

1 **Exploring the Therapeutic Potential: Malaysian *Channa striatus* Water Extract Enriched with**  
2 **Arachidonic Acid for Wound Healing in Human Foetal Lung Cells (IMR-90)**

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14  
15 **Abstract**

16 *Channa striatus*, commonly known as *haruan*, has been traditionally employed in Malaysia for its  
17 wound-healing and pain-reducing properties. This study aimed to assess the wound healing potential  
18 of *Channa striatus* water extract (CSWE) and identify key compounds promoting fibroblast cell growth.  
19 Freshly obtained *Channa striatus* samples were meticulously deboned to maximize fish fillet retention.  
20 CSWE was obtained through aqueous extraction, and various physicochemical properties were  
21 analyzed, including pH, rheological characteristics, moisture content, amino acid composition, and the  
22 presence of the essential arachidonic acid compound. The effective concentration (EC<sub>50</sub>) of the sample  
23 was determined using a conventional 2-D cell culture system with ordinary human fibroblast cells  
24 (IMR-90) over a three-day incubation period. Results revealed a near-neutral pH of CSWE (6.34 ±  
25 0.01) with high moisture content (97.3 ± 0.01%). The extract exhibited Newtonian fluid behavior, as  
26 indicated by a viscosity value of 1.50 ± 0.31 mPa.s. CSWE contained essential amino acid glycine and  
27 arachidonic acid, known for their roles in the wound healing process, albeit in relatively low  
28 concentrations. However, the low concentrations of glycine and arachidonic acid in CSWE did not  
29 positively influence the growth of normal IMR-90 cells ( $p>0.05$ ) compared to the untreated control.

30 Consequently, the EC<sub>50</sub> values in the 2-D cell culture system for CSWE were deemed invalid and  
31 indeterminable due to the over-diluted extract resulting from a high fish weight-to-solvent ratio.  
32 Despite this, the IMR-90 cell growth rate remained consistent across different CSWE concentrations,  
33 with no observed mortality during the three-day incubation period. Nevertheless, IMR-90 cells  
34 exhibited insignificant growth profiles, even in the presence of arachidonic acid and glycine at  
35 extremely low concentrations during each cell treatment.

36

37 **Keywords:** Aqueous Extraction, *Channa striatus*, IMR-90 Cell, Physicochemical Characteristics,  
38 Wound Healing

39

## 40 **1.0 Introduction**

41 The snakehead fish, commonly referred to as *haruan*, is a carnivorous freshwater species  
42 belonging to the *Channidae* family and is indigenous to numerous tropical and subtropical regions,  
43 including Malaysia [1]. In Malaysia, locals traditionally incorporate *haruan* into their diet as a  
44 therapeutic remedy post-surgery, for the treatment of injuries resulting from road accidents, and for  
45 postpartum care in caesarian mothers [2]. Typically consumed by dry-frying, grilling, boiling, or in the  
46 form of porridge, *haruan* is recognized for its healing properties [3]. Throughout history, animal  
47 extracts have been utilized in treating various ailments [4], with the snakehead fish (*Channa striatus*)  
48 extract emerging as the most commonly used animal extract due to its unique medicinal attributes,  
49 including potent anti-inflammatory and analgesic properties [4, 6, 7]. These properties effectively  
50 alleviate pain, inflammation, and enhance the wound healing process [6]. The stability of bioactive  
51 compounds in snakehead extract within a pH range of 6 to 8, resistance to high temperatures (100 °C),  
52 and resilience to enzymes such as  $\alpha$ -amylase, protease, and lipase further contribute to its medicinal  
53 appeal [8].

