**Borneo Journal of Pharmacy**
Vol 8 Issue 1 February 2025
Pages 95 – 106

*https://journal.umpr.ac.id/index.php/bjop/article/view/8182*
DOI: https://doi.org/10.33084/bjop.v8i1.8182
e-ISSN: 2621-4814

*Research Article*

# Analysis of Amino Acid Profiles in Bone, Oil, and Muscle Tissue and Wound Healing Activity of *Pangasius hypophthalmus* Oil in *Rattus norvegicus*

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**Keywords**:
Bone fish
Muscle Tissue
*Pangasius hypophthalmus*
Oil fish
Wound healing

## Abstract

Patin (*Pangasius hypophthalmus*), a commercially important freshwater fish, is a rich source of protein containing both essential and non-essential amino acids, crucial for various biological functions. This study aimed to determine the amino acid composition of muscle tissue, oil, and bones of *P. hypophthalmus* and evaluate the wound healing potential of its oil extract in *Rattus norvegicus*. High-Performance Liquid Chromatography was employed to quantify the levels of essential and non-essential amino acids in each tissue. Results revealed that *P. hypophthalmus* oil exhibited the highest total amino acid content, particularly rich in histidine, arginine, and lysine. The wound healing activity of *P. hypophthalmus* oil extract was assessed in a rodent model, demonstrating a significant reduction in wound area (0.62-0.84 mm) and an impressive wound healing percentage (79-84.45%). These findings suggest that different parts of *P. hypophthalmus* possess valuable nutritional and therapeutic properties. Notably, the high amino acid content of *P. hypophthalmus* oil, particularly essential amino acids, highlights its potential as a promising source for developing pharmaceutical products, including amino acid supplements, wound healing agents, and formulations for metabolic support. Further research is warranted to fully explore the therapeutic potential of *P. hypophthalmus* oil and its bioactive components.

*Received*: September 10th, 2024
*1st Revised*: January 15th, 2025
*Accepted*: January 30th, 2025
*Published*: February 28th, 2025



## INTRODUCTION

The past few decades have witnessed a substantial surge in the utilization of natural-based pharmaceutical products, a trend largely driven by increasing public awareness regarding their perceived safety, efficacy, and the availability of environmentally sustainable raw materials compared to synthetic alternatives[1]. Among these natural sources, fish oil, plant extracts, and bioactive compounds derived from animals have garnered significant scientific interest due to their diverse therapeutic potential[2]. For instance, fish oil is a well-established source of omega-3 polyunsaturated fatty acids, crucial for cardiovascular health and neurological function. Furthermore, emerging research highlights the significant role of other animal-derived bioactive compounds, including proteins, peptides, and amino acids, in processes such as wound healing and tissue regeneration[3].

**How to cite:** Hidayaturrahmah, Suprayogi A, Hanif N, Roosita K, Darusman HS, Aprilyana L. Analysis of Amino Acid Profiles in Bone, Oil, and Muscle Tissue and Wound Healing Activity of Pangasius hypophthalmus Oil in Rattus norvegicus. Borneo J Pharm. 2025;8(1):95-106. doi:10.33084/bjop.v8i1.8182

Within the exploration of natural resources, *Pangasius hypophthalmus* or *patin* in Indonesian, a prevalent freshwater fish species in Southeast Asia, presents a promising yet underexplored source of bioactive compounds[4]. While *P. hypophthalmus* is recognized for its high-quality protein, healthy fats, and essential mineral content, comprehensive research into the pharmaceutical potential of its various components—including bones, oil, and flesh—remains limited[5]. Notably, significant portions of the fish, often considered by-products, are currently underutilized. Innovative approaches to extracting and characterizing bioactive compounds from *P. hypophthalmus* could pave the way for the development of more accessible and sustainable pharmaceutical products[6].

Although the wound-healing properties of fish oil from various sources have been acknowledged, specific investigations focusing on *P. hypophthalmus* oil within a pharmaceutical context are notably scarce[7]. A significant challenge in this research area is the current lack of standardized data concerning the amino acid profiles of different *P. hypophthalmus* components (bones, oil, and flesh). Such detailed profiles are essential for identifying specific bioactive compounds that could contribute to biological activities relevant to pharmaceutical applications, such as enhanced wound healing, inflammation modulation, and tissue regeneration[8].

