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

(Meneroka Potensi Terapeutik: Ekstrak Air Channa striatus Malaysia yang Diperkaya dengan Asid Arachidonic untuk Penyembuhan Luka dalam Sel Paru-paru Janin Manusia (IMR-90))

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Abstract

Channa striatus, also known as haruan, is traditionally used in Malaysia for its wound-healing properties. This study evaluated the wound healing potential of Channa striatus water extract (CSWE) and identified key compounds promoting fibroblast cell growth. Channa striatus were deboned to maximize fillet retention. CSWE was obtained through aqueous extraction, and its physicochemical properties were analyzed, including pH, rheological characteristics, moisture content, amino acid composition, and arachidonic acid presence. The effective concentration (EC_{50}) of the sample was determined using a 2-D cell culture system with human fibroblast cells (IMR-90) over three days. Results showed CSWE had a near-neutral pH (6.34 ± 0.01) and high moisture content (97.3 ± 0.01%). The extract displayed Newtonian fluid behavior with a viscosity of 1.50 ± 0.31 mPa.s. CSWE contained essential amino acid glycine and arachidonic acid, important for wound healing, but in low concentrations. These low concentrations did not significantly promote IMR-90 cell growth ($p>0.05$) compared to the control. Consequently, EC_{50} values for CSWE were invalid due to over-dilution from a high fish weight-to-solvent ratio. Despite this, IMR-90 cell growth rates remained consistent across different CSWE concentrations, with no observed mortality during the three-day incubation. Overall, IMR-90 cells exhibited insignificant growth, even with arachidonic acid and glycine at low concentrations during treatment.

Keywords: Aqueous extraction; Channa striatus; IMR-90 cell; Physicochemical characteristics; Wound healing

Abstrak

Channa striatus, juga dikenali sebagai haruan, digunakan secara tradisional di Malaysia untuk sifat penyembuhan lukanya. Kajian ini menilai potensi penyembuhan luka ekstrak air Channa striatus (CSWE) dan mengenal pasti sebatian utama yang mempromosikan pertumbuhan sel fibroblast. Channa striatus dibuang tulangnya untuk memaksimumkan pengekalan fillet. CSWE diperoleh melalui pengekstrakan berair, dan sifat fisiko-kimianya dianalisis, termasuk pH, ciri-ciri reologi, kandungan lembapan, komposisi asid amino, dan kehadiran asid arakidonik. Kepekatan berkesan (EC_{50}) sampel ditentukan menggunakan sistem kultur sel 2-D dengan sel fibroblast manusia (IMR-90) selama tiga hari. Hasil menunjukkan CSWE mempunyai pH hampir neutral (6.34 ± 0.01) dan kandungan lembapan tinggi (97.3 ± 0.01%). Ekstrak menunjukkan kelakuan bendalir Newton dengan kelikatan 1.50 ± 0.31 mPa.s. CSWE mengandungi asid amino penting glisin dan asid arakidonik, yang penting

untuk penyembuhan luka, tetapi dalam kepekatan rendah. Kepekatan rendah ini tidak secara signifikan mempromosikan pertumbuhan sel IMR-90 ($p>0.05$) berbanding kawalan. Oleh itu, nilai EC_{50} untuk CSWE tidak sah kerana pencairan berlebihan daripada nisbah berat ikan kepada pelarut yang tinggi. Walaupun demikian, kadar pertumbuhan sel IMR-90 kekal konsisten di seluruh kepekatan CSWE yang berbeza, tanpa kematian yang diperhatikan selama tiga hari inkubasi. Secara keseluruhan, sel IMR-90 menunjukkan pertumbuhan yang tidak signifikan, walaupun dengan kehadiran asid arakidonik dan glisin pada kepekatan rendah semasa rawatan.

