UNIVERSITI PUTRA MALAYSIA

ANALYSIS OF FLAVONOIDS, PHENOLIC COMPOUNDS AND ESSENTIAL OILS IN *CURCUMA AERUGENOSA* ROXB. (ZINGIBERACEAE)

LOO WAI SUM

FBSB 2006 28

ANALYSIS OF FLAVONOIDS, PHENOLIC COMPOUNDS AND ESSENTIAL OILS IN *CURCUMA AERUGENOSA* ROXB. (ZINGIBERACEAE)

LOO WAI SUM

MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA

2006

ANALYSIS OF FLAVONOIDS, PHENOLIC COMPOUNDS AND ESSENTIAL OILS IN *CURCUMA AERUGENOSA* ROXB. (ZINGIBERACEAE)

By

LOO WAI SUM

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

June 2006

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

ANALYSIS OF FLAVONOIDS, PHENOLIC COMPOUNDS AND ESSENTIAL OILS IN *CURCUMA AERUGENOSA* ROXB. (ZINGIBERACEAE)

By

LOO WAI SUM

June 2006

Chairman: Associate Professor Radzali Muse, PhD

Faculty: Biotechnology and Biomolecular Sciences

Curcuma aerugenosa Roxb. or locally known as "Temu Hitam" has been used as medicine. It contains useful secondary metabolites. The amount of total phenolic content detected in *Curcuma aerugenosa* is ranged from 4.5 – 50.9 mg pyrogallol equivalent per g dry weight. Leaf and root extracts exhibited strong antioxidant activity in ferric thiocyanate method (FTC), thiobarbituric acid method (TBA), scavenging effect of the 2,2-diphenyl-1-picyl-hydrazyl (DPPH) radical and conjugated diene formation.

In this study, flavonoids and phenolic compounds were determined by using high-performance liquid chromatography (HPLC). The highest content of rutin (1704.7 ± 68.2 μg/g dry weight) and quercetin (1992.4 ± 92.2 μg/g dry weight) were found in leaf unhydrolized and hydrolized extracts respectively. Tuber extract exhibited the highest content of total phenolic compounds (988.6 ± 22.7 μg/g dry weight). The main phenolic

compound detected was catechol (615.4 ± 8.2 μg/g dry weight). The essential oils of *C. aerugenosa* were obtained by hydrodistillation. They were analysed by a gas chromatography mass spectrometry (GC-MS). The major compounds were camphor, 1,8-cineole, β-pinene and camphene. Rhizome oil was found to contain the highest percentage of 1,8-cineole (45.9%) and camphor (15.9%).

In this study, Gamborg B5 media with combination of 0.2 mg/L (w/v) 2,4-dichlorophenoxyacetic acid (2,4-D) and 2.0 mg/L (w/v) 6-benzylaminopurine (BAP) was found as the best for initiation of rhizome bud and *in vitro* grown seedling-leaf callus. A different combination was obtained in callus induction of *in vitro* grown seedling-petiole and root which was 0.2 mg/L (w/v) of 2,4-D and 1.0 mg/L (w/v) of 6-furfurylaminopurine (kinetin). The callus induced in this study was friable and light yellow in colour.

Study on the effect of sucrose concentrations [1, 2, 3 and 4% (w/v)] showed that optimum callus growth was achieved with 3% of sucrose. The highest callus growth of *in vitro* seedling-leaf and root derived callus were achieved in 0.060 and 0.020 mg/L (w/v) of 2,4-D respectively. Both callus produced 6.382 ± 0.408 and 6.440 ± 0.193 g fresh weight /culture respectively. Dicamba [0.150 mg/L (w/v)] produced the highest production of biomass in rhizome bud (5.603 ± 0.263 g fresh weight /culture) and *in vitro* grown seedling-petiole (5.458 ± 0.246 g fresh weight / culture) derived callus.

Elicitors, yeast extract [20, 40, 60, 80 and 100 mg/L (w/v)] and chitosan [20, 30, 40, 50 and 60 mg/L (w/v)] exhibited different effects on monoterpene (1,8-cineole, linalool, β-pinene and camphor) production. The highest content of 1,8-cineole (0.459 ± 0.020 µg/g fresh weight) and camphor (0.067 ± 0.003 µg/g fresh weight) were produced in 40 and 60 mg/L (w/v) yeast extract added to *in vitro* grown seedling-petiole derived callus respectively. At 50 mg/L (w/v) of chitosan revealed the highest production of linalool (0.374 ± 0.015 µg/g fresh weight) and β-pinene (0.495 ± 0.021 µg/g fresh weight) *in vitro* grown seedling-petiole derived callus.

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

ANALISIS SEBATIAN FLAVONOID, FENOLIK DAN MINYAK PATI DI DALAM *Curcuma aerugenosa* ROXB. (ZINGIBERACEAE)

Oleh

LOO WAI SUM

Jun 2006

Pengerusi: Profesor Madya Radzali Muse, PhD

Fakulti: Bioteknologi dan Sains Biomolekul

Curcuma aerugenosa Roxb. atau dikenali sebagai temu hitam oleh masyarakat tempatan telah digunakan sebagai ubat. Ia mengandungi pelbagai metabolik sekunder. Kajian telah dijalankan untuk menentukan kandungan keseluruhan fenol. Julat nilai yang terhasil ialah 4.5 – 50.9 mg pirogalol / g berat kering. Ekstrak daun dan akar memberi kesan antioxidan yang baik di dalam kaedah ferik tiosianat (FTC), kaedah asid tiobarbiturik (TBA), pelupusan radikal 2,2-difenil-1-picil-hidrazil (DPPH) dan pembentukkan diene konjugat.

