DIVERSITY AND CHEMOTAXONOMY OF SEAWEEDS IN THE WATERS OF PULAU GAYA

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CONSERVATION BIOLOGY PROGRAMME
FACULTY OF SCIENCE AND NATURAL RESOURCES
UNIVERSITI MALAYSIA SABAH

2014

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THIS DISSERTATION SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF SCIENCE WITH HONOURS

CONSERVATION BIOLOGY PROGRAMME
FACULTY OF SCIENCE AND NATURAL RESOURCES
UNIVERSITI MALAYSIA SABAH

2014

DECLARATION

I hereby declare that this thesis is my original and genuine work except for some caption and quotation that have been explained the sources.

26th May 2014

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ACKNOWLEDGEMENTS

I would like to take this opportunity to express my gratitude and appreciation to the Institute for Tropical Biology and Conservation and Sabah Parks to have provided the necessary facilities, materials, and instruments to finish this study. I am grateful to Prof. Dr. Charles Santhanaraju Vairappan, my project supervisor, for his guidance and advice throughout this research.

Besides, I would like to express my gratitude to the staff of Institute For Tropical Biology and Conservation and the seniors in the Natural Product Chemistry Laboratory especially Kishneth, Thilahgavani, Tulasiramanan, Hazami, and Steve for their kind help and support.

I also want to thank my fellow course mates especially Yeong Hong, King Ming, Sam, Ting Hui, Junia, Shalini, Mathavan, Soo Voon, Kin Man, and Chin Soon for their encouragement and cooperation.

My deepest appreciation would be to my love especially my parents, sibling, and girlfriend for their continuous encouragement and faith on me. Last but not least, I would like to thank everyone and I wish all of you best of luck and happy always.

VERIFICATION

Signature

SUPERVISOR

(Prof. Dr. Charles Santhanaraju Vairappan)

ABSTRACT

Tunku Abdul Rahman Marine Park holds a wealth of marine flora and fauna of enormous importance for the health of marine ecosystem in Sabah. Coastal waters in the vicinity of Pulau Gaya are perhaps the most important niche for the existence of seaweed and corals. The objectives of this study are to study the diversity of seaweeds and its distribution in the waters of Pulau Gaya and to investigate the presence of chemotaxonomical markers of selected species of seaweeds. Eight transect lines surrounding Pulau Gaya were provided to study the diversity of seaweeds and its distribution. A total of 47 samples were collected and 30 samples were further subjected into chemotaxonomy to investigate the presence of chemotaxonomical markers. Herbariums were made and voucher specimens were deposited at BORNEENSIS Herbarium (BORH), Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah. The seaweeds composition observed at the eight transect lines set up around Pulau Gaya are 44.91% Rhodophyta, 34.15% Ochrophyta (Phaeophyta), and 20.95% Chlorophyta. According to Simpson's Diversity Index (D), both Base Camp Reef (Transect 2) and Polish Reef (Transect 8) show highest value which is D = 0.89 whereas Tg. Wokong Reef (Transect 4) is the lowest among the eight transects which is D = 0.77. The Shannon-Wiener Index (H') shows that both Sapi Reef (Transect 1) and Polish Reef (Transect 8) have the highest value which is H' = 2.35. Moreover, Tg. Wokong Reef (Transect 4) and Malohom Reef (Transect 5) are both the lowest with a value of H' = 1.92. Also, Tg. Wokong Reef (Transect 4), Malohom Reef (Transect 5), and Tavajun Reef (Transect 6) show abundance of genus *Eucheuma* which may due to the anthropogenic impact on the environment. On the other hand, 30 samples were subjected to Thin Layer Chromatography to show the chemical profile in the crude extract collected. Three samples of *Caulerpa serrulata* (S46), *Caulerpa racemosa* (S47), and *Caulerpa sertularioides* (S48) were further subjected into High Performance Liquid Chromatography and show the presence of caulerpin compound at retention time of 17.4 min. This compound was further subjected into Nuclear Magnetic Resonance and the structure was elucidated and proven according to previous literature review. It can be concluded that caulerpin found in this research was the chemotaxonomical markers for the genus *Caulerpa*.

