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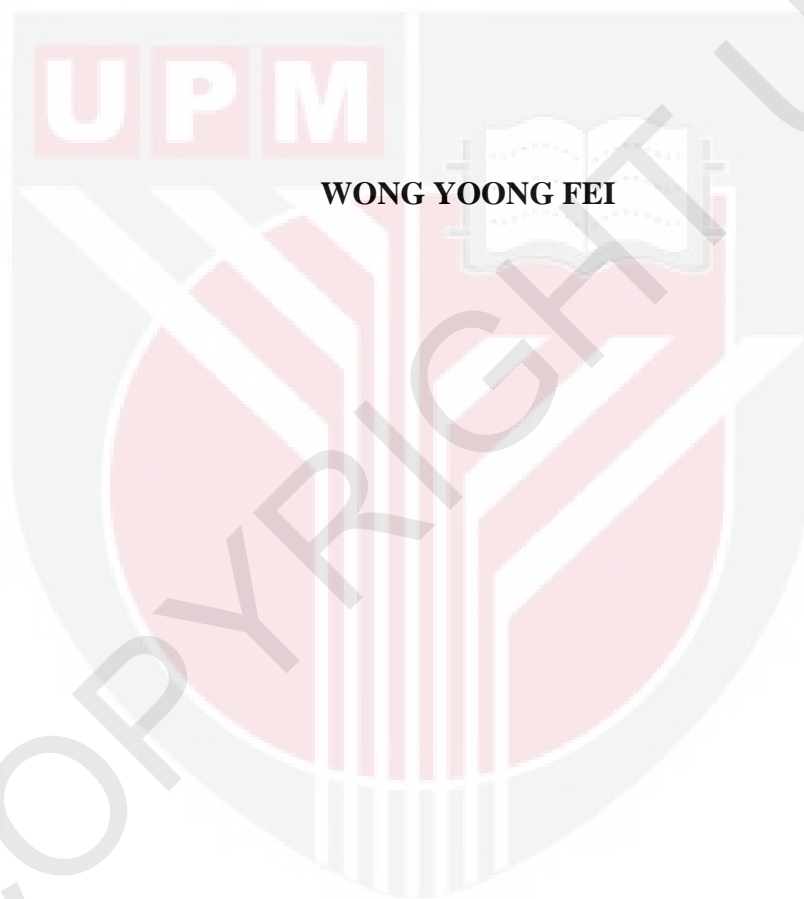
**THE ASSESSMENT OF CHOLINESTERASE FROM THE LIVER OF
ANABAS TESTUDINEUS FOR DETECTION OF METAL IONS**

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**DEPARTMENT OF BIOCHEMISTRY
FACULTY OF BIOTECHNOLOGY AND BIOMOLECULAR SCIENCES
UNIVERSITI PUTRA MALAYSIA**

2015

**THE ASSESSMENT OF CHOLINESTERASE FROM THE LIVER OF
ANABAS TESTUDINEUS FOR DETECTION OF METAL IONS**

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161209

**Thesis Submitted in Partial Fulfillment of the Requirement
For the course BCH 4999 (Project) in the Department of Biochemistry,
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PENGESAHAN

Dengan ini adalah disahkan bahawa laporan yang bertajuk penilaian kolinesteres daripada hati ikan Puyu (*Anabas testudineus*) sebagai pengesanan logam ion telah disiapkan serta dikemukakan kepada Jabatan Biokimia, Fakulti Bioteknologi dan Sains Biomolekul, Universiti Putra Malaysia oleh Wong Yoong Fei (161209) sebagai syarat untuk kursus BCH 4999 (Projek).

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ABSTRACT

Climbing Perch or its scientific name, *Anabas testudineus* is classified as one of the freshwater fishes belonging to the family of Anabantidae. It is locally known as 'Ikan Puyu' and is widely distributed in ponds, swamps and estuaries in Asia. In this study, cholinesterase (ChE) was partially purified from the liver of *A. testudineus* through ion exchange chromatography. This purification method provided recovery yield of 5.35% with a purification fold of 6.6. The optimum conditions for ChE assay were identified to be 2.5 mM butyrylthiocholine iodide (BTC) with pH 8.0 in 0.1 M Tris-HCl buffer at 40°C. Substrate specificity profile also indicated that ChE favours BTC as substrate because it records the highest catalytic efficiency (V_{\max}/K_m). Metal ion inhibition tests were conducted and mercury (Hg) was found to show the highest inhibition effect (87.30%) whereas lead (Pb) showed the lowest inhibition effect (28.01%). The half maximal inhibitory concentration (IC_{50}) value of mercury for partially purified ChE was 0.071 ppm. Result obtained from protein analysis through Native-PAGE has shown ion exchange chromatography as an effective method in partially purified ChE. All these findings showed that partially purified ChE from the liver of *A. testudineus* is suitable to be used as a bioindicator to detect the presence of metal ions.