Wound healing is a complex physiological process involving a dynamic interplay of epithelial cells, the extracellular matrix, and various growth factors. Amino acids, as fundamental building blocks of proteins, play a critical role in all phases of this process, encompassing the inflammatory, proliferative, and remodeling stages. Beyond their structural function, specific amino acids regulate key molecular mechanisms. For example, arginine supports fibroblast proliferation, enhances collagen synthesis, and promotes nitric oxide production, which aids in vasodilation and tissue repair[9,10]. Similarly, the non-essential amino acid (NEAA) glutamine exhibits significant immunomodulatory properties and can accelerate wound healing by promoting the proliferation of epithelial cells and lymphocytes[11]. Furthermore, methionine has been shown to facilitate tissue regeneration through its involvement in the synthesis of sulfur-containing compounds crucial for DNA methylation and repair processes[12].

Conventional pharmaceutical approaches to wound healing, while established, are often associated with significant costs and the potential for adverse side effects, prompting a growing interest in natural-based alternatives. These natural compounds are increasingly being investigated for their potentially safer, more affordable, and efficacious properties in promoting tissue repair and regeneration. This shift towards exploring nature-derived therapeutics stems from a recognition of their complex bioactive constituents, which may offer multifaceted mechanisms of action that address various aspects of the wound healing process[13]. In this context, the utilization of fish oil including from *P. hypophthalmus* as an active ingredient to support tissue regeneration and accelerate wound healing holds significant relevance.

To optimize these natural-based approaches, a promising strategy involves a comprehensive investigation of all components of *P. hypophthalmus*—from bones to oil and flesh—to fully explore their potential bioactive compounds. *Pangasius hypophthalmus* oil, for instance, is known to contain high levels of essential omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)[14]. Moreover, proteins derived from *P. hypophthalmus* are a potential source of bioactive peptides with antioxidant, anti-inflammatory, and wound-healing promoting activities[15]. A holistic research approach integrating these components could maximize the pharmaceutical innovation potential of *P. hypophthalmus*[16].

In the context of pharmaceutical research, establishing a robust literature framework is crucial for formulating sound hypotheses. Previous studies have indicated that fish oil from various species exhibits anti-inflammatory properties and can enhance fibroblast proliferation during wound healing[17]. For example, Huang *et al.*[18] reported that omega-3 fatty acids present in fish oil can effectively reduce local inflammation and accelerate granulation tissue formation in rat wound models. However, specific literature focusing on *P. hypophthalmus* in this context remains limited, highlighting a significant research gap that warrants investigation.

This study aims to address this gap by comprehensively analyzing the amino acid profiles of *P. hypophthalmus* bones, oil, and flesh, and by evaluating the effects of *P. hypophthalmus* oil on wound healing in a rat (*Rattus norvegicus*) model. The novelty of this research lies in its integrated approach, encompassing detailed characterization of bioactive compounds from multiple *P. hypophthalmus* components and clinically relevant preclinical pharmacological testing. By combining laboratory analysis with *in vivo* experimentation, this study seeks to provide a foundation for the development of innovative, safe, effective, and sustainable pharmaceutical products derived from *P. hypophthalmus*.

## MATERIALS AND METHODS

### *Materials*

Specimens of *P. hypophthalmus* were randomly sampled from the Riam Kanan Reservoir, South Kalimantan, Indonesia, for this study. The species identity was confirmed at the Basic Laboratory of Universitas Lambung Mangkurat (Certification Number: 293/LB.LABDASAR/XI/2024). To ensure representative sampling of the entire organism, thirty individual fish were utilized to obtain bone, oil, and muscle tissue samples. The laboratory equipment employed in this research included a rotary evaporator for solvent removal, an analytical balance for precise mass measurements, a laboratory grinder for tissue homogenization, and a high-performance liquid chromatography (HPLC) system equipped with a C18 column and a UV detector set at 254 nm for compound analysis.

### *Methods*

*Sample collection*

Fresh *P. hypophthalmus* specimens were initially cleaned to remove any external debris and then thinly sliced to facilitate subsequent processing. To prepare the fish for oil extraction, the sliced samples were immersed in water and boiled for 30 minutes at a controlled temperature of 60°C. Crude oil was then recovered from the fish muscle tissue using a modified wet rendering procedure. This involved mechanical separation of the oil through centrifugation at 10,000 rpm under refrigerated conditions (4°C). The extracted crude oil was carefully collected and stored under appropriate conditions until further analysis. Notably, the oil extraction process was conducted at 60°C without any additional purification steps to maintain the crude oil's native composition.

