

**CRUDE EXTRACT OF *Xestospongia* sp. AND IT'S
ANTIMICROBIAL ACTIVITY ON PATHOGENIC BACTERIA**

SURAYA BINTI GUNAWAN

**FACULTY OF MARITIME STUDIES AND MARINE SCIENCE
UNIVERSITI MALAYSIA TERENGGANU**

2008

1100061871

In 6446

LP 50 FMSM 1 2008



1100061871

Crude extract of *xestospongia* sp. and its antimicrobial activity on pathogenic bacteria / Suraya Gunawan.



PERPUSTAKAAN SULTANAH NUR ZAHIRAH
UNIVERSITI MALAYSIA TERENGGANU (UMT)
21030 KUALA TERENGGANU

1100061871

Lihat sebelah

HAK KILIK
PERPUSTAKAAN SULTANAH NUR ZAHIRAH UNT



JABATAN SAINS MARIN
FAKULTI PENGAJIAN MARITIM DAN SAINS MARIN
UNIVERSITI MALAYSIA TERENGGANU

PENGAKUAN DAN PENGESAHAN LAPORAN
PROJEK PENYELIDIKAN I DAN II

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: Crude Extract of *Xestospongia* sp. and It's Antimicrobial Activity on Pathogenic Bacteria oleh Suraya Binti Gunawan, No.Matrik UK 12594 telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Marin sebagai memenuhi sebahagian daripada keperluan memperolehi Ijazah Sarjana Muda Sains (Biologi Marin), Fakulti Pengajian Maritim dan Sains Marin, Universiti Malaysia Terengganu.

Disahkan oleh:

Penyelia Utama **DR. AHMAD SHAMSUDDIN BIN AHMAD**
Nama: Lecturer
Cop Rasmi Department of Marine Science
Faculty of Maritime Studies and Marine Science
Universiti Malaysia Terengganu (UMT)
21030 Kuala Terengganu.

Tarikh: **4.5.2008**

Penyelia Kedua (jika ada)

Nama: **DR. ZAINUDIN BACHOK**
Cop Rasmi Lecturer
Faculty of Maritime Studies and Marine Science
Universiti Malaysia Terengganu (UMT)
21030 Kuala Terengganu.

Tarikh: **4.5.2008**



Ketua Jabatan Sains Marin

Nama: **DR. RAZAK ZAKARIYA**

Ketua Jabatan Sains Marin

Cop Rasmi: **Fakulti Pengajian Maritim dan Sains Marin**
Universiti Malaysia Terengganu
(UMT)

Tarikh: **12/5/08**

ACKNOWLEDGEMENTS

First of all, I would like to give all thanks and praise to Allah s.w.t for His blessing of good health and strength for me to accomplish this final year project. I would like to gratefully acknowledge the peer reviewers who pointed out glaring errors, eliminated trivia, and added useful information. They were my first supervisor, Dr Ahmad Shamsuddin and my co-supervisor Dr Zainudin Bachok.

I would like to take this opportunity to give my sincere thanks to the individuals who helped me completed this final year project paper, through their files and generously provided illustrative materials especially, Dr Najiah and Dr Habsah. A special thank to Encik Luqman Hakim and En. Che Mohd. Zan Husin for their help during laboratory work and advices.

A special thank for my beloved father, Gunawan bin Pono, for his sincerely accompaniment prayers and support. For my sweet siblings, thanks for your understanding of my future undertaking and I really appreciate your cares. I would like to thank my fellow coursemate especially, Kumari Geetha, Siti Asma', Shafarini, Sellinna, Kamaliah and to all individuals who had help directly or indirectly lend me a helping hand.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
LIST OF TABLES	v
LIST OF FIGURES	vi
LIST OF ABBREVIATIONS	vii
LIST OF APPENDICES	viii
ABSTRACT	ix
ABSTRAK	x
CHAPTER 1: INTRODUCTION	1
1.1 Introduction	1
1.2 Importance of Study	3
1.3 Objectives	3
CHAPTER 2: LITERATURE REVIEW	4
2.1 Marine sponges	4
2.2 Antimicrobial activity of marine sponges	6
2.3 Marine sponges of genus <i>Xestospongia</i> sp.	8
2.4 Antimicrobial Test	9
2.4.1 Disc diffusion method	9