54

55 Previous studies have explored the use of *Channa striatus* water extract (CSWE) emulsified in  
56 specialized polymers to create an aerosol spray for regulating wound healing [9]. Application of the  
57 formulated emulsion onto abrasion areas in Sprague Dawley animal models resulted in the  
58 development of a thin film containing CSWE, significantly accelerating the healing process [10, 11].  
59 This emulsion, acting as a spray dressing, not only reduced discomfort and pain but also prevented  
60 microbial infections as it rejuvenated cells. The efficacy of cetrimide, palm oil-based emulsified  
61 CSWE, and a combination of CSWE and cetrimide was evaluated in abrasion treatment, showing a  
62 significant improvement in wound healing efficacy compared to the untreated control. Particularly  
63 noteworthy was the positive healing efficacy of the palm oil-based cream formulated with CSWE,  
64 demonstrating greater tensile strength on rat abrasion areas than other commercially available creams.  
65 Consequently, CSWE has demonstrated substantial facilitation of the wound healing process, offering  
66 a faster and stronger outcome compared to chemically synthesized medicines available in the market.

67 Recent studies have identified the presence of amino acids and fatty acids in snakehead fish,  
68 suggesting their potential as wound healing agents [3]. Amino acids and fatty acids are pivotal  
69 components in the wound healing process, with their absence believed to impede recovery [3]. The  
70 hypothesis that the wound healing efficacy of CSWE is influenced by its high glycine content (amino  
71 acid) and arachidonic acid (fatty acid) was explored. Both components play crucial roles in the wound  
72 healing process by initiating reactions leading to collagen formation, re-epithelization from injuries,  
73 and the induction of wound contraction [12, 13]. To accurately mimic cellular behavior during the  
74 healing process, fibroblast origin cell lines such as IMR-90 were employed. Fibroblast cells regulate  
75 the secretion of Extracellular Matrix (ECM) products, controlling fiber-forming proteins in acute  
76 wounds, such as collagen, fibrin, and fibronectin. This study was conducted to investigate the wound  
77 healing efficacy of CSWE and the essential compounds facilitating fibroblast cellular growth, utilizing  
78 normal fibroblast cell lines (IMR-90).

79

## 80 **2.0 Materials and Methods**

### 81 **2.1 Raw Materials**

82 In this investigation, *C. striatus* (haruan) served as the primary material, as illustrated in Figure 1(a).  
83 The fish specimens were procured from local markets in Bandar Baru Bangi and a wet market in  
84 Kajang, Selangor, Malaysia. Subsequently, the fish species underwent a visual inspection and  
85 validation by the State Fisheries Department, Ministry of Agriculture (MOA), Malaysia. Upon the  
86 collection of the fish samples, a thorough cleansing process was conducted, involving the removal of  
87 bones, livers, stomachs, and viscera. Only the consumable muscles with skin (boneless fillet) were  
88 utilized in the course of this experiment (Figure 1b).

89

### 90 **2.2 Chemicals**

91 The analytical chemicals employed in this research comprised hydrochloric acid (HCl) (Friendemann  
92 Schmidt),  $\alpha$ -aminobutyric acid (HmbG® Chemicals), performic acid (HmbG® Chemicals), bromic  
93 acid (HBr) (HmbG® Chemicals), hexane (LiChrosolv®), methanol (J.T.Baker), and potassium  
94 hydroxide (KOH) (R&M Chemicals). All reagents utilized for the chemical analyses adhered to  
95 analytical grade standards unless otherwise specified. Dulbecco's Modified Eagle Medium: Nutrient  
96 Mixture F-12 (DMEM/F12), Phosphate Buffered Saline (PBS), Fetal Bovine Serum (FBS), and  
97 Antibiotic-Antimycotic (AA) were sourced from GIBCO™. PrestoBlue Cell Viability Reagent™ was  
98 procured from Invitrogen™, and normal human fibroblasts (IMR-90: Catalogue number CCL-186)  
99 were acquired from the American Type Culture Collection (ATCC).

