

ดีเอ็นเอบาร์โคดและรูปแบบทางเคมีของพืชสกุล *Aristolochia* สำหรับการตรวจสอบสมุนไพร  
ไคร้เครือ

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สาขาวิชาเภสัชเวท ภาควิชาเภสัชเวทและเภสัชพฤกษศาสตร์  
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย  
ปีการศึกษา 2557  
ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย



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DNA BARCODES AND CHEMICAL PROFILES OF *ARISTOLOCHIA* PLANTS FOR  
EXAMINATION OF KRAI-KRUA HERBS

Miss Piroonrat Dechbumroong



ห้องสมุดคณะเภสัชศาสตร์  
จุฬาลงกรณ์มหาวิทยาลัย

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By Miss Piroonrat Dechbumroong

Field of Study Pharmacognosy

Thesis Advisor Associate Professor Surattana Amnuoypol, Ph.D.

Thesis Co-Advisor Associate Professor Suchada Sukrong, Ph.D.

---

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



..... Dean of the Faculty of Pharmaceutical Sciences  
(Assistant Professor Rungpetch Sakulbumrungsil, Ph.D.)

THESIS COMMITTEE

..... Chairman

(Professor Kittisak Likhitwitayawuid, Ph.D.)

..... Thesis Advisor


(Associate Professor Surattana Amnuoypol, Ph.D.)

..... Thesis Co-Advisor

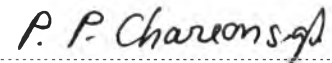
(Associate Professor Suchada Sukrong, Ph.D.)

..... Examiner

(Assistant Professor Taksina Chuanasa, Ph.D.)

..... Examiner

(Associate Professor Jessada Denduangboripant, Ph.D.)



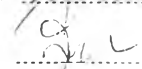
..... External Examiner

(Piyarat Parinyapong Chareonsap, Ph.D.)

พิรุณรัตน์ เดชบำรุง : ดีเอ็นเอบาร์โคดและรูปแบบทางเคมีของพืชสกุล *Aristolochia* สำหรับการตรวจสอบสมุนไพรร้อยเอ็ด (DNA BARCODES AND CHEMICAL PROFILES OF *ARISTOLOCHIA* PLANTS FOR EXAMINATION OF KRAI-KRUA HERBS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ภญ. ดร.สุรตนา อำนวยผล, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ. ร.ต.อ.หญิง ภญ. ดร.สุชาดา สุขห่อง, 140 หน้า.

พืชสกุล *Aristolochia* จัดอยู่ในวงศ์ *Aristolochiaceae* หรือวงศ์กระเช้าสีดา สารหลักที่พบในทุกส่วนของพืชสกุลนี้คือ aristolochic acid I (AAI) และ AAI ซึ่งเป็นสารก่อมะเร็งในมนุษย์ ในปี พ.ศ. 2556 คณะกรรมการยาจึงมีมติให้ตัดเครื่องยาที่ได้จากพืชสกุลนี้ออกจากทะเบียนตำรับยาที่ได้ขึ้นทะเบียนไว้แล้ว รากแห้งของพืชสกุลนี้ 3 ชนิดถูกใช้เป็นแหล่งของเครื่องยา “ไคร้ไคร้อ” ได้แก่ กระเช้าถ่วงทอง หนอนตาย และกระเช้าผิมด อย่างไรก็ตามไคร้ไคร้อยังได้จากรากแห้งของข้าวสารดอกใหญ่ พืชสกุล *Jasminum* และรากชี่กาขาวชี่กาแดงเช่นกัน การพิสูจน์เอกลักษณ์ของไคร้ไคร้อด้วยสัณฐานวิทยาเป็นไปได้ค่อนข้างยากจึงอาจทำให้เกิดความสับสนในการใช้สมุนไพรรักษา ดังนั้นเพื่อความปลอดภัยของผู้บริโภค เครื่องมือในการระบุเอกลักษณ์ของวัตถุดิบสมุนไพรที่เชื่อถือได้และมีประสิทธิภาพจึงได้รับการพัฒนาอย่างต่อเนื่อง ในการศึกษาที่ใช้การประเมินทางพันธุกรรมของพืชสกุล *Aristolochia* จำนวน 11 ชนิด โดยอาศัยเทคนิค DNA barcode ของดีเอ็นเอ 4 บริเวณ ได้แก่ *rbcl matK ITS* และ *trnH-psbA* ความแตกต่างของลำดับนิวคลีโอไทด์ของดีเอ็นเอทุกบริเวณดังกล่าวสามารถใช้ในการจำแนกชนิดของพืชสกุล *Aristolochia* ทั้ง 11 ชนิดนี้ได้ ข้อมูลลำดับนิวคลีโอไทด์บริเวณ internal transcribed spacers 2 (ITS2) นี้ได้ถูกนำไปใช้ใน multiplex PCR ร่วมกับการประเมินรูปแบบองค์ประกอบทางเคมีโดยใช้วิธี high-performance thin layer chromatography (HPTLC) โดยใช้ AAI เป็นสารมาตรฐาน เพื่อใช้ระบุเอกลักษณ์สมุนไพรร้อยเอ็ดวิธีการนี้สามารถประยุกต์ใช้เพื่อการทดสอบเบื้องต้นสำหรับสาร AAI ในอุตสาหกรรมสมุนไพรและการบังคับใช้กฎหมายได้ ผลจากการศึกษาในครั้งนี้แสดงให้เห็นว่าการประเมินทางพันธุกรรมร่วมกับการประเมินรูปแบบองค์ประกอบทางเคมีสามารถพิสูจน์เอกลักษณ์ของพืชสกุล *Aristolochia* และจำแนกชนิดสมุนไพรร้อยเอ็ดได้

