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DR. TAKAHIRO ISHII
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# CHEMICAL PROFILING AND BIOLOGICAL ACTIVITIES OF SPONGES FROM KUDAT

PREMILA A/P MOHAN



CONSERVATION BIOLOGY
SCHOOL OF SCIENCE AND TECHNOLOGY
UNIVERSITI MALAYSIA SABAH

2010

# CHEMICAL PROFILING AND BIOLOGICAL ACTIVITIES OF SPONGES FROM KUDAT

PREMILA A/P MOHAN

THIS IS A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT
FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE
WITH HONOURS



CONSERVATION BIOLOGY
SCHOOL OF SCIENCE AND TECHNOLOGY
UNIVERSITI MALAYSIA SABAH

2010

## 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.

PREMILA A/P MOHAN
(BS 07110170)

5 MAY 2010

**VERIFIED BY**

**Signature**

1. **SUPERVISOR**

   **(DR.TAKAHIRO ISHII)**

2. **EXAMINER 1**

   **(ASSOC.PROF.DR.HOW SIEW ENG)**

3. **EXAMINER 2**

   **(EN.RUZAIDI AZLI MOHD.MOKHTAR)**

   Pensyarah
   Institut Penyelidikan Bioteknologi

4. **DEAN**

   **(PROF.DR.MOHD.HARUN ABDULLAH)**

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# ABSTRAK

## PROFIL KIMIA DAN AKTIVITI BIOLOGI

## BUNGA KARANG DARI KUDAT

Bunga karang adalah invertebrata yang hidup di laut dan ia dikenali sebagai salah satu daripada beberapa pengeluar utama hasilan semulajadi daripada laut. Organisma ini merupakan pemakan turas yang sangat dikenali dan ia digolongkan dalam filum Porifera. Projek ini mengkaji tentang profil kimia serta aktiviti biologi bunga karang. Terdapat lima sampel bunga karang kesemuanya yang dikaji (S1-*Haliclona* sp., S2-*Phyllospongia* sp., S3-*Phyllospongia lamellose*, S4-*Stylotella aurantium* dan S5-*Aka* sp.). Peratusan pecahan ekstrak yang diperolehi daripada bunga karang tersebut adalah 0.12% dari pecahan ekstrak metanol dan 0.11% dari pecahan ekstrak heksana untuk Sampel 1, 0.21% dari pecahan ekstrak metanol dan 0.14% dari pecahan ekstrak heksana untuk Sampel 2, 0.32% dari pecahan ekstrak metanol dan 0.42% dari pecahan ekstrak heksana untuk Sampel 3, 0.15% dari pecahan ekstrak metanol dan 0.13% dari pecahan ekstrak heksana untuk Sampel 4 dan 0.09% dari pecahan ekstrak metanol dan 0.07% dari pecahan ekstrak heksana untuk Sampel 5. Hasil ekstrak kasar untuk bunga karang yang dikaji menunjukkan ciri-ciri bioaktif yang penting dengan merencatkan pertumbuhan bakteria patogen, membawa kepada pemencilan satu bahagian daripada sebatian metanol mentah dari Sampel 5. Oleh kerana maklumat yang diperolehi daripada kajian ini adalah terhad, struktur bagi sebatian yang dikaji tidak dikenalpasti. Penyelidikan dan kajian tentang hasilan semulajadi laut menunjukkan pertahanan antimikrob oleh organisma hidupan laut terhadap persekitaran. Hasilan semulajadi laut bukan sahaja penting dalam penyelidikan, malahan ia juga turut menyumbang dalam bidang bioteknologi serta bioperubatan.

## ABSTRACT

### CHEMICAL PROFILING AND BIOLOGICAL ACTIVITIES OF SPONGES FROM KUDAT

Sponges are marine living invertebrates and they are denoted as one of the few primary producers of natural products in the ocean. These organisms are well-known filter feeders and they are classified under the phylum Porifera. This project studied on the chemical profiling as well as biological activities of the sponges. There were altogether five samples of sponges studied (S1-*Haliclona* sp., S2-*Phyllospongia* sp., S3-*Phyllospongia lamellose*, S4-*Stylotella aurantium* and S5-*Aka* sp.). The percentage of fractions obtained from the sponges were 0.12% of methanol fraction and 0.11% of hexane fraction for Sample 1, 0.21% of methanol fraction and 0.14% of hexane fraction for Sample 2, 0.32% of methanol fraction and 0.42% of hexane fraction for Sample 3, 0.15% of methanol fraction and 0.13% of hexane fraction for Sample 4 and 0.09% of methanol fraction and 0.07% of hexane fraction for Sample 5. The yield of fractions for the studied sponges displayed prominent bioactive properties by inhibiting the growth of pathogenic bacteria, leading to the isolation of one part of the compound from the methanol fraction of Sample 5. Due to limited information obtained during the study, the structures of the compounds were not elucidated. Research and several studies on marine natural products show antimicrobial defense of marine living organism towards the environment. The marine natural products are not only important for the purpose of research, but also to contribute to the field of biotechnology and biomedicine.

