UNIVERSITI PUTRA MALAYSIA

THE EFFECT OF DIFFERENT EXTRACTION METHODS ON ANTIOXIDANT ACTIVITY OF *ANNONA MURICATA*, *CLINACANTHUS NUTANS* AND *PERSICARIA HYDROPIPER*

NOOR SYAHIRAH ABDULLAH

FBSB 2015 157

THE EFFECT OF DIFFERENT EXTRACTION METHODS ON ANTIOXIDANT ACTIVITY OF *ANNONA MURICATA*, *CLINACANTHUS NUTANS* AND *PERSICARIA HYDROPIPER*

NOOR SYAHIRAH BINTI ABDULLAH

BACHELOR OF SCIENCES (HONS.)

UNIVERSITI OF PUTRA MALAYSIA

2015

THE EFFECT OF DIFFERENT EXTRACTION METHODS ON ANTIOXIDANT ACTIVITY OF *ANNONA MURICATA*, *CLINACANTHUS NUTANS* AND *PERSICARIA HYDROPIPER*

By

NOOR SYAHIRAH BINTI ABDULLAH

Thesis Submitted to the Department of Cell and Molecular Biology,

Faculty of Biotechnology and Biomolecular Sciences,

Universiti Putra Malaysia,

In Fulfilment of the Requirement for the Degree of

Cell and Molecular Biology

June 2015

Abstract of thesis presented to the Department of Cell and Molecular Biology in fulfilment of the requirement for the degree of Cell and Molecular Biology

THE EFFECT OF DIFFERENT EXTRACTION METHODS ON ANTIOXIDANT ACTIVITY OF *ANNONA MURICATA, CLINACANTHUS NUTANS* AND *PERSICARIA HYDROPIPER*

By

NOOR SYAHIRAH BINTI ABDULLAH

June 2015

Chair: Dr. Nik Mohd Afizan Bin Nik Abd Rahman, PhD

Faculty: Biotechnology and Biomolecular Sciences

Antioxidants are compound that helps to scavenge the reactive oxygen species (ROS) found in human body. The ROS are not naturally found in human body, but they can enter human cells through radiation, consumption of foods, life style or even from the environment. Reaction from the reactive oxygen species may cause cell and tissue damage which may leads to cancer formation. Ancient traditional medicine uses herbs to treat several cancers, internal and external wounds although they did not possess any scientific knowledge regarding the scavenging activity presents in those herbs. Plant basically produces active compounds that are required for their metabolisms and catabolisms. Antioxidant activity is a way of controlling ROS through scavenging activity which are also produced naturally in plants. Extraction for the antioxidant activity on *Annona muricata, Clinacanthus nutans* and *Persicaria hydropiper* is performed by using different extraction methods; methanol, ethanol,

water and hexane in order to determine which extraction methods will give the highest yields. The crude samples obtained from the extraction process will then further analysed for its scavenging activity to determine which plants contains the highest antioxidant activity. The data obtained shows that methanol extract of A. muricata gives the highest yield of active compounds.

Abstrak tesis yang dikemukakan kepada Jabatan Biologi Sel dan Molekul

Sebagai memenuhi keperluan untuk ijazah Biologi Sel dan Molekul

KESAN KAEDAH EKSTRAK YANG BERBEZA BAGI AKTIVITI ANTIOKSIDAN DALAM *ANNONA MURICATA, CLINACANTHUS NUTANS* DAN *PERSICARIA HYDROPIPER*

Oleh

NOOR SYAHIRAH BINTI ABDULLAH

Jun 2015

Pengerusi : Dr. Nik Mohd Afizan Bin Nik Abd Rahman, PhD

Fakulti : Bioteknologi dan Sains Biomolekul

Antioksidan adalah sejenis molekul yang membantu dalam menghapuskan radikal bebas yang terdapat dalam tubuh badan manusia. Radikal bebas tidak terdapat di dalam badan manusia secara semula jadi, sebaliknya ia boleh mengganggu system badan manusia melaui radiasi, pola pemakanan, cara hidup ataupun melalui persekitaran sekeliling yang tidak sihat. Hasil tindak balas daripada radikal bebas boleh mengakibatkan kerosakan sel dan tisu badan dan berupaya mengakibatkan penghasilan sel-sel kanser. Penggunaan ubat-ubatan tradisional yang berasaskan herba telah lama digunakan untuk merawat kanser serta luka dalaman dan luaran walaupun tiada pengetahuan saintifik wujud mengenai penghapusan radikal bebas

