BIOASSAY GUIDED ISOLATION OF BIO-ACTIVE METABOLITES FROM SEVERAL SPECIES OF MALAYSIAN SOFT CORAL

JEGA DIVAN S/O SUNDRAMOORTHY

THIS THESIS IS SUBMITTED AS A PART OF REQUIREMENT TO OBTAIN BACHELORS OF SCIENCE WITH HONORS

INDUSTRIAL CHEMISTRY PROGRAMME

SCHOOL OF SCIENCE AND TECHNOLOGY

UNIVERSITY MALAYSIA SABAH

FEBRUARY 2005

PUMS 99:1

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS@

JUDUL: Bioassay Guided Isolation of Bio-Active Metabolite From Malaysian Soft Coral

Ijazah: Degree of Bachelor with Honours

SESI PENGAJIAN: ~~2004/2005~~ 2001/2002.

Saya Jega Divan A/L Sundramoorthy

(HURUF BESAR)

mengaku membenarkan tesis (LPS/Sarjana/Doktor Falsafah)* ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:

1. Tesis adalah hakmilik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. **Sila tandakan (/)

✓	SULIT	(Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972)
	TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)
	TIDAK TERHAD	

Disahkan oleh

(TANDATANGAN PENULIS)

(TANDATANGAN PUSTAKAWAN)

Alamat Tetap: 36, Jalan 1, Desa Jaya, 52100 Kepong, Selangor.

Nama Penyelia

Tarikh: 26/3/05

Tarikh: ____________

CATATAN: * Potong yang tidak berkenaan.

** Jika tesis ini SULIT atau TERHAD, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai SULIT dan TERHAD.

@ Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana secara penyelidikan, atau disertasi bagi pengajian secara kerja kursus dan penyelidikan, atau Laporan Projek Sarjana Muda (LPSM).

DECLARATION

I here by declare that this thesis is my original work except the quotations and reference that have clearly noticeable sources.

21 February 2005

JEGA DIVAN S/0 SUNDRAMOORTHY

HS2001-2061

APPROVAL

Signature

1. SUPERVISOR

School of Science and Technology

(Dr. Charles.S.Vairappan) ------------------------------------

2. EXAMINER-1

School of Science and Technology

(Associate Professor Dr Mashitah Mohd Yusof) ------------------------------------

3. EXAMINER-2

School of Science and Technology

(En Jahimin Asik) ------------------------------------

4. DEAN

School of Science and Technology

(Associate Professor Dr Amran Ahmed) ------------------------------------

ACKNOWLEDGEMENT

First and foremost, I would like to take this opportunity to express my sincere gratitude to my supervisor Dr. Charles S. Vairappan for his guidance, encouragement and motivation throughout the course of this research. He has spent a lot of time and energy in guiding me completing my research work with the expected time plan. Besides that, throughout the research work, he have always made sure that we had enough chemicals and all the instruments that is necessary for the research is in good shape. It has been an honor working with a great researcher like him.

Besides that, I would like to send my warm regards to all the people in Marine Borneo Research Institute. They have always been friendly and supportive and created a great research environment throughout my research work there.

Last but not least I would like to thank my friends Kak Rosnita, Tan Kung Han, Ang May Yen, Goh Pei Nee, Cheng Yi, Kayethrie, Lechmanan and my parents. All have them have supported this research by various ways towards making my research to progress and finally complete.

All the love and guidance showed by these people towards me will always be in my mind. Hope they will be always great as what they are now. Thanks!

ABSTRACT

The research was done on four species of soft corals collected at Tanjung Aru beach at Kota Kinabalu, Sabah. The species were identified as, 1) *Sinularia*. sp1, 2) *Sinularia*.sp2, 3) *Lobophyton*. sp1 and 4) *Sarcophyton*. sp2. This soft corals possess bioactivity towards some laboratory bacteria strains. Bioactivity-guided fractionation of hexane extract from *Sinularia*. sp1 and *Sarcophyton*. sp2 resulted in isolation of 5 bioactive metabolites . The bioactive metabolites isolated from *Sinularia*. sp1 are **(1)** 9-Fatty Acid Methyl Esters (FAME) which are C6:0, C14:0, C16:0, C16:1, C18:ln9t, C18:ln9c, C17:0, C18:2n6t and C24:1 together with a **(2)** Sterol. Bioactive isolation of metabolites from *Sarcophyton*. sp2 resulted in isolation of 3 bioactive metabolites which are **(3)** Compound A, **(4)** Compound B and **(5)** Compound C. The Fatty Acid Methyl Esters and Sterol isolated were found to be active against the environmental bacteria strain Clostridium *cellobioparum* while Compound A, Compound B and Compound C were active towards the environmental bacteria strain *Clostridium sordelli*.

