UNIVERSITI PUTRA MALAYSIA

EXTRACTION OF PHENOLIC COMPOUNDS FROM EARTHWORMS LUMBRICUS RUBELLUS AND EUDRILUS EUGENIAE (AUTHORITY) AND EVALUATION OF THEIR ANTIOXIDANT ACTIVITIES FOR COSMECEUTICAL USE

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FPAS 2013 23

EXTRACTION OF PHENOLIC COMPOUNDS FROM EARTHWORMS *Lumbricus rubellus* AND *Eudrilus eugeniae* (Authority) AND EVALUATION OF THEIR ANTIOXIDANT ACTIVITIES FOR COSMECEUTICAL USE

By

QASIM MOHAMMED MATROOD ALDARRAJI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

July 2013

DEDICATION

To

The soul of my Father

My dear Mother

My wife

Abstract of dissertation submitted to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Master of Science.

EXTRACTION OF PHENOLIC COMPOUNDS FROM EARTHWORMS *Lumbricus rubellus* AND *Eudrilus eugeniae* (Authority)AND EVALUATION OF THEIR ANTIOXIDANT ACTIVITIES FOR COSMECEUTICAL USE

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July 2013

Chairman: Normala Halimoon, PhD

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Synthetic materials used in cosmetics products have adverse effects on the skin causing skin irritation. Natural bioactive compounds have no adverse effects on the skin or any other parts of the body. Cosmeceuticals are materials having natural bioactive compounds that have medicinal benefits. Unlike drugs, Cosmeceuticals do not have any adverse biological effects on the living tissues. The objectives of this study were to extract the phenolic compounds present in the earthworm paste (EP) of red worm (*Lumbricus rubellus)* and African night crawler (*Eudrilus eugeniae)* and evaluation its antioxidant activity for Cosmeceutical use. The Folin-Ciocalteu procedure was used to quantify the phenolic compounds. Identification of the phenolic compounds in the earthworm paste was achieved by using high-performance liquid chromatography (HPLC), followed by confirmation of compounds using liquid chromatography mass spectroscopy (LC/MS). Tow types of

solvent materials were used in the experiment: methanol and ethanol with different concentration levels 80% and 75%. Type of solvent used at different concentration levels had no effects on the total phenolic contents of the earthworm paste. In this study, the earthworm paste from the red worm extract yielded higher amounts of phenolic compounds than that of the African night crawler extract. HPLC analysis of the phenolic compounds revealed the presence of phenolic acids, cinnamic and benzoic acid derivatives, which were measured at 270, 280, 290, 300 and 325 nm. These compounds included gallic, chlorogenic, salicylic, caffeic, syringic and *p*-coumaric acids. The red worm extract had more phenolic acids than that of the African night crawler extract. LC/MS confirmation showed two compounds in the methanolic extract of the earthworm paste of the African night crawler. These compounds were sinapic and caffeic acids. In the methanolic extract of the earthworm paste of the red worm, four phenolic acids were confirmed according to their molecular weight. The compounds were syringic, *p*- coumaric, gallic and ferulic acids. The compounds identified were phenolic acids, which showed significant antioxidant activity. The antioxidant activity of each extract was assessed using the DPPH assay. All the extracts showed a strong antioxidant activity. The 75% methanolic extract of the earthworm paste of the red worm exhibited stronger antioxidant activity than that of 80% methanol and 80% ethanol. In contrast, 80% methanolic extract of the earthworm paste of the African night crawler revealed the strongest antioxidant activity. Earthworm extract of African night crawler revealed a stronger antioxidant activity than that of the red worm extract. In conclusion,The results shown that earthworms paste had notable amounts of phenolic compounds with strong antioxidant activity.

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

PENGEKSTRAKAN SEBATIAN FENOLIK DARIPADA CACING *Lumbricus rubellus* **DAN** *Eudrilus eugeniae* (Pihak berkuasa) **SERTA PENILAIAN AKTIVITI ANTIOKSIDAN UNTUK KEGUNAAN KOSMESEUTIKAL**

