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Metabolomic analysis of marine and mud crabs based on antibacterial activity

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ABSTRACT

Isolated compounds from marine invertebrates are being increasingly known to possess various pharmacological activities with which many useful drugs have been developed. Crabs contain bioactive compounds including antibacterial, antifungal and antiviral metabolites, isolated from various tissues and organs that have revolutionized treatment of serious diseases. The present study represents the first attempt to investigate and compare the natural antibacterial properties from whole extract of marine blue swimmer crab, *Portunus pelagicus*, and mud crab, *Scylla tranquebarica*, against fish pathogenic bacteria. Liquid chromatography/mass spectrometry utilizing a time-of-flight (TOF) mass analyser (LC/MS-QTOF) based metabolomics approach was used to characterize the variation in secondary metabolite production in *P. pelagicus* and *S. tranquebarica* crab habitats in Malaysia. Different metabolites are evaluated in both crab species using LC/MS-QTOF. Initially a total of 75 metabolites were identified and only 19 metabolites satisfied the P-Corr cut-off point of less than 0.01 and at least 2-fold change. These metabolites, which contain anti-inflammatory and antibacterial properties, were down regulated in *S. tranquebarica* samples and up regulated in *P. pelagicus* samples. *In vitro* bioassay of methanolic *P. pelagicus* extracts showed the best antimicrobial response against Gram positive bacteria, *Streptococcus agalactiae*, and Gram negative bacteria, *Vibrio alginolyticus, Klebsiella pneumoniae,* and *Escherichia coli,* with a statistically significant difference ($P < 0.05$) of *P. pelagicus* extracts as compared to *S. tranquebarica*. The results indicate that both types of crab extracts are bactericidal at higher concentrations and bacteriostatic at lower concentrations. This manuscript reports the role of marine and mud crabs with specific emphasis on their secondary metabolites, and discusses current and future developments in both the production of desired crab metabolites and their potential uses in pharmaceutical industries.

1. Introduction

Marine natural products have attracted the attention of biologists and chemists worldwide. The marine world represents a large, unexplored, wealthy resource; thus, there is great potential for the discovery of chemotherapeutic agents in such ecosystem. The recent research on multi-drug-resistant bacteria proposes that animals living in unsanitary conditions have developed ways of protecting themselves against pathogenic microorganisms (Yoneyama and Katsumata, 2006). Among these organisms, crustaceans are emphasized due to their high potential to provide valuable nutritive products (Oliveria et al., 2017). Marine invertebrates defend against pathogenic organisms using their innate immune system (Li and Nikaido, 2009) which includes both humoral and cellular responses. Humoral immunity is characterized by antimicrobial agents present in blood cells and plasma, while cellular immunity is based on cellular defence reactions such as encapsulation, nodule formation, and phagocytosis (Wright, 1981). Moreover, numerous marine organisms live in complex habitats exposed to extreme conditions. These challenges allows them to adapt to their new environmental surroundings and produce a variety of secondary bioactive metabolites that cannot be found in other organisms (Rasmussen and Morrissey, 2007). Marine organisms offer the potential to understand and develop treatments for disease based on the normal physiological role of their secondary metabolites. It has been noted by several researchers that specific proteins, peptides, and secondary metabolites found in marine invertebrates possess antibacterial, antiparasitic, antiviral, and anticancer activities (Trivedi et al., 2003; Simmons et al., 2005; Chalupniak et al., 2014). Furthermore, several marine invertebrate-based isolated compounds have biological and pharmacological activities that can interfere with the pathogenesis of diseases; in addition, aid in the discovery of bioactive compounds, primarily for diseases including cancer, acquired immunodeficiency

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syndrome (AIDS), and osteoporosis (Senthilkumar and Kim, 2013).

Several researchers reported that marine crabs contain various bioactive compounds isolated from various organs and tissues (Veeruraj et al., 2008; Anbuchezhian et al., 2009; Priya et al., 2014; Lekshmi et al., 2015). Likewise, biologically active products were also derived from crab shells as described in the works of Varadharajan and Soundarapandian (2013) and Ghousia (2015). Chitin is an important substance that contains numerous properties that makes them attractive for a wide variety of health applications as antibacterial, antifungal, and antiviral agent. Chitin is also non-toxic and hypoallergenic, rendering it very useful in experimental and pharmaceutical research (Vongchan et al., 2003). Another medical compound found in crab shells was Glucosamine, which is used in the treatment of osteoarthritis (Pham et al., 2007). Furthermore, recent studies indicate that astaxanthin, a keto-carotenoid, isolated from shells in three crab species, *Portunus sanguinolentus* (Three Spotted Crab), *Callinectes sapidus* (Blue Crab), and *Paralithodes brevipes* (Spiny King Crab), have promising antioxidant and antimicrobial activities which can be used in the food and pharmaceutical industries (Suganya and Asheeba, 2015).