Kata kunci: Pengekstrakan berair; Channa striatus; Sel IMR-90; Ciri-ciri fisiko-kimia; Penyembuhan luka

INTRODUCTION

The snakehead fish, commonly referred to as haruan, is a carnivorous freshwater species belonging to the Channidae family and is indigenous to numerous tropical and subtropical regions, including Malaysia (Mohsin & Ambak 1983). It is present throughout tropical and subtropical Asian nations, spanning from Pakistan and India to Southeast Asia and Southern China. *Channa striatus* are opportunistic carnivores. Their diet varies depending on the availability of prey but can include small fish, amphibians, and aquatic insects (Roy et al. 2020). In Malaysia, locals traditionally incorporate haruan into their diet as a therapeutic remedy post-surgery, for the treatment of injuries resulting from road accidents, and for postpartum care in caesarian mothers (Rahmawanty et al. 2013). Typically consumed by dry-frying, grilling, boiling, or in the form of porridge, haruan is recognized for its healing properties (Jais et al. 1994). Throughout history, animal extracts have been utilized in treating various ailments (Zakaria, Somehit & Sulaiman 2004), with the snakehead fish (*Channa striatus*) extract emerging as the most commonly used animal extract due to its unique medicinal attributes, including potent anti-inflammatory and analgesic properties (Jais et al. 1994; Zuraini et al. 2004; Somchit et al. 2004). These properties effectively alleviate pain, inflammation, and enhance the wound healing process (Zuraini et al. 2004). The stability of bioactive compounds in snakehead extract within a pH range of 6 to 8, resistance to high temperatures (100 ºC), and resilience to enzymes such as α-amylase, protease, and lipase further contribute to its medicinal appeal (Dambisya, Sathivulu & Jais 1999).

Previous studies have explored the use of *Channa striatus* water extract (CSWE) emulsified in specialized polymers to create an aerosol spray for regulating wound healing (Febriyenti & Baie 2008). Application of the formulated emulsion onto abrasion areas in Sprague Dawley animal models resulted in the development of a thin film containing CSWE, significantly accelerating the healing process (Baie & Sheikh 2000). This emulsion, acting as a spray dressing, not only reduced discomfort and pain but also prevented microbial infections as it rejuvenated cells. Consequently, CSWE has demonstrated substantial facilitation of the wound healing process, offering a faster and stronger outcome compared to chemically synthesized medicines available in the market.

Recent studies have identified the presence of amino acids and fatty acids in snakehead fish, suggesting their potential as wound healing agents (Jais et al. 1994). Amino acids and fatty acids are pivotal components in the wound healing process, with their absence believed to impede recovery (Jais et al., 1994). The hypothesis that the wound healing efficacy of CSWE is influenced by its high glycine content (amino acid) and arachidonic acid (fatty acid) was explored. Both components play crucial roles in the wound healing process by initiating reactions leading to collagen formation, re-epithelization from injuries, and the induction of wound contraction (Shafri & Abdul manan, 2012; Tracy et al., 2016). To accurately mimic cellular behavior during the healing process, fibroblast origin cell lines such as IMR-90 were employed. Fibroblast cells regulate the secretion of Extracellular Matrix (ECM) products, controlling fiber-forming proteins in acute wounds, such as collagen, fibrin, and fibronectin. This study was conducted to investigate the wound healing efficacy of CSWE and the essential compounds facilitating fibroblast cellular growth, utilizing normal fibroblast cell lines (IMR-90).

MATERIALS AND METHODS

Raw materials

In this investigation, *Channa striatus* (haruan) served as the primary material, as illustrated in Figure 1(a) with an estimated weight and length (from head to tail) of 200 to 230 g and 35 to 40 cm respectively. The fish specimens were procured from local markets in Bandar Baru Bangi and a wet market in Kajang, Selangor, Malaysia. Subsequently, the fish species underwent a visual inspection and validation by the State Fisheries Department, Ministry of Agriculture (MOA), Malaysia. Upon the collection of the fish samples, a thorough cleansing process was conducted, involving the removal of bones, livers, stomachs, and viscera. Only the consumable muscles with skin (boneless fillet) were utilized in the course of this experiment (Figure 1b).

