UNIVERSITI PUTRA MALAYSIA

EFFECTS OF DRYING METHODS AND SOLVENT TYPES ON ANTIOXIDANT PROPERTIES AND ANTI-INFLAMMATORY POTENTIAL OF TWO GINGER VARIETIES IN MALAYSIA

ISWAIBAH BINTI MUSTAFA

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By

ISWAIBAH BINTI MUSTAFA

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

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Dedicated to,

My beloved father and mother, Mustafa Yusoff and Nafisah Abd. Rahman who take a big role in completing this journey. Thank you so much for your continuous love and support in many ways.

Also to all my siblings Kak, Abe, Yah, Ijad, Adam and Adik, thank you for your endless support. All of you are my priceless treasure in this duniya. I love you for ∞ (eternity)

ALHAMDULILLAH

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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May 2017

Chair: Professor Ir. Chin Nyuk Ling, PhD
Faculty: Engineering

Ginger (*Zingiber officinale*) is one the finest medicinal herbs in the world with more than 50% of the world production coming from Asia. In Malaysia, ginger ranks among top ten potential herbs based on its remarkable medicinal properties. However, most usage of ginger from its fresh form which cause huge of postharvest loss. The objective of this study was to systematically evaluate the antioxidant properties and anti-inflammatory activity of Malaysian gingers, Halia Bentong (*Zingiber officinale* Roscoe) and Halia Bara (*Zingiber officinale* var. Rubra) as affected by drying methods of shade-, sun-, oven-, vacuum-, and freeze-drying with different extraction solvents of hotwater, 80% aqueous ethanol and ethanol. The changes in dried ginger extracts were analyzed for proximate analysis, phytochemical contents and antioxidant activities using FRAP, TAA by phosphomolybdenum, ability to scavenge $ABTS^{\bullet+}$, $DPPH^{\bullet}$ and $OH^{\bullet}$ radicals. Chelation on ferrous ion was then evaluated for metal binding capacity. Anti-inflammatory activity was done using cell culture method against inhibition of NO production in LPS-stimulated RAW 264.7 cells.

The results show that drying methods have helped to reduce the moisture content of ginger whilst preserving the ginger's nutritional value, phytochemical contents as well as their antioxidant properties. Phenolic contents of dried ginger, with the highest value of 20.91 mg GAE/g extract for Halia Bentong and 20.07 mg GAE/g extract for Halia Bara were obtained in freeze-dried from ethanol extract and the lowest in fresh ginger with 8.03 mg GAE/g extract and 10.53 mg GAE/g extract, respectively. Flavonoid contents also were highest in dried gingers, which were ranged from 42.98 to 67.82 g RE/100g extract for Halia Bentong and 47.32 to 69.32 g RE/100g extract for Halia Bara when compared to fresh ginger. Meanwhile, ascorbic acid in fresh and dried gingers were less than 1.5 mg AA/g extract, which indicate that both gingers contain less amount of ascorbic acid. Shade-dried of Halia Bentong and sun-dried of Halia Bara from ethanol extracts gave the highest total antioxidant activity of ginger which were 75.23 g AA/100g extract and 81.12 g AA/100g extract, respectively. Sun-dried from ethanol extracts had

the highest antioxidant activity as exhibited by FRAP and $ABTS^{\bullet+}$ scavenging activity. The activity was increased by 15.35-fold and 3.5-fold for dried Halia Bentong and 3.95-fold and 2.07-fold for dried Halia Bara compared to its fresh form. However, different methods of drying did not affect the activity of inhibition of $DPPH^{\bullet}$, $OH^{\bullet}$ as well as the ascorbic acid contents.

