THE STUDY OF SECONDARY METABOLITES OF SOFT CORALS FROM THE MANTANANI ISLANDS

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I hereby declare that thesis is my original research work except for quotations, summeries, references and other sources of findings reviewed have been duly acknowledged.

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ABSTRACT

Soft corals (phylum Cnidaria) are known to produce diverse marine secondary metabolites which play a vital role for their defence, communication and often found to exhibit pharmacological activity. Less research has conducted on soft corals that found in Mantanani Islands. Therefore, investigation of secondary metabolites (especially terpenoids) for three soft corals; *Lobophytum*, *Sarcophyton* and *Sinularia* sp. from Mantanani Islands were carried out. These samples were undergoes extraction and solvent-solvent partition to get crude extract that contained mainly of secondary metabolites which were later subjected to chemical profiling, followed by fractionation, isolation and purification to obtained seven different compounds. The isolated metabolites were subjected to NMR for several 1D and 2D spectral analysis to elucidate the structure of these natural products. Thus, a total seven compounds were isolated from three different samples. Three cembrane diterpenoids and a cholesterol were found in *Lobophytum* sp. As for *Sarcophyton* sp., two different cembrane diterpenoids were isolated, plus a cholestorol was also present in this sample. Whereas, a known steroid was found in the *Sinularia* sp. All the isolated compounds were conducted for antibacterial bioassay. In conclusion, only compound **4** exhibited positive activities against *Escherichia coli* and *Staphylococcus aureus*. Out of the seven compounds, five compounds were found to be known.

ABSTRAK

*Karang lembut (filum Cnidaria) dikenali untuk menghasilkan pelbagai kompaun yang memainkan peranan yang penting untuk pertahanan dan komunikasi. Selain itu, mereka juga mempunyai aktiviti farmakologi. Kurang penyelidikan dijalankan ke atas karang lembut di Pulau Mantanani. Oleh itu, penyiasatan metabolit sekunder (terutamanya "terpenoid") telah dijalankan untuk tiga karang lembut yang berbeza; Lobophytum, Sarcophyton and Sinularia sp. dari Pulau Mantanani. Pengekstrakan dan pelarut-pelarut pembahagian telah dijalankan untuk semua sampel untuk mendapatkan ekstrak mentah yang mengandungi terutamanya ialah metabolit sekunder. Kemudiannya tertakluk kepada profil kimia, mengikuti fraksinasi, pengasingan kimia dan penulenan untuk diperolehi tujuh kompaun yang berlainan. Kompaun ini tertakluk kepada NMR untuk analisis spektrum 1D dan 2D, untuk menentukan struktur kimia ini. Oleh itu, seramai tujuh kompaun telah diasingkan daripada tiga sampel yang berbeza. Tiga "diterpenoids cembrane" dan kolesterol yang ditemui di Lobophytum sp. Manakala untuk Sarcophyton sp., dua "diterpenoids cembrane" yang berbeza telah diperolehi, dan satu kolestorol yang turut hadir dalam sampel ini. Selain itu, steroid yang dikenali telah ditemui di sp Sinularia itu. Kesimpulannya, hanya kompaun **4** menunjukkan aktiviti positif terhadap Escherichia coli dan Staphylococcus aureus. Daripada tujuh kompaun, lima kompaun didapati diketahui.*

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LIST OF ABBREVIATIONS

C-X	Compound number X
CC	Column chromatography
$CDCl_3$	Deuterated chloroform
$CHCl_3$	Chloroform
DEPT	Distortionless Enhancement by Polarization Transfer
EtOAc	Ethyl acetate
F	Fraction
GGPP	Geranylgeranyl diphosphate
H_2O	Distilled water
Hex	Hexane
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear Single Quantum Coherence
HSQCTOCSY	Heteronuclear Single quantum Coherence-Total Correlation Spectroscopy
MeOH	Methanol
MeCN	Acetonitrile
Na_sSO_4	Anhydrous sodium sulphate
NMR	Nuclear magnetic resonance
NOESY	Nuclear Overhauser Effect Spectroscopy
TLC	Thin Layer Chromatography
Tol	Toluene
PTLC	Preparative Thin Layer Chromatography
TMS	Tetramethylsilane
UV-Vis	Ultra violet - visible
^{1}H-^{1}H COSY	Proton-Proton Correlation Spectroscopy
^{1}H-NMR	Proton nuclear magnetic resonance
^{13}C-NMR	Carbon nuclear magnetic resonance
1D	One dimensions
2D	Two dimensions

