STUDY OF POLYPHENOLS IN BROWN ALGAE

TAN KAI YEE

THIS DISSERTATION IS SUBMITTED TO FULFILL THE REQUIREMENT FOR THE DEGREE OF BACHELOR OF SCIENCE WITH HONOURS

MARINE SCIENCE PROGRAMME
SCHOOL OF SCIENCE AND TECHNOLOGY
UNIVERSITI MALAYSIA SABAH

MARCH 2008

PUMS99:1

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS@

JUDUL: STUDY OF POLYPHENOLS IN BROWN ALGAE

IJAZAH: DEGREE OF BACHELOR OF SCIENCE WITH HONOURS

SAYA TAN KAI YEE (HURUF BESAR) SESI PENGAJIAN: 2005

mengaku membenarkan tesis (LPSM/Sarjana/Doktor Falsafah) ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:-

1. Tesis adalah hakmilik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institutsi pengajian tinggi.
4. Sila tandakan (/)

☐ SULIT (Mengandungi maklumat yang berdarjah keselamatan atau Kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972)

☐ TERHAD (Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)

☐ TIDAK TERHAD

Disahkan Oleh

(TANDATANGAN PENULIS) (TANDATANGAN PUSTAKAWAN)

Alamat Tetap: 78, JALAN 8, TAMAN SENTOSA, 34000 TAIPING, PERAK

Nama Penyelia

Tarikh: 30 APRIL 2008 Tarikh:

CATATAN:- *Potong yang tidak berkenaan.

**Jika tesis ini SULIT atau TERHAD, sila lampirkan surat daripada pihak berkuasa /organisasi berkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai SULIT dan TERHAD.

@Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana secara penyelidikan atau disertai bagi pengajian secara kerja kursus dan Laporan Projek Sarjana Muda (LPSM).

DECLARATION

I hereby declare that this dissertation contains my original research work. Sources of findings reviewed herein have been duly acknowledged.

27 March 2008

TAN KAI YEE

HS2005-2406

VERIFIED BY

Signature

1. SUPERVISOR
(DR. CHARLES S. VAIRAPPAN)

2. CO-SUPERVISOR
(DR. PUSHPALATHA M. PALANIAPPAN)

3. EXAMINER 1
(DR. HOW SIEW ENG)

4. EXAMINER 2
(DR. SUJJAT AL-AZAD)

5. DEAN
(SUPT/KS. ASSOC. PROF. DR. SHARIFF A.K. OMANG)

ACKNOWLEDGEMENTS

I am grateful to Dr. Charles S. Vairappan for giving me the opportunity of work under his supervision, and explore the fascinating world of natural product chemistry. I am thankful for his guidance and advice. I would also like to thank my co-supervisor, Dr. Pushpalatha M. Palaniappan for her advice and care.

I would like to acknowledge Borneo Marine Research Institute for providing boat facilities and diving equipments for my project's sampling. I would also like to acknowledge the Natural Product Chemistry Laboratory of Institute for Tropical Biology and Conservation for providing the facilities essential for the completion of this project.

I also owe a deep gratitude to the seniors in the Natural Product Chemistry Laboratory: Tan Kai Lee, Sangeetha Priya Anangdan, Kishneth Palaniveloo, and Jacqueline Vincent for their guidance and support. I thank my lab-mate, Ruth Yap for her help in the laboratory.

I am grateful to my parents for their love, encouragement and inspiration throughout the 3 years study and in this project. Last but not least, I wish to express my appreciation to those who had played a part in helping me to succeed in this study which I failed to mention.

Please accept my heartfelt gratitude and I wish all of you be well, successful and happy always.

Thank you.