Extraction solvent has a significant influence ($p < 0.05$) on the extraction of antioxidant compounds from ginger. Ethanol extracts of ginger gave the highest level of antioxidant activities as exhibited by phytochemical contents, FRAP, total antioxidant activity (TAA), $ABTS^{\bullet+}$ scavenging and IC_{50} values, while hotwater extracts showed the least potent solvent extraction. However, hotwater was found as the most effective solvent in giving the strongest of ferrous ion chelating power with the ranged of 69.45 to 145.47 mg EDTA/g extract for Halia Bentong and 60.73 to 159.17 mg EDTA/g extract for Halia Bara. The extractive yield indicates that aqueous ethanol of 80% which ranged from 2.39 to 17.79% for Halia Bentong and 3.57 to 15.21% for Halia Bara, gave the maximum yield amongst the other extracts. Results suggested that not only the polarity of the solvent affected the effectiveness of extraction, the chemical structure or nature of the solvent also lead to different antioxidant capability.

The enhanced phytochemicals and antioxidant activities of dried ginger were also supported by anti-inflammatory studies against inhibition of NO production, which is known as inflammation mediator. Dried ginger from ethanol extracts were recorded as a potent inhibitor of NO at the concentrations 100 µg/mL, while fresh ginger was less potent. At the concentration of 100 µg/mL extracts, NO-inhibitory activity of dried ginger had caused inhibition with ranged from 40.85 to 50.88% for Halia Bentong and 29.16 to 45.13% for Halia Bara. While inhibitory activity of fresh ginger on NO production was 8.65% and 18.23%, respectively. Drying has demonstrated an improved post-harvest treatment of gingers' bioactivities in regard to enhanced final ginger quality.

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

KESAN KAEDAH PENGERINGAN DAN JENIS PELARUT TERHADAP CIRI-CIRI ANTIOKSIDAN DAN ANTI-RADANG DARIPADA DUA JENIS HALIA MALAYSIA

Oleh

ISWAIBAH BINTI MUSTAFA

Mei 2017

Pengerusi: Profesor Ir. Chin Nyuk Ling, PhD
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Halia adalah salah satu herba perubatan terbaik di dunia dengan lebih daripada 50% daripada pengeluaran dunia adalah dari Asia. Di Malaysia, halia berada di kedudukan sepuluh herba berpotensi berdasarkan sifat-sifat perubatannya. Walau bagaimanapun, kebanyakan penggunaan halia adalah dalam keadaan segar yang menyebabkan kerugian yang besar selepas proses menuai. Oleh itu, objektif kajian ini adalah untuk mengkaji sifat-sifat antioksidan dan aktiviti anti-radang halia Malaysia iaitu Halia Bentong dan Halia Bara yang dipengaruhi oleh kaedah pengeringan yang berbeza dengan megunakan pelarut pengekstrakan yang berbeza. Kaedah pengeringan yang digunakan ialah teduhan, matahari, ketuhar, vakum dan beku. Manakala, pelarut pengekstrakan yang digunakan ialah air panas, 80% etanol akueus dan etanol. Perubahan dalam ekstrak halia kering telah dianalisis untuk komposisi proksimat, kandungan fitokimia dan aktiviti antioksidan dengan menggunakan FRAP, phosphomolybdenum (TAA), $ABTS^{\bullet+}$ kation radikal, $DPPH^{\bullet}$ dan $OH^{\bullet}$ radikal. Perebutan pada ion Fe^{2+} telah dinilai untuk activiti pengikatan logam. Aktiviti anti-radang telah dilakukan dengan menggunakan kaedah kultur sel terhadap pengurangan bagi pengeluaran NO yang dirangsang oleh LPS pada sel RAW 264.7.

Hasil kajian menunjukkan bahawa kaedah pengeringan bukan saja membantu untuk mengurangkan kandungan lembapan halia, tetapi mengekalkan nilai nutrisi, kandungan fitokimia dan sifat antioksidan. Pengeringan beku dari pelarut etanol memberi nilai fenol yang paling tinggi iaitu 20.91 mg GAE/g ekstrak untuk Halia Bentong dan 20.07 mg GAE/g ekstrak untuk Halia Bara, dan halia segar masing–masing dengan nilai yang paling rendah iaitu 8.03 mg GAE/g ekstrak dan 10.53 mg GAE/g ekstrak. Kandungan flavonoid juga lebih tinggi dalam halia kering, yang antara 42.98-67.82 g RE/ekstrak 100g untuk Halia Bentong dan 47.32-69.32 g RE/ekstrak 100g untuk Halia Bara berbanding halia segar. Sementara itu, asid askorbik dalam halia segar dan halia kering adalah kurang daripada 1.5 mg AA/g ekstrak, yang menunjukkan halia mengandungi kandungan asid askorbik yang rendah. Halia Bentong dari kaedah pengeringan teduhan

