# DIVERSITY OF HALOGENATED METABOLITES IN RED ALGAE, *Laurencia snackeyi*, COLLECTED FROM DINAWAN AND LANKAYAN ISLAND

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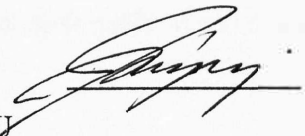

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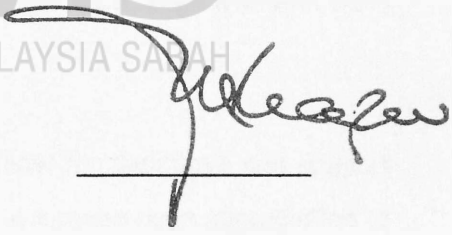

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# ABSTRACT

Diversity of halometabolites in red algae *Laurencia snackeyi* collected from two different locations, Dinawan and Lankayan Island was investigated through chemical analysis. A total of four pure compounds were isolated from the Dinawan Island's sample which identified as the **(1)** Palisadin A, **(2)** 5-acetoxypalisadin B, **(3)** Palisain B, and **(4)** Aplysistatin. These compounds were synthesized in significant quantity, 14.66%, 9.42%, 3.67% and 2.14% of 5.18 g of crude extracts, respectively. However, *Laurencia snackeyi* from Lankayan Island was found to contain five compounds such as compounds **(1)**, **(2)**, **(3)**, **(4)** with additional compound **(9)** which was identified as 5β-hydroxypalisadin B. These compounds were found in significant quantity, 5.53%, 6.50%, 7.08%, 2.69% and 4.51% of 7.90 g of crude extracts, respectively. From this study, it is concluded that the diversity of halogenated metabolites in *Laurencia Snackeyi* from Lankayan Island is richer than the Dinawan Island.

# ABSTRAK

Kepelbagaian sebatian-sebatian berhalogen dalam alga merah spesies *Laurencia snackeyi* yang dikumpul dari dua lokasi, Pulau Dinawan dan Pulau Lankayan telah dikaji melalui analisi kimia. Sebanyak empat jenis sebatian telah dipencilkan daripada *Laurencia snackeyi* yang berasal dari Pulau Dinawan dan sebatian-sebatian ini telah dikenalpasti sebagai **(1)** Palisadin A, **(2)** 5-acetoxypalisadin B, **(3)** Palisain B, and **(4)** Aplysistatin. Peratusan kandungan sebatian-sebatian ini yang disintesiskan oleh alga merah adalah 14.66%, 9.42%, 3.67% dan 2.14% daripada 5.18 g ekstrak kasar, masing-masing. Manakala, *Laurencia snackeyi* yang disampel dari Pulau Lankayan pula mengandungi 5 jenis sebatian. Sebatian-sebatian ini termasuklah sebatian **(1)**, **(2)**, **(3)**, **(4)** dan satu sebatian tambahan iaitu sebatian **(9)**, 5β-hydroxypalisadin B. Peratusan kandungan sebatian dalam sample ini ialah 5.53%, 6.50%, 7.08%, 2.69% dan 4.51% daripada 7.90 g ekstrak kasar, masing-masing. Berdasarkan penyelidikan ini, disimpulkan bahawa kepelbagaian sebatian-sebatian berhalogen dalam *Laurencia Snackeyi* dari Pulau Lankayan Island adalah lebih kaya daripada Pulau Dinawan.

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# LIST OF ABBREVIATIONS & SYMBOLS

| | |
|---|---|
| sp | species |
| SCUBA | self-contained underwater breathing apparatus |
| GPS | global positioning system |
| AR | analytical grade |
| MeOH | methanol |
| $dH_2O$ | distilled water |
| $NaSO_4$ | sodium sulphate |
| TLC | thin layer chromaography |
| $CHCl_3$ | chloroform |
| He | hexane |
| EtoAc | ethyl acetate |
| UV | ultra violet |
| HPLC | high-performance liquid chromatography |
| MeCN | acetonitrile |
| CC | column chromatography |
| NMR | nuclear magnetic resonance |
| $^1$H-NMR | proton nuclear magnetic resonance |
| $^{13}$C-NMR | carbon-13 nuclear magnetic resonance |
| FTIR | Fourier transformed infrared spectroscopy |
| MHz | mega hertz |
| TMS | tetramethylsilane |
| $CDCl_3$ | deuterated chloroform |
| $R_f$ | retardation factor or ration to front |

# CHAPTER 1

# INTRODUCTION

## 1.1 Marine Environment

Three-fourths of our earth's surface is covered by water. Hence, it is reasonable that the name of our Earth would be known as the ocean planet (Miller, 2007). With 71% of majority water surface, the aquatic environment is accounted as the largest part of the biosphere in terms of area and plays a very significant role in the earth's biological productivity. Generally, the aquatic environment can be further divided into the saltwater and freshwater environment. About 97% of the earth's water is made of saltwater while less than 1% is freshwater (Miller, 2007).