LIST OF SYMBOLS

v/v	volume to volume
%	percent
°C	degree Celsius
cm	centimetre
Hz	hertz
g	gram
J	coupling constant
kg	kilogram
L	litre
MHz	mega hertz
mg	milligram
ml	millilitre
mm	millimetre
nm	nanometre
ppm	part per million
vol	volume
$\delta\ ^{1}H$	pronton chemical shift
$\delta\ ^{13}C$	carbon chemical shift
µl	microlitre
µg	microgram

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CHAPTER 1

INTRODUCTION

1.0 Introduction

According to the Natural Environment Research Council in 2007, about 29% of the Earth's surface is covered by the land and the rest of the Earth's surface is covered by the ocean which is about 71%. Thus, ecosystem services provided by the oceans are essential to our earth. One of the reasons are because ocean harbour a large number of diverse marine organisms especially invertebrates which consisted of about 60% of them. Among of them are belong to the phyla Annelida, Arthropoda, Bryozoa, Cnidaria, Echinodermata, Mollusca, Platyhelminthes, Porifera and sub-phylum Tunicata (Leal *et al.*, 2012b). Most of them are sessile and soft bodied, hence to survive in this harsh and competitive environment them have to rely on the chemical defences or secondary metabolites which have evolved through their history to deter predators, to paralyze prey, to make themselves bitter taste or to poison their competitors.

Terrestrial plants have been extensively exploited for natural products since the late 19th century (Brahmachari, 2011). But recently, marine organisms have been explored as a source of natural products. In 2004, total 716 new marine compounds were discovered and further 812 novel compounds were found in 2005 (Harvey, 2007).

1.2 Mantanani Island

The Mantanani Islands are surrounded by South China Sea. The Mantanani Islands are included the three islands that can be found at the north-west coast of the Sabah state, Malaysia (Mari Mari Mantanani, n.d.). The Mantanani Islands consisted of one large island called Mantanani Besar and two small island called Mantanani Kecil and Lungisan. The diverse marine organisms such as soft corals can be found in the Mantanani Islands (Rowthorn *et al.*, 2008).

1.3 Soft Coral

Soft corals are from phylum Cnidaria which have about 90 genera that belong to 23 families that are distributed around the world but especially rich in the Indo-Pacific region (Chanmethakul, 2010; Lim, 2010). Soft corals are sessile organisms that living in harsh and competitive marine environment, therefore they produced many structural diverse and complex natural products or secondary metabolites through the evolutionary pressure such as predation or competition for space and resources. Many of these natural products cannot be found in terrestrial organisms and proved to have wide range of biological activities (Putra, 2012).

Soft corals have soft bodies that contained huge number of eight-fold symmetry tentacles in their polyps with fleshy tissue. Despite their soft bodies, their tissues have small hard sclerites which provided support to their bodies. Other characteristic of soft corals are the mutual relationship between soft corals and photosynthetic dinoflagellate called zooxanthellae (Lim, 2010). Whereas symbiotic relationship between soft coral and algae are called zooxanthellate. These symbionts are lived inside the tissues of soft corals, thus soft corals provide them with shelter and wastes, in return the soft corals gained photosynthetic foods from them.

Soft corals often found in the dim habitats such as under rock formations or grow erect like trees in order to protect themselves. Generally, soft corals can be found in the intertidal zones and few are lived at the depths of 650 feet or 200 m or more. On the surface of earth, corals occupied 1.1% of the oceans and 0.3% of the salt water (Madina, 2006).

1.4 Secondary metabolite

In the ocean, marine organisms such as soft corals are the source of diverse structurally unique secondary metabolites due to the great diversity of their life strategies, defence strategies and evolutionary reasons. Example of secondary metabolites such as terpenoids are the monoterpenes (C_{10}) derived from two isoprene units, sesquiterpenes (C_{15}) are formed from three isoprene units, diterpenes (C_{20}) synthesized by four isoprene units and less common sesterterpene (C_{25}) constructed from five isoprene units (In addition, other larger terpenoids are triterpenes (C_{30}) and tetraterpenes (C_{40}) (Agostini-Costa *et al.*, 2012).

The secondary metabolites or natural products are organic compounds that are not use for normal growth, development or reproduction of an organism. In addition, an organism would not immediately death in the absence of secondary metabolites. However, absence of secondary metabolites might lead to long-term impairment of organism ability to defence and survive such as anti-predator, anti-feeding, antimicrobial, anti-fouling or to adapt the environment or stress tolerance. Besides that, allelechemical compounds are accumulated and released by the soft coral to interact another organism such competitor in a way that affected their growth and survival (Agostini-Costa *et al.*, 2012). Many of these compounds show variety of biological activities especially pharmacological activities that are potential for drug leads discovery. Moreover, many compounds isolated from the microorganisms, algae and invertebrates such as soft corals have used to produce many life saving drugs (Jha & Xu, 2004).