dan Halia Bara dari kaedah pengeringan matahari, yang masing-masing dari ekstrak etanol menunjukkan jumlah aktiviti antioksidan yang paling tinggi halia iaitu dengan 75.23 g AA/100g ekstrak dan 81.12 g AA/100g ekstrak. Manakala kaedah pengeringan matahari dari ekstrak etanol mempunyai aktiviti antioksidan yang paling tinggi seperti yang ditunjukkan oleh FRAP, dan $ABTS^{\bullet+}$ kation radikal. Aktiviti ini telah meningkat sebanyak 15.35 dan 3.5 kali ganda untuk Halia Bentong kering; dan 3.95 dan 2.07 kali ganda untuk Halia Bara kering berbanding halia segar. Walaubagaimanapun, kaedah pengerigan yang berbeza tidak menunjukkan sebarang perubahan dalam activiti $DPPH^{\bullet}$, $OH^{\bullet}$ radikal dan juga kandugnan asid askorbik.

Kesan pengekstrakan pelarut mempunyai pengaruh yang ketara ($p < 0.05$) terhadap antioksidan dari halia. Ekstrak halia dari pelarut etanol memberikan nilai aktiviti antioksidan yang tinggi seperti yang ditunjukkan oleh kandungan fitokimia, FRAP, TAA, $ABTS^{\bullet+}$ dan nilai IC_{50}. Manakala pengekstrakan meggunakan air panas memberikan activity yang paling minimun. Walau bagaimanapun, air panas didapati sebagai pelarut yang paling berkesan dalam activiti pengikatan ion ferus dengan nilai 69.45-145.47 mg EDTA/g ekstrak untuk Halia Bentong dan 60.73-159.17 mg EDTA/g ekstrak untuk Halia Bara. Hasil ekstrak menunjukkan bahawa 80% etanol akueus memberikan hasil yang maksimum iaitu antara 2.39-17.79% untuk Halia Bentong dan 3.57-15.21% untuk Halia Bara. Kajian menunjukkan bahawa bukan sahaja kekutuban pelarut mempengaruhi kesan pengekstrakan, struktur kimia atau sifat pelarut juga membawa kepada keupayaan antioksidan yang berbeza.

Kandugan fitokimia dan aktiviti antioksidan halia kering juga disokong oleh kajian anti-radang terhadap pengurangan bagi pengeluaran NO, yang juga dikenali sebagai pengantara kepada sifat keradangan. Halia kering dari ekstrak etanol menunjukkan sebagai jumlah keluaran NO yang sedikit pada 100 μg/mL, manakala halia segar menunjukkan jumlah pengeluaran NO yang banyak. Pada kepekatan ekstrak 100 μg/mL, aktiviti pengurangan NO untuk halia kering adalah antara 40.85-50.88% untuk Halia Bentong dan 29.16-45.13% untuk Halia Bara. Manakala pengurangan NO untuk halia segar masing-masing pada 8.65% dan 18.23%. Kaedah pengeringan telah menunjukkan peningkatan yang lebih baik terhadap activiti biologi halia.

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I certify that a Thesis Examination Committee has met on 18 May 2017 to conduct the final examination of Iswaibah binti Mustafa on her thesis entitled "Effects of Drying Methods and Solvent Types on Antioxidant Properties and Anti-Inflammatory Potential of Two Ginger Varieties in Malaysia" 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.