100

### 101 **2.3 Aqueous Extraction of *C. striatus* Water Extract (CSWE)**

102 A boneless fish fillet sample underwent a cleaning process with distilled water, followed by weighing,  
103 and placement into a pressure cooker (Khind PC-600, Malaysia). Distilled water, with a fish to water  
104 volume ratio of 1:4, was introduced, and the pressure cooker was set to 100 °C for two hours [3].

105 Distilled water was incrementally added to maintain the initial volume every 30 minutes during the  
106 cooking period. Upon completion of the cooking process, the resultant liquid extract was gathered,  
107 subjected to filtration using a filter paper (Sartorius Stedim Biotech, diameter = 125 mm, 84 g/m<sup>2</sup>),  
108 and subsequently stored at 4 °C for subsequent physicochemical analyses (Figure 2). The fish fillet  
109 itself was then discarded.

110

#### 111 **2.4 pH Analysis**

112 The determination of the CSWE's pH was carried out using a PHM210 standard pH meter (MeterLab<sup>®</sup>,  
113 Radiometer Analytical S.A., France). Calibration of the pH meter was conducted before each analysis  
114 to guarantee the precision of the readings. About 20 mL of the extract was utilized for the analysis [9],  
115 and measurements were taken on three samples ( $n = 3$ ), with the results reported as mean  $\pm$  standard  
116 deviation.

117

#### 118 **2.5 Flesh Water Content via Gravimetric Analysis**

119 The water content in the flesh of CSWE was determined using the oven drying method [14, 15].  
120 Initially, empty aluminum plates were cleaned and subjected to overnight drying in the oven (Fisher-  
121 Isotemp<sup>™</sup> Nema 5-15 Oven Drying, United States) at 105 °C. Afterward, they were cooled in a  
122 desiccator, and the weight of each empty aluminum plate was recorded. Subsequently, 2.0 g of the  
123 sample was placed on the aluminum plate and transferred to a Protech Force Air Convection Oven  
124 FAC-138SS (Malaysia) at 105 °C for 16 hours. Upon removal from the oven and cooling in the  
125 desiccator, the weight of the dried sample on the aluminum plate was recorded. The calculation for the  
126 flesh water content of the CSWE sample was then performed as follows:

127

$$128 \text{ Water content (\%)} = \frac{W_1 - W_2}{W_1} \times 100 \quad (1)$$

129 where,

130 W1 = weight of sample before drying (g)

131 W2 = weight of sample after drying (g)

132

## 133 **2.6 Rheological Attributes Analysis**

134 The rheological properties, specifically Newtonian viscosity, of the CSWE were assessed at a room  
135 temperature of  $25 \pm 1$  °C using a Physica MCR 301 rheometer (Anton Paar, Graz, Austria) equipped  
136 with a CC27 concentric cylinder measuring system. The instrument operated under standard settings  
137 and a flow profile during the evaluation. Measurements were conducted on three samples ( $n = 3$ ) and  
138 the results are presented as the mean  $\pm$  standard deviation.

139

## 140 **2.7 Amino Acid Compositional Profiles**

141 The determination of the amino acid composition in CSWE utilized High-Performance Liquid  
142 Chromatography (HPLC) with an AccQ•Tag<sup>TM</sup> amino acid analysis column (dimensions = 3.9 mm x  
143 150 mm; packing material = silica base bonded with C18; particle size = 4  $\mu$ m). Sample preparation  
144 followed the guidelines provided by the supplier (Waters Corp., USA). The volumetric preparation ( $\mu$ l)  
145 of the sample solution, control, and amino acid standard analysis were as follows: Borate buffer (70 to  
146 80), sample/standard (10), and AccQ-Fluor<sup>TM</sup> reagent (20), respectively [17].

