

STRUCTURAL ELUCIDATION AND BIOLOGICAL ACTIVITIES OF
HALOGENATED SECONDARY METABOLITES OF *Laurencia similis* AND
Laurencia nangii COLLECTED FROM THE WATERS OF SABAH

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THIS THESIS IS SUBMITTED AS A REQUIREMENT TO OBTAIN BACHELOR
DEGREE IN SCIENCE WITH HONOURS

BIOTECHNOLOGY PROGRAM
SCHOOL OF SCIENCE AND TECHNOLOGY
UNIVERSITY MALAYSIA SABAH

MARCH 2004

BORANG PENGESAHAN STATUS TESIS@

UDUL: Structural elucidation and biological activities of
 halogenated secondary metabolites of Laurencia similis and
L. nangii collected from the waters of Sabah.
 azah: Sarjana muda sains

SESI PENGAJIAN: 2001 / 2002

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CONFESSTION

I hereby to admit that this whole report is my sole work except for the reference I already explained the sources.

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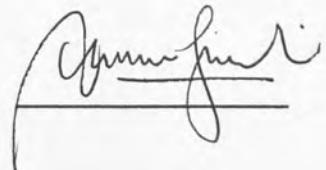
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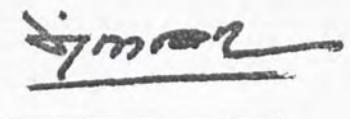
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ACKNOWLEDGEMENT

This project would not be succeeded without the contributions of several people. First and foremost, I would like to take this golden opportunity to thank the Faculty of Science and Technology, Sabah University, Malaysia for providing a variety of facilities for me in order to complete this project successfully.

I'm particularly grateful to my supervisor, Dr. Charles S. Vairappan for his valuable guidance and directions. He has been such a wonderful supervisor throughout the year who he has sacrificed his time and energy in order to make this project a success. Therefore, his willingness to help me is greatly appreciated.

Not forgetting my friends who co-ordinate with me throughout the project. I sincerely acknowledge for their kind suggestions. Mainly, I would like to thank my friend, Ang May Yen, Julenah, Chow Ling, Lee Ee Hong, Bee shan, Chen Fong and Ghee Siew.

I'm also indebted to both my dad and mum for their love and support. Their love to me will never be forgotten. Thank you once again.



ABSTRAK

Laurencia similis dan *Laurencia nangii* merupakan sejenis makroalga dari divisi *Rhodophyta* (alga merah). Alga merah mempunyai banyak keunikan dari segi menghasilkan kompaun yang dipercayai mempunyai kepentingan dalam bidang keubatan. *Laurencia similis* adalah unik dalam menghasilkan dua kompaun yang merupakan polibromida indol. Kedua-dua kompaun ini adalah 1-metil 2,3,5,6-tetrabromoindole (1) and 2,3,5,6-tetrabromoindole (2) dan kedua-dua struktur telah ditentukan. *Laurencia nangii* yang dikutip dari Pulau Langkayan menghasilkan tiga kompaun yang dikenali sebagai Palisadin A serta dua kompaun lagi adalah kompaun C₁₅-Acetogenin. Peratusan bagi 1-metil 2,3,5,6-tetrabromoindole adalah 29% manakala peratusan bagi 2,3,5,6-tetrabromoindole adalah 30% dimana kedua-duanya diperolehi daripada *Laurencia similis*. Bagi ketiga-tiga kompaun dari *Laurencia nangii* pula, peratusan bagi Palisadin A adalah 2% serta dua C₁₅ Acetogenin kompaun lagi mempunyai peratusan masing-masing adalah 6% dan 2%. Kesebelah bakteria ialah *Escherichia coli*, *Pseudomonas aeurogenes*, *Enterococcus faecalis*, *Salmonella* sp. , *Proteus mirabilis*, *Clostridium cellobioparum*, *Clostridium sordelli*, *Clostridium novyi*, *Proteus vulgaris*, *Vibro anginolyticus* dan *Vibro parahaemolyticus*. Antara sebelas spesis bacteria, lima jenis bacteria adalah patogenik and enam jenis bacteria adalah dari bakteria persekitaran. Kedua-dua Kompaun 1 dan Kompaun 2 dari *Laurencia similis* tidak menunjukkan aktiviti antibakteria terhadap bakteria patogenik. Ujian antibakteria telah dijalankan keatas Kompaun 2 dari *Laurencia similis* dan ini menunjukkan aktiviti antibakteria adalah kuat iaitu sebanyak 65% ke atas empat jenis bakteria persekitaran iaitu *Clostridium cellobioparum*, *Clostridium novyi*, *Vibro anginolyticus* and *Vibro parahaemolyticus* manakala Kompaun 1 hanya menunjukkan aktiviti antibakteria sebanyak 40% ke atas dua bacteria persekitaran iaitu *Clostridium cellobioparum* dan *Clostridium novyi*. Berdasarkan kajian ini serta kajian yang terdahulu, ini telah menunjukkan bahawa kedua-dua kompaun dari *Laurencia similis* mempunyai potensi untuk menjadi antibiotik.



