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Amino and fatty acid compositions in Haruan traditional extract (HTE)

[Composición de aminoácidos y ácidos grasos en extractos de "Haruan" tradicional]

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Abstract

Evaluation of amino and fatty acids compositions in Haruan Traditional Extracts (HTE) was done using HPLC and GC methods. The HTE contained at least 17 amino acids with glutamic acid, glycine, leusine, aspartic acid, proline, alanine and arginine are the most, 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, 11.38 - 35.25 mg/g and 5.99 - 21.79 mg/g, respectively. Meanwhile, the highest percentage of fatty acids is palmitic acid; 3.54 - 26.84 % of total protein. The others major fatty acids are stearic acid, oleic acid and linoleic acid with values 3.25 - 15.90 %, 1.40 - 27.68 %, 0.51 - 7.82 % of total protein, respectively. HTE also found to have 4 extra bioactive compounds labelled as 1 to 4 on chromatographic tracing which in line with previously finding. It is concluded that the HTE is containing all the important amino acids plus some fatty acids, which is the basis to conduct antioxidant composition in both fresh Haruan and the HTE which was claimed to have wound healing properties. Comparative study was also carried out in various other extraction protocols, including commercial product.

Keywords: Haruan *Channa striatus, Haruan Traditional Extract (HTE), biochemical analysis, amino acid and fatty acid profiles*

Resumen

Evaluación de las composiciones de aminoácidos y ácidos grasos en Haruan Extractos tradicional (HTE) se realizó mediante métodos de HPLC y GC. La HTE contenía al menos 17 aminoácidos con ácido glutámico, glicina, leucina, ácido aspártico, prolina, alanina y arginina como mayoritarios, con valores de 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, 11.38 - 35.25 mg/g and 5.99 - 21.79 mg/g, respectivamente. Mientras tanto, el mayor porcentaje de ácidos grasos es el ácido palmítico; 3.54 - 26.84 % de la proteína total. Otros ácidos grasos importantes son el ácido esteárico, ácido oleico y ácido linoleico con valores de 3.25 - 15.90 %, 1.40 - 27.68 %, 0.51 - 7.82 % de la proteína total, respectivamente. HTE también encontró cuatro compuestos bioactivos adicionales etiquetados de 1 a 4 en el seguimiento cromatográfico que está de acuerdo con resultados previos. Se concluye que la HTE contiene todos los aminoácidos importantes además de algunos ácidos grasos, que es la base para llevar a cabo la composición antioxidante, tanto en fresco Haruan y la HTE que se afirma poseen propiedades curativas. Estudios comparativos se llevaron a cabo con otros protocolos de extracción, incluido el producto comercial.

Palabras claves: Haruan *Channa striatus*, extracto de Haruan tradicional (HTE), análisis bioquímicos, los aminoácidos y los perfiles de ácidos grasos

List of abbreviations: HTE – Haruan Traditional Extract

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INTRODUCTION

Fish is a major component in daily diet in Malaysia, and it is traditionally a major source of protein. According to the Malaysian Agricultural Directory and Index (1993/1994), fish contributed about 60 - 70 % of protein intake and the annual per capita consumption of fish is estimated to be 39 kg, and expected to be increased up to 45 kg in year 2000 (Osman *at el*., 2001). During the past few years, fish have been proven having good sources of monounsaturated, polyunsaturated fatty acids and amino acid constituents in the high quality of proteins. Together with vitamins and minerals compositions, providing potentials benefits in alleviating various health diseases and disorders such as rheumatoid arthritis and inflammatory disorder has been successfully demonstrated (Gosh and Dua, 1997). Omega-3 and 6 polyunsaturated fatty acids (PUFA) have been shown to have positive effects on cardiovascular diseases and cancer (Conner, 1997).

A fish rich in n-3 fatty acids has shown favourable results in mitigating health risk (von Schacky *et al*., 1999; Christensen *et al*., 2001), and consumption of 300 - 600 g daily of freshwater fish showed lowering blood pressure, lower plasma lipid concentration and increased plasma concentrations of EPA and DHA. All the beneficial health results are parallel to the marine fish consumption in Japan (Yamada *et al*., 2000; Ackman *et al*., 2002).

For many decades, Haruan is well known to many in parts of the world, especially to it native regions such as Malaysia, Indonesia, Brunei, Thailand and other tropical climate areas of Asia. The fish is always consumed because the ability of the fish in reduces pain and inner inflammation (Mat Jais *et al*., 1997). Haruan is believed by rural peoples to having good medicinal qualities and as well as easy to access naturally. Today, in Malaysia, Haruan is popularly used by local peoples to treat mother after birth if the hospital is far from the village. The Haruan oil and essence's remedies also help to promote healing process of many traumas conditions especially after surgical intervention or among caesarean mothers, after giving birth or childbirth, alleviates post-operative pain, road accidents and discomfort (Mat Jais *et. al*., 1998).

The present of arachidonic acid (AA), a precursor of prostaglandins, in Haruan have been demonstrated by Mat Jais *et al*. (1994), in an unusual amount in fresh fillet is believed played major responsible in blood clotting as well as tissue growth (Bowman and Rand, 1980). Together with omega-3 polyunsaturated fatty acid (PUFAs) (Mat Jais *et al*., 1994) and essentials amino acids promote wound healing processes. Haruan fresh fillet also contained high glycine (Mat Jais *et al*., 1994, 1998; Zakaria *et al*., 2007), one of the important components responsible in formation of collagen in human skin body.

In addition, Mat Jais *et al*. (1998) also demonstrated the presence of unsaturated fatty acids, particularly oleic acid (C18:1) and linoleic acid (C18:2), as a major components of Haruan mucus extract. Other than that, roe of Haruan was also have been investigated to have unsaturated fatty acid (MUFA and PUFA) as major component, compared with the mucus. Interestingly, the components of mucus together with fillet have been shown to have antinociceptive activity (Mat Jais *et al*., 1997). In an extension study, Somchit *et al*. (2004), has reported that *Channa sp.* indeed possess analgesic or antinociceptive properties. Since, the many marine and freshwater fish have been proven to have antioxidant activity (Ekanayake *et al*., 2004, 2005; Kaur *et al*., 2005; Wu, 2006 Wu & Bechtel, 2008), it is interestingly to establish the amino and fatty acids profiles of all the extraction protocols from *C. striatus*, (Haruan). Therefore, all the data will be used as reasons to support the antioxidant activity analysis in this fish species.