As the largest aquatic system, the saltwater environment is also described as the marine environment. In the marine ecosystem, the organisms occupy the habitat based on

several abiotic factors such as the depth of water, degree of light penetration, distance from shore and so on. This largest marine biome contains more than 500,000 species of flora and fauna. In general, the marine dwelling organisms are divided into four main groups like plankton, nekton, benthos, and the decomposers. Each group of organisms is playing a key role to support the food webs in the marine ecosystems. However, according to aquatic scientists, we have too little understanding of the marine environment. Therefore, in depth scientific investigation is necessary in order to discover and study the vast number of poorly understood marine species and their interactions.

## 1.2 Marine algae

The algae are chlorophyll bearing, non-vascular plant with predominant aquatic distribution. Some scientists suggest that the alga can be considered as a simple lower plant due to the alga has several common features with other plants biochemically and physiologically. For instance, the algae are the photoautotrophic organisms or primary produces which have the same biochemical pathways as the higher plants as they produce starch as the end products (Bilgrami & Saha, 2004). Unlike the terrestrial plants, the multicellular wall around the sporangia is absent within the algae.

Algae are ubiquitous for the marine habitats. They are distributed in the marine either in form of free-floating, attaching to the rocks, or attaching to submerged vegetation. The marine algae are commonly called seaweeds and divided into macroalgae and microalgae. There are three main recognized divisions of macroalgae based on their ornament photosynthetic pigments respectively which include the green algae

(Chlorophyta), brown algae (Phaeophyta) and red algae (Rhodophyta) (Stern *et al.*, 2007). The red algae, Rhodophyta are those seaweeds with complex life cycles that involve three different types of thallus structures. They are abundant in Malaysian waters especially in the coastal waters of Borneo.

Algae are ecological and economically important. The most significant ecological role of algae is as serving as the primary food for fishes and other aquatic small animals. In addition, the photosynthetic algae function in providing the oxygen to the marine ecosystems. The production of oxygen is achieved by the algae during photosynthesis process. Furthermore, some seaweeds are utilized as the fertilizers of agriculture activity due to their ability to increase the water holding capacity.



## 1.3 Division of Rhodophyta

Phylum Rhodophyta includes about 500 genera and 6,000 species of organisms which commonly known as the red algae (Rajan, 2001). Majority of the red algae are marine dwellers. The red algae occupy the marine environment at all latitudes. As compared to brown and green algae, they favor the warmer and deeper waters where they receive only blue-green wavelengths of light (Karleskint, 1998).

Like the other macroalgae, red algae are multicellular with the major body parts like blades, thallus, and holdfast. Each of these body parts has similar function as in the terrestrial green plants, the blades serve as the leave, the filamentous thallus acts as the

branch and the holdfast functions like the root. As their name would suggest, the red algae are red in colour because of the pigments, phycoerythrin (McClintock & Baker, 2001). Yet, somes do not appear as reddish instead they look violet, yellow, brown or green due to the chromatic adaptation.

The red algae have the widespread economic importance. The red algae produce cell-wall polysaccharides that have many industrial uses. For instance, the carrageenan are used in stabilizing paints, cosmetics and other commercial products is extracted from the red algae *Chondrus crispus*. Besides, some halogenated metabolites from *Laurencia* were reported have shown the antibacterial activity against marine bacteria as well as the terrestrial bacteria (Vairappan *et al.*, 2001). Also, these secondary metabolites are used for the chemotaxonomy purpose (Carvalho & Roque, 2004).



## 1.4 Genus *Laurencia*

According to the taxonomical classification, red alga genus *Laurencia* is derived from the phylum Rhodophyta which only has one class under it, Rhodophyceae. The genus *Laurencia* is derived from the family Rhodomelaceae which belongs to the subclass Florideophyceae, and order Ceramiales (Masuda *et al.*, 1997; Masuda *et al.*, 2002).

The red algae are distributed widely in Malaysian waters. Particularly the genus *Laurencia*, it is one type of the red macro marine algae that are abundant in Malaysian waters. Its habitat mostly ranges from 1 to 12m depths of the seawater floor (Vairappan *et al.*, 2001). For instance, *Laurencia snackeyi* is a type of red alga that is rich in Malaysian waters especially in Sabah.

