Chapter

Haruan Extract (*Channa striatus*) as an Effective Mediator in Promoting Wound Healing

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Abstract

Wound healing remains a major issue in surgery. None of the existing treatment modalities in caring for wounds can yet claim to be the holy grail of wound management. *Channa striatus*, locally known in Malaysia as *Haruan*, is a freshwater air-breathing carnivorous fish that is proven to influence the different phases of wound healing. As a medicinal fish, not only does *Haruan* have a high content of amino and fatty acids, which are essential in collagen fibre synthesis during wound recovery, it also abounds in arachidonic acid and polyunsaturated fatty acids that promote prostaglandin synthesis, a vital component of the healing process. Moreover, its antinociceptive effects could potentially reduce wound pain, an important factor in wound healing. Proteomic studies show that a quarter of the total protein detected in freeze- and spray-dried *C. striatus* extract are actin, myosin and tropomyosin – all molecules that play a role in the wound healing process. Proteomic profiling also reveals that *Haruan* possesses two types of collagen namely collagen type-I and type-II that confer tensile strength during the healing process. It is proven that collagen along with other components of the extracellular matrix form the granulation tissue which, when contracted, closes the wound and concomitantly aligns the collagen fibres in the extracellular matrix. Hence, it is inferred that *Haruan* promotes the maturation of granulation tissue, thereby expediting the wound healing process itself. Consequently, it could mediate a faster recovery from surgical wound coupled with a lower incidence of wound infection due to an improved and accelerated wound healing process. Additionally, *Haruan* has demonstrated its ability in promoting angiogenesis and cell proliferation in wound bed preparation for skin grafting. Furthermore, a *Haruan* aerosol concentrate can act as a wound dressing at the donor site thereby enhancing the healing process while simultaneously exhibiting some antinociceptive properties. *Haruan*'s exceptional ability in promoting wound healing together with its potential use in skin grafting would be instrumental in the field of surgery. In essence, the cumulated benefits from all the processes involved would translate into a significant reduction of hospitalisation cost; that would immensely benefit not only the patient, but also the government.

Keywords: *Haruan*, *Channa striatus*, wound healing, proteomic studies, skin grafting, economic burden

1. Introduction

A wound is a mechanical injury to the body leading to disruption of the normal anatomical structure and function. It can be classified into acute and chronic wounds. Acute wounds normally proceed through the reparative process in an orderly and timely manner to restore anatomical and functional integrity. Conversely, wounds that demonstrate signs of delayed and interrupted healing and fail to go through the normal healing process are termed chronic wounds [1–3].

Wound healing reflects a cascade of complex, highly regulated biological events to restore the body's anatomical function back to its pre-injured state. Unlike acute wounds that heal by primary intention where the edges of the wound are apposed and held together with minimal scarring, chronic wounds heal by secondary intention [4, 5]; they form granulation tissue which fills the wound defects.

2. History of wound care and wound dressing

Wound management involves providing an optimum environment to promote healing, control bleeding and prevent infection. The history of wound care traces its origin to the Sumerians, a civilisation believed to be older than 2,000 BC [6]. In their manuscript, three healing gestures – cleansing the wounds, making the plasters and bandaging the wounds – were identified [7].

The ancient Egyptians and Greeks also contributed to the evolution of wound management. The Egyptian medical papyri documented the principle of wound closure to aid healing and the utilisation of honey, grease and lint as the main constituents of the most common plaster. It was believed that lint, a derivative of vegetable fibre, serves an absorbent role; grease or animal fat forms a barrier against bacteria; and honey, the most frequently cited ingredient in multiple topical wound preparations, possesses various healing and antibacterial properties favourable for wound healing [8, 9].

Interestingly, the Greeks were the first to recognise the difference between infected and uninfected wounds, using terms such as "fresh" or "non-healing" to describe wounds [10, 11]. Galen of Pergamum (120–201 AD) is a Greek surgeon who made remarkable contributions to wound and haemorrhage management. He emphasised the maintenance of wound moisture and the application of styptics consisting of basic elements with antibiotic properties for optimum wound healing [12, 13]. Despite advances in modern technology, Galen's basic principles are still incorporated into the development of current wound dressings.

Additionally, the Hippocratic collection discussed the addition of wine to obstinate ulcer for maximal wound healing [13]. Indeed, in ancient times, a number of magical and mythological agents were utilised as wound dressings; they include honey, plaster, wine and milk. While some of them demonstrate significant pharmacological roles, others merely have ritualistic meanings [13].

In the modern era, a wide array of dressings and wound care products with their properties tailored to special wound care needs were invented. In fact, Winter's study [10–13], which concluded that moisturised wounds heal quicker than dry wounds, sparked an explosive burst in the evolution of wound dressings. Thanks to modern technology, novel techniques such as the adoption of growth factors, bioengineered tissue, negative pressure therapy and hyperbaric oxygen therapy are nowadays implemented in wound management. Nonetheless, none of the existing modalities can claim to be the holy grail of wound management.