147

### 148 **2.7.1 Acid Hydrolysis**

149 Approximately 0.05 g of CSWE underwent hydrolysis with 5.0 mL of 6.0 N HCl within a borosilicate  
150 test tube. The tightly sealed test tube was placed in the oven at 110 °C for 24 hours. After cooling, the  
151 hydrolyzed sample was transferred to a 100 mL volumetric flask, and 0.4 mL of the internal standard  
152  $\alpha$ -aminobutyric acid (AABA) was introduced into the flask [17]. Deionized water was then added to  
153 the solution until reaching a volume of 100 mL, after which the solution underwent filtration using a  
154 syringe filter (25 mm in diameter). The resultant filtered solution was collected into a microcentrifuge

155 tube.

156

### 157 **2.7.2 Performic Acid Hydrolysis**

158 About 0.05 g of CSWE was transferred to a borosilicate test tube and placed in an ice bath for 30  
159 minutes. Following this, 2.0 mL of a cold performic acid solution was added to the test tube, and the  
160 mixture was left in the freezer for 16 hours. Subsequently, around 0.4 mL of HBr was introduced, and  
161 the solution underwent an additional 30-minute freezing period [17]. After the freezing step, the  
162 mixture was concentrated to an almost dry state using a hot plate. The resulting dried sample was  
163 hydrolyzed with a 6.0 N HCl solution, following the same procedure as described in the acid hydrolysis  
164 section. All eluents ( $\mu\text{l}$ ) were prepared according to the supplier's instructions, as outlined in Table 1,  
165 before being injected into the HPLC. Separations were conducted using an AccQ-Tag amino acid  
166 analysis column (dimensions = 3.9 mm x 150 mm). The column temperature was set to 36 °C for the  
167 acid hydrolysate and 31 °C for the oxidized performic acid hydrolysate. Approximately 10  $\mu\text{L}$  of  
168 standard or hydrolyzed sample was injected into the HPLC at a flow rate of 1 mL/min. The  
169 fluorescence detector was configured with 250 nm excitation and 395 nm emission wavelengths.

170

### 171 **2.8 Fatty acid (Arachidonic Acid) Determination**

172 A quantity of 2.0 g of CSWE was dissolved in 2.0 mL of hexane and 4.0 mL of 2.0 M methanolic KOH  
173 within a 20 mL test tube. The test tube was sealed, subjected to vortexing for 2 minutes at room  
174 temperature, and then centrifuged at 4000 rpm for 10 minutes. The resulting clear supernatant (upper  
175 layer) was transferred into a 2.0 mL autosampler vial for Gas Chromatography (GC) analysis [17]. The  
176 GC-17A Gas Chromatography (Shimadzu, Japan), equipped with a split-splitless injector, electronic  
177 pressure controller, and Flame Ionisation Detection (FID) system, was utilized to separate the Fatty  
178 Acid Methyl Esters (FAMES). A Trajan SGE BPX-70 column (dimensions = 60 m x 0.25 mm (internal  
179 diameter) x 0.15  $\mu\text{m}$  polyethylene glycol film) was employed in this investigation. The oven

180 temperature was initially set at 50 °C for 1 minute, increased to 175 °C at a rate of 4 °C/min, and  
181 finally elevated to 230 °C for 5 minutes. The injector and detector temperatures were set at 250 °C and  
182 260 °C, respectively, with a split ratio of 100:1 and a column temperature of 200 °C. Approximately  
183 1.0 µL of the sample was injected into the GC. Helium gas served as the carrier gas for the system with  
184 a flow rate of 1.7 mL/min, maintained at a pressure of 103.4 kPa. The hydrogen and air pressures for  
185 the FID were set to 275.6 kPa. A standard FAMES was employed to ascertain the presence and quantity  
186 of arachidonic acid.