MATERIALS AND METHODS

Fish Preparation: The medium size *Channa striatus* (250 – 400 g/fish), were collected from cultivated ponds from Naning, Melaka and Rawang, Selangor, Malaysia and directly brought alive to Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Selangor, Malaysia, for acclimatization processes for at least 24 h before the extractions procedures to carried out using the method described by Mat Jais *et al.* (1997). Pre-cleaned live *C. striatus* were weighed and sacrificing was done in hypothermal shocked with iced for about 10 to 20 minutes (Mat Jais *et al.*, 1997). Subsequently, fillets were obtained by carefully cutting the fish lengthwise along the backbone to gain maximum amount of flesh without any spine.

Haruan Traditional Extracts (HTE) (1:1 w/v): The fish fillets extract was prepared using a pressure cooker set at 100°C for 60 minutes (Mat Jais *et al*., 1994). Fresh boneless fillet was weighed and seasoned by way of Malay traditional preparation about one hour, followed by one hour cooking under pressure. Ordinary drinking water is used in fish:water volume ratios of 1:1, and the water level is kept above and submerging the fillet throughout the cooking. Occasionally, the extraction is done through steaming. At the end of extraction process, the fillet was discarded, where two phases of HTE were obtained, a solid UPPER PHASE and the liquid LOWER PHASE. The liquid extracts were collected, filtered, centrifuged and stored at 4°C before use. Under these conditions, the final concentration was approximately 50 % fish in water (w/v). The weight refers to wet fit weight.

Haruan Aqueous Extraction (1:1 w/v): The fish fillets extract was prepared using a pressure cooker set at 100°C for 60 minutes (Mat Jais *et al*., 1994). Fresh boneless fillet was weighed and soaked in pressure cooker cup with distilled water. Distilled water added in fish:water volume ratios of 1:1 (w/v), the extract was obtained through boiling as if making into essence or soup. At the end of extraction process, the fillet was discarded, while the liquid soup of extract collected and filtered with Whatman No. 1 filter paper and then centrifuged and stored at 4°C before use. Under these conditions, the final concentration has been approximately 50 % fish in water (w/v).

Haruan Chloroform Methanol Extract: Fresh fish was pre-cleaned, weighed and then slaughtered. The fish fillets were obtained as usual (Mat Jais *et al*., 1997), and cut into small portions and were placed into sterile plastic bag (15 x 30 cm). The bag was sealed and transferred to a freezer at – 80 °C for 24 h. At the end of 24 h, the bag was removed from the freezer and was cut to open its sealer. Then the wet fillets were prepared for chloroform methanol extract according to Zakaria *et al*. (2004) with slightly modification. The Haruan fillets was homogenized for 5 min in chloroform methanol solution (2:1 v/v) in the ratio of 1:20 (w/v), in a conical flask and then stirred for 48 h to mix it thoroughly. At the end of 48 h, the sample was filtered and the supernatant obtained was left for 30 min to settle down. The LIPID PHASE and AQUOUES PHASE extracts were collected and then the chloroform methanol was removed by rotary evaporation process or flushed by nitrogen gas. The yield of the extracts was stored at -20 °C before use.

Commercial Haruan Extract: A popular commercial sample of Haruan Essence was bought from pharmacy shop to be used as a comparison. The amino and fatty acids compositions of the sample were obtained as same protocol as described below.

Determination of Amino Acid Compositions in Haruan Extracts: The amino acid analysis of the extracts of Haruan including the commercial Haruan essence was performed according to the methods described by Khan *et al*. (1994) and Vidotti *et al*. (2003) with slightly modification. The extracts of Haruan (0.25 g) was hydrolysed with 15 mL of 6 Molar hydrochloric acid (HCI) in a closed test tube, shaken for 15 min and then flushed with nitrogen for 1 min prior to being put in an oven for 24 hours at 110 °C. After cooling, 10 mL of the internal standard α-aminobutyric acid (AABA) was added to each sample prior to the addition of 20 µL redrying solution (methanol: water: triethylamine, 2 : 2 : 1, v/v/v) and 20 µL derivatization reagent (methanol: triethylamine: water: phenylisocyanate, 7 : 1 : 1 : 1, v/v/v/v). The mixture was then poured into volumetric flasks and deionized water was added to a final volume of 50 mL. Five to 15 mL of the upper layer was discarded; the rest of the upper layer was filter through Whatman No 1 filter paper. The hydrolysed sample obtained after filtration was kept for up to 4 weeks at -20 °C until use.

Before injection into HPLC, the hydrolysed samples were filtered using a nylon 0.2 µm cellulose nitrate membrane filter. Then, 10 µL filtered sample was put into a vial and the same volume of internal standard was added before the sample was dried under a vacuum for 30 min. The re-drying solution (20 µL) was then added to the dried sample and the mixture was shaken vigorously for 15 minutes. The sample was dry again under vacuum for another 30 minutes, followed by the addition of 20 µL derivatization reagent. The mixture was again shaken vigorously for 15 min and then left at room temperature for 20 min before being dried again under vacuum for 30 min. The dried sample was kept at - 20 °C until analysis by HPLC.

Prior to injection into the HPLC, the sample and standard were mixed with 100 µL sample

diluents (Khan *et al*., 1994), shaken for 15 min and injected onto the HPLC in volumes of 20 and 8 µL, respectively. The free amino acid was separated using the WATER PICO-TAG column for hydrolysate amino acid (3.9 x 300 mm; Millipore Corporation, Water Chromatography Division, USA) by reversed phase HPLC, with the flow rate of 1.0 mL/min and detected using a UV detector at 254 nm. The eluent A (50 mM sodium acetate trihydrate buffer, pH 5.7) and eluent B (60 % acetonitrile in water) were used as transporters/mobiles phase. Gradient conditions were used as shown in the Table 1 below:

Table 1. The gradient table of buffer A and buffer B

Time (Min)	Buffer / Eluent A (%)	Buffer / Eluent B (%)
0	100	0
15	90	10
30	60	40
40	50	50
50	0	100
55	0	100
57	100	0
60	100	0

Determination of Fatty Acid Composition (FAME Analysis) of *C. striatus* Extracts: About 100 mg of sample was weighed in a 20 mL test tube (with screw cap) or reaction vial. The sample was then dissolved in 10 mL hexane. One hundred (100) µL of 2N KOH was added in methanol (11.2 g in 100 mL). Tube or vials were capped, and vortex for 30 seconds. The solution later was centrifuged, and the clear supernatant was transferred into a 2 mL auto sampler vial and injected to GC. Analysis made using the gas chromatography was recorded. Fatty acid composition of Haruan samples including the commercial Haruan essence was analyzed using Shimadzu Gas Chromatography (GC) GC-17A equipped with a split-splitness injector, and electronic pressure controller with flame ionization detection (FID) system used to separate and quantify each FAMEs component. FAMEs were separated using SGE BPX-70 column (60 m x 0.25 mm I.D. x 0.15 µm plyethylene glycol film).