In this study, we focused on whole crab extracts and not exclusively crab proteins, as has been studied previously by researchers. The antibacterial activity of marine and mud crab crude extracts was quantitatively assessed by determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC), respectively, to evaluate their bacteriostatic and bactericidal properties. Antibacterial activity of hemocyanin isolated from serum of mud crab, *Scylla serrate*, expressed bacteriostatic activity against pathogenic bacteria *E. coli*, *B. flexus*, *V. harveyi* (Velayutham et al., 2016; Velayutham and Munusamy 2016), *V. alginolyticus*, and *V. vulnificus* (Meiyalagan and Arumugam, 2015) due to its protein function that regulates the immunity of the crab against microbial infection (Yedery and Reddy, 2009). For the preliminary assessment of toxicity, brine shrimp lethality assay was conducted. It has been utilized for screening pharmacological activities in plant extracts. Brine shrimp lethality assay has been applied as an alternative bioassay technique to screen the toxicity of plant extracts (Meyer et al., 1982) as well as screening of marine natural products (Carballo et al., 2002).

Unfortunately, the potential of crabs as a source of biologically active compounds is largely unexplored and information regarding its metabolomics profile is poorly studied. Therefore, the application of metabolomics in this study aims to investigate the characteristic metabolites of marine and mud crabs and to obtain new information concerning biological roles of these natural products. Currently, metabolomics analysis is successfully applied in biology, medicine, toxicology and environmental research (Patti et al., 2012). Liquid chromatography/mass spectrometry utilizing a time-of-flight mass analyser (LC/MS-QTOF) offers a higher sensitivity and does not require complicated sample preparation or chemical derivatization (Theodoridis, 2012; Gowda and Djukovic, 2014).

Hence, the present study represents the first attempt to investigate antibacterial properties from secondary metabolites of whole extract in different species of crabs, marine blue swimmer crab, *Portunus pelagicus*, and mud crab, *Scylla tranquebarica*, based on the functional chemical constituents characterized by LC/MS-QTOF, and to investigate the hypothesis that whole crabs crude extract are truly a potential source of novel compounds with great biological potential.

2. Materials and methods

2.1. Sample collection and identification

Healthy species of marine crab, *Portunus pelagicus*, and mud crab, *Scylla tranquebarica*, were collected from Pulau Kambing jetty in Kuala Terengganu, Malaysia. The weight of the crabs ranged from 110 g to 220 g. The species were separated and transferred via a recirculating (seawater/mud) system to the Microbial laboratory in University Malaysia Terengganu. The crabs were identified and classified in the Institute of Tropical Aquaculture, of University Malaysia Terengganu (Figs. 1 and 2).

Fig. 1. Marine blue swimmer crab, *Portunus pelagicus*.

Fig. 2. Mud crab, *Scylla tranquebarica*.

2.2. Animal preparation and assessment

Marine crab, *Portunus pelagicus*, and mud crab, *Scylla tranquebarica*, were placed individually (for the prevention of cannibalism) in an aquarium containing well aerated seawater at 28 °C, 30 ppt salinity/mud at 28 °C, 20 ppt salinity, and a thin layer of sand and gravel. The crabs were kept under a 12 h light: 12 h dark regime. In order to assess the crabs for pre-existing systemic infection with culturable bacteria, 150 µl of hemolymph was sampled from the pericardial sinus, diluted 1:10 in sterile 10 mmol l^{-1} Hepes-buffered 2% NaCl (saline), suspended in marine agar (Merck, Germany), then overlaid onto a sterile tryptic soy agar (TSA) (Merck, Germany) microbial culture plate supplemented with 2% NaCl (w/v). Plates were kept overnight at 28 °C and examined for bacterial colony forming units (CFU). Only those crabs with no culturable bacteria present in the hemolymph were used for experimentation (Thibodeaux et al., 2009). The crabs were not fed during this experiment.

2.3. Preparation of extract

The crabs were sacrificed according to RSPCA (2006). The crabs were euthanized by thermal-shock at −20 °C for 20 min then killed by spiking to destroy the nerve center. Crab tissues were broken up into smaller pieces, and separated into two equal parts.