yang terdapat pada herba tersebut. Secara asasnya, tumbuhan menghasilkan komponen-komponen aktif yang diperlukan dalam proses metabolisma dan katabolisma. Antioksidan merupakan salah satu cara mengawal radikal bebas melalui process penghapusan radikal bebas yang turut dihasilkan secara semula jadi daripada tumbuhan. Ekstrak untuk menilai aktiviti antioxidan pada tiga jenis pokok, *Annona muricata*, *Clinacanthus nutans* dan *Persicaria hydropiper* diperoleh menggunakan etanol, metanol, hexan dan air untuk mengenal pasti cara ekstrak yang lebih berkesan. Bahan mentah yang diperoleh hasil daripada ekstak akan digunakan umtuk mengkaji kandungan antioksidan yang paling tinggi antara ketiga-tiga jenis tumbuhan tersebut. Menurut keputusan yang diperoleh, proses ekstrak menggunakan metana memberikan hasil yang lebih banyak dan analisis daripada hasil ekstrak membuktiksan bahawa kandungan antioksidan dalam *A.muricata* adalah lebih tinggi.

APPROVAL

This thesis was submitted to the Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences and has been accepted as fulfilment of the requirement for the degree of Cell and Molecular Biology. The member of the Supervisory Committee was as follows:

Name: Prof. Madya Dr. Janna Ong Abdullah, PhD

Title: Professor Madya

Faculty of Biotechnology and Biomolecular Science

Universiti Putra Malaysia

_____

(Prof. Madya Dr. Janna Ong Abdullah)

Head of Department of Cell and Molecular Biology

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

Date :

DECLARATION

Declaration by undergraduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by The Department of Cell and Molecular Biology
- written permission must be obtained from supervisor before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials.
- There is no plagiarism or data falsification/fabrication in the thesis.
- The thesis has undergone plagiarism detection software (TURNITIN)

Signature: _____ Date: _____

Name and Matric No.: Noor Syahirah Binti Abdullah (162585)

Declaration by Supervisor

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision

Supervisor,

_____

Dr. Nik Mohd Afizan Bin Nik Abd Rahman

ACKNOWLEDGEMENTS

First of all, I would like to express my appreciation and thankful to Allah, for His Most Kindness in giving the guidance, patience and strength for me to complete my final year project.

I would like to thank to my supervisor, Dr. Nik Mohd Afizan Bin. Nik Abd. Rahman for giving me advices, guidance's and moral supports throughout my project. His comments and opinions are significantly reliable and very valuable in completing this project. My next appreciation goes to my co-supervisor, Prof. Madya Dr. Noorjahan Banu Mohamed Alitheen, to laboratory assistant in the animal tissue culture lab, Mrs. Roszaimah Bt. Muhammad Sapah for their guidance and support. I also would like to give my appreciation to Agro-Biotechnology Institute for giving me the chances and permission to use all the equipment in order to perform and completes my project. My special thanks to all the research assistants and friends Dr. Wei Lun, Dr. Nadia, Firdaus, Umar, Miss Yani, Miss Rizi and Miss Rizi for all their kindness and support.

Not to forget, my parents Abdullah bin Haron and Wan Nooryah Bt. Wan Awang for always give support and endless motivation and pray. Last but not least, thank you to all my friends and individuals who has contribute directly or in directly along completing my final year project

Lastly, I hope this research can provide valuable information for future guidance.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AA	Ascorbate
$AlCl_3$	Aluminium chloride
CAT	Catalase
DHAR	Dehydroascorbate reductase
DPPH	2,2-diphenyl-2-picrylhydrazyl free radical
ELISA	Enzyme-linked immunosorbent assay
FC	Follin-Ciocalteu
FRAP	Ferric Ion Reducing Antioxidant Power
GPX	glutathione peroxidase
GSH	Glutathione
H_2O_2	Hydrogen peroxide
MDHAR	Monodehydroascorbate
mg	Milligram
nm	Nanometre
NaOH	Sodium hydroxide
$NaNO_3$	Sodium nitrate
O_2^-	Superoxide radical
O_2^1	Singlet oxygen
POX	Peroxidase
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
TPTZ	Tripyridyltriazine
%	Percentage

^{o}C	Degree Celcius
µg	Microgram
µl	Microliter
µg/ml	Microgram per milligram

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

Introduction

Annona muricata, *Clinacanthus nutans*, and *Persicaria hydropiper* are plants that can be found abundance in Malaysia and are believed to have good antioxidant activity depending on their scavenging activity and bioactive compounds. The bioactive compound or is polyphenolic contents are considered as the secondary product and produced naturally in the plants especially for metabolism and biochemical defence system. Plants secondary metabolites are also required for plant differentiation and development (Collin, 2000). Different parts of the plant have different concentration of secondary metabolites. On the other hand, production of reactive oxygen species (ROS) comes about with side effect which is detrimental to both plant and human even though it is an important mechanism for plant development.

