

THE EVALUATION OF ANTIMICROBIAL ACTIVITY FROM  
THE EXTRACTS OF *Hibiscus tiliaceus*

NOOR DALILA BINTI JAFFAR

PL  
26  
PPSMS  
1  
2014

SCHOOL OF MARINE SCIENCE AND ENVIRONMENT  
UNIVERSITI MALAYSIA TERENGGANU

2014

9843

1100093374



PL 26 PPSMS 1 2014



1100093374

The evaluation of antimicrobial actracts of hibiscus tiliaceus / by  
Noor Dalila Jaffar.

PUSAT PEMBELAJARAN DIGITAL SULTANAH NUR ZAHIRAH  
UNIVERSITI MALAYSIA TERENGGANU (UMT)  
21030 KUALA TERENGGANU

1100093374		

Lihat Sebelah

**THE EVALUATION OF ANTIMICROBIAL ACTIVITY FROM THE  
EXTRACTS OF *Hibiscus tiliaceus***

**By**

**Noor Dalila Binti Jaffar**

**Research Report submitted in partial fulfilment of  
the requirements for the degree of  
Bachelor of Science (Marine Biology)**

**School of Marine Science and Environment  
UNIVERSITI MALAYSIA TERENGGANU**

**2014**

This project report should be cited as:

Dalila, N. J. (2014). The evaluation of antimicrobial activity from the extracts of *Hibiscus tiliaceus* .Undergraduate Thesis, Bachelor of Science (Marine Biology), School of Marine Science and Environment, Universiti Malaysia Terengganu, Terengganu. P, 39.

***No part of this project report may be reproduced by any mechanical, photographic or electrical process, or in the form of phonographic recording, nor may it be stored in retrieval system, transmitted or otherwise copied for public or private use, without written permission from the author and the supervisor(s) of the project.***



**SCHOOL OF MARINE SCIENCE AND ENVIRONMENT  
UNIVERSITI MALAYSIA TERENGGANU**

**DECLARATION AND VERIFICATION REPORT**

**FINAL YEAR RESEARCH PROJECT**

It is hereby declared and verified that this research report entitled The Evaluation of Antimicrobial Activity from the Extracts of *Hibiscus tiliaceus* by Noor Dalila Binti Jaffar, Matric Number UK 25725 have been examined and all errors identified have been corrected. This report is submitted to the School of Marine Science and Environment as partial fulfilment towards obtaining Degree of Bachelor of Science (Marine Biology), Universiti Malaysia Terengganu.

Verified by:

.....

First Supervisor

Name: Dr. Yosie Andriani HS

Official stamp:

**DR. YOSIE ANDRIANI HS**  
Pensyarah  
Institut Bioteknologi Marin  
Universiti Malaysia Terengganu  
21030 Kuala Terengganu

Date: 12/06/2014



## ACKNOWLEDGEMENTS

Praise to Allah for giving me the opportunities and easiness to finish up my Final Year Research Project.

First of all, I would to express my gratitude to my supervisor Dr. Yosie Andriani HS for giving me all guidelines, support and advices while works on this projects. Same goes to Dr. Hing Lee Siang, thank you giving me guidelines on writing this report. Without them, I will face many difficulties to finish up this project. Aside, I would like to address special thanks to all UMT's staff and Lab Assistants especially from Laboratory of Marine Biotechnology, Laboratory of Biodiversity and Laboratory of Marine Oceanography of Schools of Marine Science and Environment and Institute of Marine Biotechnology, Universiti Malaysia Terengganu for helping to prepare and provide the materials and apparatus, taught on me on how to used instruments and spend their time to allow me to used the laboratory during holiday and after office hour.

Not forgetting, to all my team-mate, Noor Zulaikha Binti Shamsudin, Jasmin Jaafar and Rodhiyah Binti Yahya, colleagues, and friends, thank you for all your support, help, advices and cooperation. To my family at hometown, thanks for your support, pray and understanding.

## TABLE OF CONTENTS

	<b>Page</b>
<b>DECLARATION AND VERIFICATION REPORT</b>	i
<b>ACKNOWLEDGEMENTS</b>	ii
<b>TABLE OF CONTENTS</b>	iii
<b>LIST OF TABLES</b>	vi
<b>LIST OF FIGURES</b>	iii
<b>LIST OF ABBREVIATIONS</b>	iii
<b>LIST OF APPENDICES</b>	ix
<b>ABSTRACT</b>	x
<b>ABSTRAK</b>	xii
<b>CHAPTER 1: INTRODUCTION</b>	
1.1 Background of Study	1
1.2 Importance of Study	4
1.3 Objectives of Study	4
<b>CHAPTER 2: LITERATURE REVIEW</b>	
2.1 <i>Hibiscus tiliaceus</i>	5
2.1.1 Taxonomy of <i>Hibiscus tiliaceus</i>	5
2.1.2 Characteristics of <i>Hibiscus tiliaceus</i>	6
2.1.3 Uses of <i>Hibiscus tiliaceus</i>	6
2.1.4 Chemical content	7
2.2 Bacteria Strains	7
2.2.1 Bacterial infections	8

