Protein & Peptide Letters, 2020, *27*, 48-59

RESEARCH ARTICLE

Bioactive Proteins in *Channa striata* Promote Wound Healing through Angiogenesis and Cell Proliferation

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Abstract: ***Background*:** *Channa striata* are speculated to contain bioactive proteins with the ability to enhancing wound healing. It is commonly consumed after surgery for a faster recovery of the wound.

***Objective*:** To identify the bioactive proteins and evaluate their ability in cell proliferation and angiogenesis promotion.

***Material and Methods*:** Freeze-Dried Water Extracts (FDWE) and Spray-Dried Water Extracts (SDWE) of *C. striata* were tested with MTT assay using EA.hy926 endothelial cell line and *ex-vivo* aortic ring assay. Later the proteins were fractionated and analysed using an LC-QTOF mass spectrometer. The data generated were matched with human gene database for protein similarity and pathway identification.

***Results*:** Both samples have shown positive cell proliferation and pro-angiogenic activity. Four essential proteins/genes were identified, which are collagen type XI, actin 1, myosin light chain and myosin heavy chain. The pathways discovered that related to these proteins are integrin pathway, Slit-Robo signalling pathway and immune response C-C Chemokine Receptor-3 signalling pathway in eosinophils, which contribute towards wound healing mechanism.

***Conclusions*:** The results presented have demonstrated that *C. striata* FDWE and SDWE protein fractions contain bioactive proteins that are highly similar to human proteins and thus could be involved in the wound healing process *via* specific biological pathways.

ARTICLE HISTORY

Received: April 09, 2019
Revised: June 17, 2019
Accepted: June 25, 2019

DOI:
10.2174/0929866526666190730121711

Keywords: Angiogenesis, bioactive, bioinformatics, cell proliferation, *Channa striata*, protein, wound healing.

1. INTRODUCTION

Channa striata is a freshwater snakehead fish that is abundantly available in the South East Asia region, particularly Malaysia [1]. For generations, the locals have been passing on traditional knowledge regarding the fish on wound healing enhancement. In the past decade, the fish has successfully caught the interest of the researchers across the region due to its popularity among the locals. Various researches have been conducted to solve and explore the compounds within the fish [2-4]. The outcome has indicated that the fish does exhibit wound healing enhancement properties. In a recent development, Rahayu *et al.* has highlighted a *C. striata* fraction that contains bioactive proteins which accelerate wound healing, yet the identity of those bioactive proteins and how they involve in the wound healing mechanism are still poorly understood [5].

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The tide has turned with the advancement of technologies in areas such as genomics and proteomics; they enable many bioactive secondary metabolites to be identified in all these natural products [6]. Proteins are one of the primary targets for drug discovery. The rise of proteomics technologies has enabled protein identification and validation from natural resources and research into the development of drug and medicine. These proteins can be transformed into potential therapeutic targets to treat the patient's illness or diseases.

Recently, many researchers have taken the initiative to apply network approach for wound healing discovery, involving gene expression and proteomics profiling in the regular clinical experiments [7, 8]. According to Arodz *et al.* [9], software packages can be applied for establishing and analysing networks proceeding from data found in a database search or report or sourced from experimental metabolic, gene expression and proteomics data. Network approach analysis helps to identify genes/proteins involved during the recovery process and their roles in specific pathways or modules.

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The focus of this research is to explore the bioactive proteins available within *Channa striata* and determine their roles in wound healing enhancement *via* bioinformatics analysis. The proteins within the samples were tested on different assays to validate their wound healing activity. It will be appreciated to explore and discover the bioactive compounds within the species and the wound healing pathways involved for a more in-depth application on wound healing that plays a vital role in wound care management.

2. MATERIALS AND METHODS

2.1. Sample Preparation and Protein Extraction

Two different samples sourced from *Channa striata*, namely the Freeze-Dried Water Extracts (FDWE) and Spray Dried Water Extract (SDWE), were produced from a fish farm situated in Sik, Kedah, Malaysia. Briefly, 10 litres of *C. striata* water extracts were inserted into a freeze dryer (Labconco, MO, USA) and freeze-dried under vacuum state and temperature of -40 °C until the samples are fully dried. Another 10 litres of *C. striata* water extracts were loaded into a spray dryer machine (Yakin Gigih, Malaysia). The ultrasonic frequencies were set at 40 and 120 kHz. The dryer was operated at 100±5 °C (inlet) and 65±5 °C (outlet) at a flow rate of 100 mL/hour. Protein extraction was conducted on the samples, according to Gam *et al.* [10] with minor modifications. 30 mg of samples from each group were mixed into 1 mL of 40 mM Tris-Hydrochloric Acid (HCl) (pH 8.8) (Sigma Aldrich, MO, USA) extraction buffer, respectively. Each mixture was added with 1 mM phenylmethane sulfonyl fluoride (PMSF) (Sigma Aldrich, MO, USA) and incubated for 20 minutes under regular vortex. Sample mixtures were then centrifuged at the speed of 12,000 x g for half hour and the supernatants were recovered for subsequent analysis. Bradford assay was carried out according to Bradford [11] to determine the total protein concentration.