CHAPTER 3: METHODOLOGY	11
3.1 Sampling	11
3.2 Extraction	11
3.2.1 Extraction using methanol	11
3.2.2 Extraction using ethanol	12
3.2.3 Extraction using chloroform	12
3.3 Microorganisms	13
3.4 Bioassay procedures	13
3.4.1 Preparation of assay medium for bacteria	13
3.4.2 Disc diffusion method	14
 CHAPTER 4: RESULTS	16
4.1 Identification of <i>Xestospongia</i> sp.	16
4.2 Zone of inhibition for impregnated disc	16
4.2.1 Zone of inhibition at concentration 100 mg/ml	16
4.2.2 Zone of inhibition at concentration 200 mg/ml	17
4.2.3 Zone of inhibition at concentration 500 mg/ml	18
 CHAPTER 5: DISCUSSION	25
 CHAPTER 6: CONCLUSION	29
 REFERENCES	30
 APPENDICES	36
 CURICULUM VITAE	43

LIST OF TABLES

Table		Page
4.1	Diameter zone of inhibition in millimeter at concentration 100 mg/ml	19
4.2	Diameter zone of inhibition in millimeter at concentration 200 mg/ml	20
4.3	Diameter zone of inhibition in millimeter at concentration 500 mg/ml	21
4.4	Diameter of inhibition growth in millimeter for positive control	22

LIST OF FIGURES

Figure		Page
4.1	Zone of inhibition	23
4.2	Diameter zone of inhibition (15 mm for methanol extract at concentration 500 mg/ml which inhibit the growth of Gram-positive bacteria <i>Bacillus subtilis</i>)	24
4.3	Diameter zone of inhibition (14 mm for methanol extract at concentration 500 mg/ml which inhibit the growth of Gram-negative bacteria <i>Escherichia coli</i>)	24

LIST OF ABBREVIATIONS

UMT	-	University Malaysia Terengganu
g	-	gram
mg/ml	-	milligram/milliliter
μg	-	microgram
$^{\circ}\text{C}$	-	degree
mm	-	millimeter
ml	-	milliliter
NA	-	Nutrient agar
CFU/ml	-	colony forming units/milliliter
PBS	-	Phosphate buffered saline
NCCLS	-	National Committee for Clinical Laboratory Standards

LIST OF APPENDICES

Appendix		Page
1	Map of sampling site	36
2	Taxonomy of <i>Xestospongia</i> sp.	37
3	Sample of <i>Xestospongia</i> sp.	38
4	Type of extract	39
5	Evaporation process using rotary evaporator at 40 °C	40
6	Product (extracted)	41
7	Anova two-ways output	42

ABSTRACT

Antimicrobial activities of *Xestospongia testudinaria* on pathogenic bacteria were investigated. The sponge has been collected from Pulau Bidong and extracted using methanol, ethanol and chloroform. The antimicrobial activity was carried out using Disc Diffusion Methods on four strains of Gram-positive bacteria, *Bacillus cereus*, *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis* and four Gram-negative bacteria, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, and *Klebsiella pneumonia*. Bacteria inhibition process depends on the types of the crude extract and the types of the bacteria. The methanol and chloroform extracts were found to inhibit the growth of three Gram-positive bacteria and one Gram-negative bacteria while the ethanol extracts inhibit the growth of two gram positive bacteria and one gram negative bacteria. The methanol extracts at concentration of 500 mg/ml showed the highest activities against the pathogenic bacteria. Range of diameters for the extracts inhibition zones for bacteria growth are between 7 to 15 mm for concentration of 100, 200 and 500 mg/ml. *Xestospongia testudinaria* has been approved to contained some bioactive compound which positive against pathogenic bacteria.

PENGESKTRAKKAN MENTAH *Xestospongia* sp. DAN AKTIVITI ANTIBAKTERIANYA KE ATAS PATOGENIK BAKTERIA

ABSTRAK

Kajian mengenai aktiviti antibakteria *Xestospongia testudinaria* telah dijalankan. Sampel telah dikumpul dari Pulau Bidong dan diekstrak dengan menggunakan metanol, etanol dan klorofom. Ujian antibakteria dijalankan dengan menggunakan kaedah pembauran cakera ke atas empat bakteria gram positif, *Bacillus cereus*, *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis* dan empat bakteria gram negatif, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, and *Klebsiella pneumonia*. Proses perencatan pertumbuhan bakteria bergantung kepada jenis ekstrak mentah dan jenis bakteria. Ekstrak metanol dan klorofom didapati berjaya merencat pertumbuhan tiga bakteria gram positif dan satu bakteria gram negatif sementara ekstrak etanol berjaya merencat pertumbuhan dua bakteria gram positif dan satu bakteria gram negatif. Ekstrak metanol pada kepekatan 500 mg/ml menunjukkan aktiviti paling tinggi dalam melawan patogenik bakteria. Julat diameter bagi zon perencatan pertumbuhan bakteria antara 7 hingga 15 mm bagi kepekatan 100, 200 dan 500 mg/ml. *Xestospongia testudinaria* telah dibuktikan mempunyai kompound bioaktif di mana positif melawan patogenik bacteria.