Alongside the cosmetic advancement in the past decades, skin grafting – a source of epithelium for both acute and chronic wounds – has become increasingly prevalent [14, 15]. However, quite surprisingly, skin grafting is not a new concept; for the past 3500 years, it has been extensively practised by a string of renowned physicians. These include Aulus Cornelius Celsus (25 BC - 50 AD), the Roman author of the first systematic treatise on Medicine; Claudius Gelenus (129 AD - 210 AD) popularly known as Galen, a prominent Greek physician; Jaques-Louis Reverdin (1842–1929), the Swiss surgeon who performed the first "fresh skin" allograft; and George David Pollock (1817–1897), a British surgeon known as a pioneer of skin grafts [16–24]. Throughout the years, the roles and functions of skin grafting have expanded. Nowadays, skin graft is an indispensable therapy in burn reconstruction, major traumatic injuries and surgical defects [25, 26]. Nonetheless, it still suffers from major drawbacks such as compromised skin grafts, skin graft rejection and skin graft contractions particularly in elderly patients, immunocompromised individuals and those on immunosuppressant medications [27–35].

Meanwhile, TIME – a concept that stands for Tissue, Infection or Inflammation, Moisture, and Epithelial edge advancement – is a new framework of wound bed preparation initiated by Schultz and his team in 2003 to achieve optimal wound healing [36, 37]. As the freshwater fish *Haruan* is naturally gifted with numerous antinociceptive and antimicrobial capabilities, high water content and ample amounts of amino acids and polyunsaturated fatty acids essential for granulation tissues formation and epithelialisation, it fits the components of Tissue, Infection and Moisture in the TIME framework. Therefore, we can postulate that *Haruan* fish also has the potential to function as an effective wound dressing.

3. Stages of wound healing

The phases of wound healing is a continuum that encompasses homeostasis, inflammatory, proliferative and maturation phases under stringent regulation of growth factors, cytokines, and chemokines [38]. Admittedly, the various phases of the wound healing process can overlap and go awry anytime. The inflammatory phase is the shortest of all phases and, if arrested, wound healing will be delayed and fibrosed tissue may be formed. Meanwhile, in the proliferative phase, the wound is shrunken in size until the maturation phase. Despite the surface of the wounds being closed completely, full tensile strength might take up to twelve months to develop [3, 4, 38–40]. **Table 1** below describes the different stages, mechanisms and molecules at interplay during the wound healing process.

Unfortunately, despite the enormous efforts made in skin repair, a wound can never achieve the maximum tensile strength of a normal skin. Additionally, owing to its tight regulation by a multitude of factors, proper wound healing can be easily impeded. Indeed, chronic non-healing wounds are a common phenomenon. **Figure 1** describes both the intrinsic and extrinsic factors that affect wound healing.

Stages	Mechanism	Main molecules
Homeostasis	• Vasoconstriction • Initiation of coagulation cascade • Formation of clot at the site of injury	• Complement prostaglandins • Vascular endothelial transforming growth factors • Nitric oxide • Cytokines • Platelets
Inflammatory	• Vasodilatation • Recruit and activation of neutrophils and macrophages for phagocytosis • Synthesis of wound exudate	• Cytokines • Growth factors • Neutrophils • Monocytes and macrophages
Proliferative	• Formation and proliferation of granulation tissues • Fibroblasts and collagen production • Formation of vascular network via angiogenesis • Contraction – contractile cells pull the wound margins together • Epithelialisation – growth of epidermal cells over the surface of the granulation tissue	• Fibroblasts • Macrophages • Collagen • Extracellular ground substance • Growth factors • Proteases • Epidermal cells
Maturation	• Fibroblasts reduce in number • Vascularisation decreases • Tensile strength increases	• Fibroblasts • Collagen • Matrix metalloproteinases (MMPs) and inhibitors

Table 1.
Stages of wound healing [3, 4, 38–40].

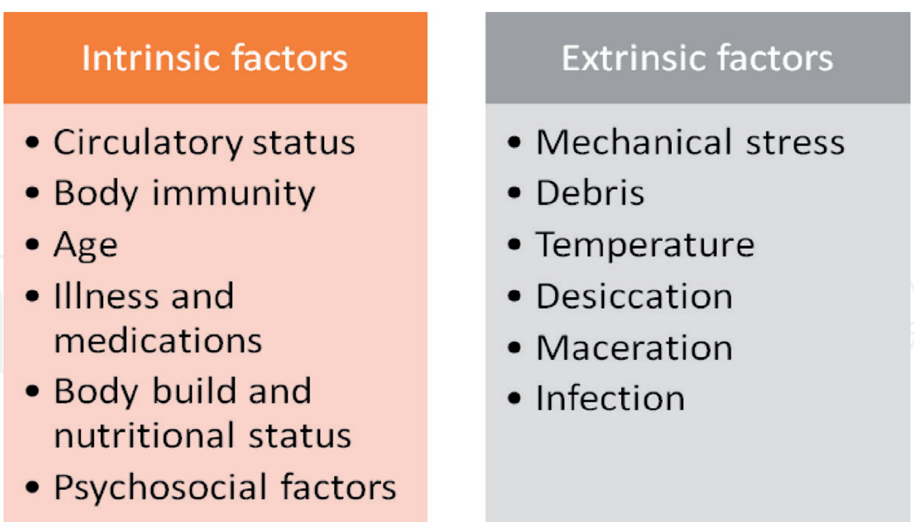

Figure 1.
Factors that affect wound healing.

