Drying kinetics and anticoagulant activity of microwave-vacuum, dehumidified-air and freeze-dried african night crawler (Eudrilus eugeniae kinberg)

Abstract. In this study, the drying kinetics and anticoagulant activity of dried earthworm Eudrilus eugeniae were compared using microwave-vacuum, dehumidified-air, and freeze-drying. Data showed that microwave-vacuum and dehumidified-air drying methods have relatively short drying time, higher drying rate, and have low operational cost compared with freeze-drying. The Two-term, Midilli, and Diffusion models fit the best in describing drying kinetics under microwave-vacuum, dehumidified-air, and freeze-drying, respectively. The water activity of the dried sample was in the acceptable value for safe storage through the dehumidified-air dried sample was in the critical range. Proximate analysis showed that dried earthworms have high protein content ranging from 60 to 70 percent of its total dry weight. The anticoagulant assay showed that fresh earthworm is potent as heparin showing no coagulation. Microwave-vacuum dried earthworm exhibited the strongest anticoagulant activity compared with other drying methods though weaker than the fresh sample. This study suggests that earthworms have anticoagulant activity, and microwave-vacuum and dehumidified-air drying could be an alternative method for drying the heat-sensitive sample.

1. INTRODUCTION
The Eisenia fetida and Eudrilus eugeniae Kinberg are the most familiar commercially cultured earthworms in the temperate and tropics countries respectively (Kale, 2004). In the 1970s, Pheretima asiatica from Taiwan was presented by the private sector in the Philippines. In that same year, the culture of earthworms started in the country (Guerrero, 2009), and matters with beneficial properties were found (Elicano, 2004). Earthworms have high protein and more minerals which explained its antibacterial, anti-aging, mitogenic (inhibit rapid mitosis), anti-ulcer, fibrinolytic (blood clot factor), anti-inflammatory, and anti-oxidative activity (Syariah, Sains, Nilai, & Sembilan, 2013). Since then, therapeutic properties of earthworms have been used in numerous countries and cultures. In the 1980s, scientific awareness in earthworm pharmaceutical usage started. Through the use of technology, certain compounds from earthworms in producing pharmaceutical and cosmetic products were made promising.

Recently, it has been reported that earthworm powder has a mechanism for treating thrombolytic disorder (Fu et al., 2013). Deep venous thrombosis is one of the common indications of a thrombolytic disorder. Thrombosis is the development of a blood clot inside a blood vessel hindering the flow of blood through the circulatory system. Medication of thrombembolic vascular ailment presently relies on medicines having a variety of fibrinolytic enzymes, such as tissue-type plasminogen activator and urokinase. However, intravenous administration of these enzymes is costly and has numerous side effects including anaphylaxis and immunoreactions (Fu, Zhang, Liu, Li, and Tian, 2013). The
fibrinolytic enzymes liquefy fibrin, the key component of blood clots. Blood clot formation and lysis is one of the significant causes contributing to cardiovascular diseases. At present cardiovascular disease is considered one of the primary causes of death throughout the world.

This study aims to determine the most suitable thin-layer drying models for three methods: microwave-vacuum, dehumidified-air and freeze-drying; to determine and compare the water activity, glass transition temperature, and drying kinetics of the dried earthworm; to compare the anticoagulant activity of dried earthworms; and to determine if the drying methods would not alter the anticoagulant activity.

2. MATERIALS AND METHODS

2.1. Mathematical Modelling of Drying Data

The moisture loss data were fitted to nine models usually used for the drying curves. The moisture ratio (MR) and drying rates were calculated using Eq. 1 and 2, respectively where

\[ M_t = \text{moisture content (g water.g dry solid }^{-1}) \text{ at time } t \text{ (min)}, \]

\[ M_{t+dt} = \text{moisture content (g water.g dry solid }^{-1}) \text{ at time } t+dt \text{ (min)}; \]

\[ M_0 = \text{initial moisture content}, \]

\[ M_e = \text{equilibrium moisture content}. \]

The best model describing the thin-layer drying characteristics of earthworms was chosen as the one with the lowest reduced root mean square error (RMSE) and the highest coefficient of determination (R^2) values (Menge et al. 2005; Goyal et al. 2006; Abalone et al. 2006; Ozbek and Dadali 2007).

\[ MR = \frac{M_t - M_e}{M_0 - M_t} \]  

\[ \text{Drying Rate} = \frac{M_{t+dt} - M_t}{dt} \]  

2.2. Determination of Water Activity (aw) of the Samples

Water activity determination of the samples was carried out through Novasina LabSwift-aw® water activity analyzer machine model GRPO: 500200 in triplicates recorded at ± 0.01. Two grams of the ground sample from each drying method was placed in the analyzer for 30-45 minutes until the readings were stabilized.