1.5 Justification

Less study or research were conducted on the secondary metabolites of soft corals from the Mantanani Islands as soft corals are known to have wide range of biological activities, yet the pharmacological functions of these metabolites are still not fully understood. The second major cause of death worldwide is due to infectious diseases (Lam, 2007). In addition, pathogens are kept evolving to acquire drug-resistant abilities. Hence, it is no doubt finding novel compounds and large scale screening of compounds to combat these drug-resistant pathogens is upmost priority or the

combination use of novel compound with existing antibiotic to compromise the development of drug-resistant by bacteria. Therefore, it is imperative to perform antibacterial bioassay of isolated metabolites from soft corals in the Mantanani Islands.

1.6 Objectives

This research aimed to study of secondary metabolites of soft corals from the Mantanani Islands. Detailed objectives are as follows:
To extract and quantify the crude extracts from the soft corals at the Mantanani Islands.

1) To quantify and perform chemical profiling of the crude extracts from the soft corals at the Mantanani Islands.

2) To isolate major compounds or secondary metabolites from the crude extracts of soft corals from the Mantanani Islands.

3) To elucidate the chemical structure from the isolated compounds.

4) To evaluate the antibacterial activity of the isolated compounds of soft corals from Mantanani Islands.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Abundance of new secondary metabolites have found in various marine organisms included soft corals in the past decades. These new key structure and promising compounds are the candidates for new therapeutic agents for many diseases. Bioprospecting for novel compounds never ceased and each year the novel compounds are continuously added to the natural product libraries. In ten years, from year 2000 to 2009, total of 5,286 of new natural products have discovered from variety of marine organisms. Among these new compounds from 2000 to 2009, terpenoids have covered 40.5% of them. Besides that, among 5,286 of new compounds from 2000 to 2009 is actually isolated from phylum Cnidaria of 33.5 % among marine invertebrates (Leal *et al.*, 2012a). Therefore, soft corals such as genus *Sinularia* have attracted a lot of attention due to their secondary metabolites that have been well known for the structural diversity and a wide range of biological activities (Cheng *et al.*, 2009).

2.2 Different classes of secondary metabolites from soft corals

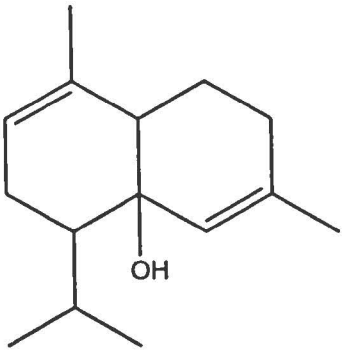
Marine organisms such as soft corals are rich in complex and diverse sterols. In addition, the bioactive metabolites such as sesquiterpenes, diterpenes and steroids

are commonly found in soft corals (Vairappan *et al.*, 2009b). Hence, soft corals are accumulated and rich in terpenoids (Jha & Xu, 2004).

Among the secondary metabolites, the largest class and most structural diverse are the terpenoids, with about 55,000 known members (Zhou, 2011). The isoprene units or 2-methyl-1,3-butadiene are important component that synthesized all terpenoids through the process of condensation (Mahmoud *et al.*, 2002). Terpenoids are classified into several groups based on the number of isoprene unit, one isoprene unit consist of five carbons. Therefore monoterpenes (C_{10}) derived from two isoprene units, sesquiterpenes (C_{15}) are formed from three isoprene units, diterpenes (C_{20}) synthesized by four isoprene units and less common sesterterpene (C_{25}) constructed from five isoprene units (In addition, other larger terpenoids are triterpenes (C_{30}) and tetraterpenes (C_{40}) (Agostini-Costa *et al.*, 2012). The terpenoids are biosynthesized from the mevalonate and methylerythritol phosphate pathways (Dewick, 2009).

