



รายงานวิจัยฉบับสมบูรณ์

การพิสูจน์เอกลักษณ์พืชสกุล *Mucuna* และผลิตภัณฑ์
โดยใช้เทคนิคดีเอ็นเอบาร์โคดและลักษณะลายพิมพ์ขององค์ประกอบทางเคมี
Combination of DNA barcode and chemical analysis
for authentication of *Mucuna* spp. and their herbal products

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สัญญาเลขที่ MRG6080203

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สังกัด

มหาวิทยาลัยเชียงใหม่
จุฬาลงกรณ์มหาวิทยาลัย

สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัยและมหาวิทยาลัยเชียงใหม่
(ความเห็นในรายงานนี้เป็นของผู้วิจัย สกว. และต้นสังกัดไม่จำเป็นต้องเห็นด้วยเสมอไป)

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ชื่อโครงการ	การพิสูจน์เอกลักษณ์พืชสกุล <i>Mucuna</i> และผลิตภัณฑ์โดยใช้เทคนิคดีเอ็นเอบาร์โคดและลักษณะลายพิมพ์ขององค์ประกอบทางเคมี
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พืชสกุลหมามุ่ย (*Mucuna*) พบในประเทศไทยทั้งหมด 14 ชนิด ซึ่งเมล็ดของหมามุ่ยชนิด *M. pruriens* ถูกนำไปใช้ในการเพิ่มสมรรถภาพทางเพศโดยเฉพาะในเพศชาย เนื่องจากในเมล็ดของหมามุ่ยชนิดนี้มีการรายงานพบสารลิโวโดปา (L-3,4-dihydroxy phenylalanine: L-DOPA) ปัจจุบันมีผลิตภัณฑ์หมามุ่ยออกจำหน่ายในท้องตลาดเป็นจำนวนมากเพื่ออ้างสรรพคุณในเรื่องเพิ่มสมรรถภาพทางเพศ แต่อย่างไรก็ตาม คณะกรรมการอาหารและยาของไทยยังไม่ได้มีการรับรองผลิตภัณฑ์นี้ให้ออกจำหน่ายได้อย่างถูกต้องตามกฎหมาย ดังนั้นการควบคุมคุณภาพเป็นสิ่งจำเป็นเพื่อความเชื่อมั่นของผู้บริโภคมีเรื่องคุณภาพ ความปลอดภัย และประสิทธิภาพ งานวิจัยนี้มีจุดประสงค์ ที่จะพิสูจน์เอกลักษณ์ของพืชสกุลหมามุ่ยและผลิตภัณฑ์ที่มีจำหน่ายในท้องตลาดในประเทศไทยโดยใช้เทคนิคดีเอ็นเอบาร์โคดและลักษณะลายพิมพ์ขององค์ประกอบทางเคมี

พืชสกุลหมามุ่ยจำนวน 12 ชนิด ทั้งหมด 40 ตัวอย่าง ถูกเก็บในประเทศไทย จากนั้นทำการศึกษาคัดเลือกตำแหน่งยีนในดีเอ็นเอจำนวน 4 ตำแหน่ง ได้แก่ *matK*, *rbcl*, *trnH-psbA* และ ITS ที่มีประสิทธิภาพในการที่จะใช้แยกชนิดของพืชสกุลหมามุ่ยได้มีประสิทธิภาพมากที่สุด นอกจากนี้เมล็ดของพืชสกุลหมามุ่ย 10 ชนิด ถูกนำมาวิเคราะห์ลักษณะลายพิมพ์ขององค์ประกอบทางเคมีและปริมาณของสารลิโวโดปา โดยเครื่อง HPLC

จากการวิเคราะห์ผลของสายนิวคลีโอไทด์ของพืชสกุลหมามุ่ย จำนวน 12 ชนิด ใน 4 ตำแหน่งยีน โดยวิธี genetic method และ tree topology พบว่าตำแหน่งยีน ITS เหมาะที่จะนำมาใช้ในการพิสูจน์ชนิดของพืชสกุลหมามุ่ยมากที่สุด จากนั้นนำผลิตภัณฑ์หมามุ่ยที่วางขายในท้องตลาดมาวิเคราะห์ผลทาง DNA โดยใช้ตำแหน่งยีน ITS พบว่าผลิตภัณฑ์ทั้งหมดมีส่วนประกอบของเมล็ดหมามุ่ยเท่านั้น นอกจากนี้ผลการวิเคราะห์ทางเคมียังพบว่าทุกผลิตภัณฑ์มีสารลิโวโดปา เพราะฉะนั้นสามารถสรุปได้ว่าการพิสูจน์เอกลักษณ์แท้ของผลิตภัณฑ์หมามุ่ยด้วยเทคนิคทางดีเอ็นเอบาร์โคด ในตำแหน่งยีน ITS และเทคนิคทางเคมี ด้วย HPLC มีประสิทธิภาพในการพิสูจน์เอกลักษณ์ของพืชสกุลหมามุ่ยและผลิตภัณฑ์ได้

คำสำคัญ หมามุ่ย, ดีเอ็นเอบาร์โคด, ลิโวโดปา, ผลิตภัณฑ์สมุนไพร, การควบคุมคุณภาพ

Abstract

Project Code:	MRG6080203
Project Title:	Combination of DNA barcode and chemical analysis for authentication of <i>Mucuna</i> spp. and their herbal products
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Project Period	2 years 6 months

Mucuna plants have been found fourteen species in Thailand. Seeds of *M. pruriens* or "Ma mui" in Thai has utilized for the improvement of sexual performance because it contains an L-DOPA (L-3,4-dihydroxy phenylalanine). There are many products available in the market to claim for aphrodisiac activity however, they have not approved from Thai FDA yet. Therefore, the quality control is indispensable for the assurance of customer in quality, safety, and efficacy. The molecular and chemical analyses were utilized for evaluating the quality of *M. pruriens* seed products in the Thai herbal market.

Twelve *Mucuna* species with forty *Mucuna* specimens were collected from Thailand. An effective DNA marker was selected from four DNA regions (*matK*, *rbcl*, *trnH-psbA*, and ITS) to appraise for authentication of *Mucuna* species. The best effective DNA marker was chosen to identify the *Mucuna* products. Moreover, ten *Mucuna* species were analyzed the chemical marker, L-DOPA by HPLC.

From the collected twelve *Mucuna* plants, the result of comparison of four candidate DNA loci revealed that from DNA sequence analysis and the two methods; (1) genetic method and (2) tree topology. The ITS region was recommended for the identification of *Mucuna* species, and *psbA-trnH* may supplement. The ITS marker was selected to authenticate their Ma mui products that claimed as *M. pruriens* for aphrodisiac activity in male. Furthermore, the *M. pruriens* seed crude drugs and herbal products were evaluated by chemical markers using HPLC. The results showed that ITS region was able to utilize for authenticate the Ma mui products and all Ma mui products composed of *M. pruriens* seed as ingredient.

