UNIVERSITI PUTRA MALAYSIA

EFFECTS OF DIFFERENT LIGHT INTENSITIES AND CO2 ENRICHMENT ON YIELD AND PHARMACEUTICAL QUALITY OF YOUNG GINGER (ZINGIBER OFFICINALE ROSCOE)

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By

ALI GHASEMZADEH DAGHIGH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Doctor Philosophy

August 2011
DEDICATIONS

This thesis is dedicated to my:

Lovely wife and son

Narges and Mani

With innermost and everlasting affection and love
EFFECTS OF DIFFERENT LIGHT INTENSITIES AND CO\textsubscript{2} ENRICHMENT ON YIELD AND PHARMACEUTICAL QUALITY OF YOUNG GINGER (\textit{Zingiber officinale Roscoe})

By

ALI GHASEMZADEH DAGHIGH

August 2011

Chairman : Associate Professor Hawa bt Jaafar, PhD

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In this study, the effect of some environmental factors (light intensity and CO\textsubscript{2} concentration) on physiological characteristics and biochemical changes of Malaysian young ginger varieties namely Halia Bentong and Halia Bara were considered. Ginger (\textit{Zingiber officinale}) is herbaceous annual plant of the family Zingiberaceae, locally known as Halia in Malaysia. It is one of the most common medicinal plants used by diverse ancient cultures and has been widely used as health food and in cosmetic industries. Information on the polyphenolic compounds (flavonoids and phenolic acids) of Malaysian young ginger and their biological activities are still scarce and such data would be useful to provide information on foods containing high levels of beneficial components. A factorial experiment was designed to determine effect of different solvent polarity on extraction yield of flavonoids and phenolic acids of Malaysian young ginger varieties namely Halia Bentong and Halia Bara. The results showed extraction solvent had significant effects on total phenolics (TP), total flavonoids (TF), quercetin, catechin and rutin
contents and antioxidant activity of Halia Bentong and Halia Bara. The highest content of TP (39.06 mg/g dry weight), TF (7.05 mg/g dry weight) and 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) assay activities (58.21%) were found in methanolic extracts compared to acetone and chloroform extracts. A factorial experiment was designed to determine effect of different light intensity (310, 460, 630 and 790 μmol/m²/s) on yield and pharmaecological quality of Halia Bentong and Halia Bara. Between the two varieties and four levels of glasshouse light intensities (310, 460, 630 and 790 μmol/m²/s) that were tested, Halia Bara exhibited highest TF content (8.45 mg/g dry weight) under 310 μmol/m²/s, while TP was high (39.06 mg/g dry weight) in this variety under a light intensity of 790 μmol/m²/s. The partitioning of TF and TP at 16 weeks after planting was: leaves > rhizomes > stems. Antioxidant activities determined by the DPPH assay and ferric reducing (FRAP) activity in both varieties increased significantly with increasing TF concentration, and high antioxidant activity 62.8% for DPPH assay, and 788.5 μmol Fe(II)/g dry weight for FRAP assay, were observed respectively in the leaves and rhizomes of Halia Bara grown under 310 μmol/m²/s. The results of HPLC analysis indicated that synthesis and partitioning of quercetin, rutin, catechin, epicatechin and naringenin were high in plants grown under 310 μmol/m²/s. The evidence from this study elucidated that the average value of flavonoids synthesis for both varieties increased (Halia Bentong 26.1%; Halia Bara 19.6%) by decreasing light intensity from 790 to 310 μmol/m²/s. However, high photosynthesis rate (12.25 μmol CO₂/m²/s in Halia Bara), plant biomass (79.47 g in Halia Bentong) and total soluble carbohydrate content (18.49 mg/g dry weight in Halia Bara) were observed at 790 μmol/m²/s. A factorial experiment was designed to determine alteration of flavonoids synthesis during growth period of