ABSTRACT

Marine brown algae are known to produce various secondary metabolites such as terpenes, steroids, and polyphenols. It is also known that phloroglucinol-based polyphenols (phlorotannins) are found only in brown algae. Present investigation delves upon the chemical profiling, phenolic quantification and bioactive potential assessment (antibacterial and antioxidant activity) in four species of brown algae (*Sargassum* sp., *Padina* sp., *Turbinaria* sp., and *Dictyota* sp.). Results from chemical profiling revealed all brown algae contain phlorotannins and various non-polar metabolites. *Dictyota* sp. had more compounds compared to the other three species of brown algae. Phenolic content for $CHCl_3$ extracts of four species of brown algae were recorded at the range of 19-37 μg P per gram of partially dried seaweed; EtOAc extracts was recorded at the range of 27-40 μg P per gram of partially dried seaweed. Phenolic content was highest in *Dictyota* sp. < *Padina* sp. < *Turbinaria* sp. < *Sargassum* sp. Results obtained by DPPH assay was comparable with FRAP assay. Percentage of scavenging from DPPH assay indicated highest antioxidant potential in *Sargassum* sp. < *Padina* sp. < *Turbinaria* sp. < *Dictyota* sp. while FRAP assay indicated *Dictyota* sp. < *Sargassum* sp. < *Turbinaria* sp. < *Padina* sp. Among the four species of brown algae, the range of percentage radical scavenging from DPPH assay was 12-35% and the range of FRAP was 4.8- 9.5 mg AA/g. All four species possessed antibacterial properties when tested with environmental and pathogenic microbes most notably *Sargassum* sp. and *Dictyota* sp.

ABSTRAK

Alga perang marin diketahui menghasilkan pelbagai jenis sebatian metabolit sekunder seperti terpenes, steroids, dan polifenols. Juga diketahui bahawa phlorotannins (poliphenols berdasarkan phloroglucinol) hanya ditemui dalam alga perang. Kajian ini menyelidik profil kimia, perhitungan isi kandungan polifenols, dan penilaian potensi bioactive (antibacteria dan antioxidant) dalam empat spesies alga perang. (*Sargassum* sp., *Padina* sp., *Turbinaria* sp., and *Dictyota* sp.). Keputusan profil kimia menunjukkan algae perang mengandungi phlorotannins and pelbagai jenis metabolit tidak berkutub. *Dictyota* sp. mengandungi jenis sebatian yang lebih tinggi berbanding dengan tiga spesies yang lain. Isi kandungan fenolic dalam ekstrak $CHCl_3$ mencatatkan julat antara 19-37 mg P per gram alga; ekstrak EtOAc mencatatkan julat antara 27-40 mg P per gram alga. Isi kandungan fenolic adalah paling tinggi dalam *Dictyota* sp. < *Padina* sp. < *Turbinaria* sp. < *Sargassum* sp. Keputasan ujian antioxidant menggunakan ujian DPPH dan ujian FRAP menunjukkan perbezaan. Ujian DPPH menunjukkan potenti antioxidant paling tinggi dalam *Sargassum* sp. < *Padina* sp. < *Turbinaria* sp. < *Dictyota* sp. manakala ujian FRAP menunjukkan *Dictyota* sp. < *Sargassum* sp. < *Turbinaria* sp. < *Padina* sp. Keempat-empat spesies alga perang menunjukkan aktiviti antibacterial terhadap bakteria persekitran dan mikrob pathogenik yang diuji terutamanya *Sargassum* sp.dan *Dictyota* sp.

CONTENTS

LIST OF FIGURES

LIST OF TABLE

Table No.

LIST OF PHOTOS

Photo No.

LIST OF ABBREVIATIONS & SYMBOLS

MeOH	methanol
EtOH	ethanol
EtOAc	ethyl acetate
H_2O	water
HCl	hydrochloric acid
H_2SO_4	sulphuric acid
NaCl	sodium chloride
Na_2SO_4	anhydrous sodium sulphate
$CHCl_3$	chloroform
CH_3COOH	acetic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
TLC	thin layer chromatography
HPLC	high performance liquid chromatography
UV	ultraviolet
R_f	mobility relative to front
v/v	volume to volume
°C	degree Celsius
nm	nanometre
Da	Dalton
mg	miligram
mL	mililitre

CHAPTER 1

INTRODUCTION

1.1 Seaweeds

Seaweeds, also known as macroalgae show a wide range of growth forms and structures. However, there are several unifying features among seaweeds. Seaweeds lack true leaves, stems, and roots. The body of seaweeds is known as the thallus, whether it is filaments, thin leafy sheets, crusty cushions, or giant kelps. The leafy, flattened portions of the thallus are known as blades. This increases the surface area and is the main photosynthetic regions. Pneumatocysts are gas filled bladders aiding in buoyancy. Some species possess stipes, from which blades originate giving structural support like a stem. The holdfast, which is a structure looks like roots, holds the thallus to the substrates (Lee, 1999).

