CHEMICAL PROFILING
AND ISOLATION OF BIOACTIVE METABOLITES OF GORGONIANS

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ABSTRACT

This project studies the bioactive properties of the Gorgonians, being carnivorous soft corals with specialised nerve cells. These organisms are intermediate between hard corals and soft corals as they contain a rigid skeleton and are classified under the phylum Cnidarians. Five samples of Gorgonians were studied (Ug#k#sp1, Ug#k#sp2, Ug#k#sp3, Ug#k#sp5, Ug#k#sp6). Yield of crude for all 5 species displayed prominent bioactive properties by inhibiting the growth of environmental bacteria, leading to the isolation of compound 1 from the MeOH crude of Ug#k#sp6. As much as 0.847 g (0.37%) of MeOH crude and 0.936 g (0.41%) of Hexane crude was obtained from the species studied. Therefore, based on spectral data Compound 1 that was isolated from Ug#k#sp6 can be assigned as $C_8H_{18}O_6$. This compound is active against *Clostridium sordelli* and *Vibrio parahaemolyticus*. Apart from this, suspected sterols were isolated from all 5 species and a pattern of similarity could be seen as they contained approximately the same Rf value based on Thin Layer Chromatography suggesting their use as biomarkers for species of Gorgonian. Studies on marine natural product shows the various defence of marine organisms towards the environment and isolation of these products can be put to beneficial use through recent advance in biotechnology.

ABSTRAK

Projek ini mengkaji ciri-ciri bio-aktif Gorgonian, sejenis batu karang karnivor yang mempunyai sel-sel saraf. Orgnisma ini mempunyai tulang belakang dan diklasifikasikan dalam filum Cnidaria. Sebanyak lima sample (Ug#k#sp1, Ug#k#sp2, Ug#k#sp3, Ug#k#sp5, Ug#k#sp6) dikaji. Ekstrak daripada semua spesis menunjukkan ciri-ciri bio-aktif yang ketara dengan menghalang penumbuhan bacteria padanya dan seterusnya membawa kepada penemuan struktur 1 dari ekstrak MeOH sample Ug#k#sp6. Sebanyak 0.847 g (0.37%) ekstrak MeOH dan 0.936 g (0.41%) ekstrak Hexane diperolehi daripada sepsis yang dikaji. Dengan berpandukan kepada data spectra, struktur 1 boleh dikatakan berformula $C_8H_{18}O_6$. Struktur ini didapati aktif terhadap *Clostridium sordelli* dan *Vibrio parahaemolyticus.* Selain ini, beberapa sterol juga telah diasingkan dan ujian kromatografi yang dijalankan menunjukkan persamaan nilai Rf pada kesemua kemungkinan sterol yang diasingkan. Ini boleh dijadikan sejenis penanda bagi membezakan kumpulan Gorgonian dengan organisma lain. Kajian berkenaan produk semulajadi marin menunjukkan pelbagai jenis mekanisma pertahanan organisma marin. Pengasingan produk- produk ini boleh disahkan kepentingannya dengan perkembangan dalam arena bioteknologi.

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LIST OF SYMBOLS AND ABBREVIATIONS

°C	Degree Celsius
BuOH	Buthanol
TLC	Thin Layer Chromatography
PTLC	Preparative Thin Layer Chromatography
MeOH	Methanol
ml	Milliliter
cm	Centimeter
UV	Ultraviolet
nm	Nanometer
MS	Mass Spectroscopy
FTIR	Fourier Transform Infrared Spectroscopy
GC	Gas Chromatography
LC	Liquid Chromatography
IR	Infrared
^{1}H-NMR	proton nuclear magnetic resonance
^{13}C-NMR	carbon-13 nuclear magnetic resonance
R_f	mobility relative to front
δ	chemical shift
dd	doublet
ddd	triplet
$CDCl_3$	chloroform-D1
$CHCl_3$	chloroform
Na_2SO_4	anhydrous sodium sulphate
Hex	hexane
H_2O	water
EtOAc	ethyl acetate

