



UNIVERSITI PUTRA MALAYSIA

***THE ASSESSMENT OF CHOLINESTERASE FROM THE BLOOD OF
Anabas testudineus AS DETECTION OF METAL IONS***

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FBSB 2015 52

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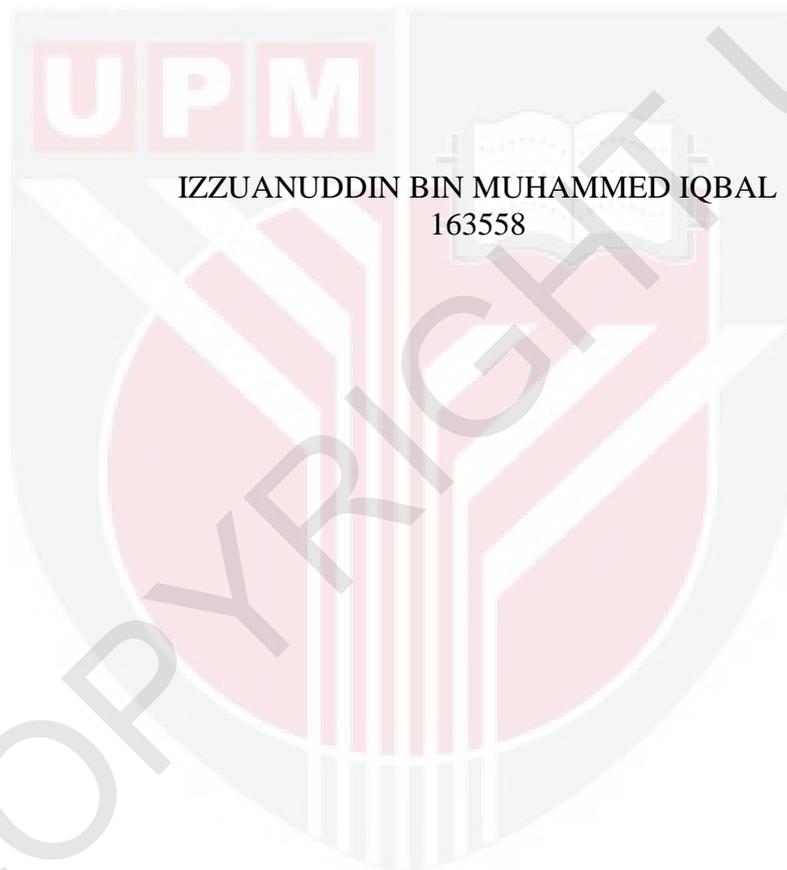


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2015

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Anabas testudineus AS DETECTION OF METAL IONS



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Dissertation submitted in partial fulfilment for the requirement for the course of
BCH 4999

Project in the Department of Biochemistry,
Faculty of Biotechnology and Biomolecular Sciences,
Universiti Putra Malaysia.

June 2015

PENGESAHAN

Dengan ini adalah disahkan bahawa tesis projek yang bertajuk “The Assessment of Cholinesterase from the Blood of *Anabas testudineus* as Detection of Metal Ions” telah disiapkan serta dikemukakan kepada Jabatan Biokimia oleh Izzuanuddin bin Muhammed Iqbal (163558) sebagai syarat untuk kursus BCH4999 Projek.

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ABSTRACT

Climbing perch, or its scientific name, *Anabas testudineus* is one of the freshwater fishes that inhabit river areas. The fish belongs to the family Anabantidae and in Malaysia, its local name is “Ikan Puyu”. Cholinesterase (ChE) was partially purified from the blood extract of *A. testudineus* in this study through ion exchange chromatography with DEAE-Cellulose as column matrix. This method of purification provided recovery yield of 47.66% and purification fold of 8.37. Analysis of protein through Native polyacrylamide gel electrophoresis (Native-PAGE) proves that ChE had been successfully partially purified by ion exchange chromatography. Thus, ion exchange chromatography can be used as one of the purification methods to partially purify ChE from *A. testudineus* blood extract. The optimum conditions for ChE assay were identified to be at 30°C and pH 8.0 in phosphate buffer with 2 mM BTC as substrate. Metal ion inhibition study was conducted and it was found that mercury showed the highest inhibition effect (89.94%) on ChE activity. IC₅₀ value of mercury towards the partially purified ChE was determined to be 1.733 ppm. These findings suggest that partially purified ChE from blood extract of *A. testudineus* is suitable to be used as bioindicator for the presence of metal ions in the environment.

ABSTRAK

Climbing perch, atau nama saintifiknya, *Anabas testudineus* merupakan salah satu ikan air tawar yang menduduki kawasan sungai. Ikan ini tergolong di dalam keluarga Anabantidae dan di Malaysia, nama tempatannya adalah “Ikan Puyu”. Kolinesteres (ChE) telah berjaya dipisahkan dari ekstrak darah *A. testudineus* di dalam kajian ini melalui kaedah kromatografi pertukaran ion menggunakan DEAE-Selulosa sebagai matriks ruangan. Kaedah penulenan ini memberi hasil perolehan sebanyak 47.66% dan gandaan penulenan sebanyak 8.37. Analisis protein melalui poliakrilamid gel elektroforesis natif (Native-PAGE) membuktikan bahawa ChE telah berjaya dipisahkan menggunakan kaedah kromatografi pertukaran ion. Lantas, kaedah kromatografi pertukaran ion boleh digunakan sebagai salah satu kaedah penulenan untuk proses penulenan separa ChE daripada ekstrak darah *A. testudineus*. Kondisi optimum bagi asai ChE telah dikenalpasti pada suhu 30°C dan pH 8.0 di dalam penimbal fosfat dengan 2 mM BTC sebagai substrat. Kajian perencatan ion logam telah dijalankan dan didapati bahawa merkuri menunjukkan kesan perencatan tertinggi (89.94%) terhadap aktiviti ChE. Nilai IC₅₀ merkuri terhadap ChE separa tulen telah ditentukan iaitu 1.733 ppm. Penemuan ini mencadangkan bahawa ChE separa tulen dari ekstrak darah *A. testudineus* adalah sesuai untuk digunakan sebagai petunjuk biologi bagi kehadiran ion logam pada alam sekitar.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my Final Year Project supervisor, Dr. Siti Aqlima Binti Ahmad. Without her guidance and dedicated involvement in every step throughout the process, this thesis would have never been completed. Thank you very much for your support and understanding.