2.3. Proximate Analysis

The proximate composition of microwave-vacuum, dehumidified-air, and freeze-dried earthworm was evaluated based on the Official Methods of Analysis of the AOAC (1993). Five grams from each sample was used for ash, fat, protein, fiber, and moisture content determination.

2.4. Anticoagulant Assay

The anticoagulation activity was measured by assimilating the clotting time of the dried earthworms using three drying methods, fresh earthworm sample, heparin as a positive control, water as a negative control, and CaCl2 as the clotting factor. Three-point eight percent (3.8%) sodium citrate solution was utilized as the anticoagulant during blood extraction. Fifty milliliters (50 mL) fresh blood was drawn from every three pigs. Blood was collected after ante-mortem inspection and prior to stunning and sticking. Blood collection was done using a blood drawing needle and Vacutainer tube with sodium citrate solution. Blood plasma was separated from other components using a refrigerated centrifuge (2400Xg, 20 mins). The coagulation was triggered by addition of 0.2 mL 1% CaCl2 to 0.5 mL citrated plasma. The absorbance of the solution was read at 700 nm for every 10 s until the absorbance reaches 0.8 (coagulation) (Swoap and Kuzienga, 1949 with some modifications).
3. RESULTS AND DISCUSSIONS

3.1. Drying Kinetics

The moisture loss data for microwave-vacuum, dehumidified-air, and freeze-dried experimental conditions are presented in figure 1. At a given microwave power level, results show that drying time was shorter in the 16.93 kPa vacuum pressure (840 mins) and significantly different at a 95% confidence level than at 33.86 kPa (870 mins) and 50.8 kPa (900 mins). Hu et al. (2006) and Orsat et al. (2007) stated that vacuum pressure has important effects on drying time. In the case of dehumidified-air drying, increasing the airflow rate caused a substantial decrease in drying time at the same drying air temperature. This is due to the faster movement of drying air that absorbs moisture as it passes through the sample. The drying time of 2.7 m min\(^{-1}\) airflow rate is significantly different compared to 2 m min\(^{-1}\) and 1.5 m min\(^{-1}\) airflow rate. Freeze drying has a long drying time (3300 mins). This may be due to the poor heat transfer rate associated with the conventional electric heating method, which transfers heat for drying by conduction (Wang et al., 2010).
Comparing the three drying processes, dehumidified-air drying has the shortest drying time, specifically at 2.7 m min\(^{-1}\) airflow rate. This may be due to the higher absorption capacity of the dehumidified-air that has a low humidity ratio compared to the ambient air. The dehumidified-air can hold more water as it passes through the materials resulting in shorter drying time. During the dehumidification process, the dry bulb temperature is cooled from 29.9 °C down to 19.9 °C that causes a decrease in the moisture content of the air through condensation. The moisture loss during the dehumidifying process is approximately 0.01 kg/kg of dry air. Microwave-vacuum drying has comparable drying time with dehumidified-air drying. This result is due to the volumetric heating where the vapor is generated inside the product, developing an internal pressure gradient that causes water to flow from the interior to the surface of the material (Lemos et al., 2015). Based on the ANOVA test, there is no significant difference in the drying time of microwave-vacuum and dehumidified-air, but these two methods are significantly different from freeze-drying.

The graphs of drying rate versus the moisture content of Microwave-vacuum, Dehumidified-air, and Freeze-drying are presented in Figure 2. The graph indicates that dehumidified-air drying has the highest drying rates compared with microwave-vacuum and freeze-drying. Dehumidified-air drying resulted in a four-to-five-fold increase in drying rates compared with microwave-vacuum and fifty-to-fifty five-fold increase with freeze-drying. Analysis of Variance shows that drying rates of Dehumidified-air, Microwave-vacuum, and Freeze drying are significantly different. The increase in drying rates using dehumidified-air is due to the continuous flow of forced air with a low moisture content that can easily absorb the available surface moisture as it passes through the sample. Among the different drying conditions in dehumidified-air drying, 2.7 m min\(^{-1}\) at 43 °C has the highest drying
rates, as shown in Figure 2. The three drying conditions in dehumidified-air drying are significantly different from each other based on the ANOVA test. Graphs of drying rate versus moisture content demonstrated a decreasing removal of water towards the end of the drying process. This occurs when the bound moisture is removed and the rate of moisture diffusion within the product decreases below that essential to replenish the moisture at the surface. A similar observation was described by Sharma et al. (2005) when the moisture is being eliminated and the product surface is drying out, succeeding energy absorption and heat penetration through the product layer decreases, which impedes the drying rate.
3.2 Fitting of Thin-Layer Drying Models

