2.64
10.	17.410	Heptacosane	$C_{27}H_{56}$	380.7335	1.25
11.	18.215	Eicosane	$C_{20}H_{42}$	282.5475	2.30
12.	18.886	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241	6.44
13.	19.062	Octadecane	$C_{18}H_{38}$	254.4943	1.23
14.	19.197	Heneicosane	$C_{21}H_{44}$	296.5741	1.08
15.	19.482	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6, 9-diene-2,8-dione	$C_{17}H_{24}O_3$	276.3707	4.14
16.	20.027	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	$C_{16}H_{22}O_4$	278.3435	5.32
17.	20.144	1-Iodo-2-methylundecane	$C_{12}H_{25}I$	296.23	3.30
18.	20.891	Heptacosane	$C_{27}H_{56}$	380.7335	1.13
19.	21.050	Heptadecane	$C_{17}H_{36}$	240.4677	4.52
20.	21.923	Tetracosane	$C_{24}H_{50}$	338.6538	6.43
21.	22.577	Octadecane	$C_{18}H_{38}$	254.4943	1.81
22.	22.753	Pentacosane	$C_{25}H_{52}$	352.6804	6.96
23.	23.550	Hexacosane	$C_{26}H_{54}$	366.7070	7.08
24.	23.953	Tetracosane	$C_{24}H_{50}$	338.6538	1.44
25.	24.137	Octadecane	$C_{18}H_{38}$	254.4943	2.03
26.	24.322	Heptacosane	$C_{27}H_{56}$	380.7335	5.51
27.	24.641	Phthalic acid, di(2-propylpentyl) ester	$C_{24}H_{38}O_4$	390.5561	6.33
28.	25.043	Tetracosane	$C_{24}H_{50}$	338.6538	4.29
29.	25.404	Octacosane	$C_{28}H_{58}$	394.7601	1.32
30.	25.580	Octacosane	$C_{28}H_{58}$	394.7601	1.16
31.	25.756	Tetracosane	$C_{24}H_{50}$	338.6538	2.54
32.	26.142	Docosane	$C_{22}H_{46}$	310.6006	1.45
33.	26.453	Tetracosane	$C_{24}H_{50}$	338.6538	1.87
34.	26.620	13-Docosenamide, (Z)-	$C_{22}H_{43}NO$	337.5829	1.09
35.	27.241	Tetracosane	$C_{24}H_{50}$	338.6538	1.10

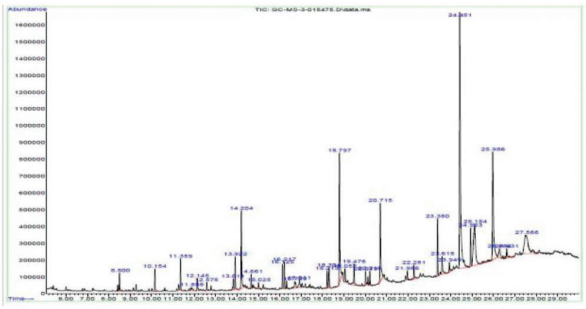
Table 5. The GC-MS of Ethyl acetate extract of *Saccharina japonica*

