

การทดสอบฤทธิ์ต้านอนุมูลอิสระ เอมส์ และไมโครนิวคลีไอของสารสกัดกวาวเครือขาว

*Pueraria mirifica* กวาวเครือแดง *Butea superba* กวาวเครือดำ *Mucuna*

*collettii* และผักปัดผี *Pueraria lobata*



นางสาว วันดี สุทธิจิตร

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

สาขาเทคโนโลยีทางชีวภาพ หลักสูตรเทคโนโลยีทางชีวภาพ

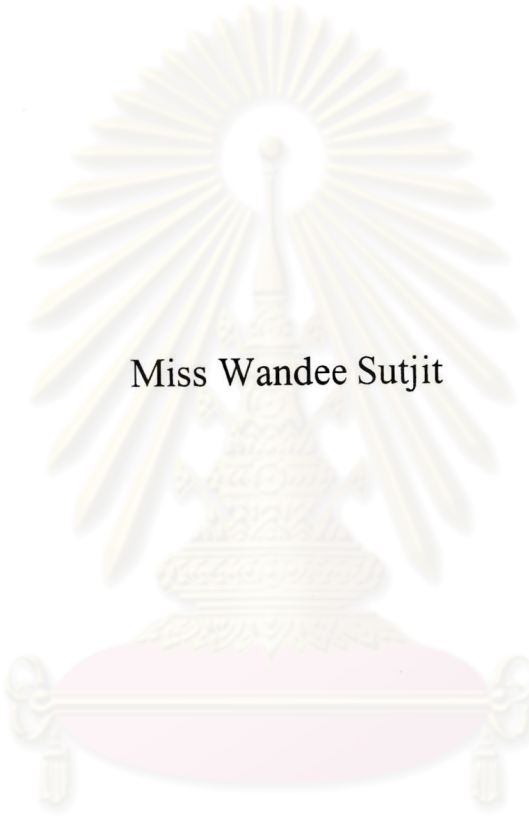
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2546

ISBN 974-17-4933-3

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

THE ANTIOXIDANT TEST, AMES' TEST AND MICRONUCLEI  
TEST OF CHEMICAL EXTRACT FROM WHITE KWAO KRUA  
*Pueraria mirifica*, RED KWAO KRUA *Butea superba*, BLACK KWAO  
KRUA *Mucuna collettii* AND KUDZU *Pueraria lobata*



Miss Wandee Sutjit

ศูนย์วิทยทรัพยากร  
จุฬาลงกรณ์มหาวิทยาลัย

A Thesis Submitted in Partial Fulfillment of the Requirements  
for the Degree of Master of Science in Biotechnology

Faculty of Science  
Chulalongkorn University  
Academic Year 2003  
ISBN 974-17-4933-3

Thesis Title            The Antioxidant test, Ames's test and Micronuclei test  
of chemical extract from White Kwao Krua *Pueraria  
mirifica*, Red Kwao Krua *Butea superba*, Black Kwao  
Krua *Mucuna collettii* and Kudzu *Pueraria lobata*

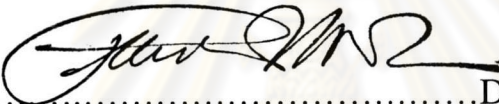
By                            Miss Wandee Sutjit

Field of study            Biotechnology


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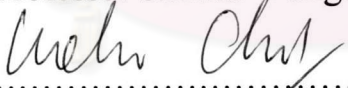
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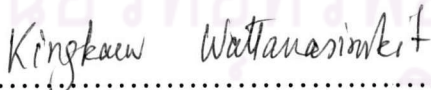
Accepted by the Faculty of Science, Chulalongkorn University  
in Partial Fulfillment of the Requirements for the Master's Degree

