



# Multifunctional biological activities of water extract of housefly larvae (*Musca domestica*)

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## ARTICLE INFO

### Chemical compounds studied in this article:

Hydrochloric acid (HCl: PubChem CID: 313)  
Methane sulfonic acid (PubChem CID: 6395)  
2,2-diphenyl-1-picrylhydrazyl (PubChem CID: 2735032)  
Vitamin C (PubChem CID: 54670067)  
Ethanol (PubChem CID: 702)  
Tris(hydroxymethyl) aminomethane (PubChem CID: 6503)  
Acetic acid (PubChem CID: 176)

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Dipeptidyl peptidase-IV inhibitory activity  
Housefly larvae water extract  
Multifunctional activities

## ABSTRACT

Many types of insects have been used as foods and protein sources. In this study, we investigated the usefulness of housefly larvae (*Musca domestica*) based on their amino acid composition and multifunctional biological activities. First, the utility of the amino acid composition of housefly larvae was evaluated by amino acid analysis. Notably, the housefly larvae contained sufficient amounts of all essential amino acids, and the amino acid composition was similar to that of hen eggs. Second, we prepared housefly larvae water extract (HLWE) using the decoction method and explored the biological activities of the extract for potential application of the extract as a functional food. HLWE showed significant antioxidant activity (75.4% at 5.00 mg/mL), angiotensin-converting enzyme (ACE) inhibitory activity (half-maximal inhibitory concentration [IC<sub>50</sub>] = 0.430 mg/mL), and dipeptidyl peptidase-IV (DPP-IV) inhibitory activity ([IC<sub>50</sub>] = 3.52 mg/mL). We found that the low-molecular-weight constituents (< 6 kDa) in HLWE contributed to antioxidant and ACE-inhibitory activities, whereas the high-molecular-weight constituents (> 6 kDa) contributed to DPP-IV inhibition. Our results suggested that housefly larvae may provide a useful source of multifunctional protein.

## 1. Introduction

Bioactive compounds have been studied for the prevention or treatment of various conditions, such as oxidative stress, hypertension, and diabetes. Oxidative stress is caused by a variety of free radicals, which can damage DNA, membranes, lipids, and proteins [1]. Moreover, oxidative stress is related to many chronic diseases, including hypertension and diabetes [1,2]. Hypertension is a chronic adult disease affecting 40% of the population worldwide [3]. Angiotensin-converting enzyme (ACE, EC 3.4.15.1) plays an important role in regulating blood pressure in the renin-angiotensin aldosterone system (RAAS). ACE hydrolyzes angiotensin I to the potent vasoconstrictor angiotensin II and destroys the vasodilator of bradykinin in the RAAS [4]; therefore, many ACE inhibitors have been developed for the treatment of hypertension. Similar to hypertension, diabetes is another prevalent disease affecting a substantial number of individuals worldwide [5]. Type 2 diabetes is the most common form of diabetes,

accounting for approximately 90–95% of all cases [6]. Dipeptidyl peptidase-IV (DPP-IV, EC 3.4.14.5) is a serine protease that hydrolyzes incretin hormones, which stimulate insulin secretion in a glucose-dependent manner. Therefore, DPP-IV inhibitors, which prevent the inactivation of incretin hormones, are expected to be effective for the management and prevention of type 2 diabetes [7].

In addition, diabetes and hypertension have been reported to share some metabolic pathways, including the RAAS, oxidative stress, and insulin resistance pathways, among others [8]. A study by Csermely et al. suggested that multitarget drugs are more efficient than single-target drugs [9]. Although many chemically synthesized antioxidants, ACE inhibitors, and DPP-IV inhibitors have been developed, it is still unclear whether these chemically synthesized antioxidants or inhibitors are sufficiently safe for long-term dosing or whether these compounds may have side effects. Therefore, bioactive compounds derived from natural sources are needed to obtain biocompatibility exceeding that of chemically synthesized drugs. Bioactive compounds are usually derived

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from plants, aquatic animals, and mammals [10]. Moreover, although insects are expected to yield high-protein products, few studies have evaluated bioactive compounds derived from insects.

The housefly is a common insect found worldwide and has high reproductive ability and a short lifecycle. Housefly larvae inhabit unhygienic environments and still manage to remain healthy, potentially because of the presence of various bioactive components in the larval body; housefly larvae prefer organic wastes, such as garbage and animal manure, and can convert these wastes into value-added biomass rich in protein, chitin, and fat [11]. As the consumption of meat increases with the rise in global population, livestock waste becomes a serious problem, which may pollute the groundwater and environment, posing a major risk to public health [12]. The treatment of livestock manure using bioconversion by housefly larvae has been attempted. Niu et al. produced housefly larvae biodiesel using housefly larvae oil obtained from bioconversion of waste food by housefly larvae [11]. Recently, a full-scale bioconversion system for value-added swine manure reduction using housefly larvae has been developed [13]. This system can harvest two products, i.e., housefly larvae and manure residue (after larvae bioconversion), which can be used to as animal feed, for research and development of chitosan and antibacterial peptide products, or as an organic fertilizer for agriculture. Moreover, several studies have shown that insects have a higher efficiency of matter assimilation than livestock; the amount of plant nutrients needed to produce 1 kg of meat is over 10-times that needed to produce the same amount of insect zoomass [14]. Hence the rearing of insects for production of insect-based food causes much less strain on ecosystem services than livestock-based food [14], which may contribute to overcoming food and feed security problems in the future. These studies have shown the potential of housefly larvae for applications with economic and environmental benefits.