187

### 188 **2.9 2-D Adherent Cell Culture Bioassay (EC<sub>50</sub>)**

189 The determination of the effective concentration (EC<sub>50</sub>) value for CSWE was conducted according to  
190 the provided protocol. Various concentrations of the sample were prepared through dilution, ranging  
191 from 0.25 (measured density of CSWE), 0.125 (Dilution Factor (DF): 2), 0.0625 (DF: 4), 0.03125 (DF:  
192 8), to 0.015625 g/mL (DF: 16) [18]. In a 96-well microtiter plate, each well was seeded with a total of  
193  $1 \times 10^4$  human fibroblast cells (IMR-90), and the experiment was performed in triplicates ( $n = 3$ ). The  
194 key characteristics of the IMR-90 cell lines considered in this study included: (a) Cell phenotype -  
195 Normal fibroblast; (b) Material source - Human Caucasian fetal lung fibroblast; (c) Culture medium -  
196 DMEM, and (d) Subculture routine - performed when 70–80% of the well space is filled with cell  
197 seeding, typically 2 to 3 x 10,000 cells/cm<sup>2</sup>, using 0.25% (v/v) trypsin when reaching the confluent  
198 stage [18]. The IMR-90 cells were maintained in a 5% carbon dioxide (CO<sub>2</sub>) environment at 37 °C.  
199 On the third day post-treatment, 10 mL of PrestoBlue Cell Viability Reagent™ was added and  
200 allowed to remain in the 96-well plate for 4 hours. Subsequently, the absorbance for each well was  
201 measured using BioTek Instruments ELx808™ Absorbance Microplate Readers at a wavelength of  
202 293 nm.

203

204

## 205 **2.10 Statistical Analysis**

206 The gathered data underwent analysis through Statistical Package for the Social Sciences (SPSS)  
207 software version 20.0 (IBM, USA). The paired sample T-test was employed to identify significant  
208 differences in CSWE, and the significance threshold was set at 95% ( $p < 0.05$ ).

209

## 210 **3.0 Results and Discussion**

### 211 **3.1 The pH of Extract**

212 The pH of CSWE was determined to be close to neutral, specifically  $6.34 \pm 0.01$ . Maintaining a neutral  
213 pH is ideal for the effective treatment of fresh wounds. In formulations intended for external use, a  
214 neutral pH helps minimize pain and irritation experienced by patients. If the formulation is designed  
215 for internal consumption, a slightly acidic condition may not pose a significant issue, as the  
216 homeostasis process naturally works to balance the pH [17].

217

### 218 **3.2 Flesh Water Content**

219 The water content in the flesh and the solid residual of CSWE were determined to be  $97.3 \pm 0.01\%$   
220 and  $2.70 \pm 0.01\%$ , respectively. These findings fell within the moisture content range observed in  
221 various commercial meat essences, such as beef, freshwater clam, hard clam, eel, and six types of  
222 chicken essence in a prior study, which varied from 91.1% to 97.5% [18].

223

### 224 **3.3 Rheological Profiles**

225 Figure 3 illustrates the rheogram depicting the correlation between the shear stress and shear rate of  
226 CSWE. The observations led to the inference that the water-based crude extract displayed Newtonian  
227 fluid behavior, demonstrating a linear relationship between shear stress and shear rate. Simultaneously,  
228 the viscosity of CSWE was quantified at  $1.50 \pm 0.31$  mPa.s. Notably, variations in shear stress did not  
229 impact the viscosity of CSWE, indicating compliance with Newtonian laws.

### 230 **3.4 Amino Acid Composition**

231 Table 2 presents the amino acid composition of CSWE. The investigation revealed the presence of at  
232 least 18 amino acid constituents, even at low concentrations. Predominant essential amino acids in  
233 CSWE from the snakehead fish included glutamic acid ( $0.18 \pm 0.00$  mg/g), glycine ( $0.15 \pm 0.00$  mg/g),  
234 and lysine ( $0.12 \pm 0.00$  mg/g). These findings highlight the presence of glycine in CSWE, a crucial  
235 amino acid contributing to the wound healing process. Glycine, working in conjunction with  
236 arachidonic acid from the fatty acid group, plays a significant role in the recovery process. Glycine, a  
237 key component of human collagen, collaborates with other amino acids like proline, alanine, arginine,  
238 isoleucine, phenylalanine, and serine, to form polypeptides that foster tissue repair and the healing  
239 process [19, 20].