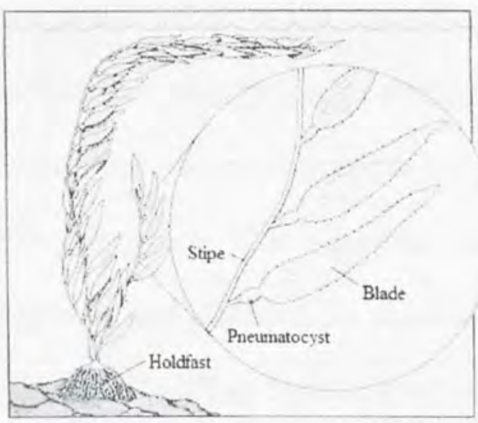


Figure 1.1 General structure of seaweed (Source:Castro and Huber, 2003)

Macroalgae consist of three groups of multicellular algae: Rhodophyta (red algae), Chlorophyta (green algae), and Phaeophyta (brown algae). Rhodophyta consists the most diverse species of marine algae compared to marine green and brown algae. They are characterized by having red pigments called phycobilins. Chlorophyta, commonly known as green algae are found mostly in freshwater and terrestrial environment. However, there are also marine species which dominated coastal waters. They can be easily identified by its green colouration as results of the chlorophyll pigments. Phaeophyta or the brown algae has a characteristic olive green to brownish colour. This is caused by the preponderance of yellow-brown pigments, the fucoxanthin over chlorophyll. Almost all species are marine, consists of some of the largest and most complex seaweeds, like the kelps.

Seaweeds have various economic importances. The obvious use is as food source. People from all over the world had discovered many edible algae. The most popular is "Nori" which is dried seaweed used in sushi wrapping and various Japanese cuisine. The farming or mariculture of seaweed is a big business in China, Japan, Korea and other nations (Lee, 1999). Besides as a food source, a few gelatinous chemicals called phycocolloids are extracted for manufacturing various products. Algin, which is a type of phycocolloids are use extensively in manufacturing dairy products. It is used as stabilizer and emulsifier in ice cream, cheese and cake toppings. It is also used in manufacturing pharmaceutical products. Carrageenan generally extracted from *Eucheuma* species can also serve the purpose of algin. Another phycocolloids, which is agar, is typically used for pharmaceutical and microbiology purposes. It is used to make culture medium for microbial culturing.

Besides the abundance of phycocolloids produced by various marine algae, most of them also produce various secondary metabolites. These metabolites do not participate directly in the growth, development and reproduction of the organisms. These compounds are usually involved in mediating a diverse array of inter- and intraspecific interactions including predation, competition, mutualism, and reproductive processes, as well as interactions between organisms and their physical environment (Stachowicz, 2001).

Many red algae such as *Laurencia*, *Plocamium* and *Asperigopsis* are prolific producers of halogenated terpenes. Green algae species also produce various kinds of terpenes. Brown algae are unique by producing polyphenolic compounds known as phlorotannins (Amsler *et al*., 1999) besides terpenes. All these terpenes and phenolics compounds possess various bioactive properties which had attracted the interest of many researchers. Various studies (Van Alstyne *et al*., 2001) and research had been done to look into the potentials in these compounds for pharmaceutical, antifouling, chemical remediation and various other purposes.

1.2 Research Objectives

The objectives of this study were:

1. To obtain extracts and perform chemical profiling.
2. To quantify polyphenolic content in seaweed tissues.
3. To evaluate the antibacterial and antioxidant properties of the samples.

CHAPTER 2

LITERATURE REVIEW

2.1 Polyphenols

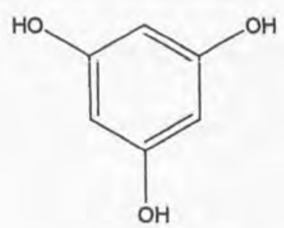
Polyphenols are compounds that are usually referred to as naturally occurring compounds containing multiple phenolic functionalities (Handique & Baruah, 2002). These compounds are commonly found in higher plants. An overview of polyphenolic compounds by Handique & Baruah (2002), suggested that polyphenols have significant chemical functions. For example, it can react with one-electron oxidants, which prevents formation of free radicals in a biological system. Polyphenols are also capable of interacting with available multiple polar functional groups for selective and unselective binding with biologically important molecules such as proteins. Because of its multiple phenol functionalities, they interact with proteins, and this results in precipitation of protein-polyphenol complexes. This process is the basis in the tanning process of leather.