ABSTRAK

Ikan Puyu atau nama saintifiknya *Anabas testudineus* diklasifikasikan sebagai satu jenis ikan air tawar yang tergolong dalam keluarga Anabantidae. Ikan Puyu ini boleh didapati secara meluas di kolam, paya dan muara di Asia. Dalam kajian ini, kolinesteres (ChE) daripada hati ikan *A. testudineus* telah berjaya ditulenkan separa melalui kaedah kromatografi pertukaran ion. Melalui kaedah penulenan ini, hasil perolehan adalah sebanyak 5.35% dengan faktor penulenan sebanyak 6.6. Keadaan optimum untuk assay ChE telah dikenalpasti di 2.5 mM Butirilthiokolin Iodide (BTC) dengan pH 8.0 dalam penimbal Tris-HCl di suhu 40°C. Profil spesifikasi substrat menunjukkan bahawa ChE lebih menggunakan BTC sebagai substrat kerana substrat ini menunjukkan kecekapan pemangkinan (V_{\max}/K_m) tertinggi. Ujian perencatan logam ion telah dijalankan dan merkuri (Hg) didapati menunjukkan kesan perencatan tertinggi iaitu sebanyak 87.30% manakala plumbum (Pb) menunjukkan kesan perencatan terendah iaitu sebanyak 28.01%. Nilai kepekatan perencatan separuh maksimum (IC_{50}) merkuri untuk ChE separa tulen ialah 0.071 ppm. Keputusan yang diperolehi daripada analisis protein melalui poliakrilamide gel elektroforesis natif (Native-PAGE) membuktikan kromatografi pertukaran ion ini sebagai kaedah yang berkesan dalam penulenan separa ChE. Semua penemuan dalam kajian ini menunjukkan bahawa penulenan separa ChE dari hati *A. testudineus* sesuai untuk digunakan sebagai biopenanda untuk mengesan kehadiran logam ion.

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LIST OF ABBREVIATIONS

| | |
|------------------|--|
| Å | Angstrom |
| °C | Degree Celsius |
| % | Percent |
| AChE | Acetylcholinesterase |
| ACh | Acetylcholine |
| ATC | Acetylthiocholine Iodide |
| BChE | Butyrylcholinesterase |
| BTC | Butyrylthiocholine Iodide |
| ChE | Cholinesterase |
| DEAE | Diethylaminoethyl |
| DTNB | 5,5'-dithiobis(2-nitrobenzoic acid) |
| <i>et al.,</i> | And others |
| IC ₅₀ | Half maximal inhibitory concentration |
| kDa | Kilo Dalton |
| K _m | Michaelis-Menten Constant |
| L | Litre |
| M | Molar |
| mg | Milligram |
| mM | Millimolar |
| μl | Microliter |
| nm | Nanometer |
| PAGE | Polyacrylamide gel electrophoresis |
| pH | -log concentration of H ⁺ ion |
| ppm | Parts per million |
| PTC | Propionylthiocholine Iodide |
| TEMED | N,N,N',N'-tetramethylethylenediamine |
| V _{max} | Maximum velocity |

CHAPTER 1

INTRODUCTION

Cholinesterases (ChE) are important enzymes that are present in both vertebrates and invertebrates. It is a family of enzymes that comprise of Acetylcholinesterase (AChE; EC 3.1.1.7), Butyrylcholinesterase (BChE; EC 3.1.1.8) and Propionylcholinesterase (PChE; EC 3.1.1.8).

In biochemistry, cholinesterases especially AChE readily hydrolyses acetylcholine, a neurotransmitter into choline and acetic acid (Mayberry *et al.*, 2015). According to Ashani *et al.*, (1991), BChE could act as a useful prophylaxis against soman poisoning in mice. Besides, BChE can degrade cocaine into inactive metabolite (Larrimore *et al.*, 2013). The function of ChE in the liver is to act as detoxifier. Liver performs an important role in biosynthesis and the ChE activity is an assessment indicator for liver function in patients with liver diseases (Meng *et al.*, 2013).

Heavy metals especially mercury, cadmium, lead, zinc, arsenic, chromium, copper and silver are known to cause abnormalities in fish. According to Fatima *et al.*, (2014), heavy metals lead to an altered physiology in fish and nuclear damage. Moreover, study from Zheng *et al.*, (2003) stated that toxic heavy metals do have the ability to interrupt normal functioning of the animal central nervous system (CNS). Therefore, to access the implication of heavy metals toward living organisms, ChE enzyme present in fish was used for the detection of heavy metals.

In this study, crude ChE was extracted from the liver of Climbing Perch (*Anabas testudineus*) or locally known as “ikan puyu”. Next, crude ChE was partially purified using ion-exchange chromatography with diethylaminoethyl-cellulose (DEAE-cellulose) as matrix. The partially purified ChE was analysed for various

parameters. The inhibitive test of heavy metals toward ChE was carried out to study the effect of heavy metals on ChE activity. Lastly, native polyacrylamide gel electrophoresis (Native-PAGE) was conducted to analyse and separate the protein in the sample.

The objectives of this study include:

1. Extraction and partial purification of cholinesterase (ChE) from the liver of *A. testudineus* using ion exchange chromatography.
2. Determination of the optimum pH and temperature of ChE
3. Determination of the inhibitory effects of metal ions toward ChE activity

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