S. No .	RT	Compound name	Molecular formula	Molecular weight	Peak area
1.	8.500	1-Decene	$C_{10}H_{20}$	140.2658	0.88
2.	10.154	Benzene, 1,3-bis(1,1-dimethylethyl)-	$C_{14}H_{22}$	190.3245	1.04
3.	11.359	1-Tetradecene	$C_{14}H_{28}$	196.3721	1.37
4.	11.888	Nonadecane	$C_{19}H_{40}$	268.5209	0.50
5.	12.145	2-Bromo dodecane	$C_{12}H_{25}Br$	249.231	0.79
6.	12.578	Tritetracontane	$C_{43}H_{88}$	605.1588	0.45
7.	13.811	Hexadecane	$C_{16}H_{34}$	226.4412	0.72
8.	13.922	5-Octadecene, (E)-	$C_{18}H_{36}$	252.4784	1.58
9.	14.204	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206.3239	4.88
10.	14.661	Hexadecane	$C_{16}H_{34}$	226.4412	0.68
11.	15.025	Octadecane	$C_{18}H_{38}$	254.4943	0.48
12.	16.125	Octadecane	$C_{18}H_{38}$	254.4943	1.08
13.	16.217	Pentafluoropropionic acid, pentadecyl ester	$C_{18}H_{31}F_5O_2$	374.4296	1.33
14.	16.709	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.3709	0.90
15.	16.911	Octadecane, 1-iodo-	$C_{18}H_{37}I$	380.3909	0.82
16.	18.213	Eicosane	$C_{20}H_{42}$	282.5475	0.74
17.	18.304	5-Eicosene, (E)-	$C_{20}H_{40}$	280.5316	1.16
18.	18.797	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241	8.14
19.	19.052	Oxalic acid, 6-ethyloct-3-yl isobutyl ester	$C_{16}H_{30}O_4$	286.41	0.98
20.	19.476	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6, 9-diene-2,8-dione	$C_{17}H_{24}O_3$	276.4	1.71
21.	20.019	Phthalic acid, nonyl pentadecyl ester	$C_{32}H_{54}O_4$	502.8	0.84
22.	20.216	13-Methyl-Z-14-nonacosene	$C_{30}H_{60}$	420.8	0.74
23.	20.715	Octadecanoic acid	$C_{18}H_{36}O_2$	284.4772	5.50
24.	21.966	1-Nonadecene	$C_{19}H_{38}$	266.5050	0.44
25.	22.281	Dodecane, 1,1'-oxybis-	$C_{24}H_{50}O$	354.6532	1.10
26.	23.380	1H-Indole, 2-methyl-	C_9H_9N	131.1745	2.69
27.	23.618	Behenyl chloride	$C_{22}H_{45}Cl$	345.046	1.60
28.	23.949	Hexadecane, 2,6,10,14-tetramethyl-	$C_{20}H_{42}$	282.5475	0.74
29.	24.451	Glycerol 1-palmitate	$C_{19}H_{38}O_4$	330.5026	24.45
30.	24.923	Naphthalene, 1,2,3,4-tetrahydro-1-methoxy-	$C_{11}H_{14}$	146.2289	1.89
31.	25.154	Cobalt, indenyl-(.eta.-4-1,2,3, 4-tetraphenylcyclohexa-1,3-diene,	$C_{39}H_{31}Co^{-9}$	558.6	9.33
32.	25.986	Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$	358.5558	10.92
33.	26.284	Methoxyacetic acid, 4-tetradecyl ester	$C_{17}H_{34}O_3$	286.4	1.39
34.	26.631	Eicosane	$C_{20}H_{42}$	282.5475	0.84
35.	27.566	Trisilane, 1,1,1,3,3,3-hexafluoro-	$C_{10}H_{27}F_3Si_4$	316.66	7.30

Figure 3. The GC-MS of of *Saccharina japonica*



Graph 3. Petroleum ether



Graph 4. Ethyl acetate

Conflict of Interest

The authors declare that there is no conflict of interest.

References

Artan, M., Li, Y., Karadeniz, F., Lee, S.H., Kim, M.M. and Kim, S.K. 2008. Anti-HIV-1 activity of phloroglucinol derivative, 6,62 -bieckol, from Ecklonia cava. *Bioorg Med Chem.* 16 : 7921–7926.

Güven, K.C., Percot, A. and Sezik, E. 2010. Alkaloids in marine algae. *Mar Drugs*. 8: 269–284.

Heo, S.J., Hwang, J.Y. and Choi, J.I. 2009. Diphlorethohydroxycarmalol isolated from Ishige okamurae, a brown algae, a potent β-glucosidase and β-amylase inhibitor, alleviates postprandial hyperglycemia in diabetic mice. *Eur J Pharmacol.* 615: 252–256.

Heo, S.J., Ko, S.C. and Kang, S.M. 2010. Inhibitory effect of diphlorethohydroxycarmalol on melanogenesis and its protective effect against UV-B radiation-induced cell damage. *Food Chem Toxicol***.** 48 : 1355–1361.

Jung, H.A., Hyun, S.K., Kim, H.R. and Choi J.S. 2006. Angiotensin-converting enzyme I inhibitory activity of phlorotannins from Ecklonia stolonifera. *Fish Sci* 72: 1292–1299.

Klejdus, B., Lojková, L., Plaza, M., Snóblová, M., St rbová D. 2010. Hyphenated technique for the extraction and determination of isoflavones in algae: Ultrasound-assisted supercritical fluid extraction followed by fast chromatography with tandem mass spectrometry. *J Chromatogr A.* 1217 : 7956–7965.

Kong, C.S., Kim, J.A., Yoon, N.Y. and Kim, S.K. 2009. Induction of apoptosis by phloroglucinol derivative from *Ecklonia cava* in MCF-7 human breast cancer cells. *Food Chem Toxicol.* 47 : 1653–1658.