2.2. Cell Proliferation Assay

The activity of the cell proliferation promotion by the *C. striata* samples was determined using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay. The MTT assay was performed in a 96-wells plate using EA.hy926 cell lines. The assay was carried out according to the method described by Mosmann [12] with minor modifications on the reference wavelength. Briefly, 100 µL Dulbecco's Modified Eagle's medium (DMEM) media which contains 5×10^3 cells were seeded into the respective well. Then, 100 µL of *C. striata* FDWE and SDWE were added to each well at different concentrations (6.25, 12.5, 25, 50, 100 and 200 µg/mL). Wells treated with DMEM served as control and wells with media but without cells as blank. After 48 hours of incubation in a 37ºC and 5% CO_2 humidified atmosphere chamber, each well was added with MTT reagent. The MTT reagent solution was prepared at 5 mg/mL in Phosphate Buffered Saline (PBS). After 4 hours of incubation, the media in each well was extracted, followed by adding in 100 µL of dimethyl sulfoxide (DMSO) to dissolve the formazone crystals in respective well. After 10 minutes of incubation at 37°C, optical density (OD) of the violet colour was measured using a microplate reader at 570 nm absorbance and a reference wavelength of 620 nm. The assay was performed in triplicate. The data was recorded to calculate the effective concentration at half maximum (EC_{50}) value for both samples.

2.3. *Ex-vivo* Rat Aorta Ring Assay

Animal ethics approval has been obtained from Universiti Sains Malaysia (USM) with the reference number USM/Animal Ethics Approval/2014/(617) before the experiment. All procedures were carried out strictly adhering to the guidelines of the USM Animal Ethical Committee. The assay was carried out according to [13] with minor modifications on the scoring system. It is an angiogenesis model that is based on organ culture, and it is applicable for angiogenic factors or inhibitors in a controlled environment [14]. Thoracic aortas were removed from euthanised male Sprague Dawley rats (12-14 weeks old) and rinsed with serum-free medium, and the fibro adipose tissues were removed. The aortas were then cross-sectioned into small rings (approximately 1 mm thickness) and placed individually in 48-wells plate. Each well was loaded with 300 µL serum-free M199 media containing 3 mg/mL fibrinogen and 5mg/mL aprotinin. Next, 10 µL thrombin (50 NIH U/mL in 1% bovine serum albumin in 0.15 M Sodium Chloride (NaCl)) was added into each well and incubated at 37 °C for 90 minutes to solidify. A second layer media (300 µL) containing M199 media supplemented with 20% Heat Inactivated Fetal Bovine Serum (HIFBS), 0.1% ε-aminocaproic acid, 1% L-Glutamine, 2.5 µg/mL amphotericin and 60 µg/mL gentamicin was loaded into the respective well. Six rings were used for each treatment. Two different concentrations (100 and 200 µg/mL) of *C. striata* crude proteins from a different group were added for screening purpose, whereas distilled water was used for untreated samples. On day four, the medium was replaced with fresh media. Photos of the aortic ring were taken on day 5 using AMG EVOS fl inverted microscope (40× magnification). Among a few parameters for scoring, two parameters which were described in Blacher *et al.* (2001), namely the maximal microvessel length (L_{max}) and area covered by the newly formed blood vessels were measured using ImageJ software. The results are presented as mean per cent proliferation ± SD, (n = 6).

2.4. Protein Fractionation and Trypsin Digestion

Next, the extracted proteins were fractionated using GELFREE 8100 fractionation system (Expedeon, CA, USA) according to a native electrophoresis protocol. Briefly, 2 mg of protein-buffer mixtures were loaded into an 8% tris-acetate cartridge. A total of 8 fractions were collected during the procedure from both samples. Bradford assay was conducted again to verify the protein concentration of each fraction. The fractions with a concentration of >50 µg/mL were shortlisted and digested by re-suspending in 100 µL of 6 M urea, 100 mM Tris buffer at 10 mg/mL. The digestion method was carried out, as mentioned in Kinter and Sherman [15].

2.5. LC-MS/MS Analysis

The digested fractions of each *C. striata* sample were mixed with 0.1% Formic Acid (FA) (Fisher Scientific, NH,

USA) and resolved by High-Performance Liquid Chromatography (HPLC). Briefly, 1 µL from each digested fraction was introduced using an autosampler. The HPLC separation was conducted on a Hypersil Gold C18 column (C18, 2.1 × 150 mm, 3 µm particles) (Thermo Fisher, MA, USA) in two different mobile phases. Mobile phase A (0.1 % FA in water) consisted of 90 %, mixing with 10% mobile phase B (0.1 % FA in acetonitrile) at a flow rate of 15 µL/min. An LC-QTOF mass spectrometer was applied for the proteomics research. The running setup was set for 5 minutes, and elution of peptides was run with a linear gradient ranging from 5 % to 95 % mobile phase B for 120 minutes. The gradient was then set to 10 % B again for the final 15 minutes before the entire running ends. Each run was separated by a single blank run to wash and re-equilibrate the column before proceeding to the next run. The mass range of *m/z* 100–2000 were acquired for the spectrum of MS and MS/MS studies. 6 V/100 Da (offset −2) of collision energy was set. The analysis was conducted at 15 µL/min, and the ion spray source was infused at 3.5 kV. Nitrogen was applied at 10 L/min at 350 °C. The fragmentor voltage and nebuliser pressure and were fixed at 110 V and 3 psig respectively. Nitrogen gas was used for the Collision-Induced Dissociation (CID) of the precursor ions. Precursors for CID were decided on the basis of abundance. A maximum of 4 precursors was selected per cycle for fragmentation.

PEAKS Studio Version 7.5 (Bioinformatics Solutions Inc., Canada) was used to perform *de novo* sequencing and database matching. *Actinopterygii* (boned fish) database updated until October 2016 from Uniprot (http://www.uniprot.org/) was selected for the database matching. Fixed modifications were carbamidomethylation and methionine oxidation, maximum missed cleavage was set at 2. Parent mass and precursor mass tolerance were set at 0.1 Da. False detection rate (FDR) less than 0.1 % and significant score (−10logP) for protein more than 20 were used as the parameters for protein acceptance. The Average Local Confidence (ALC) was set at more than 80 %. The number of the unique peptide was set at >1. The maximum variable post-translational modification was set at 4.