  
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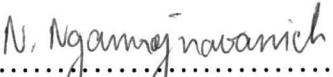
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วันดี สุทธิจิตร : การทดสอบฤทธิ์ต้านอนุมูลอิสระ เอมส์ และไมโครนิวเคลียสไอของสารสกัด กวาวเครือขาว *Pueraria mirifica* กวาวเครือแดง *Butea superba* กวาวเครือดำ *Mucuna collettii* และผักปืดผี *Pueraria lobata*. (THE ANTIOXIDANT TEST, AMES' TEST AND MICRONUCLEI TEST OF CHEMICAL EXTRACT FROM WHITE KWAO KRUA *Pueraria mirifica*, RED KWAO KRUA *Butea superba*, BLACK KWAO KRUA *Mucuna collettii* AND KUDZU *Pueraria lobata*) อ. ที่ปรึกษา : รศ. ดร. วิชัย เติตชีวีศาสตรจารย์ จำนวน 128 หน้า. ISBN 974-17-4933-3.

การวิเคราะห์ฤทธิ์ต้านอนุมูลอิสระโดยวิธี DPPH ของกวาวเครือขาวจาก 28 จังหวัด กวาวเครือแดง 23 จังหวัด กวาวเครือดำ 4 จังหวัดในประเทศไทย เปรียบเทียบกับ *Pueraria lobata* จากประเทศจีน พบว่าสารสกัดกวาวเครือดำจากจังหวัดเชียงรายให้ฤทธิ์ต้านอนุมูลอิสระสูงที่สุดในกลุ่มประชากร ( $IC_{50} = 55.53 \pm 2.66$  ไมโครกรัม/มิลลิลิตร) สารสกัดกวาวเครือแดงจากจังหวัดเลยให้ฤทธิ์ต้านอนุมูลอิสระสูงที่สุดในกลุ่มประชากร ( $IC_{50} = 227.08 \pm 0.38$  ไมโครกรัม/มิลลิลิตร) และสารสกัดกวาวเครือขาวจากจังหวัดอุทัยธานีให้ฤทธิ์ต้านอนุมูลอิสระสูงที่สุดในกลุ่มประชากร ( $IC_{50} = 2,470.38 \pm 37.81$  ไมโครกรัม/มิลลิลิตร) และ *Pueraria lobata* ให้ฤทธิ์ต้านอนุมูลอิสระต่ำ ( $IC_{50} = 2,482 \pm 66.11$  ไมโครกรัม/มิลลิลิตร) ผลการศึกษาความสัมพันธ์ระหว่างฤทธิ์ต้านอนุมูลอิสระกับปริมาณสารไอโซฟลาโวน 5 ชนิดจากหัวกวาวเครือขาว ซึ่งวิเคราะห์ผลโดยวิธี HPLC พบว่าฤทธิ์ต้านอนุมูลอิสระมีความสัมพันธ์โดยตรงกับปริมาณ Daidzein ได้ทำการเลือกกวาวเครือดำ กวาวเครือแดง และกวาวเครือขาว ที่มีฤทธิ์ต้านอนุมูลอิสระสูงที่สุดในแต่ละกลุ่มประชากร สำหรับทดสอบฤทธิ์การก่อกลายพันธู์และฤทธิ์ยับยั้งการก่อกลายพันธู์ โดยวิธีเอมส์ และทดสอบไมโครนิวเคลียส พบว่าสารสกัด กวาวเครือขาว กวาวเครือแดง กวาวเครือดำ และ *Pueraria lobata* ไม่มีฤทธิ์ก่อกลายพันธู์แต่มีฤทธิ์ในการยับยั้งการก่อกลายพันธู์ต่อแบคทีเรียซัลโมเนลลาทั้งสายพันธู์ TA98 และ TA100 ทั้งที่มีเอนไซม์ และไม่มีเอนไซม์กระตุ้น กวาวเครือขาว กวาวเครือแดง กวาวเครือดำ และ *Pueraria lobata* ไม่มีฤทธิ์ก่อกลายพันธู์ โดยไม่ก่อให้เกิดไมโครนิวเคลียสที่ความเข้มข้นสูงสุด (16 กรัม/ผงป่นแห้ง) ในช่วงเวลา 24, 48 และ 72 ชั่วโมง.