Key word: *Mucuna*, DNA barcode, L-dopa, herbal product, quality control

Introduction to the research problem and its significance

Mucuna pruriens (L.) D.C. known as cowage, velvet bean or "Ma mui" in Thai (Fabaceae-Papilionoideae) is vigorous climbing vine found in disturbed areas (hedges, roadsides) of all parts of Thailand. Ma mui's pods are known as itchy bean when once contact its hair. However, their seeds contain various chemical substances including L-3,4 dihydroxyphenyl alanine (L-DOPA), the important substance for treatment of Parkinson's disease and improving fertility, as aphrodisiac, widely used in traditional Indian systems of medicine (Ayurveda and Unani). In summer and dry season, many parts of Thailand especially rice fields affect with drought problem. Therefore, Prime Minister Prayut Chan-o-cha encouraged farmers for cultivating low-water use plants such as *M. pruriens* instead of rice. The plants were promoted for exporting to Indian herbal market and providing in domestic market. Unfortunately, in Thailand, the approval and certification of the seeds of *M. pruriens* products have yet obtained from the Thai Food and Drug Administration (FDA). Nonetheless, the products are currently affordable on the internet. The tragedy case was reported about *M. pruriens* product, causing death after taking capsules of Indian *M. pruriens* seed extract. Hence, from this evidence, the quality and safety of herbal products are an essential issues to urgently research.

Literature review

Genus *Mucuna* belongs to family Fabaceae, subfamily Papilionoideae distributed in pantropical zone and consists about 105 species around the world. Thirteen species were found in Thailand (Table 1). Twelve species is native namely *M. thailandica* Niyomdham & Wilmot-Dear, *M. oliplax* Niyomdham & Wilmot-Dear and *M. gracilipes* Craib and another is Indonesian species as ornamental plant. *Mucuna* plants has been used in agriculture as cover crop, fodder and green manure, food as vegetable, gardening and medicine. In Thai traditional medicine, *M. macrocarpa* Wall. (syn *M. collettii* Lace) or Black Kwao Khrua has been utilized as rejuvenating herb for reproductive function in males.

M. pruriens (L.) D.C. or "Ma mui" in Thai spreads across the tropical region. It is important member and exhibits highly promising economic potential in agriculture as for green manure, biological control and human consumption (coffee substitute and vegetable). Furthermore, It

has been used as an herbal medicine for the treatment of Parkinson's disease and amelioration of sexual performance. Three varieties of *M. pruriens* are found in Thailand namely var. *pruriens* Wilmot-Dear, var. *hirsuta* (Wight & Arn.) Wilmot-Dear, and var. *utilis* (Wall. ex Wight) Baker ex Burck. They are able to discriminate using morphological appearance of fruit. The indumentum of *M. pruriens* var. *utilis* fruits is fine, silky, pale, not irritant nor caducous, but, *M. pruriens* var. *pruriens* and var. *hirsuta* fruit show orange or brown irritant bristles. The differentiation of var. *pruriens* and *hirsuta* is hair covered on stems, petioles, leaves, and infructescences: var. *pruriens* shows orange-brown pubescence, while var. *hirsuta* exhibits silvery hair.

Table 1 The *Mucuna* species found in Thailand

Species		Vernacular name
Subgenus <i>Mucuna</i>		
<i>M. macrocarpa</i>		สะบ้าลิง (Saba ling)
<i>M. thailandica</i>		พวงมรกต (Phuang morakot)
<i>M. gigantea</i>	subsp. <i>gigantea</i>	หมามุ่ยช้าง (Ma mui chang)
<i>M. oligoplax</i>		หมามุ่ยไต้ (Ma mui tai)
<i>M. monosperma</i>		หมามุ่ยใหญ่ (Ma mui yai)
<i>M. stenoplax</i>		หมามุ่ยลาย (Ma mui lai)
<i>M. hainanensis</i>	subsp. <i>hainanensis</i>	หมามุ่ยไห่หนาน (Ma mui hai nan)
<i>M. revoluta</i>		สะบ้าลาย (Saba lai)
<i>M. interrupta</i>		
Subgenus <i>Stizolobium</i>		
<i>M. gracilipes</i>		หมามุ่ยเหนือ (Ma mui nuae)
<i>M. pruriens</i>	var. <i>pruriens</i>	หมามุ่ย (Ma mui)
	var. <i>hirsuta</i>	หมามุ่ยส้ม (Ma mui som)
	var. <i>utilis</i>	หมามุ่ยขนอ่อน (Ma mui khon on)
<i>M. bracteata</i>		หมาเยื่อง (Ma yueang)
<i>M. warburgii</i>		พวงโกเมน (Phuang komen)

The seeds of *M. pruriens* consist of various chemical substances such as alkaloids, triterpenes, sterols, lecithin, vernolic acid, gallic acid, and many types of protein especially L-

3,4 dihydroxyphenyl alanine (L-DOPA). L-DOPA, a precursor of neurotransmitter dopamine, shows anti-Parkinson and aphrodisiac activity. In the study on infertile obese mutant rat model, it is stated that *M. pruriens* showed a significant increasing of testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin including sperm count and motility. Ahmad KM *et al.* reported that the inhibition of lipid peroxidation, elevation of spermatogenesis, and improvement of sperm motility were able to be found after treated infertile male with velvet bean seeds. Furthermore, cowage seeds show the positive impact of fertility by its action on the hypothalamic-pituitary-gonadal axis that effect to Leydig cells in testis for increasing sexual performance and spermatogenesis, and decreasing lipid peroxidation.

The traditional identification and authentication use macroscopic and microscopic methods including organoleptic manners which depend heavily on the experience of experts. Therefore, Molecular technique has an important role for herbal differentiation from close related species, inferior substitutes, adulterants and artificial materials affects with patient safety and herbal efficacy. DNA barcode is a rapid and accurate method for species identification, classification, and authentication by using a standardized DNA region. The standard part of genome from an identified specimen (i.e. holotype) will be used as the reference barcode sequences for comparing with unknown specimen. To search for the gene regions that are appropriate to species-level identification of a great number of plants, the CBOL (the Consortium for the Barcode of Life) plant working group: an international inventive supporting, promoting, and developing DNA barcoding, recommended the combination of plastid DNA region *matK* + *rbcl*. Because some closely related species could not be differentiated by these two recommended loci, the China Plant BOL group advised to utilize nuclear ribosomal DNA ITS for supplement with the *matK* + *rbcl* combination. Gao T *et al.* reported that ITS2 is a powerful marker for discriminating Leguminous species but some species showed resemble ITS2 sequence. Thus, other DNA marker (e.g. *psbA-trnH*) should be performed for assured species identification. The usage of DNA barcode is the identification of several herbal medicines such as *Rheum officinale* (Polygonaceae), *Panax ginseng* (Araliaceae), *Dendrobium nobile* (Orchidaceae), and Chinese caterpillar fungus (*Ohiocordyceps sinensis*) so

on. Moreover, DNA barcode has been used to identify adulterants, substitution and resolve phenotypically resemble species that could easily be misidentified and mixed within herbal product. Srirama Red *et al.* demonstrated that DNA barcode can be utilized to discriminate species admixture in the raw drug trade of *Phyllanthus* sold in southern India. Furthermore, the species identification of *Sida cordifolia* collected from Indian herbal market was done by using DNA barcode. The study showed that none of the market samples belong to authentic *S. cordifolia*. For the confidence of customer in quality, safety, and efficiency of medicinal *Mucuna* products, the combination of DNA barcode and chemical analysis will be chosen to be suitable for authenticating, identifying, and authenticating the commercial crude drugs of Thai *Mucuna* species.