ABSTRACT

Laurencia similis and *Laurencia nangii* are known as macroalgae belonging to the Rhodophyta division or Red algae. *Laurencia similis* is unique in producing 2 novel polybrominated indoles compounds. The two compounds are 1-methyl 2,3,5,6-tetrabromoindole (**1**) and 2,3,5,6-tetrabromoindole (**2**) in which their structures were deduced on the basis of spectroscopic evidence. *Laurencia nangii* from Langkayan Island produces three compounds which are Palisadin A and two compounds which are known to be C₁₅-acetogenins compounds. The yield of Compound **1** was 29% whilst percentage of Compound **2** was 30% which are obtained from *Laurencia similis*. The yield of Palisadin A was 2% and the two C₁₅ Acetogenin compounds were 6% and 2% respectively. The eleven bacteria species used were *Escherichia coli*, *Pseudomonas aeurogenes*, *Enterococcus faecalis*, *Salmonella* sp., *Proteus mirabilis*, *Clostridium cellobioparum*, *Clostridium sordelli*, *Clostridium novyi*, *Proteus vulgaris*, *Vibro anginolyticus* dan *Vibro parahaemolyticus*. Out of the eleven bacteria, six are pathogenic bacteria whilst the rest is environmental bacteria. Compound **1** (1-methyl 2,3,5,6-tetrabromoindole) and Compound **2** (2,3,5,6-tetrabromoindole) of *Laurencia similis* showed no inhibition against the five pathogenic bacteria. Antibacterial test had tested on the two compounds from *Laurencia similis*. However, only Compound **2** showed 65% of inhibition against four environmental bacteria, while Compound **1** showed 40% activity against two environment bacteria that are *Clostridium novyi* and *Vibrio anginolyticus*. Based on this study and past study, compounds of *Laurencia similis* have the potential of becoming antibiotics.

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LIST OF ABBREVIATION AND SYMBOL

g	Gram
mg	Milligram
ml	Millilitre
cm	Centimetre
%	Percentage
min	Minute
M	Molar
R _f	Mobility relative to front
UV	Ultraviolet
OD	Optical density
HCl	Hydrochloric acid
TLC	Thin Layer Chromatography
°C	Degree Celsius
Hex	Hexane
CCl ₄	Chloroform
EtOAc	Ethyl acetate
H ₂ O	Water
Hex.	Hexane
MeOH	Methanol
Tol	Toluene

CHAPTER 1

INTRODUCTION

Marine biodiversity refers to the variety of biological life such as various flora and fauna that live in the ocean, sea and coastal environments as well as the ecosystem they live in which forms one big community. This particular community varies from one to the other depending on the climate and locality. It is in the sea that we find the greatest genetic diversity compared to land and fresh water. In the sea, there are multitude of mammals, fishes, corals, shrimps, bacteria and also algae. Algae are an essential constituent in the ecosystem because they are the primary producer. Without algae, most marine species mainly marine herbivour won't be able to survive.

Research done on marine algae mainly red algal genus *Laurencia* in Malaysia is still very few although Malaysian waters are rich with these species. Furthermore, only within the last decade, researchers only began to realize the biological potential of marine secondary metabolism.