Chemicals

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

Aqueous Extraction of Channa striatus Water Extract (CSWE)

A boneless fish fillet sample underwent a cleaning process with distilled water, followed by weighing, and placement into a pressure cooker (Khind PC-600, Malaysia). Distilled water, with a fish to water volume ratio of 1:4, was introduced, and the pressure cooker was set to 100 °C for two hours (Jais et al. 1994). Distilled water was incrementally added to maintain the initial volume every 30 minutes during the cooking period. Upon completion of the cooking process, the resultant liquid extract was gathered, subjected to filtration using a filter paper (Sartorius Stedim Biotech, diameter = 125 mm, 84 g/m^2), and subsequently stored at 4 °C for subsequent physicochemical analyses (Figure 2). The fish fillet itself was then discarded. In this study, the weight fillet (FDF) of haruan obtained after the drying process was 39.40 g (20.87%) of the 188.8 g wet weight of haruan fillet.

pH Analysis

The determination of the CSWE's pH was carried out using a PHM210 standard pH meter (MeterLab®, Radiometer Analytical S.A., France). Calibration of the pH meter was conducted before each analysis to guarantee the precision of the readings. About 20 mL of the extract was utilized for the analysis (Febriyenti & Baie 2008), and measurements were taken on three samples (n = 3), with the results reported as mean ± standard deviation.

Flesh Water Content via Gravimetric Analysis

The water content in the flesh of CSWE was determined using the oven drying method (Fazial, Tan & Zubairi 2018; AOAC 2019). Initially, empty aluminum plates were cleaned and subjected to overnight drying in the oven (Fisher-Isotemp™ Nema 5-15 Oven Drying, United States) at 105 ºC. Afterward, they were cooled in a desiccator, and the weight of each empty aluminum plate was recorded. Subsequently, 2.0 g of the sample was placed on the aluminum plate and

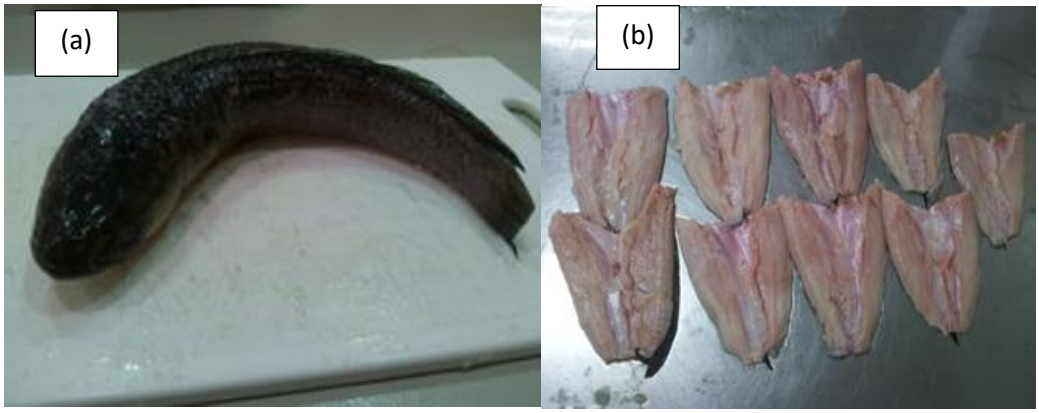

Figure 1 Photograph depicting (a) freshly captured entire *Channa striatus* (haruan) and (b) boneless fillet of *Channa striatus* (haruan) prior to the aqueous extraction procedure

Figure 2 Sample of *Channa striatus* Water Extract (CSWE) before undergoing physicochemical analysis.

transferred to a Protech Force Air Convection Oven FAC-138SS (Malaysia) at 105 ºC for 16 hours. Upon removal from the oven and cooling in the desiccator, the weight of the dried sample on the aluminum plate was recorded. The calculation for the flesh water content of the CSWE sample was then performed as follows:

Water content (%) = W1-W2/W1 x 100

where,

W1 = weight of sample before drying (g)
W2 = weight of sample after drying (g)

Rheological Attributes Analysis

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

Amino Acid Compositional Profiles

The determination of the amino acid composition in CSWE utilized High-Performance Liquid Chromatography (HPLC) with an AccQ•Tag^{TM} amino acid analysis column (dimensions = 3.9 mm x 150 mm; packing material = silica base bonded with C18; particle size = 4 µm). Sample preparation followed the guidelines provided by the supplier (Waters Corp., USA). The volumetric preparation (µl) of the sample solution, control, and amino acid standard analysis were as follows:

Borate buffer (70 to 80), sample/standard (10), and AccQ-Fluor^{TM} reagent (20), respectively (Fazil et al. 2018).