ABSTRAK

KEPELBAGAIAN DAN KIMIA TAKSONOMI RUMPAI LAUT DI PERAIRAN PULAU GAYA

Taman Marin Tunku Abdul Rahman memegang pelbagai flora dan fauna yang sangat penting untuk kesihatan ekosistem marin di Sabah. Perairan pantai di sekitar Pulau Gaya mempunyai perananan yang penting untuk kewujudan rumpai laut dan batu karang. Objektif kajian ini adalah untuk menyelidik kepelbagaian rumpai laut dan ketaburannya di perairan Pulau Gaya dan menyiasat kehadiran penanda kimia taksonomi untuk rumpai laut yang terpilih. Lapan transek garisan di sekitar Pulau Gaya telah disediakan untuk mengkaji kepelbagaian rumpai laut dan ketaburannya. Sebanyak 47 sampel telah dikumpulkan dan 30 sampel telah digunakan untuk menjalani analisasi kimia taksonomi untuk menyiasat kehadiran penanda kimia taksonomi. Herbariums telah dibuat dan specimen baucar telah disimpan di BORNEENSIS Herbarium (BORH), Institut Biologi Tropika dan Pemuliharaan, Universiti Malaysia Sabah. Komposisi rumpai laut yang telah diperhatikan pada lapan transek garisan yang disediakan sekitar Pulau Gaya adalah 44.91% Rhodophyta, 34.15% Ochrophyta (Phaeophyta), dan 20.95% Chlorophyta. Menurut Indeks Simpson Kepelbagaian (D), kedua-dua Base Camp Reef (Transek 2) dan Polish Reef (Transek 8) menunjukan nilai tertinggi iaitu D = 0.89 manakala Tg. Wokong Reef (Transek 4) adalah yang paling rendah di antara lapan transek menunjukkan nilai D = 0.77. Indeks Shannon-Wiener (H') menunjukkan bahawa kedua-dua Sapi Reef (Transek 1) dan Polish Reef (Transek 8) mempunyai nilai yang tertinggi iaitu H' = 2.35. Selain itu, kedua-dua Tg. Wokong Reef (Transek 4) dan Malohom Reef (Transek 5) adalah yang paling rendah dengan nilai H' = 1.92. Di samping itu, Tg. Wokong Reef (Transek 4), Malohom Reef (Transek 5), dan Tavajun Reef (Transek 6) menunjukkan banyak genus Eucheuma yang mungkin disebabkan oleh kesan antropogenik terhadap alam sekitar. Sebaliknya, 30 sampel telah digunakan untuk menjalani analisasi Thin Layer Chromatography untuk menunjukkan profil kimia dalam ekstrak mentah yang dikumpulkan. Tiga sampel Caulerpa serrulata (S46), Caulerpa racemosa (S47), dan Caulerpa sertularioides (S48) telah digunakan untuk menjalani analisasi High Performance Liquid Chromatography dan menunjukkan kehadiran sebatian caulerpin pada masa pengekalan 17.4 min. Sebatian ini telah digunakan untuk menjalani analisasi Nuclear Magnetic Resonance dan struktur ini telah dijelaskan dan terbukti menurut kajian ulasan perpustakaan sebelum ini. Kesimpulannya, caulerpin yang ditemui dalam kajian ini adalah penanda kimia taksonomi untuk genus Caulerpa.

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List of Symbols and Abbreviations

km	Kilometers
m	Meters
cm	Centimeter
mm	Milliliter
µm	Micrometer
nm	Nanometer
g	Gram
mg	Milligram
min	Minutes
mL	Milliliter
MHz	Megahertz
Na_2SO_4	Sodium sulphate
TLC	Thin Layer Chromatography
HPLC	High Performance Liquid Chromatography
NMR	Nuclear Magnetic Resonance
HSQC	Heteronuclear Single-Quantum Correlation Spectroscopy
HMBC	Heteronucleaer Multipe-Bond Correlation Spectroscopy
$CHCl_3$	Chloroform
$CDCL_3$	Deuterated Chloroform
Hex	Hexane
EtOAc	Ethyl acetate
MeOH	Methanol
dH_2O	Distilled water
TMS	Tetramethylsilane
%	Percentage
C	Coverage in each 50 cm x 50 cm quadrats
Σ	Summation
^oC	Degree Celsius

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CHAPTER 1

INTRODUCTION

1.1. Overview

Malaysia, separated from each other by the South China Sea into Peninsular Malaysia and East Malaysia, has a total coastline of 3432 km, with 418,000 km^2 of continental shelf. Around these two halves of Malaysia are clustered with numerous islands along the coastlines.