It is the presence of chemical compounds mainly valuable secondary metabolites in red algae that interest many scientist. Many marine organisms particularly algae that contain brominated organic compounds are said to be potential

therapeutic drugs (Kotterman *et al.*, 1998). Because of the medicinal potential that has been found in red algae, this study will focus primarily on isolation, structural elucidation and biological activities of halogenated secondary metabolites from several red algal genus *Laurencia* species collected from waters of Sabah. Sabah, one of the thirteen states of Malaysia, covers an area of 29,388 square miles with a coastline 900 miles long washed by the South China Sea on the west and north and the coastal sea of Sabah contains an enormous species of marine plants (Ismail, 1987). Hence, the possibility of isolating bioactive metabolites for this genus is high. In this project, the main focus is on red algal genus *Laurencia similis* Nam et Saitoi and *Laurencia nangii* Masuda.

Red algal genus *Laurencia similis* and *Laurencia nangii* have medical potential. Hence, research is done on these 2 species. It requires several steps in order to fulfill my objectives of study. Thus, the first objective of study is to extract halogenated secondary metabolites compounds from red algal genus *Laurencia* using methanol solvent as to yield crude extract.

My second objective is to introduce the crude extract into the Column Chromatography using Column Chromatography methods and to isolate and purify halogenated secondary metabolites compounds from red algal genus *Laurencia* using preparative thin layer chromatography (pTLC).

The pure compounds isolated from red algal genus *Laurencia* will then be brought for structural elucidation using H¹-NMR, C¹³-NMR and other spectroscopic methods which will be my next objective.

Eventually, compounds will be tested for anti-bacterial activities. Potencies of these compounds will also be determined by comparing their anti-bacterial activities against commercially available antibiotics.

CHAPTER 2

LITERATURE REVIEW

The Red algal (genus *Laurencia*) are found worldwide, both in tropical as well as temperate water. Tropical and subtropical zones are the center of distribution of this genus. One of the uniqueness of red algae is its ability to produce a wide variety of halogenated secondary metabolites with diverse structural features depending on the species and localities and it seems to be an endless source of new chemical constituents.

2.1 ALGAE

Algae are also known as seaweed which can be found both intertidally and subtidally throughout the world. Algae represent 2 kingdoms which are between protista kingdom and plantae kingdom (Figure 2.1). It is the presence of photosynthetic pigment such as chlorophylls, b and c and also colour pigment like phycocyanin (blueish), phycoerythrin (reddish), carotene (yellowish brown) and xanthophylis (brownish) that caused algae as a marine plant.

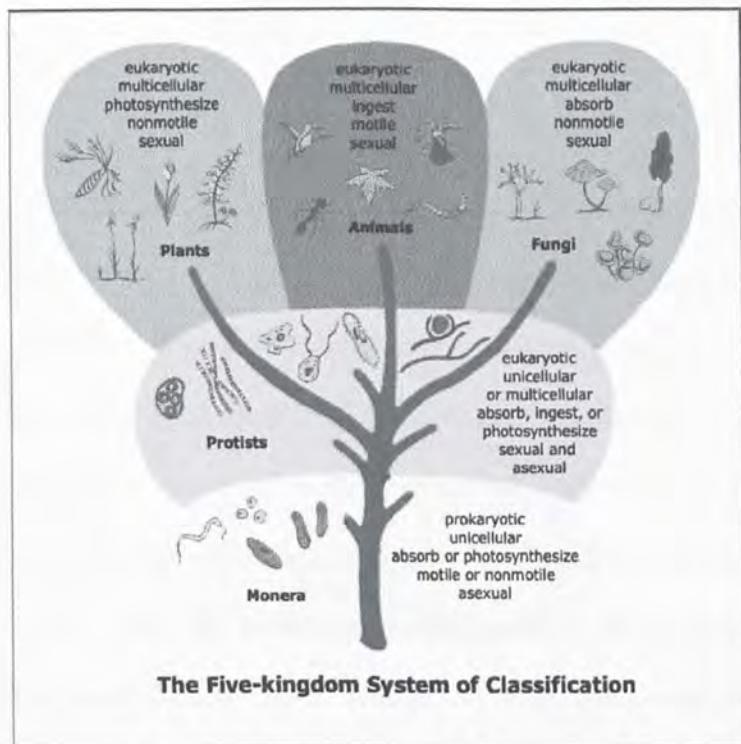


Figure 2.1 A Five-kingdom life chart. It consists of Plantae, Animalia, Fungi, Protists and Monera. Algae are between protista kingdom and plantae kingdom. Algae are also known as marine plant.