ABSTRAK

Kajian ini telah dijalankan ke atas empat spesis karang lembut yang telah dikutip di pantai Tanjung Aru, Kota Kinabalu, Sabah. Spesis-spesis ini telah dikenalpasti sebagai 1) *Sinularia.* sp1, 2) *Sinularia.*sp2, 3) *Lobophyton.* sp1 and 4) *Sarcophyton.* sp2. Karang lembut yang dikutip didapati mempunyai aktiviti biologi terhadap beberapa strain bakteria yang didapati daripada makmal. Pembahagian ekstrak hexana daripada *Sinularia.* sp1 dan *Sarcophyton.* sp2 berpandukan aktiviti biological memberi pengasingan kepada 5 metabolit yang bioaktif . Metabolik bioaktif yang diasingkan daripada *Sinularia.* sp1 adalah **(1)** 9-Asid Lemak Metil Ester (FAME) iaitu C6:0, C14:0, C16:0, C16:1, C18:ln9t, C18:ln9c, C17:0, C18:2n6t dan C24:1 bersamaan dengan **(2)** sterol. Pengasingan metabolik bioaktif dari *Sarcophyton.* sp2 membawa kepada pengasingan 3 metabolik bioaktif iaitu **(3)** metabolik A, (4) metabolik B dan (5) metabolik C. Asid Lemak Metil Ester (FAME) dan sterol didapati aktif terhadap bakteria persekitaran *Clostridium cellobioparum* manakala , metabolik A, metabolik B dan metabolik C didapati aktif terhadap bakteria persekitaran *Clostridium sordelli.*

CONTENTS

LIST OF TABLE

TABLE.NO **Page Number**

LIST OF FIGURES

LIST OF SYMBOL ABBREVATION

TLC	Thin Layer Chromatography
PTLC	Preparative Thin Layer Chromatography
UV	Ultra Violet
R_f	Mobility relative to front
g	Gram
mg	Milligram
ppm	Parts per million
FAME	Fatty Acid Methyl Ester

CHAPTER 1

INTRODUCTION

1.1 Marine Diversity

The oceans covers 71% of the earth's surface and traditionally classified into four large basins which are the Pacific ocean, Atlantic ocean, Indian ocean and finally the Arctic ocean. The Pacific ocean is the deepest and largest ocean, it is almost as large as all the other oceans combined. This is followed by the Atlantic ocean which is a bit larger than the Indian ocean and the smallest and shallowest is the Artic ocean (Castro & Huber, 2003). Because the earth is mostly covered by oceans, the majority habitat of living organism is found in the ocean.

The living organism are classified to five different system which are from the kingdom Animalia, Plantae, Fungi, Protista and Monera are found abundant in the ocean. In the kingdom Animalia, there are 16 Phylum's found so far which are the Phylum Annelida, Phylum Arthropoda, Phylum Brachiopoda, Phylum Bryozoa, Phylum Chordata, Phylum Cnidaria, Phylum Ctenophora , Phylum Echinodermata, Phylum Mollusca, Phylum Nematoda, Phylum Nermertea, Phylum Onychophora, Phylum Platyhelminthes, Phylum Phoronida, Phylum Porifera and finally

Rotifera (Hickman *et al.*, 2004). In the marine world, the Phylum Cnidaria are found abundant.

1.1.1 Phylum Cnidaria

According to Castro & Huber (2003), Cnidarians (also known as Coelenterates) are common or abundant in many marine environments. They display a wide variety of body forms and lifestyles, and include such familiar organisms as jellyfish , corals , and sea anemones. Throughout the phylum there is typically an alternation of two body forms, the polyp and medusae , however, in some groups one or the other form is reduced or absent. There are approximately 9,000 living species of Cnidarians , and they can be divided into four classes which are Hydrozoa, Scyhozoa, Anthozoa and Cubozoa.