Pulau Gaya that is in Tunku Abdul Rahman Park, nearest to Kota Kinabalu, Sabah, is one of the few islands that were gazetted under the Tunku Abdul Rahman Park and protected under the Sabah Parks Enactments. Pulau Gaya is rich in biodiversity of terrestrials and marine life because it is covered with dense virgin tropical forest and surrounded by sandy bays alternate with mudflats and mangroves. Diverse groups of marine algae are suitable to inhabit these type of niches found in Malaysia (Phang, 2006).

A tally of 377 specific and infraspecific taxa of marine algae (17 Cyanophyta, 102 Chlorophyta, 186 Rhodophyta and 72 Ochrophyta [Phaeophyta]) are found in Malaysia (Phang *et al.*, 2005). Marine algae have a great variety of forms, sizes and colours ranging from unicellular of 3-10 µm to giant kelps up to 70 m long and growing at up to 50 cm per day (Gamal, 2010). They can be divided into two major types, macro algae and micro algae. Macro algae are large algae and they be seen

with naked eyes. They have rhizoids to attach themselves to rocks and structures are more complex with variety of colours and forms. While micro algae are much smaller organisms and they can only be seen under a microscope. They are phytoplankton and found abundance in oceans where light is available. Micro algae serve an important role in the food web of the marine environments. Macro algae, more commonly known as seaweeds can be divided into red algae (Rhodophyta), green algae (Chlorophyta), and brown algae (Ochrophyta [Phaeophyta]). The different colours are due to the presence of chlorophyll that is found within seaweeds (Gamal, 2010). Green algae can be found common not only in marine but also in freshwater and even terrestrial unlike red and brown algae which are almost exclusively marine.

Seaweeds have a variety of purpose and regarded as one of the living renewable resources (Syad *et al.*, 2013) that are under research for their value for food, agricultural and horticultural, pharmaceutical, cosmetic and bioenergy applications (Stengel *et al.*, 2011). Seaweeds are consumed by people and also harvested for the extraction of alginate, agar and carrageenan, which are the gelatinous substances collectively known as hydrocolloids that serve as food additives. Other seaweeds may be used as fertilizer and they will provide nutrients and grow-promoting substances. Also, seaweeds may serve as a potential source of bioethanol that may provide an alternative bioenergy.

The interaction between wide diversity of marine organisms with the marine environment produce rich source of secondary metabolites (Wijesekara *et al.*, 2011) that act as chemical defence system that protect themselves from harm (Wright *et al.*, 2004). Seaweeds are among the marine organisms that possess a high content of polysaccharides, vitamins and minerals and they have become structurally diverse bioactive secondary metabolites (Syad *et al.*, 2013) with various biological activities (Wijesekara *et al.*, 2011). Chemotaxonomists use these fatty acids, proteins, carbohydrates, or secondary metabolites found as qualitative or quantitative data for classification to differentiate seaweeds that are morphologically the same (Frisvad *et al.*, 2007).

1.2. Objectives

The objectives of this study are:

- To study the diversity of seaweeds and its distribution in the waters of Pulau Gaya

- To investigate the presence of chemotaxonomical markers of selected species of seaweeds

1.3. Justifications

Although there are many researches that have been carried out on the diversity of seaweeds all around the world and Malaysia, the study on diversity of seaweeds and using its secondary metabolites as chemotaxonomical markers in Pulau Gaya, Sabah is less in details and not well documented. Hence, this study aims to investigate the distribution and diversity of seaweeds around Pulau Gaya together with the use of chemotaxonomical markers as a classification tools for selected species of seaweeds collected. Also, the seaweeds collected will be processed into herbarium to be kept and use as future references by other researchers.