young ginger varieties for 16 weeks. Time to harvest was observed to be an important factor for accumulation of TF in the plants. In both varieties, the concentration of flavonoids in the leaves decreased (Halia Bentong, 42.3%; Halia Bara 36.7%), and in the rhizomes it increased (Halia Bentong 59.6%; Halia Bara 60.1%) as the growth period increased from 8 to 16 weeks. A split-split plot experiment was designed to investigate effects of two level of CO$_2$ (400 and 800 µmol/mol) on production of primary metabolites (soluble sugar and starch), secondary metabolites (flavonoids and phenolic acids) and leaf gas exchange of Halia Bentong and Halia Bara for 16 weeks. The results showed that as CO$_2$ levels increased from 400 to 800µmol/mol TF, TP, total soluble carbohydrates (TSC) and starch content also increased considerably. In this study, eight flavonoids and six phenolic acids were identified. In addition, after fisetin (3.11mg/g dry weight, in Halia Bara rhizome) in CO$_2$ enriched plants, quercetin (1.33mg/g dry weight, in Halia Bara leaves), and morin (0.876mg/g dry weight, in Halia Bentong rhizome) were detected in the highest amount. Concurrently, gallic acid (0.645mg/g dry weight, in Halia Bara leaves) and vanillic acids (0.357mg/g dry weight, in Halia Bara rhizome) were the phenolic acids that were most highly detected. The most obvious finding to emerge from this study is that CO$_2$ enriched ginger plants exhibited the ability to synthesize new compounds such as vanillic acid, cinnamic acid and salicylic acid which were not detected from gingers grown under ambient CO$_2$ concentration. The current study is the first report of the isolation of flavonoid and phenolic compounds from Malaysian young ginger varieties and has shown these varieties are the major dietary source for flavonoids especially quercetin, fisetin and morin. Higher photosynthetic rate (10.05µmol CO$_2$/m$^2$/s in Halia Bara) and plant
biomass (83.4 g in Halia Bentong) were observed at 800µmol/mol CO$_2$ compare to 400µmol/mol CO$_2$. Stomatal conductance decreased and water use efficiency increased with elevated CO$_2$ concentration. Free radical scavenging power (DPPH) increased about 30% in Halia Bentong and 21.4% in Halia Bara by elevated CO$_2$ concentration and the rhizomes exhibited more enhanced free radical scavenging power, with 44.9% in Halia Bentong and 46.2% in Halia Bara. In addition, enriched Halia Bara exhibited the highest anticancer activity on MCF–7 cancer cells with IC$_{50}$ values of 25.3 and 27.31µg/ml respectively, for rhizome and leaf extract. The IC$_{50}$ values for MDA–MB–231 exhibition were 30 and 32.81µg/ml, respectively for rhizome extract of Halia Bara and Halia Bentong. These results indicate that the yield and pharmaceutical quality of Malaysian young ginger varieties can be enhanced by controlled environment production and CO$_2$ enrichment.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KESAN PERBEZAAN KEPEKATAN CAHAYA DAN PERKAYAAN CO₂ PADA HASIL DAN KUALITI FARMASEUTIKAL HALIA MUDA (ZINGIBER OFFICINALE ROSCOE)