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| | |
|---|---|
| °C | Degree Celcius |
| TLC | Thin Layer Chromatography |
| PTLC | Preparative Thin Layer Chromatography |
| Rf | mobility relative to front |
| ml | milliliter |
| L | litre |
| cm | centimeter |
| km | kilometer |
| g | gram |
| mg | milligram |
| µL | micro litre |
| % | percentage |
| µgmL$^{-1}$ | microgram per mili litre |
| UV | Ultraviolet |
| MeOH | Methanol |
| $^{1}$H-NMR | Proton Nuclear Magnetic Resonance |
| $^{13}$C-NMR | Carbon Nuclear Magnetic Resonance |

# CHAPTER 1

# INTRODUCTION

## 1.1 PREAMBLE

The ocean covers approximately 75% of the overall earth surface. It is consists of more than 300,000 known species of both marine plants as well as animals. The rich diversity of species in the coral reefs shows that there are about 1,000 species per $m^2$ in certain areas (Donia and Hamann, 2003).

In the previous years, in order to delineate the natural products of the marine plants as well as the marine animals, the marine environment has been given paramount attention from worldwide. So far, there are almost 12,000 chemical compounds that have been yielded from the marine environment. However, there are still hundreds of new compounds that are still being discovered every year. This effort eventually has lead to the yielding of several bioactive compounds that are successfully developed in the pharmaceutical industry (Donia and Hamann, 2003).

There are about 15,000 known species of sponges throughout the world. Not only that, primarily there are around 150 species of sponges that live in fresh water (Castro & Huber, 2005).

Sponges, which are the most primitive of multicellular animals (Galeano & Martinez, 2007) represent an important constitutive group of the coral reef fauna with a wide range of species (Touati *et al.*, 2007). Sponges are an important component of the benthic fauna throughout the tropical, temperate as well as the polar habitat (Bell and Smith, 2004).

Sponges are considered as simple organisms that represent the lowest metazoan group. They are amenable to cell disassociation as well as reaggregation into functional units (de Caralt *et al.*, 2003). Not only that, their cellular level of organization is also very primordial. Due to this, the cells of bioactive sponges are able to be cultured and the cultured cells will continue to produce bioactive metabolites (Friedrich *et al.*, 2001).

The class Demospongia is the largest class of the sponges group. The sponges in this particular class are characterized by the possession of a skeleton of one to four rayed siliceous spicules in conjunction with an organic skeleton of collagen. It can be secreted in the form of filaments of sponging or just simply diffuse in the mesohyl (Campbell & Reece, 2003).

The class Calcarea contains sponges with siliceous spicules and an organic collagen skeleton that is restricted to a thin veneer of living tissue. These sponges are usually supported by massive calcareous skeleton which are aragonitic or calcitic (Castro & Huber, 2005).

The last class includes the class Hexactinellidae. The sponges under this class are usually prone to living in deep water. The skeleton of the sponges from this class is made up of megascleres as well as microscleres. Not only that, their skeleton is also siliceous in nature and possesses haxactine structure. It is lack of superficial cellular pinacoderm and a mesohyl matrix (Campbell & Reece, 2003).

Many infectious diseases have evolved and eventually developed resistance against the existing pharmaceutical products. Many marine natural products have shown potential leads for pre-clinical assessment. Furthermore, emerging infectious diseases as well as the bioterrorism threat have contributed to the interest in exploiting natural oceanic products (Donia and Hamann, 2003). There are sponge farms established worldwide for production of bioactive natural products and also cosmetic products.

Sponges have generated great interest in the field of natural product chemistry mainly due to the presence of bioactive components in them (Faulkner, 2002). Besides producing a wide range of natural products, sponges also produce rich source of bioactive compounds. Those bioactive compounds that are formed usually contain a variety of properties such as cytotoxic, anti-tumour, anti-inflammatory and also antibiotics (de Caralt *et al.*, 2003).



Various microorganisms have been said to exist in sponges including cyanobacteria as well as unicellular algae (Friedrich *et al.*, 2001). A cyanobacterium is a type of symbiont of the sponges whereby it plays vital role in carbon fixation that helps in the nutrition of the sponges (Webster & Hill, 2001).

Bacteria have the potential to cause sponges' mortality as well as being able to degrade sponging tissue. They are able to do so by destroying the commercially important fibrous structures of microorganisms (Webster *et al.*, 2002).

We cannot deny the fact that sponges have created great significance in the natural product chemistry because of their tendency to be able to generate bioactive metabolites (Faulkner, 2002). Certain chemical compounds found in the sponges have been noted to have valuable pharmaceutical effect, related to the antibiotic activities and respiratory antitumor for humans (Yoo *et al.*, 2001).

## 1.2 JUSTIFICATION

Withstanding the fore mentioned, sponges are very interesting organism in terms of their vast natural products potential. They are undeniably worth investigating particularly in the varied species biodiversity of the Land Below the Wind, Sabah. Due to the diverse marine sponge biodiversity, the sponge chemistry could be regarded as an important niche area research for Sabah.