Acid Hydrolysis

Approximately 0.05 g of CSWE underwent hydrolysis with 5.0 mL of 6.0 N HCl within a borosilicate test tube. The tightly sealed test tube was placed in the oven at 110 ºC for 24 hours. After cooling, the hydrolyzed sample was transferred to a 100 mL volumetric flask, and 0.4 mL of the internal standard α-aminobutyric acid (AABA) was introduced into the flask (Fazil et al. 2018). Deionized water was then added to thesolution until reaching a volume of 100 mL, after which the solution underwent filtration using a syringe filter (25 mm in diameter). The resultant filtered solution was collected into a microcentrifuge tube.

Performic Acid Hydrolysis

About 0.05 g of CSWE was transferred to a borosilicate test tube and placed in an ice bath for 30 minutes. Following this, 2.0 mL of a cold performic acid solution was added to the test tube, and the mixture was left in the freezer for 16 hours. Subsequently, around 0.4 mL of HBr was introduced, and the solution underwent an additional 30-minute freezing period (Fazil et al. 2018). After the freezing step, the mixture was concentrated to an almost dry state using a hot plate. The resulting dried sample was hydrolyzed with a 6.0 N HCl solution, following the same procedure as described in the acid hydrolysis section. All eluents (µl) were prepared according to the supplier's instructions, as outlined in Table 1, before being injected into the HPLC. Separations were conducted using an AccQ-Tag amino acid analysis column (dimensions = 3.9 mm x 150 mm). The column temperature was set to 36 ºC for the acid hydrolysate and 31 ºC for the oxidized performic acid hydrolysate. Approximately 10 µL of standard or hydrolyzed sample was injected into the HPLC at a flow rate of 1 mL/minute. The fluorescence detector was configured with 250 nm excitation and 395 nm emission wavelengths.

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

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

Fatty acid (Arachidonic Acid) Determination

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 within a 20 mL test tube. The test tube was sealed, subjected to vortexing for 2 minutes at room temperature, and then centrifuged at 4000 rpm for 10 minutes. The resulting clear supernatant (upper layer) was transferred into a 2.0 mL autosampler vial for Gas Chromatography (GC) analysis (Fazil et al. 2018). The GC-17A Gas Chromatography (Shimadzu, Japan), equipped with a split/splitless injector, electronic pressure controller, and Flame Ionisation Detection (FID) system, was utilized to separate the Fatty Acid Methyl Esters (FAMEs). A Trajan SGE BPX-70 column (dimensions = 60 mm x 0.25 mm (internal diameter) x 0.15 µm polyethylene glycol film) was employed in this investigation. The oven temperature was initially set at 50 °C for 1 minute, increased to 175 °C at a rate of 4 °C/minute, and finally elevated to 230 °C for 5 minutes. The injector and detector temperatures were set at 250 °C and 260 °C, respectively, with a split ratio of 100:1 and a column temperature of 200 ºC. Approximately 1.0 µL of the sample was injected into the GC. Helium gas served as the carrier gas for the system with a flow rate of 1.7 mL/minute, maintained at a pressure of 103.4 kPa. The hydrogen and air pressures for the FID were set to 275.6 kPa. A standard FAMEs was employed to

ascertain the presence and quantity of arachidonic acid.

2-D Adherent Cell Culture Bioassay (EC_{50})

The determination of the effective concentration (EC_{50}) value for CSWE was conducted according to the provided protocol. Various concentrations of the sample were prepared through dilution, ranging from 0.25 (measured density of CSWE), 0.125 (Dilution Factor (DF): 2), 0.0625 (DF: 4), 0.03125 (DF: 8), to 0.015625 g/mL (DF: 16) (Kuo et al. 2020). In a 96-well microtiter plate, each well was seeded with a total of 1 x 10^4 human fibroblast cells (IMR-90), and the experiment was performed in triplicates (n = 3). The key characteristics of the IMR-90 cell lines considered in this study included: (a) Cell phenotype - Normal fibroblast; (b) Material source - Human Caucasian foetal lung fibroblast; (c) Culture medium - DMEM, and (d) Subculture routine - performed when 70–80% of the well space is filled with cell seeding, typically 2 to 3 x 10,000 cells/cm^2, using 0.25% (v/v) trypsin when reaching the confluent stage (Kuo et al. 2020). The IMR-90 cells were maintained in a 5% carbon dioxide (CO_2) environment at 37 °C. On the third day post-treatment, 10 mL of PrestoBlue Cell Viability Reagent™ was added and allowed to remain in the 96-well plate for 4 hours. Subsequently, the absorbance for each well was measured using BioTek Instruments ELx808™ Absorbance Microplate Readers at a wavelength of 293 nm.