4. What is *Haruan*

Channa striatus or snakehead murrel, commonly known as *Haruan* or *Gabus* in the Southeast Asian region, originates from the family *Channidae* or *Ophiocephalidae* [41]. It is a tropical, aerobic, carnivorous freshwater species measuring around 100 cm. It is sexually active at 30 cm, with a dark dorsal surface and sides, and mottled with a combination of black and ochre; it also has a white belly, a large head resembling a snake and a fully-toothed mouth with large scales. It preys on smaller fishes and frogs for survival. Since *C. striatus* is an obligate air-breather, it survives by burrowing in muds of lakes, rivers or canals, keeping its skin and air-breathing apparatus moist while surviving on stored fats [42, 43]. Wild *Haruan* lives a solitary life except in the spawning season. **Figure 2** shows a solitary *Haruan* in an aquarium with a glass reflection showing its white belly.

Figure 2.
Wild Haruan - Channa striatus - in an aquarium.

Pairs breed during most months of the year, laying hundreds of amber-coloured eggs. The eggs, guarded by both parents, are non-adhesive and they hatch within one to three days. The adults have compact muscles and a less bony structure which give them the desirable characteristics of a predatory fish [43]. Besides, they are highly aggressive predators with the ability to travel overland to exploit new bodies of water [42]. **Figure 3** describes the characteristics of *Channa striatus* or *Haruan*.

Figure 3.
Summary of the characteristics of Haruan species [41–43].

- Obligate air-breathing species
- Cavernous freshwater fish
- Common habitats: small ditches, ponds, rivers, lakes, rice fields
- Ideal water temperature for survival: around 20–30°C
- Depth of water: below two meters
- pH of habitat: 4.30 to 7.90

Haruan fish can be farmed though it is considered a pest or predatory species in Europe, North America and Australia, being a voracious predator and a competitor of native fish species [44, 45]. When it is reared in a controlled habitat, the parameters of the aquatic environment such as the pH, temperature and water depth should be kept as close to its natural habitat as possible. For its diet, *Haruan* fish can be fed with a wide range of food products including formulated food [46–48].

However, compared to other species such as *Keli* or *Tilapia* fish, *Haruan* fish farming is not popular for two reasons: firstly, being a predator, it can easily eat up the surrounding aquatic animals and small terrestrial rodents; secondly, its commercial benefits have not been extensively publicised to receive enough attention [49, 50].

In Malaysia, *Haruan* fish is cherished as a wholesome delicacy; it is served in a multitude of preparations ranging from steamed, grilled, spiced, fried, roasted, in the form of soup to even raw [51]. According to a study [52] conducted by Haemamalar and his team in Krau Wildlife Reserve, *Haruan* fish was reported to be one of the sources of freshwater fish among the Orang Asli (aboriginal people) tribunates.

Additionally, *Haruan* serves as a natural remedy for the local population. The National Health and Morbidity Survey carried out in 2014 [53], which looked at the prevalence of food supplements and the reasons for their intake, demonstrated that of the 0.68% of Malaysians consuming *Haruan* as a dietary supplement, 90.82% did so based on its alleged health benefits.

Thanks to the Chinese and Malay communities, *Haruan* has acquired a reputation for wound healing for the past several decades [54]. Poh *et al* did a research [55] involving a total of 134 Chinese mothers during the months of childbirth; they found out that *Haruan* fish was reported by a quarter of the participating women as either a necessary or a recommended food owing to its wound healing property [56]. Nonetheless, the wound healing effect of *Haruan* was merely anecdotal until two recently published clinical trials [57, 58] scientifically confirmed this common belief.

5. Preparation of *Haruan*

5.1 Cooking

Different cooking methods of *Haruan* fish can generate different outcomes. For instance, *Haruan* fish fillets preserve their nutritional value when grilled but absorb too much oil when fried, which can be detrimental to health [59]. Meanwhile, when prepared in soup, the time and heat utilised have to be properly adjusted for the snakehead fish to retain its nutritional value [60].

5.2 Topical agent

Haruan fish can also be converted into a topical agent in the form of spray or cream. This preparation involves the addition of a propellant (spray) or aqua cream (cream) to the *Haruan* extract [61, 62]. When *Haruan* is formulated into aerosol concentrate and sprayed on a wound, it will form a thin layer of dressing that acts as a protective barrier against the outside environment [63]. This minimises the physical pain as well as the mental suffering associated with dressing application and removal [64].

5.3 Haruan capsules

The principal author of this chapter worked collaboratively with the School of Pharmacy, Universiti Sains Malaysia, to process *Haruan* capsules for his research work together with fellow surgeons at the National Heart Institute, Kuala Lumpur, several years ago. Admittedly, oral *Haruan* supplement has a higher amount of concentrate which is believed to yield more merits compared to eating the flesh itself. Besides, for surgical or major traumatic wounds that involve multiple tissue layers, oral administration of *Haruan* extract is deemed superior to topical application [65, 66]. The detailed steps in the preparation of *Haruan* capsules are described in **Figure 4**.