In addition to oil extraction, bone samples were also processed. Following the removal of muscle tissue, the bones were thoroughly washed to eliminate any residual muscle fragments. The cleaned muscle and bone samples were then subjected to lyophilization (freeze-drying) to remove moisture, followed by pulverization into a fine powder for subsequent analyses.

*Amino acid analysis*

Amino acid quantification was performed using a HITACHI 7-LT-1.0 system (K.LP-04). The analytical procedure involved a standardized protocol encompassing sample preparation, amino acid extraction via acid hydrolysis, HPLC injection, and subsequent identification and quantification against certified reference standards. Specifically, bone, oil, and fish muscle samples underwent hydrolysis in 6 N HCl at 110°C for 24 hours. Following hydrolysis, the resulting solutions were filtered to remove particulate matter and then concentrated to approximately one-third of their original volume using a rotary evaporator. The extracted amino acids were then separated using an Agilent 1200 series HPLC system equipped with a C18 column. Detection of the separated amino acids was achieved through UV absorbance at a wavelength of 254 nm. Identification of individual amino acids in the test samples was based on the comparison of their retention times with those of authentic amino acid standards. Quantification was subsequently performed by integrating the peak areas and referencing them against calibration curves generated from the standards.

*Wound activity test*

This research was conducted following ethical approval obtained from the Research Ethics Committee of the Faculty of Veterinary Medicine and Biomedical Sciences, IPB University, under the reference number KET: 147/KEH/SKE/XII/2023. To evaluate the wound healing activity of *P. hypophthalmus* oil extract, an *in vivo* study was performed using a Complete Randomized Design (CRD). Forty-two male *R. norvegicus* were randomly assigned to one of seven treatment groups (n=6 per group): a positive control treated with Betadine® ointment, a negative control receiving no treatment, four experimental groups treated topically with *P. hypophthalmus* oil extract at varying concentrations (A: 100% *P. hypophthalmus* oil extract; B: 5% *P. hypophthalmus* oil extract in Tween 20; C: 10% *P. hypophthalmus* oil extract in Tween 20; D: 15% *P. hypophthalmus* oil extract in Tween 20), and a vehicle control group (E) treated with Tween 20 alone.

*Incision making and wound care*

Prior to surgical intervention, each rat was anesthetized via ether inhalation. Following the establishment of anesthesia, the dorsal fur of each animal was meticulously shaved after swabbing the area with 70% ethanol using sterile cotton swabs to ensure aseptic conditions. The shaved rats were then allowed a 48-hour acclimation period to minimize stress-induced

variables. Subsequently, a standardized full-thickness excisional wound was created on the dorsal surface of each rat using a sterile scalpel. Each wound measured 2 cm in length and 2 mm in depth. Immediately post-incision, the wound sites were gently cleansed with a sterile 0.9% sodium chloride solution[19]. The topical application of *P. hypophthalmus* oil extract was performed once daily for a duration of seven consecutive days. This treatment was administered directly to the wound area using a 1 mL syringe without a needle[20]. Macroscopic observations of the wound healing process were conducted and documented on days 1, 3, 5, and 7 post-wounding[21].

*Wound measurement and observation*

To quantify wound healing, each incision on the experimental rats was individually photographed using a standardized, pre-fabricated photographic frame to ensure consistent image capture. The resulting digital images were subsequently analyzed using the Macbiophotonic ImageJ software. Measurements of the wound area, initially obtained in centimeters, were then converted to millimeters for uniformity and precision in subsequent calculations. The percentage of wound healing (P%) was determined using the following **Equation 1**, where d0 represents the initial wound area on day 0, and dx represents the wound area on the specific day of measurement.

$$P\% = \frac{d0 - dx}{d0} \times 100 \quad [1]$$

### *Data analysis*

Wound area measurements in rats were obtained using Macbiophotonic ImageJ. Prior to statistical analysis, the data from the wound healing activity assays underwent Shapiro-Wilk and Levene's tests to assess normality and homogeneity of variance, respectively. Following confirmation of these assumptions, a one-way analysis of variance (ANOVA) was performed to determine significant differences between treatment groups. In cases where the ANOVA revealed statistically significant variations ($p < 0.05$), post-hoc Duncan's multiple range tests were conducted to identify specific pairwise differences between the treatment groups.

