



UNIVERSITI PUTRA MALAYSIA

**ANTIOXIDATIVE POTENTIAL OF FOUR EXTRACTS OF HARUAN,
Channa striatus (BLOCH) AN INDIGENOUS MALAYSIAN
SNAKEHEAD FISH**

CHE KU DAHLAN BIN CHE KU DAUD

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SNAKEHEAD FISH**

By

CHE KU DAHLAN BIN CHE KU DAUD

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Chair: Professor Abdul Manan Mat Jais, PhD

Faculty: Medicine and Health Sciences.

Channa striatus, Haruan is one of the most popular freshwater fish in Malaysia for many medicinal purposes since the past few decades especially in reducing pain and inflammation as well as wound healing. This study was aimed to investigate the antioxidative potentials of *C. striatus* extracts obtained by different methods of extraction including the proximate, minerals contents and to determine the amino and fatty acids compositions. Four types of extracts have been used namely Haruan Traditional Malay Extract (HTE); upper and lower phase, aqueous extract (1:1, w/v), and chloroform methanol extract. An established commercial *C. striatus* essence was used as a reference extract. Energy dispersive x-ray variable pressure scanning electron microscopy (EDX-VPSEM) and atomic absorption spectrophotometry (AAS) were used to determine and identify the mineral elements in the fresh fillet sample. Amino and fatty acids of extracts were analysed using high performance liquid chromatography (HPLC) and gas chromatography (GC) techniques, respectively. Antioxidant capacity of all samples was assessed using photosensitized chemiluminescence (PCL) method, ferric reducing antioxidant power (FRAP) and β -

carotene–linoleate bleaching (BCL) assays. The proximate analysis revealed that the moisture content, crude protein, ash and crude lipid of Haruan fresh fillet were 75 ~ 85%, 20.85%, 0.98 ~ 0.99% and 2.49%, respectively. The elemental analysis using EDX-VPSEM showed that the sample contain many important macro minerals including Ca (21.29 ± 8.36) mg/100, K (230.74 ± 22.65) mg/100, Mg (24.05 ± 1.05) mg/100 and Na (69.20 ± 14.44) mg/100, and trace minerals; Zn (7.08 ± 0.44) $\mu\text{g/g}$, Fe (17.42 ± 1.53) $\mu\text{g/g}$, Cu (0.79 ± 0.37) $\mu\text{g/g}$, Cr (0.12 ± 0.11) $\mu\text{g/g}$ and Se (7.27 ± 0.77) $\mu\text{g/g}$. The extracts contained at least 17 amino acids with the dominantly were glutamic acid, glycine, leusine, aspartic acid, proline, alanine and arginine with values 1.87 – 43.13 mg/g, 21.80 – 80.85 mg/g, 7.85 – 40.19 mg/g, 13.85 – 44.07 mg/g, 9.49 – 45.46 mg/g, 11.32 – 35.25 mg/g and 5.99 – 21.79 mg/g, respectively. The highest percentage of fatty acids present in the extracts was palmitic acid; 3.53 – 26.84% of total lipid. The others major fatty acids were stearic acid, oleic acid and linoleic acid with values 3.25 – 15.90%, 1.40 – 27.68%, 0.51 – 7.82% of total lipid, respectively. HTE also found to have extra 4 bioactive compounds which been labelled with 1, 2, 3 and 4 after direct injection to HPLC method. The antioxidant activity of the extracts based on PCL method was range between 1.14 – 1.86 $\mu\text{mol/mL}$ (ascorbic acid equivalent) and followed the order of HTE Lower phase > chloroform methanol extract > commercial Haruan essence > aqueous extract > HTE Upper phase. The ability to reduce ferric ion based on FRAP assay was not significant amongst *C. striatus* extracts with value in range 302.48 - 429.81 $\mu\text{M Fe}^{2+}/\text{g}$ reducing capacity, but significantly different to the standards used. The highest FRAP value was HTE upper phase (429.81 ± 0.88 $\mu\text{M Fe}^{2+}/\text{g}$ dry weight sample), followed by HTE Lower phase > aqueous extract (1:1 w/v) > commercial Haruan essence > chloroform methanol extract. The antioxidant activity of extracts