Table 1 shows the model constants and quality of fit in terms of $R^2$ and RMSE values for nine thin-layer drying models fitted to the moisture loss data. Good fit results were observed from the values of $R^2$ and RMSE for all models, which ranged from 0.9214 to 0.999778, 0.004412 to 0.150312, respectively.

Table 1. Values of coefficients and statistical analysis obtained from different thin-layer drying models for drying of earthworm using dehumidified-air, microwave-vacuum, and freeze-drying method

| NO | MODEL NAME       | MODEL EQUATION                                                                 | MODEL COEFFICIENTS       | $R^2$     | RMSE     |
|----|------------------|-------------------------------------------------------------------------------|--------------------------|-----------|----------|
| 1  | Henderson and Pabis | $MR = a \exp(-kt)$                                                            | $a = 0.95959$ $k = 0.008613$ | 0.991479  | 0.073896 |
| 2  | Newton            | $MR = \exp(-kt)$                                                              | $k = 0.010991$           | 0.986336  | 0.034505 |
| 3  | Page              | $MR = \exp(-kt^n)$                                                            | $k = 0.019889$ $n = 0.853937$ | 0.992913  | 0.024079 |
| 4  | Logarithmic       | $MR = a \exp(-kt)+c$                                                          | $a = 1.007972$ $c = -0.04821$ $k = 0.008991$ | 0.992308  | 0.026802 |
| 5  | Two Term          | $MR = a \exp(k_0t)+b \exp(-k_1t)$                                           | $a = 0.135266$ $b = 0.866487$ $k_0 = 0.092326$ $k_1 = 0.008129$ | 0.996833  | 0.016096 |
| 6  | Two Term Exponential | $MR = a \exp(-kt)+(1-a)\exp(-kat)$                                         | $a = 0.128243$ $k = 0.064577$ | 0.996097  | 0.01787  |
| 7  | Wang and Singh    | $MR = 1+at+bt^2$                                                              | $a = -0.00771$ $b = 0.0000152$ | 0.965206  | 0.057004 |
| 8  | Diffusion Approach | $MR = a \exp(-kt)+(1-a)\exp(-ktb)$                                          | $a = 0.866593$ $b = 11.29718$ $k = 0.00813$ | 0.996829  | 0.016105 |
| 9  | Midilli           | $MR = a \exp(-kt)+bt$                                                        | $a = 1.001641$ $b = 0.000041$ $k = 0.033098$ $n = 0.702007$ | 0.99849   | 0.011113 |

RMSE = root mean square error
Generated values of $R^2$ and RMSE were used as the selection criteria to determine the best model. The best model describing the thin-layer drying characteristics of earthworms was chosen as the one with the lowest reduced root mean square error (RMSE) and the highest coefficient of determination ($R^2$) values. In dehumidified air drying, the two-term model was the most suitable for all drying conditions except for drying with 2.7 m min$^{-1}$, 43 °C where Midilli was the best model. In the case of Microwave-vacuum drying, Midilli model was the most fitting for all drying conditions tested. Midilli exhibited the highest $R^2$ value and lowest decreased RMSE. In freeze-drying, Two-term and Diffusion approach models have the highest $R^2$ value, but the later has lower RMSE than the former. Based on the observed value, the Diffusion approach was the best model.

