**Data on the potent fibrinolytic effects of the *Lumbricus rubellus* earthworm and the *Perinereis linea* lugworm**

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**ABSTRACT**

There is technology available for anti-thrombus with earthworms, but the procedure is complex and extracts protein with inferior purity. In order to develop a simplified process with a stronger purity of protease, we investigated the *Lumbricus rubellus* earthworm and *Perinereis linea* lugworm. We purified water extracts cut off at 10 kDa of molecular weight using ultrafiltration because proteins are large biomolecules, or macromolecules, consisting of one or more long chains of amino acid residues. We purified EW1 (raw earthworm extract), EW2 (molecular weight (m.w) > 10 kDa of earthworm extract), and EW3 (m.w < 10 kDa) from the *Lumbricus rubellus* earthworm. Likewise, we purified LW1 (wild lugworm extract), LW2 (m.w > 10 kDa), and LW3 (m.w < 10 kDa) from the *Perinereis linea* lugworm. Using a fibrin assay, we found that fibrinolytic activity of the specimens had a rank order of clear zone diameter: EW2 > EW1 > EW3 > LW2 > LW1 > LW3. In particular, EW2 and LW2 showed a potent fibrinolytic effect in two different worm specimens. The protein content of each sample was detected as 2.34 (EW1), 3.03 (EW2), 2.80 (LW1), 3.71 (LW2) mg/ml.

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respectively, and their molecular weights were measured using SDS-PAGE. The samples contained the following amounts of total fatty acids: EW1, 3.61%; EW2, 0.48%; LW1, 4.96%; and LW2, 0.23%. We developed a process to increase the thrombolytic effect with a higher purity protein. The study results demonstrate this procedure and provide basic data for developing an anti-thrombolytic agent.

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Specifications table

| Subject area                      | Anti-thrombus |
|-----------------------------------|---------------|
| More specific subject area        | Fibrinolytic effects of earthworm and lugworm |
| Type of data                      | Table, graph |
| How data was acquired             | SDS-PAGE, ELISA reader, Fibrin plate assay |
| Data format                       | Raw, analyzed |
| Experimental factors              | The Lumbricus rubellus earthworm and Perinereis linea lugworm were investigated |
| Experimental features             | Potent fibrinolytic effects of the Lumbricus rubellus earthworm and Perinereis linea lugworm |
| Data source location              | Silla University, Korea |
| Data accessibility                | Data are available within this article |

Value of the data

- The data show the fibrinolytic effects of the *Lumbricus rubellus* earthworm and *Perinereis linea* lugworm.
- Lugworm (*Perinereis linea*) powder can be considered to be a new resource for fibrinolytic enzymes.
- Lugworm (*Perinereis linea*) powder may be used to treat heart disease.
- The medicinal applications and molecular biological characteristics of lugworm (*Perinereis linea*) powder may be clarified.

1. Data

Samples from the *Lumbricus rubellus* earthworm indicated fibrinolytic activity on the fibrin plates, with samples EW1 and EW2 showing much higher fibrinolytic activity than EW3. The order was EW2 > EW1 > EW3 (Fig. 1A). In addition, the samples from the *Perinereis linea* lugworm showed fibrinolytic activity in the order of LW2 > LW1 > LW3 (Fig. 1B). It should be noted that the samples of the two species were combined in order to observe the results. As shown in Fig. 1C and D, the diameter of each hydrolyzed clear zone was measured. The rank order of the clear zone diameters was EW2 > EW1 > EW3 > LW2 > LW1 > LW3. The samples EW2 and LW2 presented the potent fibrinolytic effect and had clear zone diameters of more than 28.5mm and 11.5mm in dissimilar earthworm and lugworm samples.

It characterized as the results of molecular weights measurement of the earthworm samples (Fig. 2). At the same time, the molecular weights ranged from 23.5 to 34.2 kDa, which were reported by Mihara et al. [1]. This means that all of the samples in this paper possessed fibrinolytic activity, the molecular weight act as a part of the fibrin assay but are not vital to the fibrinolytic effect.

