



***ANTIOXIDANT ACTIVITY, PHENOLICS, POLYPHENOLS AND
FLAVONOIDS IN THE RHIZOMES AND LEAVES OF ZINGIBER
OFFICINALE VAR. RUBRUM***

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By

ABDULLAHI MUHAMMAD

**Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfillment of the requirement for Degree of Master of Science**

March 2015

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DEDICATION

To my family
Mr. Muhammad Abdu and Aisha Muhammad
And all my siblings



Abstract of thesis submitted to the senate of Universiti Putra Malaysia in fulfillment of the requirement for the award of degree of Master of Science

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Zingiber officinale var rubrum is a special cultivar of ginger that has distinctive genetic and phenotypic profiles. The use of synthetic antioxidants has been reported to have negative impacts on the consumers, this leads to tremendous increase in the interest for antioxidants from natural dietary sources especially for medicinal plants consumed as foods, beverages, condiments and spices like the ginger. Rhizomes and leaves of the ginger were exhaustively studied for their antioxidant activities (DPPH and FRAP), flavonoids, phenolics, polyphenols and primary metabolites contents. The effects of different growth stages and storage temperatures on the antioxidant activity and levels of phenolics, flavonoids and polyphenols were explored. Using the technique of HPLC, the flavonoid genistein was identified as one of the individual flavonoids in the plant. It was found that the rhizome extracts have the highest antioxidant activities of up to 57.55% DPPH and 20.27mg/g Trolox FRAP concentrations for the 70% methanol extracts. The flavonoid was found to be the most abundant compound among the three (phenolics, polyphenols and flavonoids) studied in the rhizomes whereas polyphenols were the most abundant in the leaves. The rhizomes have significant sugar content of up to 176µg/g Glucose FW and relatively lower protein content 48.50µg/gBSA FW. Storage temperature was found to significantly lead to the deterioration and degradation of phenolics, polyphenols, flavonoids and antioxidant activity. For example, FRAP and DPPH values of the control in the first week were 13.0mg/g Trolox DW and 58.82% respectively, these drastically fell to 7.23mg/g Trolox DW and 7.33% for the FRAP and DPPH respectively after storage at 70°C for four weeks. Early vegetative stages of growth (8th week after germination) were found to be richer in flavonoids, phenolics, polyphenols, 18.74mg/g Quercetin DW, 4.70mg/g GA DW, and 16.6mg/gGADW respectively. The antioxidant was also higher than the later stages (12th and 16th weeks). The plant has enormous antioxidant content, genistein is identified to be a constituent flavonoid in the rhizomes of *Z. officinale* younger plants have higher antioxidant activity

then older (for example, FRAP in the eighth and twelfth weeks were 16.70 and 14.45mg/gTrolox DW respectively while the DPPH percentage inhibitions were 56.80% and 50.05% for the eighth and twelfth weeks respectively) ones and also storage temperatures have so much effect on the total antioxidant.



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

**KANDUNGAN ANTIOKSIDA, FENOLIK, POLIFENOL DAN FLAVONOID
DALAM RIZOM DAN DAUN *ZINGIBER OFFICINALE* VAR. *RUBRUM***

Oleh

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Zingiber officinale var *rubrum* merupakan kultivar halia yang mempunyai profil genetik dan fenotipik tersendiri. Penggunaan antioksidan sintetik dilaporkan mempunyai kesan negatif terhadap pengguna, ini membawa kepada peningkatan mendadak dalam kepentingan antioksidan daripada sumber semula jadi pemakanan terutamanya tumbuhan ubatan yang digunakan sebagai makanan, minuman, bahan perasa dan rempah seperti halia. Rizom dan daun halia telah dikaji secara menyeluruh bagi aktiviti antioksidan (DPPH dan FRAP), flavonoid, fenolik, polifenol dan kandungan metabolit primer. Kesan bagi peringkat pertumbuhan yang berbeza dan suhu penyimpanan terhadap aktiviti antioksidan dan tahap fenolik, flavonoid dan polifenol dikaji. Dengan menggunakan teknik HPLC, flavonoid genistein telah dikenal pasti sebagai salah satu flavonoid yang terdapat di dalam tumbuhan tersebut. Ekstrak rizom mempunyai aktiviti antioksidan yang tertinggi iaitu 57.55% DPPH dan 20.27mg / g Trolox FRAP bagi ekstrak methanol 70%. Flavonoid merupakan sebatian yang paling banyak terdapat di antara sebatian-sebatian (fenolik, polifenol dan flavonoid) yang dikaji dalam rizom manakala polifenol paling banyak terdapat dalam daun. Rizom mempunyai kandungan gula yang tinggi iaitu 176µg / g Glukosa FW dan kandungan protein yang agak rendah iaitu 48.50µg/ gBSA FW. Suhu penyimpanan mempunyai kesan yang ketara kepada pengurangan dan degradasi fenolik, polifenol, flavonoid dan aktiviti antioksidan. Sebagai contoh, nilai kawalan bagi FRAP dan DPPH untuk minggu pertama masing-masing ialah 13.0mg / g Trolox DW dan 58.82%, nilai ini jatuh secara drastik masing-masing kepada 7.23mg / g Trolox DW dan 7.33% selepas empat minggu penyimpanan pada 70°C. Kandungan yang tinggi bagi flavonoid, fenolik, polifenol iaitu masing-masing 18.74mg / g Quercetin DW, 4.70mg / g GA DW, dan 16.6mg / gGADW dikesan pada peringkat awal pertumbuhan vegetatif (minggu ke-8 selepas percambahan). Antioksidan juga lebih tinggi berbanding peringkat akhir (minggu ke-12 dan minggu ke-16). Halia mempunyai kandungan antioksidan yang sangat banyak, genistein dikenalpasti sebagai flavonoid konstituen yang terdapat dalam rizom *Z. officinale*. Rizom daripada tumbuhan