Since ancient times, herbs and medicinal plants have been used to relieve pain, cure disease, treat external or internal tissue damage and cure cancer. Usually, they consume this plant either by making tea out of the leaves; applying it directly to the wounded area or by consuming it together with their diets. Extracting the phytochemical content of the secondary metabolites requires proper extraction procedure. Different extraction methods will give different results of its antioxidant activity when subsequent antioxidant assay is performed. Methanol, ethanol, water and hexane act as the extraction solutions in which each of this solution was used to extract the active compound present in the plant samples. These different extraction processes produce various extraction yield depending on the polarity of the active compound. Total phenolic content (TPC), total flavonoid content (TFC), fluorescent

Ferric Ion Reducing Antioxidant Power (FRAP) and 2,2-diphenyl-2-picrylhydrazyl free radical (DPPH) assay were used to determine the antioxidant activity. DPPH is stable free radicals that are used to measure the scavenging activity of the bioactive compounds. The scavenging activity was measured by determining the color changes occurred from violet color into pale yellow color; the reduced form of free radicals. The color variation produced was then expressed using IC_{50}. This value was then determine the concentration of antioxidant required to scavenge 50% of the free radicals. FRAP also have the same principal, in which the antioxidant capacity of the samples were determined based on the ability to reduce ferric tripyridyli triazine (Fe^{3}TPTZ) into Fe^{2}TPTZ. Intense blue color will achieve if the samples contain high antioxidant activity (Litescu *et al*., 2010). Phenolic content are basically determined based on the reduction of phosphotungstate-phosphomolybdenum complex by phenolate ion which causes formation of blue color. When phenolic content are higher, the blue color will become darker which shows antioxidant activity are higher (Mamta *et al*., 2013). Flavonoid content was also determined using aluminium chloride colorimetric assay. The formation of stable complex formed between aluminium and hydroxyl group of flavonoids which causes the formation of dark blue intensity. This blue colour is detected using ELISA at 430 nm. As the flavonoids content is higher, the color will become darker.

Therefore, the objectives of the study are:

1. To perform different extraction methods that will give the highest yield.
2. To analyse the effect of different extraction methods on the antioxidant activity of *Annona muricata*, *Clinacanthus nutans* and *Persicaria hydropiper*.

References

Abdullin, I. F., Turova, E. N., Gaisina, G. K., & Budnikov, G. K. (2002). Use of electrogenerated bromine for estimating the total antioxidant capacity of plant raw materials and plant-based medicinal preparations. *Journal of Analytical Chemistry, 57*(6), 557-560. doi:10.1023/A:1015758221044

Apel, K., & Hirt, H. (2004). REACTIVE OXYGEN SPECIES: Metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology, 55*(1), 373-399. doi:10.1146/annurev.arplant.55.031903.141701

BandonienÄ—, D., Murkovic, M., Pfannhauser, W., Venskutonis, P., & GruzdienÄ—, D. (2002). Detection and activity evaluation of radical scavenging compounds by using DPPH free radical and on-line HPLC-DPPH methods. *European Food Research and Technology, 214*(2), 143-147. doi:10.1007/s00217-001-0430-9

Bean, H., Radu, F., De, E., Schuler, C., Leggett, R., & Levin, R. (2009). Comparative evaluation of antioxidant reactivity within obstructed and control rabbit urinary bladder tissue using FRAP and CUPRAC assays. *Molecular and Cellular Biochemistry, 323*(1-2), 139-142. doi:10.1007/s11010-008-9972-5

Bora, F. W.,Jr, & Didizian, N. H. (1974). The treatment of injuries to the carpometacarpal joint of the little finger. *The Journal of Bone and Joint Surgery.American Volume, 56*(7), 1459-1463.