Chromatography data was recorded and integrated using Chemstations Software (version 6.0). Oven temperature was held at 50 °C for 1 min, then increased to 175 °C at 4 °C/min and lastly increased to 230 °C, held for 5 min at 230 °C. Temperature for injector and detector were set at 250 °C and 260 °C, respectively. One µL of sample volume was injected with split ratio of 100:1 at column temperature 200 °C. Carrier gases that were used for the system are helium gas, 1.7 mL/min controlled at 103.4 kPa, hydrogen and air used for FID was held at 275.6 kPa. Standard of FAMEs were used to determine each type of fatty acid. Fatty acid composition of Haruan samples were determined by comparing the retention time of sample FAMEs with those of standards of FAMEs for each chromatography peak for each fatty acids.

High Performance Liquid Chromatography Profiling of HTE: Subsequently, the HTE extract was subjected to High Performance Liquid Chromatography (HPLC) according to method described by Hornby *et al.* (2001) with slight modification. We have been tried many procedures by manipulating the volume of sample injection, the flow rate of sample or absorbance wavelength, and the conditions given below was found to be the best for separation of peptides from HTE. Briefly, a 15 µL sample was injected into LC-10AT Shimadzu HPLC equipped with two slave 306 pumps and analytical reverse-phase Vydac C-18 column; 5 µm, 0.46 x 25 cm set at 40°C, with flow rate of 1.0 mL min^{-1} absorbance at wavelength of 220 nm is monitored by a wavelength UV detector. The eluent A is 15 % Acetol nitrile (ACN) and eluant B is 85 % dH_2O.

Statistical Analysis: Data collected were analyzed using SPSS (Scientific Package of Social Science) version 16.00. Descriptive statistics were used in analysing mean and standard deviation (SD) and inferential statistics involving ANOVA-One Way test with Post Hoc and Tukey's were employed to compare the differences in means of significant differences values of all the test extracts. The confidence interval of statistics was 95 % and the significance value was set at $p < 0.05$.

RESULTS

The Amino Acid and Fatty Acid Composition of Haruan Extracts: The HPLC profiling of amino acid of Haruan Extracts (HTE) is shown in Figure 1 and the compositions of each amino acid compounds of five Haruan extracts is illustrated in Table 2. All

the Haruan extracts namely, Haruan Traditional Extracts (HTE); UPPER and LOWER PHASE, Haruan Aqueous Extract, Haruan Chloroform Methanol Extract (AQUOUES PHASE) and Commercial Haruan Essence from pharmacy were found to contain at least the 17 essential amino acids and the major amino acid found are glutamic acid, glycine, leusine, aspartic acid, proline, alanine and arginine with the range of the protein between 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, 11.38 - 35.25 mg/g and 5.99 - 21.79 mg/g, respectively.

The GC profiling of fatty acids of Haruan extract is shown in Figure 2 and the fatty acid composition percentage of total fatty acid of Haruan extracts namely Haruan Traditional Extracts (HTE); UPPER and LOWER PHASE, Haruan Aqueous Extract, Haruan Chloroform Methanol Extract (LIPID PHASE) and Commercial Haruan Essence from a pharmacy were illustrated in Table 3. The highest percentage of fatty acids present in the Haruan extracts was palmitic acid with range between 3.54 - 26.84% of total protein. The other major fatty acids were stearic acid, oleic acid and linoleic acid, which those all gave approximately with range between 3.25 - 15.90%, 1.40 - 27.68%, 0.51 - 7.82% of total protein, respectively.

The HPLC Profile of Haruan Traditional Extract (HTE): At least there are four major fractions were detected in Haruan Traditional Extract shown in Figure 3 were labelled as fractions 1, 2, 3 and 4.

DISCUSSION

In the this study, five different of *Channa striatus*, Haruan extracts namely, Haruan Traditional Extract (HTE); UPPER PHASE and HTE LOWER PHASE, Aqueous extract (1:1 w/v), Chloroform Methanol extract (AQUEOUS and LIPID PHASE) and Commercial Haruan Extract (Essence) available at pharmacy have been used to evaluate the amino and fatty acids compositions present in the extracts. HTE UPPER PHASE was more watery extract but HTE LOWER PHASE was more fatty extract. All the extracts of fish in this study were based on fish muscle (fillet), which a main part of fish body and forms part of the staple diet of a large percentage of the Malaysian population.

Dominant amino acid in all extracts of the *C. striatus*, Haruan were glutamic acid, glycine, leusine, aspartic acid, proline, alanine and arginine, meanwhile the dominant fatty acids in all extracts were palmitic acid (C16 : 0), stearic acid (C18 :0), oleic acid (C18 : 1n-9) and linoleic acid (C18 : 2n -6). The comparison of amino acid compositions between HTE UPPER PHASE and HTE LOWER PHASE with AQUOUES extract (Table 2) showed an increase in glutamic acid, glycine, arginine, proline and leucine levels, but all the extracts were significant different at $p<0.05$ statistically when compared to Commercial Haruan Essence . This might be have occurred during seasoning period of preparing of HTE extracts due to chemical reactions between alpha amino and aldehyde groups present in amino acids (Vidotti *et al*., 2003) and the seasoning process cause the degradation of some muscle proteins into peptides and amino acids (Dince *et al*., 2010). All the extracts in this study showed significant different between each extracts at $p<0.05$.

Amino acids contain basic amino groups, acidic carboxyl groups and a side chain (R). The R chains influence the chemical properties of amino acids and proteins. The types of side chains may be classified into four groups; polar-uncharged (glycine, serine, threonine, cysteine, aspargine and glutamine) are generally soluble in aqueous phases, non-polar (organic) (Alanine, valine, leucine, isoleucine, phenylalanine, tryptophan and methionine) are less soluble in aqueous phase, positively charged (lysine, arginine and histidine) and finally, negatively charged (aspartic acid and glutamic acid) dicarboxylic amino acids. They are known to have significant antioxidant properties as synergists or primary antioxidants and believed to be important metal chelators present in fish and most amino acids had significant antioxidative potential in linoleic acid and methyl esters of linoleic acid system (Hultin, 1992).

The highest concentration of amino acid in all five Haruan extracts was glycine. Glycine is important in healing process together with arachidonic acid from fatty acid group. It is one of major components of human skin collagen, synergistic together with other essential amino acid proline, alanine, arginine, isoleucine, phenylalanine and serine to form a polypeptide that promote tissue repairing and healing process (Witte *et al*., 2002). Huang *et al*., (2001) have been proven and reported

that a lipoamino acid called, arachidonoylglycine is demonstrated suppressed edema and pain.