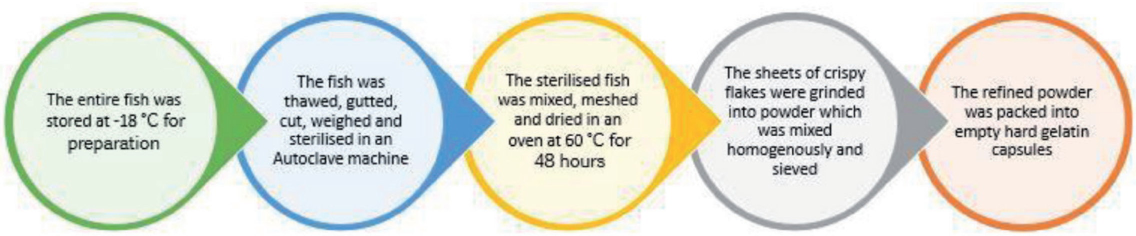

Figure 4.
Preparation of Haruan capsules [57].

6. Laboratory works

6.1 Chemical properties

Haruan is considered a crucial source of protein (78.32 ± 0.23%), lipid (2.08 ± 0.08%) and vitamin A (0.265 ± 0.013 mg) [49, 67, 68]. A proximate analysis of *Haruan* revealed that the ratio of crude protein, crude fat to crude ash in *Haruan* is 23:5.7:1.8 [67, 68]. In fact, *Haruan* is rich in amino acids and fatty acids, particularly glycine and arginine, which help minimise protein losses and enhance collagen synthesis essential for wound healing [56, 69, 70].

Interestingly, *Haruan* also synthesises polyunsaturated fatty acids, which accelerate wound healing via the mediation of prostaglandin and thromboxane synthesis [49, 68, 71–73]. Furthermore, apart from the major fatty acids such as stearic acid and linoleic acid [56, 74], *Haruan* possesses an unusually high profile of arachidonic acid (AA) and docosahexaenoic acid (DHA) which lower the risk of coronary artery disease [56, 67, 68, 75–77]. In terms of dietary nutritional elements, micronutrients such as magnesium, copper, zinc, iron, calcium and manganese and trace amounts of nickel and lead are also present in *Haruan* [78–80].

6.2 Antimicrobial effects

Exposed to an aquatic environment full of microbiota, fish usually develop their own immunity to safeguard against pathogens [81–83]. As a front-liner and paramount component of the innate immune system, fish mucus possesses a broad array of proteins and enzymes such as lysozyme, immunoglobulin, complement proteins, lectins and proteolytic enzymes that can phagocytose and digest microorganisms [84–90]. Furthermore, it constantly secretes and sloughs off the skin to avoid adherence and prolonged colonisation by parasites [84–89]. Hence, fish skin mucus is regarded as a potential antibacterial therapeutic agent [91, 92]. The multiple roles of *Haruan* mucus are described in **Figure 5**.

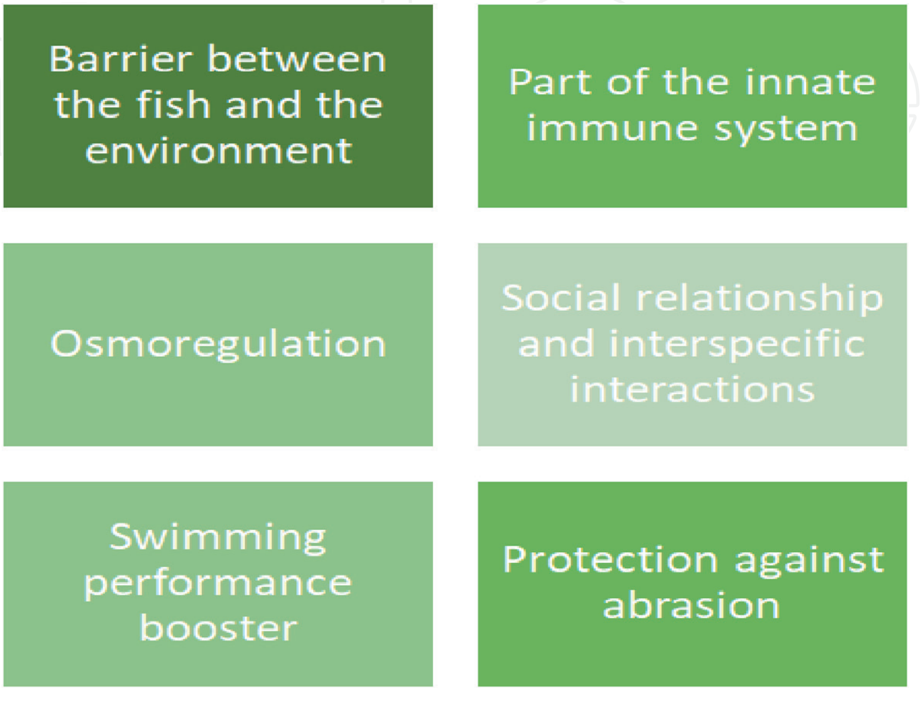

Figure 5.
Roles of the fish mucus [91, 92].