CHAPTER 1

INTRODUCTION

1.1. Ocean

The ocean sustains a great deal of life form comprising of both animal and plants. Marine organisms such as sponges, tunicates and sea hares are known to synthesize primary and secondary metabolites. Primary metabolites are compounds that are synthesised and used in the daily lives of organisms. Some examples of such are protein, carbohydrates and lipids. Secondary metabolites are compounds that are synthesised by organisms due to the various environmental conditions to the organisms. The synthesis of secondary metabolites requires additional energy, so its usage by a marine organism is minimized. These metabolites are used for various purposes such as defence and communication, but most remain unclear. These metabolites attract scientists worldwide as potential novel drugs used in pharmacology (Bruno et al, 2005). For example, *Aplysia* from sea hare is used as depilatory and essences from gastropod opercula is used in perfumes and incense (Libes, 1992).

1.1.1. Corals

Ninety- seven percent of marine organisms are invertebrates, generally known as organisms without backbones. They are soft bodied organisms, which lack internal skeleton but some do possess hard, protective calcareous coverings. Corals, jellyfishes and sea anemones are invertebrates classified under the phylum Cnidarians (Castro and Huber, 2005).

Corals are categorised as animals that are made up of polyps, which looks like upside down jellyfishes. The polyps contain stinging cells, which give protection to the corals as well as providing food by entrapping suspended particles in the water. These polyps live symbiotically with zooxanthellae that provide food to the polyps through photosynthesis. Zooxanthellae are algae that give colour to the corals (Koenig, 2004).

Basically, corals are divided into hard and soft corals. Hard corals are reef builders that sustain a biodiversity of organisms. They are important to human as a source of food, shore breaks to reduce the impacts caused by wave action onto coastal zones, being a source for pharmaceutical products and plays a major role in boosting the tourism industry. Hard corals exist in different growth forms such as coral branching, tabular, encrusting, massive, sub-massive, foliose, the free living and columnar (Hill, 2003).

Another form of coral is the soft coral. These are not reef builders as their skeleton is made from soft tissue that is supported internally like re-enforced concrete by a matrix of calcareous elements. Most soft corals can be differentiated from hard corals by wafting the water column and the whole structure will move. Soft corals are known as octocorals with polyps of eight tentacles, unlike hard corals which has polyps of six tentacles (Hill, 2003).

1.1.2. Gorgonian

Gorgonian, are soft coral with rigid skeleton. Being carnivorous and having specialised nerve cells, this organisms are categorised under the phylum Cnidaria (Castro and Huber, 2005). Gorgonians can be fan-shaped, whip-like or branching. They are usually anchored onto substratum by a holdfast and sway with the movement of the water current. Gorgonian form forests on some nearshore hard bottom areas, and even the bottoms of channels and canals. Its distribution and abundance depend on environmental factors such as substrate, light, temperature as well as current. Generally, gorgonians are found on hard ocean bottom substrate of boulders from 10 - 3,500 meters deep, at temperatures of 3.0° C and above (Gotthardt, 2005).

Gorgonians are octocoral with polyps of eight tentacles which produce a skeleton consisting of protein, made up of a wood-like core that is surrounded by a softer layer called the rind. Coral polyps are embedded in this rind and extend their bodies through openings in order to trap zooplanktons as feed (Matley, 1998).

Gorgonian can reproduce sexually or asexually. Sexual reproduction happens when the sperms of the male polyps fertilizes the eggs released by the female polyps.

Fertilized eggs develop into planula larvae that eventually grow into young polyps. Most polyps do not survive resulting from unfavourable substrate, eaten by predators or smothered by sediment. Some gorgonian species are capable of asexually reproduction whereby the colony continues to grow by budding polyps and secreting skeletal material (Gotthardt, 2005).

Gorgonians are also a source of marine natural products (eg; Sung et al, 2003; Fenical, 1987; Reddy et al, 2005; Rajeev and Ji-rong, 2004) as researches have illustrated that the gorgonians contained high levels of secondary metabolites. Gorgonians are recorded to acetogenins, sesquiterpenoids, diterpenoids, and even some highly functionalized steroids (Fenical, 1987). Some of such metabolites extracted have even made its way into the cosmetic marketplace, for instance, ambergris is used as a perfume fixative (Kijjoa and Sawangwong, 2004).