Findings have proven that certain peptides (Montecucchi *et al*., 1979; 1981) like mentioned (arachidonylglycine) (Huang *et al*., 2001) expected to be part of building block of various types of short peptide compounds. Glycine, which is converted to serine mainly in the kidney, is also needed for the synthesis of other important body compound, including creatine, heme/porphyrins, sarcosine and the bile salt glycocholate. Glycine and serine are produced from one another in a reversible reaction requiring folate. Otherwise, serine is used for the synthesis of ethanolamine and choline for phospholipid (Sareen *et al*., 2004).

Arginine and histidine catabolised to form proline and glutamate in which occurs mostly in the liver and kidney, arginine is used glycine in the first reaction of the creatine synthesis and histidine may combine with β-alanine to generate carnosine (a nitrogen-containing compound) and they can be found in neurons, in cells of gastric mucosa and in mast cells. Histamine release causes dilation of capillaries (flushing of the skin), constriction of bronchial smooth muscle, gastric secretions and play important roles in allergic mechanism (Sareen *et al*., 2004). Glutamate, which an excretory amino acid was found in high amount in all Haruan extracts, synthesis occurs within all tissues, including adipose and brain (Kowalski and Watford, 1994), but especially large amounts are produced by the muscle and lungs.

In extra hepatic tissues, ammonia (NH_3) or ammonium ion (NH_4^+) generated in the cell from amino acid glutamate to form glutamine. Glutamine functions to carry the generated ammonia safely out of the cell because too much free ammonia is toxic to cells. Glutamate also a dominant excitatory neurotransmitter of the mammalian central nervous system, seems to be a key effectors in diseases reflecting long-term plastic changes in the central nervous system, such as chronic pain and pain-related neurotoxicity (Bennett, 2000). Glutamate is found in nerve terminals on spinal nociceptive neurons (Broman and Adahl, 1994) and is released in the spinal cord after stimulation of peripheral nociceptors (Ueda *et al*., 1994). In an acidic environment, the used of glutamine for the urea cycle diminishes and the liver releases glutamine into the blood for transporting to and uptake by the kidney. It is justify to remember that *Channa striatus*, Haruan a fresh water fish, air breather with pH 5.5 (acidic state) to 7.5 (neutral state) where the water temperature is ranging from 71 - 82 °F (Wee, 1982; Mohsin and Ambak, 1983), the reason why glycine was high in amino acid composition of the Haruan (Table 2) above.

Table 4. The amino acid compositions of *C. Striatus* obtained by Zakaria *et al*., 2007 and Zuraini *et al*., 2006.

Amino Acids	% of Total Protein ± SD
Aspartic acid	8.53 ± 1.14
Glutamic acid	4.59 ± 0.07
Serine	3.4 ± 0.25
Glycine	35.77 ± 0.58
Histidine	1.61 ± 0.11
Arginine	4.09 ± 0.22
Threonine	4.07 ± 0.08
Alanine	1.19 ± 1.27
Proline	6.86 ± 0.78
Tyrosine	1.10 ± 0.05
Valine	2.18 ± 0.07
Methionine	1.53 ± 0.10
Cystine	0.9 ± 0.15
Isoleucine	1.28 ± 0.04
Leucine	2.91 ± 0.02
Phenylalanine	2.48 ± 0.07
Lysine	9.44 ± 0.56

Aspartic acid, which is grouped as excitatory amino acids, is found in high amount in the Haruan too (Table 2). Recently, this amino acid is proven involved in antioxidant mechanism of the neuronal injury during stroke and *N*-methyl-D-aspartic acid (NMDA) receptor activation (Salvatore *et al*., 2001). Based on theoretical considerations and previous evidence showing that reperfusion injury in the central nervous system is associated with activation of NMDA receptors, which then triggers the production of $O^{\bullet-}_2$ and NO• (Beckman, 1991). Aspartic and glutamic acids were also demonstrated have role in pain inhibition together with gamma-amino butyric acid (GABA) which works together with NMDA receptor in brain. There is now indirect evidence to show that NMDA receptor activation is associated with a marked increase in $HO^{\bullet-}$ like

Figure 1. The amino acids profiling of Haruan extracts.

