



UNIVERSITI PUTRA MALAYSIA

***MORPHOLOGICAL AND MOLECULAR
CHARACTERIZATION OF MUD LOBSTER AND
DETERMINATION OF ITS ANTIOXIDANT AND
ANTI-INFLAMMATORY ACTIVITIES***

NUR NADIAH ZAKARIA

FSPM 2020 3



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LOBSTER AND DETERMINATION OF ITS ANTIOXIDANT AND ANTI-
INFLAMMATORY ACTIVITIES**

By

NUR NADIAH BINTI ZAKARIA

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Master of Science**

August 2020

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia, in fulfillment of the requirement for the degree of Master of Science

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By

NUR NADIAH BINTI ZAKARIA

August 2020

Chairman : Amy Halimah binti Rajaee, PhD
Faculty : Agriculture and Food Science, Bintulu Campus

Mud lobsters (*Thalassina* spp.) are locally referred to as 'udang ketak' or 'ketam busut' are considered as an important organism in the mangrove ecosystem for its burrowing activities and the role of its mounds or burrows as home to other animals. In Malaysia and Thailand, mud lobsters are eaten by locals as treatment for asthma. It is traditionally believed that they are effective in reducing the number of asthma attacks and severity of asthma symptoms. However, the therapeutic potential of mud lobster remains unclear and has not been fully elucidated or reported in scientific study. Present study builds on that knowledge to determine the species of mud lobster and test the potential of mud lobster as natural antioxidant and anti-inflammatory. The objectives of this study are to identify species of mud lobster through analysis of morphological traits and molecular gene markers (PEPCK and CO1), to analyse sexual dimorphism and length-weight relationship of mud lobster and to investigate antioxidant and anti-inflammatory potential of mud lobster extracts and determine the bioactive compounds involved. All mud lobster samples (n=70) collected from four distinct regions in Sarawak (Kuala Tatau, Kuala Balingian, Sarikei and Lingga) were identified as *Thalassina anomala* through morphological characteristics and molecular gene markers (PEPCK and CO1). Maximum likelihood tree analysis of CO1 gene sequences showed that *T. anomala* from all populations were closely related with each other with 100% bootstrap supporting evidence. For morphometric variations between sex, specimens with damaged or missing cheliped were not included in analysis. Sexual dimorphism of *T. anomala* can be observed where males are significantly longer for carapace and left chelae propodus compared to females ($p < 0.05$). Meanwhile, the abdominal width was significantly larger in females. The length-weight relationship of *T. anomala* showed that males had isometric growth rate for carapace length-weight (CL/W), total length-weight (TL/W) and abdominal length-weight (ABL/W) relationships. However, females had negative allometric growth in CL/W and ABL/W relationship and isometric growth in TL/W relationship.

Antioxidant potential of *T. anomala* extracts (methanol, chloroform and hexane) were analysed through DPPH, ABTS and FRAP assays. The chloroform extract was identified to have higher antioxidant activity except in DPPH assay in which the methanol extract exhibited higher antioxidant activity. The results suggested that all *T. anomala* extracts have antioxidant potential and this finding was confirmed by presence of radical scavenging activity in antioxidant assays. For anti-inflammatory investigations, the hexane extract of *T. anomala* showed anti-inflammatory activity by significantly inhibit the LPS-induced production of nitric oxide, prostaglandin PGE₂, interleukin- (IL-) 6, IL-1 β and tumor necrosis factor-alpha (TNF- α) in a concentration-dependent manner. GC-MS analysis of the hexane extract revealed the presence of 19 putative compounds with anti-inflammatory activities. The peaks of putative compounds were identified by comparing the retention times with data from NIST and WILEY libraries. Highest peaks detected are cholesterol (C₂₇ H₄₆ O) and butyric acid (C₄ H₈ O₂). Overall, results from this study showed evolutionary relationship, sexual dimorphism and growth pattern of *T. anomala* from Kuala Tatau, Kuala Balingian, Sarikei and Lingga, Sarawak. This present study also suggested that *T. anomala* extracts have antioxidant and anti-inflammatory potential.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia, sebagai memenuhi keperluan untuk ijazah Master Sains

