



UNIVERSITI PUTRA MALAYSIA

***NUTRITIONAL AND BIOACTIVITY PROPERTIES OF SELECTED
EARTHWORM SPECIES FOR POTENTIAL COSMECEUTICAL
APPLICATIONS***

NURHAZIRAH BINTI AZMI

IPPH 2013 5



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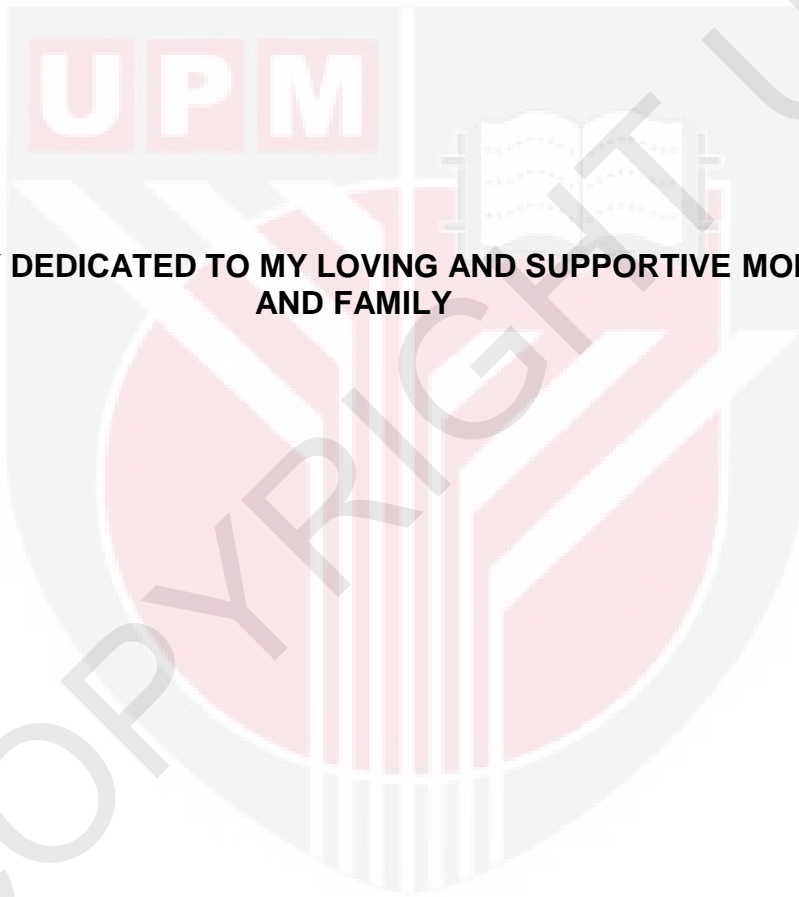
**MASTERS OF SCIENCE
UNIVERSITI PUTRA MALAYSIA**

2013

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**ESPECIALLY DEDICATED TO MY LOVING AND SUPPORTIVE MOM, DAD
AND FAMILY**

Abstract of thesis presented to the Senate of University Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

**NUTRITIONAL AND BIOACTIVITY PROPERTIES OF SELECTED
EARTHWORM SPECIES FOR POTENTIAL COSMECEUTICAL
APPLICATIONS**

By

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December 2013

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Earthworms are invertebrates that have been widely applied to environmental and land study, however, a new chapter opens opportunities of research in cosmetic and medicine. The objectives of the experiment were to evaluate the nutritional and antioxidant properties from the earthworms *Eudrilus euginae*, *Eisenia fetida* and *Lumbricus rubellus* and to examine the bioactivity properties of the earthworms extract in tyrosinase, matrix metalloproteinase-1, and elastase inhibitory activities for cosmeceutical applications. The analyzed components include moisture, available carbohydrates, total lipids, dietary fiber, crude protein, amino acids, ash, and minerals. The amino acids profile of the earthworm extracts was evaluated using High Performance Liquid Chromatography (HPLC). Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-Page) was used to determine and separate the protein based on size. The results showed *E. fetida* had slightly higher amount of protein and ash than *L. rubellus* and *E. euginae*. All the earthworm extracts showed high amounts of minerals ranging from 3-22% in calcium, potassium and phosphorus. All the extracts had the least amount of the amino acid cysteine, ranging from 0.17-0.21 mg/100 g protein, while the highest concentration is leucine, being 1.0-2.5 mg/100 g protein. Lysine and methionine, which were the limiting amino acids in most feedstuff, were present in all the extracts, ranging from 0.29-1.16 mg/100 g proteins and 0.3-0.57 mg/100 g protein, respectively. Molecular weights of earthworm extracts showed from 13-430 kDa under the optimal conditions of sodium dodecyl

