



UNIVERSITI PUTRA MALAYSIA

***EFFICACY OF BETEL LEAVES, Piper betle L. CRUDE EXTRACT ON
Vibrio
alginolyticus IN CULTURED ASIAN SEABASS, Lates calcarifer
(Bloch, 1790)***

AHMAD BAIHAQI BIN OTHMAN

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By

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

May 2018

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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

EFFICACY OF BETEL LEAVES, *Piper betle* L. CRUDE EXTRACT ON *Vibrio alginolyticus* IN CULTURED ASIAN SEABASS, *Lates calcarifer* (Bloch, 1790)

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May 2018

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Production of Asian seabass, *Lates calcarifer* is facing a crucial problem due to vibriosis caused by *Vibrio alginolyticus*. The current treatment using commercial antibiotic is not encouraged due to the issue of antibiotic resistant bacteria. Betel leaf, *Piper betle* has been known to contain various active compounds that are effective against bacterial infection and maintain the fish health.

This study used several compounds for extraction medium of the active compounds of betel leaf. Ethanol has been concluded as the best solvent for the extraction of the active ingredients of betel leaf. The ethanoic crude extract showed inhibition zone of 21 mm compared with aquous extraction with 9 mm ($p < 0.05$). Results of the minimum inhibitory concentration (MIC) test indicate that the extract could inhibit *Vibrio alginolyticus* at a concentration of 0.2 mg/mL and could kill the bacteria at concentration of 0.39 mg/mL (MBC).

The palatability test revealed that the seabass could consume 100% of the crude extract as high as 200mg/kg of feed for over 7 days without showing abnormality. Next, the challenge test was done in two trials to test the crude extract as preventive and as treatment against vibriosis due to *Vibrio alginolyticus*. For the preventive test, the fish were fed with crude extract at 100mg/kg of feed before being infected with *Vibrio alginolyticus*. For the treatment test, the fish were initially infected with *Vibrio alginolyticus* before being treated with crude extract at the same dose of 100mg/kg of feed. The results revealed that both preventive and treatment trials produced significantly ($p < 0.05$) higher survival rate compared with oxytetracycline. These results showed that the betel leaf crude extract is highly potential to be used as preventive measure against vibriosis.

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

**KEBERKESANAN EKSTRAK MENTAH DAUN SIREH, *Piper betle* L. KE
ATAS *Vibrio alginolyticus* PADA IKAN SIAKAP, *Lates calcarifer*
(Bloch, 1790)**

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Pengeluaran ikan siakap, *Lates calcarifer* menghadapi masalah genting berhubung jangkitan vibriosis oleh *Vibrio alginolyticus*. Rawatan sekarang yang menggunakan antibiotik tidak digalakkan kerana mengakibatkan kemunculan bakteria rintang antibiotik. Sireh, *Piper betle* telah dikenalpasti mengandungi pelbagai bahan aktif yang mampu melawan jangkitan bakteria pada ikan.

Kajian ini telah menggunakan beberapa bahan untuk mengekstrak bahan aktif dari daun sireh. Hasil menunjukkan yang etanol merupakan pelarut terbaik bagi mengekstrak daun sireh. Ekstrak etanol sireh menunjukkan zon perencatan 21 mm berbanding ekstrak air sireh dengan 9 mm ($p < 0.05$). Keputusan kepekatan perencatan minimum (MIC) menunjukkan ekstrak sireh dapat merencat *Vibrio alginolyticus* pada kepekatan 0.2 mg/ml dan membunuh bakteria pada kepekatan 0.39 mg/ml (MBC).

Hasil dari kajian kebolehterimaan juga menunjukkan ikan boleh memakan 100% ekstrak mentah setinggi 200mg/kg makanan untuk selama 7 hari tanpa menunjukkan ketidaknormalan. Ujian cabaran dijalankan dalam dua cara untuk menguji ekstrak mentah sebagai pencegahan dan juga sebagai rawatan. Untuk ujian pencegahan ikan terlebih dahulu diberi makan ekstrak mentah sebanyak 100mg/kg makanan sebelum dijangkitkan dengan inokulum *Vibrio alginolyticus*. Untuk ujian rawatan, ikan terlebih dahulu dijangkitkan dengan *Vibrio alginolyticus* sebelum diberi rawatan ekstrak mentah pada kadar yang sama. Keputusan menunjukkan kedua-dua pencegahan dan rawatan ekstrak mentah daun sireh menghasilkan kadar hidup yang tinggi berbanding rawatan oxytetracycline. Dari hasil yang diperolehi menunjukkan ekstrak mentah daun sireh lebih berpotensi digunakan sebagai pencegahan kerana menunjukkan kadar hidup yang lebih tinggi berbanding ujian rawatan.

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I certify that a Thesis Examination Committee has met on 21 May 2018 to conduct the final examination of Ahmad Baihaqi bin Othman on his thesis entitled "Efficacy of Betel Leaves, *Piper betle* L. Crude Extract on *Vibrio alginolyticus* in Cultured Asian Seabass, *Lates calcarifer* (Bloch, 1790)" 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.