Housefly larvae have been an important resource in traditional Chinese medicine since the 14th century [15] and are thought to represent a high-quality protein source that could be used as a livestock feed [16]. Recently, some reports on housefly larvae extracts or peptide fractions have shown that they possess useful bioactivities, including antioxidant, antibacterial, and antitumor activities [15,17–19]. However, it is unclear whether these bioactive compounds possess other multifunctional activities, and insects possessing multifunctional activities, such as antioxidant, ACE inhibitory, and DPP-IV inhibitory activities, have not been reported.

In this study, we evaluated the amino acid composition of housefly larvae and investigated their biological activities, including antioxidant, ACE inhibitory, and DPP-IV inhibitory activities. We performed amino acid analysis of housefly larvae and then prepared a housefly larvae water extract (HLWE) using a decoction method, i.e., an extraction method for active ingredients from herbs in traditional Chinese medicine. We then explored the antioxidant, ACE inhibitory,

and DPP-IV inhibitory activities of the HLWE. We found that the HLWE possessed high multifunctional activities, providing important insights into the biological activities and applications of housefly larvae.

## 2. Materials and methods

### 2.1. Materials

Dried housefly larvae were kindly provided by E's Inc. (Tokyo, Japan). 2,2-Diphenyl-1-picrylhydrazyl (DPPH, D4313) and fluorescent substrate H-(2)Abz-Acp(6)-Ala-Phe(4-NO<sub>2</sub>)-Leu-OH (N09830) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan) and Watanabe Chemical Industries, Ltd. (Hiroshima, Japan), respectively. ACE from rabbit lungs (EC 3.4.15.1, A6778,  $\geq 2$  U/mg protein), porcine DPP-IV (EC 3.4.14.5, D7052,  $\geq 10$  U/mg protein), and Gly-Pro-pNA (G0513) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Centricon Plus-70 centrifugal filter units were purchased from Merck Millipore Co. (Tokyo, Japan). Quick-CBB PLUS and all other chemicals were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

### 2.2. Amino acid analysis

Dried housefly larvae were smashed using a mixer grinder, and the powder was hydrolyzed using 6 M HCl for 24 h at 110 °C in vacuum tubes sealed under a nitrogen atmosphere. After hydrolysis, the HCl was removed *in vacuo* and the powder was redissolved in 0.02 M HCl. HLWE and its fractions were also hydrolyzed under the same conditions as the housefly larvae powder. To determine the tryptophan (Trp) contents of housefly larvae, we used a 4 M methane sulfonic acid solution to hydrolyze the powder under the same conditions. After hydrolysis, 4 M sodium hydroxide solution was used to neutralize the hydrolysis solution to pH 2–3. After neutralization, the hydrolysate solution was filtered to remove the insoluble matter. Amino acid analyses were performed using an automatic analyzer (L-8800; Hitachi, Tokyo, Japan). The amount of each amino acid was calculated based on the peak area in comparison with that of the standard. The experiments were performed in duplicate, and the results are presented as means.

### 2.3. Preparation of HLWE

In this study, we prepared HLWE using the decoction method [20] to extract the bioactive components from housefly larvae. The decoction method (Fig. 1) was performed as follows. First, dried housefly larvae powder (0.5 g) and deionized water (25 mL) were added to an earthen pot, and the housefly larvae were immersed for 30 min. The samples were then simmered for 10 min (heat was reduced after the water began to boil), after which the heat was turned off, and the

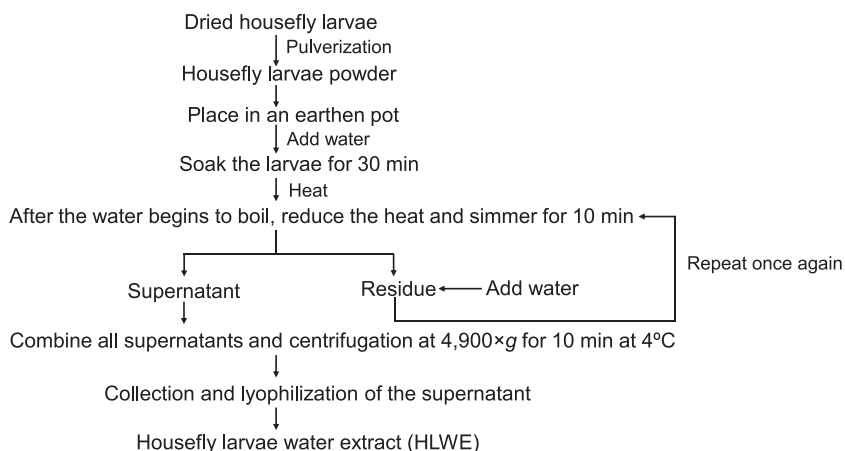


Fig. 1. Extraction procedures for housefly larvae water extract (HLWE).

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