Campbell, J. K., King, J. L., Harmston, M., Lila, M. A., & Erdman, J. W. (2006). Synergistic effects of flavonoids on cell proliferation in hepa-1c1c7 and LNCaP

cancer cell lines. *Journal of Food Science, 71*(4), S358-S363. doi:10.1111/j.1750-3841.2006.00013.x

Christodouleas, D., Fotakis, C., Nikokavoura, A., Papadopoulos, K., & Calokerinos, A. (2014). Modified DPPH and ABTS assays to assess the antioxidant profile of untreated oils. *Food Analytical Methods,* , 1-9. doi:10.1007/s12161-014-0005-6

Collin, H. A. (2001). Secondary product formation in plant tissue cultures. *Plant Growth Regulation, 34*(1), 119-134. doi:10.1023/A:1013374417961

Elzaawely, A., & Tawata, S. (2012). Antioxidant capacity and phenolic content of rumex dentatus L. grown in egypt. *Journal of Crop Science and Biotechnology, 15*(1), 59-64. doi:10.1007/s12892-011-0063-x

Formagio, A. N., Kassuya, C. L., Neto, F., Volobuff, C. F., Iriguchi, E. K., Vieira, M., & Foglio, M. (2013). The flavonoid content and antiproliferative, hypoglycaemic, anti-inflammatory and free radical scavenging activities of annona dioica st. hill. *BMC Complementary and Alternative Medicine*, *13*(1), 1-8. doi:10.1186/1472-6882-13-14

Gaikwad, P., Barik, A., Priyadarsini, K. I., & Rao, B. S. M. (2010). Antioxidant activities of phenols in different solvents using DPPH assay. *Research on Chemical Intermediates, 36*(9), 1065-1072. doi:10.1007/s11164-010-0220-8

Goto-Yamada, S., Mano, S., & Nishimura, M. (2014). The role of peroxisomes in plant reproductive processes. In H. Sawada, N. Inoue & M. Iwano (Eds.), (pp. 419-429) Springer Japan. doi:10.1007/978-4-431-54589-7_35

Gupta, S. D., & Datta, S. (2003). Antioxidant enzyme activities during in vitro morphogenesis of gladiolus and the effect of application of antioxidants on plant regeneration. *Biologia Plantarum, 47*(2), 179-183. doi:10.1023/B:BIOP.0000022248.62869.c7

Habu, J., & Ibeh, B. (2015). In vitro antioxidant capacity and free radical scavenging evaluation of active metabolite constituents of newbouldia laevis ethanolic leaf extract. *Biological Research*, *48*(1), 1-10. doi:10.1186/s40659-015-0007-x

Hebelstrup, K., & MÃ¸ller, I. (2015). Mitochondrial signaling in plants under hypoxia: Use of reactive oxygen species (ROS) and reactive nitrogen species (RNS). In K. J. Gupta, & A. U. Igamberdiev (Eds.), (pp. 63-77) Springer International Publishing. doi:10.1007/978-3-319-10079-1_4

Huq Moyeenul A.K.M, Jamal Jamia Azdina and Stanlas Johnson. (2014). Ethnobotanical, phytochemical, pharmacological, and toxicological aspects of persicaria hydropiper (L.) delarbre., 11 pages.

Ibraheim, Z. Z., Nafady, A. M., Mostafa, M. A., & Amin, F. M. (2015). Antioxidant activity and total flavonoids content of aerial parts of ficus pyriformis hook. & arn.(moraceae) cultivated in egypt. *American Journal of Chemistry, 5*(1), 23-27.

Klepacka, J., Gujska, E., & Michalak, J. (2011). Phenolic compounds as cultivar- and variety-distinguishing factors in some plant products. *Plant Foods for Human Nutrition, 66*(1), 64-69. doi:10.1007/s11130-010-0205-1

Klepacka, J., Gujska, E., & Michalak, J. (2011). Phenolic compounds as cultivar- and variety-distinguishing factors in some plant products. *Plant Foods for Human Nutrition, 66*(1), 64-69. doi:10.1007/s11130-010-0205-1

Koren, E., Lipkin, J., Klar, A., Hershkovitz, E., Ginsburg, I., & Kohen, R. (2009). Total oxidant-scavenging capacities of plasma from glycogen storage disease type ia patients as measured by cyclic voltammetry, FRAP and luminescence techniques. *Journal of Inherited Metabolic Disease, 32*(5), 651-659. doi:10.1007/s10545-009-1242-5

Kurek-GÃ³recka, A., Rzepecka-Stojko, A., GÃ³recki, M., Stojko, J., Sosada, M., & Åšwierczek-ZiÄ™ba, G. (2013). Structure and antioxidant activity of polyphenols derived from propolis. *Molecules, 19*(1), 78-101. doi:10.3390/molecules19010078

Litescu, S., Eremia, S., & Radu, G. (2010). Methods for the determination of antioxidant capacity in food and raw materials. In M. Giardi, G. Rea & B. Berra (Eds.), (pp. 241-249) Springer US. doi:10.1007/978-1-4419-7347-4_18