## RESULTS AND DISCUSSION

### *Amino acid profile description*

Amino acid analysis of *P. hypophthalmus* muscle tissue, oil extract, and bone revealed distinct concentrations of both essential amino acids (EAAs) and NEAAs across these different anatomical compartments within the same fish. The specific EAA profiles identified in the muscle tissue, oil extract, and bone are visually presented in **Figures 1**, **2**, and **3**, respectively. Notably, the amino acid profile of the fish oil exhibited a remarkably high concentration of histidine (410.58% w/w) relative to its other EAA components, followed by lysine (137.75% w/w) and methionine (80.81% w/w) (**Table I**). In contrast, the concentrations of individual EAAs in both muscle tissue and bone were considerably lower. Regarding NEAAs (**Table II**), *P. hypophthalmus* oil displayed the highest concentration of arginine (332.48% w/w) compared to muscle tissue and bone. Muscle tissue, however, exhibited the highest glutamate concentration (11.91% w/w) among the NEAAs. In contrast, glycine (4.22% w/w) was the most abundant NEAA identified in *P. hypophthalmus* bone. These findings underscore the differential distribution of amino acids, both essential and non-essential, within various tissues of *P. hypophthalmus*, suggesting potential functional roles associated with these distinct compositional profiles.

Quantitative analysis of the amino acid composition, detailed in **Table III** revealed a markedly elevated concentration of both EAAs and NEAAs in the *P. hypophthalmus* oil extract compared to its muscle and bone tissues. Specifically, the oil exhibited a substantial EAA concentration of 695.15% w/w and a NEAA concentration of 355.26% w/w. In contrast, the muscle tissue presented significantly lower concentrations, with EAAs at 26.25% w/w and NEAAs at 40.1% w/w. The bone tissue demonstrated the lowest amino acid content among the analyzed samples, registering EAA and NEAA concentrations of 8.95% w/w and 18.37% w/w, respectively. These findings unequivocally indicate that the *P. hypophthalmus* oil extract possesses a considerably higher overall amino acid content compared to the muscle and bone tissues of the same organism.