240

### 241 **3.5 Essential Compound for Cellular Growth: Fatty Acid (Arachidonic Acid)**

242 Table 3 displays the overall content of arachidonic acid in CSWE. The determined quantity of  
243 arachidonic acid was notably low ( $3.72 \times 10^{-4} \pm 0.02$   $\mu$ g) in comparison to findings from a prior study  
244 [21]. This discrepancy might be attributed to the over-diluted sample utilized in this study, featuring a  
245 high fish weight-to-solvent ratio. Nonetheless, the results affirm the existence of arachidonic acid (C20:  
246 4n-6) in the extract, albeit at a low concentration.

247

### 248 **3.6 The EC<sub>50</sub> Value (2-D Adherent Cell Culture Growth)**

249 In this investigation, the EC<sub>50</sub> value was determined to indicate the predictive concentration at which  
250 the substrate would elicit a 50% effect on cell number, whether positive (cell growth) or negative (cell  
251 death). The expectation was an increase in the number of IMR-90 cells with rising substrate  
252 concentration. Figure 4 illustrates the growth profile of IMR-90 cells treated with CSWE over three  
253 days, with the inset diagram depicting cell morphology after the same treatment duration.

254 Although the number of IMR-90 cells initially exhibited gradual fluctuations with increasing

255 sample concentration, followed by a rise at a concentration of 0.125 g/mL, the actual cell count showed  
256 no significant difference ( $p>0.05$ ) compared to the control sample. In essence, normal IMR-90 cells  
257 demonstrated no discernible positive growth compared to the untreated control, despite the escalating  
258 substrate concentration. The lack of significant growth in IMR-90 cells was attributed to the notably  
259 low concentrations of arachidonic acid and glycine during each cell treatment.

260 Despite employing a similar sample preparation ratio as recent studies, the determination of  
261 the CSWE sample's  $EC_{50}$  value proved challenging due to the minute amount of active ingredients  
262 present in the extract before cell treatment. While the sample preparation ratio of fish weight to water  
263 volume (1:4, w/v) aligned with a previous study, they concentrated the snakehead essence later using  
264 the spray drying method to produce *haruan* encapsulated powder [22]. The preparation of CSWE with  
265 a 1:4 ratio was also applied in a previous study with variations in cooking duration [23].

266 The extremely high dilution was identified as a factor hindering the determination of  $EC_{50}$   
267 values for the sample. Consequently, further concentration of the sample is recommended to observe  
268 the growth of IMR-90 cells, with a concentration of 1 g/mL suggested as a benchmark for future  
269 research, as previously used in other studies [24, 17]. Challenges in determining  $EC_{50}$  values also  
270 encompassed the short incubation period for IMR-90 cells, the three-day treatment duration, and the  
271 extensively diluted concentration range used in the cell culture study. To obtain accurate  $EC_{50}$  values,  
272 it is suggested that at least five data points should be incorporated into the graph to signify continuous  
273 cell growth [25].

274 Despite these challenges, this study is deemed a successful preliminary exploration, as no prior  
275 research has delved into the proliferation of IMR-90 cells using snakehead essence. Previous studies  
276 related to CSWE have primarily focused on other cell types, such as *Helacyton gartleri* (HeLa)  
277 (cervical cancer cells), adenocarcinoma colon-rectal (HT-29) cells [22, 26], and adrenal  
278 pheochromocytoma (PC-12), with the use of normal human fibroblast cells (IMR-90) not previously  
279 reported. Moreover, most studies evaluating the function of CSWE were conducted using Sprague-