sulphatepolyacrylamide gel electrophoresis (SDS-Page). *E. fetida* exhibited wide molecular weight distribution compared to *L. rubellus* and *E. eugeniae* which consisted of 11, 10 and 6 prominent bands, respectively. In addition, *E. fetida* had the highest antioxidant activity (91.76%) using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay compared to *E. eugeniae* and *L. rubellus*. At 25 mg/ml concentration, the antioxidant activity of *E. fetida* using DPPH assay was almost comparable to ascorbic acid (10mg/ml) which is usually used as antioxidant in most pharmaceutical products. All extracts demonstrated excellent inhibitory effects on tyrosinase. Extract from *E. fetida* gave the highest tyrosinase inhibition activity (80.12%) compared to *L. rubellus* (72.02%) and *E. eugeniae* (71.28%). However, their inhibition was still lower than the standard L-cysteine. In conclusion, this study provides nutritional compositional of earthworms as well as new findings on bioactivity properties having antioxidant, anti-tyrosinase and anti-aging properties. All the earthworms demonstrated valuable properties in antioxidant, anti-aging and whitening inhibition capacity despite the species. As such, it can be a potential candidate for application and utilization in cosmeceutical products.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Master Sains

CIRI-CIRI NUTRISI DAN BIOAKTIVITI SPESIES CACING TANAH TERPILIH UNTUK POTENSI APLIKASI KOSMESEUTIKAL

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Cacing tanah adalah invertebrata yang digunakan secara meluas dalam kajian berkaitan alam sekitar dan tanah, namun terdapat dimensi baru dalam ruang penyelidikan dalam penggunaan cacing tanah dalam bidang kosmetik dan perubatan. Objektif eksperimen adalah untuk mengenal pasti komposisi pemakanan daripada ekstrak cacing tanah daripada *Eudrilus euginae*, *Eisenia fetida* dan *Lumbricus rubellus* serta untuk mengkaji bioaktif yang berpotensi daripada ekstrak cacing tanah untuk aktiviti antioksidan, anti-penuaan dan pemutihan kulit untuk kegunaan kosmeseutikal. Komponen yang dianalisis terdiri daripada jumlah kelembapan, jumlah lipid, protein, asid amino, abu, dan mineral. Profil asid amino dari ekstrak cacing tanah telah dinilai menggunakan Kromatografi Cecair Prestasi Tinggi (HPLC). Selain itu, saiz protein daripada ekstrak cacing tanah juga telah dikaji menggunakan Polyakrilamide Sulfat Natrium dodesil elektroforesis gel (SDS-Page). Analisis dari kajian menunjukkan *E. fetida* mempunyai jumlah yang lebih tinggi untuk protein dan abu daripada *L. rubellus* dan *E. euginae*. Semua ekstrak cacing tanah menunjukkan jumlah yang tinggi dalam mineral iaitu di antara 3-22% dalam kalsium, kalium dan fosforus. Selain itu, semua ekstrak juga menunjukkan kepekatan tertinggi untuk asid amino leusin dengan julat kadar 1-2.5 mg/100 g protein manakala kepekatan terendah untuk asid amino sistin dengan julat kadar 0.17-0.21 mg/100 g protein. Lisin dan methionine masing-masing hadir dalam ketiga-tiga ekstrak cacing tanah dengan kadar julat di antara 0.29-1.16 mg/100 g protein dan 0.3-0.57 mg/100 g protein. Berat molekul protein yang terdapat di dalam

ekstrak cacing tanah menunjukkan kadar di antara 13-430 kDa di bawah keadaan optimum menggunakan SDS Page. *E. fetida* menunjukkan pengedaran berat molekul yang lebih luas berbanding *L. rubellus* dan *E. eugeniae* yang terdiri daripada 11, 10 dan 6 band. Selain itu, ekstrak *E. fetida* juga menunjukkan aktiviti antioksidan yang tertinggi (91.8%) dengan menggunakan ujian 2,2-diphenyl-1-picrylhydrazyl(DPPH) berbanding dengan *E. eugeniae* dan *L. rubellus*. Pada kepekatan 25 mg/mL, aktiviti antioksidan *E. fetida* adalah hampir setanding dengan asid askorbik (10 mg/mL) yang biasanya digunakan sebagai antioksidan komersial di dalam kebanyakan produk farmaseutikal. Semua ekstrak menunjukkan aktiviti perencatan yang cemerlang terhadap enzim tyrosinase. Ekstrak dari *E. fetida* memberikan perencatan aktiviti tertinggi (80.12%) berbanding dengan *L. rubellus* (72.02%) dan *E. eugeniae* (71.28%), namun perencatan ketiga-tiga ekstrak cacing tanah tersebut masih rendah jika berbanding dengan L-sistin yang digunakan sebagai rujukan. Kesimpulannya, kajian ini menunjukkan komposisi analisis pemakanan dari cacing tanah serta penemuan baru yang bernilai tinggi pada bioaktiviti dalam antioksidan, anti-tyrosinase dan aktiviti anti-penuaan. Oleh itu, ia berpotensi untuk menjadi calon ramuan dalam produk kosmeseutikal pada masa hadapan.