Mamta, Misra, K., Dhillon, G., Brar, S., & Verma, M. (2014). Antioxidants. In S. K. Brar, G. S. Dhillon & C. R. Soccol (Eds.), (pp. 117-138) Springer New York. doi:10.1007/978-1-4614-8005-1_6

Mittler, R., Vanderauwera, S., Gollery, M., & Van Breusegem, F. (2004). Reactive oxygen gene network of plants. *Trends in Plant Science, 9*(10), 490-498. doi:http://dx.doi.org/10.1016/j.tplants.2004.08.009

Moure, A., Franco, D., Sineiro, J., DomÃnguez, H., NÃºÃ±ez, M. J., & Lema, J. M. (2000). Evaluation of extracts from gevuina avellana hulls as antioxidants. *Journal of Agricultural and Food Chemistry, 48*(9), 3890-3897. doi:10.1021/jf000048w

Nazeer, R. A., & Naqash, S. (2013). In vitro antioxidant activity of two molluscs, loligo duvauceli orbigny and donax cuneatus linnaeus, by solvent extraction methods. *Mediterranean Journal of Nutrition and Metabolism, 6*(1), 17-21. doi:10.1007/s12349-011-0088-1

Ohashi, S., Okada, N., Abdul Azim, A., Siripatanadilok, S., Veenin, T., Yahya, A., & Nobuchi, T. (2014). Vessel feature changes as a tool for detecting annual rings in tropical trees. *Trees, 28*(1), 137-149. doi:10.1007/s00468-013-0936-3

Park, C., Kim, M., Han, J., Jeong, T., & Sok, D. (2009). Lysophosphatidylcholine exhibits selective cytotoxicity, accompanied by ROS formation, in RAW 264.7 macrophages. *Lipids, 44*(5), 425-435. doi:10.1007/s11745-009-3286-6

PÄ™kal, A., & Pyrzynska, K. (2014). Evaluation of aluminium complexation reaction for flavonoid content assay. *Food Analytical Methods, 7*(9), 1776-1782. doi:10.1007/s12161-014-9814-x

Ravid, M., Robson, M., & Kedar, I. (1977). Prolonged colchicine treatment in four patients with amyloidosis. *Annals of Internal Medicine, 87*(5), 568-570.

Reyes-Jurado, F., Franco-Vega, A., RamÃrez-Corona, N., Palou, E., & LÃ³pez-Malo, A. (2014). Essential oils: Antimicrobial activities, extraction methods, and their modeling. *Food Engineering Reviews*, , 1-23. doi:10.1007/s12393-014-9099-2

Routray, W., & Orsat, V. (2012). Microwave-assisted extraction of flavonoids: A review. *Food and Bioprocess Technology*, *5*(2), 409-424. doi:10.1007/s11947-011-0573-z

Routray, W., & Orsat, V. (2013). Preparative extraction and separation of phenolic compounds. In K. G. Ramawat, & J. MÃ©rillon (Eds.), (pp. 2013-2045) Springer Berlin Heidelberg. doi:10.1007/978-3-642-22144-6_55

Sakdarat, S., Shuyprom, A., Pientong, C., Ekalaksananan, T., & Thongchai, S. (2009). Bioactive constituents from the leaves of clinacanthus nutans lindau. *Bioorganic & Medicinal Chemistry*, *17*(5), 1857-1860.

Vermerris, W., & Nicholson, R. (2006). Chemical properties of phenolic compounds. (pp. 35-62) Springer Netherlands. doi:10.1007/978-1-4020-5164-7_2

Vermerris, W., & Nicholson, R. (2006). Chemical properties of phenolic compounds. (pp. 35-62) Springer Netherlands. doi:10.1007/978-1-4020-5164-7_2

Viacava, G., Roura, S., & AgÃ¼ero, M. (2015). Antioxidant activity of butterhead lettuce: Evaluation of significant factors affecting antioxidant extraction and quantification. *Journal of Food Measurement and Characterization*, *9*(2), 206-214. doi:10.1007/s11694-015-9225-8

Wu, J., Shang, Z., Wu, J., Jiang, X., Moschou, P. N., Sun, W., . . . Zhang, S. (2010). Spermidine oxidase-derived H2O2 regulates pollen plasma membrane hyperpolarization-activated Ca2+-permeable channels and pollen tube growth. *The Plant Journal, 63*(6), 1042-1053. doi:10.1111/j.1365-313X.2010.04301.x