Faculty of Resource Science and Technology

Antimicrobial and Antioxidant Properties in Tissues of Mud Crab from *Scylla olivacea* and *Scylla paramamosain*

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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Signature

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ABSTRACT

Mangrove crab (genus Scylla) is a kind of mud crab that has long been recognized as a rich source of protein. Several antimicrobial peptides from mud crabs have recently been identified by their antibacterial activity against Gram-positive and Gram-negative bacteria. Meanwhile, antioxidant capabilities found in mud crabs have been linked to cell protection from free radicals. Unfortunately, to date, little is known about the antimicrobial and antioxidant activities of *Scylla olivacea* and *Scylla paramamosain***.** This study investigates the natural antibacterial and non-enzymatic antioxidant properties from whole tissue extract of two mud crab species, *S. olivacea*, and *S. paramamosain*. The data from this study may offer the basis for much future research. In this study, extraction through maceration was utilized to produce tissue extract from *S. olivacea* and *S. paramamosain*. The tissue extracts were diluted and tested against five different bacteria through microdilution analysis. These extracts were further characterized through non-enzymatic antioxidant activity. The identities of chemical constituents in the tissue extracts were then identified using Gas Chromatography-Mass Spectrometry (GC-MS). The antibacterial activities were determined using the broth microdilution method. The methanol extract of *S. olivacea* and *S. paramamosain* was found to inhibit the growth of *Staphylococcus aureus, Klebsiella pneumoniea, Pseudomonas aeruginosa***,** *Escherichia coli,* and *Salmonella typhi* with minimum inhibitory concentration (MIC) and minimum bacteriostatic concentration (MBC) ranging from 1.56-6.25 μg/μL. Additionally, it was revealed that the highest Diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging activity was in the extracts of *S. olivacea* and *S. paramamosain*, which were 1.23 mg/mL and 1.28 mg/mL, respectively. In terms of ferric reducing antioxidant potential (FRAP), *S. paramamosain* extracts in methanol, ethyl acetate, and n-hexane solvent showed better

results than the *S. olivacea* extracts. The FRAP value obtained was similar at 1 mg/mL, which is 0.033±0.004 Fe (II)/mg for both methanol and ethyl acetate extract of *S. paramamosain***.** It was observed that the highest nitric oxide scavenging activity for both species was in ***n*-**hexane extract with the EC50 values of 0.35 mg/mL and 0.36 mg/mL, respectively. The extract contained 225 compounds with up to 40 reported from previous studies to exhibit antimicrobial, antioxidant, or anti-inflammatory properties.

Keywords: *Scylla olivacea*, *Scylla paramamosain*, antibacterial, antioxidant, Gas Chromatography-Mass Spectrometry.

***Sifat Antimikrob dan Antioksidan dalam tisu Ketam Lumpur dari* Scylla olivacea *dan* Scylla paramamosain**

ABSTRAK

Ketam bakau (genus Scylla) adalah sejenis ketam lumpur yang telah lama dikenali sebagai sumber protein yang kaya. Beberapa peptida antimikroba dari ketam lumpur baru-baru ini dikenal pasti mempunyai aktiviti antibakteria terhadap bakteria Gram-positif dan Gram-negatif. Sementara itu, keupayaan antioksidan yang terdapat pada ketam lumpur telah dikaitkan dengan perlindungan sel dari radikal bebas. Namun, setakat ini, sedikit yang diketahui mengenai aktiviti antimikroba dan antioksidan Scylla olivacea *dan* Scylla paramamosain. *Oleh itu, kajian ini menyiasat sifat antioksidan antibakteria dari ekstrak keseluruhan tisu dari dua spesies ketam lumpur,* S. olivacea *dan* S. paramamosain. *Data dari kajian ini dapat menawarkan peluang sebagai dasar bagi banyak penelitian terapan di masa depan. Dalam kajian ini, pengekstrakan melalui maserasi digunakan untuk menghasilkan ekstrak tisu dari* S. olivacea *dan* S. paramamosain. *Ekstrak tisu dicairkan dan diuji terhadap lima bakteria yang berbeza melalui analisis mikrodilusi. Ekstrak ini kemudian dicirikan melalui aktiviti antioksidan nonenzimatik. Identiti unsur kimia dalam ekstrak tisu kemudian dikenal pasti menggunakan Kromatografi Gas dan Spektometri Jisim. Kegiatan antibakteria ditentukan menggunakan kaedah mikrodilusi*. *Ekstrak metanol* S. olivacea *dan* S. paramamosain *didapati menghalang pertumbuhan* Staphylococcus aureus, Klebsiella pneumoniea, Pseudomonas aeruginosa, Escherichia coli, *dan* Salmonella typhi *dengan kepekatan perencatan minimum (KPM) dan kepekatan minimum bakteriostatik (KMB) mulai dari 1.56 -6.25 μg / μL. Selain itu, dinyatakan bahawa aktiviti pemuliharaan radikal bebas* Diphenyl-2-picryl-hydrazyl (DPPH) *tertinggi terdapat pada ekstrak* S. olivacea *dan* S. paramamosain, *masing-masing 1.23 mg / mL dan*