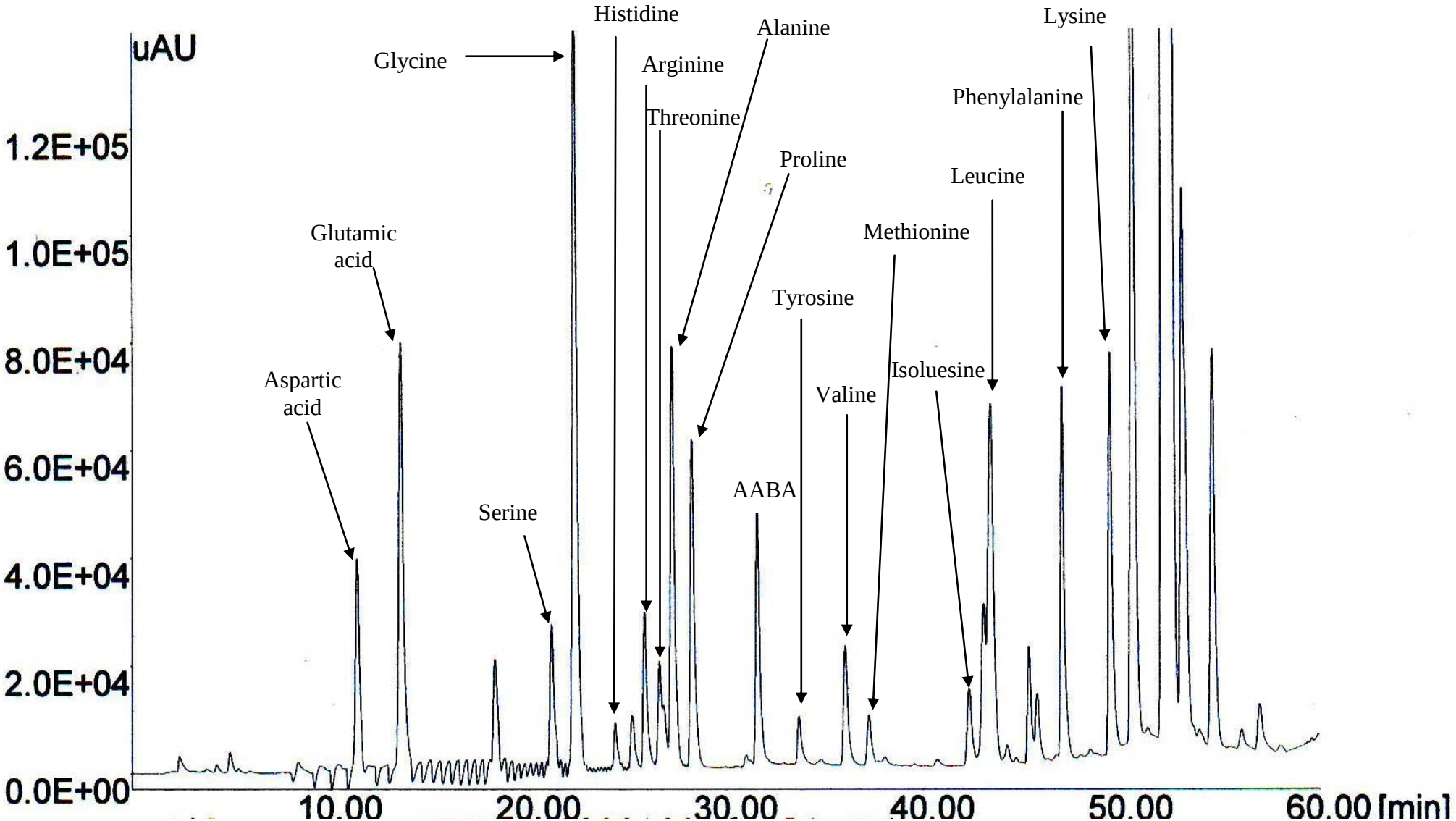


Table 2. The amino acids compositions of Haruan extracts.

Amino acid	HTE Upper phase	HTE Lower phase	Aquoues Extract (1:1 w/v)	Chloroform methanol extract (Aqueous Phase)	Commercial Haruan Essence
Aspartic acid	18.19 ± 1.21[b]	18.03 ± 1.42[b]	22.95 ± 0.98	13.85 ± 1.28	44.07 ± 1.47
Glutamic acid	42.53 ± 1.04[a]	43.13 ± 1.39[a]	29.41 ± 1.22	23.77 ± 1.31	1.87 ± 1.36
Serine	7.72 ± 0.27	8.37 ± 0.25[e]	8.19 ± 0.17[e]	5.60 ± 0.31	13.01 ± 0.29
Glycine	25.43 ± 2.08	28.81 ± 2.35	21.80 ± 1.93	42.10 ± 2.13	80.85 ± 2.44
Histidine	3.67 ± 0.34	4.03 ± 0.46	5.37 ± 0.39	19.86 ± 0.44	15.69 ± 0.32
Arginine	11.35 ± 1.42	13.82 ± 1.36	10.50 ±1.19	5.99 ± 1.47	21.79 ± 1.28
Threonine	8.94 ± 0.66[d]	10.09 ± 0.89	9.39 ± 0.73	8.43 ± 0.81[d]	17.21 ± 0.72
Alanine	12.29 ± 1.19[c]	14.40 ± 1.26	12.04 ± 1.16[c]	11.32 ±1.18	35.25 ± 1.19
Proline	15.29 ± 1.02	17.81 ± 1.33	9.49 ± 1.26	12.27 ± 1.39	45.46 ± 1.41
Tyrosine	3.70 ± 0.29[g]	3.96 ± 0.37[g]	4.84 ± 0.25	3.07 ± 0.26	6.96 ± 0.49
Valine	7.94 ± 0.76[f]	9.08 ± 0.80	10.77 ± 0.87	7.46 ± 0.94[f]	217.88 ± 0.72
Methionine	3.13 ± 0.30[h]	3.54 ± 0.32[h]	4.02 ± 0.26	2.17 ± 0.37	9.27 ± 0.19
Cystine	0.65 ± 0.03	0.76 ± 0.06[i]	0.75 ± 0.09[i]	0.21 ± 0.02	0.50 ± 0.11
Isoleucine	5.23 ± 0.46	5.76 ± 0.52	7.98 ± 0.59	4.71 ± 0.63	13.69 ± 0.39
Leucine	29.62 ± 0.29	40.19 ± 0.23	27.58 ± 0.27	7.85 ± 0.19	23.66 ± 0.31
Phenylalanine	7.18 ± 0.86	10.11 ± 0.77	11.60 ± 0.71	6.08 ± 0.85	14.41 ± 0.69
Lysine	2.51 ± 1.38	12.50 ± 1.21	17.73 ± 1.28	11.49 ± 1.31	28.54 ± 1.49

All the values are in mg/g of sample; values with same lower case letter are statistically no significant different ($p<0.05$).

Figure 2. The fatty acid profiling of Haruan extracts.