Members of the Thesis Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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ABTS	2,2'-azinobis (3-ethyl-benzothiozoline-6-sulfonic acid) disodium salt
ANOVA	Analysis of variance
AOAC	Official methods of analysis
BHA	Butylated hydroxyanisole
DCPIP	2,6-dichlorophenolindophenol
DMEM	Dulbecco's modified eagle's medium
DPPH	1,1- diphenyl-1-picrylhydrazyl
EDTA	Ethylenediaminetetraacetic acid
FBS	Fetal bovine serum
FRAP	Ferric reducing/antioxidant power
LPS	Lipopolysaccharide
NO	Nitric oxide
PM	Phosphomolybdenum
TAA	Total antioxidant activity
TPTZ	2,4,6-tripyridyl-s-triazine
Trolox	6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Ginger, a rhizome of the plant *Zingiber officinale* is one the most valuable medicinal herbs in the world with its innumerable uses in food and medicines. The fresh ginger has been used from ancient times as food and medicine and has gained considerable awareness for various functional benefits such as dietary supplement, food antioxidants, and as a food product for preventing diseases (Ho & Su, 2016; Yeh et al., 2013). In Malaysia, *Zingiber officinale* Roscoe and *Zingiber officinale* var. Rubra respectively known as Halia Bentong and Halia Bara are widely used by the locals as a major herb, health food and as an important flavouring and traditional medicine (Mojani & Ghasemzadeh, 2014). Due to its nutritive value and presence of potent bioactive compounds, the rhizome ginger are gaining importance for its health benefits and are emerging as a potential ingredients among the nutrition market. Analysis of nutritive value of ginger has been performed by several researchers (Butt & Sultan, 2011; Ghosh, 2011). The rhizome gingers are rich in minerals, vitamin, carbohydrate and other proximate compositions. Apart from being nutritious, ginger are rich sources of bioactive compounds which have shown to be effective in scavenging free radicals and to treat inflammation (Dugasani et al., 2010; Tsai, Tsai, & Ho, 2005).

However, the fresh ginger are sometimes seasonal, highly perishable and have high content of moisture. High moisture content can cause ginger to be prone to microorganism spoilage such as enzymatic reactions and other detrimental changes (Akdaş & Başlar, 2015; Roshanak, Rahimmalek, & Goli, 2016). Drying is a common traditional preservation method which significantly reduces the amount of moisture contents, and also extent the shelf-life of ginger. Despite improving the shelf life of ginger, drying processing are also recognized as one of the major factors leading to changes of natural phytochemical present in foods (Kamiloglu et al., 2016).

The health-promoting behavior of ginger is attributed to its rich natural phytochemicals, which have been widely discovered as having antioxidants and anti-inflammatory properties, protecting human from oxidative stress and inflammation related disorders. Oxidative stress is related to an imbalance between the production of free radicals and antioxidant defense system. Free radicals and their uncontrolled production will initiate and propagate the oxidation chain reactions and lead to inflammation development (Sagrin & Chong, 2013). Inflammation, which is a functionally defense response of living tissue to harmful stimuli, can be associated with uncontrolled or prolonged inflammatory responses that develop when the body's regulation of inflammation is dysfunctional (Cheung et al., 2013; Shin et al., 2013). Since a number of complex reactions take place during drying processing, the final quality of ginger may be the results of degradation of natural phytochemicals and/or formation of new compounds of phytochemicals that further affect the therapeutic value in the dried ginger when compared to fresh ginger.

A work done on green tea leaves reported that drying processing enhanced the final product than fresh samples in terms of phytochemical contents, antioxidant activity and vitamin C (Roshanak et al., 2016). Hossain et al. (2010) observed that an increase in phytochemical contents and antioxidant activity through drying treatment of Lamiaceae herbs (Hossain, Barry-Ryan, Martin-Diana, & Brunton, 2010). Joshi et al. (2011) and Chan et al. (2013) suggested that drying treatment was able to preserve phenolic content and enhance antioxidant activity of apple and herbs, respectively (Chan, Lye, Eng, & Tan, 2013; Joshi, Rupasinghe, & Khanizadeh, 2011). In contrast, phenolic content in dried tomato (Gümüşay, Borazan, Ercal, & Demirkol, 2015) and dried mandarin (Akdaş & Başlar, 2015) were found to decrease approximately 60% and 40% from the fresh samples, respectively. The above findings have proven the drying methods may affect the natural compounds in either ways.

