

การวิเคราะห์เชิงปริมาณไอโซฟลาโวนอยด์โดยใช้เอชพีแอลซีและฤทธิ์ทางชีวภาพ
ของกวาวเครือขาว (*Pueraria mirifica*) ในแปลงปลูก



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**QUANTITATIVE HPLC ANALYSIS OF ISOFLAVONOIDS AND
BIOASSAYS OF THE FARM-GROWN WHITE KWAO KRUA**

Pueraria mirifica

Miss Suttijit Sriwatcharakul

**A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Biotechnology**

Faculty of Science

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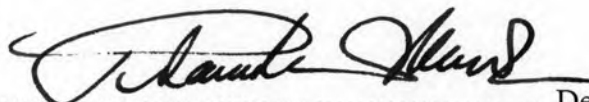
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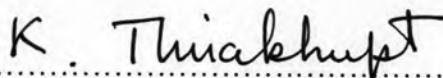
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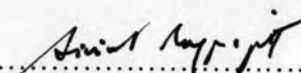


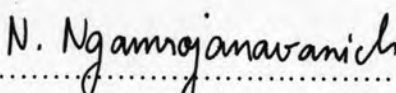
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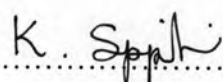
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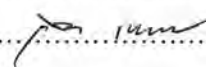

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สุทธิจิต ศรีวัชรกุล: การวิเคราะห์เชิงปริมาณไอโซฟลาโวนอยด์โดยใช้เอชพีแอลซีและฤทธิ์ทางชีวภาพของกวาวเครือขาว *Pueraria mirifica* ในแปลงปลูก (QUANTITATIVE HPLC ANALYSIS OF ISOFLAVONOIDS AND BIOASSAYS OF THE FARM-GROWN WHITE KWAO KRUA *Pueraria mirifica*) อ. ที่ปรึกษา: รศ.ดร.วิชัย เชิดชูชีวิต, 217 หน้า.