## 1.3 OBJECTIVES

The purpose of this research is to study the chemical properties as well as the biological activities of sponges. In order to accomplish the purpose, definite objectives have been set to plot the route of this research. The objectives are:

i) To extract and quantify bioactive extracts of five sponges from Tigabu Island, Kudat.

ii) To perform chemical profiling on Thin Layer Chromatography (TLC) of the crude extracts.

iii) To determine the antimicrobial potential of the crude extract from sponges.

iv) To isolate and characterize the bioactive metabolites in the sponges.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 A GENERAL IDEA OF SPONGES POTENTIAL

Sponges are classified into the phylum Porifera. The sponges usually live in an attached manner towards the solid substrates. Not only that, they are also well-known as filter feeders. The Porifera are divided into three distinctive classes, namely the Demospongia, the Calcarea and the Hexaclinellida (Faulkner, 2002).

Sponges have pores system which generates networks of water conducting canals (Imhoff & Stohr, 2003). All the way through the pores, the sponges confine bacteria as well as some detritus components which are brought close to the sponges by water currents. The water currents are formed by a specialized cell in the sponges, known as the choanocyte's flagellum.

An open up exploration by Scheuer (1983) verifies that marine organisms inevitably produced secondary metabolites which were usually uncommon and absent in the terrestrial atmosphere (Abas *et al.*, 1999).

Sponges are known to produce very large number and also diverse range of chemical compounds. Until now, more than 5000 different compounds have been isolated from approximately 500 species of sponges (Rifai *et al.*, 2005). Besides, up to 800 antibiotics have been isolated from marine sponges (Touati *et al.*, 2007).

A very well-known instance on the significance of microalgae in the typical marine natural products can be clearly seen in sponges from genus *Halichondria*. The examples include *Halichondria okadai* and *Halichondria melanodocia*. Those two *Halichondria* species have protein phosphate inhibitor okadaic acid in them (Simmons *et al.*, 2005).

The halichondrins are vastly becoming the aim of the chemical synthesis. This is due to the fact that they have an enormous structural complexity as well as a unique biological activity especially in killing cancer cells (Simmons *et al.*, 2005).

Apart from that, the sponges known from the genus *Oceania* sp. are present regularly in the Pacific as well as the Indopacific Oceans. During the bioassay, the pyridoacridine alkaloid (Figure 2.1) of the kuanoniamine type, which inevitably are also accountable for the prominent red color of certain sponges, is said to be major deterrent constituents of *Oceanapia* sp. (Proksch *et al.*, 2003).

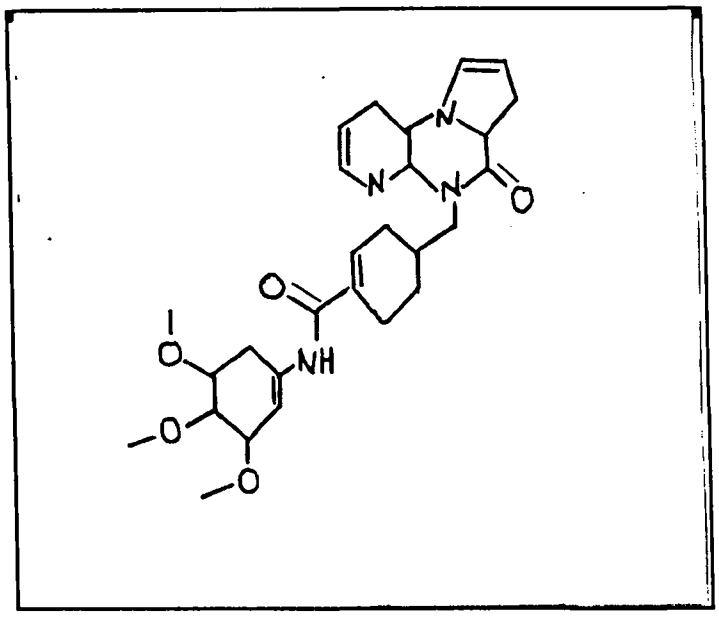


**Figure 2.1** Structure of pyridoacridine alkaloid (Proksch *et al.*, 2003).

Furthermore, according to Berlinck (1996), the compound known as halitoxin from the marine sponge called *Amphimedon viridis*, possessed quite a large pharmalogical properties. It was therefore tested in terms of its neurotoxicity.

Another example would be *Amphimedon terpensis*, a type of marine sponge from the same genus as *Amphimedon viridis*. This particular *Amphimedon terpensis* metabolizes a strange fatty acid which has a general scheme of structure, but with a stupendous reaction against free radicals (Berlinck, 1996).

The sponge known as *Theonella swinhoei* which is usually found in the islands of Philippines, are known to contain the macrolide swinholide (Figure 2.2) (Berlinck, 1996). This particular component in that sponge is proven to demonstrate *in vitro* cytotoxicity activity and has a remarkable antimalarial activity against the clones of *P. falciparum*.

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