1.28 mg / mL. Dari segi potensi pengurangan antioksidan besi (FRAP), ekstrak S. paramamosain *dalam metanol, etil asetat, dan pelarut n-heksana menunjukkan hasil yang lebih baik daripada ekstrak* S. olivacea. *Nilai FRAP yang diperoleh adalah serupa pada 1 mg / mL, iaitu 0,033 ± 0,004 Fe (II) / mg untuk ekstrak metanol dan etil asetat* S. paramamosain. *Telah diperhatikan bahawa aktiviti pengumpulan oksida nitrat tertinggi bagi kedua-dua spesies tersebut adalah ekstrak n-heksana dengan nilai* EC_{50} *masing-masing 0.35 mg / mL dan 0.36 mg / mL. Ekstrak itu mengandungi 225 sebatian dengan hingga 40 yang dilaporkan dari kajian sebelumnya menunjukkan sifat antimikroba, antioksidan, atau anti-radang.*

***Kata kunci*:** Scylla olivacea, Scylla paramamosain, *antibakteria, antioksidan, Kromatografi Gas dan Spektrometri Jisim*

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LIST OF ABBREVIATIONS

%	Percentage
°C	Degree of Celcius
µg/mL	Microgram per mililitres
AMP	Antimicrobial peptides
G- bacteria	Gram-negative bacteria
CFU/mL	Colony-forming units per milliliter
cm	Centimetres
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
EC_{50}	Half maximal effective concentration
$FeCl_3$	Iron(iii) chloride
g/mL	Gram per milliliters
G+ bacteria	Gram-positive bacteria
GC/MS	Gas chromatography-mass spectroscopy
gL^{-1}	Gram per liters
HCl	Hydrochloric acid
LPS	Lipopolysaccharides
LTA	Lipoteichoic acid
MBC	Minimum bactericidal concentration
mg/mL	Miligram per mililitres

MHA	Mueller Hinton agar
MHB	Mueller Hinton broth
MIC	Minimum inhibitory concentration
mL	Milliliters
mmol	Millimole
NIST	National institute of standards and technology's
OH	Hydroxide
PBS	Phosphate-buffer saline
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
rpm	Rotations per minutes
SPCA	Society for the prevention of cruelty to animals
TPC	Total phenolic content
TPTZ	2,4,6-tris(2-pyridyl)-s-triazine
w:v	Weight/ volume

CHAPTER 1

INTRODUCTION

Mangrove crabs or mud crabs are marine organisms that live in mangrove swamps and can be found in various species and families (Azra & Ikhwanuddin, 2016). In many regions, these crabs are prepared as soup in meals because of the delectable taste. Apart from having tasty meat, it is also rich in nutrition (Paul et al., 2015). Mud crabs have been mentioned to demonstrate their potential as antioxidants and antimicrobial agents (Salaenoi et al., 2006). In Malaysia, older people prefer to eat mud crab soup as a common remedy and folk medicine to reduce dengue fever symptoms (Wan Yusof et al., 2017).

Antioxidants are critical for various nutritional effects and thus tend to be used to treat a variety of health problems. Natural antioxidants have received increased attention in recent years (Lourenço et al., 2019). These antioxidants are abundant in nature as bioactive compounds or can be extracted as bioactive peptides, and marine species are one of the valuable resources for bioactive peptides. This crab is loaded with bioactive peptides that perform critical biological functions, one of which is of interest to antioxidants (Sujeetha et al., 2015).

Antimicrobial peptides (AMPs) are a class of host immune response molecules that play a crucial role in demonstrating antimicrobial interest in fighting against invading microorganisms (Wan Yusof et al., 2017). AMPs in marine animals have piqued scientists' interest since 1982 due to their role in developing promising antibacterial remedies (Tanaka S. et al., 1982; Ohashi K. et al., 1984). Nakamura et al. (1988) characterized an AMP in crabs, Tachyplesin. Tachyplesin is a peptide traced from a combination of 18 amino acids isolated from the hemocytes of the Japanese horseshoe crab *Tachypleus*

tridentatus. The AMPs are thin and come in various shapes, lengths, and sequences (primarily less than 10 kDA).

Additionally, many fish and marine invertebrates are highly susceptible to bacterial infection in the water environment (Pandey et al., 2010). Thus, AMPs contribute to the innate immunity of the host against pathogens and diseases. AMPs are also thought to have antibacterial, antifungal, antiprotozoal, anti-yeast, and antiviral properties (Prakash et al., 2011). Countless antimicrobial peptides in the crab have been discovered and published over the last few years. Therefore, the present research aims to study mud crabs' antimicrobial and antioxidant properties, *S. olivacea* and *S. paramamosain*.