2.6. Bioinformatics Analysis

Software Tool for Researching Annotations of Proteins (STRAP) was applied to match the proteins shortlisted with annotations from the latest Uniprot and European Bioinformatics Institute QuickGO databases [16]. Biological process, cellular component and molecular function for each protein were generated based on the databases referred to. The amino acid sequence of each shortlisted protein was matched against *Homo sapiens* database using Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) (Version 10.5) [17]. The confidence score was set at the highest (0.900) for the protein-protein interaction network. Proteins displayed in the STRING database was then searched against GeneCards® (human gene database) to identify the pathway involved for each protein detected [18].

3. RESULTS AND DISCUSSION

3.1. Analysis of *C. striata* FDWE and SDWE Samples on Promoting Cell Proliferation

Both samples were tested on Ea.hy926 human endothelial cells to determine the effectiveness of these samples on wound healing enhancement. As cell proliferation is one of the crucial steps in wound healing, the results obtained serves to provide an insight into the efficiency of the *C. striata* samples. All the samples have shown positive proliferation activity on the treated cells (shown in Figure **1**). The results were analysed with Duncan's test, and it has shown that SDWE gave a significant change on the cell proliferation activity.

Whereas for FDWE, there was no significant proliferate activity observed on the cell at a low level of concentration (6.25 µg/ml – 12.5 µg/ml). The significant cell proliferation induced by SDWE has suggested that the bio-active proteins that enhanced the wound healing process can withstand high temperature. As the process of spray-drying involves considerable high temperature, some proteins may be malfunctioned or destroyed due to high heat and the stresses caused by the air interface [19]. A protein that has denatured refers to the possible destruction and disruption of the entire structures of the mentioned protein. If a protein is denatured during spray drying, it will lose its healing ability and also other necessary features [20]. However, based on our observation on the treated cell, both FDWE and SDWE were effective in promoting cell proliferation. Besides proteins available within the samples, minor fatty acids might exist as well in the water extracts [5, 21], though the study on the role of fat in wound healing is still scarce [22]. Most of the fatty acid's involvement is on anti-inflammatory and antinociceptive activities of wound healing [21]. Effective concentration at half maximum (EC_{50}) provides the concentration of a drug/compound that induces 50% of the response between baseline and maximum after a specific exposure time. It is used to determine the performance of the tested samples. Both samples have a similar EC_{50} value as well, indicating both can be considered as similarly effective on enhancing the wound healing process.

3.2. Analysis of Different *C. striata* Samples on Promoting Angiogenesis

The samples were applied on rat aorta ring assay to screen the capabilities of the respective sample on supporting the growth of blood capillaries. The calculation was based on the maximal microvessel length (L_{max}) and area covered by the newly formed blood vessels (as shown in Figure **2** & **3**) against the untreated ring. The growth promoted by both FDWE and SDWE were comparable to the MTT assay which showed positive activity on cell proliferation as well. As angiogenesis is one of the indispensable events occurred during wound healing, the assay gave a strong indication that the bio-active proteins are within the two samples.

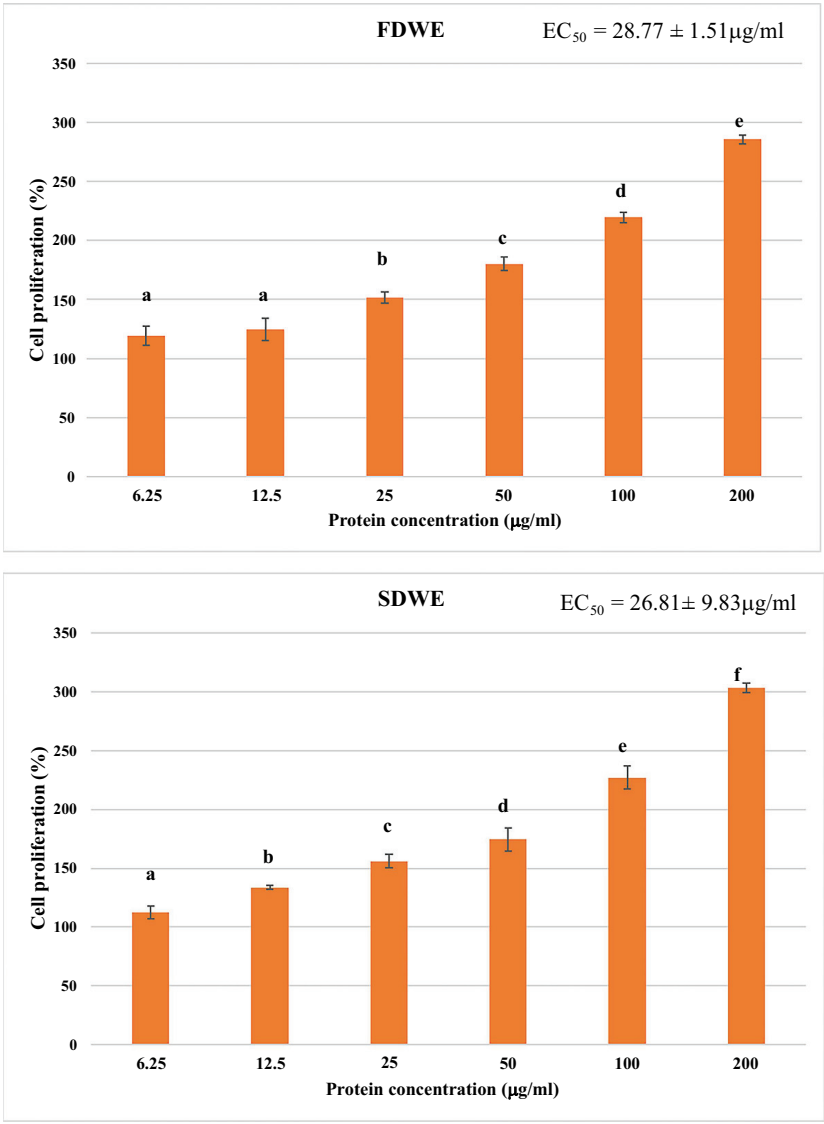

Figure 1. Effects of proteins in *C. striata* Freeze-Dried Water Extracts (FDWE) [top] and Spray-Dried Water Extracts (SDWE) [bottom] on the proliferation of EA.hy926 cell lines. Both samples promote cell proliferation of EA.hy926 cell lines in a dose-dependent manner. Bars and values presented are mean ± SD (n=3). Different letters on top of each bar indicated the values are significantly different at $p<0.05$ level of significance, according to Duncan's test (n=3).