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สาขาวิชา เกษศาสตร์  
ปีการศึกษา 2557

ลายมือชื่อนิสิต   
ลายมือชื่อ อ.ที่ปรึกษาหลัก   
ลายมือชื่อ อ.ที่ปรึกษาร่วม 

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PIROONRAT DECHBUMROONG: DNA BARCODES AND CHEMICAL PROFILES OF ARISTOLOCHIA PLANTS FOR EXAMINATION OF KRAI-KRUE HERBS. ADVISOR: ASSOC. PROF. SURATTANA AMNUOYPOL, Ph.D., CO-ADVISOR: ASSOC. PROF. SUCHADA SUKRONG, Ph.D., 140 pp.

The genus *Aristolochia* belongs to the Aristolochiaceae family. The major chemical constituents in the whole part of *Aristolochia* plant are aristolochic acids I (AAI) and AAI1, which are classified as human carcinogens. In Thailand, 2013, The National Drug Committee have issued an order that demands the removal of crude drugs derived from *Aristolochia* plants from all registered formulas. Dried roots of *A. pothieri* Pierre ex Lecomte, *A. pierrei* Lecomte and *A. tagala* Cham., have been reported as sources of medicinal crude drugs called "Krai-Krue". However, Krai-Krue can also be derived from dried roots of *Raphistemma pulchellum* (Roxb) Wall, *Jasminum* spp and *Gymnopetalum integrifolium* Kurz. Authentication of Krai-Krue by morphological examination is quite difficult and can cause confusion. For the protection of consumer's safety, reliable and effective tools for identification of raw herbal materials have been continuously developed. In this study, genetic assessment of 11 *Aristolochia* plants by DNA barcoding technique was conducted based on four DNA regions including *rbcL*, *matK*, ITS and *trnH-psbA*. The nucleotide variations of the four regions are useful to differentiate the eleven *Aristolochia* species. Multiplex PCR based on nucleotide sequences of ITS2 region combining with HPTLC using AAI as standard substance were used for the identification of Krai-Krue herbs. This method can be used as a preliminary AAI-screening test for safety control by the herbal industries as well as the regulatory authorities. The results from these studies indicated that the combination of genetic and chemical assessment would be useful for the identification and discrimination of *Aristolochia* plants and Krai-Krue herbs.

Department: Pharmacognosy and

Student's Signature

*Prof. Duly*

Pharmaceutical Botany

Advisor's Signature

*S. Amoypol*

Field of Study: Pharmacognosy

Co-Advisor's Signature

*Suchada Sukrong*

Academic Year: 2014



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## LIST OF ABBREVIATIONS

AA	=	aristolochic acid
AAN	=	Aristolochic Acid Nephropathy
AFLPs	=	amplified fragment length polymorphisms
AL(s)	=	aristolactam(s)
ARMS	=	amplification-refractory mutation system
bp	=	base pair
CBOL	=	Consortium for the Barcode of Life
CHN	=	Chinese herb nephropathy
°C	=	degree Celsius
DNA	=	deoxyribonucleic acid
dNTP	=	deoxyribonucleotide triphosphate
GC	=	gas chromatography
GC-MS	=	gas chromatography-mass spectroscopy
H <sub>2</sub> O	=	water
HPLC	=	high-performance liquid chromatography
HPLC-MS	=	high-performance liquid chromatography- mass spectroscopy
HPTLC	=	high-performance thin-layer chromatography
IARC	=	International Agency for Research on Cancer
ITS	=	internal transcribed spacer
Kb	=	kilobase
MARMS	=	multiplex amplification refractory mutation system
<i>matK</i>	=	maturase K
min	=	minute(s)
NIR	=	near infrared
PCR	=	polymerase chain reaction
ppm	=	part(s) per million
RAPD	=	random amplified polymorphic DNA
<i>rbcl</i>	=	large subunit of ribulose-bisphosphate carboxylase
rDNA	=	ribosomal deoxyribonucleic acid



RFLP	=	restriction fragment length polymorphism
SCAR	=	sequence characterized amplified region
sp	=	species (singular)
spp	=	species (plural)
SSR	=	simple sequence repeat
TLC	=	thin-layer chromatography
<i>trnH-psbA</i>	=	<i>trnH-psbA</i> intergenic spacer region
UV	=	ultraviolet
V	=	voltage