1.1 Problem Statements

The emergence of new diseases, resistant pathogenic microorganisms, and development of life-threatening viruses has increased the need for novel bioactive compounds to be explored (Fair & Tor, 2014). Apart from this, the search for natural antioxidants as safe alternatives has become an important aspect of the food industry because of the potential health hazards caused by synthetic antioxidants (Ngo et al., 2011). Many recent studies have revealed that antioxidants in crustaceans play a vital role in antioxidant responses caused by oxidative stress. When living in water environments exposed to microorganisms, innate immunity plays a part in preventing infections in the host. Little research on the antimicrobial and antioxidant activity of the species *S. olivacea* has been reported to date. In the meantime, a few papers were written on antioxidants and antimicrobial peptides for *S. paramamosain***,** but no study was undertaken in Sarawak, Malaysia. The findings of this study may provide a foundation for much future applied research.

1.2 Objectives

The objectives of this study are:

i. To establish an optimal method to extract the bioactive compound from the tissue of mud crab *S. olivacea* and *S. paramamosain* using three different solvents selected based on polarity;

ii. To characterize the antimicrobial properties of *S. olivacea* extracts and *S. paramamosain* extracts tissue against five bacterial strains; and

iii. To characterize the antioxidant properties of *S. olivacea* extracts and *S. paramamosain* extracts.

1.3 Hypothesis

Bioactive compound with antimicrobial and antioxidant properties is present in tissues of *S. olivacea* and *S. paramamosain.*

CHAPTER 2

LITERATURE REVIEW

2.1 Crustaceans

A diverse community of invertebrate species includes active animals such as crabs, lobsters, shrimp, krill, copepods, amphipods, and more sessile organisms such as barnacles are crustaceans (Kennedy, 2020).

Crabs belong to a group called the Brachyura and decapod crustaceans. They have a shallow "tail" projection, and their narrow abdominals are entirely concealed under the thorax. There are about 7,000 valid crab species, and there are more than 800 freshwater crabs. Water crabs, shore crabs, crayfish, and mangrove crabs are included among the crab types (Marine Education Society of Australasia, 2020).

All crabs have four pairs of walking legs and one pair of pincers (chelipeds). The first pair of crab legs are the chelipeds, which they use to catch and carry food, dig, break clecks, and repel predators. The carapace covers the internal organs of the head, thorax, and gills. The eyes are on the ends of small stalks and have a series of short legs that specialize in food processing and chewing operations that form the base of the mouth. The abdomen is thin and holds tightly under the body. Crabs have distinct sexes and are distinguished by the size of the belly (Marine Education Society of Australasia, 2020).

2.2 Mud Crab

Mud crabs are primarily found in mangrove environments. Due to its high nutritional value, it is consumed by both sea animals and humans. Mud crabs are widely

grown in many Asian countries, including Malaysia, Indonesia, and China, due to their commercial importance (Azra & Ikwanuddin, 2016). In mangrove areas with an optimal salinity of 20 to 30 gL^{-1}, mud crab of the genus Scylla can be commonly found (Nurdiani & Zeng, 2007).

Four different members in the Scylla genus are *Scylla paramamosain*, *Scylla serrata*, *Scylla olivacea*, and *Scylla transquebarica*, based on genetic evidence and morphological character. The frontal lobe spines and the chelipeds are two morphological characters that help identify the genus (Keenan et al., 1998). Ikhwanuddin et al. (2011) reported on mud crab biological details and population characteristics in the Sematan mangrove forest, Malaysia, the South China Sea coastal waters, and the famous mud crab species in the South China Sea was *S. olivacea* and *S. transquebarica*.

2.2.1 Taxonomy of mud crab

The use of DNA sequencing has identified the taxonomy of mud crab, allozyme electrophoresis, and morphometrics to classify mud crab species obtained from the Red Sea to the Indo-Pacific (Keenan et al., 1998). *S. serrata*, also known as the giant mud crab, is the first species identified, followed by *S. olivacea*; orange mud crab, *S. tranquebarica*; purple mud crab, and *S. paramamosain*; green mud crab (Ikhwanuddin et al., 2011). Table 2.1 below describe the taxonomy of mud crab adapted from Shelley and Lovatelli (2011).

Table 2.1: Taxonomy of mud crab adapted from Shelley and Lovatelli (2011)

Kingdom	Animalia
Phylum	Arthroposa
Subphylum	Crustacea
Class	Malacostraca
Order	Decapoda
Infraorder	Branchyura
Family	Portunidae
Genus	*Scylla*

Besides the colour, the four species of mud crab can be identified through the structure of carapace (Figure 2.1a) and cheliped (Figure 2.1b), as shown below. Based on Figure 2.1a, the carapace of *Scylla serrata* is observed to be in blunted point and high. Whereas *Scylla tranquebarica* also has a part of the blunted carapace, it is moderate in appearance. Meanwhile, *Scylla paramamosain* has a rounded and low part of carapace, and *Scylla olivacea* has a triangular and intermediate part of the carapace.