2.2.1 Sesquiterpene

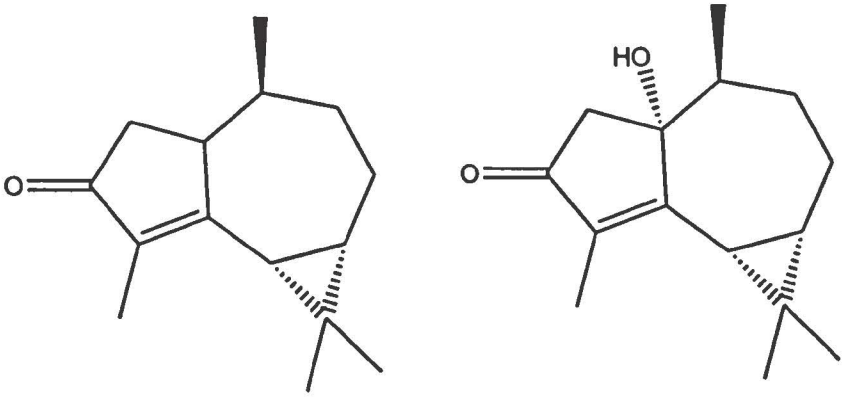
Sesquiterpenes are formed from three isoprene units. The term of sesquiterpenes are derived from the Latin prefix where sesqui mean one and a half time. Sesquiterpenes with fifteen carbon atoms have three possible cyclization modes such as mono-, bi-, and tri-cyclic structures. Thus, the amount of known natural sesquiterpenoids are more than known natural monoterpenoids (Dewick, 2009). The presence of fifteen carbons and four methyls are the two significant characteristics that indicated a sesquiterpenoid. Based on spectroscopic data, a bicyclic cadinane-type sesquiterpenoid characterized as 6-hydroxy -α- muurolene (**1**) was elucidated which isolated from the Red Sea soft coral *Heteroxenia fuscescens* as shown in Figure 2.1 (Mohammed *et al.*, 2012).



(1)

Figure 2.1 : A cadinane-type sesquiterpenoid was isolated from the Red Sea soft coral *Heteroxenia fuscescens*.

Two oxygenated tricyclic sesquitepenoids were isolated from a soft coral *Nephthea* species of Andaman and Nicobar coasts of Indian ocean. The structure of these compounds were elucidated through spectroscopic data. Among the two compounds, one of the oxygenated tricyclic sesquitepenoids were a new compound which characterized as 1α-hydroxy-(+)-cyclocolorenone or 4-aromadendrene - 1α-ol-3-one (**2**) and along the known compound which characterized as (+)-cyclocolorenone or 4-aromadendrene-3-one (**3**) as shown in Figure 2.2 (Rao *et al.*, 2003).

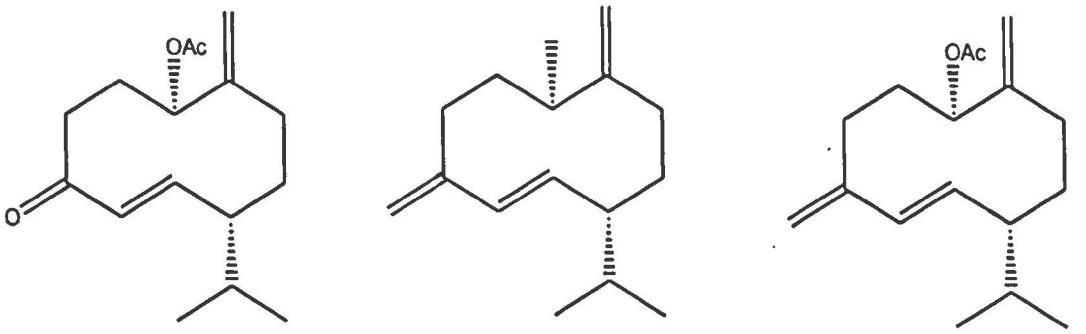


(2) **(3)**

Figure 2.2 : Two oxygenated tricyclic sesquitepenoids were isolated from a soft coral *Nephthea* species of Andaman and Nicobar coasts of Indian ocean.

2.2.2 Norsesquiterpene

Three monocyclic germacrane-type norsesquiterpenoids were isolated from a Bornean soft coral genus *Nephthea*, a new germacrane-type norsesquiterpenoid was characterized known as 1-acetoxy-germacra-5*E*,10(14)-diene-4-one (**4**), while the other two known germacrane-type norsesquiterpenoids were characterized as germacra-4(15),5*E*,10(14)-trien-1-ol (**5**) and [9,15,16], 1-acetoxy-germacra-4(15),5*E*,10(14)-triene (**6**) as shown in Figure 2.3 (Vairappan *et al.*, 2009b). The structure elucidation was based on spectroscopic methods.



(**4**) (**5**) (**6**)

Figure 2.3 : Three germacrane-type norsesquiterpenoids isolated from Bornean soft coral genus *Nephthea.*

2.2.3 Diterpene

Diterpenes arise from geranylgeranyl diphosphate (GGPP) that was formed from four isoprene units. Phytol (**7**) is one of the most basic among the diterpenoids as shown in Figure 2.4 (Dewick, 2009). It was one of the components of chlorophylls such as phytol formed the lipophilic side chain for chlorophyll *a*. Diterpenoids with twenty carbon atoms have five possible possible cyclization modes such as acyclic, mono-, bi-, tri-, and tetra-cyclic structures. Soft corals are rich in structural diverse diterpenoids such as the cembrane, norcembrane, eunicellin, germacrane and cubitane-type diterpenoids.

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