3.3. Protein Profiling and Determination of Bio-active Proteins

From a total of 16 fractions harvested from two different samples of *C. striata*, four fractions were shortlisted to proceed with the mass spectrometry. The fractions with the concentration of >50 µg/mL (data not displayed) were shortlisted for the analysis. One fraction from FDWE (the third fraction obtained from FDWE, will be named as FDWE3 subsequently) and three fractions from SDWE (first, fifth and sixth fractions obtained from SDWE, will be named as SDWE1, SDWE5 and SDWE6 subsequently) were analysed and the mass spectra generated were matched with *Actinopterygii* (boned fish) database using PEAKS studio 7.5. Overall, there were 5, 17, 8 and 4 proteins detected in fraction FDWE3, SDWE1, SDWE5 and SDWE6, respectively. Most of the proteins detected were uncharacterised (50%) and structural proteins (32%) (presented as supplementary materials).

Among the proteins identified, most of them are common proteins (structural proteins) which can be found in many eukaryotes. However, there were some uncharacterised proteins detected as well, suggesting the available database was not comprehensive enough to confirm the identity of all the extracted proteins. To know and understand the biological processes of every single protein detected, a comparative analysis was conducted according to Gene Ontology (GO) [23]. Software Tool for Researching Annotations of Proteins (STRAP), developed at the Cardiovascular Proteomics Centre of Boston University School of Medicine (Boston, MA), was applied to

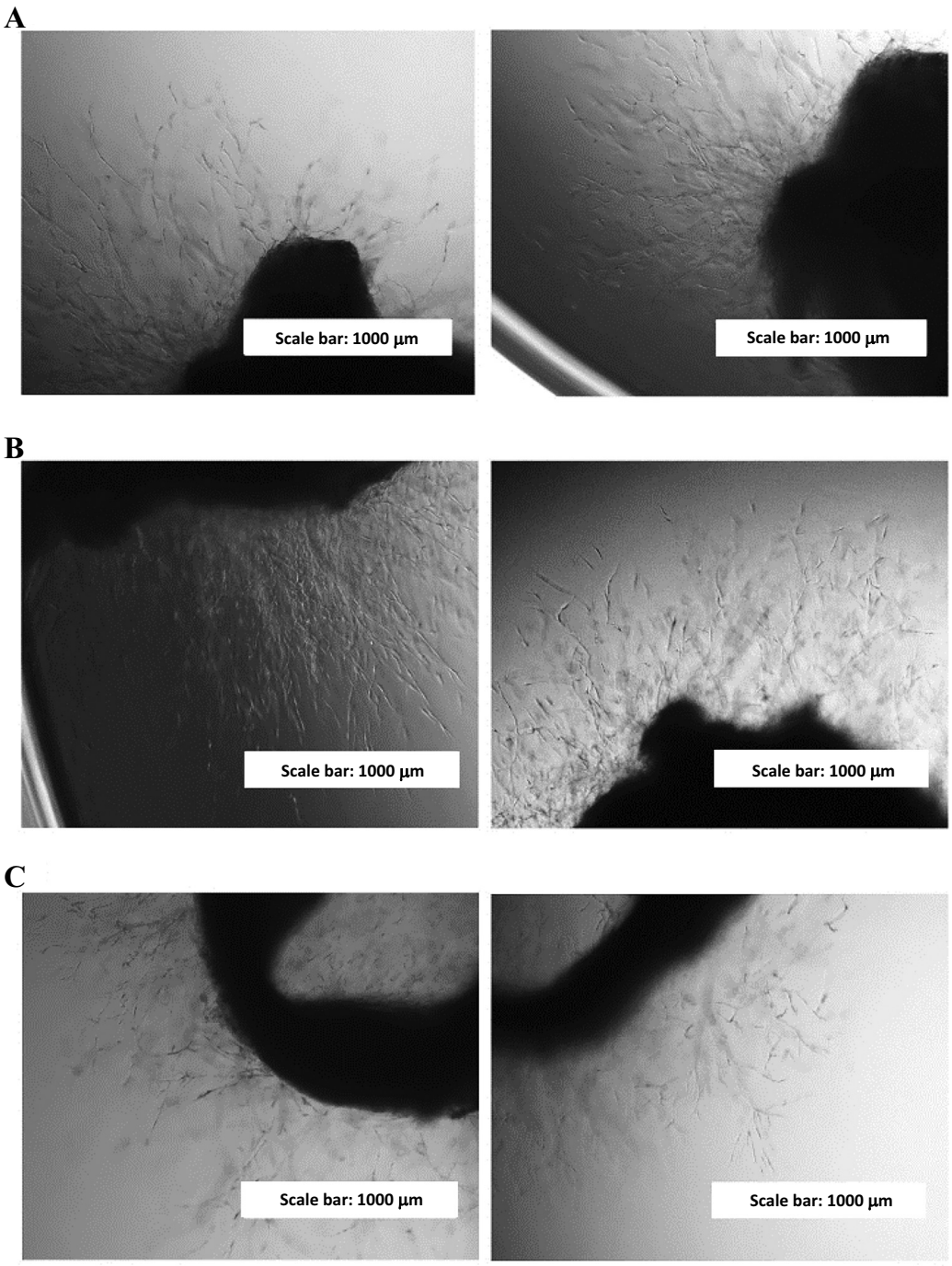

Figure 2. Images of angiogenesis property induced by proteins within *C. striata* FDWE on the rat aorta ring. **A**: Rings treated with 100 µg/mL; **B**: Rings treated with 200 µg/mL. **C**: Rings that were untreated. The images were taken with 40× magnification.

automatically match annotations from the Uniprot and and European Bioinformatics Institute QuickGO databases [16]. The graph generated is shown in Figure **4**.