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LIST OF ABBREVIATIONS

%	Percentage
°C	Degree celsius
µl	Microliter
API	Analytical Profile Index
BHI	Brain heart infusion
CFU	Colony-forming unit
FAO	Food and Agriculture Organization
g	Gram
LD ₅₀	Lethal dose of the amount of an ingested substance that kills 50 percent of a test sample
mg	Miligram
mL	Milliliter
mm	Milimeter
MS-222	Tricaine Methanesulfonate
PCR	Polymerase Chain Reaction
rpm	Rotation per minute
TCBS	Thiosulfate-citrate-bile salts-sucrose
TSA	Trypticase soy agar
TSB	Trypticase soy broth
USDA	United States Department of Agriculture



CHAPTER 1

INTRODUCTION

Aquaculture is an important industry Malaysia. It is a major source of protein for the population, and one of the factors that strengthen the economy of the country. The fish culture or aquaculture helps to fulfill the increasing demand for fish as fish landings from seas are insufficient while the stocks have reached the limit of production (Mustafa, 2000). According to Shoko (2009), aquaculture contributes to the fisheries industry as the natural sources of the fish are declining, while the population of the global is increasing and demanding more output from fisheries.

Asian seabass or Barramundi, *Lates calcarifer* is one of the major fish species cultured in Southeast Asia (Ali, 1986). Seabass is in the family Centropomidae, an euryhaline species that can tolerate a wide range of salinity. This ability makes the fish easier to be cultured compare with other marine fishes (Giffard *et al.*, 2008). Asian seabass has become a preferable species to be culture not only in marine but also in freshwater condition (Kandan, 2015).

Fish diseases are caused by the interactions between the host, the pathogen and the environment (Scholthof, 2006). According to Faruk and Zahan (2017), the common fish diseases in hacery are caused by ciliate, protozoan, worms, myxosporodians fungi and bacteria. Pathogens are readily present in the ecosystem, but without stress factors the immune system of fish can tolerate the situation and prevent infection. However, modern aquaculture practices that lead to high-density culture and intensification have increased the stress levels of fish resulting to disease outbreaks and high mortality (Toranzo *et al.*, 2005).

For seabass, among the common fatal bacterial infections is vibriosis. This disease causes high mortality and significant economic losses (Abdel-Aziz *et al.*, 2013). The etiological agents include *Vibrio alginolyticus*, *Vibrio parahaemolyticus*, *Vibrio harveyi* and *Vibrio vulnificus* (Kumaran *et al.*, 2010; Caipang *et al.*, 2012; Abdel-Aziz *et al.*, 2013).

Vibrio is a genus of Gram-negative bacterium, with a curved rod or comma shaped. It is classified under the family Vibrionaceae, a facultative anaerobe with positive oxidizing test. Since *Vibrio* is a Gram-negative bacterium, this means that it has another layer of membrane covering the peptidoglycan layer. This protective layer makes this bacterium more resistant to antibiotics. Nevertheless, *Vibrio* is pathogenic to marine and brackish water fishes and rarely found infecting freshwater fish (Reed *et al.*, 1996). According to Osunla and Okoh (2017), *Vibrio* species are prevalent in the surface waters around the world, and is influenced by location, season and analytical method used.

Uncontrolled use of synthetic antibiotics such as oxytetracycline has resulted in development of antibiotic-resistant bacterial (Ventola, 2015). To overcome this problem, new antibacterial agents have to be developed. Herbs are considered to be the best alternative because herbs contain various antimicrobial properties (Harikrishnan and Balasundaram, 2005). One of the herbs that possess strong antioxidant and antibacterial properties is betel plant, *Piper betle*.

Betel plant have been used for a long time in traditional medicine to treat various diseases of humans (Guha, 2006). However, there are not many studies being carried out on the extract of this plant against pathogenic bacterial infection in fishes, such as vibriosis. As expected, no study was done on the toxic effects of betel plant extract on fishes. Therefore, the overall effects of betel plant as an antimicrobial substance for fish remain unknown. Thus, the objectives of this study were:

1. To determine the best extraction method for preparation of *Piper betle* leaf crude extract for aquaculture use.
2. To analyse the optimum *in vitro* dose of *Piper betle* leaf crude extract against *Vibrio alginolyticus*.
3. To evaluate the effectiveness of *Piper betle* leaf in treatment and prevention of vibriosis in cultured seabass.

The hypotheses of this study were:

1. Extraction of betel leaf using ethanol is more suitable compared with methanol and distilled water.
2. The ethanol betel leaf extract shows equivalent or better antimicrobial effect against *Vibrio alginolyticus* than the commercial antibiotics.
3. Betel leaf extract should be able to be consumed by the fish and give protection to Asian seabass against *Vibrio alginolyticus* when given as treatment or preventive measures.

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