Oleh

ALI GHASEMZADEH Daghhigh

Ogos 2011

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Dalam kajian ini, beberapa faktor persekitaran (keamatan cahaya dan kepekatan CO₂) telah memberi kepada perubahan fisiologikal dan biokimia pada dua variati halia Malaysia iaitu Halia Bentong dan Halia Bara. Halia (Zingiber officinale) ialah tanaman herba dalam family Zingiberaceae, yang juga dikenali sebagai “Halia” di Malaysia. Ianya adalah salah satu pokok ubatan yang paling biasa digunakan oleh pelbagai budaya dan telah digunakan secara meluas sebagai makanan kesihatan dan industri kosmetik. Maklumat tentang sebatian polifenolik (asid flavonoid dan fenolik) di dalam halia serta aktiviti biokimianya masih kurang dan data ini akan menyediakan maklumat tentang khasiat halia yang mengandungi banyak komponen berguna untuk makanan kesihatan. Satu eksperimen factorial telah direkabentuk untuk menentukan kesan pelbagai pelarut berikutub pada pengekstrakan asid flavonoid and fenol pada dua jenis varieti halia iaitu bentong dan bara Keputusan menunjukkan pelarut mempunyai kesan significant pada jumlah fenolik (TP), jumlah flavonoid (TF), quercetin, catechin dan kandungan rutin dan aktiviti antioksida dari
Halia Bentong dan Halia Bara. Kandungan tertinggi TP (39.06mg/g berat kering), TF (7.05mg/g berat kering) dan aktiviti assay 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) (58.21%) telah ditemui dalam ekstrak metanolik dibandingkan dengan aseton dan klorofom. Satu eksperimen factorial telah direkabentuk untuk menentukan kesan pelbagai kepekatan cahaya pada (310, 460, 630 and 790μmol/m²/s) pada hasil dan kualiti Halia bentong dan bara. Diantara dua dua varieti dan empat tahap kepekatan cahaya yang telah diuji, Halia Bara menunjukkan kandungan TF tertinggi (8.45mg/g berat kering) dibawah 310μmol/m²/s, manakala TP adalah paling tinggi (39.06mg/g berat kering) dalam varieti ini di bawah kepekatan cahaya sebanyak 790μmol/m²/s. Pembahagian TF dan TP pada minggu ke 16 selepas penanaman adalah tertingi dalam daun > rizom > batang. Aktiviti antioksida yang ditentukan dengan assay DPPH dan aktiviti penurunan ferric (FRAP) di dalam kedua-dua varieti meningkat secara signifikan dengan peningkatan kepekatan TF, dan aktiviti antioksida yang tinggi 62.8% untuk assay DPPH, dan 788.5μmol Fe(II)/g berat kering untuk assay FRAP, telah diperhatikan masing-masing pada daun dan rizom Halia Bara ditanam di bawah 310μmol/m²/s. Keputusan daripada analisis HPLC menunjukkan bahawa sintesis dan pembahagian quercetin, rutin, catechin, epicatechin dan naringenin adalah tinggi dalam tanaman yang ditanam pada kepekatan cahaya pada 310μmol/m²/s. Bukti yang diperolehi dari kajian ini menghuraikan bahawa nilai purata untuk sintesis flavonoid untuk kedua-dua variati telah meningkat (Halia Bentong 26.1%; Halia Bara 19.6%) dengan penurunan keamatan cahaya dari 790 kepada 310μmol/m²/s. Walaubagaimanapun, kadar fotosintesis (12.25μmolCO₂/m²/s dalam Halia Bara), biomass tanaman (79.47g dalam Halia Bentong) dan jumlah karbohidrat terlarut tertinggi (18.49mg/ g berat kering in Halia Bara) telah didapati