Marine natural products, which are obtained from the bioactive metabolites in marine organisms, were synthesized due to such major variability in environmental conditions. It covers a wide range of temperature; below freezing temperatures in Antarctic waters to about 350°C in deep hydrothermal vents, pressure range of 1 to 1000 atmosphere, nutrient range from oligotrophic to eutrophic and it has extensive photic and non-photic zones. Formation of bioactive metabolites in the gorgonians is a result of adaptations to the various unique environmental conditions in the water. These compounds or drugs are found abundantly in the marine environment compared to the terrestrial environment (Rajeev and Zi-Rong, 2004).

It has long been proven that gorgonians are source of prostaglandins (PG) since Weinheiner found PG-A_2 in Plexaura in 1969. Thanks to much availability, research into the use of marine natural products as medical agents is very active due to ethno-medical history and extensive collection of these sources. On the other hand, modern technologies have opened up new applications for the extraction of biomedical compounds from them.

1.1.3. Biomarkers

Synthesis of sterols is species specific. Dinoflagellates are the only organisms that produce sterol dinosterol. Only sponges are known to synthesize poriferasterol and spongosterol and gorgonians with gorgosterols (Libes, 1992). These compounds which synthesised by its respective organisms are called biomarkers. Such compounds are biological identities for a specific organism. Biomarkers are considered indicators of the presence of a particular organism. It is the biological production of an organism. To be concise, biomarkers is an identity, which is available to a specific group of organism and cannot be inherited by others (Peakall and Shugart, 1993).

1.1.4. Objectives

The major objective of this project is to find secondary metabolites in the Gorgonians studied.

The specific objectives are ;

1. To do a chemical profiling such as Thin Layer Chromatography on the crude extract of the Gorgonian studied
2. To isolate the bioactive metabolites from the studied gorgonian
3. To structurally identify any active metabolites available in the particular sample.
4. To investigate the presence of biomarkers for Gorgonians as a whole

CHAPTER 2

LITERATURE REVIEW

2.1. Secondary Metabolites

Gorgonians, in general are known to possess a wide range of secondary metabolites, which points out to be rather useful in various fields. To date, of all the 7000 marine natural products, which have been isolated from organisms, approximately 18 percent was contributed by the phylum Cnidaria. Through various researches that have been carried out (Sung et al, 2003; Fenical, 1987; Reddy et al, 2005; Rajeev and Ji-rong, 2004), it is shown that Gorgonians contain compounds that can inhibit the settlement of algae and both adult and larval form of barnacles. In addition, the extracts of Gorgonians even show antibacterial properties as well. Apart from these, there were a series of compounds that had been isolated from this particular group of corals namely terpenoids, steroids, eucinin, and peridinin. Terpenoids, for instance were known for its capability to cause tissue necrosis when it comes in contact with neighbouring corals and the cembranoid diterpenes from these organisms shows antifouling properties (Koh et al, 2002).

2.1.1 Genus Junceella (Appendix A to D)

Gorgonian corals of the genus *Junceella* are extensively distributed on the subtropical and tropical waters of the Indo-Pacific Ocean. Research had been done onto corals of this genus and a series of novel secondary metabolites had been isolated (Sung et al, 2003). Among the secondary metabolites that were isolated includes 39 briarane-type diterpenoids, three steroids, six *N*-acylsphingosines and an amine derivative, triacetonamine (TAA).

According to Sung, seven briarane- type derivatives, junceellolides A-D (1-4) and E-G (5-7) were successfully isolated from *J.fragilis* that was collected from the Taiwanese waters and the South China Sea. Samples collected from Indonesian waters provided four diterpenoids with briarane carbon skeleton. These compounds were named (-)-4-deacetyljunceellolide D (**8**), (+)-11α, 20α-epoxyjunceellolide D (**9**), (-)-11α, 20α-epoxy-4-deacetyljunceellolide D (**10**) and (-)-11α, 20α-epoxy-4-deacetoxyjunceellolide D (**11**). The other compounds isolated from this species of Gorgonian had similarity with compounds from other organisms for instance junceellin A (**13**), praelolide (**14**) and umbraculolide A (**15**).