Algae are the most abundant species which constitute 94% out of the total amount of species found in the ocean. Besides, it can also be found in the rivers, lakes and in the sea. Basically, algae can be divided into 2 types: micro algae and macro algae. Macro algae are then further subdivided into 3 main division based on the colour they have: *Rhodophyta* (Red algae), *Chlorophyta* (Green algae) as well as *Phaeophyta* (Brown algae). Out of the 3 division, *Rhodophyta* is exploded extensively. There are approximately 2,500 species that are classified under *Rhodophyta*. It is followed by *Phaeophyta* having approximately 1,000 species. The green algae, *Chlorophyta* has the most species. That is estimated 7,000 species under green algal division (Ismail, 1995).

2.1.1 Red Algae

Red algae are also known as *Rhodophyta* consisting of phycocyanin, phycoerythrin and chlorophyll pigments. It is the presence of phycoerythrin that gives its name. This type of algae has colour ranging from dark brown to reddish purple. Red algae absorb blue light reflecting red light. Blue light can penetrate water to a greater depth and hence, red algae can grow up to 200 m depth compared to brown or green algae. One of the main characteristic of red algae is they store photosynthetic product in the form of floridean starch. There are about 550 – 600 genera in *Rhodophyta* with 5,500 morphological species. *Rhodophyta* produced 908 metabolites with 46% of them isoprenoids, 38% acetogenins, 9% amino acids, 5% shikimates. Most of the red algae have thick filament and the arrangement of the filament is varied (Ismail, 1995). Red seaweed attaches to substrates like rocks, pilings, and shells which helps it to absorb adequate sunlight for photosynthesis. There are various genera that fall in this category such as *Laurencia*, *Gigartina*, *Chondrus*, *Euchema* and many more. Red algal genus *Laurencia similis* and red algal genus *Laurencia nangii* originate from division of *Rhodophyta*, family *Rhodophyceae*, order *Ceramiales*, genus *Laurencia* and species *nangii* and *similis*.

2.1.2 Green Algae

Green algae are also known as *Chlorophyta*. It contains pigments like chlorophyll a and b that cause this type of algae looks green in colour. *Caulerpa*, *Codium* and *Halimeda* are examples of genera of green algae.

2.1.3 Brown Algae

Another name for brown algae is *Phaeophyta*. This kind of algae appears to be brown in colour due to the presence of pigment like xanthophylls. Brown algae are found lavishly in the off coast of Sabah which serves as the major source for alginates. Alginates obtained from brown algal genus *Sargassum* can be used as gelling agent (Ismail, 1987).