A comparison between the experimental and the predicted values from Midilli, Two terms, and Diffusion approach was presented in Figure 3. Although these models slightly over-predicted or under-predicted the experimental data, they were still satisfactory in describing the thin-layer drying behavior of earthworms under the microwave-vacuum, dehumidified-air, and freeze-drying conditions. The results of nine drying models are also in good agreement with other reported drying models, including the results presented by Izli and Gunasekaran (2014).
(c) Microwave-vacuum (50.8 kPa, 43 °C)

(d) Dehumidified-air (2.7 m min⁻¹, 43 °C)

(e) Dehumidified-air (2 m min⁻¹, 43 °C)
Figure 3. Experimental and predicted moisture ratio vs. drying time of microwave vacuum (a,b,c), dehumidified-air (d,e,f), and freeze-dried earthworm (g)

3.3. Cost of Operation
Table 2 shows that freeze-drying has the highest power consumption compared with microwave-vacuum and dehumidified-air drying. The high power consumption could be attributed to long drying time and slow drying rate, as shown in Tables 1 and 2, respectively. Wang et al. (2010) reported that freeze-drying is a costly method of drying due to long drying time and high energy consumption. On the other hand, the power consumption of microwave-vacuum and dehumidified-air drying is quite numerically similar and much lower compared with freeze-drying. ANOVA test indicates that there is no significant difference between the drying methods. Hence, microwave-vacuum and dehumidified-air drying offered the advantages of short drying time and increased drying rate.

Table 2. The power consumption of Microwave-vacuum, dehumidified-air, freeze-drying operation.

| DRYERS                  | POWER CONSUMPTION (kW-hr) |
|-------------------------|---------------------------|
| Microwave-vacuum        |                           |
| 16.93 kPa, 204 W        | 6.3                       |
| 33.86 kPa, 204 W        | 6.53                      |
| 50.8 kPa, 204 W         | 6.75                      |
| Dehumidified-air        |                           |
| 2.7 m min⁻¹, 43 °C     | 6.13                      |
| 2 m min⁻¹, 43 °C        | 7.39                      |
| 1.5 m min⁻¹, 43 °C      | 9.16                      |
| Freeze                 |                           |
| 0.0749 kPa, -43 °C     | 25.2                      |
It is important to note that as the airflow rate in the dehumidified-air drying increases, the power consumption decreases. While in microwave-vacuum, power consumption increases when the pressure increases.

3.4. Quality of Dried Earthworms

Freeze-dried samples were as light brown as the fresh earthworms, unlike the microwave-vacuum dried products, which showed darkening and the dehumidified-air dried samples, which were all brown. The discoloration of the samples can be attributed to the higher drying temperature (43°C) of microwave-vacuum and dehumidified-air compared with freeze-dried (-43°C) since the sample is heat sensitive. All dried samples were soft, except for the freeze-drying method, where the products were more brittle and easily crumbled. Freeze-dried earthworms were very light in weight compared with microwave-vacuum dried and dehumidified-air dried samples. Microwave-vacuum and dehumidified-air dried samples also showed apparent deformation, unlike the one that has undergone freeze-drying. In general, the freeze-drying method produced the most visually appealing dried earthworm, unlike the other two drying methods, which resulted in products with deformed and darkened appearance.

Table 3. Physical attributes of Microwave-vacuum, Dehumidified-air, and Freeze-dried African Night Crawler.

| Product          | Property  | Microwave-vacuum Drying          | Drying Method          | Freeze Drying          |
|------------------|-----------|----------------------------------|------------------------|------------------------|
| African Night Crawler | Color     | Darker compared with the fresh sample | Slightly darker compared with the fresh sample | Similar to fresh samples |
|                   | Texture   | Rubbery/gummy                    | Rubbery/gummy but harder | Brittle               |
|                   | Weight    | Light                            | Light                  | Very light             |
|                   | Appearance| Totally deformed                 | Deformed               | Similar to fresh samples |

3.5. Water Activity

Knowledge of the water activity and moisture of foods can aid in their proper storage in order to inhibit the growth of spoiling agents. A water activity between 0.6 and 0.7 represents the lower bound at which most bacterial and fungal growth can persist, and the moisture content associated with this critical range may, therefore, be interpreted as the maximum allowable level to limit microbial degradation (Bonner and Kenney, 2012). Hence, food materials need to be kept in temperatures below this, or they are dehydrated to moisture contents corresponding to values below the $A_w$ range so that mold and bacterial growth is prevented. Table 4 shows that the water activity of dried earthworm is below the critical range wherein chemical reactions, physical changes, microbial proliferation, and enzymatic activity may occur except for dehumidified-air dried earthworm that falls within the critical range or maximum allowable level. This means that the dried earthworms are in stable condition, especially the freeze-dried and microwave-vacuum dried *Eudrilus eugeniae*. Product stability of dried *Eudrilus eugeniae* can be prolonged by lowering $A_w$ or moisture content to some extent where harmful and spoiling microbes can survive, temperature control, preservatives, and the use of modified atmosphere packaging (MAP).