ACKNOWLEDGEMENTS

Bismillahirrahmanirrahim

The author would like to thank Allah swt for His Guidance, Mercy and Blessings, without which the author would never have completed this study. The author wishes to express her gratitude and thanks to Dr. Puziah Hashim, En. Dzulkifly Mat Hashim, and Dr. Normala Halimoon for their supervision, constructive criticism and guidance in carrying out this study.

The author is also indebted to the support and co-operation given by the staff of Halal especially to the late Prof. Dato' Dr. Yaakob Che Man and Prof. Dr Shuhaimi Mustaffa from Halal Products Research Institute, Universiti Putra Malaysia. Last but not least, the author would like to express her warmest gratitude to her beloved parent, Hj. Azmi Abdul Wahab and Hjh. Hazilah Abdullah and her siblings, Ammar, Amir, and Atikah for their encouragement, love, endless support, understanding and patience during the course of this study.

Expandable gratitude and thanks also to colleagues and friends, Nadia, Alia, Nisa, Shafiqah, Wani, Umi, Norra, Aina, Azira, Illi, Yanti, Irmey, Aimi, Ima and Aisyatul for their co-operation and constant motivation through their knowledge and insight throughout my research study.



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LIST OF ABBREVIATIONS

DPPH	1,1-diphenyl-2-picrylhydrazyl
EE	Earthworm extract
EFE	Earthworm fibrinolytic enzyme
EGCG	Epigallocatechin
EWP	Earthworm powder
HCl	Hydrochloric acid
H ₂ SO ₄	Sulphuric acid
HNO ₃	Nitric acid
HPLC	High Performance Liquid Chromatography
NNGH	N-Isobutyl-N-(4-methoxyphenylsulfonyl) glycol hydroxamic acid
ROS	Reactive oxygen species
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
TCA	Trichloroacetic acid

CHAPTER 1

GENERAL INTRODUCTION

There are about 4500 species of worms in the world and 2,700 different types of earthworms alone. In the classification system, earthworms are the largest members of the Oligochaeta in the phylum Annelida (Campbell and Reece, 2002). Earthworms are invertebrates, which lacks a skeleton; and are hermaphrodites, possessing both male and female sexual systems for reproduction (Ismail, 1997; Edwards and Bohlen, 1996). This unique characteristic made earthworms reproduced very easily and contributes to well environmental adjustment. They can be classified and identified based on several features such as morphological, physiological, phylogenicity, ecological, as well as their behavioral characteristics. The main families of earthworm are *Lumbricidae*, *Moniligastridae*, *Megascolecidae*, *Eudrilidae*, and *Glossoscolecidae*. Earthworms are found all over the world. They survived over a million years due to their ability of adjusting to different environmental conditions. However, they are rarely found in extreme areas such as deserts, land with constant ice and snow, areas with low vegetation, mountain ranges as well as areas lacking soil (Edwards and Bohlen, 1996). They will burrow themselves in deeper layer of the soil to protect from low temperature during the winter and the summer dryness to protect from dehydration (Brusca and Brusca, 2003).

The dorsal side of the worm is dark while the ventral side is paler. Earthworms breathe through their skin and they are very sensitive on changing the temperature, the light and on the touch, so it is essential for its skin to be moist and live constantly in damp environments such as soil enriched with organic substances as to ensure successful gas exchange for respiration. It will eventually suffocate to death if the skin dries out. They possessed segmented bodies as their muscle system is built with circular and longitudinal muscles and with their combination of shrinkage and spread made the earthworms able to move. The earthworms' body is covered with small fluffs, which is important in environmental adjustment and for search of the food in the soil. Their size ranges from an inch to two yards (Pechenik, 2009).