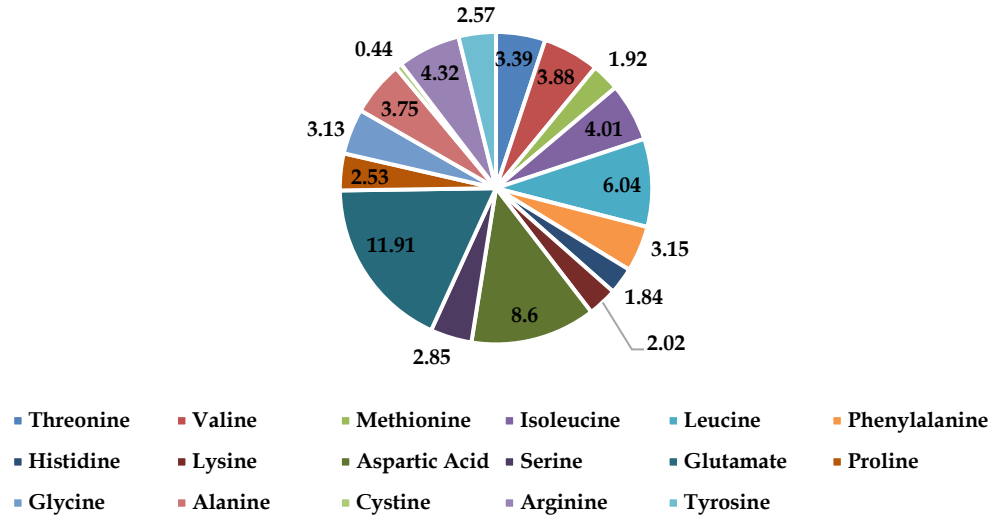


**Figure 1.** Amino acid types (%w/w) of *P. hypophthalmus* muscle tissue.

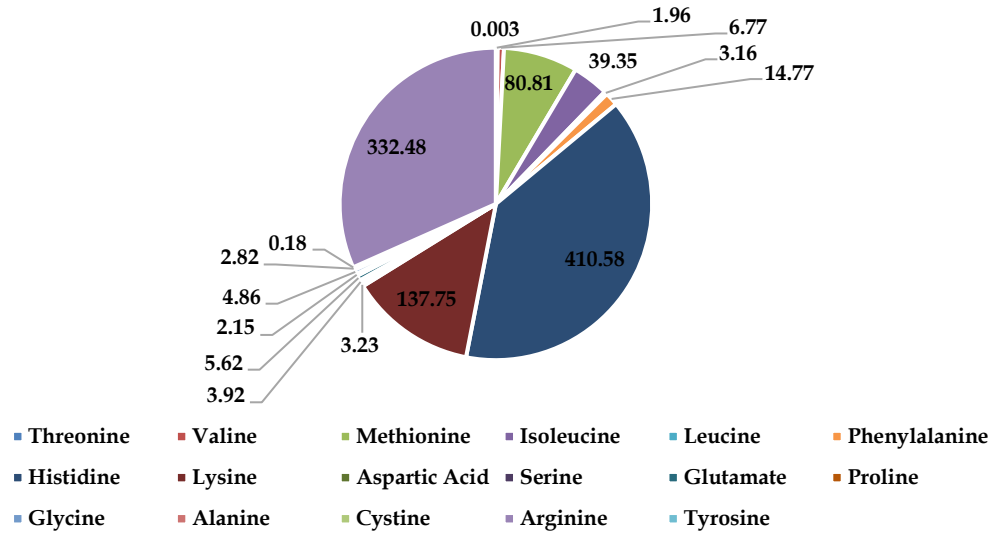


**Figure 2.** Amino acid types (%w/w) of *P. hypophthalmus* oil.

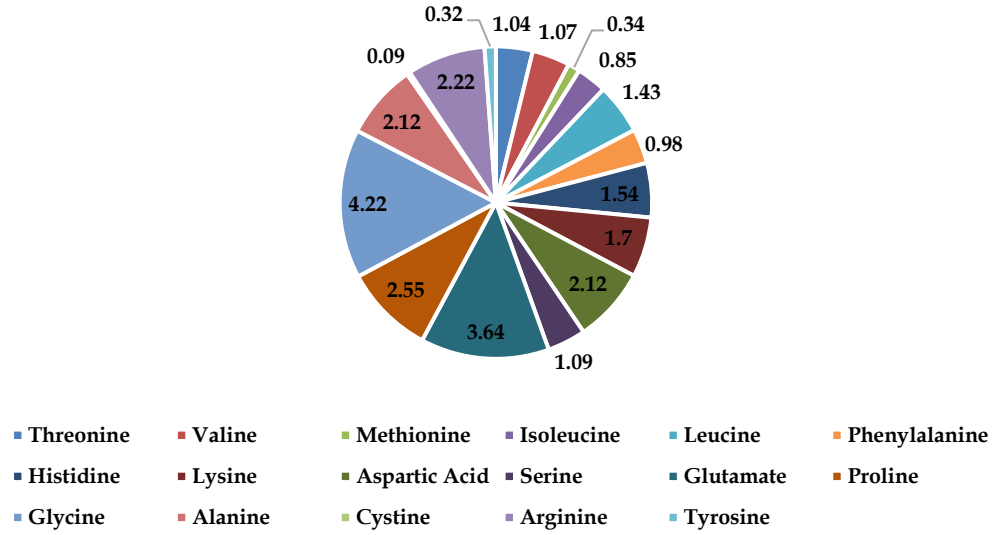


**Figure 3.** Amino acid types (%w/w) of *P. hypophthalmus* bones.

**Table I.** Essential amino acid profile of *P. hypophthalmus* parts.

| Type of amino acid | Amino acid (%W/W) in *P. hypophthalmus* parts | | |
|---|---|---|---|
| | Muscle tissue | Oil | Bones |
| Threonine | 3.39 | 1.96 | 1.04 |
| Valine | 3.88 | 6.77 | 1.07 |
| Methionine | 1.92 | 80.81 | 0.34 |
| Isoleucine | 4.01 | 39.35 | 0.85 |
| Leucine | 6.04 | 3.16 | 1.43 |
| Phenylalanine | 3.15 | 14.77 | 0.98 |
| Histidine | 1.84 | 410.58 | 1.54 |
| Lysine | 2.02 | 137.75 | 1.7 |

**Table II.** Non-essential amino acid profile of *P. hypophthalmus* parts.

| Type of amino acid | Amino acid (%W/W) in *P. hypophthalmus* parts | | |
|---|---|---|---|
| | Muscle tissue | Oil | Bones |
| Aspartic Acid | 8.6 | 3.23 | 2.12 |
| Serine | 2.85 | 3.92 | 1.09 |
| Glutamate | 11.91 | 5.62 | 3.64 |
| Proline | 2.53 | 2.15 | 2.55 |
| Glycine | 3.13 | 4.86 | 4.22 |
| Alanine | 3.75 | 2.82 | 2.12 |
| Cystine | 0.44 | 0.18 | 0.09 |
| Arginine | 4.32 | 332.48 | 2.22 |
| Tyrosine | 2.57 | <0.003 | 0.32 |