Table 4. Water Activity, Moisture Content, and Temperature of dried earthworms using three drying methods.

| SAMPLE                  | WATER ACTIVITY ($A_w$) | MOISTURE CONTENT (%) | TEMPERATURE (°C) |
|-------------------------|------------------------|----------------------|------------------|
| Microwave-vacuum        | 0.582                  | 10                   | 27               |
| Dehumidified-air        | 0.692                  | 14.84                | 26.8             |
| Freeze                  | 0.451                  | 7.51                 | 27.1             |

values shown are mean values of three replicates

3.6. Proximate Composition

In general, the proximate contents of Microwave-vacuum, Dehumidified-air, and Freeze-dried earthworms are numerically similar to each other, as shown in table 5. The dry matter content of earthworms has been found to be between 17 and 21 percent of the fresh weight. Dry matter content of earthworms contains 60-67 % protein, 4-10% fat, 7-10 % ash, and 1.5-2 % fiber. Sharma et al. (2005) obtained comparable results of earthworm's dry matter between 20 and 25 percent of the fresh weight and contained around 60 % protein, 7-10 % fat, and 8-10 % ash. Results show that microwave-vacuum, dehumidified-air, and freeze-drying methods maintained the high protein content of *Eudrilus eugeniae*. The results showed further that the protein content of *Eudrilus eugeniae* is comparable with
a reported protein content of earthworm species like *Eisenia fetida*. Because of its favorably helpful protein, it has been observed to be economically feasible to culture earthworms. The isolated active matter from earthworms can be developed into vermiceautical or pharmaceutical products as medication for certain human diseases. It has been established that the presence of anti-blood-clotting action of a crude extract from earthworms used by indigenous people to thin the blood in the elderly (Syariah et al., 2013). The dehumidified-air dried sample obtained the highest crude protein and is statistically different from the two drying methods.

Table 5. Proximate composition of the total dry weight of *Eudrilus eugeniae*.

| SAMPLE         | % MOISTURE | % ASH | % CP  | % CFI | % CFA |
|----------------|------------|-------|-------|-------|-------|
| Microwave-vacuum| 10         | 7.21  | 61.79 | 1.49  | 9.26  |
| Dehumidified-air | 14.84      | 7.62  | 66.11 | 1.59  | 8.97  |
| Freeze         | 7.51       | 9.43  | 59.86 | 2.09  | 3.96  |

Values shown are mean values of three replicates.

3.7. Glass Transition

Microwave-vacuum, dehumidified-air, and freeze-dried earthworms showed no apparent glass transition based on the graph generated by Differential Scanning Calorimetry (DSC). The glass transition temperature of the product was not detected within the set temperature range of 20 °C – 150 °C, which means that the glass transition temperature of the product is not within the set temperature range. Bhandari and Howes (1999) mentioned that the Glass transition temperature (T_g) of very high molecular weight food polymers such as starches and proteins could not be determined experimentally as they start decomposing before reaching T_g. It could be the main reason in the absence of the glass transition temperature since dried earthworms used in this study have relatively high protein content that ranged between 60 and 67 % of its total weight. Though T_g was not determined, denaturation of protein was detected in dried *Eudrilus eugeniae* except for dehumidified-air dried samples wherein no data could support that the glass transition temperature is within the specified temperature range. In microwave-vacuum dried samples, onset denaturation temperature was recorded at 67.77 °C and the point of maximum denaturation at 127.39 °C, while those were respectively at 36.36 °C and 76.24 °C in freeze-dried samples. Graphs of Protein denaturation created by Differential Scanning Microscopy were shown in figure 4.
Figure 4. Protein denaturation of freeze, microwave-vacuum, and dehumidified-air dried earthworm generated by Differential Scanning Microscopy

3.8. Anticoagulant Activity

Anticoagulation of blood is important to prevent high blood pressures and stroke accompanying it. Fu et al. (2013) stated that cardiovascular disease caused by thrombosis is remarkably prevalent in older people and resorted to disability and death. Treatment of such disease becomes much vital than ever before. Thus, the anticoagulant activity of fresh and dried earthworms was evaluated.