Oleh

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Bahan sintetik yang digunakan dalam produk-produk kosmetik mempunyai kesan buruk pada kulit yang menyebabkan kerengsaan kulit. Sebatian bioaktif semula jadi tidak mempunyai kesan buruk pada kulit atau mana-mana bahagian badan. Kosmeseutikal ialah bahan-bahan yang mempunyai sebatian bioaktif semulajadi yang mempunyai manfaat untuk perubatan. Kosmeseutikal juga tidak mempunyai apa-apa kesan buruk biologi pada tisu-tisu hidup. Objektif kajian ini adalah untuk mengekstrakk sebatian fenolik yang terdapat dalam pes cacing (EP), iaitu cacing merah *(Lumbricus rubellus*) dan Afrikan night crawler *(Eudrilus eugeniae*) dan juga untuk menilai aktiviti antioksida untuk kegunaan kosmeseutikal. Prosedur Folin-Ciocalteu telah digunakan untuk mengukur sebatian fenolik. Pengenalpastian sebatian fenolik dalam pes cacing tanah telah dicapai dengan menggunakan cecair kromatografi berprestasi tinggi (HPLC), diikuti oleh pengesahan sebatian yang

menggunakan cecair kromatografi spektroskopi massa (LC / MS). Dua jenis bahan pelarut telah digunakan dalam eksperimen, iaitu metanol dan etanol dengan berlainan tahap kepekatan pada 80% dan 75%. Jenis pelarut yang digunakan pada tahap kepekatan yang berbeza tidak memberi kesan pada jumlah kandungan fenolik daripada pes cacing tanah. Dalam kajian ini, pes cacing tanah daripada ekstrak cacing merah menghasilkan jumlah sebatian fenolik yang lebih tinggi daripada ekstrak Afrikan night crawler. Analisis HPLC dari sebatian fenolik menunjukkan kehadiran asid fenolik, cinnamik dan benzoik acid derivatives, yang diukur pada 270, 280, 290, 300 dan 325nm. Sebatian-sebatian ini termasuk gallik, klorogenik, salisilik, caffeik, syringik dan asid *p*-coumarik. Ekstrak cacing merah mempunyai asid fenolik yang lebih tinggi berbanding dengan ekstrak African night crawler. Pengesahan LC/MS menunjukkan dua sebatian fenolik dalam ekstrak metanol daripada pes cacing African night crawler. Sebatian-sebatian ini ialah asid sinapik dan asid caffeik. Dalam ekstrak metanol daripada pes cacing tanah, empat asid fenolik telah disahkan mengikut keberatan molekul-molekulnya. Sebatian-sebatian itu ialah syringik, *p*-coumarik, gallik dan asid ferulik. Sebatian yang dikenalpasti adalah asid fenolik yang menunjukkan aktiviti antioksidan yang secara ketara. Aktiviti antioksidan bagi setiap ekstrak telah dinilai dengan menggunakan cerakin DPPH. Semua ekstrak menunjukkan aktiviti antioksida yang kuat. 75% ekstrak methanol daripada pes cacing merah menunjukkan aktiviti antioksida yang lebih kuat daripada 80% metanol dan 80% etanol. Sebaliknya, 80% ekstrak metanol daripada pes cacing tanah *African night crawler* mendedahkan aktiviti antioksida yang paling kuat. Ekstrak cacing *African night crawler* mendedahkan aktiviti antioksida yang lebih kuat daripada ekstrak cacing merah. Hasil kajian menunjukkan pes cacing tanah

mempunyai jumlah sebatian fenolik secara ketara yang mana ia berkaitan dengan asid-asid fenolik dengan aktiviti antioksida yang kuat.

ACKNOWLEDGEMENTS

Firstly, my gratitude goes to Allah Almighty who helped me complete my study. I thank merciful Allah for his gifts of patience, wisdom and perseverance to pursue my dreams of attaining academic success.

I would like to express my sincere gratitude and thanks to the chairman of my supervisory committee, Dr. Normala Halimoon for the continuous support of my study and research, for her patience, motivation, enthusiasm, and immense knowledge. Her guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my study. I would also like to thank the Prof. Dato' Dr. Nik Ab. Muhamad Nik Majid for his encouragement and comments.

My sincere thanks also go to all laboratory staffs of the Faculty of Environmental Studies especially, Miss. Siti Norlela Bt. Talib, the HPLC Operator, Mr. Zuber Mohd Saad. Mr, Haji Abdul Rashid Haron and Mr. Ahmad Ithanin for their help and permission to do my study and use of facilities in their laboratories.

My appreciation and gratitude goes to my dear brothers, Jassim and Yasser for their constant support, love and patience. My wholehearted thanks go to my mother for her support and encouragement. My sincere thanks also go to my wife and to all my children for their patience and encouragement. I have no suitable words that can describe their everlasting love and patience. I hope the accomplishment of this

dissertation represents a small token of my appreciation. I would like to thank my best friend Jaafar Al-Frajat for his help and encouragement. I appreciate all my friends for their kindness and their invaluable assistance.