With respect to the total protein content, the earthworm samples (EW1 and EW2) from the *Lumbricus rubellus* earthworm had persistent fibrinolytic effects than specimens (LW1 and LW2) of the *Perinereis linea* lugworm. However, there were two species earthworm samples with fibrin assays with molecular weights of more than 10kDa, samples EW2 and LW2, which had the most potent fibrinolytic effects. Combined with the protein content (Fig. 3A and B), higher protein was verified in EW2 (3.03 mg/ml) and
LW2 (3.71 mg/ml), whereas other enzyme samples supplied less than the two specimens did. Samples EW2 and LW2 played a meaningful role in the thrombolysis assay with the uppermost protein.

There are several remarkable features of the total fatty acid composition syncretized preceding the results of the fibrinolytic activity in samples EW1, EW2, LW1, and LW2, which were chosen for the measurement of fatty acids because of their great disparity in fibrinolytic effects. The total fatty acid

| Sample | Clear zone diameter (mm) | Mean ± SD |
|--------|--------------------------|-----------|
| P0     | 16.50                    | 17.00     | 16.00 | 16.50±0.5 |
| EW1    | 26.00                    | 26.00     | 26.00 | 26.00±0.0 |
| EW2    | 28.50                    | 29.00     | 28.00 | 28.50±0.5 |
| EW3    | 12.50                    | 13.00     | 12.00 | 12.50±0.5 |
| LW1    | 5.00                     | 5.00      | 5.00  | 5.00±0.0  |
| LW2    | 11.50                    | 11.00     | 12.00 | 11.50±0.5 |
| LW3    | 3.25                     | 3.00      | 3.50  | 3.25±0.3  |

Fig. 1. A. Fibrin Plate Assay (Lumbricus rubellus earthworm). B. Fibrin Plate Assay (Perinereis linea lugworm). C. Mean diameters of the hydrolyzed clear zone data. D. Mean diameters of the hydrolyzed clear zone. P0: Plasmin; EW1: Crude earthworm extract; EW2: Molecular weight >10 kDa of earthworm extract; EW3: Molecular weight <10 kDa of earthworm extract; LW1: Wild lugworm extract; LW2: Molecular weight >10 kDa of lugworm extract; LW3: Molecular weight <10 kDa of lugworm extract. All values are expressed as means ± S.D. (n = 3). The letters a, b, c, d, and e above the bars stand for significantly different (p < 0.05) groups by one-way ANOVA, followed by Duncan’s multiple test.
contents of EW1, EW2, LW1, and LW2 were 3.61%, 0.48%, 4.96% and 0.23% respectively (Table 1), with roughly accumulated and found the total fatty acid ratio of sample EW1 (3.61%) was significantly (7.5-fold) higher than EW2 (0.48%). Another difference was sample LW1 (4.96%), which had a more intense (21.6-fold) higher total fatty acid proportion than LW2 (0.23%) under the same survey.

In the light of the adequate data on fatty acids that can be conveyed, sample EW1 (from *Lumbricus rubellus*) consists mostly of saturated fatty acids like myristic acid, lauric acid, and stearic acid. However, the fatty acids of specimen LW1 (from *Perinereis linea*) were grouped as palmitic acid and stearic acid, which belong to saturated fatty acids. The effect of them on the risk of disease is controversial, but many reviews suggest these are linked to higher risks of cardiovascular diseases and diabetes. All the crucial fatty acids (EW1 and LW1) that yield and their ratios are summarized in Table 1, but the demonstrated specimens EW2 and LW2 have contained only limited total fatty acids at the 0.48% and 0.23% level (Table 1).