Earthworms need to live near their food source which includes leaves and grasses. These types of vegetation contain bacteria, algae and fungi which contribute to a healthy diet for the earthworm. Other types of diet are plants, fruits and vegetables as well as dirt (Mihara *et al.*, 1996). One of the uses of earthworms in soil and farming can be seen by manipulating their population in the soil. This technique can improve soil quality by enhancing soil structure, turnover and act as fertilizer (Brown *et al.*, 1999). Earthworms play a fundamental role in the subject of inoculation programs for the restoration of

degraded lands (Butt, 1999) and metal-contaminated soils (Dickinson, 2000). This is mainly due to the role of earthworms itself in soil formation at such fields (Frouz *et al.*, 2007). In addition, the waste products of earthworm diet enrich the soil with nutritive substances, which stimulate the growth of plants. However, the earthworms are very important source of diet for numerous animals in the soil. The earthworms are the major decomposers of dead and decomposing organic matter, and acquire their nutrition from the bacteria and fungi that grow upon these materials. They decompose the organic matter and make the major contributions to recycling the containing nutrients. The knowledge of the use of common earthworm powder has been known and utilized since ancient times for treating various diseases from common disease like flu, inflammation to cancer and heart attack. Scientific interest, specifically pharmaceutical use of earthworms had been initiated since the 1980s. Mihara *et al.* (1983) was the first to isolate and study *Lumbricus rubellus*'s proteases for their fibrinolytic activity, followed by isolation and purification by gel filtration, affinity chromatography, ion exchange chromatography and high pressure liquid chromatography (HPLC).

In Malaysia, earthworms have been used in vermicomposting (Hamydy, 2008), and as fish feed (Zakaria *et al.*, 2012). Such utilization is now gaining attention in agriculture and livestock industry where many local studies had shown the benefits of earthworms in having a quality amount of valuable nutritional properties such as proteins and fibers. In addition, it can generate a profitable income for entrepreneurs and also regarded as green technology as it provides sustainable options for the human and environment.

1.1 Statement of Problem

Cosmetics are defined as substances or preparations for external use on the human body such as skin, hair, nails, lips, teeth and oral cavity for the purpose of cleaning, fragrance, facial skin, body odor, protect or maintain in good condition. Those goods are synonyms with women especially young adults and teenagers, but nowadays, the use of cosmetics has been used not limited to women only, but to nearly all strata of society that men, adolescents, young adults and senior citizens. The global cosmetics industries had more than \$270 billion in sales in 2006. These industries are outpacing most others. The largest global segment is skin care and is estimated to have more than \$31 billion during this same time period. Euromonitor International forecasts annual global growth of 3.7% to reach international sales of more than \$700 billion by 2014. According to market researcher, the sales in the cosmetics and personal care sectors are nearly \$5 billion globally where the highest adoption rates are in countries like Malaysia and Indonesia.

It is known that majority of the Muslims depends on the cosmetics products made by the non-Muslims where the Muslims seem have to take the back seat where the cosmetics industry was dominated and monopolized by the non-Muslims. Thus, the issue of halal ingredients in cosmetics products showed serious challenges among the Muslims in order to find a suitable cosmetic product that is permissible under Shariah (Islamic law), a parameter designed for health, safety and benefit of all mankind regardless of age, faith or culture. In the scope of Halal cosmetics, the concept covers critical aspects of production including sourcing of Halal ingredients and usage of permissible substances-all must be manufactured, stored, packaged, and delivered in conformity with Shariah requirements.

On the other hand, they just sincerely did not know and realize that most cosmetics and personal care products on store shelves today contain five major categories of toxic ingredients which were carcinogens ingredients, precursors of or "hidden" carcinogens; endocrine or hormonally disruptive; penetration enhancers; and allergens. Notwithstanding the critical concern of authenticity of halal ingredients, the issue now has extended to toyyibah aspect which encompasses the safety and cleanliness of the products. The most paramount factor in deciding a product or ingredient as Halal is based on the premise of preserving human health. This creates an increase in consumers' awareness and demands for value-added contains halal, natural, and organic-based cosmetics.

However, the natural, organic and halal- based cosmetics are still lacking in the market. One of the alternatives being looked into is the study of bioactive components with medicinal value from earthworms as to meet the increasing demand for natural-based cosmeceuticals throughout the global. Since very little information is available on the use of earthworms in cosmeceutical applications, it is important to conduct further research. Therefore, the objectives of this study were:

- a) To evaluate the nutritional and antioxidant properties from the earthworms *Eudrilus eugeniae*, *Eisenia fetida* and *Lumbricus rubellus*.
- b) To examine the bioactivity properties of the earthworms extract in tyrosinase, matrix metalloproteinase-1, and elastase inhibitory activities for cosmeceutical applications.

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