I certify that a Thesis Examination Committee has met on 16 July 2013 to conduct the final examination of Qasim Mohammed Matrood Al-Darraji on his thesis entitled "Extraction of Phenolic Compounds from Earthworms *Lumbricus rubellus* and *Eudrilus eugeniae (*Authority) and Evaluation of Their Antioxidant Activities for Cosmeceutical Use" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.

QASIM MOHAMMED MATROOD ALDARRAJI

Date: 16 July 2013

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LIST OF ABBREVIATIONS

AAPH	2, 2 azobis (2-amidinopropane) dihydrochloride
ABTS	2, 2 azino-bis (3-ethylpbenothiazolin-6-sulphonat)
AHA	Alpha hydroxy acids
ALA	Alpha lipoic acids
ANOVA	Analysis of variance
BBC	British Broadcasting Corporation
CAT	Catalase
DNA	deoxy ribonucleotide
DPPH	1, 1 diphenyl 1-2 –picrylhydrazl
EC_{50}	Efficient concentration index
EDTA	Ethylenediaminetetraacetic acid
EFE	Earthworm fibrinolytic enzymes
EP	Earthworm paste
ESI	Electroscopy ionization
FC	Folin-Ciocalteu
FDA	Food and Drug Administration
FRAP	Ferric ion reducing antioxidant power
G-90	Glycolipoprotien
GAE	Gallic acid equivalent
GP_x	Glutathione peroxidase
GSH	Reduced Glutathione

H_2O_2	Hydrogen peroxide
HPLC	High Performance Liquid Chromatography
KG	Kilogram
L	Liter
LC/MS	Liquid Chromatography-Mass Spectroscopy
LC/MS/MS	Liquid chromatography with tandem mass spectrometry
M/Z	Mass to charge ratio
MEH	Melanocytes stimulating hormone
MG	Milligram
ML	Milliliter
MS	Mass spectrometry
NG	Nanogram
NMR	Nuclear magnetic resonance
OCH_3	Methoxy group
OH	Hydroxy group
ORAC	Oxygen Radical Absorbance Capacity
PMA	Phosphmolybdic acid
PPM	Parts per million
PTA	Phosphotungestic acid
R-HPLC	Reversed-High-Performance Liquid Chromatography
ROS	Reactive oxygen species
SOD	Superoxide dismutase

TEAC	Trolox equivalent antioxidant capacity
TPC	Total phenolic content
UV	Ultraviolet
W/V	Weight-volume
WHO	The World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background

Bioactive compounds are secondary metabolites found in living organisms. Bioactive compounds are also called nutraceuticals which was coined by Stephen De Felice in 1989 from nutrition and pharmaceutical (Biesalski *et al.,* 2009). The most important feature of bioactive compounds is the antioxidant activity. Antioxidant materials play a vital role in reducing the damage of the body by radicals in the body (Zheng *et al.,* 2001*).* Physiological and biochemical activities in the body of human beings are primarily responsible for producing these unwanted products that can cause oxidative damage to essential molecules such as protein, lipids and DNA. Consequently, the damage can produce many ailments mainly, aging, diabetes and cancer (Choi *et al*., 2002). Phenolic compounds act as free radical scavenging molecules. Antioxidant compounds have many medicinal characteristics such as anticarcinogenic, antitumor, antiatherclerosis, antibacterial, anti-inflammatory and antiviral (Halliwell, 1994). Therefore, consuming of natural antioxidant compounds may reduce the threats of cancer and other diseases related to aging.

The earthworm is one of the potential natural sources of antioxidant. Earthworm paste or extracts display antioxidant activity by prohibiting the oxidative damage. A high amount of enzymes in the tissues of earthworms is responsible for the radical scavenging activity of earthworms. Glycolipoprotein isolated from the earthworm species *Eisenia fetida* exhibited tissue repairing capacity, which represents

antioxidant ability, when it was cultured *in situ* in human fibroblast and epithelial cells (Grdisa *et al.,* 2001).

However, the antioxidant activity is directly attributed to phenolics which can quench the free radicals owing to the presence of hydroxyl groups (Balamurugan *et al*., 2007). The polyphenol content in earthworm paste is higher than other components and is responsible for the anti-inflammatory and antioxidative activities (Prakash *et al*., 2007).

Cosmeceutical science is a study of drugs and cosmetics. Cosmeceutical includes both the therapeutical properties of drugs and the appearance properties of cosmetics. Cosmeceuticals are added to cosmetic products to achieve two purposes: improving the appearance of skin texture, and achieving pharmacological influence. Cosmeceuticals are the most important class of the natural personal-care industry (Kligman, 2005).