ภาควิชา.....-.....ลายมือชื่อนิสิต.....วันดี สุทธิจิตร  
สาขาวิชา.....เทคโนโลยีชีวภาพ.....ลายมือชื่ออาจารย์ที่ปรึกษา.....ดร. เติตชีวี  
ปีการศึกษา.....2546.....ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....-

###4472401323 : MAJOR : BIOTECHNOLOGY

**KEY WORD:** *P. mirifica* / *B. superba* / *M. collettii* / *P. lobata* / Antioxidant activity / Mutagenicity / Antimutagenicity / Micronucleus test

**WANDEE SUTJIT: THE ANTIOXIDANT TEST, AMES' TEST AND MICRONUCLEI TEST OF CHEMICAL EXTRACT FROM WHITE KWAO KRUA *Pueraria mirifica*, RED KWAO KRUA *Butea superba*, BLACK KWAO KRUA *Mucuna collettii* AND KUDZU *Pueraria lobata*. THESIS ADVISOR: ASSOC. PROF. DR. WICHAJ CHERDSHEWASART. 128 pp. ISBN 974-17-4933-3**

Antioxidant activity assay by DPPH scavenging method was submitted to *Pueraria mirifica* from 28 provinces, *Butea superba* from 23 provinces and *Mucuna collettii* from 4 provinces in comparison with *Pueraria lobata* from China. Our studies revealed that *Mucuna collettii* from Chiang Rai exhibited the strongest activity ( $IC_{50} = 55.53 \pm 2.66 \mu\text{g/ml}$ ) in their population, *Butea superba* from Loei exhibited the strongest activity in their population ( $IC_{50} = 227.08 \pm 0.38 \mu\text{g/ml}$ ), *Pueraria mirifica* from Uthai Thani exhibited the strongest activity in their population ( $IC_{50} = 2,470.38 \pm 37.81 \mu\text{g/ml}$ ), *Pueraria lobata* showed the weakest activity ( $IC_{50} = 2,482 \pm 66.11 \mu\text{g/ml}$ ). The correlation study between five isoflavone contents in tubers analysed by HPLC and antioxidant activity, it was found that the antioxidant activity was directly correlated to daidzein content. The highest antioxidant activity plants in each group was analysed for the mutagenic and antimutagenicity by Ames' test and micronucleus test. The results revealed that *Pueraria mirifica*, *Butea superba*, *Mucuna collettii* and *Pueraria lobata* exhibited no mutagenicity in TA98 and TA100 strains either in the absence and presence of the activation enzyme. *Pueraria mirifica*, *Butea superba*, *Mucuna collettii* and *Pueraria lobata* exhibited antimutagenic activity in TA98 and TA100 strains in the absence and presence of the activation enzymes. The mutagenic activity of *Pueraria mirifica*, *Butea superba*, *Mucuna collettii* and *Pueraria lobata* were also examined in animals using the micronucleus test. The result revealed that *Pueraria mirifica*, *Butea superba*, *Mucuna collettii* and *Pueraria lobata* at highest dose (16 g/powder) had no mutagenic effect in the 24, 48 and 72 hour test period.

Department .....-.....Student's signature.....

Field of study.....Biotechnology.....Advisor's signature.....

Academic year.....2003..... Co-advisor's signature.....-.....

## ACKNOWLEDGEMENTS

I would like to express my heartfelt appreciation and gratitude to my advisor, Associate Professor Dr. Wichai Cherdshewasart, for his accompany with my thought and experience, with full understanding, exhibiting throughout the period of my study.

I would like to express my sincere gratitude to Associate Professor Punya Temcharoen for his valuable suggestion, guidance and expert recommendations with full patience, on the techniques of Ames' test for *in vitro* and micronucleus test for *in vivo*.