### 2. Experimental design, materials, and methods

Mature earthworms were first washed with running tap water; the cleaned worms were left at room temperature until there was no dripping. Earthworm samples weighed between 165 g and 190 g. They were mixed with distilled water at a 1:4 ratios and homogenized. The supernatants were stored for further use. The 80% earthworm solution was filtrated using an ultra-filter (Synopex micron rating PNO3N membrane, Korea) with a molecular weight cut off of 10 kDa, and distilled water was added for more complete ultrafiltration. The 20% earthworm supernatant was retained for subsequent concentration. The 30 ml earthworm ultrafiltration was frozen at −70 °C for 24 hours. The concentration was freeze-dried into powder for five days.

The processed earthworm powders were used as assaying fibrinolytic activity was determined by following the protocols of the fibrin plate method [2]. Briefly, the materials were prepared: in a 3% fibrinogen solution, a thrombin solution (1,000 U of thrombin in 1000 µl of PBS), and a plasmin solution (100 µg of plasmin in 1 ml of PBS), and then in a 10% sample solution (50 mg of sample in 0.5 ml of distilled water). Then, 10 ml of fibrinogen solution and 0.05 ml of thrombin solution were poured into a sterile petri-dish (90 mm), homogenized completely, and left for about 5 min until clotted. Each well was loaded with 10 µl of each sample, which incubated at 37 °C for 6 h. Then, the mean diameter of the hydrolyzed clear zone was measured.

The molecular weights of the samples were measured using SDS-PAGE, 12.5% polyacrylamide gel, and a standard protein marker according to a slightly modified version of Laemmli’s method [3]. The gels were stained with Gel Code Blue stain reagent.
The Coomassie Brilliant Blue protein assay, known as the Bradford assay, is widely used because of its performance, rapidity, relative sensitivity, and specificity for proteins. Bovine serum albumin (BSA) was used as the standard, 20 μl BSA solution was reserved, and the sample solution was added to each tube. Then, 1 ml of Bradford reagents were added. Next, the control and sample solutions were shaken slightly by vortex. After that, 100 μl of the control and each sample was placed into the 96-well microplate quickly. Then, the optical density was measured using an ELISA reader at 595 nm.

### Table 1

| Sample  | Optical Density | Mean ± SD   | Protein content (mg/ml) ± SD |
|---------|-----------------|-------------|-----------------------------|
| EW1     | 0.470           | 0.488       | 0.481±0.01                  | 2.34±0.08 |
| EW2     | 0.615           | 0.630       | 0.626±0.01                  | 3.03±0.08 |
| EW3     | 0.280           | 0.289       | 0.291±0.01                  | 1.43±0.09 |
| LW1     | 0.567           | 0.586       | 0.578±0.01                  | 2.80±0.08 |
| LW2     | 0.745           | 0.772       | 0.769±0.02                  | 3.71±0.14 |
| LW3     | 0.412           | 0.415       | 0.415±0.00                  | 2.02±0.05 |

**Fig. 3.** A. Protein assay of earthworm and lugworm samples data. B. Protein assay of earthworm and lugworm samples. EW1: Crude earthworm extract; EW2: Molecular weight >10 kDa of earthworm extract; EW3: Molecular weight <10 kDa of earthworm extract; LW1: Wild lugworm extract; LW2: Molecular weight >10 kDa of lugworm extract; LW3: Molecular weight <10 kDa of lugworm extract. All values are expressed as means ± S.D. (n = 3). The letters a, b, c, d, e, and f above the bars stand for significantly different (p < 0.05) groups by one-way ANOVA, followed by Duncan’s multiple test.
The desired 25 mg amount of each powdered sample of EW1 (wild earthworm extract), EW2 (molecular weight >10 kDa of earthworm extract), EW1-EW2 from Lumbricus rubellus; LW1 (wild lugworm extract), LW2 (molecular weight >10 kDa of lugworm extract), LW1-LW2 from Perinereis linea was dissolved in an alkaline solution. Analyses were conducted with an Agilent 6890 N gas chromatograph equipped with a split/splitless injector and a flame ionization detector (FID).

**Author contribution**

Yuanyuan Dong and Young Min Woo are co-first authors and contributed equally.
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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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