based on the BCL method followed the order of HTE Upper phase > commercial Haruan essence > aqueous extract (1:1 w/v) > HTE Lower phase > Chloroform methanol extract. All the samples in PCL and FRAP assays showed no significant difference but have significant difference in BCL assay at $p < 0.05$. Based on results of the three antioxidant capacity assays, antioxidant capacity all *C. striatus* extracts been studied were in the order of HTE Upper phase > HTE Lower phase > aqueous extract (1:1 w/v) > commercial Haruan essence > chloroform methanol extract. Results indicated that different assays revealed different antioxidant capacity. In conclusion, *Channa striatus* Haruan extracts were proven to have high antioxidant activities. Besides, the extracts were shown to have hydrophilic antioxidative properties. Thus the extract may recommend as biopharmaceutical product or as a food supplement which providing natural antioxidant for improving and maintaining body's health.

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

KEUPAYAAN ANTIOKSIDATIF DALAM EMPAT EKSTRAK IKAN HARUAN, *Channa striatus* (BLOCH) LELUHUR MALAYSIA

Oleh

CHE KU DAHLAN BIN CHE KU DAUD

April 2011

Pengerusi: Profesor Abdul Manan Mat Jais, PhD

Fakulti: Perubatan dan Sains Kesihatan

Channa striatus, Haruan adalah salah satu ikan air tawar yang popular di Malaysia untuk pelbagai tujuan perubatan sejak beberapa dekad yang lalu terutamanya dalam mengurangkan kesakitan dan pembengkakan serta penyembuhan luka. Kajian ini bertujuan untuk mengkaji keupayaan antioksidatif ekstrak *C. striatus* yang disediakan melalui beberapa kaedah pengekstrakan yang berbeza termasuk menentukan komposisi proksimat, kandungan mineral, asid amino dan asid lemak. Empat jenis ekstrak digunakan iaitu ekstrak Haruan Tradisional Melayu (HTE); bahagian atas dan bahagian bawah, ekstrak akues (1:1, w/v) dan ekstrak kloroform methanol. Satu ekstrak pati *C. striatus* yang komersial terkenal umum telah digunakan sebagai ekstrak rujukan. Teknik penyerakan tenaga x-ray pelbagai tekanan-imbasan mikroskop elektron (EDX-VPSEM) dan kaedah spektrofotometri penyerakan atom (AAS) telah digunakan untuk menentukan dan mengenalpasti unsur-unsur mineral dalam sampel isi segar. Asid amino dan asid lemak dari setiap ekstrak masing-masing dianalisis menggunakan teknik kromatografi cecair prestasi tinggi (HPLC) dan kromatografi gas (GC). Kapasiti antioksidan semua sampel telah

diukur menggunakan kaedah *photosensitized chemiluminescence* (PCL), asai penurunan ferik/kekuatan antioksidan (FRAP) dan asai pelunturan β -karotena–asid linoleik (BCL). Analisis proksimat menunjukkan bahawa kandungan air, protein kasar, abu dan lemak mentah masing-masing adalah 75 ~ 85%, 20.85%, 0.98 ~ 0.99% dan 2.49%. Analisis unsur mineral dengan EDX-VPSEM menunjukkan bahawa sampel mengandungi mineral-mineral penting termasuk makromineral seperti Ca (21.29 ± 8.36) mg/100, K (230.74 ± 22.65) mg/100, Mg (24.05 ± 1.05) mg/100 dan Na (69.20 ± 14.44) mg/100, manakala mineral surih; Zn (7.08 ± 0.44) ug/g, Fe (17.42 ± 1.53) ug/g, Cu (0.79 ± 0.37) ug/g, Cr (0.12 ± 0.11) ug/g dan Se (7.27 ± 0.77) ug/g. Semua ekstrak didapati mengandungi sekurang-kurangnya 17 asid amino dengan asid glutamik, glisin, leusine, asid aspartik, prolin, alanin dan arginin dengan nilai masing-masing 1.87 - 43.13 mg/g, 21.80 – 80.85 mg/g, 7.85 - 40.19 mg/g, 13.85 - 44.07 mg/g, 9.49 – 45.46 mg/g, 11.32 – 35.25 mg/g dan 5.99 – 21.79 mg/g. Asid lemak tertinggi hadir dalam ekstrak adalah asid palmitik; 3.53 – 26.84% daripada jumlah keseluruhan lipid. Asid lemak utama lain termasuklah asid stearik, asid oleik dan asid linoleik dengan nilai masing-masing 3.25 – 15.90%, 1.40 – 27.68%, 0.51 – 7.82% daripada jumlah keseluruhan lipid. HTE juga didapati mengandungi 4 sebatian bioaktif tambahan yang telah dilabel sebagai 1, 2, 3 dan 4 selepas ekstrak HTE disuntik terus kepada alatan HPLC. Aktiviti antioksidan ekstrak berasaskan kaedah PCL adalah antara 1.14 – 1.86 $\mu\text{mol/mL}$ (ekuivalen asid askorbik) dan mengikut urutan fasa HTE bahagian bawah > ekstrak kloroform metanol > pati Haruan komersial > ekstrak akues > HTE bahagian atas. Kemampuan untuk mengurangkan ion ferum berdasarkan asai FRAP adalah tidak berbeza secara signifikan pada semua ekstrak *C. striatus* dengan nilai keupayaan penurunan adalah antara 302.48 - 429.81 $\mu\text{M Fe}^{2+}/\text{g}$, tetapi berbeza signifikan kepada standard yang