The classification of natural polyphenols is based on the types of building blocks that appear as repeated units. They are namely proanthocyanidin derivatives, galloyl and hexa hydroxydiphenyl ester derivatives, hydroxycinnamic acid derivatives, and phloroglucinol derivatives.

In this study, focus is placed on the phloglucinol derivatives because polyphenols of marine brown algae are from this origin. Phloroglucinol derivatives are also known as phlorotannins.

2.2 Phlorotannins

Phlorotannis are secondary metabolites, polyphenolic compounds that are found only in marine brown algae (Paul *et al.*, 2001). Secondary metabolites are chemicals with no obvious function in the primary metabolic demands of the cell (Stachowicz, 2001). These polyphenolic compounds may function like terrestrial tannins by binding proteins and other macromolecules. However, structurally they are different compounds as compared to terrestrial tannins. They are complex polymers derived from a simple aromatic precursor, phloroglucinol (1,3,5-trihydroxybenzene) (**1**). They are formed from the condensation of both C-C and C-O coupling (Handique & Baruah, 2002). These metabolites are often termed phlorotannins to distinguish them from the terrestrial tannins (Paul *et al.*, 2001).



(**1**) Phloroglucinol

Like many secondary metabolites, phlorotannins have multiple roles, serving as cell wall strengtheners, feeding deterents, digestability reducers, antimicrobial agents, act as metal chelators, as well as absorbing UV radiation (Targett *et al.*, 1992).

2.2.1 Biosynthesis

Phenolic compound can be formed from primary metabolites of the cell by either the shikimate pathway or the polyketide pathway. In the shikimate pathway, carbohydrate is used as the starter, as for the polyketide pathway, it starts from acetyl and malonyl coenzyme A instead. When it comes to the production of phloroglucinol, which is the basic structure for various other phlorotannins compounds (Harborne, 1994), it is produced by the polyketide pathway (Figure 2.2). This pathway produces phenols with hydroxyl groups in the 1,3,5-positions.

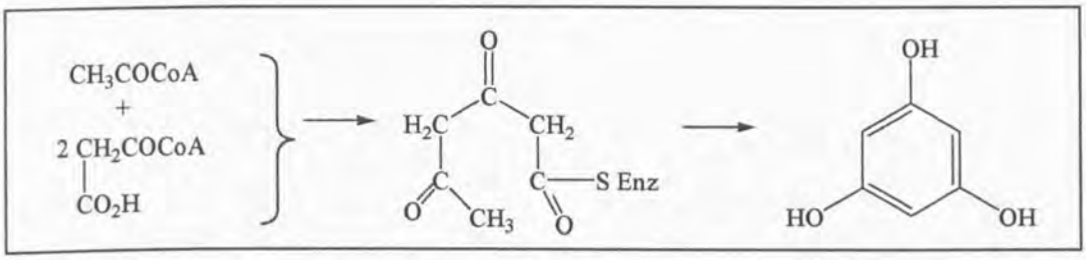
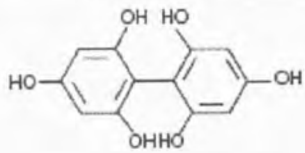


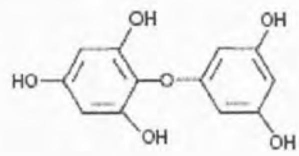
Figure 2.1 Biosynthesis of phloroglucinol by the polyketide pathway.

2.2.2 Characteristic and Occurrence of Phlorotannins

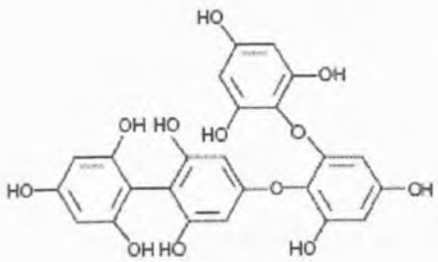
Phlorotannins span a wide range of molecular sizes, from 126Da to 650k Da. In some cases, phlorotannins may constitute up to 20% of a brown alga's dry mass. It can be divided into six specific groups (Fig. 2.3): fucols (**2**), phlorethols (**3**), fucophlorethols (**4**), fuhalols (**5**), isofuhalols (**6**) and eckols (**7**) (Targett *et al.*, 1992). Specific groups of phlorotannins are often characteristic of specific algal genera, for example, fucols in *Fucus* and eckols in *Ecklonia*. The characterization is based on the differences in the nature of the structural linkages binding the phlorotannin polymers and in the number of hydroxyl groups present.