2.3	Antimicrobial Activity	8
	2.3.1 Antimicrobial agents	8
2.4	Antimicrobial Test	9
	2.4.1 Disc diffusion method	9
	2.4.2 Minimum inhibitory concentration (MIC)	10
	2.4.3 Kinetic study	10
<b>CHAPTER 3: METHODOLOGY</b>		
3.1	Sampling	13
3.2	Plant materials	13
3.3	Extraction of <i>Hibiscus tiliaceus</i>	13
3.4	Antimicrobial Assay	14
	3.4.1 Disc diffusion method (Kirby-Bauer disc diffusion)	14
	3.4.2 Minimum Inhibitory Concentration (MIC)	15
	3.4.3 Kinetic study of bacteria	16
<b>CHAPTER 4: RESULTS</b>		
4.1	Disc Diffusion Test	17
4.2	Determination of Minimum Inhibitory Concentration (MIC)	20
4.3	Kinetic study	22
<b>CHAPTER 5: DISCUSSION</b>		
5.1	Disc Diffusion Test	25
5.2	Minimum Inhibitory Concentration of Selected Bacteria	26
5.3	Kinetic Study of Selected Bacteria	27
<b>CHAPTER 6: CONCLUSION AND RECOMMENDATIONS</b>		
		28



<b>REFERENCES</b>	30
<b>APPENDICES</b>	34
<b>CURRICULUM VITAE</b>	39

## LIST OF TABLES

<b>Table</b>		<b>Page</b>
4.1	Inhibition zone of Flower extracts from <i>Hibiscus tiliaceus</i>	18
4.2	Inhibition zone of Leaf extracts from <i>Hibiscus tiliaceus</i>	18
4.3	Inhibition zone of Seed extracts from <i>Hibiscus tiliaceus</i>	19
4.4	Minimum inhibitory concentration of different extracts from <i>Hibiscus tiliaceus</i> based on Disc diffusion method	20

## LIST OF FIGURES

Figure		Page
2.1	Flower, Leaf and Seed of <i>Hibiscus tiliaceus</i>	6
2.2	The differences between gram positive bacteria and gram negative bacteria	7
2.3	Growth curve for hypothetical population	11
4.1	Micro-dilution test on <i>Bacillus subtilis</i> with water extracts from the flower, leaf and seed of <i>Hibiscus tiliaceus</i>	21
4.2	Micro-dilution test on <i>Pseudomonas aeruginosa</i> with water extracts from the flower, leaf and seed of <i>Hibiscus tiliaceus</i>	21
4.3	Kinetic study of antimicrobial activity of aqueous extract from flower of <i>Hibiscus tiliaceus</i> against <i>Pseudomonas aeruginosa</i> (a) and <i>Bacillus subtilis</i> (b).	22
4.4	Kinetic study of antimicrobial activity of aqueous extract from leaves of <i>Hibiscus tiliaceus</i> against <i>Pseudomonas aeruginosa</i> (a) and <i>Bacillus subtilis</i> (b)	23
4.5	Kinetic study of antimicrobial activity of aqueous extract of seed from <i>Hibiscus tiliaceus</i> against <i>Pseudomonas aeruginosa</i> (a) and <i>Bacillus subtilis</i> (b)	24

## LIST OF ABBREVIATIONS

CFU	-	Colony forming unit
MDR	-	Multidrug resistant
L	-	Liter
mg/ml	-	Milligram per milliliter
MIC	-	Minimum inhibitory concentration
mL	-	milliliter
mm	-	millimeter
nm	-	nanometer
No.	-	Number
μ	-	Micro
°C	-	Degree Celsius
OD	-	Optical density
DMSO	-	Dimethyl sulfoxide

## LIST OF APPENDICES

<b>Appendix</b>		<b>Page</b>
1	Disc diffusion test for determining the MIC	34
2	The absorbance reading for <i>Pseudomonas aeruginosa</i>	35
3	Growth Pattern of <i>Pseudomonas aeruginosa</i>	36
4	The absorbance reading for <i>Bacillus subtilis</i>	37
5	Growth Pattern of <i>Bacillus subtilis</i>	38

## ABSTRACT

The antimicrobial activity from flower, leaf, and seed of *Hibiscus tiliaceus* was screened on *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus*. Methanol, hexane, ethyl acetate and water were used as extractive solvent. The antimicrobial effect of the extract was tested using Disc diffusion method with concentration of 20 mg/ml for each extracts. Based on the result, it reveals that most of the extracts only gave positive result on *Bacillus subtilis* and *Pseudomonas aeruginosa*. All of the extracts were do not effective on *Escherichia coli* and *Staphylococcus aureus* as it only exhibited trace and no inhibition zone at all. Hence, we can conclude that the extracts showed high activity as antimicrobial toward *Pseudomonas aeruginosa* and *Bacillus subtilis* only. The result also showed that the water extract from flower (WF), leaf (WL) and seed (WS) of *Hibiscus tiliaceus* was the best extractive solvent for their antimicrobial properties. The water extracts exhibited the widest zone of inhibition which range from 8.0 mm until 11.0 mm. It then followed by the ethyl acetate (EF) and methanol (MF) extract from the flower and methanol (MS) extract from the seed. The ranges on inhibition zone are 7-10 mm, 7-9 mm and 8 mm respectively. Moreover, the Minimum Inhibitory Concentration test was only done for those extracts that showed widest inhibition zone in the disc diffusion test. For *Bacillus subtilis*, the minimum inhibitory concentration showed by water extracts of flower, leaf and seed is 1.25 mg/ml,