Objectives

This project aims to authenticate and identify the Thai *Mucuna* plants using the combination of DNA barcode and chemical analysis and their application to evaluate medicinal products. The particular objectives of the study were:

- To conserve the genetic diversity of Thai *Mucuna* species especially Thai *M. pruriens*.
- To authenticate and identify *M. pruriens* and related species using chemical marker (L-DOPA) analysis and DNA barcode and search a correlation between L-DOPA content and Thai *Mucuna* phylogeny.
- To apply chemical marker analysis and DNA barcoding method for testing the quality of medicinal *Mucuna* products.

Materials

Plant materials and products

Eleven of all fourteen *Mucuna* species distributed in Thailand were collected, as shown in table 2. For DNA extraction, healthy fresh leaves of twelve *Mucuna* species collected all regions of Thailand (Fig. 1) were collected and desiccated in silica gel. Furthermore, the seeds of nine *Mucuna* species (Fig. 2), except *M. gracilipes* as a rare species, and *M. warburgii* as a species for garden decoration were assembled from authentic species and purchased

from Thai herbal markets for determination of L-DOPA. Voucher samples were identified using morphological characters of flowers and fruits (Fig. 1) by Wannaree Charoensup, a botanist of Faculty of Pharmacy, Chiang Mai University and Wittaya Pongamornkul, botanist in charge of Queen Sirikit Botanic Garden (QBG) Herbarium. Voucher specimens were deposited in the herbarium of Faculty of Pharmacy, Chiang Mai University (CMU) and Forest herbarium (BKF) under Department of National Parks, Wildlife and Plants Preservations. Ten commercially aphrodisiac products (MPP1-10) that claimed the composition made from the seeds of *M. pruriens* as a single ingredient and multicomponent were purchased from online stores (Fig. 3).

Table 2 Collected *Mucuna* species distributed in Thailand

<i>Mucuna</i> species	Code	Date	Location	Accession number			
				ITS	<i>rbcL</i>	<i>matK</i>	<i>trnH-psbA</i>
1. <i>M. pruriens</i> var. <i>pruriens</i>	MPR1	25/09/2016	Faculty of Pharmacy, Chiang Mai University	LC494602	LC494390	LC494401	LC494612
	MPR2	25/09/2016	Faculty of Pharmacy, Chiang Mai University				
	MPR3	25/11/2016	Faculty of Pharmacy, Chiang Mai University				
	MPR4	07/06/2016	Faculty of Pharmaceutical Sciences, Chulalongkorn University				
	MPR5	24/05/2017	Maesot, Tak				
	MPR6	24/05/2017	Maesot, Tak				
2. <i>M. pruriens</i> var. <i>utilis</i>	MPR11	25/11/2016	Faculty of Pharmacy, Chiang Mai University				
	MPR12	25/06/2018	Ban Tak, Tak	LC494601	-	LC494400	LC494613
3. <i>M. bracteata</i>	MBT1	20/05/2017	Wiang Pa Pao, Chiang Rai	LC494604	LC494392	LC494403	LC494615
	MBT2	20/05/2017	Doi Saket, Chiang Mai				
	MBT3	29/09/2017	Faculty of Agriculture, Khon Kean University				
	MBT4	01/10/2017	Faculty of Agriculture, Khon Kean University				
	MBT5	18/03/2018	Umphang, Tak				
	MBT6	18/03/2018	Umphang, Tak				
	MBT7	25/03/2018	Mae Sai, Chiang Rai				
4. <i>M. gigantea</i>	MGG1	24/11/2017	Pak Pa Yun, Pattalung	LC494611	LC494399	LC494410	LC494622
	MGG2	24/11/2017	Pak Pa Yun, Pattalung				
	MGG3	24/11/2017	Kuan Niang, Songkhla				

<i>Mucuna species</i>	Code	Date	Location	Accession number			
				ITS	<i>rbcL</i>	<i>matK</i>	<i>trnH-psbA</i>
5. <i>M. gracilipes</i>	MGP1	18/03/2018	Umphang, Tak	LC494603	LC494391	LC494402	LC494614
	MGP2	18/03/2018	Umphang, Tak				
6. <i>M. hainanensis</i>	MHN1	19/05/2018	Chae Hom, Lampang	LC494608	LC494397	LC494407	LC494620
	MHN2	19/05/2018	Chae Hom, Lampang				
7. <i>M. interrupta</i>	MIT1	10/10/2017	Mae Sariang, Mae Hong Son	LC494607	LC494396	LC494406	LC494619
	MIT2	10/10/2017	Mae Sariang, Mae Hong Son				
	MIT3	25/03/2018	Mae Sai, Chiang Rai				
8. <i>M. macrocarpa</i>	MMC1	06/10/2017	Mae Tang, Chiang Mai	LC494605	LC494393	LC494404	LC494616
	MMC2	27/10/2017	Hang Chat, Lampang				
	MMC3	12/08/2017	Mae Sot, Tak				
	MMC4	11/03/2018	Chom Thong, Chiang Mai				
	MMC5	11/03/2018	Chom Thong, Chiang Mai				
9. <i>M. monosperma</i>	MMM1	24/05/2017	Mae Sot, Tak	LC494606	LC494395	LC494405	LC494618
	MMM2	25/06/2017	Mae Sot, Tak				
10. <i>M. revoluta</i>	MRT1	01/04/2018	Noen Maprang, Phitsanulok	LC495171	LC495173	LC495170	LC495172
	MRT2	01/04/2018	Noen Maprang, Phitsanulok				
11. <i>M. thailandica</i>	MTC1	11/03/2018	Chom Thong, Chiang Mai	LC494609	LC494394	LC494408	LC494617
	MTC2	11/03/2018	Chom Thong, Chiang Mai				
	MTC3	11/03/2018	Chom Thong, Chiang Mai				
12. <i>M. warburgii</i>	MWG1	16/07/2017	Sansai, Chiang Mai	LC494610	LC494398	LC494409	LC494621
	MWG2	16/07/2017	Sansai, Chiang Mai				
	MWG3	04/10/2017	Faculty of Agriculture, Chiang Mai University				
	MWG4	04/10/2017	Faculty of Agriculture, Chiang Mai University				
	MWG5	16/10/2017	Umphang, Tak				

Genomic DNA Extraction

Total DNA was extracted from approximately 100 mg of leaves specimen or 25 mg of products using a DNA Plant Mini Kit (Qiagen®, Germany) following the manufacturer's protocol with minor modification. For commercial products, about 20 mg of sample powder was extracted by using cetyltrimethylammonium bromide (CTAB) following the protocol described by Healey *et al.* 2014 with minor modification, then the obtained genomic DNA was purified using a GENECLAN® Kit following manufacturer's protocol.