Kandungan flavonoid dan bahan fenol telah dianalisis dengan menggunakan teknik kromatografi turus berprestasi tinggi (HPLC). Ekstrak daun mencatatkan kandungan rutin (1704.7 ± 68.2 μg/g berat kering) dan kuercetin (1992.4 ± 92.2 μg/g berat kering) yang paling tinggi. Ekstrak umbisi mencatatkan kandungan bahan fenol yang paling tinggi (988.6 ± 22.7 μg/g berat kering). Bahan fenol utama yang di dalamnya ialah katekol

(615.4 ± 8.2 μg/g berat kering). Minyak pati telah dikaji menggunakan teknik kromatografi gas spektometik jisim (GC-MS). Kamfor, 1,8-cineole, β-pinen dan kamfen telah dikesan sebagai bahan utama di dalam minyak pati. Rizom mencatatkan peratusan 1,8-cineole (45.9%) dan kamfor (15.9%) tertinggi.

Media Gamborg B5 bersama dengan penambahan 0.2 mg/L (w/v) 2,4-diklorofinoksi asetik (2,4-D) dan 2.0 mg/L (w/v) benzilaminopurin (BAP) adalah media yang baik bagi pembentukan kalus tunas dan daun anak pokok media. Manakala, kalus batang dan akar anak pokok media terhasil dengan penambahan 0.2 mg/L (w/v) 2,4-D dan 1.0 mg/L (w/v) 6-furfurilaminopurin (kinetin). Kalus yang terbentuk berwarna kuning cerah.

Kajian ke atas kesan kepekatn sukrosa [1, 2, 3 dan 4% (w/v)] mendapati 3% (w/v) menggalakkan pertumbuhan kalus yang maksimum. Penambahan 2,4-D yang berkepekatan 0.060 dan 0.020 mg/L (w/v) menggalakkan pertumbuhan kalus daun dan akar anak pokok media yang maksimum. Biomas kalus yang terhasil ialah 6.382 ± 0.408 dan 6.440 ± 0.193 g/tiub kultur berat basah masing-masing. Manakala, penambahan 0.150 mg/L (w/v) dikamba mencatatkan pembentukan biomas kalus yang maksimum bagi tunas (5.603 ± 0.263 g/tiub kultur berat basah) dan batang anak pokok media (5.458 ± 0.246 g/tiub kultur berat basah).

Elisitor seperti ekstrak yis [20, 40, 60, 80 and 100 mg/L (w/v)] dan kitosan [20, 30, 40, 50 and 60 mg/L (w/v)] memberi kesan yang berlainan ke atas penghasilan monoterpen (1,8-cineole, kamfor, β-pinen dan kamfen). Penambahan 40 dan 60mg/L (w/v) ekstrak

yis ke dalam media kalus batang mencatatkan kandungan 1,8-cineole (0.459 ± 0.020 μg/g berat basah) dan kamfor (0.067 ± 0.003 μg/g berat basah) yang paling tinggi. Kalus batang yang dirawat dengan 50 mg/L (w/v) kitosan telah mencatatkan penghasilan linalol (0.374 ± 0.015 μg/g berat basah) dan β-pinen (0.495 ± 0.021 μg/g berat basah) tertinggi di kalus batang.

ACKNOWLEDGEMENTS

I would like to thank my supervisors, Associate Professor Dr. Radzali Muse, Prof. Dr. Maziah Mahmood, Prof. Dr. Suhaila Mohamed and Associate Professor Dr. Mohd Aspollah Sukari for their guidance, encouragement and patience towards the completion of this thesis.

Special thanks should be granted to my mother, Madam Teoh Siew Yook and my lovely sister, Loo Wai Yee for their supports and sacrifices throughout the course.

I greatly appreciate the support from Department of the Biochemistry, Universiti Putra Malaysia for giving me the opportunity to use all available facilities.

Lastly, thanks to all friends throughout my study.

I certify that an Examination Committee has met on ____9th June 2006___ to conduct the final examination of Loo Wai Sum on her Master of Science thesis entitled "Analysis of Flavonoids, Phenolic Compounds and Essential Oils in *Curcuma aerugenosa* ROXB. (Zingiberaceae)" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Norhani Abdullah, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Mohd Arif Syed, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Johari Ramli, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Shaida Fariza Sulaiman, PhD
Associate Professor
School of Biological Science
Universiti Sains Malaysia
(External Examiner)

_____
HASANAH MOHD GHAZALI, PhD
Professor/ Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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

Radzali Muse, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Maziah Mahmood, Ph.D
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Mohd Aspollah Sukari, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

Suhaila Mohamed, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

_____
AINI IDERIS, PhD
Professor/ Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 12 OCTOBER 2006

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 degree at UPM or other institutions

_____

LOO WAI SUM

Date:

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

NAA	α-naphthalene acetic acid
TFA	trifluoroacetic acid
GC	gas chromatography
HPLC	high performance liquid chromatography
2,4-D	2,4-Dichlorophenoxyacetic acid
BAP	6-benzylaminopurine
Dicamba	3,6-dichloro-o-aniscis acid
Kinetin	6-furfurylaminopurine
min	Minute
v/v	Volume over volume
w/v	Weight per volume
BHT	Butylated hydoxytoluene
FTC	Ferric thiocyanate
TBA	Thiobarbituric acid
HCl	Hydrochloric acid
μg	Microgram
R_t	Retention time
NaOH	Sodium hydroxide
FW	Fresh weight
DW	Dry weight