CIRI-CIRI MORFOLOGI DAN MOLEKULAR UDANG LUMPUR DAN PENILAIAN ANTIOKSIDA DAN ANTIKERADANGANNYA

Oleh

NUR NADIAH BINTI ZAKARIA

Ogos 2020

Pengerusi : Amy Halimah binti Rajaee, PhD
Fakulti : Sains Pertanian dan Makanan, Kampus Bintulu

Udang lumpur (*Thalassina* spp.) dikenali oleh penduduk tempatan sebagai ‘udang ketak’ atau ‘ketam busut’ dianggap sebagai organisma penting dalam ekosistem paya bakau ekoran aktivitinya menggali tanah dan peranan sarang mereka sebagai perumah kepada organisma lain. Di Malaysia dan Thailand, udang lumpur dimakan oleh penduduk tempatan sebagai penawar kepada asma. Secara tradisional, ia dipercayai berkesan untuk mengurangkan kekerapan serangan asma serta mengurangkan kesan teruk simptom serangan asma. Walau bagaimanapun, potensi terapi ekstrak udang lumpur masih tidak jelas dan belum diterangkan atau dilaporkan sepenuhnya dalam kajian saintifik. Kajian ini dijalankan untuk menentukan spesies udang lumpur dan menguji potensi udang lumpur sebagai antioksidan dan antikeradangan semula jadi. Objektif kajian ini adalah untuk mengenal pasti spesies udang lumpur melalui analisis morfologi dan penanda gen molekul (PEPCK dan CO1), analisis seksual dimorfisme dan hubungan antara panjang-berat udang lumpur dan menyiasat potensi antioksidan dan antikeradangan ekstrak udang lumpur dan menentukan sebatian aktif yang terlibat. Semua sampel udang lumpur (n=70) yang dikumpulkan dari empat kawasan di Sarawak (Kuala Tatau, Kuala Balingian, Sarikei and Lingga) telah dikenal pasti sebagai *Thalassina anomala* dengan merujuk kepada ciri-ciri morfologi dan penanda gen molekul (PEPCK dan CO1). Analisis pokok kemungkinan maksimum daripada gen CO1 menunjukkan *T. anomala* dari semua populasi berkait rapat antara satu sama lain dengan 100% sokongan daripada bukti butstrap. Untuk variasi morfometri antara jantina, specimen yang rosak dan hilang cheliped tidak termasuk dalam analisis. Seksual dimorfisme *T. anomala* boleh diperhatikan secara jelas dimana jantan mempunyai karapas dan chelae propodus bahagian kiri yang lebih panjang berbanding betina ($p < 0.05$). Sementara itu, betina mempunyai lebar perut yang lebih besar. Hubungan antara panjang-berat *T. anomala* telah menunjukkan jantan mempunyai kadar pertumbuhan isometrik bagi hubungan panjang karapas-berat (CL/W), jumlah panjang-berat (TL/W) dan panjang perut-berat (ABL/W). Namun demikian, betina pula mempunyai negatif pertumbuhan allometrik bagi hubungan CL/W dan ABL/W