Fig. 1 Inflorescences and fruits of *Mucuna* spp. collected in Thailand



A. *M. pruriens* var. *pruriens* (L.) DC.; B. *M. pruriens* var. *utilis* (Wall. ex Wight) Baker ex Burck; C. *M. bracteata* DC. ex Kurz; D. *M. gigantea* (Willd.) DC.; E. *M. gracilipes* Craib; F. *M. hainanensis* hayata subsp. *multilamellata* Wilmot-Dear; G. *M. interrupta* Gagnep.; H. *M. macrocarpa* Wall.; I. *M. monosperma* DC. ex Wight; J. *M. revoluta* Wilmot-Dear; K. *M. thailandica* Niyomdham & Wilmot-Dear; L. *M. warburgii* Lauterb. & K. Schum. (The photo taken by Aekkhaluck Intharuksa, Wittaya Pongamornkul and Wannaree Charoensup)

Fig. 2 List of *Mucuna* seeds

<i>Mucuna</i> species	Sample ID	Collected site	Collected dates
<i>M. pruriens</i> var. <i>pruriens</i> 	XS1	Sao Hai, Saraburi	13 May 2016
	XS2	Sao Hai, Saraburi	29 Aug 2016
	XS3-5	Sam Chai, Kalasin	6 May 2016
	XS11	Chiang Dao, Chiang Mai	9 May 2017
	XS12	Muang, Pichit	18 Feb 2018
<i>M. pruriens</i> var. <i>utilis</i> 	XS43	Ban Tak, Tak	25 Jun 2018
<i>M. bracteata</i> 	XS23	Umphang, Tak	19 Mar 2018
	XS24	Mae Sai, Chiang Rai	24 Mar 2018
	XS25	Wiang Pa Pao, Chiang Rai	25 Mar 2018
<i>M. gigantea</i> 	XS18	Khuan Niang, Songkhla	24 Nov 2017
	XS19-20	Pak Pa Yun, Pattalung	24 Nov 2017
<i>M. hainanensis</i> 	XS42	Chae Hom, Lampang	19 May 2018
<i>M. interrupta</i> 	XS17	Mae Sariang, Mae Hong Son	10 Jun 2017
<i>M. macrocarpa</i> 	XS26	Chom Thong, Chiang Mai	22 Sep 2018
<i>M. monosperma</i> 	XS13-14	Mae Sot, Tak	24 May 2017
	XS15	Mae Sot, Tak	25 Jun 2017
<i>M. revoluta</i> 	XS21	Noen Maprang, Phitsanulok	31 Mar 2018
<i>M. thilandica</i> 	XS27	Chom Thong, Chiang Mai	23 May 2018

Fig. 3 Commercially Herbal Products claimed as *M. pruriens* as an ingredient for desired aphrodisiac activity



PCR Amplification, Electrophoresis and Sequencing

The nuclear and plastid DNA regions as core DNA barcode: ITS, *trnH-psbA*, *rbcL* and *matK* were amplified by PCR. PCR reaction system contained total DNA as the template 100-120 ng mixed with 12.5 μL of 2x PCR Buffer for KOD FX Neo (ToYoBo[®], Japan), 5 μL of each deoxyribonucleotide triphosphate (dNTP) (0.4 mM), 0.75 μL of each universal primer (0.15 μM) (Table 3) and 0.5 μL of KOD FX Neo DNA polymerase (0.5 unit). PCR amplification was carried out with a thermocycler using the following program: 35 cycles of denaturation at 98 $^{\circ}\text{C}$ for 10 s, annealing at 57 $^{\circ}\text{C}$ for 30 s, and extension at 72 $^{\circ}\text{C}$ for 1 min 30 s; and a final elongation at 72 $^{\circ}\text{C}$ for 2 min. The PCR product was examined on 1.8% agarose gel electrophoresis and visualized by staining with RedSafe[™] nucleic acid staining solution (iNtRON Biotechnology, Korea) under UV light radiation. Then, the PCR products were subsequently sequenced by capillary sequencing (Biobasic. Inc, Canada) using the amplification primer for each region (Table 3).

Table 3 Primers Used in this Study

DNA region	Primer name	Sequence (5'→3')	Reference
Primer used for DNA barcoding			
ITS	ITS1	CCT TAT CAT TTA GAG GAA GGA G	Stanford et al., 2000
	ITS4	TCC TCC GCT TAT TGA TAT GC	White et al., 1990
<i>trnH-psbA</i>	<i>trnH</i> (GUG)	ACT GCC TTG ATC CAC TTG GC	Hamilton, 1999
	<i>psbA</i>	CGA AGC TCC ATC TAC AAA TGG	Hamilton, 1999
<i>rbcL</i>	<i>rbcL</i> 1F	ATG TCA CCA CAA ACA GAA AC	Fay et al., 1997
	<i>rbcL</i> 724R	TCG CAT GTA CCT GCA GTA GC	Fay et al., 1997
<i>matK</i>	<i>matK</i> -1RKIM-f	ACC CAG TCC ATC TGG AAA TCT TGG TTC	CBOL plant working Group, 2009
	<i>matK</i> -3FKIM-r	CGT ACA GTA CTT TTG TGT TTA CGA G	CBOL plant working Group, 2009

DNA analyses and validation of barcode region

The successfully bidirectional sequences of four regions were aligned by using Clustal W and MUSCLE in Molecular Evolutionary Genetics Analysis (MEGA) version X software with manual adjustment. The Basic Local Alignment Search Tool (BLAST) analysis for species identification was conducted by using NCBI nucleotide database (www.blast.ncbi.nlm.nih.gov/Blast.cgi). Assessment of genetic distance was calculated by Kimura 2-Parameter (K2P) distance model. Furthermore, intraspecific and interspecific divergences were evaluated by using MEGA software. Then, the consensus sequences were deposited to the DDBJ/EMBL/GenBank database, and their accession number are shown in Table 1. The genetic distance method using Kimura2-parameter (K2P) distance matrices and tree topology analysis based on the maximum likelihood (ML) method based on different loci in MEGA version X with the K2P distance model and 1000 bootstrap replications are the method for selecting the powerful DNA marker to identify and authenticate the *Mucuna* species including their products.

Phylogeny Reconstruction

The aligned nucleotide sequences of each *Mucuna* species in all regions were analyzed by using Molecular Evolutionary Genetic Analysis (MEGA) software version X. Neighbor-Joining (NJ) calculation was performed using Kimura two-parameter model with 1000

bootstep replications. *Glycine max* L. of the same tribe Phaseoleae in Fabaceae family was used as outgroup species for phylogenetic tree rooting.

Determination of L-DOPA content

The determination of L-DOPA content was conducted following the protocol of Dhanani *et al.* with minor adjustment. The dried matured seeds were pulverized using electric grinder and sieved 100 mesh. The powderized seed sample and Ma mui product was mixed with petroleum ether for separating lipid in their seeds and refluxed. The solid residue will be added by deionized water pH 2.5 and refluxed for 5 hours. Liquid extract was discriminated from solid residue by vacuum filtration and concentrated using a vacuum rotary evaporator.