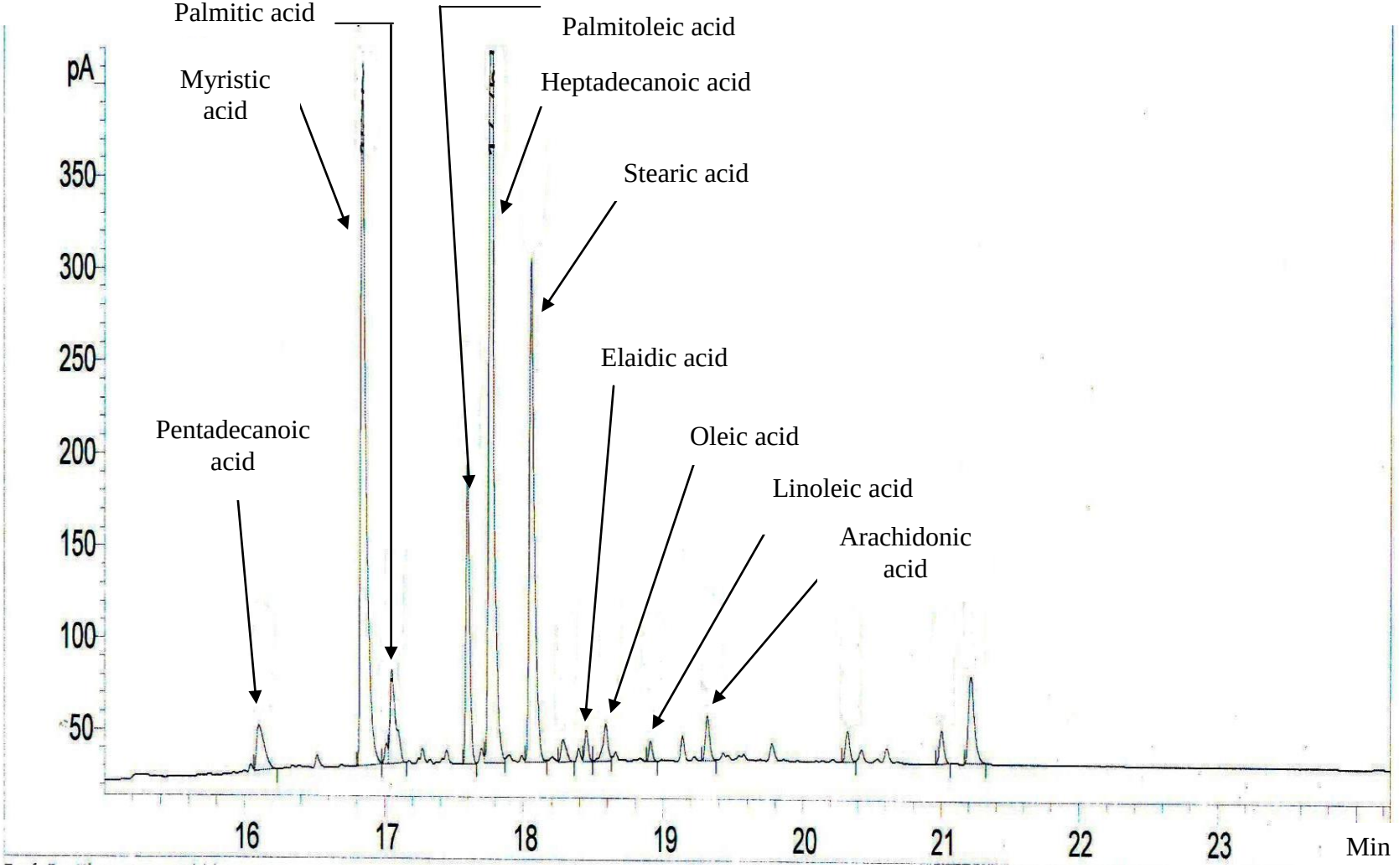
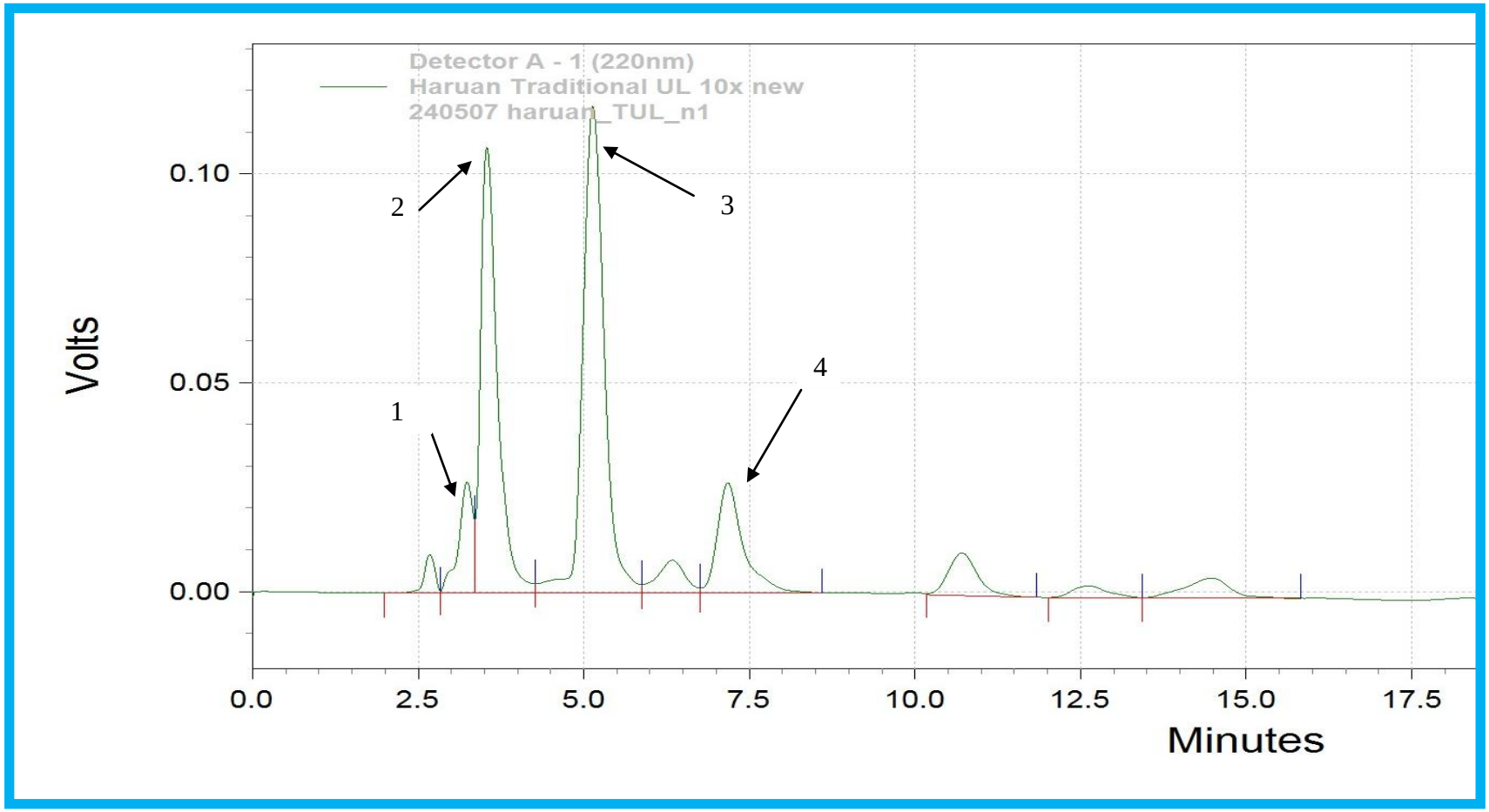


Table 3. The fatty acids compositions of Haruan Extracts.