The genus *Laurencia* were reported to prolific syntheses of halogenated secondary metabolites like the diterpenes, sesquiterpenes, and $C_{15}$ acetogenins. These halogenated metabolites are suspected to act as the deterrence to protect the algae from being eaten by the predators (Iliopoulou *et al.*, 2002).



## 1.5 Scope of Study

To investigate the chemical diversity of halogenated secondary metabolites of red algae *Laurencia snackeyi* collected from two different locations, Dinawan Island and Lankayan Island. Dinawan Island is located at the Western Coast of Sabah. On the other hand, the Lankayan Island is situated at the North-Eastern Coast of Sabah.

## 1.6 Significant of Research

Up to now, very few studies have been carried out on the halogenated metabolites of red algae genus *Laurencia* from Malaysian waters. The *Laurencia snackeyi* species has no complete documentation or investigation as compared with *Laurencia similis*. Thus, a scientific investigation of halogenated secondary metabolites of *Laurencia snackeyi* from Sabah's waters is essential.

## 1.7 Research Objectives

The objectives of the investigation are:



1. To obtain crude extract and chemical profiling of *Laurencia snackeyi*.

2. To isolate and elucidate the pure compound of *Laurencia snackeyi.*

3. To compare the structural diversity of halogenated secondary metabolites of *Laurencia snackeyi* from two different locations, Dinawan Island and Lankayan Island.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Halogenated Secondary Metabolites of Red Algae

The division Rhodophyta is well-known to produce metabolites particularly the halogenated secondary metabolites. However, each of the metabolites has different function respectively. For the primary metabolites, they are essential to the primary metabolites pathway of the organism such as growth, development, and reproduction (Kaufman *et al.*, 1999). On the other hand, the secondary metabolites do not take part in the primary metabolism. As reported in some scientific studies, these halogenated secondary metabolites serve as the deterrence of predators and diseases (Vairappan *et al.*, 2005).

Mostly, the red macroalgae are rich in bromoperoxidases which the haloterpenes are commonly synthesized as well as the mono and oligophenols. In genus *Laurencia*, brominated sesquiterpenes are commonly found. These compounds exhibit a series of structures which can be used as the taxonomy markers to identify the certain species (Carvalho & Roque, 2004). The secondary metabolites of red algae genus *Laurencia* are located at the intracellular "*corps en cerise*" where is the synthesis site of the metabolites. Besides, the "*corps en cerise*" in superficial cortical cells are suspected to be acting as the site of storage of the halometabolites. If the "*corps en cerise*" is absent in a particular *Laurencia* species, this indicates that there is no halogenated metabolites are produced (Howard *et al.*, 1980; Suzuki *et al.*, 2002; Vairappan *et al.*, 2001).



## 2.2 Chemical Diversity in Red Algae Genus *Laurencia* from Malaysian Waters

Based on the available information, there are seven known *Laurencia* species that can be found in Malaysian waters. So far, the reported species of red algae genus *Laurencia* form Malaysian waters are including *Laurencia crib*, *Laurencia majuscula*, *Laurencia nanggii*, *Laurencia pannosa*, *Laurencia papilossa*, *Laurencia similis* and *Laurencia sncakeyi* (Suzuki *et al.*, 2001; Vairappan *et al.*, 2001; Vairappan, 2003; Vairappan *et al.*, 2005; Vairappan & Tan, 2005; Vairappan *et al.*, 2007).

### 2.2.1 *Laurencia majuscula* Harvey (1935)

As reported by Vairappan *et al.* (2001), two major halogenated metabolites were isolated from Malaysian *Laurencia masjucula* from northern Borneo. This species of red algae synthesized a total of two halogenated sesquiterpenes, elatol (**1**) and iso-obtusol (**2**). Besides, the chemical composition of this species of genus *Laurencia* bears a resemblance to the chemical composition of Gran Canarian *Laurencia masjucula* (Vairappan *et al.*, 2001; Vairappan, 2003).




**Figure 2.1** Halogenated secondary metabolites (**1-2**) of *Laurencia majuscula* from Sabah's water (Vairappan, 2003).

### 2.2.2 *Laurencia pannosa* Zanardini (1872)

The specimen of *Laurencia pannosa* collected from the waters of Talang-talang Island of Sarawak yielded the two halogenated sesquiterpenes and one $C_{15}$ acetogenin. The two halogenated sesquiterpenes are pannosaaol (**3**) and pannosane (**4**). While (3Z)-