I would like to extend my deepest gratitude to my seniors, Mohd Khalizan Bin Sabullah and Nursabrina Binti Mohd Hayat for their constant supervisions and continuous suggestions that helped me tremendously in improving my methods and results. I would also like to thank them for all the knowledge and experiences that they have taught and shared with me. Without their assistance, the completion of my project would never have been possible.

An expression of appreciation is also extended for my colleagues; Ain Aqilah, Shakirah, Low Weini and Wong Yoong Fei for their helps and supports through all the difficulties of completing this project. You all have been very patient with my knowledge gaps on the subject. Thanks for tolerating me for the past year. Your contributions will not be forgotten.

I would also like to give recognition to the academic and technical staffs of the Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia for providing me with necessary knowledge and facilities required for the completion of my project.

As a final token of gratitude, I would like to thank my family, especially my mother, Shura Bibi binti Mohammad Kunju who offered her encouragement through phone calls - despite my own limited devotion to correspondence. You have stood by me through thick and thin. Every time I was about to quit, you did not let me and I am forever grateful. This thesis stands as a testament to your unconditional love and encouragement.

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LIST OF ABBREVIATIONS

°C	Degree Celsius
%	Percent
µL	Microlitre
ACh	Acetylcholine
AChE	Acetylcholinesterase
APS	Ammonium persulfate
As	Arsenic
ATC	Acetylthiocholine iodide
BCh	Butyrylcholine
BChE	Butyrylcholinesterase
BSA	Bovine serum albumin
BTC	Butyrylthiocholine iodide
ChE	Cholinesterase
Cl	Chlorine
cm	centimetre
Co	Cobalt
Cr	Chromium
Cu	Copper
DEAE	Diethylaminoethyl
DTNB	5,5'-dithiobis(2-nitrobenzoic acid)
<i>et al.</i>	And friends
g	Gram
Hg	Mercury
IC ₅₀	Half maximal inhibitory concentration
kDa	Kilo Dalton
K _m	Michaelis-Menten constant
L	Litre
M	Molar
mg	Miligram
min	Minutes
ml	Millilitre
mM	Milimolar

Ni	Nickel
nm	Nanometre
PAGE	Polyacrylamide gel electrophoresis
Pb	Lead
PCh	Propionylcholine
PChE	Propionylcholinesterase
pH	$-\log$ concentration of H^+ ion
ppm	Part per million
PTC	Propionylthiocholine iodide
rpm	Revolution per minute
TEMED	N,N,N',N'-tetramethyl-ethylenediamine
U	Unit activity
V	Volt
V_{max}	Maximum velocity
Zn	Zinc

CHAPTER 1

INTRODUCTION

Cholinesterases (ChEs) are enzymes that are found everywhere in nature. ChEs can be found in mammalian red blood cell membranes, in blood plasma or in haemolymph, as well as in other organs (Rickwood and Galloway, 2004). ChEs can be classified into two groups, namely acetylcholinesterase (AChE), which hydrolyses acetylcholine (ACh), and butyrylcholinesterase (BChE), the one able to hydrolyse bigger molecules such as butyrylcholine (BCh) (Bajda *et al.*, 2013). AChE has a vital function in the working of the neuromuscular system by avoiding continuous muscular contraction (Matozzo *et al.*, 2005).

The structure of AChE was initially discovered in 1991 by Sussman, who achieved to crystallise the enzyme from the electric organ of electric ray, *Torpedo californica* (Bajda *et al.*, 2013). X-ray structures of AChE co-crystallised with different ligands gave perpectives into the fundamental structural elements and motifs vital to its mechanism of catalysis and ACh processing mode (Berg *et al.*, 2011).

Climbing perches, also known as climbing gouramis are a species of fish in the Anabantidae family, a family of perciform fishes. Climbing perches are well known for their ability to breathe air, and their resiliency to survive out of water in moist air for up to six days.

ChE is useful for detecting the presence of heavy metals. ChE assays are simple and rapid, making it suitable to be used outside in the field. ChE from fish is even more useful as there are a lot of freshwater bodies and also due to the substantial aquaculture industry in Malaysia (De La Torre *et al.*, 2002).

In this study, crude ChE was extracted from the blood of climbing perch (*Anabas testudineus*), one of the local freshwater fishes, also known as “Ikan Puyu”. Through ion exchange chromatography, crude ChE was partially purified. Native polyacrylamide gel electrophoresis (Native-PAGE) was conducted for the separation and protein analysis of the sample.

Thus, in this study, three major objectives will be accomplished. They are:

1. To extract and purify ChE from the blood extract of Climbing Perch (*A. testudineus*).
2. To determine the optimum pH and temperature conditions as well as substrate specificity of partially purified ChE sample.
3. To study the effects of various metal ions on the enzymatic activity of ChE.

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