pada kepekatan cahaya 790µmol/m²/s. Satu eksperimen faktorial telah dijalankan untuk mengenalpasti pengubahsuaian sintesis flavonoid pada tumbesaran variety halia selama 16 minggu. Masa menuai telah diperhatikan sebagai faktor penting untuk pengumpulan TF dalam tanaman. Dalam kedua-dua variati, kepekatan flavonoid dalam daun menurun (Halia Bentong, 42.3%; Halia Bara 36.7%), dan ianya meningkat dalam rizom (Halia Bentong 59.6%; Halia Bara 60.1%) apabila tempoh pertumbuhan meningkat dari minggu ke 8 hingga minggu ke 16. Eksperimen dalam rekabentuk spit-split plot telah dijalankan untuk menyiasat kesan dua tahap kepekatan CO2 (400 dan 800µmol/mol) pada pembentukan metabolit primer (gula terlarut dan kanji), metabolit sekunder (asid flavonoid dan fenol) serta fotosintesis Halia Bentong dan Bara selama 16 minggu. Keputusan menunjukkan apabila tahap CO2 meningkat dari 400 kepada 800µmol/mol TF, TP, jumlah karbohidrat terlarut (TSC) dan kandungan kanji juga sangat meningkat. Dalam kajian ini, lapan flavonoid dan enam asid fenolik telah dikenalpasti. Selain itu, fisetin (3.11mg/g berat kering, dalam rizom Halia Bara), quercetin (1.33mg/g berat kering, dalam daun Halia Bara), and morin (0.876mg/g berat kering, dalam rizom Halia Bentong) telah ditemui dalam amaun tertinggi apabila diperkayakan pada kepekatan CO2 yang tinggi. Dalam masa yang sama, asid gallic (0.645mg/g berat kering, dalam daun Halia Bara) dan asid vanillic (0.357mg/g berat kering, dalam rizom Halia Bara) adalah komponen asid fenolik yang paling banyak dikesan. Penemuan yang paling ketara daripada kajian ini adalah tanaman halia yang dikayakan dengan CO2 menunjukkan kebolehan untuk mensintesis komponen baru seperti asid vanillic, asid cinnamic dan asid salicylic yang mana tidak dapat dikesan dalam halia yang ditanam dalam keadaan kawalan (400µmol/mol). Kajian ini adalah laporan pertama pengasingan komponen asid
flavonoid dan fenolik dari varieti Halia Malaysia dan menunjukkan bahawa varieti tersebut boleh menjadi sumber utama asid flavonoid utama seperti kuersetin, fisetin dan Morin. Kadar fotosintesis yang lebih tinggi (10.05 µmol CO₂/m²/s dalam Halia Bara) dan biomass tanaman (83.4g dalam Halia Bentong) telah diperhatikan pada kepekatan CO₂ sebanyak 800 µmol/mol CO₂ jika dibandingkan dengan 400 µmol/mol CO₂. Kekondusksian stomata menurun dan kecekapan penggunaan air meningkat dengan peningkatan kepekatan CO₂. Aktiviti DPPH meningkat sebanyak 30% dalam Halia Bentong dan 21.4% dalam Halia Bara dengan peningkatan kepekatan CO₂ dan rizom menunjukkan lebih peningkatan DPPH, dengan 44.9% dalam Halia Bentong dan 46.2% dalam Halia Bara. Keputusan ujian antikanser menunjukkan bahawa CO₂-diperkaya Halia Bara yang diperkaya dengan kepekatan CO₂ tinggi mempamerkan aktiviti antikanser yang paling tinggi pada sel MCF–7 kanser dengan nilai IC₅₀ sebanyak 25.3 dan 27.31 µg/ml masing-masing untuk ekstrak rizom dan daun. Nilai IC₅₀ untuk MDA–MB–231 adalah 30 and 32.81 µg/ml, masing-masing untuk ekstrak rizom Halia Bara dan Halia Bentong. Keputusan menunjukkan hasil dan kualiti farmaseutikal variati halia muda boleh ditingkatkan dengan produksi persekitaran terkawal dan perkayaan CO₂.
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Finally, I offer thanks to my family: my parents for their patience and support. Especially, I am very much indebted to my lovely wife, Narges, who always helps me out and pray for me.