muda mempunyai aktiviti antioksida yang lebih tinggi berbanding yang lebih tua (contohnya, FRAP dalam minggu kelapan dan kedua belas masing-masing masing-masing ialah 16.70 dan 14.45mg / gTrolox DW manakala peratusan perencatan DPPH masing-masing adalah 56.80% dan 50.05% untuk minggu kelapan dan kedua belas masing-masing) dan juga yang suhu penyimpanan mempunyai begitu banyak kesan ke atas jumlah antioksidan



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LIST OF ABBREVIATIONS

$^{\circ}\text{C}$	Degree Celsius
μL	Micro liter
AlCl_3	Aluminum chloride
ANOVA	Analysis of Variance
BSA	Bovine serum albumin
CAT	Catalase
CD	Copalyl diphosphate
DPPH	1,1-Diphenyl-2-Picrylhydrazil
DW	Dry weight
FRAP	Ferric reducing antioxidant potential
GAE	Gallic acid equivalent
HCl	Hydrochloric acid
HIV	Human Immune Virus
HPLC	high performance liquid chromatography
L	Liter
ml	milliliter
MPa	Mega Pascal
Na_2CO_3	Sodium carbonate
NaNO_3	Sodium Nitrite
NaOH	Sodium hydroxide
NO	Nitric Oxide
Nm	Nanometer
OH	Hydroxyl
POX	Proline Oxidase
RAPD	Random Amplified Polymorphic DNA
ROS	Reactive oxygen species
R_T	Retention time
SOD	Superoxide Desmutase
TPTZ	2,4,6-tri (2-pyridyl)-s-triazine
UV	Ultraviolet

CHAPTER ONE

INTRODUCTION

1.1 General Introduction

Ginger (*Zingiber officinale*) is a widely used spice, condiment for foods and beverages and medicinal plant for over 3000 years in Southeast Asian countries of India and China (Vermin and Parkanyi, 2005). It is thought to have originated from Indo-Malayan regions and then spread to other parts of the world through trading, including Africa, Asia Mediterranean and Pacific Islands (Kizhakkayil & Sasikumar, 2011). Ginger is a member of the genus *Zingiber*, in a family Zingiberaceae. There are roughly 1,200 species of Zingiberaceae belonging to 18 genera world over, out of which 160 species from all the 18 genera are found to be dominant in Malaysian Peninsular (Holttum, 1950).

Medicinal plants are plants that possess therapeutic properties or exert beneficial pharmacological effect on animals. It has been established that the plants which naturally synthesize and accumulate secondary metabolites such as alkaloids, glycosides, tannins, phenols, volatile oils, flavonoids and vitamins possess medicinal properties. These medicinal plants which are often called traditional medicines need to be evaluated, given due recognition and develop as to improve their safety, efficacy, availability and wider application and low cost; [according to World Health Organisation (WHO) reports (Maydel, 1986)].

Surendran, et al, (2004) reported that there are four main cultivars of ginger in Malaysia, Halia Bara (red ginger), Halia Padi, Halia Betel (real ginger) and Halia Udang. (Surendran, et al., 2004). Different cultivars contain varied quantity of essential oils, the pungent compounds (6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol) and thus give a basis for classification and/or differentiation (Salmon et al., 2012). Halia bara can out rightly be differentiated from other ginger types, through genetic and phenotypic studies. Using Phenotypic data, Halia Bara rhizome is reddish, smaller in size and has more pungent smell (Sivasothy et al., 2011). It has red petiole during early stages of growth and the base of its leaf shoot is also reddish. It was reported that using RAPD (Random Amplified Polymorphic DNA), the four main types of ginger found in Malaysia differ in three operon primers: OPA1, OPA8 and OPA20 (Ibrahim and Hussin, 2007). Chromatographic fingerprinting of gingers from five ginger producing countries (Malaysia, Vietnam, China, India and Thailand) was carried out to ascertain the alleged origin of ginger. Using the parameters, hierarchical cluster analysis, principal component analysis, linear discriminate analysis, the ginger profiles were grouped and separated into five (Yudthavorasit et al., 2014).