Based on Figure **4a**, it was evident that most of the proteins identified in the SDWE fractions involved in cellular process, except for a small number involving in other methods. Myosin and tropomyosin are motor proteins which regularly engaged in the cellular movement [24]; thus the cellular process was the dominant biological process detected. The high number of motor/muscle proteins detected was in line with the GO result presented in Figure **4b** showing cytoskeleton as the main cellular component of all the proteins identified in these four fractions. Cytoskeleton refers to a broad three-dimensional network formed by the nucleus, organelles, fibrous systems and membranes that function in coordinated systems [25]. The cytoskeleton is an essential part of wound healing, needed for actomyosin contraction, recruitment of repair machinery,

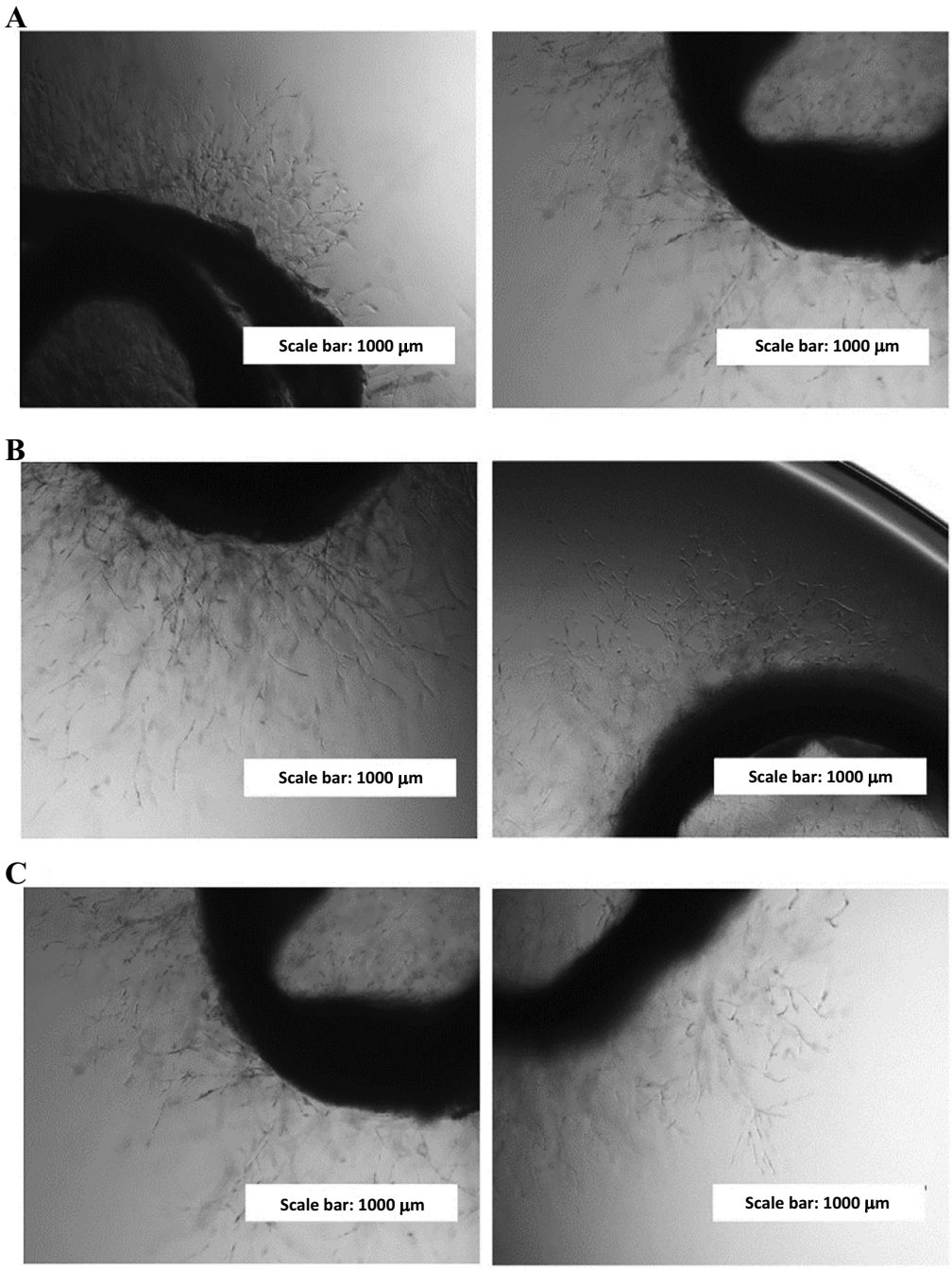

Figure 3. Images of angiogenesis property induced by proteins within *C. striata* SDWE on the rat aorta ring. **A**: Rings treated with 100 µg/mL; **B**: Rings treated with 200 µg/mL. **C**: Untreated Rings. The images were taken with 40× magnification.

and cell migration [26]. The actin and tropomyosin networks identified are suspected to be the driving force for the repair process, possibly promoting angiogenesis and cell proliferation.