The concentration of L-DOPA was quantified in the extract samples using HPLC (Prominence, Shimadzu[®], Japan). The chromatographic separation was performed on RP-18 GP (250 x 4.6 mm, 5 μ m) (Supelco, Sigma-Aldrich[®], USA). The mobile phase used as HPLC-grade organic solvent is a mixture of 0.1 N formic acid and methanol (98:2). The solvent flow rate run 1.0 mL/min. The injection volume is 10 μ L. The photodiode array detector wavelength is 280 nm for the determination of L-DOPA in the extract of *M. pruriens*. The data obtained with samples were compared with the calibration curves of the standard and reported in terms of mg/100 mg of extract. L-DOPA standard showed good linearity with correlation coefficients (R^2) as 1.

Result and discussion

Sequencing analysis

The nucleotide sequences of all *Mucuna* samples were submitted in DDBJ/EMBL/GenBank database. Nucleotide differences of four candidate DNA markers, where selected nucleotides were compared with each other, are summarized in Table 4-13. The parameters for sequence data analysis were compared among candidate DNA barcode markers as shown in Table 14. Among the analyzed DNA barcode markers, the region that contains site with the lowest variability was *rbcl* followed by *matK*, *trnH-psbA*, and ITS. The 720 bp of coding chloroplast *rbcl* exhibited 20 variable sites among twelve *Mucuna* species.

Furthermore, *M. pruriens* and *M. gracilipes* cannot be differentiated by the *rbcl* region. In ITS region, the length ranged from 631-661 bp. The length of *M. pruriens* var. *pruriens* and *M. pruriens* var. *utilis* showed the same length, 651 bp. There were 217 variable sites in the twelve species: 199 sites were single nucleotide polymorphisms (SNPs) and 18 sites were indels. On comparing the variety of *M. pruriens*, there was only one base difference in the ITS2 region. Analyzed the chloroplast DNA region, the length of aligned nucleotide sequences of *matK*, *rbcl*, and *trnH-psbA* region were 784, 720, and 328 bp respectively. In the *trnH-psbA* region of *M. pruriens* var. *pruriens* and var. *utilis*, there are two different bases occurred at nucleotide no. 312 and 317. While, six base insertion (ATTGTA) of *M. pruriens* var. *utilis* at nucleotide no. 26-31 in *matK* region.

Sequencing base composition of *Mucuna* species collected from Thailand

A total of 47 closely related sequence belonging to *Mucuna* plants collected from Thailand were analyzed (Table 1). The alignment of all the sequences required the additional of a gap, excepted in *rbcl*. The aligned sequences were calculated to have total length of 689, 784, 328, and 720 nucleotides in ITS, *matK*, *trnH-psbA*, and *rbcl*, respectively. Based on the parsimony criterion, 140 of 199, 40 of 52, 33 of 42, and 14 of 20 variable sites in ITS, *matK*, *trnH-psbA*, and *rbcl*, respectively. Regarding the percentage of singleton sites, a site containing at least two types of nucleotides with one occurring multiple times, *rbcl* manifested the lowest % singleton site (0.83), followed by *matK* (1.53), ITS (8.85), and *trnH-psbA* (10.06) (Table 14).

Table 4 Variation in ITS1 of 11 *Mucuna* species

Species	Nucleotide position																											
	ITS1																											
	20	23	26-7	29-34	39-40	44	46	50-2	54	57	62-76	82-4	86-7	90	92-5	97-8	100-1	106-10	112	116-9	123-4	126	129-30	133-44	147	149		
<i>M. pruriens</i> var. <i>pruriens</i>	T	C	--	ACAACA	CG	G	C	CCC	-	C	CAGGACAAGTTCGAT	CTG	CT	G	----	GA	CT	----T	G	ACCG	CC	T	TC	CGA-CCC	GAGCT	C	A	
<i>M. pruriens</i> var. <i>utilis</i>	*	*	--	*****	**	*	*	***	-	*	*****	***	**	*	----	**	**	----	*	*	****	**	*	**	*****	*	*	
<i>M. bracteata</i>	A	*	CA	*****	**	*	*	***	-	*	-----CGCGTGC	*CA	*C	*	----	*C	*C	CTTGC	*	*A**	**	*	**	**G-*****C	*C	*	G	
<i>M. gigantea</i>	*	T	CA	-----	T*	T	T	T*T	-	T	T---C**-CCCTC*C	*A	G*	T	-GCT	TT	T*	----	C	***T	T*	*	*T	TC-----CG	*T*	*	G	
<i>M. gracilipes</i>	*	*	--	*****	*A	*	*	***	-	*	*****C*****	***	**	*	----	**	A*	----	*	****	**	*	**	***C*****	*	*	*	
<i>M. hainanensis</i>	*	*	CA	****TC	**	*	T	T*T	-	T	T---C**-CCCTC*C	*CA	G*	*	----	TT	T*	----	*	**GC	**	*	*T	TC-----CG	*T*	*	G	
<i>M. interrupta</i>	*	*	CA	****TC	**	*	T	T*T	-	T	T---C**-CCCTC*C	*CA	G*	*	----	TT	T*	----	*	*T**	**	*	*T	TC-----CG	*T*	*	G	
<i>M. macrocarpa</i>	A	*	CG	*****C	**	*	*	***	T	T	T---CT*-CGC*C*C	*C*	**	*	----	*C	*C	GGTC*	*	GA**	**	*	C*	TCGG---*G	*T*	*	G	
<i>M. monosperma</i>	*	T	CA	****TC	**	*	T	TYT	-	T	T---C**CCCCTC*C	S*A	G*	*	GGTT	TT	T*	----	C	**AT	TY	*	*T	TCGGTT*G	*T*	*	G	
<i>M. revoluta</i>	*	*	CA	*****C	**	*	T	T*T	-	T	T---C**-CCCTC*C	*CA	GC	*	----	TT	**	----	*	****	**	G	*T	TC-----*G	*T*	*	G	
<i>M. thailandica</i>	A	*	CG	*****C	**	*	*	**T	T	T	T---C**-CGCTC*C	*C*	**	*	----	*C	**	GTTCC	*	*A**	**	*	C*	*CG-----*G	*T*	C	G	
<i>M. warburgii</i>	*	T	CA	****TC	T*	*	T	T*T	-	T	T---C**-CCCTC*C	*A	G*	*	-GCT	TT	T*	----	C	***T	**	*	*T	TC-----CG	*T*	*	G	

Asterisk (*) indicates the same nucleotide as top sequence; hyphen (-) indicates nucleotide deletion; R denotes A and G; S denotes G and C; and Y denotes C and T.

Table 5 Variation in ITS1 of 12 *Mucuna* species (cont.)