dan pertumbuhan isometrik bagi hubungan TL/W. Potensi antioksidan ekstrak *T. anomala* (metanol, kloroform dan heksana) telah dikaji melalui pencerakinan DPPH, ABTS dan FRAP. Ekstrak kloroform menunjukkan aktiviti antioksidan yang lebih tinggi kecuali bagi pencerakinan DPPH di mana ekstrak metanol menunjukkan aktiviti antioksidan yang lebih tinggi. Data dalam kajian ini mencadangkan bahawa semua ekstrak *T. anomala* mempunyai potensi antioksidan dan dibuktikan melalui kewujudan aktiviti perencatan radikal bebas dalam pencerakinan antioksidan. Bagi aktiviti antikeradangan, ekstrak heksana *T. anomala* menunjukkan aktiviti antikeradangan dengan merencatkan secara jelas pengeluaran nitrik oksida, prostaglandin PGE₂, interleukin- (IL-) 6, IL-1 β dan nekrosis tumor faktor-alfa (TNF- α) yang teraruh oleh LPS dalam keadaan yang bergantung kepada kepekatan. Analisis GC-MS ekstrak heksana menunjukkan kehadiran 19 jenis sebatian putatif yang mempunyai aktiviti antikeradangan. Puncak sebatian putatif telah dikenal pasti dengan cara membezakan masa pengekalan dengan data daripada NIST dan WILEY. Puncak tertinggi dikesan adalah kolesterol (C₂₇ H₄₆ O) dan butyric acid (C₄ H₈ O₂). Keseluruhannya, data dalam kajian ini menunjukkan hubungan evolusi, seksual dimorfisme dan corak pertumbuhan *T. anomala* dari Kuala Tatau, Kuala Balingian, Sarikei dan Lingga, Sarawak. Kajian ini juga mencadangkan bahawa ekstrak *T. anomala* mempunyai potensi antioksidan dan antikeradangan.

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I certify that a Thesis Examination Committee has met on 11 August 2020 to conduct the final examination of Nur Nadiah binti Zakaria on her thesis entitled "Morphological and Molecular Characterization of Mud Lobster and Determination of its Antioxidant and Anti-Inflammatory Activities" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Shahrulrazid Sarbini, PhD

Associate Professor
Faculty of Agriculture and Food Science (Bintulu Campus)
Universiti Putra Malaysia
(Chairman)

Ina Salwany binti Md. Yasin, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Rozida binti Mohd Khalid, PhD

Senior Lecturer
Faculty of Science and Technology
National University of Malaysia
Malaysia
(External Examiner)

ZURIATI AHMAD ZUKARNAIN, PhD

Professor Ts. and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 03 November 2020

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Amy Halimah binti Rajae, PhD

Senior Lecturer
Faculty of Agriculture and Food Science
Universiti Putra Malaysia Bintulu Campus
(Chairman)

Masnindah binti Malahubban, PhD

Senior Lecturer
Faculty of Agriculture and Food Science
Universiti Putra Malaysia Bintulu Campus
(Member)

Sharida binti Fakurazi, PhD

Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

ZALILAH MOHD SHARIFF, PhD

Professor and Dean
School of Graduate Studies
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Signature: _____
Name of Chairman
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Committee: Dr. Amy Halimah Binti Rajae

Signature: _____
Name of Member
of Supervisory
Committee: Dr. Masnindah Binti Malahubban

Signature: _____
Name of Member
of Supervisory
Committee: Professor Dr. Sharida Binti Fakurazi

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LIST OF ABBREVIATIONS

ABL	Abdomen length
ABL/W	Abdomen length-weight relationship
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
ABW	Abdomen width
ANOVA	Analysis of variance
BLAST	Basic local alignment system tool
CL	Carapace length
CL/W	Carapace length-weight relationship
CPH	Chelae propodus height
CPL	Chelae propodus length
CPW	Chelae propodus width
CW	Carapace width
CO1	Mitochondrial-coding gene, cytochrome c oxidase subunit I
DMEM	Dulbecco's modified eagle medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPPH	1, 1-diphenyl-2-picrylhydrazyl
ELISA	Enzyme-linked immunosorbent assay
FRAP	Ferric reducing antioxidant power
LPS	Lipopolysaccharide
g	Gram
GC-MS	Gas chromatography-mass spectrometry
IL1 β	Interleukin 1 β
IL 6	Interleukin 6

kPa	Kilopascal
MC	Chloroform extract of <i>T. anomala</i>
MH	Hexane extract of <i>T. anomala</i>
mg	Milligram
mg trolox/g	Milligram trolox per gram
ml	Milliliter
MM	Methanol extract of <i>T. anomala</i>
mmol/l	Millimoles per liter
ms	Millisecond
NED	N-1-naphthylethylenediamine dihydrochloride
nm	Nanometer
NO	Nitric oxide
PBS	Phosphate-buffered solution
PCR	Polymerase chain reaction
PEPCK	Phosphoenolpyruvate carboxykinase
PGE2	Prostaglandin E2
rpm	Revolutions per minute
SD	Standard deviation
TL	Total length
TL/W	Total length-weight relationship
TNF- α	Tumor necrosis factor alpha
μ l	Microliter
μ m	Micrometer
WW	Wet weight