280 Dawley rats and did not specifically target the wound healing process.

281

#### 282 **4.0 Conclusion**

283 In conclusion, the physicochemical analysis of CSWE demonstrated favorable characteristics,  
284 including an almost neutral pH, high water content, and Newtonian fluid behavior with a viscosity of  
285  $1.50 \pm 0.31$  mPa.s. The presence of essential amino acids, particularly glycine, and arachidonic acid at  
286 low concentrations in CSWE suggested potential benefits for the wound healing process. However,  
287 the determination of EC<sub>50</sub> values for CSWE using normal IMR-90 fibroblast cells proved challenging  
288 due to the excessive dilution of the prepared samples with a fish weight-to-water volume ratio of 1:4  
289 (w/v). Additionally, the concentrations of glycine and arachidonic acid in the aqueous extract were  
290 insufficient for effective growth of normal IMR-90 cells. The short incubation period (three days) and  
291 the low concentrations used in the cell culture work further contributed to the inability to determine  
292 EC<sub>50</sub> values. Despite these challenges, the growth rate of IMR-90 cells remained consistent across  
293 different CSWE concentrations, with no observed mortality during the three-day incubation period.  
294 This study provides valuable insights into the physicochemical properties of CSWE, laying the  
295 groundwork for future research to optimize concentrations and explore its potential wound healing  
296 effects on normal human fibroblast cells.

297

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301

#### 302 **6.0 Data Availability Declaration**

303 No data were used elsewhere to support this study and it was entirely a new set of data.

304

305 **7.0 Declaration of Interests**

306 The authors declare that they have no known competing financial interests or personal relationships  
307 that could have appeared to influence the work reported in this paper.

308

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## Tables and Figures

**Table 1:** Preparation of sample solution, control, and amino acid standard analysis in terms of volume

Solution	Sample	Control	Standard
Borate buffer (μl)	70	80	70
Sample/standard (μl)	10	-	10
AccQ-Fluor reagent (μl)	20	20	20

**Table 2:** Yield of amino acid composition in *Channa striatus* Water Extract (CSWE)

Amino acid	Compositional yield (mg/g)
Aspartic acid	0.11 ± 0.00
Glutamic acid	0.18 ± 0.00
Serine	0.05 ± 0.00
Glycine	0.15 ± 0.00
Histidine	0.02 ± 0.00
Arginine	0.10 ± 0.00
Treonine	0.04 ± 0.00
Alanine	0.10 ± 0.00
Proline	0.07 ± 0.00
Tyrosine	0.02 ± 0.00
Valine	0.04 ± 0.00
Methionine	0.02 ± 0.00
Cysteine	ND
Isoleucine	0.03 ± 0.00
Leucine	0.07 ± 0.00
Phenylalanine	0.04 ± 0.00
Lycine	0.12 ± 0.00
Hydroxyproline	0.05 ± 0.00

ND = Not Detected. Values represent the mean values of duplicates ( $n = 2$ )

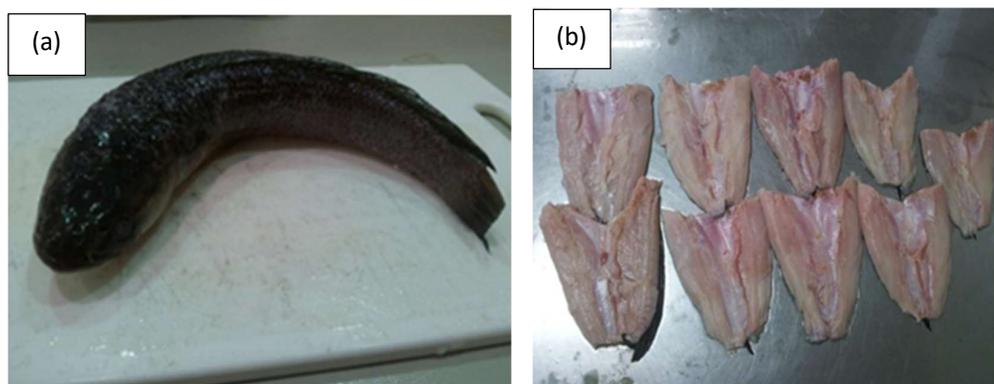
419 **Table 3:** Fatty acid content, specifically arachidonic acid, in *Channa striatus* Water Extract (CSWE)