Therefore, this research aims to identify the photochemical contents of fresh and dried rhizome ginger as subjected to different drying treatments evaluated in terms of antioxidant and anti-inflammatory activities.

1.2 Problem Statement

Ginger has been identified as an herbal medicinal product with beneficial bioactivity effects (Yeh et al., 2013). Ginger contains a large number of active ingredients and shows a therapeutic role in the treatment of many diseases that involve inflammation and those which caused by oxidative stress (Dugasani et al., 2010). Previous study reported that some bioactive constituents present in ginger plays a crucial role in antioxidant, anti-inflammatory, anti-tumour, anti-microbial, anti-diabetic, anti-emetic, and etc. (Rahmani, Al, & Aly, 2014). In traditional practices, ginger is used to treat a wide range of ailments, including nausea and vomiting, colds and flu symptoms, stomachaches and rheumatic disorders (Kubra & Rao, 2012). Currently, two varieties of ginger rhizomes, Halia Bentong and Halia Bara are available in Malaysia and found to have potential source for natural antioxidants (Mojani & Ghasemzadeh, 2014).

Unfortunately, ginger postharvest period is relatively short due to their high moisture contents. High moisture content is highly susceptible to oxidize by external (atmospheric) and internal (enzymatic action) degradation (Roshanak et al., 2016), which in turn causes hygienic and qualitative problems. This problem also affects its phytochemicals compounds and potential bioactivity of ginger. It has been demonstrated that the health benefits of ginger against oxidative stress and inflammation diseases are mainly attributed to the occurrence of bioactive phytochemicals of phenolic and flavonoids compounds (Dugasani et al., 2010). Oxidative stress is related to an imbalance between the production of free radicals and antioxidant defense system. Free radicals and their uncontrolled production will initiate and propagate the oxidation chain reactions and lead to inflammation development (Lu & Yen, 2015) In inflammatory disorders, nitric oxide (NO) is secreted excessively and overproduction or prolonged of NO has resulted the diseases developments. The extracts of ginger were reported to possess scavenging properties against free radicals, and exhibit anti-inflammation by inhibiting NO (Dugasani et al., 2010; Tsai et al., 2005). Phenolic and flavonoids are major contributors to its protective role against oxidative damage and its consequences such as inflammation and others, thus contributing to the overall health-protective effects of food samples.

Hence, drying is applied to prolong the shelf life of ginger and to preserve their natural phytochemical efficiency (Gümüşay et al., 2015). More recently, improvement of ginger in terms of its potential bioactivity by drying processing has been well documented (Chumroenphat, Khanprom, & Butkhup, 2011; Guo, Wu, Du, Zhang, & Yang, 2014). Drying inhibits the microbial growth and prevents the changing of certain biochemical components that might alter the antioxidant activity. Though ginger has been used by local communities for cuisines and medicine, drying processes information is still lack especially on antioxidant and anti-inflammatory activities, in particular the Malaysian ginger. Currently, there has been an increased interest to identify therapeutic potentials of natural antioxidants from plant sources which are known with their pharmacology effect with less or no side effects. As plants counteract oxidative stress caused by environmental stressor, they present a potential source of natural compounds with antioxidant activity to themselves (Azmir et al., 2013; Sudan, Bhagat, Gupta, Singh, & Koul, 2014). Antioxidant of their phytochemicals could give an additional support to the human health.