CHAPTER 1

INTRODUCTION

Mud lobsters (*Thalassina* spp.) are nocturnal organisms, belong to Order Decapoda that are rarely seen due to their habitat which is far underneath the ground. Their existence is acknowledged by the presence of their mounds. Mud lobster is considered as an important organism in the mangrove ecosystem for its burrowing activities. Their nests provide habitat for many of the mangrove organism (Macintosh et al., 2002) and the mud lobster themselves help in recycling nutrients by bringing nutrients from underground to the upper level of the sediment via their burrowing activities (Kartika & Patria, 2012). Previous research on mud lobsters were focused on its morphology (Dworschak et al., 2012; Sakai & Türkay, 2012; Moh & Chong, 2009; Ngoc-Ho & Laurent, 2009; Sakai, 1992) , gill mechanism (Batang & Suzuki, 1999), relations and effects on mangrove succession (Havanond et al., 1999; Havanond, 1987), phylogenetics (Moh et al., 2013; Tsang et al., 2008) as well as taxonomy and ecology (Hassan et al., 2015; Dubey et al, 2012). In Sarawak, Malaysia, mud lobsters are consumed by locals as medicine for asthma. It is believed that an asthma patient can either be cured or experience less asthma attack after eating them. This theory has already existed for generations, although the truth about its effectiveness has never been proven or reported in scientific studies. According to Holthuis (1991), people in Thailand used the mud lobster as a remedy for asthma although not to be eaten directly. The mud lobsters were either dried, grinded into powder form and drank with water or dissolved in alcoholic liquor for a couple of days before being drunk. Mud lobster is sought by asthma patients as the local believe for generations that it can reduce asthma attacks. However, the therapeutic potential of this crustacean has not been well documented. Previous studies revealed that both oxidative stress and inflammation play a significant role in the pathogenesis of asthma, in which the reduction of both activities can be a crucial strategy for controlling asthma. The potential use of antioxidant and anti-inflammatory to reduce oxidative stress and inflammation respectively in asthma treatment have been discussed in many studies such as Rajizadeh et al. (2019); Zhu et al. (2017) and Chen et al. (2014).

In this present study, the antioxidant and anti-inflammatory properties of mud lobster are investigated. Prior to this, the species of mud lobster are identified via morphology and molecular work. Sexual dimorphism and their length-weight relationship are also determined. The outcomes of this study contributed basic biological information of mud lobster, which would be beneficial for future planning in terms of its potential use for aquaculture and management of the species for conservational purposes.

The study aims to identify species of mud lobster and to investigate antioxidant and anti-inflammatory potential of mud lobster extracts. The specific objectives of this study were to:

1. Identify species of mud lobster through analysis of morphological traits and molecular gene markers (PEPCK and CO1)
2. Analyse sexual dimorphism and length-weight relationship of mud lobster
3. Investigate antioxidant and anti-inflammatory potential of mud lobster extracts and determine the bioactive compounds involved



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BIODATA OF STUDENT

Nur Nadiah Binti Zakaria was born in Kuala Lumpur, Malaysia on September 1987. She received her Bachelor of Science in Biomedical Sciences at University Putra Malaysia, Serdang in 2010. She is currently pursuing a Master of Science in Molecular Biotechnology in University Putra Malaysia, Bintulu Campus, Sarawak.

As a post graduate student, she has conducted and assisted an experiment for final year project which focused on the genetic variation of mud lobster (*Thalassina anomala*) using RAPD markers.

Besides, she also has published one article in Pertanika Journal of Tropical Agricultural Science and she has submitted one article for publication in Tropical Life Sciences Research.