Statistical Analysis

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

RESULTS

Currently available in the market are haruan extract products, which are offered either in liquid form for culinary applications or in cream form for skincare treatment. However, liquid haruan essence often poses certain issues such as limited shelf life and challenges in handling. This study aimed to evaluate the physicochemical characteristics of aqueous extracts derived from haruan fish (*Channa striatus*). These properties encompassed pH levels, moisture content, rheological behavior, as well as the composition of amino acids and fats.

The pH of CSWE was determined to be close to neutral, specifically 6.34 ± 0.01 as shown in Table 2. One of the most basic and important analytical procedures that can be performed on food products is the assessment of total moisture content. The water content in the flesh and the solid residual of CSWE were determined to be 97.3 ± 0.01% and 2.70 ± 0.01%, respectively. Figure 3 illustrates the rheogram depicting the correlation between the shear stress and shear rate of CSWE. The observations led to the inference that the water-based crude extract displayed Newtonian fluid behavior, demonstrating a linear relationship between shear stress and shear rate. Simultaneously, the viscosity of CSWE was quantified at 1.50 ± 0.31 mPa.s. Notably, variations in shear stress did not impact the viscosity of CSWE, indicating compliance with Newtonian laws.

Table 2 pH value of *Channa striatus* Water Extract (CSWE)

Sample	pH
CSWE	6.34 ± 0.01[a]

Values represent the mean values of triplicate (n = 3)

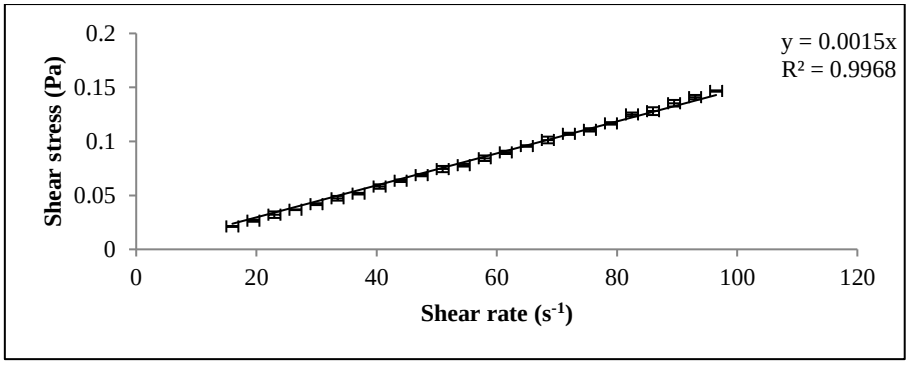

Figure 3 The Newtonian fluid dynamic representation illustrating the relationship between shear stress and shear rate for *Channa striatus* Water Extract (CSWE) (*n* = 3)

Table 3 presents the amino acid composition of CSWE. The investigation revealed the presence of at least 18 amino acid constituents, even at low concentrations. Predominant essential amino acids in CSWE from the snakehead fish included glutamic acid (0.18 ± 0.00 mg/g), glycine (0.15 ± 0.00 mg/g), and lysine (0.12 ± 0.00 mg/g). These findings highlight the presence of glycine in CSWE, a crucial amino acid contributing to the wound healing process.

Table 3 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 duplicate (*n* = 2).

Table 4 displays the essential fatty acid content of arachidonic acid in CSWE. The determined quantity of arachidonic acid was notably low ($3.72 \times 10^{-4} \pm 0.02$ µg) in comparison to findings from a prior study (Dahlan et al. 2010). This discrepancy might be attributed to the over-diluted sample utilized in this study, featuring a high fish weight-to-solvent ratio.