Species	Nucleotide position																			Length		
	ITS1																					
	154-84	186	189-90	192	194-5	198-200	213-5	219	229-31	233	237-8	242-3	245	250-1	253	259	266	271	274-6		288-90	286-287
<i>M. pruriens</i> var. <i>pruriens</i>	CGGTCGCC-----CCGCGCA--CCT	C	TC	C	AA	CAC	TTC	C	CTC	-	CT	AA	T	TG	T	G	G	C	CCC	C--	CAAC	276
<i>M. pruriens</i> var. <i>utilis</i>	*****-----*****_***	*	**	*	**	***	***	*	***	-	**	**	*	**	*	*	*	*	***	*--	****	276
<i>M. bracteata</i>	*A-----AAA	*	**	*	**	***	CA*	*	***	-	*C	M*	C	CC	S	*	*	*	***	*--	A***	262
<i>M. gigantea</i>	T***T**T-----*T*T**T--***	*	*T	T	-G	*TA	***	T	T*T	A	T*	CG	*	*T	*	A	C	T	TTT	*--	*G--	266
<i>M. gracilipes</i>	*****-----*****_***	*	**	*	**	***	***	*	***	-	**	C*	C	*C	*	*	*	*	***	*--	****	279
<i>M. hainanensis</i>	T*****-----T**T**TGC***	*	C*	T	-G	**A	***	T	T*T	-	T*	CG	*	*T	*	A	*	T	T*T	*--	*G--	269
<i>M. interrupta</i>	T*****-----T**T**T--G**	*	C*	T	-G	**A	***	*	T*T	-	T*	*G	*	*A	*	A	*	T	T*T	*--	*G--	267
<i>M. macrocarpa</i>	*****-----*****G--**C	*	C*	T	-G	***	***	*	***	-	T*	CG	*	*C	C	*	*	*	T**	T--	*G--	274
<i>M. monosperma</i>	T***T***-----T**TC*T--***	*	C*	T	-G	MTA	**Y	T	T*T	A	A*	GG	*	*T	*	A	C	T	T*T	*--	*G--	278
<i>M. revoluta</i>	T***G***-----T**T**TG--**C	G	C*	T	-G	**A	***	*	T*T	-	T*	GG	*	*T	*	A	*	T	T*T	*--	*G--	269
<i>M. thailandica</i>	*****-----*****G--**C	*	C*	T	-G	***	***	*	***	-	T*	CG	*	*C	C	*	*	*	T**	T--	*G--	273
<i>M. warburgii</i>	T***T**TCAGCTCTTGTG*TAT**T--***	*	CT	T	-G	*TA	***	T	TCT	A	T*	CG	*	*T	*	A	C	T	T*T	*CA	*G--	285

Asterisk (*) indicates the same nucleotide as top sequence; hyphen (-) indicates nucleotide deletion; M denotes A and C; S denotes G and C; and Y denotes C and T.

Table 6 Variation in 5.8S of 12 *Mucuna* species

Species	Nucleotide position		
	5.8 S		
	312	443-4	Length
<i>M. pruriens</i> var. <i>pruriens</i>	T	CC	166
<i>M. pruriens</i> var. <i>utilis</i>	*	**	166
<i>M. bracteata</i>	C	**	166
<i>M. gigantea</i>	*	TT	166
<i>M. gracilipes</i>	*	**	166
<i>M. hainanensis</i>	*	TT	166
<i>M. interrupta</i>	*	TT	166
<i>M. macrocarpa</i>	*	**	166
<i>M. monosperma</i>	*	TT	166
<i>M. revoluta</i>	*	TT	166
<i>M. thailandica</i>	*	**	166
<i>M. warburgii</i>	*	*T	166

Asterisk (*) indicates the same nucleotide as top sequence; hyphen (-) indicates nucleotide deletion.

Table 7 Variation in ITS2 of 12 *Mucuna* species

Species	Nucleotide position																
	ITS2																
	477-8	482-3	486-8	494	496	498-9	501	505-8	519	521	524	532	534	536-45	547	550	558
<i>M. pruriens</i> var. <i>pruriens</i>	TA	CA	TGC	C	T	AC	T	TGC-	T	C	A	C	A	--CATCGT--	T	T	TG
<i>M. pruriens</i> var. <i>utilis</i>	**	**	***	*	*	**	*	***_	*	*	*	*	*	_*****_	*	*	**
<i>M. bracteata</i>	C*	**	A**	*	*	GA	C	C**A	C	*	G	*	*	--**C***--	*	*	**
<i>M. gigantea</i>	**	T*	GT-	T	*	*T	*	*TT-	A	T	*	T	G	--*CA**A--	*	C	*A
<i>M. gracilipes</i>	**	**	A**	*	*	**	*	***_	*	*	*	*	*	CA**C***--	*	*	**
<i>M. hainanensis</i>	**	T*	GC-	*	*	*T	*	**T-	A	*	*	T	G	--*CA**A--	*	C	**
<i>M. interrupta</i>	**	T*	GC-	*	*	*T	*	**T-	A	*	*	T	G	--*CA**A--	*	C	**
<i>M. macrocarpa</i>	CG	*C	A**	*	*	**	*	***_	A	*	*	*	G	--*GA***--	C	C	C*
<i>M. monosperma</i>	**	T*	GCA	T	*	*T	*	**T-	A	*	*	T	G	--GCA**ACT	*	C	**
<i>M. revoluta</i>	**	T*	GC-	*	*	*T	C	**T-	A	*	*	T	G	--*CA**A--	*	C	**
<i>M. thailandica</i>	CG	*C	A**	*	*	**	*	***_	A	*	*	*	G	--*GA***--	C	C	C*
<i>M. warburgii</i>	**	T*	GC-	T	C	*T	*	**T-	A	*	*	*	G	---CAA*A--	*	C	**

Table 8 Variation in ITS2 of 12 *Mucuna* species (cont.)

Species	Nucleotide position																								Length	Length	
	ITS2																										
	572-3	576-7	579	581-3	585	588	601-2	594	603	607-10	621-2	628-32	634	636	638-45	652-54	657	660	662-64	667	669-76	680	683	685-6			688
<i>M. pruriens</i> var. <i>pruriens</i>	GC	CC	A	CTC	T	C	CA	A	T	CGAT	AG	CCGAA	C	G	CCAAGGTC	CCC	C	T	CAC	G	----C--CAC	C	T	CC	A	209	651
<i>M. pruriens</i> var. <i>utilis</i>	**	**	*	***	*	*	**	*	*	****	**	***G*	*	*	*****	***	*	*	***	*	----*--***	*	*	**	*	209	651
<i>M. bracteata</i>	**	**	*	*C*	C	*	**	*	*	*A*C	**	---*G	*	*	**G*C*C*	***	A	C	***	*	---C*AG***	*	C	G*	G	211	639
<i>M. gigantea</i>	AT	TT	*	AAT	C	T	T*	*	*	*AT*	*A	TGCT*	T	A	TT**TT*T	*TT	*	-	G*A	A	----*--*T*	T	*	**	*	205	637
<i>M. gracilipes</i>	**	**	*	***	*	*	**	*	*	****	**	**C**	*	*	*****	***	*	*	***	*	----*--***	*	*	**	G	209	654
<i>M. hainanensis</i>	AT	TT	*	AAT	C	T	T*	*	*	**T*	*A	TGC**	T	*	T***TT*T	TT*	*	-	GTT	*	----*--**T	*	*	**	*	207	642
<i>M. interrupta</i>	AT	TT	*	AAT	C	T	T*	*	*	**T*	*A	TGC**	T	*	T***TT*T	TT*	*	-	ATT	*	----*--*G*T	*	*	*G	*	207	640
<i>M. macrocarpa</i>	*T	**	G	TG*	C	*	T-	M	C	***C	GA	*GC**	*	C	T*CGCA**	***	*	C	***	*	----*--*G*	*	*	*G	*	209	649
<i>M. monosperma</i>	A*	TT	*	AAT	C	T	T*	*	*	**T*	*A	TGCT*	T	A	TT**TT*T	TTT	*	-	G*T	*	----*--*T*	*	*	**	*	210	654
<i>M. revoluta</i>	AT	TT	*	AAT	C	T	T*			T*T*	*A	TGCG*	T	*	T***TT*T	AT*	*	A	AT*	*	----*--***	*		**	*	208	643
<i>M. thailandica</i>	*T	**	G	TG*	C	*	T-	*	C	***C	GA	*GC**	*	C	T*CGC***	***	*	C	***	*	----*--*G*	*	*	*G	*	209	648
<i>M. warburgii</i>	AT	TT	*	AAT	C	T	T*	*	*	*AT*	*A	TGCT*	T	A	*T**AT*T	*TT	*	-	G*T	A	CCTC*--*T*	T	*	**	*	210	661