การวิเคราะห์ปริมาณสารไอโซฟลาโวนอยด์ของสารสกัดห้วกวาวเครือขาว 5 สายพันธุ์ ใน 3 จุด จากแปลงปลูก จังหวัดราชบุรี ในระยะเวลา 1 ปี ด้วยเทคนิคเอชพีแอลซี พบว่า สารไอโซฟลาโวนอยด์หลัก 5 ชนิด ได้แก่ ฟิราอริน ไดด์ซิน เจนีสติน ไดด์เซอิน และเจนีสเตอิน ในห้วกวาวเครือขาวที่เก็บเกี่ยวในฤดูต่างๆมีปริมาณแตกต่างกัน ในการศึกษาความสัมพันธ์ของปริมาณน้ำฝนและการเปลี่ยนแปลงของอุณหภูมิต่อปริมาณสารไอโซฟลาโวนอยด์ในห้วกวาวเครือขาว พบว่า ปริมาณเจนีสติน อัตราส่วนระหว่างไกลโคไซด์ต่ออะไกลโคไซด์ อัตราส่วนระหว่างอะไกลโคไซด์และไกลโคไซด์คือ ฟิราอริน มีความสัมพันธ์ในทิศทางเดียวกับปริมาณน้ำฝนในฤดูฝนและฤดูหนาว แต่ในฤดูฝนอัตราส่วนระหว่างอะไกลโคไซด์ต่อไกลโคไซด์มีความสัมพันธ์ในทิศทางตรงข้ามกับปริมาณน้ำฝน ส่วนการเปลี่ยนแปลงของอุณหภูมิไม่มีความสัมพันธ์กับปริมาณสารไอโซฟลาโวนอยด์ สรุปได้ว่าปริมาณน้ำฝนมีผลต่อการสะสมของปริมาณสารไอโซฟลาโวนอยด์ในห้วกวาวเครือขาว กวาวเครือขาวทั้ง 5 สายพันธุ์มีฤทธิ์กระตุ้นและยับยั้งการเจริญของเซลล์มะเร็งเต้านม MCF-7 ต่างกัน โดย PM-I, PM-II และ PM-V ที่เก็บเกี่ยวในฤดูร้อน และ PM-III ที่เก็บเกี่ยวในฤดูหนาว มีฤทธิ์กระตุ้นการเจริญของเซลล์มะเร็งเต้านม ส่วน PM-II ที่เก็บเกี่ยวในฤดูหนาว มีฤทธิ์ยับยั้งการเจริญของเซลล์มะเร็งเต้านม ส่วนห้วกวาวเครือขาวที่เก็บเกี่ยวในฤดูฝนไม่มีทั้งฤทธิ์กระตุ้นและยับยั้งการเจริญของเซลล์มะเร็งเต้านม แสดงว่าฤดูกาลในการเก็บเกี่ยวมีผลต่อฤทธิ์กระตุ้นและยับยั้งการเจริญของเซลล์มะเร็งเต้านม เอนไซม์ S9 มีผลเพิ่มฤทธิ์กระตุ้นการเจริญของเซลล์มะเร็งเต้านมในฤดูร้อนและฤดูฝน ปริมาณไดด์เซอิน เจนีสเตอิน และปริมาณอะไกลโคไซด์ มีผลต่อฤทธิ์เอสโตรเจนิกของกวาวเครือขาว ในการศึกษาฤทธิ์เอสโตรเจนิกของกวาวเครือขาวในหนูแรทเพศเมียตัดรังไข่ โดยให้สารแขวนลอยกวาวเครือขาวขนาด 100 และ 1000 มก./กก.น.ตัว เปรียบเทียบกับกลุ่มที่ป้อนน้ำกลั่น 0.7 มล./วัน และกลุ่มที่ฉีด 17 β -estradiol การทดลองแบ่งออกเป็น 3 ระยะ คือ ระยะก่อนการทดลอง 14 วัน ระยะการทดลอง 14 วัน และระยะหลังการทดลอง 7 วัน โดยใช้ดัชนีชี้วัด 5 ชนิด ผลจากวันแรกที่ปรากฏการเปลี่ยนแปลงของเซลล์ที่ผนังช่องคลอดเป็น cornified cell และจำนวนวันที่มี cornified cell ในระยะทดลองและระยะหลังการทดลอง พบว่ากวาวเครือขาวแสดงฤทธิ์เอสโตรเจนิกตามขนาดที่ให้ นั่นคือ กวาวเครือขาวในขนาด 1000 มก./กก.น.ตัว สามารถกระตุ้นการเจริญของเซลล์ที่ผนังช่องคลอดได้เร็วและนานกว่าขนาด 100 มก./กก.น.ตัว และการเจริญของเซลล์ผนังช่องคลอดสัมพันธ์กับการเพิ่มน้ำหนักของมดลูก จากดัชนีชี้วัดทั้ง 5 พบว่า ในฤดูร้อนและฤดูหนาว กวาวเครือขาวมีฤทธิ์เอสโตรเจนิกสูงกว่าในฤดูฝน โดยกวาวเครือขาวสายพันธุ์ PM-III มีฤทธิ์เอสโตรเจนิกสูงกว่าสายพันธุ์อื่น โดยพบว่าปริมาณสารไอโซฟลาโวนอยด์ในห้วกวาวเครือขาวที่เก็บเกี่ยวในแต่ละฤดูมีผลต่อฤทธิ์เอสโตรเจนิก โดยเฉพาะปริมาณของอะไกลโคไซด์ (ไดด์เซอินและเจนีสเตอิน) และไกลโคไซด์ (ไดด์ซินและเจนีสติน) ผลการทดลองสรุปได้ว่าปัจจัยทางกายภาพ และพันธุกรรมพืช มีผลต่อฤทธิ์เอสโตรเจนิกของห้วกวาวเครือขาว ดังนั้นสามารถนำช่วงเวลาการเก็บเกี่ยวที่เหมาะสมและการคัดเลือกสายพันธุ์กวาวเครือขาวที่มีฤทธิ์เอสโตรเจนิกสูงเป็นดัชนีชี้วัดในการคัดเลือกวัตถุดิบที่มีคุณภาพดีเพื่อใช้ในระดับอุตสาหกรรมต่อไป

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SUTTIJIT SRIWATCHCHARAKUL: QUANTITATIVE HPLC ANALYSIS OF ISOFLAVONOIDS AND BIOASSAYS OF THE FARM-GROWN WHITE KWAO KRUA *Pueraria mirifica*. THESIS ADVISOR: ASSOC. PROF. WICHAI CHERDSHEWASART, D.Sc., 217 pp.