Gorgonian species *Junceella gemmacea* that was collected from the Australian waters was a source for three types of briaranes ; (1*R*, 2*R*, 5*Z*, 7*R*, 8*S*, 9*R*, 10*R*, 11*R*, 14*R*, 17*S*)-2, 14-diacetoxy-8, 17:11, 20-bis-epoxy-9-hydroxybriara-5-en-18-one (**16**), (1*R*, 2*R*, 5*Z*, 7*R*, 8*S*, 9*R*, 10*R*, 11*Z*, 14*R*, 17*S*)-2, 14-diacetoxy-8, 17-epoxy-9-hydroxybriara-5, 11-dien-18-one (**17**) and (1*R*, 2*R*, 5*Z*, 7*R*, 8*S*, 9*R*, 10*R*, 12*R*, 14*R*, 17*S*)-2, 14-diacetoxy-8, 17-epoxy-9, 12-dihydroxybriara-5, 11(20)-dien-18-one (**18**).

On the other hand, six highly oxidized diterpenoids named gemmacolides A-F (19-24) were obtained from this species from Micronesia (Sung et al, 2003).

Next, from the gorgonian species ***Junceella juncea*** of the Red Sea, was isolated diterpenoids juncins A-F (25-30). Then, from the Indian Ocean species, two new briarane-type metabolites, denoted as juncins G (**31**) and H (**32**), along with the antipodal derivatives of the known gemmacolides A and B, briaranes **33** and **34**, and three polyhydroxyl steroids, (24*S*)-24-methylcholestane-3β, 5α, 6β-triol, 25-monoacetate (**35**), (24*S*)-24-methyl- cholestane-3β, 5α, 6β, 25-tetrol (**36**), (22*E*, 24*R*)-24-methylcholesta- 7, 22-dien-3β, 5α, 6β-triol (**37**), were isolated. On the other hand, five briaranes ; juncenolides A-D (**38-41**) and junceellolide C (**3**) was isolated from *J. Juncea* of Taiwanese waters (Sung et al, 2003).

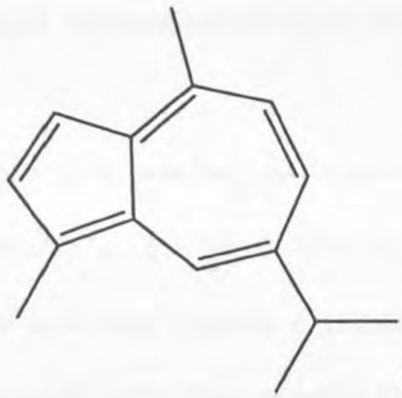
Finally, from *J. Squamata* collected from the South China Sea, two new briarane-type metabolites, junceellins A (**13**) and B (**42**), along with praelolide (**14**) and a bioactive substance, triacetonamine (TAA) (**43**) as well as six *N*-acylsphingosines **44–49** were isolated (Sung et al, 2003).

2.1.2 Genus Euplexaura

Apart from the various compounds isolated from gorgonian genus Junceella, in 1980, a team of researches from Japan isolated the guaiazulene compound from the gorgonian *Euplexaura* erecta. This is indeed interesting because guaiazulene has commonly been found in essential oils of terrestrial plants and in the marine red alga.

Therefore, this isolation of guaiazulene (**50**) from the particular gorgonian is considered the first isolation from the coral

Figure 2.1 Structure of Guaiazulene



(50)

because previous records only show of guaiane skeleton containing sesquiterpenes being isolated (Fusetanil, 1981). In relation to the guaizulenes, this compound has positive reaction in a variety of activities including antifungal, antitumor, antibacterial, immuno-regulatory, and anti-proliferative effects on fertilized sea urchin and ascidian eggs.