Fatty acid	CSWE ( $\mu\text{g}$ )
Arachidonic acid	$3.72 \times 10^{-4} \pm 0.02$

420 Values represent the mean  $\pm$  S.D. of duplicate experiments ( $n = 2$ )

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424 **Figure 1:** Photograph depicting (a) freshly captured entire *Channa striatus* (haruan) and (b) boneless  
425 fillet of *Channa striatus* (haruan) prior to the aqueous extraction procedure

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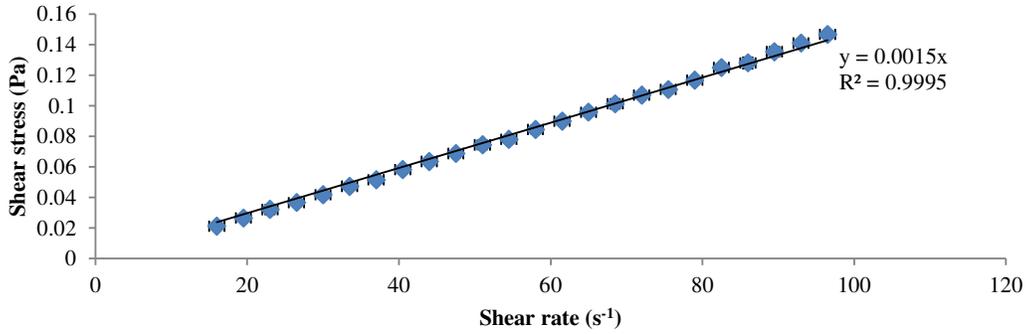


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428 **Figure 2:** Sample of *Channa striatus* Water Extract (CSWE) before undergoing physicochemical  
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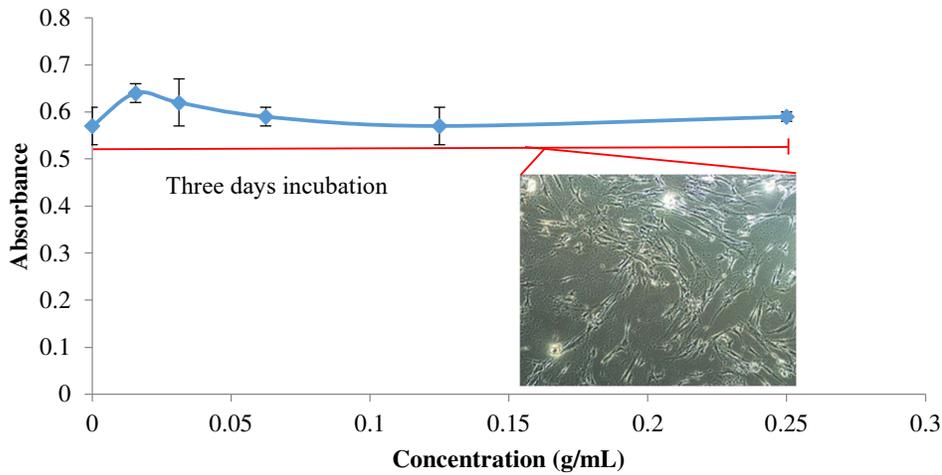
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**Figure 3:** The Newtonian fluid dynamic representation illustrating the relationship between shear stress and shear rate for *Channa striatus* Water Extract (CSWE) ( $n = 3$ )

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**Figure 4:** Proliferation of IMR-90 cells subjected to varied dilutions of CSWE, spanning from 0.25, 0.125, 0.0625, 0.03125, to 0.015625 g/mL, during a three-day incubation period. Inset diagram: Morphology of robustly viable IMR-90 fibroblast cells post-treatment with CSWE for three days.

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Absorbance values denote the average of triplicate measurements ( $n = 3$ )