Fatty acid (% Total fatty acid)	HTE Upper Phase	HTE Lower Phase	Haruan Aqueous Extract (1:1 w/v)	Chloroform Methanol Extract (Lipid Phase)	Haruan Commercial Essence
Pentadecanoic acid (C15:0)	-	-	-	23.74 ± 0.21	7.58 ± 0.03
Myristic acid (C14:0)	-	-	9.40 ± 0.01	2.33 ± 0.05	-
Myristoleic acid (C14:1)	14.20 ± 0.42	17.05 ± 0.09	8.23 ± 0.14	-	-
Palmitic acid (C16:0)	19.08 ± 0.21[a]	19.01 ± 0.14[a]	18.39 ± 0.07[a]	3.53 ± 0.06	26.84 ± 0.07
Palmitoleic acid (C16:1)	3.58 ± 0.58	-	5.12 ± 0.01	7.40 ± 0.07	-
Heptadecanoic acid (C17:0)	12.82 ± 0.21	-	8.53 ± 0.04	36.75 ± 0.07	-
Stearic acid (C18:0)	3.24 ± 0.07	4.58 ± 0.07	10.88 ± 0.01	15.89 ± 0.14	5.49 ± 0.07
Elaidic acid (C18:1n-9)	4.68 ± 0.07[c]	10.02 ± 0.06	4.26 ± 0.20[c]	0.72 ± 0.02	25.27 ± 0.07
Oleic acid (C18:1)	7.00 ± 0.26	9.48 ± 0.12	3.75 ± 0.01	1.40 ± 0.14	27.68 ± 0.07
Linoleic acid (C18:2)	7.82 ± 0.26[b]	7.50 ± 0.07[b]	3.76 ± 0.03	0.50 ± 0.07	-
Linolenic acid (C18:3)	5.70 ± 0.06	11.67 ± 0.07	-	-	-
Arachidonic acid (C20:4)	6.19 ± 0.05	-	2.88 ± 0.02	7.51 ± 0.02	-

All the values are percentage of total fatty acid ± SD
(-) Not Detected. Value with same lower case letter are significantly no different at $p<0.05$

Figure 3. The HPLC profiling of the HTE demonstrated the present at least four major fractions labelled as 1, 2, 3 and 4.



activity in the brain (blocked by inhibition of NOS), which is presumably due to $ONOO^-$ generation (Hammer *et al*., 1993).

Phenylalanine (Phe) also the important amino acid present in all Haruan extracts. Phe together with tyrosine are catabolised to acetoacetate and thus are partially ketogenic from partially glucogenic in amino acid carbon skeleton cycle (Chinsky *et. al*., 1994). Phenylalanine will convert to tyrosine by an enzyme called hepatic phenylalanine hydroxylase or also called a monooxygenase (Sareen *et al*., 2004). A genetic absence of phenylalanine hydroxylase activity will cause genetic disorder phenylketonuria (PKU) and necessitates a phenylalanine-restricted diet (Chinsky *et al*., 1994).

Cysteine is the lesser amino acid present in all Haruan extracts in this study (Table 2). According to a report by Roger *et al*. (1986) and review by Sareen *et al.* (2004), cysteine is one of the amino acid grouped in sulphur (S)-containing essential amino acid and easily to degrade compared to others amino acid. This statement should be a reason why the levels of cystein in samples were less. Cysteine is required for synthesis of protein and the non-protein nitrogen containing compound glutathione and maybe further metabolized to form cystine and another amino acid, taurine (Sareen *et al.*, 2004).

Referring to Table 3, the fatty acids in the *C. striatus* extracts can be grouped to saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA). The SFA in the extracts are trideconoic, pentadecanoic, myristic, palmitic, heptadecanoic and stearic acids, MUFA are myristoleic, palmitoleic and oleic acids, meanwhile, PUFA acid linoleic, linolenic and arachidonic acids. Most of the fatty acids listed (Table 3) were present significantly higher in HTE and Chloroform methanol extract LIPID PHASE. The higher level of palmitic and palmitoleic acids has been described as a characteristic of freshwater fish (Ackman, 1967). Higher levels of palmitoleic, palmitic and oleic acids have already been related for Mandi fish, Brazilian freshwater fish by Andrade and Lima (1979). These fatty acids have been demonstrated have ability to bind with cannabinoid receptors in antinociceptive activity of *Cannabis sativa.*

Table 5. The fatty acids compositions of *C. striatus*, Haruan extracts obtained by Mat Jais *et al.*, 1994; Zakaria *et al.*, 2007; Zuraini *et al*., 2006)

Fatty acid	**Fatty acid(% Total fatty acid)**
Myristic acid (C14:0)	2.15 ± 0.11
Myristoleic acid	ND
Palmitic acid (C16:0)	35.93 ± 0.63
Palmitoleic acid (C16:1)	1.86 ± 0.32
Heptadecanoic acid (C17:0)	2.90 ± 0.56
Stearic acid (C18:0)	15.31 ± 0.33
Elaidic acid (C18:1n-9)	ND
Oleic acid (C18:1)	22.96 ± 0.40
Linoleic acid (C18:2)	11.45 ± 0.31
Linolenic acid (C18:3)	ND
Arachidonic acid (C20:4)	7.44 ± 0.83
Eicosapentanoic acid (EPA) (C20:5)	1.29 ± 0.07
Docosahexanoic acid (DHA) (C22:6)	15.18 ± 1.12

ND, Not determined

The major fatty acids of HTE extracts were observed to be palmitic, myristoleic and oleic acids (Table 3). This observation was typical similar to fatty acid composition values of Sardine (De-Leonardis and Macciola, 2004) because palmitic acid is the key metabolite in fish (Andrade *et al*., 1995). There are slightly significant differences ($p<0.05$) between the Haruan extracts in palmitic, stearic, oleic and linoleic acids. It is because the differences way of extraction processes of each extracts. The best result of fatty acids in this study based on the present of fatty acids was chloroform methanol extract or Bligh and Dyer (1959) method with slightly modification performed by Mat Jais *et al.* (1994).

The oleic and stearic acids have been reported to attenuate polymorphonuclear leukocytes activity and undoubtedly influence membrane fluidity, thus suppressed inflammatory processes

(Crocker *et al*., 2001). In this study, the chloroform methanol extract LIPID PHASE showed a very interesting fatty acid profile, especially in consistently present of Arachidonic Acid (AA), which similar to previous study (Mat Jais *et al*., 1994; 1997; 1998; Zuraini *et al*., 2005; Zakaria *et al*., 2007) but with slightly different in value and present of fatty acid compounds. Previously as mentioned that the extraction method for fatty acid profiling of Chloroform methanol of this study was carried out using different ratio of Haruan fillet to solvent (Chloroform methanol 2:1 v/v) was 1:20 w/v, slightly different to Zakaria *et al.* (2007) which was 1:2 (w/v) fillet to solvent and not directly extractions of Haruan fillet as reported in previously studies (Mat Jais *et al*., 1994; 1998 and Zuraini *et al*., 2006).