1.4. Scope of Study

This research will only study the diversity of seaweeds and its distribution in the waters of Pulau Gaya collected from the eight marines transect points that set up around Pulau Gaya using line transect and quadrats methods. Additionally, only the selected major colonies of seaweeds observed from these eight marines transect points will be classified using chemotaxonomy tools to investigate the presence of chemotaxonomical markers.

CHAPTER 2

LITERATURE REVIEW

2.1. Morphology of Seaweeds

Seaweeds may have specialized tissues and growth forms due to environment stress. Some are simple colonies with many cells, and some may be filamentous, tubular, meshed, membranous or saccate algae. Also, some might be more delicate and complex. However, the internal structures are composed of similar cells which undergo simple differentiation that gives them different forms (Lobban and Harrison, 2000).

Seaweeds have a diverse functional-form group of structure assembled which contrasts strongly with uniformity in vascular plants. Among the seaweeds, functional-form group such as sheet group morphologically looks thin, tubular, and sheet-like (foliose). It has uncorticated, one to several cells thick underlying inside the sheet structure. Sheet group gives a soft texture to the seaweeds. Moreover, filamentous group are delicately branched (filamentous) morphologically and composed of uniseriate, multiseriate, or lightly corticated cells. It also gives a soft texture to the seaweeds. While coarsely branched group gives an irregularly shaped branched vertically. The internal anatomy of this group is corticated and it gives a kind of fleshy-wiry texture (Lobban and Harrison, 2000).

Furthermore, thick and leathery functional-form group have thick blades and branches. The cells are differentiated, heavily corticated, and thick walled. It gives a leathery and rubbery texture to the seaweeds. For jointed calcareous group, it has articulated and calcareous branching upright. The internal anatomy is calcified genicula, flexible intergenicula with parallel cell rows. This will gives a stony texture. Additionally, crustose group are lying stretched out and covered with a hard surface layer morphologically. Its internal anatomy is calcified or uncalcified parallel rows of cells. Therefore it gives a stony or tough texture (Lobban and Harrison, 2000). Table 2.1 shows the morphology of seaweeds.

Table 2.1: Morphology of Seaweeds

Functional-form group	External morphology	Internal anatomy	Texture
Sheet group	Thin, tubular, and sheet-like (foliose)	Uncorticated, one to several cells thick	Soft
Filamentous group	Delicately branched (filamentous)	Uniseriate, multiseriate, or lightly corticated	Soft
Coarsely branched group	Coarsely branched, upright	Corticated	Fleshy-wiry
Thick, leathery group	Thick blades and branches	Differentiated heavily corticated, thick-walled	Leather, rubbery
Jointed calcerous group	Articulated, calcareous, upright	Calcified genicula, flexible intergenicula with parallel cell rows	Stony
Crutose group	Prostrate, encrusting	Calcified or uncalcified parallel rows of cells	Stony or tough

(Source: Lobban and Harrison, 2000)

2.2. Classification of Seaweeds

Marine algae (seaweed) are a group of photoautotrophic, multi-cellular algae that can be found in marine environments. The differences between seaweeds when comparing the photosynthetic pigments, reserve foods, composition of the cell wall, mitosis, position of the flagella, morphology, and life histories are evident and crucial keys for taxonomic classification. Table 2.2 shows the diagnostic characteristics of seaweeds.

2.2.1 Rhodophyta (Red Algae)

Red algae are exclusively marines except for few species. Red algae vary in size and shape. Red algae are eukaryotic cells and they lack of flagellar structures. They are red in colours because of the presence of phycoerythrin and phycothcyanin that masks the other pigments (Gamal, 2010). The photosynthetic product of this group is floridean starch. Pectin built up the outer layer and cellulose was the inner layer of the cell wall for this group. Their cell walls contain agar and carrageenan. They are generally found from the intertidal to the deep limits of the photic zone.

2.2.2 Chlorophyta (Green Algae)

Green algae are found in both fresh and marine habitats. They come in a wide variety of morphologies, ranging from unicellular and filaments to blades and fleshy thalloid forms. The cell structure of green algae is eukaryotic and most of them are uninucleated while only few of them are multinucleated. They are green in colours because of the presence of chlorophyll a and chlorophyll b that masks others (Gamal, 2010). The photosynthetic product of this group is starch. The cell walls are composed of an outer layer of pectin and an inner layer of cellulose. In general, they can be found in the intertidal zone and lower subtidal region.