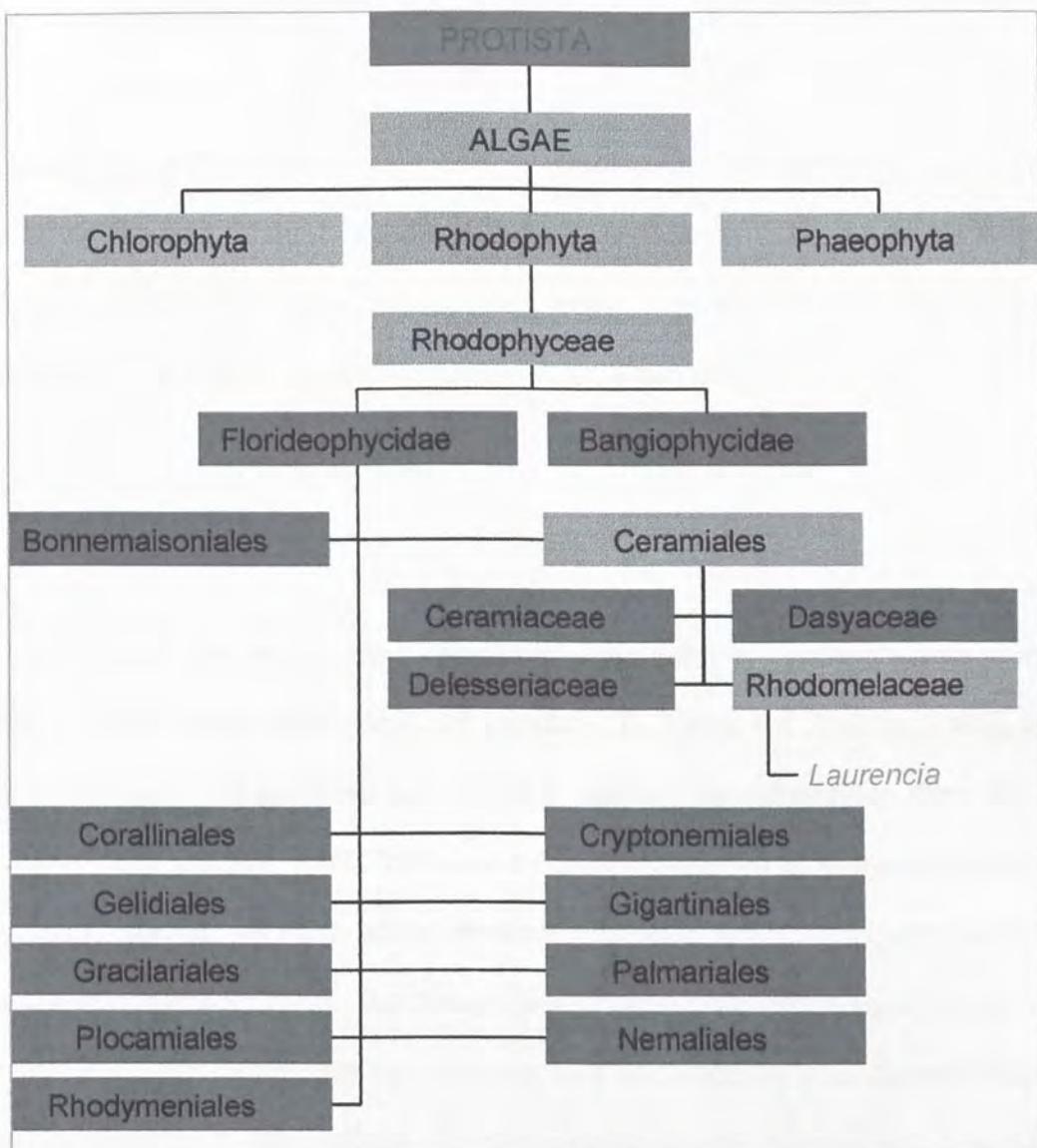


Figure 2.2 A seaweed chart. Algae can be divided into *Rhodophyta*, *Chlorophyta* and *Phaeophyta*. *Laurencia* falls into the *Rhodophyta* category and the family of *Laurencia* is *Rhodophyceae* and the order *Ceramiales*.

REFERENCES

- Ahmad Ismail, 1995. *Rumpai Laut Malaysia*. Dewan Bahasa dan Pustaka, Kuala Lumpur.
- Carter, G. T., Rinehart, K. L., Jr., Li, L. H., Kuentzel, S. L. and Connor, J. L., 1978. Brominated indoles from *Laurencia bronniartii*. *Tetrahedron letters* **46**, 4479-4482.
- Damonte, E., Neyts, J., Pujol, C. A., Snoeck, R., Andrei, G., Ikeda, S., Witvrouw, M., Reymen, D., Haines, H., Matulewicz, M. C., Cerezo, A., Coto, C. E. and De Clerco, E., 1994. Antiviral activity of a sulphated polysaccharide from the red seaweed *Nothogenia fastiglata*. *Biochemical Pharmacology* **47** (12), 2187-2192.
- De Ruiter, G. A. and Rudolph, B., 1997. Carrageenan biotechnology. *Trends in Food Science and Technology* **8** (12), 389-395.
- Erickson, K. L., 1983. Constituents of *Laurencia*. In: Scheuer, P. J. (Ed.) *Marine Natural Products: Chemical and Biological Perspectives V*. Academic Press, New York, 131-257.
- Emilio, L. G., 1993. *Detection, Isolation of Bioactive Natural Product and Structural Determination*. CRC Press, London, 10-48.
- Fenical, W., 1975. Halogenation in the *Rhodophyta*. A review. *Journal Phycology* **11**, 245-259.
- Fleurence, J., 1999. Seaweed proteins: biochemical, nutritional aspects and potential uses. *Trends in Food Science & Technology* **10** (1), 25-28.