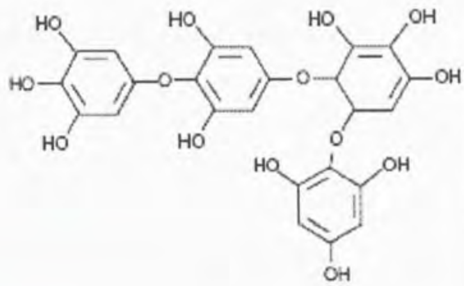
(**2**) fucols



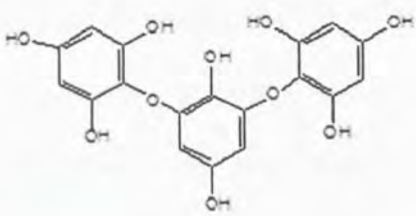
(**3**) phlorethols



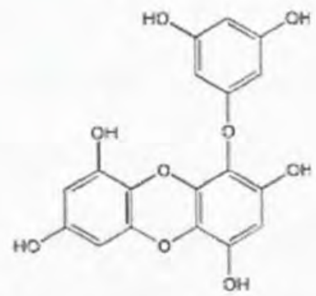
(**4**) fucophlorethol



(**5**) fuhalols



(**6**) isofuhalols



(**7**) eckols

Figure 2.2 Six groups of phlorotannins.

A review by Targett & Arnold (2001) pointed out the major structural characteristics of all six types of phlorotannins. In fucols, phloroglucinol units are linked through aryl-aryl bonds. Fucols most often occur in Fucales and Ectocarpales. Phlorethols is characterized by its diaryl ether bonds linked between phloroglucinol units. It occurs in Fucales as well as Laminariales. Fucophlorethols as its names suggests occur primarily in Fucales and sporadically in Laminariales. They are dehydrooligomers of phloroglucinol which contain both direct carbon-carbon and diaryl ether bonds. Another primary type of phlorotannins occur in Fucales is fuhalols.

It is ether-linked phloroglucinol units with an extra hydroxyl group on one unit, where the ether bonds are oriented *para* and *ortho*. Besides fuhalols, isofuhalols also form similar bonds and linkages, but vary a little where the ether bonds are *para*- and *meta*-orientated. Its occurrence is limited to the Laminariales. Lastly, eckols is characterised by its dehydrooligomerization of three phloroglucinol units and found only in Laminariales.

Based on this review, it can be concluded that Fucales consist the most diverse variety of phloroglucinol. This statement is further strengthened after another study by Glombitza & Hauperich (1997), 33 phlorotannins isolated from brown algae *Cystophora torulosa* from the order Fucales. These polyphenols includes compounds that belong to the group of fucols, branched fucophlorethols, difucol-containing fucophlorethols, and two entirely new subclasses, namely hydroxylated branched fucophlorethols and *bis*-fucophlorethols lacking a 1,2,3-tryphenoxy-5-acetoxybenzene moiety. In *Sargassum spinuligerum*, other than fucophlorethols, a variety of fuhalols had been isolated (Glombitza *et al*., 1997).

A study was conducted by Shibata *et al.* (2002a, b) on brown alga *Eisenia bicyclis* in the Japanese waters where phlorotannins were isolated by column and thin-layer chromatography. The isolated compounds (Fig. 2.4) were phloroglucinol (**1**), eckol (**7**), dieckol (**8**), 8, 8'-bieckol (**9**), and phlorofucofuroeckol A (**10**).

REFERENCES

Ahn, G., Kim, K., Cha, S., Song, C., Lee, J., Heo, M., Yeo, I., Lee, N., Jee, Y., Kim, J., Heu, M., & Jeon, Y., 2007. Antioxidant activities of phlorotannins purified from *Ecklonia cava* on free radical scavenging using ESR and H_2O_2-mediated DNA damage. *Eur Food Res Technol*. **226**: 71-79

Amsler, C.D., Iken, K.B., McClintock, J.B., & Baker, B.J., 1999. Secondary metabolites from Antartic Marine Organisms and their ecological implications. In: McClintock, J.B. and Baker, B.J. (eds.). *Marine Chemical Ecology*. CRC Press, Boca Raton, 267-300.