The class Anthozoa includes the forms commonly known as sea anemones, corals, sea fans, and sea pens . There are both solitary and colonial forms that have no medusoid stage. Most are benthic and sessile, although some forms are capable of limited locomotion. The polyps reproduce both sexually and asexually. There are about 6,000 species of Anthozoans , all are marine(Harvell *et al.*,1988). Corals are colonial anthozoans and in tropical waters may be the dominant reef-building organisms. Many corals , however, do not take part in reef formation, and some forms are solitary polyps (Castro & Huber, 2003) .

The general characteristics of the Cnidaria are, radially symmetrical, sometimes with biradial symmetry, and an oral-aboral axis . Diploblastic , having ectoderm and entoderm , with a mesoglea or mesenchyme layer between. Digestive cavity with only one opening and which is also the only body cavity. Have unique structures, cnidae , which are stinging or adhesive structures produced by cells called cnidocytes . No specialized circulatory, respiratory, or excretory systems. Basic condition of alternating polypoid and medusoid body forms, but many forms have reduced or lost one or the other of these two forms. A diffuse nervous system as a simple nerve net and usually have a ciliated planula larvae (Hickman *et al.*, 2004).

1.2 Significance of the study

Like many other soft-bodied reef animals, soft corals avoid predation by storing toxic chemicals compounds in their tissue. This makes them highly unpalatable or even poisonous to most potential predators in the marine habitate. This discovery have interested marine scientist to study the chemistry of soft coral which made them free from a lot of marine predators. From the research done, secondary metabolites are found to play a key part in soft coral weapon against predators (Maida *et al.*, 1993).

It is also found that soft corals are rich source of terpenoids with diverse structures and various biological activity (Coll *et al.*,1985). They are known also to produce large amount of sesquiterpene and diterpenes with a wide variety of carbon skeletons(Athar *et al.*,2003). Among 30 species studied from different area of chemistry, cembroid type of diterpenes emerges the most. Most of them has been characterized as defensive, competitive and reproductive, which plays a functional roles in their survival (Longeon *et al.*,2002).

1.3 Objective

From the literature studies it was found that soft corals are known to produce secondary metabolites where some of them posses bioactivity. The varieties of secondary metabolites in soft corals are terpenes, diterpenes, cembroid structures, sterol, steroids, fatty acids and many more. The main aim of this research is to identify bioactive metabolites and isolate them from the respective organism. To achieve the research goal there are 3 main objectives.

a) To obtain crude extracts in different forms from soft corals species collected after identification. The crude extracts will be in 3 different forms based on their polarity which are hexane, 90% Methanol and n- Butanol.

b) Bioassay guided separation to identify and purify the bio-active metabolites with laboratory bacteria strains that involves many chromatographic methods. This step is important to isolate the pure bioactive metabolite from soft coral species.

c) Final step in achieving the research goal is to characterize the pure bioactive metabolite isolated. This is done with various spectroscopic methods that are available such as NMR measurement and gas chromatography measurement. This is important so that structure elucidation of bio-active metabolites isolated is possible. Based on the objective listed, it is hoped that the research will be successfully done without any problems.

CHAPTER 2

LITERATURE REVIEW

2.1 SOFT CORALS

Soft Corals are conspicuous and colourful components of coral reef throughout the Indo-Pacific (Terista *et al*., 1998). As their name suggest, soft corals have no internal skeleton of calcium carbonate like the reef-building Scleractinian corals. They are colonies of animals called polypoid cnidarians. These polyps rarely exceed 5mm in diameter and are arranged in soft fleshy, irregular shaped colonies up to 1 meter in size . All soft corals bodies consist of standard poly, although in some of them the basic shape may be very obscure (Coll *et al*., 1992).

Taxonomically the soft corals belong to the subclass alcyonaria of the class anthozoa. These animals are also known by the name Octocorals which consist of Soft Corals, Sea Pans, Gorgonians, pipe-organ corals and the blue corals. Soft Corals in the ocean feed by trapping prey with their tentacles. Stringing cells in the tentacles, called nematocyst, immobilize microscopic plankton and transfer it into the body cavity for digestion. However, many shallow water soft corals that are prominent on the reef also posses symbiotic algae known zooxanthelle. The zooxanthelle live in coenenchyme of the soft coral and use photosynthesis to produce sugars, fatty acids and amino acids that help them feed the colony. In return the zooxanthelle receive protection and nutrients such as nitrogen and carbon dioxide from the polps (Kokke *et al*., 1981).