- Ghazally Ismail, 1987. Marine algal resources in Malaysia with particular emphasis on Sabah state. *Science and Natural Resources* **57** (1), 63-73.
- Glickman, M., 1987. Utilisation of seaweed of hydrocolloids in the food industry. *Hydrobiologia* **151** (152), 31-47.
- Guiseley, K. B., 1989. Chemical and physical properties of algal polysaccharides used for cell immobilization. *Enzyme and Microbial Technology* **11** (11), 706-716.
- Harris, P. J. and Ferguson, L. R., 1993. Dietary fibre: its composition and role in protection against colorectal cancer. *Mutation Research/ Fundamental and Molecular Mechanisms of Mutagenesis* **290** (1), 97-110.
- Hashim, M. A. and Chu, K. H., 2004. Biosorption of cadmium by brown, green and red seaweeds. *Chemical Engineering Journal* **97** (2-3), 249-255.
- Harbone, J. B., 1984. *Methods of Plant Analysis Phytochemical Methods*. A guide to modern techniques of plant analysis. London: Chapman and Hall.
- Iliopoulou, D., Vagias, C., Harvala, C. and Roussis, V., 2002. C₁₅ acetogenins from the red alga *Laurencia obtusa*. *Phytochemistry* **59** (1), 111-116.
- Jesefsson, B., 1980. Separation Techniques. In Scheuer, P. J. (Ed.) *Marine Natural Products: Chemical and Biological Perspectives III*. Academic Press, London, 22-24 and 67-68.
- Jiménez-Escríg, A. and Sánchez-Muniz, F. J., 2000. Dietary fibre from edible seaweeds: chemical structure, physicochemical properties and effects on cholesterol metabolism. *Nutrition Research* **20** (4), 585-598.
- Kotterman, M., Veen, I. V. D., Hesselingen, J. V., Leonards, P., Osinga, R. and Boer, J. D., 1998. Preliminary study on the occurrence of brominated organic

- compounds in Dutch marine organisms. *Biomolecular Engineering* **20** (4-6), 425-427.
- Kurata, K., Taniguchi, Y., Agatsuma and Suzuki, M., 1998. Diterpenoid feeding deterrents from *Laurencia saitoi*. *Phytochemistry* **47** (3), 363-369.
- Kurosawa, E. and Suzuki, M., 1979. Halogenated and non-halogenated aromatic sesquiterpenes from the red alga *Laurencia okamurae* Yamada. *Bulletin of the Chemical Society of Japan* **52** (11), 3352-3354.
- Liu, J. N., Yoshida, Y., Wang, M. Q., Okai, Y. and Yamashita, U., 1997. B cell stimulating activity of seaweed extracts. *International Journal of Immunopharmacology* **19** (3), 135-142.
- Manriquez, C. P., Souto, M. L., Gavin, J. A., Norte, M. and Fernandez, J. J., 2001. Several new squalene-derived triterpenes from *Laurencia*. *Tetrahedron* **57**, 3117-3123.
- Masuda, M., Kawaguchi, S. and Phang, S. M., 1997. Taxonomic notes on *Laurencia similis* and *L. papillosa* (*Ceramiales, Rhodophyta*) from the Western Pacific. *Botanica Marina* **40**, 229-239.
- Masuda, M., Kawaguchi, S., Takahashi, Y., Okamoto, K. and Suzuki, M., 1999. Halogenated secondary metabolites of *Laurencia similis* (*Rhodomelaceae, Rhodophyta*). *Botanica Marina* **42**, 199-202.
- Mihopoulos, N., Vagias, C., Mikros, E., Scoullos, M. and Roussis, V., 2001. Prevezols A and B: new brominated diterpenes from the red alga *Laurencia obtusa*. *Tetrahedron Letters* **42**, 3749-3752.
- Mohd Hani Norziah and Chio, Y. C., 2000. Nutritional composition of edible seaweed *Gracilaria changgi*. *Food Chemistry* **68** (1), 69-76.