digunakan. Nilai FRAP tertinggi adalah HTE bahagian atas ($429.81 \pm 0.88 \mu\text{M Fe}^{2+}/\text{g}$ berat sample), diikuti oleh fasa HTE bahagian bawah > ekstrak akues (1:1 w/v) > Pati Haruan komersial > ekstrak kloroform metanol. Aktiviti antioksidan setiap ekstrak pada asai BCL adalah mengikuti urutan; HTE bahagian atas > pati Haruan komersial > ekstrak akues (1:1 w/v) > HTE bahagian bawah > ekstrak kloroform metanol. Semua ekstrak dalam asai PCL and FRAP menunjukkan tiada perbezaan signifikan tetapi berbeza secara signifikan pada asai BCL pada $p < 0.05$. Berdasarkan keputusan tiga asai antioksidan, kapasiti antioksidan untuk semua ekstrak *C. striatus* disusun mengikut urutan berikut; HTE bahagian atas > HTE bahagian bawah > ekstrak akues (1:1 w/v) > pati Haruan komersial > ekstrak kloroform metanol. Keputusan ini menunjukkan bahawa asai yang berbeza memberikan nilai kapasiti antioksidan yang berbeza. Sebagai kesimpulan, ekstrak *Channa striatus* Haruan telah dibuktikan mempunyai aktiviti antioksidan yang tinggi. Di samping itu, ekstrak-ekstrak tersebut menunjukkan pemilikan antioksidatif hidrofilik. Sehubungan itu, ekstrak ini adalah dicadang sebagai produk biofarmaseutikal atau sebagai makanan tambahan yang membekalkan antioksidan semulajadi bagi meningkatkan dan mengekalkan kesihatan badan.

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APPROVAL

I certify that a Thesis Examination Committee has met on 18th April 2011 to conduct the final examination of Che Ku Dahlan bin Che Ku Daud on his thesis entitled “Antioxidative Potential of Four Extracts of Haruan, *Channa striatus* (Bloch) an Indigenous Malaysian Snakehead Fish” in accordance with the Universities and University Collage 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.

Members of the Thesis Examination Committee were as follows:

Sabrina binti Sukardi, PhD

Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Roslida binti Abd Hamid@Abdul Razak, PhD

Senior Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Shuhaimi bin Mustafa, PhD

Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Mohd Khan Ayob, PhD

Associate Professor
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
(External Examiner)

NORITAH OMAR, PhD.

Associate Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

APPROVAL

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Abdul Manan bin Mat Jais, PhD

Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Abdah binti Md Akim, PhD

Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

Zuraini binti Ahmad, PhD

Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

Aishah binti Adam, PhD

Professor
Faculty of Pharmacy
Universiti Teknologi Mara (UiTM)
(Member)

HASANAH MOHD GHAZALI, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work expect for quotations and citations which have been duly acknowledged. I also declare that is has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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CHE KU DAHLAN BIN CHE KU DAUD

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