2.2 Secondary metabolites

2.2.1 Secondary metabolites of Anthozoans

According to McClintock & Baker (2001), the phylum which consist of four classes: Hydrozoa, Scyphozoa, Cubozoa, and Anthozoa, the two promate classes, ,Scyphozoa and Cubozoa have very less reports on chemistry where they only represent 3% of the natural product reported from cnidarians. According to the statistics reports of this secondary metabolites by McClintock & Baker on the year 2001, it has been noticed that almost 98% of the metabolites from cnidarians are from the largest class, Anthozoa. The subclass Octocorallia accounts for 87% of all cnidarian compounds. Here it was found that terpenoids dominate across the Octocorallia, accounting from 92% of their reported metabolites. The order Alcynacea accounts for half of the metabolites within the subclass.

True soft corals (order Alcyonacea) and gorgonians(Gorgonacea) are chemically similar, where both product a vast array of isoprenoids, namely sesqui- and diterpenoids, although cembrane diterpenes are the most common group (McClintock & Baker, 2001). Alcynacean and gorgonian cembroids differ from each other by the fact that gorgonians cembroids usually have 1R configuration, while alcyonacean cembroid tend to possess 1S configuration (Coll, 1992). The distribution of diterpenes and sesquiterpenes varies across families; however volatile sesquiterpene hydrocarbons have been successfully used as chemotaxanomic markers for the Gorgonacea (Gerhart *et al*., 1983).

Anthozoans are also known to produce an extensive range of structurally interesting compounds with high bio-medical potential. Many anthozoans metabolites are assumed to be defense allemones, as predation is an important factor affecting the natural selection of marine organisms. Consequently, they have developed multiple defensive defense mechanisms, both chemical and structural, probably due to the inability of a single defense to avoid predators (McClintock & Baker, 2001). A tough, horny, axial skeleton is found in several anthozoans, especially gorgonians, and is composed of gorgonian , a horn-like protein-polysaccharide complex .

According to Mc McClintock & Baker (2001), there is a strong negative correlation between the degree of physical defense and the diversity of natural products. Where the swimming cnidarians such as jellyfish, the relative mobile sea anemones, and some solitary hydroids generally lack a rich complement of secondary metabolites and primarily produce neuropeptides and protein venoms. Where else, the most chemically prolific cnidarians are the soft-bodied colonial forms, such as corals, gorgonians, and zoanthids. Recent studies are now being concentrated on understanding the evolution of chemical defense and other chemical interactions among these organisms. As a result, many cnidarians were shown to have predator deterrent, antifouling, and overgrowth inhibitory activities (McClintock & Baker, 2001).

The ability of octocorals to avoid fouling has been attributed to the production of toxic secondary metabolites, particularly diterpenoids. It has been shown that soft corals release large quantities of terpenes into the surrounding water to kill neighbouring organism. (Coll *et al*., 1985). Terpene concentration, distribution, and functions in the colony appear to be very complex. In *Sinularia flexibilis*, 11,12-deoxyflexibilide was reported to serve as an ichthyotoxin, and sinulariolide as algicide, while flexibilide was involved in interference

REFERENCE

Aceret, T.L., Sammarcob, P.W. and Cell, J.C., 1995. Toxic effects of alcyonacean diterpenes on scleractinian corals. *Journal of Experimental Marine Biology and Ecology* **188,** 63-78.

Anjanayulu, A.S.R., Venugopal, M. J. R.V., Sarada, P., Rao, G.V., Clardy, J. and Lobkovsky, E., 1998. *Sarcophyton*, A novel Tetracyclic Diterpenoid from Indian Ocean Soft Coral *Sarcophyton elegens.Tetrahedron Letters* **39**, 135-138.

Atallah F. Ahmed., Shiue, R.T., Wang, G.H., Dai, C.F., Kuo, Y.H. and Sheu, J. H., 2003. Five novel norcembranoids from Sinularia leptoclados and S. parva. *Tetrahedron* **59,** 7337-7344.

Athar Ata., Ackerman, J. and Radhika, P., 2003. Cladioxazole: a novel sesquiterpene from a marine soft coralof genus *Cladiella. Tetrahedron Letters* **44,** 6951–6953.

Castro, P. and Huber, M.E., 2003. *Marine Biology*. Ed.4th. McGraw-Hill, New York.

Coll, J.C., 1992. The chemistry and chemical ecology of octocorals (Coelenterata, Anthozoa, Octocorallia). *Chemical Review* **92**, 613-631.