Skin aging is enhanced by the activity of reactive oxygen species (ROS). The most important features of anti-aging are wrinkles and pigmentation (Masaki, 2010). Melanin represents a principal pigment which is responsible for coloration of the skin. The melanin pigment is produced by the activity of melanocyte cells in the basal of the epidermis. After subjecting to the effect of certain elements, such as sun exposure, dark skin discoloration (melasma) or other diseases, melanin pigmentation may increase (Briganti *et al.*, 2003). The main enzyme which catalyses melanin, leading to hyperpigmentation, is tyrosinase.

Phenolics are one of the important constituents in plants. These compounds have shown tyrosinase inhibitory activity (Sugumaran, 2002). Furthermore, since the phenolic constituents have a close chemical structure of tyrosine, they can be utilized as depigmentation factors (Boissy and Manga, 2004).

Phenolics have gained more consideration due to their antioxidant properties. Natural antioxidant constituents are better than those of synthetic origins because of many factors such as safety, lack of side effects, harmless, and tolerance which will lead to increased consumer preference (Dai and Mumper, 2010). However, most researchers have focused on phenolic compounds extracted from the plant kingdom. Earthworms have notable profiles of phenolic compounds, which are available in the tissues of earthworms (Prakash *et al.*, 2007).

1.2 Significance of the Study

The cosmeceutical products are one of the growing trends in the cosmetics and beauty marketplace. Breakthroughs in biochemistry and skin physiology have made it possible to narrow the lines between cosmetics and medicine, and, based on British Broadcasting Corporation (BBC), the anti-aging global market was worth around $ 162 billion in 2008. This trend is expected to increase to $ 247.5 billion by 2013 (Dilip, 2009). The antioxidants present the largest class of cosmeceutical materials Recently, phenolic compounds have attracted more consideration and interest because of their antioxidant activity, which is considered an essential activity against reactive oxygen species (ROS). ROS has caused many severe ailments such as

cancer and aging (Dai and Mumper, 2010). Phenolic compounds extracted from earthworms can offer a new source of natural organic compounds in skin care.

1.3 Problem statement

Cosmetics products have synthetic compounds that have an adverse effect on the skin after using for a long time and cause dermatological disorders (Masse *et al.*, 2001). These compounds, such as hydroquinone and kojic acid, which are widely used in cosmetic material for skin-lightening have an unfavourable effect on the skin by causing skin irritation (Garcia and Fulton, 1996; Giroir *et al.,* 1991). Therefore, natural bioactive compounds have no adverse effects on the skin or any other parts of the body (Rivers, 2009). Moreover, most of the studies focused on the extraction of phenolic compounds from plant sources. They also focused on extraction of glycolipoprotein from the tissue and earthworm paste. Therefore, Literatures pertaining to the extraction and purification of phenolic compounds are absent. The earthworm is potential candidate for cosmeceutical materials.

1.4 Objectives of the study

The objectives of this present research are to study the phenolic compounds extracted from earthworm species as antioxidant materials. The specific objectives of the study are to:

1) Determine the total phenolic content in the extract of earthworms using different solvents

2) Verify phenolic compounds in earthworms using high pressure liquid chromatography (HPLC) and liquid chromatography-mass spectroscopy (LC/MS).

3) Evaluate the antioxidant activity of earthworm extracts

1.5 Scope of the study

The study focused on identification of phenolic compounds from earthworm paste of two species. The research work limits itself to use two species of earthworm because earthworm species are expensive and it is out of the scope of the study to raise them in vermicompost. Analysis depends on the extraction of earthworm paste from that two species.

There are no data about extraction and identification of phenolic compounds from earthworm. Most of the previous literatures focused only on the extraction of phenolic compounds from plant. Moreover, the study is limited by using several standards compound in HPLC since these compounds are expensive.

1.6 Thesis organization

This thesis consists of five chapters. The chapters have been organized as follows:

Chapter two discusses about earthworm and their products, bioactive compounds, cosmeceutical products, extraction of phenolic compounds , identification and confirmation of phenolic compounds and assessment of antioxidant activity.

Chapter three presents the research methodology. This chapter has been divided into three parts, namely extraction and quantification of phenolic compounds, identification and confirmation of phenolic compounds, and evaluation of the antioxidant activity of earthworm paste.

Chapter four presents the results and discussion about quantification of phenolic compounds, identification of phenolic compounds and assessment of antioxidant activity.

Chapter five presents a conclusion of the study and highlights recommendations for further study.

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Qasim Mohammed Aldarraji, Normala Halimoon, Nik M. Majid "Antioxidant activity and total phenolic content of earthworm paste of *Lumbricus rubellus* (red worm) and *Eudrilus eugenia* (African night crawler). Journal of Entomology and Nematology, Vol. 5 (3), pp. 33-37, May 2013.