Table 4: The essential fatty acid content of arachidonic acid, in *Channa striatus* Water Extract (CSWE)

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

Values represent the mean ± S.D. of duplicate experiments (*n* = 2)

In this investigation, the EC_{50} value was determined to indicate the predictive concentration at which the substrate would elicit a 50% effect on cell number, whether positive (cell growth) or negative (cell death). The expectation was an increase in the number of IMR-90 cells with rising substrate concentration. Figure 4 illustrates the growth profile of IMR-90 cells treated with CSWE over three days, with the inset diagram depicting cell morphology after the same treatment duration. Although the number of IMR-90 cells initially exhibited gradual fluctuations with increasing sample concentration, followed by a rise at a concentration of 0.125 g/mL, the actual cell count showed no significant difference (p>0.05) compared to the control sample. In essence, normal IMR-90 cells

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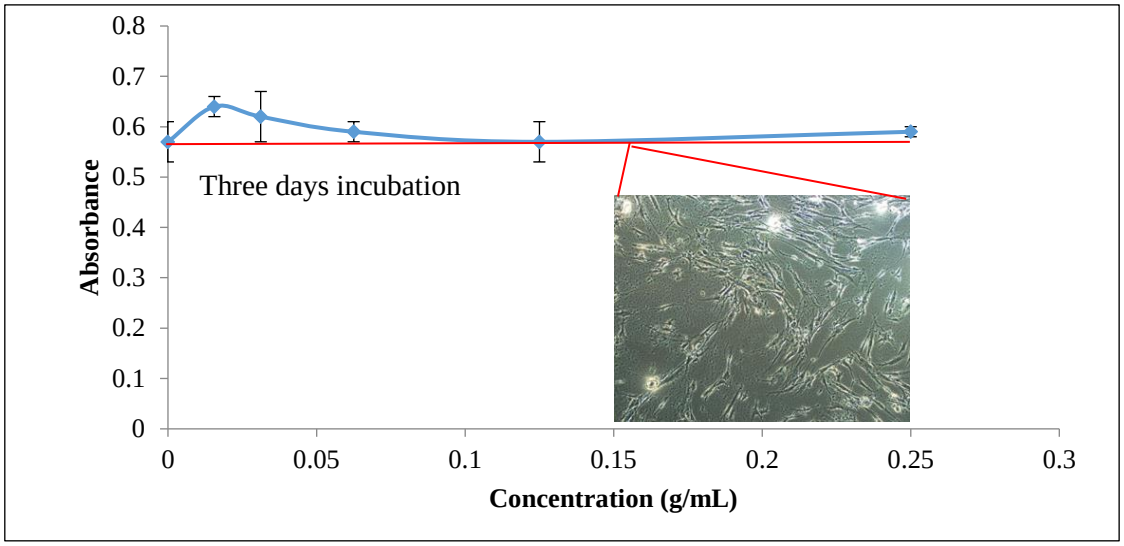

Figure 4 Proliferation of IMR-90 cells subjected to varied dilutions of CSWE, spanning from 0.25, 0.125, 0.0625, 0.03125, to 0.015625 g/mL, during a three-day incubation period. Inset diagram: Morphology of robustly viable IMR-90 fibroblast cells post-treatment with CSWE for three days. Absorbance values denote the average of triplicate measurements (n = 3).

demonstrated no discernible positive growth compared to the untreated control, despite the escalating substrate concentration. The lack of significant growth in IMR-90 cells was attributed to the notably low concentrations of arachidonic acid and glycine during each cell treatment.

Despite employing a similar sample preparation ratio as recent studies, the determination of the CSWE sample's EC_{50} value proved challenging due to the minute amount of active ingredients present in the extract before cell treatment. While the sample preparation ratio of fish weight to water volume (1:4, w/v) aligned with a previous study, they concentrated the snakehead essence later using the spray drying method to produce haruan encapsulated powder (Ngui et al. 2017). The preparation of CSWE with a 1:4 ratio was also applied in a previous study with variations in cooking duration (Ngui et al. 2017).