Castro, P., & Huber, M.E., 2003. *Marine Biology*. 4th Ed. McGraw-Hill, Boston.

Chan, E.W.C., Lim, Y.Y., & Omar, M., 2007. Antioxidant and antibacterial activity of leaves of *Etlingera* species (Zingiberaceae) in Peninsular Malaysia. *Food Chemistry*. **104**: 1586-1593

Chandini, S.K., Ganesan, P., & Bhaskar, N., 2008. *In vitro* antioxidant activities of three selected brown seaweeds of India. *Food Chemistry*. **107**: 707-713

Cronin, G., Paul, V.J., Hay, M.E., & Fenical, W., 1997. Are tropical herbivores more resistant than temperate herbivores to seaweed chemical defenses? Diterpenoid metabolites from *Dictyota acutiloba* as feeding deterrents for tropical versus temperate fishes and urchins. *Journal of Chemical Ecology*. **23** (2): 289-302

Dummermuth, A.L., Karsten, U., Fisch, K.M., König, G.M., & Wiencke, C., 2003. Responses of marine macroalgae to hydrogen-peroxide stress. *Journal of Experimental Marine Biology and Ecology*. **289**: 103-121

Glombitza, K., & Hauperich, S., 1997. Phlorotannins from the brown alga *Cystophora torulosa*. *Phytochemistry*. **46** (4): 735-740

Glombitza, K., Keusgen, M., & Hauperich, S., 1997. Fucophlorethols from the brown algae *Sargassum spinuligerum* and *Cystophora torulosa*. *Phytochemistry*. **46** (8): 1417-1422

Handique, J.G., & Baruah, J.B., 2002. Polyphenolic compounds : an overview. *Reactive & Functional Polymers*. **52**: 163-188

Harborne, J.B., 1994. Phenolics. In: Mann, J., Davidson, R.S., Hobbs, J.B., Banthorpe, D.V., & Harbone, J.B. (eds.). *Natural products: their chemistry and biological significance*. Longman Group UK, Hong Kong, 361-388.

Kang, H.S., Chung, H.Y., Kim, J.Y., Son, B.W., Jung, H.A., & Choi, J.S., 2004a. Inhibitory phlorotannins from the edible brown algae *Ecklonia stolonifera* on total reactive oxygen species (ROS) generation. *Arch Pharm Res*. **27** (2): 194-198

Kang, H.S., Kim, H.R., Byun, D.S., Son, B.W., Nam, T.J., & Choi, J.S., 2004b. Tyrosinase inhibitors isolated from the edible brown alga *Ecklonia stolonifera*. *Arch Pharm Res*. **27** (12): 1226-1232

Kang, K.A., Lee, K.H., Chae, S., Zhang, R., Jung, M.S., Lee, Y., Kim, S.Y., Kim, H.S., Joo, H.G., Park, J.W., Ham, Y.M., Lee, N.H., & Hyun, J.W., 2005. Eckol isolated from *Ecklonia cava* attenuates oxidative stress induced cell damage in lung fibroblast cells. *FEBS Letters*. **579**: 6295-6304

Lee, R.E., 1999. *Phycology*. 3rd Ed. Cambridge University Press, Cambridge.

Martinez, E.A., 1996. Micropopulation differentiation in phenol content and susceptibility to herbivory in the Chilean kelp *Lessonia nigrescens* (Phaeophyta, Laminariales). *Hydrobiology*. **326/327**: 205-211

Nagayama, K., Iwamura, Y., Shibata, T., Hirayama, I., & Nakamura, T., 2002. Bactericidal activity of phlorotannins from the brown alga *Ecklonia kurome. Journal of Antimicrobial Chemotherapy.* **50**: 889-893

Nagayama, K., Shibata, T., Fujimoto, K., Honjo, T., & Nakamura, T., 2003. Algicidal effect of phlorotannins from brown algae *Ecklonia kurome* on red tide microalgae. *Aquaculture.* **218**: 601-611

Paul, V.J., Cruz-Rivera, E., & Thacker, R.W., 2001. Chemical mediation of macroalgal-herbivore interactions: ecology and evolutionary Perspectives. In: McClintock, J.B. and Baker, B.J. (eds.). *Marine Chemical Ecology.* CRC Press, Boca Raton, 227-265.

