



UNIVERSITI PUTRA MALAYSIA

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

LOO WAI SUM

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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



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fulfilment of requirement for the degree of Master of Science

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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-picryl-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 $\mu\text{g/g}$ dry weight) and quercetin (1992.4 ± 92.2 $\mu\text{g/g}$ dry weight) were found in leaf unhydrolyzed and hydrolyzed extracts respectively. Tuber extract exhibited the highest content of total phenolic compounds (988.6 ± 22.7 $\mu\text{g/g}$ dry weight). The main phenolic



compound detected was catechol ($615.4 \pm 8.2 \mu\text{g/g}$ dry weight). The essential oils of *C. aeruginosa* 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-furfurylamino purine (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 ($0.459 \pm 0.020 \mu\text{g/g}$ fresh weight) and camphor ($0.067 \pm 0.003 \mu\text{g/g}$ 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 ($0.374 \pm 0.015 \mu\text{g/g}$ fresh weight) and β -pinene ($0.495 \pm 0.021 \mu\text{g/g}$ 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 aeruigenosa* ROXB. (ZINGIBERACEAE)**

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Jun 2006

Pengerusi: Profesor Madya Radzali Muse, PhD

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Curcuma aeruigenosa 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 antioksidan yang baik di dalam kaedah ferik tiosianat (FTC), kaedah asid tiobarbiturik (TBA), pelupusan radikal 2,2-difenil-1-picil-hidrazil (DPPH) dan pembentukan 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 $\mu\text{g/g}$ berat kering) dan kuercetin (1992.4 ± 92.2 $\mu\text{g/g}$ berat kering) yang paling tinggi. Ekstrak umbisi mencatatkan kandungan bahan fenol yang paling tinggi (988.6 ± 22.7 $\mu\text{g/g}$ berat kering). Bahan fenol utama yang di dalamnya ialah katekol



($615.4 \pm 8.2 \mu\text{g/g}$ berat kering). Minyak pati telah dikaji menggunakan teknik kromatografi gas spektrometik 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 kepekatan 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 ($0.459 \pm 0.020 \mu\text{g/g}$ berat basah) dan kamfor ($0.067 \pm 0.003 \mu\text{g/g}$ berat basah) yang paling tinggi. Kalus batang yang dirawat dengan 50 mg/L (w/v) kitosan telah mencatatkan penghasilan linalol ($0.374 \pm 0.015 \mu\text{g/g}$ berat basah) dan β -pinen ($0.495 \pm 0.021 \mu\text{g/g}$ berat basah) tertinggi di kalus batang.



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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 aeruginosa* 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:

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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

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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 hydroxytoluene
FTC	Ferric thiocyanate
TBA	Thiobarbituric acid
HCl	Hydrochloric acid
μ g	Microgram
R _t	Retention time
NaOH	Sodium hydroxide
FW	Fresh weight
DW	Dry weight