2.1.3 Genus Briareum (Appendix Table E)

On the other hand, several compounds have also been isolated from gorgonian octocorals of the genus Briareum. These are organisms classified under the order Alycyonacea and Gorgonacea. The very first briarane-type natural product, briarane A, was isolated from this genus through the octocoral *Briareum asbestinum* since 1977 and natural products from this genus was the centre of attraction due to the complexity and biological activity related to such compounds (Sung et al, 2006).

REFERENCE

Barnes, M., 2002. Fourier Transform Infrared Spectroscopy. *Practicing Oil Analysis* Magazine. March 2002

Ben-Yosef, D.Z. and Benayahu, Y., 1999. The gorgonian coral Acabaria biserialis: life history of a successful colonizer of artificial substrata. *Marine Biology* (1999) 135: 473-481

Bheemasankar, R.Ch., Ramana, K.V., Venkata, R.D., Fahy, E., and Faulkner, D.J., 1988. Metabolites of the Gorgonian *Isis Hippuris* from India. *Journal of Natural Products,* Vol. 51, No. 5, pages. 954-958.

Castro, P. and Huber, M.E., 2005. *Marine Biology*, 5^{th} edition. McGraw-Hill, New York.

Dube, D., Kim, K., Alker, A.P., Harvell, D.C., 2002. Size structure and geographic variation in chemical resistance of sea fan corals Gorgonia ventalina to a fungal pathogen. *Marine Ecology Progress Series* Vol. 231: 139–150.

Faulkner, D.J., 2000. Marine pharmacology. *Antonie van Leeuwenhoek* 77: 135–145.

Fenical, W., 1987. Marine Soft Corals of the Genus *Pseudopterogorgia:* A Resource For Novel Anti-Inflammatory Diterpenoids. *Journal of Natural Products* Vol. 50. NO. 6. pp. 1001-1008, NGV-DK.

Fleurya, B.G., Collb, J.C., Sammarcoc, P.W., Tentorid, E. and Duquesned, S., 2004. Complementary (secondary) metabolites in an octocoral competing with a scleractinian coral: effects of varying nutrient regimes. *Journal of Experimental Marine Biology and Ecology* 303 (2004) 115– 131.

Fusetanil, N., Matsunaga, S. and Konosu, S., 1981. Bioactive marine metabolites I. Isolation of guaiazulene from the gorgonian *Euplexaura erecta*. *Experientia* 37 (1981), Birkhauser Verlag, Basel (Schweiz)

Garrison, T., 2004. *Oceanography : An Invitation to Marine Science*, 5th edition. Brooks and Cole, United States of America.

Gotthardt T, 2005. Gorgonian Corals. www.natureserve.org/explorer.

Hill. J, 2003. www.reefcheck.org

Intertek Group plc, 2007. Fourier Transform Infrared Laboratories. http://www.intertek-cb.com/newsitetest/news/ftirlab.shtml

Kijjoa, A., and Sawangwong, P., 2004. Drugs and Cosmetics from the Sea. *Marine Drugs* 2, 73-82.

Kim, K., 1994. Antimicrobial activity in gorgonian corals (Coelenterata, Octocorallia) *Coral Reefs* (1994) 13:75 80.

Kim, K., Kim, P.D., Alker, A.P., Harvell, C.D., 2000. Chemical resistance of Gorgonian Corals against fungal infections. *Marine Biology* (2000) 137: 393- 401.

Koenig P, 2004. http://coralreefwatch.noaa.gov/

Koh, L.L., Tan, T.K., Chou, L.M., Goh, N.K.C., 2002. Antifungal properties of Singapore gorgonians: a preliminary study. *Journal of Experimental Marine Biology and Ecology* 273 (2002), 121– 130.

Lages, B.G., Fleury, B.G., Ferreira, C.E.L., Pereira, R.C., 2006. Chemical defense of an exotic coral as invasion strategy. *Journal of Experimental Marine Biology and Ecology*. 328 (2006) 127– 135

Libes, S., 1992. *An Introduction to Marine Biogeochemistry*. John Wiley and Sons, Canada.

Maia, L.F., Epifanio, R.A., Eve, T., Fenical, W., 1999. New Fish Feeding Deterrents, Including a Novel Sesquiterpenoid Heterogorgiolide, from the Brazilian Gorgonian *Heterogorgia uatumani* (Octocorallia, Gorgonacea). *Journal of Natural Product* 1999, *62,* 1322-1324.