In recent years, extensive work has been conducted to analyse the antibacterial effects of the mucus of fish species [93–97], including the *Channa* species. Several research studies [48, 98–104] were performed over the years to evaluate the antibacterial and antifungal activities of *Haruan*. Most of the studies [99–104] revealed that *Haruan* displays some antimicrobial activities, except two studies [98, 99] which detected negligible inhibitory effects against *Staphylococcus aureus* and *Escherichia coli* strains respectively. As wound infection and dehiscence – two disastrous yet frequent complications of surgical wounds – are the common factors of delayed wound healing, the antimicrobial activity of *Haruan* is an added merit for wound dressing [105–107].

6.3 Antinociceptive properties

Pain can have a deleterious impact on wound healing [108, 109]. Coupled with chronic inflammation, prolonged pain can trigger a vicious cycle that hinders wound healing [110]. Fortunately, appropriate wound dressings with sufficient pain control can enormously improve wound healing outcomes, with accelerated wound healing and, consequently, a shorter hospital stay [111].

The antinociceptive effects of *Haruan* in postoperative and traumatic patients have long been discussed and reported. For instance, an earlier *Haruan* study [112] conducted abdominal constriction and tail flick test on mice and found that *Haruan* not only possesses peripherally-acting antinociceptive activity, but its extracts could also act synergistically with other painkillers such as morphine to relieve postoperative pain and discomfort. These findings are supported by another study conducted by Solihah *et al* [113] who presented a similar positive result. The underlying mechanism is thought to be attributed to the presence of fatty acids and amino acids, particularly arginine, glycine and arachidonic acid, in addition to the involvement of the L-arginine-nitric oxide-cGMP pathway [42, 114]. In fact, the antinociceptive effects *Haruan* remain relatively stable in a wide range of temperature and pH; this allows the essence to be extracted and processed safely for future use [115].

6.4 Wound healing capabilities

Freshwater fish constitute 60–70% of the animal protein intake in Malaysia [116]. Previous studies demonstrated that *Haruan* contains a high content of albumin which promotes the formation of collagen [117–119]. Moreover, it has a considerable amount of copper and zinc that can help accelerate wound healing by maintaining cell stability, besides promoting wound remodelling and the formation of blood vessels and fibrosis or scar [119–124]. Additionally, the presence of elements such as hydroxyproline, glycine, arachidonic acid and arginine in *Haruan* is another essential source of collagen [124–126].

It is therefore no surprise that the collagen content in *Haruan* is relatively high. Indeed, collagen plays a determining role in expediting wound healing via various mechanisms. According to Kwan *et al* who performed proteometric profiling of *Haruan*, two types of collagen, namely type I and type II collagen, were detected [127, 128]. Both of them increase the tensile strength [129]. In fact, when the increased tensile strength and glycosaminoglycan of *Haruan* was examined and compared to cetrimide, it was reported that *Haruan* is superior to cetrimide in improving wound contraction and the fibroblastic phase of wound healing [61, 130, 131]. Furthermore, it was found that the collagen in *Haruan* can help promote the maturation of granulation tissue which accelerates wound healing. *Haruan* can act in synchrony with the other components of the extracellular matrix to form a granulation tissue which subsequently contracts and seals the wound. Concurrently, it aligns the collagen fibres in the extracellular matrix. If the area of involvement is wide enough, the granulation tissue could be reserved for split skin grafting. The various steps of collagen involvement in wound healing are described in **Figure 6**.

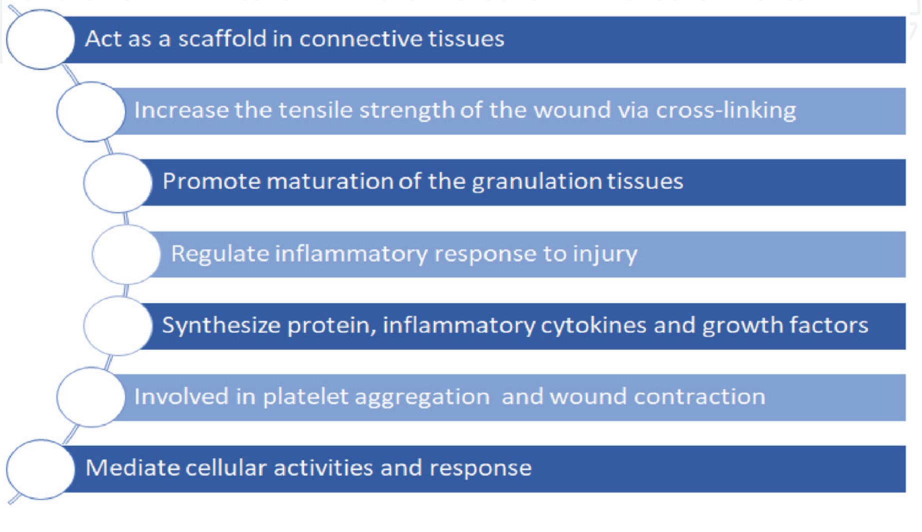

Figure 6.
Wound healing effects of collagen [132–135].