I would like to acknowledge to my committees, Professor Dr. Siriwat Wongsiri, Associate Professor Kingkaew Wattanasirmkit, Associate Professor Dr. Sirirat Rengpipat, Assistant Professor Dr. Nattaya Ngamrojanavanich for valuable comments in my research.

I am also very thankful to Associate Professor Dr. Supanee Changbumrung, Head of Department of Tropical Nutrition and Food Science, Faculty of Tropical Medicine, Mahidol University for her kindness and full encouragement throughout the period of my graduate program.

Appreciation is expressed to Associate Professor Lakana Himakoun, Pranom Puchadapirom and Suraphol Kongtim, Department of Pathology, Faculty of Science, Mahidol University for their suggestion, comment and helpful suggestion on inspecting of slide micronucleus test.

Special thanks are also extending to Somchai Phudoung and Apanchanid Thepouyporn, Department of Tropical Nutrition and Food Science, Faculty of Tropical Medicine, Mahidol University for her comments and helpful suggestions of Ames' test techniques.

Thank to National Science and Technology Development Agency (NSTDA) for 2 years grant support.

Finally, I feel very indebted and heart thanks to all members in my family for their love, care and never leave me alone. To the warm feeling sent from my far away home, especially my parents.

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 ศูนย์วิทยทรัพยากร  
 จุฬาลงกรณ์มหาวิทยาลัย

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## ABBREVIATIONS

Abbreviation or symbol	Term
AF <sub>2</sub>	2-[2-furyl]-3-[5-nitro-2-furyl]acrylamide
ATP	adenosine triphosphate
B(a)P	benzo(a)pyrene
B.W.	body weight
<i>B. superba</i>	<i>Butea superba</i>
co.	company
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
<i>et. al.</i>	at all
etc.	<i>et cetera</i>
G6P	glucose-6-phosphate
g/kg B.W.	gram per kilogram body weight
hr.	hour
<i>his</i>	histidine producing gene
<i>his</i> <sup>-</sup>	histidine auxotrophy
<i>his</i> <sup>+</sup>	histidine prototrophy
i.p.	intraperitoneal
IC <sub>50</sub>	Inhibit concentration dose for 50%
KCl	potassium chloride
K <sub>2</sub> HPO <sub>4</sub>	potassium phosphate dibasic anhydrous
LD <sub>50</sub>	Lethal dose for 50%
<i>M. collettii</i>	<i>Mucuna collettii</i>
pH	log concentration of H <sup>+</sup>
μg	microgram
μg/ml	microgram per milliliter
μl	microlitre
mg/ml	milligram per millilitre
MGA	minimal glucose agar

## ABBREVIATIONS (continued)

Abbreviation or symbol	Term
MgCl <sub>2</sub>	magnesium chloride
MgSO <sub>4</sub>	magnesium sulfate
mg/kg B.W.	milligram per kilogram body weight
ml	millilitre
mm	millimeter
min	minute
mM	milimolar
M	molar or mole per litre
MNPCE	micronucleated polychromatic erythrocyte
MW.	molecular weight
NaCl	sodium chloride
NADP	nicotinamide adenine dinucleotide hosphate
NaH <sub>2</sub> PO <sub>4</sub>	sodium hydrogen ammonium phosphate
NaOH	sodium hydroxide
NB	nutrient broth
NCEs	normochromatic erythrocytes
No.	number
N <sub>o</sub>	number of observed revertant
N <sub>t</sub>	number of induced revertant
PCEs	polychromatic erythrocytes
PI	percent inhibition
<i>P. mirifica</i>	<i>Pueraria mirifica</i>
<i>P. lobata</i>	<i>Pueraria lobata</i>
rpm	round per minute
sec	second
S.E	standard error of mean
S9	postmitochondrial supernatant (9000g supernatant)

**ABBREVIATIONS (continued)**

<b>Abbreviation or symbol</b>	<b>Term</b>
uvB	ultraviolet B radiation
VB	Vogel-Bonner medium E
w/v	weight by volume



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จุฬาลงกรณ์มหาวิทยาลัย