UNIVERSITI PUTRA MALAYSIA

SOME PHYSICAL AND CHEMICAL PROPERTIES OF THE BIOACTIVE COMPONENTS RESPONSIBLE FOR ANTINOCICEPTIVE ACTIVITY OF HARUAN (CHANNA STRIATUS) EXTRACTS

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By

ZAINUL AMIRUDDIN B. ZAKARIA

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January 2001

DEDICATION

This research project is dedicated to all the following people who have inspired me in a special way that only I understand:

MYSELF ~ For being what you are!!!

MOM and DAD ~ For me being here!!!

SHARIAH LOH LONG ~ For your eternal love and patience!!!

MY FAMILY ~ For being there!!!

"It is better to burn out than to fade away…

Peace. Love. Empathy."

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science.

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Chairman: **Associate Professor Abdul Manan B. Mat Jais, Ph.D.**

Faculty: **Medicine and Health Science**

Haruan (*Channa striatus*) fillet and mucus extracts have been proven to exhibit a dose-dependent antinociceptive activity and enhanced morphine effect.

This current study is to establish some of the physical and chemical properties of the bioactive components responsible for haruan antinociceptive activity. Five types of solvents (water, methanol, ethanol, chloroform and chloroform:methanol) were used to extract the respective components from freeze-dried haruan fillet. Various salts (NaCl, KCl, $CaCl_2$, $MgCl_2$ and their combinations (MS)) were used to study the involvement of ionic components in mediating the antinociceptive activity of haruan extract. The aqueous portion obtained after a chloroform:methanol extraction of fresh haruan fillet was filtered using three types of Millipore filters (30,000, 10,000 and 5,000 Nominal Molecular Weight Limit (NMWL)), respectively. Lastly, the aqueous portion, filtered using the 5,000 NMWL Millipore filters, was purified using the High

Performance Liquid Chromatography (HPLC) procedure. All of the prepared solutions were then subjected to antinocieptive activity evaluation using the abdominal constriction test. All the data obtained were analysed using the Student's t-test or One-Way Analysis of Variance (ANOVA) to determine the presence of respective activity with $P<0.05$ as the limit of significance.

From the data obtained, the components appear to be a combination between water-soluble and fat-soluble compounds, which may act alone, as in distilled water (DH_2O) and chloroform extracts, or act together, as in chloroform:methanol, methanol and ethanol extracts. Furthermore, the ability to exhibit the activity after extraction in polar, moderately polar and non-polar solvents suggested the presence of peptide compounds.

The activity was improved in the presence of Na^+, K^+, Ca^{2+} and Mg^{2+} or their combination (MS) in a concentration-dependent manner and can be increased without increasing the extract concentration. Furthermore, Ca^{2+}, Mg^{2+} and MS have greater influence on the antinociceptive activity of haruan extract compared to Na^+ and K^+.

The components also appear to be of two molecules. The first molecule is believed to have a molecular weight (MW) less than 5,000 MW while the second molecule is thought to have a molecular weight between 10,000 and 30,000 MW, respectively.

The HPLC fraction of aqueous extract, filtered using the 5,000 NMWL filters, was also found to exhibit a dose-dependent activity, thus, indicating the presence of molecule with molecular weight less than 5,000 MW.

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

SEBAHAGIAN DARIPADA SIFAT FIZIKAL DAN KIMIA KOMPONEN BIOAKTIF YANG BERTANGGUNGJAWAB TERHADAP AKTIVITI ANTINOSISEPTIF PADA EKSTRAK HARUAN (*CHANNA STRIATUS*)

Oleh

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Januari 2001

Pengerusi: **Profesor Madya Abdul Manan B. Mat Jais, Ph.D.**

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Ekstrak-ekstrak daging dan mukus haruan (*Channa striatus*) telah dibuktikan mempunyai kesan antinosiseptif berkadaran dengan peningkatan dosnya dan juga meningkatkan kesan morphine.

Kajian terbaru ini bertujuan menentukan sifat-sifat fizikal dan kimia komponen bioaktif yang bertanggungjawab keatas aktiviti antinosiseptif haruan. Lima jenis pelarut (air, metanol, etanol, kloroform dan kloroform:metanol) telah digunakan untuk mengekstrak komponen bioaktif terbabit dari filet haruan yang telah dibeku-keringkan. Pelbagai garam (NaCl, KCl, $CaCl_2$, $MgCl_2$ dan kombinasi diantara mereka (MS)), telah digunakan untuk mengkaji penglibatan komponen-komponen ionik sebagai perantara aktiviti antinosiseptif ekstrak haruan. Bahagian akues yang didapati selepas proses pengekstrakan filet haruan segar menggunakan kloroform:metanol telah menjalani proses penapisan menggunakan tiga jenis penapis Millipore (30,000, 10,000