Besides, there were proteins involving in the cytoplasmic component, suggesting these proteins may contribute to individual cell healing, repairing broken cell membrane before the cellular contents leak out [27]. Single cells wound healing can involve a few pathways, including actin-myosin purse-string contraction around the wound and membrane patching by rapid fusion of exocytotic vesicles [28]. Meanwhile, the molecular functions of these proteins, as shown in Figure **4c**, are mainly catalytic activity and binding activity. Parvalbumin, as a calcium-binding protein, fits the criteria of binding activity. Intriguingly, all the proteins detected in FDWE3 falls under the category of catalytic activity only.

In order to explore the relations of the identified fish proteins with human genes, STRING V10.5, an online platform that connects known and predicted protein-protein interactions, was applied. The interactions include direct (physical) and indirect (functional) associations; they stem

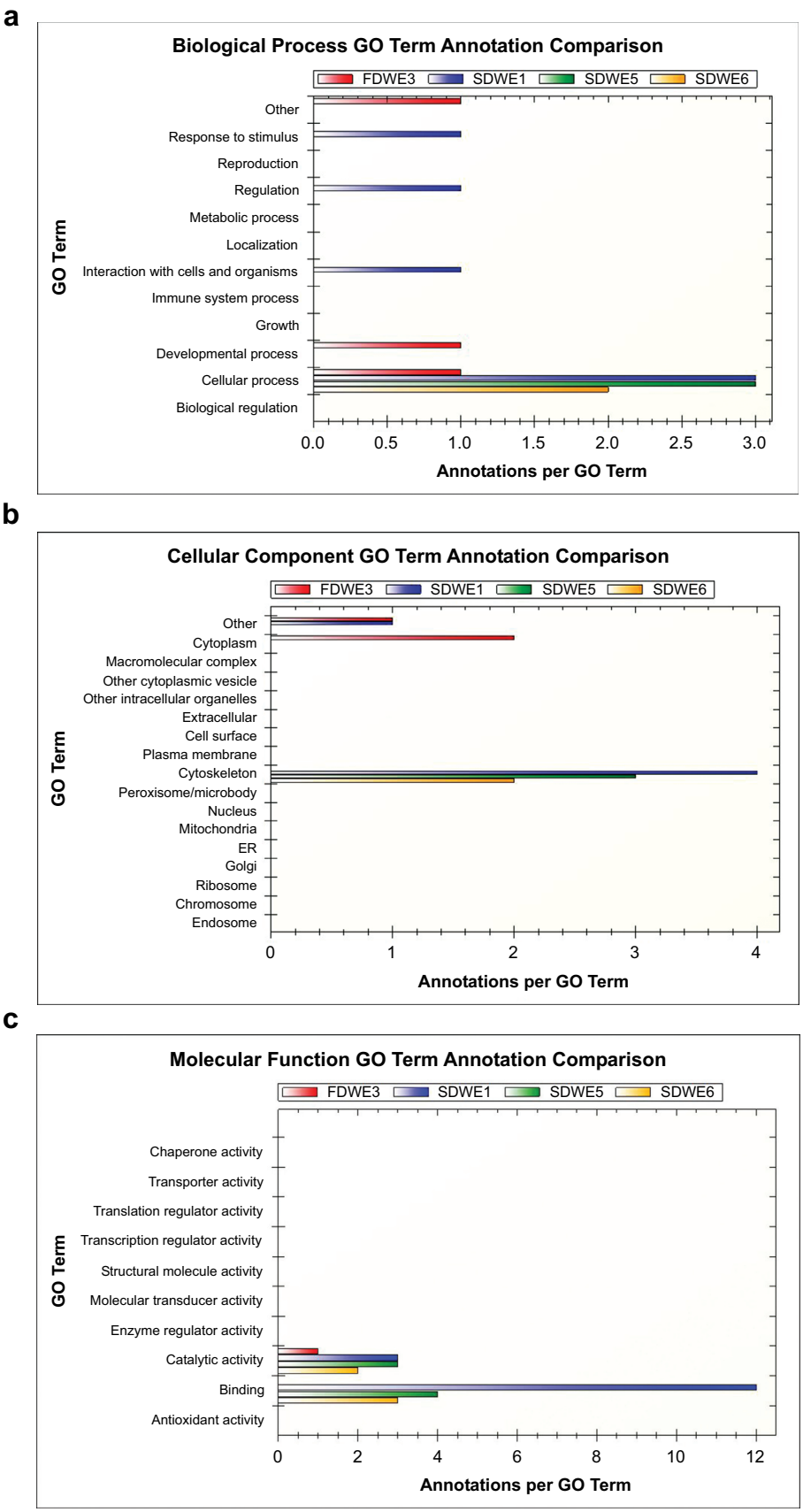

Figure 4. Information of the shortlisted fractions harvested in FDWE and SDWE. Gene Ontology (GO) terms were assigned to proteins for comparison of molecular functions using an open source program, the Software Tool for Rapid Annotation of Proteins, STRAP. **a**: Biological process; **b**: Cellular component; **c**: Molecular function.