Martin, M.C., 2007.Characteristic IR Band Position. http://infrared.als.lbl.gov/IRbands.html

Martins, D.L. and Epifanio, A.R., 1998. A New Germacrane Sesquiterpene from the Brazilian Endemic Gorgonian *Phyllogorgia dilatata* Esper. *Journal of the Brazilian Chemical Society* vol.9 no.6.

Matley, A., 1998. Gorgonian are Corals Too. http://www.fknms.nos.noaa.gov/news/gorgon.html

Millero, F.J., 1996. *Chemical Oceanography*, 2nd Edition. CRC Press: LLC, United States of America.

Peakall, D. and Shugart, L., 1993. *Biomarkers, Research and Application in the Assessment of Environmental Health*. Springer-Verlag Berlin Heidelberg, Germany.

Pordesimo, E.O., Schmitz, F.J., Ciereszko, L.S., Hossain, M.B., and Helm, D., 1991. New Briarein Diterpenes from the Caribbean Gorgonians *Erythropodium caribaeorum* and *Briareum* sp. *J. Org. Chem.* 1991, 56, 2344-2357.

Rajeev, K.J., and Zi-rong, X., 2004. Biomedical Compounds from Marine organisms. *Mar. Drugs* 2004, *2*, 123-146.

Reddy, N.S., Reed, J.K., Longley, R.E., and Wright, A.E., 2004. Two New Cytotoxic Linderazulenes from a Deep-Sea Gorgonian of the Genus *Paramuricea*. *J. Nat. Prod.* 2005, *68,* 248-250.

Rodriguez, A.D., 1995. The Natural Product Chemistry of West Indian Gorgonian Octocorals. *Tetrahedron* 0040-4020(95)00216-2

Salter-Cid, L. and Bigger, C.H., 1991. Alloimmunity in the Gorgonian Coral Swiftia exserta. *Biology Bull* 181: 127-134.

Sa´nchez, J.A. and Lasker, H.R., 2003. Growth following partial mortality in corals. *The Royal Society.*

Sung, P.J., Chen, Y.P., Hwang, T.L., Hu, W.P., Fang, L.S., Wu, Y.C., Lia, J.J. and Sheuc, J.H., 2006. Briaexcavatins C–F, four new briarane-related diterpenoids from the Formosan octocoral Briareum excavatum (Briareidae). *Tetrahedron* 62 (2006) 5686–5691

Sung, P.J., Gwo, H.H., Fan, T.Y., Li, J.J., Dong, J., Han, C.C., Wu, S.L., Fang, L.S., 2002. Natural product chemistry of gorgonian corals of the genus *Junceella. Biochemical Systematics and Ecology* 32 (2004) 185–196.

Targett, N. M., Bishop, S. S., McConnell, O. J., and Yoder, J. A., 1982. Antifouling agents against the benthic marine diatom, *Navicula salinicola* Homarine from the gorgonians *Leptogorgia virgulata* and *L. setacea* and analogs. *Journal of Chemical Ecology* Volume 9, Number 7, 817 – 829.

Tissue, B.M., 2000. Introduction to Mass Spectrometry (MS). *The Chemistry Hypermedia Project*. http://www.chem.vt.edu/chem-ed/ms/ms-intro.html

Vairappan, C.S., 2003. Potent antibacterial activity of halogenated metabolites from Malaysian red algae, Laurencia majuscula (Rhodomelaceae, Ceramiales). *Biomolecular Engineering*. 20 (2003) 255- 259.

Wikipedia, 2007. Infrared spectroscopy correlation table. http://en.wikipedia.org/wiki/Infrared_spectroscopy_correlation_table

Wikipedia, 2007. Nuclear Magnetic Resonance. http://en.wikipedia.org/wiki/Nuclear_Magnetic_Resonance

Young, P.R., 1997. Mass Spectroscopy. http://www.chem.uic.edu/web1/OCOLII/WIN/SPEC.HTM. Brooks/Cole Publishing Company