DISCUSSION

Different stages of wound healing may require environments of different pH. Acidic micro surroundings could improve fibroblast proliferation, whereas neutral and alkaline environments with a pH range of 7.21–8.34 are better for re-epithelialization (Kuo et al. 2020). Fibroblasts underwent exposure to platelet lysates after preincubation at varying pH levels (5.0, 7.1 and 7.6), revealing that platelets preincubated at a lower pH (pH 5.0) stimulated the most significant fibroblast proliferation during the wound healing process (Liu et al. 2002). Previous research reported that platelets preincubated at low pH (pH 5.0) induced the highest degree of fibroblast proliferation (Liu et al. 2002). Certain solutions like buffered saline or Ringer's lactate solution (with a pH of around 6.5-7.4) are already used for wound irrigation and cleansing. These solutions may create a more favourable environment for wound healing (Kow et al. 2002). Maintaining a neutral pH is ideal for the effective treatment of fresh wounds. In formulations intended for external use, a neutral pH helps minimize pain and irritation experienced by patients. If the formulation is designed for internal consumption, a slightly acidic condition may not pose a significant issue, as the

homeostasis process naturally works to balance the pH (Fazil et al. 2018).

Excessive moisture within fleshing residuals may result in spoilage and the proliferation of microorganisms. Maintaining an optimal moisture content range is crucial in mitigating these concerns and prolonging the shelf life of the product. The specific optimum moisture content range for fleshing residuals can vary depending on several factors, including source of the fleshing, intended use of the residual and for the storage conditions. These findings fell within the moisture content range observed in various commercial meat essences, such as beef, freshwater clam, hard clam, eel, and six types of chicken essence in a prior study, which varied from 91.1% to 97.5% (Dahlan et al. 2010).

Glycine, working in conjunction with arachidonic acid from the fatty acid group, plays a significant role in the recovery process. Glycine, a key component of human collagen, collaborates with other amino acids like proline, alanine, arginine, isoleucine, phenylalanine, and serine, to form polypeptides that foster tissue repair and the healing process (Dahlan et al. 2010; Aizad et al. 2021). Glutamine is essential for rapidly dividing cells, including white blood cells. This amino acid promotes fibroblast proliferation, aiding in wound closure (Rahayu et al. 2016). The potential benefits of glutamic acid for wound healing likely depend on factors like dosage and form (oral supplementation versus topical application).

Arachidonic acid (AA) performs diverse functions in cellular growth, serving as both a structural component and a signalling molecule. Insufficient concentrations of AA may restrict the synthesis of crucial phospholipids and growth-promoting eicosanoids. Depending on the severity of the deficiency, this can result in impaired cell growth or, in severe cases, cell death (Cantonero et al. 2020). While not essential for all cells, sustaining sufficient levels of AA can substantially influence cellular growth, especially in rapidly proliferating cells. Nonetheless, the results affirm the existence of arachidonic acid (C20: 4n-6) in the extract, albeit at a low concentration. In contrast to AA, glucose, and amino acids (which are other commonly studied solutions in cell culture) are indispensable nutrients. Cells are unable to produce them internally and depend entirely on external sources for their supply (Hamilton & Klett 2021).

The extremely high dilution was identified as a factor hindering the determination of EC_{50} values for the sample. Consequently, further concentration of the sample is recommended to observe the growth of IMR-90 cells, with a concentration of 1 g/mL suggested as a benchmark for future research, as previously used in other studies (Mohd Shafri et al. 2011; Know et al. 2002). Challenges in determining EC_{50} values also encompassed the short incubation period for IMR-90 cells, the three-day treatment duration, and the extensively diluted concentration range used in the cell culture study. To obtain accurate EC_{50} values, it is suggested that at least five data points should be incorporated into the graph to signify continuous cell growth (Musa & Min 2022). Despite these challenges, this study is deemed a successful preliminary exploration, as no prior research has delved into the proliferation of IMR-90 cells using snakehead essence. Previous studies related to CSWE have primarily focused on other cell types, such as Helacyton gartleri (HeLa cervical cancer cells), adenocarcinoma colon-rectal (HT-29) cells (Cantonero et al. 2020; Hui et al. 2010), and adrenal pheochromocytoma (PC-12), with the use of normal human fibroblast cells (IMR-90) not previously reported. Moreover, most studies evaluating the function of CSWE were conducted using Sprague-Dawley rats

and did not specifically target the wound healing process.

CONCLUSION

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

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