Extraction is an important step involved in the discovery of bioactive phytochemicals compounds from plant materials. A successful determination of bioactive components from plant materials most often involves type of solvent used in the extraction procedures. Many researchers on the extraction of bioactive compounds from ginger using various solvents are found despite no explanation forthcoming on their choice of selection. They include using methanol, ethanol, acetone, hexane, ethyl acetate, petrol, water, chloroform, benzene, and butanol. Among all the solvents used, ethanol, water, and their combinations (aqeous organic solvent) are the most commonly used solvent as they are considered to be GRAS solvents. However, it was well documented that different solvent will exhibit different potentiality in extracting bioactive compounds from plant materials and possess different potentiality in bioactivities (Loo, Jain, & Darah, 2008). Lapornik et al. (2005) who studied antioxidant activity of berries in ethanol and water extracts reported that ethanol extracts contain twice more bioactive phytochemicals of anthocyanins and polyphenols than water extracts. While, Benjakul et al. (2014) reported that water extraction produces higher yield of lead seed (*Leucaena leucocephala*) extracts, whereas lower yield of extracts were obtained in ethanol extracts. Additionally, water was suggested as a good solvent in metal chelating activity, as reported by Sowndhararajan et al. (2010). Metal chelating capacity is evaluated as one of the important mechanisms of antioxidant activity. Thus, the reported bioactivity in plant extracts using various extraction solvents are quite different. Despite the fact that some few recent studies have reported the extraction of bioactive compounds from ginger (Ho & Su, 2016; Li et al., 2011; Oboh, Akinyemi, & Ademiluyi, 2012), there is not any study on the extraction of bioactive compounds from Malaysian ginger using different solvents such as water, ethanol, and their combinations (mixtures of water-ethanol). In realizing this void, this research has served as an initial exploration for extracting antioxidant compounds from Malaysian ginger with different types of solvents and evaluated in-terms of their antioxidant activity.

1.3 Objectives

The general objectives of this research are to study the effects of different drying methods and solvents extraction on extract yields, proximate compositions, phytochemicals contents, antioxidant and anti-inflammatory activities of two Malaysian ginger varieties which are Halia Bentong and Halia Bara. The specific objectives are:

1. To compare the effectiveness of different drying methods of shade-, sun-, oven-, vacuum-, and freeze-drying on proximate composition of dried ginger; and phytochemical contents and antioxidant activities of extract ginger of both Halia Bentong and Halia Bara.

2. To evaluate the antioxidant activities of dried ginger extracted from three different solvents; hotwater, aqueous ethanol and ethanol, in terms of extract yields, phytochemical contents and antioxidant activities

3 To study anti-inflammatory activity of ethanolic extracts from sun-, oven-, and freeze-dried ginger by inhibiting the nitric oxide (NO) production in LPS-stimulated RAW 264.7 cells.

1.4 Research Scope

The research covers the scope of investigation on the effects of drying methods on antioxidant and anti-inflammatory activities of two Malaysian ginger varieties, Halia Bentong and Halia Bara. Five different drying methods applied were the shade-, sun-, oven-, vacuum-, and freeze-drying. Their changes in antioxidant activities were evaluated and compared with fresh ginger. Subsequently of the drying process, the proximate analyses of ginger were analyzed. The proximate analysis involved moisture, fat, crude fibre, protein, ash and carbohydrate. Different types of solvents were also evaluated to examine their potentiality in extraction of bioactive compounds from ginger. To achieve this target, three different solvents, hotwater, aqueous ethanol (80%, w/v) and ethanol were used. The solid-liquid extraction (maceration technique) was adopted in this study during extraction process to extract bioactive compounds from the ginger rhizome. Phytochemical content (*i.e.* phenolic, flavonoid and ascorbic acid contents), reductive ability (FRAP), total antioxidant activity by phosphomolybdenum assay (TAA), radical scavenging activity (*i.e.* $ABTS^{\bullet+}$, $DPPH^{\bullet}$ and $OH^{\bullet}$) and metal chelating activity (Fe^{2+}) were carried out in this study to examine the effects of drying methods and solvent extraction. The phytochemical and antioxidant activities of dried ginger extracts were supported by anti-inflammatory studies with cell culture approach on inhibition of NO production in LPS-stimulated RAW 264.7 cells.

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