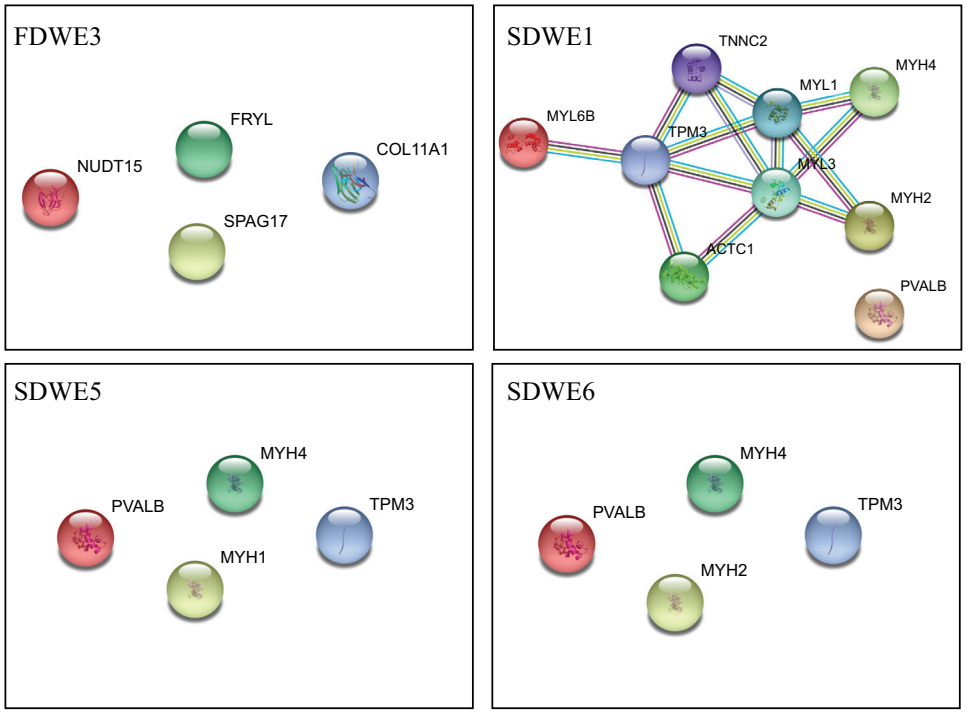

Figure 5. Protein-protein interactions within each shortlisted fraction. There was no significant interaction among proteins within all fractions except for SDWE1.

Table 1. Summary of identified proteins and their associated pathways as found in GeneCards (human gene database).

Protein Name & Gene Code	Annotation	Pathways Involved	Available in Fraction(s)
Collagen, type XI, alpha 1 (COL11A1)	May play an important role in fibrillogenesis by controlling lateral growth of collagen II fibrils.	Integrin pathway	FDWE3
Actin, alpha, cardiac muscle 1 (ACTC1)	Highly conserved proteins that are involved in various types of cell motility and are ubiquitously expressed in all eukaryotic cells.	Development Slit-Robo signalling	SDWE1
Myosin, light chain 1 (MYL1)	Alkali, smooth muscle and non-muscle; Regulatory light chain of myosin.	Immune response C-C Chemokine Receptor-3 (CCR3) signalling in eosinophils	SDWE1
Myosin, light chain 3 (MYL3)	Alkali, smooth muscle and non-muscle; Regulatory light chain of myosin.		SDWE1
Myosin, heavy chain 2 (MYH2)	A major contractile protein which converts chemical energy into mechanical energy through the hydrolysis of ATP.		SDWE1, SDWE6
Myosin, heavy chain 4 (MYH4)	Shares the common features of ATP hydrolysis (ATPase enzyme activity), actin binding and potential for kinetic energy transduction.		SDWE1, SDWE5, SDWE6

from computational prediction, from knowledge transfer between organisms, and interactions aggregated from other (primary) databases. The amino acid sequence identified within the *C. striata* fractions were compared with *Homo sapiens* database to search for any similar sequence between the two species. The protein-protein interactions are displayed in Figure **5**.

Under the highest confidence (0.900), we have noticed that there was no significant interaction among proteins detected within all fractions except for SDWE1. Such observation has suggested that the proteins do not work with each other within the same fraction, but perhaps involve in certain pathways *in-vivo*. Besides, proteins available in SDWE1, SDWE5 and SDWE6 were similar and mainly consisted of myosin, tropomyosin and parvalbumin. The

proteins reflected have shown that there are high similarities among those proteins (in terms of amino acid sequence) within *C. striata* and *H. sapiens*. Surprisingly, proteins detected in FDWE3 have shown similarity with Sperm-associated antigen 17 in the human body. The protein may play a role in endochondral bone formation, most likely because of a function in primary cilia of chondrocytes and osteoblasts. However, there is no indication that it involves any pathway known for wound healing. All the proteins were then searched against human gene database stored within GeneCards (Human Gene Database) to explore any of the genes within involved with pathways related to wound healing (shown in Table **1**).

3.4. Integrin Pathway

Collagen belongs to a family of at least 29 structural proteins synthesised from over 40 human genes [29]. The interactions of collagens are crucial in a variety of biological processes, including cell adhesion, cell migration, cell growth and cell differentiation [30]. Type XI collagen that was identified in the current work is mainly found as a quantitatively minor fibril constituent in tissues that contain collagen type I. The integrins are a family of heterodimeric receptor proteins that regulate the communications of many cell types with a wide range of extracellular matrix, cell membrane and soluble proteins [31]. The integrins that bind to RGD (R: arginine; G: glycine; D: aspartic acid) sequences have been linked with angiogenesis and thrombosis, and also activation of fibrosis and Transforming Growth Factor Beta (TGF-β) [32-35]. The collagen-binding integrins have largely been neglected in the past until recent data that reported the relationship of the collagen-binding integrins with fibroblast function in wound recovery [36]. The interrelationship between collagen and integrin have been largely discussed in Zeltz and Gullberg [37]. Integrins play a central role in the formation of new blood vessels [38]. One of the collagen-binding integrin, α2β1, is expressed constitutively in the epidermis and upregulated in wounds. A study conducted by Zweers *et al.,* has suggested that the integrin is required for regulation on wound angiogenesis but not related to cell reepithelialisation [39]. A clear correlation on how the presence of collagen introduced into the wound area contributes towards wound healing has yet to be established. However, it is certain that the integrin pathway plays a key role in wound healing mechanism, particularly angiogenesis.