Coll, J.C., Bourden, B.F., Heaton., Scheur, P.J., Li, M.K.W., Clardy, J., Schulte, G.K. and Fine-Mooore, J., 1989. Structure and possible functions of epoxypukalide and pukalide. *Journal of Chemical Ecology* **15**, 1177-1189.

Coll, J.C., Bowden, B.F., Tapiolas, D.M., Willis., R.H., Djura, P., Streamer, M. and Trott, L., 1985. Studies of Australian soft corals-XXXV: the terpenoid chemistry of soft corals and its implication. *Tetrahedron* **41**, 293-297.

Coll, J.C., Leone, P.A., Bowden, B.F., Carroll, A.R., Koenig, G.M., Heaton, D.N., Maida, M., Alino, P.M., Willis, R.H., Babcock, R.C., Florian, Z., Clayton, M.N., Miller, R.L. and Alderslade, P.N., 1995. Chemical aspects of mass spawning in corals.II. (-)-*Epi-thunbergol*, the sperm attractant in the eggs of the soft coral *Lobophytum crassum. Marine Biology* **137**, 137-142

Dai, M.C., Garson, M.J. and Coll. J. C., 1991. Biosysnthetic process in soft corals. I.A comparison of terpene biosynthesis in Alcyonium molle (Alycyoniidae) and *Heteroxenia sp. Comparative Bio-chemical Physiology* **99B**, 775-779.

Duh, C.Y. and Hou, R.S., 1996. Cytotoxic cembroids from the soft coral *Sinularia gibberosa* and *Sarcophyton trocheilophorum. Journal of Natural Products* **59**, 595-598.

Duh, C.Y., Wong, S., Chia, M.C. and Chiang, M.Y., 1999. A novel cytotoxic norditerpenoid from Formosan soft coral *Sinilaria inelegans. Tetrahedron Letter* **40,** 6033-6035

Faulkner, D.J., 1995. Marine natural products. *Natural Product Report* **12**, 223-269.

Gerhart, D.J. and Coll, J.C., 1993. Pukalide, a widely distributed octocoral diterpenoid, induces vomiting in fish. *Journal of Chemical Ecology* **19**, 2697-2704.

Goud, T.V., Reddy, N.S., Krishnaiah, P. and Y. Venkateswarlu, Y., 2001. Spathulenol: a rare sesquiterpene from soft coral *Sinularia kavarattiensis. Biochemical Systematics and Ecology* **30,** 493–495.

Harvell, C.D., Fenical, W. and Greene, C.H., 1988. Chemical and structural defenses of Caribbean Gorgonians (*Pseudopterogorgia spp*). I. Development of *in situ* feeding assay. *Marine Ecology Progress* **129**, 141-148.

Hickman, C.P., Roberts, L.S., Larson, A. and I'Ansan, H., 2004. *Integrated Principles of Zoology.* Ed.12th. McGraw-Hill, New York.

Iwagawa, T., Masuda, T., Okamura, H. and Nakatani, M.,1996. New *Xenia* diterpenoids from a *Xenia* species soft coral. *Tetrahedron* **52**, 13121-13124.

Iwashima, M., Okamoto, K. and Iguchi,K.,1999.Clavirins, a new type marine oxylipins with a growth- inhibitory activity from Okinawan soft coral, *Clavularia viridis. Tetrahedron Letters* **40**, 6455-6459.

Iwashima, M., Watanabe, K., and Iguche, K., 1997. New marine prostanoids, preclavulone lactones, from the Okinawan soft coral *clavularia viridis. Tetrahedron Letters* **38**, 8319-8323.

Koh, E.G.L., 1997. Do scleractian corals engage in chemical warfare against microbe?. *Journal of Chemical Ecology* **23**, 379-342.

Kokke, W.C.M.C., Fenical,W., Bohlin. and Djerassis, C., 1981. Sterol synthesis by cultured zooxanthellae; implication concerning metabolism in the host-symbiont a association in Carribbean gorgonians. *Comparative Biochemical Physiology* **68B**, 281-285.

Koljak, R., Lopp, A., Pehkf, T.B., Varvas, K., Miiiirisepp, A.M., Jiirving, I. and Samel, N., 1998. New Cytotoxic Sterols from the Soft Coral *Gersemiu fruticosa. Tetrahedron* **54**, 179-1 86.

Lewis, R., Gaffin, D., Hoefnagels, M. and Parker, B., 2004. *Life*. Ed.5th. McGraw-Hill, New York.