and 5,000 NMWL), masing-masing. Akhir sekali, bahagian akues tersebut, ditapis menggunakan penapis Millipore 5,000 NMWL, telah ditulenkan menggunakan kaedah Kromatografi Cecair Performans Tinggi (HPLC). Kesemua larutan yang telah disediakan itu kemudiannya dinilai aktiviti antinosiseptifnya menggunakan ujian pencerutan abdominal. Kesemua data yang telah diperolehi dianalisa menggunakan *Student's t-test* atau *One-Way Analysis of Variance (ANOVA)* untuk menetukan kehadiran aktiviti terbabit dengan $P<0.05$ sebagai had signifikan.

Daripada data-data yang telah diperolehi, komponen-komponen tersebut mungkin terdiri daripada kombinasi diantara bahan larut air dan larut lemak yang mungkin bertindak bersendirian, seperti didalam ekstrak-ekstrak air suling (DH_2O) dan kloroform, atau bertindak bersama-sama, seperti didalam ekstrak-ekstrak chloroform:methanol, methanol dan ethanol. Disamping itu, kehadiran aktiviti selepas pengekstrakan didalam pelarut-pelarut organik bersifat polar, separa polar dan tidak polar, mencadangkan kehadiran bahan peptide.

Aktiviti tersebut juga didapati meningkat dibawah kehadiran Na^+, K^+, Ca^{2+} and Mg^{2+} atau kombinasi diantara mereka (MS) berkadaran dengan peningkatan kepekatan ion-ion tersebut dan boleh ditingkatkan tanpa perlu menambahkan kepekatan ekstrak terbabit. Disamping itu, Ca^{2+}, Mg^{2+} dan MS lebih mempengaruhi aktiviti antinosiseptif ekstrak haruan berbanding Na^+ dan K^+.

Komponen tersebut mungkin juga terdiri dari dua molekul. Molekul pertama dipercayai mempunyai berat molekul kurang daripada 5,000 MW manakala komponen kedua dijangkakan mempunyai berat molekul diantara 10,000 dan 30,000 MW, masing-masing.

Fraksi HPLC, dari ekstrak akues yang ditapis menggunakan penapis 5,000 NMWL, juga menunjukkan aktiviti yang berkadaran dengan dosnya, seterusnya mencadangkan kehadiran molekul dengan berat molekul kurang daripada 5,000 MW.

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All gratification are referred to ALLAH S.W.T."

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I certify that an Examination Committee met on 27th January 2001 to conduct the final examination of Zainul Amiruddin B. Zakaria on his Master of Science thesis entitled "Some Physical and Chemical Properties of the Bioactive Components Responsible for Antinociceptive Activity of Haruan (*Channa striatus*) Extracts" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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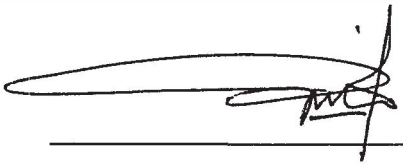
DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



ZAINUL AMIRUDDIN B. ZAKARIA

Date: February 10, 2001

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12 The Antinociceptive Activity of HPLC Fraction of Filtered Aqueous Extract of Haruan against Control Group 146

LIST OF ABBREVIATIONS

AA	Arachidonic acid
ADP	Adenosine diphosphate
AIDS	Auto-immune deficiency syndrome
AMP	Adenosine monophosphate
ANOVA	Analysis of variance
ATP	Adenosine triphospahte
C 1	Control group 1
C 2	Control group 2
Ca	Calcium
Cl	Chloride
CNS	Central nervous system
DH_2O	Distilled water
DMSO	Dimethylsulfoxide
EFA's	Essential fatty acids
EPA	Eicosapentanoic acid
G	Gravity
HPLC	High performance liquid chromatography
Hyp	Hydroxyproline
IMR	Institute of Medical Research
i.p.	Intraperitoneal
IP_3	Inositol triphosphate
i.t.	Intrathecal
K	Potassium/Kalium
kDa	kilo Dalton
LD_{50}	Median lethal dose
Mg	Magnesium
mM	milliMolar
MS	Mixed solutions
MSA	Mass spectrophotometry analysis
MW	Molecular weight
Na	Natrium/Sodium
NF	Number of Fundamental
α-NH_2	Free α amino group
NM	Nanometer
NMR	Nuclear Magnetic Resonance
NMWL	Nominal molecular weight limit
NSAID's	Non-steroidal anti-inflammatory drugs
PCE	Protein conversion efficiency
PGE_2	Prostaglandin E_2
PKC	Protein kinase C