Peckol, P., Krane, J.M., & Yates, J.L., 1996. Interactive effects of inducible defense and resource availability on phlorotannins in the North Atlantic brown alga *Fucus vesiculosus. Mar. Ecol. Prog. Ser.* **138**: 209-217

Pedersen, A., 1984. Studies on phenol content and heavy metal uptake in fucoid. *Hydrobiology.* **116/117**: 498-504

Shibata, T., Fujimoto, K., Nagayama, K., Yamaguchi, K., & Nakamura, T., 2002a. Inhibitory activity of brown algal phlorotannins against hyaluronidase. *International Journal of Food Science and Technology.* **37**: 703-709

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

Shibata, T., Nagayama, K., Tanaka, R., Yamaguchi, K., & Nakamura, T., 2003. Inhibitory effects of brown algal phlorotannins on secretory phospholipase A2s, lipoxygenases and cyclooxygenases. *Journal of Applied Phycology.* **15**: 61-66

Shibata, T., Kawaguchi, S., Hama, Y., Inagaki, M., Yamaguchi, K., & Nakamura, T., 2004. Local and chemical distribution of phlorotannins in brown algae. *Journal of Applied Phycology.* **16**: 291-296

Siamopoulou, P., Bimplakis, A., Iliopoulou, D., Vagias, C., Cos, P., Berghe, D.V., & Roussis, V., 2004. Diterpenes from the brown algae *Dictyota dichotoma* and *Dictyota linearis*. *Phytochemistry.* **65**: 2025-2030

Stachowicz, J.J., 2001. Chemical ecology of mobile benthic invertebrates: predator and prey, allies and competitors. In: McClintock, J.B. and Baker, B.J. (eds.). *Marine Chemical Ecology*. CRC Press, Boca Raton, 157-194.

Steinberg, P.D., 1989. Biogeographical variation in brown algal polyphenolics and other secondary metabolites: comparison between temperate Australasia and North America. *Oecologia.* **78**: 373-382

Steinberg, P.D., 1986. Chemical defenses and the susceptibility of tropical marine brown algae to herbivores. *Oecologia.* **69**: 628-630

Steinberg, P.D., 1988. Effects of quantitative and qualitative variation in phenolic compound on feeding in three species of marine invertebrate herbivores. *J. Exp. Mar. Biol. Ecol.* **120**: 221-237

Swanson, A.K., & Druehl , L.D., 2002. Induction, exudation and the UV protective role of kelp phlorotannins. *Aquatic Botany.* **73**: 241-253

Targett, N.M., & Arnold, T.M., 2001. Effects of secondary metabolites on digestion in marine herbivores. In: McClintock, J.B. and Baker, B.J. (eds.). *Marine Chemical Ecology*. CRC Press, Boca Raton, 391-411.

Targett, N.M., Coen, L.D., Boettcher, A.A., & Tanner, C.E., 1992. Biogeographic comparisons of marine algal polyphenolics: evidence against a latitudinal trend. *Oecologia.* **89**: 464-470

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., and Byrne, D.H., 2006. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis*. **19**: 669-675

Vairappan, C.S., Daitoh, M., Suzuki, M., Abe, T. and Masuda, M., 2001. Antibacterial halogenated metabolites from Malaysian *Laurencia* species. *Phytochemistry*. **58**: 291-297.

Van Alstyne, K.L., Dethier, M.N., & Duggins, D.O., 2001. Spatial patterns in macroalgal chemical defenses. In: McClintock, J.B. and Baker, B.J. (eds.). *Marine Chemical Ecology*. CRC Press, Boca Raton, 301-324.

Van Alstyne, K.L., McCarthy III, J.J., Hustead, C.L., Duggins, D.O., 1999. Geographic variation in polyphenolic levels of Northeastern Pacific kelps and rockweeds. *Marine Biology*. **133**: 371-379

Wisespongpand, P., & Kuniyoshi, M., 2003. Bioactive phloroglucinols from brown alga *Zonaria diesingiana*. *Journal of Applied Phycology*. **15**: 225-228

Yan, X., Nagata, T., & Fan, X., 1998. Antioxidative activities in some common seaweeds. *Plant Foods for Human Nutrition*. **52**: 253-262

Yuan, Y.V., & Walsh, N.A., 2006. Antioxidant and antiproliferative activities of extracts from a variety of edible seaweeds. *Food and Chemical Toxicology*. **44**: 1144-1150