The early study of Haruan sample, Mat Jais *et al.* (1994; 1998) and Zuraini *et al.* (2005) have been claimed that Haruan have such highly amount of arachidonic acid and (AA), eicosapentanoic acid (EPA) (C20:5), similarity with finding by Zuraini *et al.* (2006) but with highly amount of docosahexanoic acid (DHA) (C22:6) (Table 5). In this current study, three out of five *C. striatus* extracts namely Haruan Traditional Extract (HTE) UPPER PHASE, Haruan Traditional Extract (HTE) LOWER PHASE and Haruan chloroform methanol extract AQUOUES PHASE posses the present of AA, but no DHA and EPA detected in all samples (Table 3) which contradicts with the report of Mat Jais *et al.* (1994; 1998) and Zuraini *et al.* (2006). On the other hands, all the extracts in this study were remained to be used as crude extracts without going through to freeze drying process. Previously study performed by Mat Jais *et al*. (1994; 1998) had shown that, the pre-treatment freeze drying process of Haruan sample found to cause a total loss of arachidonic acid when compared to the non-freeze dried samples.

Furthermore, there are some fatty acids were not detected in this study when compared to previous report (Mat Jais *et al*., 1994; 1998) which contains chloroform, methanol and water (3 : 48 : 47, v/v/v), the lipid-soluble molecules was dissolved in chloroform and methanol or in chloroform or in methanol, but the others molecules or compounds dissolved in the water phase (Shahidi and Wanasundara, 1998; Cristie, 1982). The compositions of fatty acids in fish are also highly depending on feeds, habitats, climates and cultivated or non cultivated (Mohsin and Ambak, 1983).

In the previously study (Zuraini *et al*., 2006), samples were caught from wild in selected areas of Peninsular Malaysia, but not ours, and that might be the reasons why the DHA was not detected. The differences in lipid and fatty acids composition between carnivore, herbivorous and omnivorous fish in different region of tropical and others have been reported, should be considered but Zenebe *et al.* (1998) have argued that variation in tissue lipid and fatty acids in herbivorous fish is greater than in those carnivore fish due to diversity of their food habit. The major carp species are all omnivorous (Chakrabarti *et al*., 1995) and the proportional of plant and animal food will influent the accumulation of fatty acids (Domaizon *et al*., 2000).

However, the recent reports indicated that the freshwater fish contain relatively large amounts of EPA and DHA (Wang *et al*., 1990). The diet enrichment with EPA could protect organism from thrombosis (Gutierrez & Silva, 1993). Based on the present of fatty acid compounds, EPA and DHA in previously study using methods Mat Jais *et al*. (1994; 1998) and Zuraini *et al.* (2006), this present modified study method for fatty acid compositions, fillet to chloroform methanol (2:1 v/v), 1:20 (w/v) ratios is not the best extraction method to extract fatty acid compounds of *C. striatus*, Haruan.

Arachidonic acid (C20:4n-6) has been regarded with some suspicion with respect to risks in cardiology, but together with DHA is an “essential” fatty acid in its own right at all times (Wander & Patton, 1991), as well as in pregnancy and infant nutrition (Ghebremeskel *et al*., 2000). It is also a precursor of prostaglandins that may induce platelet aggregation to initiate blood clotting especially for tissues injuries, then the prostaglandins that have been released will involved in pain sensation, inflammation and wound healing (Katzung, 1995). The prostaglandins from AA have important effects on the maintenance of blood pressure and immune system (Jonnalagadda *et al*., 1996). A study on Indian fresh water fish found that, the fish having high concentration of AA has demonstrated that their effect in prevention of cardiovascular diseases is minimal (Ghosh & Dua, 1997).

The World Health Organization (WHO) recommended leucine and isoleucine requirements for adults of 14 and 19 mg amino acid/kg body weight per day (FAO, 1986). All the *C. striatus* extracts showed the present of leucine in range of

7.85 - 40.19 mg/g and isoleucine between 4.17 - 13.69 mg/g of protein samples. Interestingly, previous studies showed that the *C. striatus* contain all the essential amino acids to our body (Table 4) (Mat Jais *et al.*, 1994; 1998a, Zuraini *et al*., 2005 and Zakaria *et al.*, 2007) and fatty acid (Table 5) (Mat Jais *et al*., 1994; 1998a, Zuraini *et al*., 2005 and Zakaria *et al*., 2007), which help to prove it medicinal activities likes analgesic and anti-inflammatory (Somchit *et al*., 2004) and antinociceptive (Zakaria *et al*., 2007) and as the dietary medicine in healing process (Mat Jais *et al*., 1997; Baie & Sheikh, 2000a; 2000b). However Akoh and Hearnsberger (1991) verified that a consumption of 1 - 2 g/day of n-3 polyunsaturated fatty acids (PUFA) prolonged significantly bleeding and clotting times. Therefore, fish have been suggested as a key component for a healthy diet in humans (Abdul Rahman *et al*., 1995). Furthermore the basic amino acids (histidine, lysine and arginine) are known to produce the most effective antioxidant products with sugars or glico-amino acid (Frankel, 1998). A range of protein hydrolyzates of fish have been found to exhibit antioxidant activities. A polar fraction of krill extract, which was identified as a mixture of 13 - 20 free amino acids, was reported as having strong antioxidant activity.

As mentioned previously above, there are such peptides or short-chain peptides can act as antioxidant compounds in different ways of antioxidative biological systems. Upon separation, in the Haruan Traditional Extract, the liquid portion was well settling into two distinct parts, the UPPER PHASE transparent and the LOWER PHASE cloudy brownish colour, followed by HPLC separation of the analytes from early eluating impurities. A chromatographic run was completed in 10 min with chromatographic separation Genesis C_{18}. Peak broadening occurred with C18 revealed the presence of four major fragments (Figure 4.3). The chromatographic profile of HTE, after HPLC procedure represented four major peaks indicated the presence of at least four major fragments (Figure 3) could suggest extra candidates for bioactive compounds.

All the peaks were labelled as 1, 2, 3, and 4, the highest was peak 3, the peaks 1 and 4 look like same in height. Although there were four peaks obtained from the Traditional Haruan Extraction (HTE), were actually similar to previous analysis done using chloroform methanol 1:2 ratio (v/v) (Mat Jais *et al*., 1994; 1997; 1998). Findings have proven that certain peptides (Montecucchi *et al*., 1979; 1981) or lipo-amino acids, such as arachidonylglycine (Huang *et al*., 2001) expected to be part of building block of various types of short peptide compounds and a high amount of AA that may conjugated with glycine to form the called lipoamino acid. The presence of all essential amino acids in all *C. straitus* extracts in this study is strongly believe that 4 major fractions are peptides the contributed synergistic for antioxidative activities that will be investigated further.

CONCLUSION

As a conclusion, the *C. striatus* extracts HTE contains the major amino acids especially glutamic acid, glycine, leusine, aspartic acid and revealed some dominant fatty acid namely palmitic acid, myristoleic acid, oleic acid, linoleic acid and arachidonic acid. All the important amino acids and fatty acids present in HTE extract are suggested to play an important role to human body as many finding before.

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