HPLC isoflavonoid analysis of 5 clones of *Pueraria mirifica* tubers in a field trial at Ratchaburi province collected in 3 seasons revealed that the 5 isoflavonoids; puerarin, daidzin, genistin, daidzein and genistein were varied. Correlation analysis of the amount of rain and temperature change with tuberous isoflavonoid contents indicated the correlation between genistin, the ratio of glycoside/aglycoside, the ratio of aglycoside and glycoside/puerarin with the rainfall amount in rainy season and winter. In rainy season, the ratio of aglycoside/glycoside was negatively correlated with the rainfall amount. Whereas, temperature change had no correlation with isoflavonoid contents. We can conclude that the rainfall amount had influence on isoflavonoid storage in *P. mirifica* tubers. The 5 plants exhibited different proliferation effects on MCF-7. PM-I, PM-II and PM-V collected in summer and PM-III collected in winter exhibited proliferation while PM-II collected in winter exhibited antiproliferation effect. MCF-7 proliferation assay was much influenced by the presence of S9 mixture which could increase the proliferation response. The studies in ovariectomized rats treated with 100 and 1,000 mg/kg body weight (BW) of plant powder dissolved in 0.7 ml of distilled water, compared to rats fed with 0.7 ml of distilled water only and rats injected with a single dose of 17 β -estradiol. The experiments were set into 3 phases, pre-treatment period for 14 days, treatment period for 14 days and post-treatment period for 7 days, with 5 parameters. From the first day of appearance of vaginal cornified cell in the treatment period and the total day of appearance of cornified cells in the treatment period and post-treatment period including, it was found that the estrogenic activity of *P. mirifica* was a dose dependent. The vaginal cornification in rats treated with 1000 mg/kg BW occurred faster and longer than those of rats treated with 100 mg/kg BW. Differentiation of vaginal cells after *P. mirifica* treatment agreed with the increased uterine weight. From the 5 analyzed parameters, Samples collected in summer and winter showed stronger estrogenic activity than in rainy season. PM-III showed stronger estrogenic activity than other clones. Isoflavonoid activity, especially aglycoside (daidzein and genistein) and glycoside (daidzin and genistin) in differ seasonal collected *P. mirifica* had influence on estrogenic activities. The conclusion from this study is the physical factors and plant genetics exhibited influences on the plant estrogenic activities. Therefore, the right harvest season and plant clone selection for high estrogenic activity could be applied as parameters to establish high quality raw materials for industry demand.

Field of study...BiotechnologyStudent's signature.....S. Sriwatchcharakul.....
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LIST OF ABBREVIATIONS

AU	Absorbance Unit
BW	Body weight
Co	Cornified cell
DW	Distilled water
Day	Day of study period
D	Day of treatment period
D'	Day of posttreatment
E ₂	17 β -Estradiol
ER	Estrogen Receptor
ER β	Estrogen Receptor Beta
ER α	Estrogen Receptor Alpha
FSH	Follicle stimulating hormone
g	Gram
h	Hour
IC ₅₀	Median Inhibitory Concentration
kg	Kilogram
L	Litre
L	Leucocyte cell
LH	Lutinizing hormone
mg	Milligram
mm	Millimetre
ml	Millilitre
M	Molar
O	Nucleated cell
OVX	Ovariectomy
PM	<i>Pueraria mirifica</i>
S.E.M.	Standard Error of Mean
μ g	Microgram
μ l	Microliter
μ M	Micromolar
$^{\circ}$ C	Degree Celsius