20 mg/ml and 5 mg/ml respectively. While for *Pseudomonas aeruginosa*, the minimum inhibitory concentration showed by water extracts of flower and leaf is 10 mg/ml and for seed is 20 mg/ml. Lastly, the study on the effect of plant extracts on the growth of *Bacillus subtilis* and *Pseudomonas aeruginosa* when compare to the its control growth pattern showed that the water extracts of flower, leaf and seed exhibit different reaction on both types of bacteria. Compared to flower, the water extracts from leaf and seed showed the lower growth on both bacteria. It is more effective to inhibit the growth of the bacteria compared to flower extracts. As for further study, the isolation and purification of bioactive compounds from the aqueous extract are also warranted.

# PENILAIAN TERHADAP AKTIVITI ANTIBAKTERIA DARIPADA EKSTRAKS *Hibiscus tiliaceus*

## ABSTRAK

Aktiviti antibakteria dari bunga, daun dan biji benih *Hibiscus tiliaceus* telah diuji pada *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* dan *Staphylococcus aureus*. Metanol, heksana, etil asetat and air di gunakan sebagai pelarut ekstraks. Kesan antibakteria daripada kesemua ekstraks telah diuji dengan menggunakan kaedah Penyerapan Disk dengan kepekatan ekstraks adalah 20 mg/ml bagi setiap ekstraks. Daripada keputusan yang terhasil, ianya merungkai bahawa kesemua ekstraks memberi kesan positif terhadap *Bacillus subtilis* dan *Pseudomonas aeruginosa* sahaja. Kesemua ekstraks tidak menunjukkan kesan yang efektif kepada *E.coli* dan *Staphylococcus aureus* kerana ianya hanya menunjukkan “jejak” dan tiada zon perencatan terhasil. Oleh itu, disimpulkan bahawa kebanyakan ekstraks hanya menunjukkan aktiviti antibakteria yang tinggi terhadap *Pseudomonas aeruginosa* dan *Bacillus subtilis* sahaja. Melalui keputusan yang terhasil, ianya juga menunjukkan bahawa ekstraks air daripada bunga, daun dan biji benih daripada *Hibiscus tiliaceus* merupakan pelarut terbaik bagi ciri-ciri kesan antibakteria pada bakteria yang di uji. Ekstraks air daripada bunga, daun dan biji benih *Hibiscus tiliaceus* mempamerkan zon perencatan yang terbesar, di mana julatan bagi zon perencatan tersebut adalah di antara 8.0 mm hingga 11.0 mm. Ianya kemudian, diikuti oleh ekstraks etil asetat dan metanol daripada bunga dan ekstraks methanol daripada biji benih tumbuhan tersebut. Julat bagi zon perencatan masing-masing adalah diantara 7-10 mm, 7-9 mm and 8 mm. Seterusnya, bagi ujian Kepekatan Minimum untuk Kesan

Perencatan , ianya hanya dilakukan bagi ekstrak-ekstraks yang menunjukkan kesan zon perencatan yang paling terbesar pada ujian Penyerapan Disk. Bagi *Bacillus subtilis*, kepekatan minimum bagi kesan perencatan daripada ekstrak air adalah 1.25 mg/ml untuk bunga, 20 mg/ml untuk daun dan 5 mg/ml untuk biji benih. Manakala untuk *Pseudomonas aeruginosa*, kepekatan minimum bagi kesan perencatan adalah 10 mg/ml bagi bunga dan daun dan 20 mg/ml bagi biji benih. Akhir sekali, bagi kajian untuk kesan ekstrak daripada tumbuhan terhadap pertumbuhan bagi *Bacillus subtilis* dan *Pseudomonas aeruginosa* berbanding lengkung pertumbuhan yang normal bagi bakteria-bakteria tersebut, ianya menunjukkan bahawa ekstrak air daripada bunga, daun dan biji benih mempamerkan tindak balas yang berbeza terhadap bakteria-bakteria tersebut. Jika dibandingkan dengan ekstrak bunga, ekstrak air daripada daun dan biji benih menunjukkan pertumbuhan bakteria yang rendah. Ianya lebih efektif untuk merencatkan pertumbuhan bakteria tersebut berbanding ekstrak daripada bunga. Bagi kajian lanjut, pengasingan dan penulenan sebatian bioaktif daripada ekstrak air adalah disarankan.