2.2.3 Ochrophyta [Phaeophyta] (Brown Algae)

Brown algae are exclusively marine forms. Brown algae size range from microscopic filaments to the largest algae ever known. Generally, their eukaryotic cells are uninucleate except only a few have medullary cells. They are brown in colours because of the presence of xanthophylls and fucoxanthin that masks over others (Gamal, 2010). Their photosynthetic products are laminarian and manitol. The cell walls are made up of an outer layer of align and an inner layer of cellulose. Brown algae make up the majority of the biomass in the intertidal and upper subtidal zones.

Table 2.2: Diagnostic Characteristics of Seaweeds

Division	Diagnostic Characteristics
Rhodophyta	• Chlorophyll a and d; r-phycoerythrin and r-phycocycnin • Floridean starch and floridioside • Cell walls contain agar and carrageenan • Absence of flagella; no motile stages
Chlorophyta	• Chlorophyll a and b • Amidon starch • Cellulose cell walls • Motile stages with 2-4 acronematic flagella
Ochrophyta (Phaeophyta)	• Chlorophyll a and c; fucoxanthin and xanthophylls • Laminarin and mannitol • Alginic and fucinic acids in cell wall • Motile stages pear-shaped with 1 acronematic and 1 pantonematic flagella

(Source: Hunt, 2013)

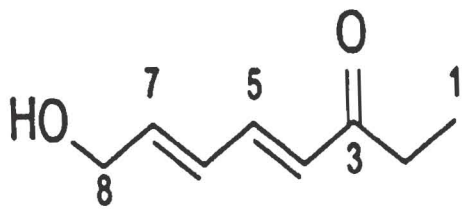
2.3. Metabolites

Generally there are two types of metabolites, which are primary metabolite and secondary metabolite. Primary metabolite is a kind of metabolite that is essential for reproduction, development, and normal growth. It usually plays vital roles in performing physiological function in the organisms. Primary metabolites typically can be found in many organism or cell.

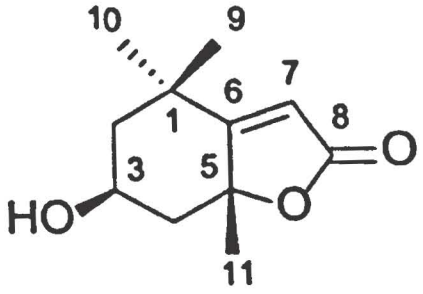
On the other hand, secondary metabolites are organic compounds that are not directly involved in the reproduction, development, and normal growth of an organism. But secondary metabolites produced often involve in destroying or blocking the normal growth of others as one of the strategies to protect the organism itself. Secondary metabolites also have contribution to new drugs discovery.

2.3.1 Secondary Metabolites of Seaweeds

According to a research done by Lu *et al.* (2011), they discovered a new secondary metabolite, 8-hydroxy-4E,6E-octadien-3-one (1), isolated from the seaweed *Gracilaria lemaneiformis* together with other uncharacterized secondary metabolites loliolide (2), 3β-hydroxy-5α,6α-epoxy-7-megastigmen-9-one (3), N-phenethylacetamide (4), squamolone (5), and 2-ethylidene-4-methylsuccinimide (6). The structures of these secondary metabolites were elucidated based on the analysis of spectroscopic data. Compounds (i) and (iii) were found to show a moderate allelopathic effect on the growth of red tide alga *Skeletonema costatum*. However, other compounds do not show any particularly toxic reaction to *Skeletonema costatum*. Figure 2.1 shows the chemical structures of compounds 1-6.