The Fresh earthworm sample, Microwave-vacuum dried, Dehumidified-air dried, and Freeze-dried earthworms were subjected to anticoagulant activity evaluation. The estimated clotting times are shown in Table 6 based on the change in absorption of the test solution at 700 nm.

At a 95% confidence interval, the fresh sample is significantly different from all other treatments. The fresh earthworm sample is as potent as heparin but not the other treatments.

Drying methods such as microwave-vacuum, dehumidified-air, and freeze-drying significantly reduced the anticoagulant activity of *Eudrilus eugeniae* compared with a fresh earthworm. The decrease in the anticoagulant activity of dried earthworms is due to the inactivation of biologically active enzymes during drying. Fu et al. (2013) reported that active ingredients like fibrinolytic
enzymes might inactivate during the drying process. It was observed that microwave-vacuum dried earthworms exhibited the strongest anticoagulant activity compared with dehumidified and freeze-dried earthworms. However, the anticoagulant activity of Microwave-vacuum, Dehumidified-air, and Freeze-dried *Eudrilus eugeniae* is insignificantly different from each other. The result implies the availability of drying methods that can give similar results aside from freeze-drying.

The change in the absorbance of the test solutions at 700nm for every 10-second interval was depicted in Figure 5. It can be observed that the sharp change in the slope is the start of coagulation. It could be noticed that there is no significant increase in the slope of heparin-treated and fresh-earthworm-treated plasma, and showing no signs of coagulation. This indicates that the biologically active and naturally present compounds in the fresh *Eudrilus eugeniae* are comparable with heparin.

### Table 6. Clotting time of Blood Plasma and other test solutions.

| TREATMENT                      | CLOTTING TIME (SECONDS) |
|--------------------------------|--------------------------|
| Heparin (positive)             | No clotting*             |
| Blood plasma (negative)        | 40*                      |
| Fresh earthworm sample         | No clotting*             |
| Microwave-vacuum dried         | 250*                     |
| Freeze-dried                   | 230*                     |
| Dehumidified-air dried         | 200*                     |

*a = significant at 95% confidence level; values shown are mean values of three replicates*

![Figure 5. Absorbance at 700 nm of test solutions for anticoagulant assay](image)

### 4. Summary and Conclusions

In the current work, the drying kinetics, water activity, glass transition, and anticoagulant activity of dried earthworm, *Eudrilus eugeniae* using microwave-vacuum, dehumidified-air, and freeze-drying methods were evaluated. The results validated that Dehumidified-air and microwave – vacuum drying gives a more promising drying rate and drying time and has relatively low operational cost compared with freeze-drying. Freeze drying has relatively longer drying time, resulting in a higher operational cost. The two-term and Midilli models were considered the best models to describe the thin-layer drying behaviors of earthworm samples in Dehumidified-air drying conditions. Of the nine mathematical drying models tested, the Midilli model was considered the best model in Microwave-vacuum drying conditions while in freeze-drying, the Diffusion approach was the most suitable. The water activity of the dried earthworms is within the recommended or acceptable value for safe storage.
Still, the water activity of dehumidified-air dried is in the critical range or extreme tolerable water content, wherein a slight increase in the moisture content would allow mold, bacteria, and fungus to persevere.

Dried earthworms used in this study have a high crude protein based on the proximate analysis. Proximate composition of dried Eudrilus eugeniae is comparable with the reported proximate composition of different earthworm species like Eisenia fetida. Glass transition temperature was not detected experimentally using Differential Scanning Calorimetry due to the high protein content of the samples. The presence of anticoagulant property of fresh earthworm Eudrilus eugeniae was substantiated, and results showed that it is comparable with commercially available anticoagulants like heparin. It was demonstrated that drying methods altered the anticoagulant activity of earthworms but still exhibited a relatively high anticoagulant activity. The results herein revealed that microwave-vacuum and dehumidified-air drying are feasible alternatives for drying heat-sensitive samples like earthworms. The study also validated the presence and potential anticoagulant activity of earthworm Eudrilus eugeniae.

5. Recommendations

1. The bacteriological test should be done to determine if the sample is contaminated during the drying processes.
2. Other biological activity assessments, such as anti-inflammatory, antibacterial, and anti-ulcer tests, should also be conducted.
3. Alternative/different methods in extracting the active compound from fresh earthworms should be studied.

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