Asterisk (*) indicates the same nucleotide as top sequence; hyphen (-) indicates nucleotide deletion; M denotes A and C.

Table 9 Variation in *matK* of 12 *Mucuna* species

Species	Nucleotide position																																					
	<i>matK</i>																																					
	1	26-37	66-7	78	99	111	114-5	117	152	168	180	195-96	199	253	255	272	285	306	329	339	370	375	400	412	437	444-5	453	478	483	485-86	502	504	555-56	567	574	577	586	
<i>M. pruriens</i> var. <i>pruriens</i>	T	-----ATTGTA	AA	C	T	T	CT	G	T	G	C	AA	A	G	A	A	A	T	A	A	A	A	T	C	G	CA	C	T	G	AC	C	A	AA	G	C	G	G	
<i>M. pruriens</i> var. <i>utilis</i>		ATTGTA*****	**	*	*	*	**	*	*	*	*	**	*	*	*	*	*	*	*	*	*	*	*	*	*	**	*	*	*	*	**	*	*	**	*	*	*	*
<i>M. bracteata</i>	*	-----*****	**	T	*	*	**	*	*	*	*	**	*	*	*	*	*	*	*	*	*	*	*	*	*	**	*	*	A	**	*	*	**	A	*	*	*	
<i>M. gigantea</i>	*	-----	CC	T	G	A	**	C	C	T	T	**	T	*	*	G	*	C	*	G	*	T	A	*	A	TG	G	C	*	GT	*	G	TC	*	G	*	*	
<i>M. gracilipes</i>	*	-----*****	**	*	*	*	**	*	*	*	*	**	*	*	*	*	*	*	*	*	*	*	*	*	*	**	*	*	*	*	**	*	*	**	*	*	*	*
<i>M. hainanensis</i>	C	-----	**	T	*	A	**	*	C	T	*	C*	T	c	*	*	*	C	G	G	*	T	A	*	*	TG	G	C	*	GT	*	G	TC	*	*	*	*	
<i>M. interrupta</i>	*	-----	**	T	*	A	**	*	C	T	*	C*	T	*	*	*	*	C	G	G	*	T	A	*	*	TG	G	C	*	GT	*	G	TC	*	*	*	C	
<i>M. macrocarpa</i>	*	-----	**	T	*	*	AG	*	C	T	*	C*	T	*	*	*	T	C	*	*	T	T	*	T	*	*G	*	*	A	GT	*	G	**	*	*	A	*	
<i>M. monosperma</i>	*	-----	**	T	*	*	A*	*	C	T	*	**	T	*	*	*	T	C	*	*	T	T	*	T	*	*G	*	*	*	GT	*	G	**	*	*	*	*	
<i>M. revoluta</i>		-----*****	**	T	*	*	**	*	*	*	*	**	*	*	*	*	*	*	*	*	*	*	*	*	*	**	*	*	*	**	*	*	AA	A	*	*	*	
<i>M. thailandica</i>	*	-----	**	T	*	*	*G	*	C	T	*	CC	T	*	C	*	T	C	*	*	T	T	*	T	*	*G	*	*	A	GT	A	G	**	*	*	A	*	
<i>M. warburgii</i>	*	-----	C*	T	G	A	**	*	C	T	*	**	T	*	*	G	*	C	*	G	*	T	A	*	*	TG	G	C	*	GT	*	G	TC	*	G	*	*	

Asterisk (*) indicates the same nucleotide as top sequence; hyphen (-) indicates nucleotide deletion.

Table 10 Variation in *matK* of 12 *Mucuna* species (cont.)

Species	Nucleotide position											Length
	matK											
	591	598	601	623	638	651	660	708	721	742		
<i>M. pruriens</i> var. <i>pruriens</i>	T	C	C	C	T	T	A	G	T	T	778	
<i>M. pruriens</i> var. <i>utilis</i>	*	*	*	*	*	*	*	*	*	*	784	
<i>M. bracteata</i>	*	T	*	*	*	C	*	*	*	*	778	
<i>M. gigantea</i>	A	*	T	*	*	C	G	A	G	G	772	
<i>M. gracilipes</i>	*	*	*	*	*	*	*	*	*	*	778	
<i>M. hainanensis</i>	A	*	T	*	*	C	G	*	G	*	772	
<i>M. interrupta</i>	A	*	T	A	G	C	G	*	G	*	772	
<i>M. macrocarpa</i>	A	*	T	*	*	C	G	*	*	*	772	
<i>M. monosperma</i>	A	*	T	*	*	C	G	*	*	*	772	
<i>M. revoluta</i>	*	T	*	*	*	C	*	*	*	*	778	
<i>M. thailandica</i>	A	*	T	*	*	C	G	*	*	*	772	
<i>M. warburgii</i>	A	*	T	*	*	C	G	A	G	G	772	

Table 11 Variation in *rbcL* of 12 *Mucuna* species

Species	Nucleotide position																			Length
	<i>rbcL</i>																			
	153	156	195	201	207	276	285	321	381	393	402-3	441	450	483	486	570	618	660-1		
<i>M. pruriens</i> var. <i>pruriens</i>	C	G	A	C	G	A	T	T	C	G	AC	G	C	T	C	C	A	TA	720	
<i>M. bracteata</i>	*	*	*	*	*	*	*	G	T	*	**	*	*	*	*	A	*	GC	720	
<i>M. gigantea</i>	T	C	G	*	C	*	C	*	G	*	G*	*	G	*	*	*	T	**	720	
<i>M. gracilipes</i>	*	*	*	*	C	*	*	*	*	*	**	*	*	*	*	*	*	**	720	
<i>M. hainanensis</i>	T	C	G	T	C	*	C	*	G	A	G*	*	G	C	*	*	T	**	720	
<i>M. interrupta</i>	T	C	G	*	C	G	C	*	G	*	G*	A	G	C	*	*	T	**	720	
<i>M. macrocarpa</i>	*	*	G	*	*	*	*	*	*	*	GA	*	G	*	G	T	T	GC	720	
<i>M. monosperma</i>	T	C	G	*	C	*	C	*	G	*	G*	*	G	*	*	*	T	**	720	
<i>M. revoluta</i>	T	C	G	*	C	*	C	*	G	*	G*	A	G	C	*	*	T	**	720	
<i>M. thailandica</i>	*	*	G	*	*	*	*	*	*	*	G*	*	G	*	*	T	T	GC	720	
<i>M. warburgii</i>	T	C	G	*	C	*	C	*	G	*	G*	*	G	*	*	*	T	**	720	

Asterisk (*) indicates the same nucleotide as top sequence; hyphen (-) indicates nucleotide deletion.