Kim, S.Y., Kim, E.A. and Kang, M.C. 2014. Polyphenol-rich fraction from *Ecklonia cava* (a brown alga) processing by-product reduces LPS-induced inflammation *in vitro* and *in vivo* in a zebrafish model. *Algae.* 29: 165-174.

Kim, A.R., Shin, T.S. and Lee, M.S. 2009. Isolation and identification of phlorotannins from *Ecklonia stolonifera* with antioxidant and anti-inflammatory properties. *J Agric Food Chem.* 57 : 3483–3489.

Kang, H.S., Chung, H.Y., Jung, J.H., Son, B.W. and Choi, J.S. 2003. A new phlorotannin from the brown alga *Ecklonia stolonifera*. *Chem Pharm Bull*. 51: 1012–1014.

Kellogg, J., Grace, M.H. and Lila, M.A. 2014. Phlorotannins from Alaskan seaweed inhibit carbolytic enzyme activity. *Mar Drugs.* 12 : 5277-5294.

Ko, S.C., Jung, W.K. and Kang, S.M. 2015. Angiotensin I-converting enzyme (ACE) inhibition and nitric oxide (NO)-mediated antihypertensive effect of octaphlorethol A isolated from Ishige sinicola: *In vitro* molecular mechanism and *in vivo* SHR model. *J Funct Foods.* 18 : 289–299.

Lee, J.H., Han, J.W. and Ko, J.Y. 2016. Protective effect of a freshwater alga, *Spirogyra* sp., against lipid peroxidation in vivo zebrafish and purification of antioxidative compounds using preparative centrifugal partition chromatography. *J Appl Phycol.* 28: 181-189.

Lee, S.H., Kang, N. and Kim, E.A. 2014. Antidiabetogenic and antioxidative effects of octaphlorethol a isolated from the brown algae Ishige foliacea in streptozotocin-induced diabetic mice. *Food Sci Biotechnol.* 23 : 1261–1266.

Mancini-Filho, J., Novoa, A.V. and González, A.E. 2009. Free phenolic acids from the seaweed Halimeda monile with antioxidant effect protecting against liver injury. *Z Naturforsch C* 64 : 657-663.

Onofrejová, L., Vaší´ková, J. and Klejdus, B. 2010. Bioactive phenols in algae: The application of pressurized-liquid and solid-phase extraction techniques. *J Pharm Biomed Anal.* 51 : 464-470.

Santoso, J., Yoshie, Y. and Suzuki, T. 2002.The distribution and profile of nutrients and catechins of some Indonesian seaweeds. *Fish Sci.* 68 : 1647-1648.

Shibata, T., Yamaguchi, K., Nagayama, K., Kawaguchi, S. and Nakamura, T. 2002. Inhibitory activity of brown algal phlorotannins against glycosidases from the viscera of the turban shell *Turbo cornutus*. *Eur J Phycol.* 37: 493-500.

Sabeena Farvin, K.H. and Jacobsen, C. 2013. Phenolic compounds and antioxidant activities of selected species of seaweeds from Danish coast. *Food Chem.* 138 : 1670–1681.

Sugiura, Y., Matsuda, K. and Yamada, Y. 2006. Isolation of a new anti-allergic phlorotannin, phlorofucofuroeckol-B, from an edible brown alga, Eisenia arborea. *Biosci Biotechnol Biochem.* 70: 2807-2811.

Thomas, N.V. and Kim, S.K. 2011. Potential pharmacological applications of polyphenolic derivatives from marine brown algae. *Environ Toxicol Pharmacol.* 32 : 325-335.

Wijesekara, I., Pangestuti, R. and Kim, S.K. 2011. Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae. *Carbohydr Polym.* 84: 14–21.

Yoshie, Y., Wang, W., Petillo, D. and Suzuki, T. 2000. Distribution of catechins in Japanese seaweeds. *Fish Sci.* 66 : 998–1000.

Yoon, N.Y., Chung, H.Y., Kim, H.R. and Choi, J.S. 2008. Acetyl- and butyrylcholinesterase inhibitory activities of sterols and phlorotannins from *Ecklonia stolonifera*. *Fish Sci.* 74: 200-207.

Zeng, L.M., Wang, C.J. and Su, J.Y. 2001. Flavonoids from the red alga *Acanthophora spicifera*. *Chin J Chem* 19: 1097-1100.