- Mukesh, K., Sahni, M. Z., Wahrman, V. B. and Sharma, G. M., 1998. Secondary metabolites of marine organisms. In: Gal, L. and Halvorson (Ed.) *New Developments in Marine Biotechnology*. Plenum Press, New York, 41-47.
- Nam, K. W. and Saito, Y., 1991. *Laurencia similis* (*Ceramiales, Rhodophyta*), a new species from Queensland, Australia. *British Phycological Journal* **26**, 375-382.
- Nam, K. W. and Saito, Y., 1995. Vegetative and reproductive anatomy of some *Laurencia* (*Ceramiales, Rhodophyta*) species with a description of *L. maris-rubri* sp.nov. from the red sea. *Phycologia* **34** (2), 157-165.
- Norte, M., Fernández, J. J., Souto, M. L. and García-Grávalos, M. D., 1996. Two new antitumoral polyether squalene derivatives. *Tetrahedron Letters* **37** (15), 2671-2674.
- Paul, V. J. and Fenical, W., 1980. Palisadins A, B and related monocyclofarnesol derived sesquiterpenoids from the red marine alga *Laurencia* cf. *palizada*. *Tetrahedron Letters* **21**, 2787-2790.
- Ruperez, P., 2002. Mineral content of edible marine seaweeds. *Food Chemistry* **79** (1), 23-26.
- Siddhanta, A. K., Mody, K. H., Ramawat, B. K. and Chauhan, V. D., 1994. A new sulfated polysaccharide with potent blood anti-coagulant activity from the red seaweed *Grateloupia indica*. *International Journal of Biological Macromolecules* **16** (5), 279-280.
- Spieler, R., 2002. Seaweed compound's anti-HIV efficacy will be tested in southern Africa. *The Lancet* **359** (9318), 1675.
- Su, J. Y., Zhong, Y. L., Zeng, L. M., Wu, H. M. and Ma, K., 1995. Terpenoids from *Laurencia karlae*. *Phytochemistry* **40** (1), 195-197.

- Suzuki, M. and Kurosawa, E., 1987. (3E)-Laureatin and (3E)-isolaureatin, halogenated C₁₅ non-terpenoid compounds from the red alga *Laurencia nipponica* Yamada. *The Chemical Society of Japan* **60**, 3791-3792.
- Suzuki, M., Takahashi, Y., Matsuo, Y., Guiry, M. D. and Masuda, M., 1997. Scanlonenyne, a novel halogenated C₁₅ acetogenin from the red alga *Laurencia obtusa* in Irish waters. *Tetrahedron* **53** (12), 4271-4278.
- Suzuki, M., Nakano, S., Takashi, Y., Abe, T. and Masuda, M., 1999. Bisezakyne-A and -B, halogenated C₁₅ Acetogenins from a Japanese *Laurencia* species. *Phytochemistry* **51** (5), 657-662.
- Suzuki, M., Takahashi, Y., Mitome, Y., Itoh, T., Abe, T. and Masuda, M., 2002. Brominated metabolites from an Okinawan *Laurencia intricata*. *Phytochemistry* **60**, 861-867.
- Vairappan, C. S., Daitoh, M., Suzuki, M., Abe, T. and Masuda, M., 2001. Antibacterial halogenated metabolites from the Malaysian *Laurencia* species. *Phytochemistry* **58** (2), 291-297.
- Vairappan, C. S., Suzuki, M., Motomura, T. and Ichimura, T., 2001. Pathogenic bacteria associated with lesions and thallus bleaching symptoms in the Japanese kelp *Laminaria religiosa* Miyabe (*Laminariales, Phaeophyceae*). *Hydrobiologia* **445**, 183-191.
- Vairappan, C. S., 2003. Potent antibacterial activity of halogenated metabolites from Malaysian red algae, *Laurencia majuscula* (*Rhodomelaceae, Ceramiales*). *Biomolecular Engineering* **20** (4-6), 255-259.
- Wise, M. L., Rorrer, G. L., Polzin, J. J. and Croteau, R., 2002. Biosynthesis of marine natural products: isolation and characterization of a Myrcene Synthase from

cultured tissues of the marine red alga *Ochtodes secundiramea*. *Biochemistry and Biophysics* **400** (1), 125-132.

Wong, C. K., Ooi, V. E. C. and Ang, P. O., 2000. Protective effects of seaweeds against liver injury caused by carbon tetrachloride in rats. *Chemosphere* **41** (1-2), 173-176.

Young, D. N., Howard, B. M. and Fenical, W., 1980. Subcellular localization of brominated secondary metabolites in the red alga *Laurencia snyderae*. *Journal Phycology* **16**, 182-185.