(1)



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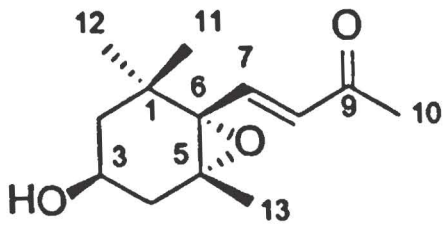

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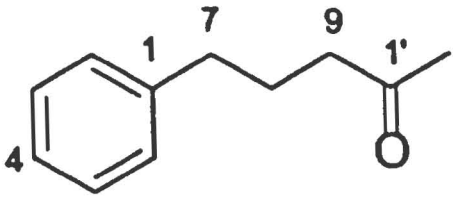

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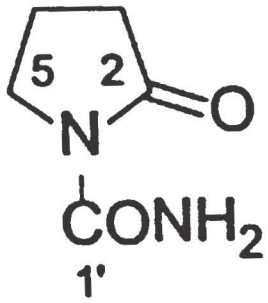

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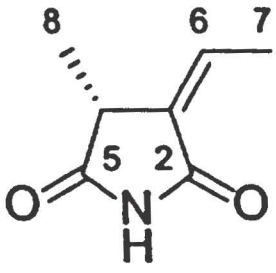

(6)

Figure 2.1: Chemical structures of compounds 1-6 (Source: Lu ***et al.***, 2011)

On the other hand, seaweeds are one of the main sources of food for the marine organisms. Foraging on seaweeds by herbivores can be very intense with nearly 100% of production being consumed in some habitats (Duffy and Hay, 1990). Therefore, seaweeds adapted themselves to the environment and developed chemical defence system against herbivory activities (Wright *et al.*, 2004).

Secondary metabolites produced by seaweeds as consequences of developing chemical defence system such as terpenes, aromatic compounds, acetogenins, amino acid derived substances, and phlorotannin polyphenolics show a high potential in deterring pathogens and fouling organisms although these compounds are known to play a role in defending the seaweeds against herbivory activities (Duffy and Hay, 1990).

2.4. Diversity of Seaweeds

According to Guiry *et al.* (2013), dynamic species counts show that there are about 6407 species of Rhodophyta, 5408 species of Chlorophyta, and 1836 species of Ochrophyta (Phaeophyta) were found and recorded.

2.4.1 Langkawi Island

According to a research done by Phang *et al.* (2005), 84 taxa of seaweeds (One Cyanophyta, 25 Chlorophyta, 62 Rhodophyta and 14 Ochrophyta [Phaeophyta]) were identified from the Langkawi Islands. It is relatively high diversity of seaweeds found in the Langkawi Islands but the biomass is low except for some green seaweed like the *Caulerpa* species that grow abundantly on the fish cage nets in the estuaries.

Based on the Sorensen's Coefficient of Similarity (S), at the species level, the seaweed flora of Langkawi is quite distinct from that of Peninsular Malaysia and East Malaysia. But the seaweed flora of Langkawi show more similarity to the seaweed flora of west coast Peninsular Malaysia and east coast Peninsular Malaysia than to west Sabah, east Sabah, and Sarawak at genus level. In conclusion, at genus level, Langkawi seaweeds show moderate similarity as compared to the total checklist for Malaysia, whereas at species level, it shows a low similarity (Phang *et al.*, 2005).

2.4.2 Other Regions

According to a research done by Satheesh and Wesley (2012), 32 seaweed taxa observed from the Kudankulam region (Nine belonging to Rhodophyta, 15 to Chlorophyta, and eight to Ochrophyta [Phaeophyta]). Whilst, Gan *et al.* (2006) recorded 81 taxa of Rhodophytes, 56 taxa of Chlorophyta, 34 taxa of Ochrophyta (Phaeophyta), and 26 taxa of Cyanophyta in Johor.

2.5. Distribution of Seaweeds

Seaweeds can be found everywhere and they are widely distributed all around the world (Gamal, 2010). However, different type of seaweeds may grow in different places and the environmental factors affect the colonies of seaweeds that would be found. Generally, green seaweeds are usually found in upper littoral zone, brown seaweeds are usually found in the middle littoral zone, and red seaweeds are usually found in the lower littoral zone. This is due to the different accessory pigments possessed by the seaweeds respectively.

Wong *et al.* (2012) found out that the occurrence and difference in seaweed communities between two distinct sites, Tanjung Batu and Kampung Kuala Nyalau in Sarawak, Malaysia are influenced by the topography and wave physical forces together with a combination of some environmental (physical and chemical) factors.

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