**Table III.** Non-essential amino acid profile of *P. hypophthalmus* parts.

| Amino acid category | Amino acid (%W/W) *P. hypophthalmus* parts | | |
|---|---|---|---|
| | Muscle tissue | Oil | Bones |
| Essential | 26.25 | 695.15 | 8.95 |
| Non-essential | 40.1 | 355.26 | 18.37 |
| **Total** | **66.35** | **1050.41** | **27.32** |

Our analysis reveals that *P. hypophthalmus* oil contains substantial quantities of the EAAs histidine, lysine, and methionine, suggesting its potential for medicinal applications. Notably, histidine, the most abundant amino acid in the oil, plays a crucial role in immunoregulation and metabolism and serves as a precursor to histamine[22]. While rapeseed oil has also been identified as a rich source of histidine[23], the significant concentration in *P. hypophthalmus* oil underscores its potential as a valuable ingredient in dietary supplements and pharmaceuticals targeting immune and metabolic functions. The remarkably high levels of lysine, an amino acid critical for protein synthesis and calcium absorption[24], further suggest the potential utility of *P. hypophthalmus* oil in metabolic and bone health supplements. Conversely, the amino acid profile of *P. hypophthalmus* muscle tissue exhibits lower and more balanced levels compared to the oil. This distinct profile, with reduced concentrations of critical amino acids, may be advantageous in the development of collagen-based products or nutritional supplements where a more balanced amino acid composition is desired[25]. The relatively high glutamate content in the muscle tissue[26], an amino acid known for its involvement in neurobiological function and metabolism[27], indicates a potential role for *P. hypophthalmus* muscle in products aimed at supporting brain health. While our findings align with existing literature highlighting fish oil as a rich source of essential nutrients, the specific abundance of histidine in *P. hypophthalmus* oil contrasts with some reports indicating it is not a prominent amino acid in fish oil from various species[28]. Furthermore, the lower levels of lysine and methionine in *P. hypophthalmus* oil compared to the significantly higher amounts observed in other fish oils[29] warrant further investigation. The substantial presence of arginine in *P. hypophthalmus* oil, consistent with previous findings, suggests its potential as a beneficial component for cardiovascular health, given arginine's established importance in endothelial function and cardiovascular well-being[30,31]. It is important to acknowledge that variations in amino acid profiles, including histidine and arginine, can be influenced by factors such as diet and extraction methods[32]. Therefore, future research should explore the impact of these variables on the amino acid composition of *P. hypophthalmus* oil and muscle to optimize its utilization in various applications.

### *Wound activity test*

Evaluation of wound healing activity in rats treated with *P. hypophthalmus* oil extract revealed promising results. By day 7 post-wounding, treatments A and D, involving topical application of the *P. hypophthalmus* oil extract, exhibited wound area

values comparable to the positive control group. Furthermore, statistical analysis indicated that the reduction in wound area observed across all extract-treated groups (A, B, C, and D) was not significantly different from that of the positive control. Notably, treatment D, employing a specific concentration or formulation of the *P. hypophthalmus* oil extract, demonstrated the most substantial wound area reduction (0.62 mm) in comparison to the other treatment groups. Conversely, the vehicle control group (Treatment E), receiving Tween 20, showed no significant difference in wound area reduction when compared to both the negative and positive control groups. A comprehensive overview of these wound healing outcomes is presented in **Table IV**. These findings suggest a significant potential for *P. hypophthalmus* oil extract in promoting wound closure, warranting further investigation into the specific bioactive compounds and underlying mechanisms responsible for this observed efficacy.