According to the researchers [128] who performed proteomic profiling of *Haruan* extract at the Analytical Biochemistry Research Centre, Universiti Sains Malaysia, the proportion of actin, myosin and tropomyosin to the total protein in freeze-dried and spray-dried *Haruan* water extract are 25% and 26% respectively. While it is known that these three structures play a significant role in muscle contraction in the sliding filament theory, many were unaware that that they also work hand-in-hand in the wound healing process. Tropomyosin can help control cell functioning of actin while actin can regulate vital cellular functions during re-epithelisation involving cell division, cytokinesis and cell signalling, seal the embryonic wound as well as interact with myosin in the regulation of cell motility [132–134]. The functions of actin, myosin, and tropomyosin during the wound healing process are diagrammatically summarised in **Figure 7**.

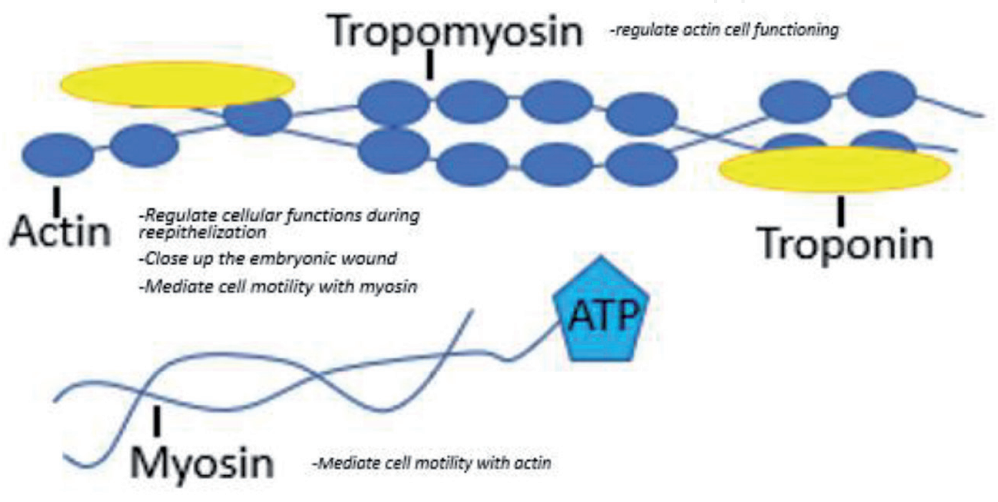

Figure 7.
Functions of actin, myosin, and tropomyosin in wound healing [57, 128].

7. Clinical trials on *Haruan*

To the best of our knowledge, only two clinical trials have reported the effects of *Haruan* in wound healing to date.

One study [57] was conducted by the principal author of this chapter. The researchers performed a double-blinded, randomised, controlled trial in 2018 at the National Heart Institute to look at the effects of *Haruan* fish extracts on the chest and leg surgical wounds of 253 patients after they have undergone coronary artery bypass surgery (CABG). Noting the detrimental impact of wound pain on wound healing and the interplay between wound pain, morbidity, quality of life and hospital stay [135–137], parameters such as wound pain and healing, mobilisation and quality of life were specifically evaluated. It was found that the wound scoring system favoured those patients who received *Haruan* capsules instead of placebo at day-6, six-weeks and three months postoperatively. They also discovered that *Haruan* extracts could alleviate wound pain, and improve quality of life with respect to energy, pain, emotion, sleep and physical level except social level based on the Nottingham Health Profile questionnaire that assesses the quality of life [57]. Therefore, *Haruan* extract is considered a cost-effective solution in wound healing because it decreases the percentage of wound infection thereby reducing the cost of hospitalisation. Moreover, with the patient's surgical recovery hastened, it can tremendously reduce the economic burden not only to the hospital but to the country as well.

Meanwhile, a similar study [58] conducted by Wahab *et al* targeted 76 post-lower segment Caesarean Section women, a study population which are the dominant consumers of *Haruan* in Malaysia due to old beliefs and food taboos [55, 56]. The study concluded that *Haruan* could improve the wound's cosmetic appearance and, accordingly, patient's satisfaction. These findings are in line with a randomised controlled trial [138] which reported that *Haruan* extract consumers harboured a remarkably higher rate of uterine involution than the placebo group. Conversely, in the same study, the outcomes of wound healing and pain were noted to be comparable; this can be attributed to the interval used for the pain score assessment and the concomitant use of analgesics.

8. Potential role of *Haruan* in skin grafting

Skin grafting is the transfer of cutaneous tissue to cover large wounds. It can take two forms: split skin grafting, which involves the epidermis and a portion of the dermis, and full-thickness skin grafting which involves the epidermis and the entire dermis [139]. While deliberating on the pros and cons of split skin grafting as compared to full thickness skin grafting is beyond the scope of this paper, it is noteworthy that a split skin graft does not have its own blood supply; it relies on the wound bed. This is where *Haruan* might play an important role. In their seminal work on the bioactive proteins in *C. striatus*, Kwan *et al* [140] have shown that the fish proteins promote angiogenesis and cell proliferation. The stable, healthy and well-vascularised wound bed potentiated by *Haruan* action allows for skin grafts to be well taken.