It has been reported that in Malaysia, *Zingiber officinale* is generally used in the treatment of ailments related to female diseases. These ailments include; post-natal

symptoms after birth, and post-partum ailments (Ibrahim, and Hussin, 2007). *Zingiberaceae* is also reported to be effective in enhancing blood circulation and the vicious contraction of the uterus during the postpartum (Jamal, et al., 2011). The most widely used methods for treatments with ginger in Malay culture include tonics, decoctions, and ointments. In the Malay folklore, Halia Bara is widely used in the treatments of joint pains, taken as juice and or applied topically as ointment made with vinegar (Ibrahim, and Hussin, 2007). It is also used in the treatment of arthritis (Jamal et, al., 2011; Ramadan, et al., 2011). For time immemorial, ginger has being used in the treatment of ailments like inflammation, rheumatic disorders, motion sickness, colds and gastrointestinal problems (Kimura, et al., 2005).

Antioxidants are substances or nutrients in the diets which have the capacity of preventing or slowing down the oxidative damage posed to the body by free radicals produced during normal cellular respiration and oxidations. Antioxidants act as "free radical scavengers" and hence prevent and repair damage caused by these free radicals. Many ailments are said to be caused and/or triggered by free radicals which include, diabetes, macular degeneration, heart diseases, and even cancers. Antioxidants may also strengthen immunity of the body against many diseases as such can prevent and lower the risk of cancers and many other infections (Brambilla et al., 2008). These free radicals, also called reactive oxygen species, singlet oxygen, hydrogen peroxide, superoxide anion, and hydroxyl radical are produced during normal metabolism and growth in the biological systems (Dragišić Maksimović et al., 2013). Antioxidants are molecules, ions or atoms that are capable of reversing the deterioration of cells caused by reactive oxygen species (ROS). ROS are known to be directly involved in reversing cell degeneration most especially in brain cells (Oboh et al., 2012). Most of the dietary plants that possess antioxidant capacities, have interestingly, demonstrated promising anti-inflammatory properties and thus prevention against oxidative damage (Conforti et al., 2009).

The interest in the studies of antioxidants has increased tremendously over the years due to the health benefits of the antioxidants. This leads to the explorations of several food and medicinal plants for their antioxidant activity. Synthetic antioxidant compound are often consumed for the antioxidant activity they display, but this often comes with side and adverse effects. This necessitates their replacements from naturally derived dietary sources.

1.2 General Objectives

The main aim of this research is to study the antioxidant properties, the secondary metabolites of the rhizomes and leaves of *Zingiber officinale* var. *rubrum* (*Halia Bara*).

1.3 Specific Objectives

1. To study the effects of different solvents on the extraction of antioxidant activity
2. To study the effects of storage temperatures on the degradation and stability of flavonoids, phenolics, polyphenols and their corresponding antioxidant capacities.
3. To study the flavonoids profiles of the rhizome extracts to determine the participatory flavonoids(s) in the antioxidant using RP-HPLC technique and to determine the primary metabolites profile of the plant.

1.4 Problem Statement

Antioxidants are found to protect the body against various diseases caused by reactive oxygen species (ROS) produced during cellular metabolism. Side and adverse effects through the use and consumptions of synthetic antioxidants lead to the need of their replacements from natural sources and most importantly from plants often consumed as foods, spices, condiments and as beverages (e.g ginger). This tremendous increase in interest and the need for antioxidant from natural sources warrant the study on foods and medicinal plants. This study will help explore the antioxidant properties of *Halia bara* and subsequently encourage or discourage the consumption of *Z. officinale* as an important source of antioxidant.

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LIST OF PUBLICATIONS

Conference Proceedings

1. **Abdullahi Muhammad**, Maziah Mahmood and Syahida Ahmad. *Zingiberacea officinale* var *rubrum* (halia bara): In vitro antioxidant, flavonoids profiling, Effects of temperature and growth stages on the synthesis of total antioxidants activity. Malaysian society of molecular biology and biotechnology conference 2014. Monash University Malaysia,
2. **Abdullahi Muhammad**, Maziah Mahmood and Syahida Ahmad. In vitro antioxidant and flavonoids profiling of *Zingiberacea officinale* var. *rubrum* (**halia** bara). International Conference on Advances in Plant Biochemistry and Biotechnology (APBB), 2014.
3. **Abdullahi Muhammad**, Maziah Mahmood and Syahida Ahmad. Antioxidant, Phenolics, Polyphenols and Flavonoids Contents in *Zingiber Officinale* Var. *Rubrum* Rhizomes and Leaves. 22nd Biotech Colloquim 2014

Publications

1. **Abdullahi Muhammad**, Maziah Mahmood and Syahida Ahmad. Effects of growth stages and storage temperatures on the total antioxidants activity of *Zingiber officinale* var. *rubrum*. Pakistan Journal of Pharmaceutical Sciences. Submitted
2. **Abdullahi Muhammad**, Maziah Mahmood and Syahida Ahmad. In vitro antioxidant, total flavonoids, phenolics and polyphenols of *Zingiber officinale* var. *rubrum* leaf and rhizome extracts. *Food Chemistry*. Submitted



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