**Table IV.** Average length of wound area (mm) in rats (mean±SD).

| Treatment | Length of wound (mm) on day | | | |
|---|---|---|---|---|
| | 1 | 3 | 5 | 7 |
| + | 2.9 ± 0.33[a] | 2.14 ± 0.6[a] | 1.11 ± 0.35[a] | 0.57 ± 0.13[a] |
| - | 2.9 ± 0.45[a] | 2.12 ± 0.43[a] | 1.47 ± 0.37[a] | 1.16 ± 0.32[b] |
| A | 2.9 ± 0.47[a] | 2.35 ± 1.24[a] | 1.81± 1.02[a] | 0.66± 0.28[a] |
| B | 2.9 ± 0.67[a] | 2.32 ± 0.54[a] | 2.28 ± 1.28[a] | 0.84 ± 0.23[a] |
| C | 2.91 ± 0.75[a] | 2.28 ± 0.61[a] | 1.9 ± 0.67[a] | 0.83 ± 0.57[a] |
| D | 2.92 ± 0.44[a] | 1.34 ± 0.43[a] | 1.16 ± 0.18[a] | 0.62 ± 0.12[a] |
| E | 2.93 ± 0.56[a] | 2.27 ± 0.45[a] | 1.36 ± 0.18[a] | 1.14 ± 0.83[ab] |

Note: Different superscript letters indicate statistically significant differences. + (Betadine®), - (None), A (100% *P. hypophthalmus* oil extract), B (5% *P. hypophthalmus* oil extract + 20% Tween 20), C (10% *P. hypophthalmus* oil extract + 20% Tween 20), D (15% *P. hypophthalmus* oil extract + 20% Tween 20), E (20% Tween 20).

### *Wound healing percentage*

Evaluation of wound healing progression in rats, assessed by the percentage of wound closure, revealed comparable efficacy between specific *P. hypophthalmus* oil extract treatments and the positive control on day 7. Specifically, treatments A and D exhibited wound healing percentages closely approximating that of the positive control group. Furthermore, across all treatment groups receiving *P. hypophthalmus* oil extract (A, B, C, and D), a consistent increase in the percentage of wound healing was observed, with no statistically significant difference compared to the positive control. Notably, treatment D, involving the administration of *P. hypophthalmus* oil extract, demonstrated a superior wound healing percentage (84.45%) compared to the positive control group. In contrast, the vehicle control group (Treatment E, receiving Tween 20) showed no significant difference in wound healing percentage when compared to both the negative and positive control groups. Detailed quantitative data on the percentage of wound healing for each treatment group are presented in **Table V**.

**Table V.** Average healing percentage (%) in rats (mean±SD).

| Treatment | Healing percentage (%) on day | | | |
|---|---|---|---|---|
| | 1 | 3 | 5 | 7 |
| + | 27.45 ± 8.43[a] | 46.4 ± 15.07[a] | 72.18 ± 8.76[a] | 85.65 ± 3.35[b] |
| - | 27.35 ± 11.5[a] | 46.95 ± 10.82[a] | 63.21 ± 9.31[a] | 70.91 ±8.11[a] |
| A | 27.35 ± 11.8[a] | 41.91 ± 31.05[a] | 54.73 ± 25.57[a] | 83.36 ± 7.17[b] |
| B | 27.35 ± 16.82[a] | 41.91 ± 13.55[a] | 43.03 ± 32.25[a] | 79 ± 5.9[b] |
| C | 27.15 ± 18.95[a] | 43.01 ± 15.28[a] | 52.43 ± 16.83[a] | 79.16 ± 14.46[b] |
| D | 26.9 ±11.11[a] | 66.31 ± 10.85[a] | 70.98 ± 4.61[a] | 84.45 ± 3.05[b] |
| E | 26.7 ± 14.25[a] | 43.15 ± 11.46[a] | 65.95 ± 4.57[a] | 71.4 ± 20.75[ab] |

Note: Different superscript letters indicate statistically significant differences. + (Betadine®), - (None), A (100% *P. hypophthalmus* oil extract), B (5% *P. hypophthalmus* oil extract + 20% Tween 20), C (10% *P. hypophthalmus* oil extract + 20% Tween 20), D (15% *P. hypophthalmus* oil extract + 20% Tween 20), E (20% Tween 20).

The significant role of the high arginine content in *P. hypophthalmus* oil in promoting wound healing observed in this study aligns with existing literature. Arginine, a conditionally EAA, becomes critically important during physiological stress such as tissue injury. Its function as a precursor for nitric oxide (NO) is well-established, where NO enhances vasodilation and oxygen delivery to the wound site, thereby fostering collagen synthesis and mitigating inflammation[33]. Prior research has consistently demonstrated that arginine supplementation can accelerate wound repair and bolster the immune response, leading to improved healing outcomes[34]. The mechanism involves increased NO production, which not only improves blood flow but also directly stimulates fibroblast proliferation and migration, crucial processes for new tissue formation[35].