The main challenge to ensure that *Haruan* play its magical role in promoting angiogenesis and cell proliferation lies in the wound bed preparation before skin grafting. To ensure that the wound bed is healthy, wound debridement is of utmost importance. This could be done in several ways: via a scalpel, a dermatome, or even by using a hydro-surgery device until the wound bed is really clean and healthy with some bleeding at the wound bed [141]. It has to be stressed here that without a clean and healthy wound bed, and wound edges cleared from any necrotic or purulent tissue, the added value of *Haruan* would be lost and the skin graft will not have a proper healing.

When the wound bed is well prepared, skin grafts will normally go through three different stages as follows:

a. Imbibition: Oxygen and nutrients from the wound bed are passively absorbed by the skin graft [142].

b. Inosculation: A vascular connection is established between the cut vessels on the underside of the skin graft and the wound bed [143].

c. Revascularisation: Neoangiogenesis or new blood vessels grow into the graft from the wound bed [144].

After undergoing these stages, the skin grafts will usually need another five to seven days to adhere to the wound bed followed by the process of maturation that could last from several months to years; this includes pigmentation changes, softening and flattening [139]. As described earlier, *Haruan* abounds in amino acids in such as glycine, lysine and arginine, and fatty acids such as arachidonic acid, palmitic acid and docosahexaenoic acid – they all help to enhance wound healing through the initiation of several pathways including the remodelling of collagen that gives the strength to the wound, besides stimulating wound contraction [145].

Having discussed at length regarding the recipient site, we should also look at whether *Haruan* plays any role in wound healing at the donor site. Theoretically, the donor site requires wound care only in terms of wound dressing. Since the wound edges are not approximated at the donor site and are left to heal via secondary intention, they will be filled by granulation tissue matrix [146] and can be covered by a simple dressing only. Presumably this is another area in skin grafting where *Haruan* dressing might play a role. An aerosol concentrate containing *Haruan* water extract was formulated in an aerosol system to produce a thin film over the wound bed and serve as a dressing at the donor site [147]. The aerosol concentrate that would form a thin layer of dressing over the wound could enhance the healing process at the donor site as proven in an animal model [148], besides showing pronounced antinociceptive properties [149].

9. Other usages of *Haruan*

Apart from its aforementioned desirable features, *Haruan* has also been reported to confer feasible outcomes in a myriad of diseases.

Osteoarthritis is a degenerative joint disease characterised by synovial inflammation and articular cartilage degradation that leads to chronic pain and inflammation [150, 151]. In osteoarthritis, a wide variety of inflammatory mediators are secreted and activated [140]. After several previous studies which demonstrated the anti-inflammatory capabilities of *Haruan*, the role of *Haruan* in osteoarthritis has been extensively explored [152–154]. Few scientific reports on the efficacy of *Haruan* in osteoarthritic patients revealed promising outcomes where *Haruan* was shown to be superior in reducing inflammatory changes in the synovial membrane, improving the pain, symptoms and quality of life of osteoarthritic sufferers while maintaining the structure of the cartilage of the control group [155–159]. As osteoarthritis is a common complication of major traumatic wound injury, which necessitates skin grafting owing to the disfigurement and disabling condition, oral administration of *Haruan* can exert a double action, improving both wound healing and osteoarthritis.

Other functions of *Haruan* mentioned in the medical literature include its usage in allergic rhinitis [160, 161], dermatitis [162, 163], gastric ulcer [164, 165], cancer [166, 167], hypertension [168, 169] and depression [170, 171]. Unfortunately, due to the paucity of studies to date, further high-powered studies are warranted to clarify and define the role of *Haruan* in these diseases.

10. Discussion

Hong *et al* [65] did a scoping review on the effectiveness of *Haruan* extracts on wound healing; they concluded that current evidence favours the use of *Haruan* extracts to expedite wound healing. Indeed, optimal wound bed preparation and proper wound closure are the two fundamental goals of skin grafting regardless of the graft type [172–174]. With its extraordinary antimicrobial, antinociceptive and anti-inflammatory properties, *Haruan* is undeniably a handy tool for skin grafting. From a psychological perspective, *Haruan* can minimise post-operative pain and discomfort, achieve satisfactory aesthetic wound effect and improve patient postoperative quality of life. For skin grafting that covers a wound area only partially, *Haruan* can promote wound closure since it encourages the epithelialisation of wound. When a wound recovery is sped up with less wound infection, the duration of hospital stay will also be shortened. Consequently, expenses related to skin grafting will be cut down.