Lindquist, N., Lobkovsky, E. and Clardy, J., 1996. Tridentatols A-C. A novel natural products of the marine hydroid *Tridentate marginata. Tetrahedron Letters* **37**, 9131-9142.

Longeon, A., Kondracki, M.L.B. and Guyot, M., 2002. Two new cembrane diterpenes from a Madagascan soft coral of the genus *Sarcophyton. Tetrahedron Letters* **43,** 5937-5939.

Maida, M., Caroll, A.R. and Coll, J.C., 1993. Variability of terpene content in the soft coral *Sinularia flexibilis* (Coelenterata: Octocorallia), and its ecological implication. *Journal of Chemical Ecology* **19**, 2285-2291.

McClintock, J.B. and Baker, B.J (eds.), 2001. *Marine Chemical Ecology*. CRC Press, Florida.

Miyaoka, H., Mitome, H., Nakano, M. and Yamada, Y., 2000. A New Xenicane-type Diterpenoid from the Okinawan Soft Coral, Xenia sp.; Absolute Configurations of Xeniaoxolane, Xeniolide-A and Xenialactol. *Tetrahedron* **56,** 7737-7740.

Papastephanou, C. and Anderson, D.G., 1982. Crassin acetate biosynthesis in a cell-free homogenate of zooxanthellae from *Plexaura porosa* (Houttyun). *Biology Bulletein* **138**, 334-341.

Ramesh, P., Reddy, N.S. and Venlmteswarlu, Y., 1998. Rameswaralide, A Novel Diterpenoid from the Soft Coral *Sinularia dissecta*. *Tetrahedron Letters* **39,** 8217-8220.

Rice, J.R., Papastephanou, C. and Anderson, D.G., 1970. Isolation, localization and biosynthesis of crassin acetate in *Pseudoplexoura porosa* (Houttyun). *Biology Bulletein* **138**, 334-338.

Rodriguez, A.D. and Boulanger, A., 1997. Novel natural products of the marine hydroid *Tridenta marginata*. *Journal of Natural Products* **60**, 207-209.

Schmitz, F.J., Krauss, K.W., Ciereszko, L.S., Sifford, D.H., and Weinheimer, A.J., Ancepsenolide., 1966. A novel bisbutenolide from gorgonian *Pterogorgia anceps*. (Pallas). *Tetrahedron Letters* **97**, 2234-2236.

Seo,Y., Jung, J.H., Rho, J.R.and Shin, J.,1995. Isolation of Novel Bioactive Steroids from the Soft Coral *Alcyonium gracillimum. Tetrahedron* **51**, 2497-2506.

Skoog, D.A., Holler, F.J. and Nieman, T.A., 1998. *Principles of Instrumental Analysis.* Ed.5th. Harcourt College Publishers, Florida.

Slattery, M., Hines, G.A., Starmer, J., and Paul, V.J., 1996. Chemical signals in gametogenesis, spawning and larval settlement and defense of the soft coral *Sinularia polydacta. Coral Reefs* **18**, 75-79.

Teresita L. A., Coll, J.C., Uchio, Y. and Sammarco, P.W., 1998. Antimicrobial activity of the diterpenes flexibilide and sinulariolide derived from *Sinularia flexibilis* Quoy and Gaimard 1833 (Coelenterata: Alcyonacea, Octocorallia). *Comparative Biochemistry and Physiology Part C* **120,** 121-126.

Watanabe, K., Sekine, M., Takahashi, H. and Iguchi, K., 2001.New Halogenated Marine Prostanoids with Cytotoxic Activity from the Okinawan Soft Coral *Clavularia viridis. Journal of Natural Product* **64,** 1421-1425.

Won, W., Rideout, J.A., and Chalker, B.E., 1995. Isolation and structure elucidation of a novel mycosporine-like amino acid from reef-building corals *Pocillopora damicornis* and *Stylophora pistillat., Tetrahedron Letters* **36**, 5255-5263.

Yamashiro, H., Oku, H., Higa, H., Chinen, I and Sakai, K., 1999. Composition of lipids, fatty acids and sterols in Okinawan corals. *Comparative Biochemistry and Phisiology Part* **B,** 397-407.

Zhang, W.H., Williams, I.D. and Chun-Tao Che., 2001. Chabrolols A, B and C, three new orditerpenes from the soft coral *Nephthea chabroli. Tetrahedron Letters* **42**, 4681–4685.