Consequently, the elevated arginine levels in *P. hypophthalmus* oil likely contributed substantially to the observed reduction in wound area and the enhanced percentage of wound healing in the *R. norvegicus* model.

Beyond arginine, the presence of other amino acids in *P. hypophthalmus* oil, namely histidine, lysine, and methionine, further supports its wound-healing potential. Histidine, an EAA, is recognized for its antioxidant and anti-inflammatory properties, which can protect tissues from further damage and expedite the healing cascade[36]. Its involvement in tissue growth and repair, as well as its role as a precursor to histamine (a key mediator of immune responses), underscores its therapeutic significance[37]. Moreover, histidine contributes to angiogenesis, re-epithelialization, and collagen production at the wound site[38]. Lysine, another EAA, is directly involved in collagen synthesis, providing structural integrity to newly formed skin and accelerating wound closure[39]. Methionine, a sulfur-containing amino acid, plays a vital role in protein synthesis and the generation of other essential molecules involved in tissue regeneration and overall wound healing[40]. The significant improvements in wound healing observed in the *P. hypophthalmus* oil extract treatment groups, particularly the substantial wound area reduction and high healing percentage in treatment D, strongly suggest that the synergistic action of this rich amino acid profile contributes to its efficacy as a natural wound-healing agent.

The notable abundance of histidine and arginine in *P. hypophthalmus* oil positions it as a promising candidate for pharmaceutical product development. Histidine's diverse involvement in biochemical processes makes it valuable for supplements and medications targeting metabolic and hematologic health[41]. Similarly, the high arginine content in fish oil, including *P. hypophthalmus* oil, presents an opportunity for its use as a raw material in formulations aimed at supporting cardiovascular health[42]. Furthermore, the broader potential of natural amino acids in vaccine and drug development warrants further exploration, as highlighted by Hwang *et al.*[43]. The presence of glutamate in fish muscle has also led to its use in dietary supplements and products aimed at enhancing mental and cognitive well-being, a concept supported by the work of Tardy *et al.*[44], suggesting a future role for *P. hypophthalmus*-derived amino acids in cognitive and mental health applications.

Collectively, these findings highlight the potential of the unique amino acid composition of *P. hypophthalmus* oil, particularly its high levels of histidine, lysine, and arginine, in the development of novel therapeutic interventions. The diverse medicinal applications suggested by its amino acid profile, ranging from wound healing to metabolic and potentially cognitive support, warrant further comprehensive investigation. Future research should focus on clinical evaluations and formulation studies to fully elucidate the therapeutic benefits of these amino acids and their practical implications for human health. Exploring the optimization of amino acid profiles to achieve tailored health solutions represents a promising avenue for future scientific inquiry. The evidence presented strongly supports the potential of *P. hypophthalmus* as a natural source of valuable amino acids for pharmaceutical and nutraceutical applications, with *P. hypophthalmus* oil representing a particularly rich source of these bioactive compounds for the development of innovative healthcare products, including wound healing agents and metabolic support formulations.

## CONCLUSION

This study revealed a significantly higher concentration of both essential (695.15% w/w) and non-essential (355.26% w/w) amino acids in the *P. hypophthalmus* oil sample compared to its muscle tissue (essential: 26.25% w/w; non-essential: 40.1% w/w) and bone (essential: 8.95% w/w; non-essential: 18.37% w/w). Furthermore, *in vivo* wound healing assays conducted on *R. norvegicus* demonstrated the potential of *P. hypophthalmus* oil extract to promote wound closure, exhibiting a reduction in wound area ranging from 0.62 to 0.84 mm and a corresponding percentage of wound healing between 79.00% and 84.45%. These findings suggest that *P. hypophthalmus* oil represents a rich source of amino acids and possesses promising wound-healing properties, warranting further investigation into its specific bioactive components and potential therapeutic applications.

## ACKNOWLEDGMENT

The authors gratefully acknowledge the financial support provided by the Indonesian Education Scholarship (BPI), managed by the Center for Education Financial Service (PUSLAPDIK) in collaboration with the Higher Education

Financing Center (BPPT) of the Ministry of Education, Culture, Research, and Technology, and the Education Fund Management Institution (LPDP) of the Ministry of Finance. This research was made possible through grant number 01541/J5.2.3./BPI.06/9/2022 and grant number 202209092017.

## AUTHORS' CONTRIBUTION

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## DATA AVAILABILITY

None.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this study.

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