Sincerely

ALI GHASEMZADEH DAGHIGH
I certify that a Thesis Examination Committee has met on 16 August 2011 to conduct the final examination of Ali Ghasemzadeh Daghigh on his thesis entitled "Effects of Different Light Intensities and CO₂ Enrichment on Yield and Pharmaceutical Quality of Young Ginger (Zingiber officinale Roscoe)" 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 relevant degree of Doctor of Philosophy.

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xiii
This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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DECLARATION

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

____________________________________
ALI GHASEMZADEH DAGHIGH

Date: 16 August 2011
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>iii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>vii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>xi</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>xiii</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>xv</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xix</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xxii</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xxiv</td>
</tr>
</tbody>
</table>

## CHAPTER

1. **INTRODUCTION**
   - 1.1 General introduction 1
   - 1.2 General objectives 7
   - 1.3 Specific objectives 7
   - 1.4 Hypothesis 8

2. **LITERATURE REVIEW**
   - 2.1 Traditional medicine 9
   - 2.2 Medicinal plants 10
   - 2.3 Important chemical compounds in medicinal plants 11
     - 2.3.1 Primary and secondary metabolites 11
     - 2.3.2 Phenolic asids 13
     - 2.3.3 Flavonoids 15
     - 2.3.4 Medicinal properties of flavonoids 17
       - 2.3.4.1 Antioxidant properties of phenolics and flavonoid 17
       - 2.3.4.2 Anticancer properties of phenolics and flavonoids 19
     - 2.3.5 The function of flavonoids in plants 20
     - 2.3.6 Phenolics and flavonoids biosynthesis in plants 21
   - 2.4 Ginger (*Zingiber officinale* Roscoe) 23
     - 2.4.1 Botanical characteristics of *Zingiber officinale* 23
     - 2.4.2 *Zingiber officinale* var Halia Bentong 24
     - 2.4.3 *Zingiber officinale* var Halia Bara 24
     - 2.4.4 Chemical compounds of ginger 25
     - 2.4.5 Ginger production 26
     - 2.4.6 The use of ginger (*Zingiber officinale*) as a traditional medicinal 27
       - 2.4.6.1 Antioxidant activities of ginger 27
       - 2.4.6.2 Anticancer activity of ginger 28
   - 2.5 Enhancing quality of food and future perspectives 29
   - 2.6 Effects of environmental factors on plant growth and 30
secondary metabolites

2.6.1 Effects of light intensity 30
2.6.2 CO₂ enrichment 32
2.6.3 Effects of CO₂ enrichment on synthesis of secondary metabolites 33
2.6.4 Effects of CO₂ enrichment on leaf gas exchanges 36
2.6.5 Effects of CO₂ enrichment on water use efficiency 38

3 GENERAL MATERIALS AND METHODS 39

4 EFFECTS OF SOLVENT TYPE ON PHENOLICS AND FLAVONOIDS CONTENT IN YOUNG GINGER (ZINGIBER OFFICINALE ROSCOE) EXTRACTS 53

Article 1 53

5 TO INVESTIGATE AND DETERMINE CHANGES IN THE ACCUMULATION AND PROFILING OF TOTAL PHENOLIC AND FLAVONOID CONTENTS AND ANTIOXIDANT ACTIVITY WITH CHANGES IN LIGHT INTENSITY AND GROWTH PERIOD IN YOUNG GINGER. 71

Article 2 71
Article 3 84
Article 4 102

6 TO INVESTIGATE AND DETERMINE THE CHANGES IN THE YIELD AND LEAF GAS EXCHANGE IN YOUNG GINGER WITH CHANGES IN FLAVONOID SYNTHESIS AND LIGHT INTENSITY. 121

Article 5 121

7 TO EVALUATE THE EFFECT OF CARBON DIOXIDE ENRICHMENT ON YIELD, LEAF GAS EXCHANGE AND ACCUMULATION OF FLAVONOIDS, PHENOLIC ACIDS AND ANTIOXIDANT ACTIVITIES IN YOUNG GINGER. 146

Article 6 146
Article 7 167

8 TO DETERMINE ANTICANCER ACTIVITIES OF CO₂-ENRICHED YOUNG GINGER. 190
### Article 8

#### 9 SUMMARY, GENERAL CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>REFERENCES</td>
<td>217</td>
</tr>
<tr>
<td>APPENDIX</td>
<td>249</td>
</tr>
<tr>
<td>BIODATA OF STUDENT</td>
<td>283</td>
</tr>
<tr>
<td>LIST OF PUBLICATIONS</td>
<td>284</td>
</tr>
</tbody>
</table>