3.5. Slit-Robo Signalling Pathway

Slits are referring to the proteins that connected to the Roundabout (Robo) receptors [40]. Slit-Robo signalling pathway is responsible for regulating the axon repulsion available in the developing nervous system [41]. The *Slit-Robo* gene was initially found in D*rosophila melanogaster* [42], and throughout years of observation, the functions of Slit-Robo signalling has been associated to the formation of blood vessels, neurons and cancer progression among other processes [40]. Cytoplasmic kinases, actin and microtubule cytoskeleton are required to broadcast Robo-triggered signalling responses. The cytoplasmic kinase Abelson (Abl) does affect both the microtubule cytoskeleton and actin [40]. Vertebrate Robo4 is known to control the formation of the blood vessel and its permeability [43] Besides Robo4, endothelial cells also synthesised Robo1 and Robo2 receptors which presenting pro-angiogenesis features through enhancing endothelial cell polarity and motility [44, 45]. As the expression pattern of Robo1, Robo2 and Robo4 overlapped, it is likely that Slit functions through receptor heterodimers without direct binding to Robo4. Zhang *et al.* [46] have highlighted that the absence of Slit3 disrupts angiogenesis during embryogenesis. It was also discovered that the Slit-Robo signalling is highly context-dependent, establishing a multifunctional platform for cell-cell or cell-matrix interactions influencing multiple physiological and pathological processes [40].

As can be observed in Table **1**, actin is involved in the part of the pathway mentioned. Wu *et al.,* [47] have highlighted that actin remodelling affects cell-cell interactions and Slit-Robo signalling regulates cell motility through actin. The actin networks provide the driving force for the repair process and serve as traffic for other components to reach the wound [24]. It is dynamic and remodelled in response to generating the mechanical forces necessary for changes in cell contraction, adhesion and motility for wound healing [48]. As actin serves as the key player of the signalling propagation, an assumption that with the external actin introduced into the wounded area, it enhances the Slit-Robo signalling for the reorganisation of the actin cytoskeleton, hence promotes angiogenesis at a faster rate on the wounded area.

3.6. Immune Response C-C Chemokine Receptor-3 (CCR3) Signalling in Eosinophils

Eosinophils are a type of white blood cells that are involved in the inflammatory infiltration associated with the healing of cutaneous wounds [49]. It has been characterised that eosinophils to be pro-angiogenic [50], thus the pathways of CRR3 signalling in eosinophils which involving the proteins identified is likely to be a key factor in wound healing enhancement. Initially, chemo-attractants/ chemokines are generated at the wounded area, inducing migration of eosinophils from the vasculature into the affected tissue. Chemotactic response of eosinophils is mostly mediated by CCR3, a member of the G-protein-coupled receptor family, which activates G-protein alpha-i family [50]. With the presence of eosinophils, they synthesise, store and release stem cell factor (SCF), which is responsible for cell differentiation, survival, proliferation, maturation, chemotaxis and adhesion [51].

Myosin is a common protein isoform in non-muscle cells. The role of myosin is controlled by the Myosin light chain (MLC) phosphorylation step. It is important for the expression of a well-developed actin-myosin motor unit. As shown in Table **1**, the myosin heavy chain (MHC) and MLC are involved in part of the CCR3 signalling for actomyosin fibre formation. Actomyosin, also known as stress fibre, are connected to focal adhesions to facilitate cell adhesion and cell migration. Mechanical tension during wound closure also induces stress fibre to gather in the epithelial cells, which then differentiate into myoepithelial cells [52]. The inhibition of myosin II will lead to the disassembly of stress

fibres, thus affecting wound recovery [53]. This detailed correlation of MHC and MLC in the pathway have been described in the works of Adachi *et al.* [54]. Ultimately, the presence of MHC and MLC in the proteins detected have given us the indication that they are related to this pathway that stimulates angiogenesis in the wound healing mechanism.

CONCLUSION

The *Channa striata* have been known for its capabilities in enhancing wound recovery. The assays conducted have demonstrated the positive activity promoted by the proteins within the fish. The proteins identified in the fractions of FDWE and SDWE using proteomics approach have allowed us to have a better understanding of the proteins available within. Through the network analysis, proteins have been identified to be associated with wound healing mechanism in a human system. It was revealed that these proteins involve in certain biological pathways that contributed towards the wound healing process. As the water extracts possibly contain minor fatty acids, more in-depth research can be conducted in future using isolated and purified bioactive protein to confirm the exact role and the criticality of each of the protein highlighted.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study on animals is approved by the ethics committee of Universiti Sains, Malaysia (USM) with the reference number USM/Animal Ethics Approval/2014/(617).

HUMAN AND ANIMAL GUIDELINES

No humans were used for the studies that are the basis of this research. The reported experiments on animals were performed in accordance with the guidelines of Universiti Sains Malaysia (USM) Institutional Animal Care and Use Committee.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIAL

Not applicable.

FUNDING

This study was supported by Universiti Sains Malaysia through the USM Bridging Grant 2017 (304.CABR.6316042) and Fundamental Research Grant Scheme, Ministry of Higher Education Malaysia (203.CABR. 6711674).

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the financial support from Fundamental Research Grant Scheme, Ministry of Higher Education Malaysia.

SUPPLEMENTARY MATERIALS

1. Proteins detected in *C. striata* fraction FDWE3. Five proteins were detected. False discovery rate (FDR) was set at <0.1%.

2. Proteins detected in *C. striata* fraction SDWE1. Seventeen proteins were detected. False discovery rate (FDR) was set at <0.1%.

3. Proteins detected in *C. striata* fraction SDWE5. Eight proteins were detected. False discovery rate (FDR) was set at <0.1%.

4. Proteins detected in *C. striata* fraction SDWE6. Four proteins were detected. False discovery rate (FDR) was set at <0.1%.

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