It is still inconclusive which particular biomolecules play a role in the wound healing property. However, with the advancement of technology especially in the field of proteomics, we have managed to conduct a more comprehensive protein profiling [175, 176]. Although proteomics helps us to understand the interactions between the proteins in the fish and the wound, the previous protein profiling [177] were not as accurate as the new one due to the lower sensitivity of the old equipment. Conversely, the current work using Gel Elution Liquid Fractionation Entrapment Electrophoresis (GELFREE) system can maximise protein profiling [127]. The researchers at the Analytical Biochemistry Research Centre of the Universiti Sains Malaysia [127, 128] also looked at the post-translational modifications (PTMs) of proteins which might be involved in the wound healing process to complement the protein profiling results. PTMs, as the name suggests, occurs following the translation of amino acids in the later part of the protein biosynthesis. They play an important role in protein regulation and are also involved in the regulation of a number of physiological functions. This helps us to appreciate how the consumption of *Haruan* contributes to the wound healing mechanism.

It is a known fact that structural proteins such as actin, myosin and tropomyosin are vital in the formation of muscle tissue within an organism. From the protein profiling, it was shown that 37% of all the proteins detected in the fish meat are structural proteins which play a specific role in enhancing wound healing. For example, actin gives rise to the formation of myofibroblasts which differentiated from fibroblasts containing bundles of actin microfilaments with contractile proteins such as non-muscle myosin [178–180]. On the one hand, both fibroblasts and myofibroblasts regulate traction force and coordinate contraction during wound closure [181]. On the other hand, tropomyosin, has been reported to regulate cell migration, particularly fibroblast and myofibroblasts [182]. This results in the promotion of rapid wound healing whenever tropomyosin is manipulated in the wound area [183, 184]. Hence, the abundant presence of structural proteins in the fish meat could be a key reason why it helps in the wound healing process.

Apart from structural proteins, *Haruan* meat also possesses numerous enzymes including trypsin. Trypsin has been shown to enhance the healing process by potentiating fibrocyte differentiation [185]. Trypsin has also been used as a biomedicine for treating wound [186]. A clinical study conducted by Gudmunssdsdóttir *et al* [175] showed that native-proteins were digested by cold-adapted cod trypsin and produced an encouraging effect on the wound. These findings supported the idea that the abundant level of trypsin in *Haruan* meat helps in facilitating the wound healing process.

Collagen, which is essential for wound healing, is also present in the *Channa striatus* meat with Collagen Type-I being the most abundant [127]. Collagen is required in the different stages of wound healing including the binding process to fibronectin that helps in platelet aggregation [187], triggering angiogenesis by transforming myocytes into macrophages [188], in addition to giving support to budding capillaries [189]. A recent study by Helary *et al* [190] has also shown that apoptosis was prevented during chronic wound treatment by the use of concentrated collagen hydrogel that promotes cell proliferation and protects fibroblasts. Recently, mammalian collagen has been replaced by fish collagen [191] which is considered a regenerative medicine [192], a sign that the abundant collagen found in fish meat does help to advance the wound healing process.

Results from the proteomic study [127] also show that *C. striatus* meat is rich in calcium related proteins such as calmodulin and parvalbumin. We are aware that calcium (Ca^{2+}) plays a major role in maintaining homeostasis of the skin and is considered a key signalling molecule during wound healing [193, 194]. Ca^{2+} binding proteins are also known to assist in Ca^{2+} signalling and skin intracellular trafficking, which includes calmodulin and calmodulin-like proteins [195]. It is also known that

Calmodulin assists in keratinocytes maturation [196], proving its significant role in the wound healing process. The important role played by both calmodulin and parvalbumin in the wound healing cascade deserves to be highlighted. Expression of parvalbumin in ependymal cells has been shown to assist in tissue remodelling and wound closure [197]. Hence, it is clear that both parvalbumin and calmodulin help to transfer Ca2+ to the affected area, thereby promoting wound healing.

Proteomic profiling also revealed that more than 50% of the total proteins detected in *C. striatus* are uncharacterised proteins [128]. The functions of these proteins are still unknown due to the paucity of research. These proteins have been labelled as such due to the absence of any detectable homology to those proteins of known functions at both the sequence and structural level [198]. However, it is possible that one or more of the uncharacterised proteins found in *Haruan* play a role in the wound healing process. Indeed, the high quantity of uncharacterised proteins detected via proteomics, that is, the proteome database for *C. striatus*, is far from complete. At this point in time, we can safely say that while existing data have given us an insight into the proteins of *Haruan,* more rigorous effort must be made into the research of the uncharacterised proteins that might be involved in accelerating the wound healing process – the indisputable characteristic of *C. striatus* or *Haruan.*

11. Conclusion

As a wound cosmetic enhancer as well as an antimicrobial, anti-inflammatory and antinociceptive agent, *Haruan* fish is a promising medicinal food product for wound healing. Current evidence has illustrated the effectiveness of *Haruan* in wound healing, particularly in postoperative patients. This book chapter has highlighted the wonders of *Haruan* in wound healing associated with skin grafting. Unfortunately, in spite of the emerging role and increasing popularity of *Haruan* in wound healing, the use of *Haruan* extracts in skin grafting remains insufficient. When its merits have been fully explored, *Haruan* extracts could become a viable alternative to the current wound dressing regimen in skin grafting in the near future.

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