Table 12 Variation in *trnH-psbA* of 12 *Mucuna* species

Species	Nucleotide position																															
	<i>trnH-psbA</i>																															
	1	5	7	12-3	20	25	27-37	40	43-5	50	53	55	66-71	73	75	80	88	90-2	95-6	110-21	123	145-6	153-4	164-9	173-4	188-9	191	197-200	203	205-7	214-9	
<i>M. pruriens</i> var. <i>pruriens</i>	T	C	A	GT	T	C	TCTATTCTATT	T	TCT	T	C	T	-----	T	A	G	C	TCC	TT	ATATATATATAC	T	AA	CT	TAAAAT	CC	AA	A	ATCA	A	TTA	-----	
<i>M. pruriens</i> var. <i>utilis</i>	*	*	*	**	*	*	*****	*	***	*	*	*	-----	*	*	*	*	***	**	*****	*	**	**	*****	**	**	*	****	*	***	-----	
<i>M. bracteata</i>	*	*	*	*G	*	T	**-----**	*	***	*	A	C	-----	*	*	*	*	***	**	*C-----	*	**	**	*****	**	C*	*	****	*	***	-----	
<i>M. gigantea</i>	C	T	C	TG	*	T	**-----**	C	**	A	*	*	AATAAA	A	G	A	T	--*	CC	T*-----	A	C*	**	-----*	TA	TT	*	T*TC	T	***	-----	
<i>M. gracilipes</i>	*	*	*	**	*	*	*****	*	***	*	*	*	-----	T	*	*	*	***	**	*****	*	**	**	*****	**	**		****	*	***	-----	
<i>M. hainanensis</i>	T	T	*	TG	*	T	**-----**	C	*--	A	*	*	AATAAA	*	G	A	T	--*	CC	T*-----	A	C*	**	-----*	TA	TT	*	*AT*	T	GG*	-----	
<i>M. interrupta</i>	T	T	*	TG	*	T	**-----*G*	C	**	A	*	*	AATAAA	*	G	A	T	--*	CC	T*-----	A	C*	**	-----*	TA	TT	G	*AT*	T	GG*	-----	
<i>M. macrocarpa</i>	T	*	*	TG	G	T	-----C	*	C**	*	*	*	AAAAAA	G	*	*	*	***	**	T*-----	A	**	**	A*****	T*	T*	*	TAA*	T	G**	TAATAA	
<i>M. monosperma</i>	C	T	*	TG	*	T	**-----**	C	**	A	*	*	AATAAA	A	G	A	T	--*	CC	T*-----	A	C*	**	-----G	TA	TT	*	T*TC	T	**C	-----	
<i>M. revoluta</i>	*	T	C	TG	*	T	**-----**	*	**	A	*	*	AATAAA	A	G	A	T	--*	CC	T*-----	A	C*	AA	-----T	TA	TT	*	*AT*	T	GG*	-----	
<i>M. thailandica</i>	T	*	*	TG	*	T	**-----**	*	*--	*	*	*	AAAAAA	G	*	*	*	***	**	T*-----	A	**	**	A****G	T*	T*	*	T*A*	T	G**	-----	
<i>M. warburgii</i>	C	T	*	TG	*	T	**-----**	C	**	A	*	*	AATAAA	A	G	A	T	--G	CC	T*-----	A	CC	**	-----*	TA	TT	*	T*TC	T	***	-----	

Asterisk (*) indicates the same nucleotide as top sequence; hyphen (-) indicates nucleotide deletion.

Table 13 Variation in *trnH-psbA* of 12 *Mucuna* species (cont.)

Species	Nucleotide position																			Length	
	<i>trnH-psbA</i>																				
	230	234	237	244	249-51	252-3	262	264	266	269	278	288-9	297-9	302-5	308	310	312-3	317-8	320-1		324
<i>M. pruriens</i> var. <i>pruriens</i>	A	G	T	A	AAA	TG	C	T	T	T	C	TT	AAA	TTAT	A	T	GT	-T	-A	T	314
<i>M. pruriens</i> var. <i>utilis</i>	*	*	*	*	***	**	*	*	*	*	*	**	***	****	*	*	A*	A*	-*	*	314
<i>M. bracteata</i>	*	*	*	*	***	**	*	*	*	*	*	**	***	****	*	*	**	A*	-*	*	300
<i>M. gigantea</i>	*	T	C	C	TTT	**	A	*	*	*	G	G*	C**	****	*	*	**	A*	-*	*	297
<i>M. gracilipes</i>	*	*	T	*	***	**	*	*	*	*	*	**	***	****	*	*	**	A*	-*	*	314
<i>M. hainanensis</i>	*	T	C	C	TTT	**	A	*	*	*	G	G*	C**	****	*	*	**	A*	-*	*	297
<i>M. interrupta</i>	C	T	C	C	TTT	**	A	*	G	-	G	G*	CGG	AGGC	G	C	*C	GA	-C	G	296
<i>M. macrocarpa</i>	*	*	C	*	***	*T	*	C	*	-	*	*A	***	**T*	T	*	**	A*	-*	*	306
<i>M. monosperma</i>	*	T	C	*	***	G*	A	*	*	*	G	G*	C**	****	*	*	**	A*	-*	*	297
<i>M. revoluta</i>	*	T	C	C	TTT	**	A	*	*	*	*	G*	C**	****	*	*	**	A*	A*	*	297
<i>M. thailandica</i>	*	*	C	*	***	**	*	C	*	*	*	**	T**	****	*	*	**	-A	-*	*	302
<i>M. warburgii</i>	*	T	C	C	TTT	**	A	*	*	*	G	G*	C**	****	*	*	**	A*	-*	*	297

Asterisk (*) indicates the same nucleotide as top sequence; hyphen (-) indicates nucleotide deletion.

Table 14 Sequencing data analysis of single marker DNA barcodes of *Mucuna* species

Marker	ITS	<i>matK</i>	<i>rbcl</i>	<i>trnH-psbA</i>
Length range (bp)	637-661	772-784	720	296-314
Aligned length (bp)	689	784	720	328
C (%)	472 (68.51)	726 (92.60)	700 (97.22)	246 (75.00)
V (%)	199 (28.88)	52 (6.63)	20 (2.78)	75 (22.86)
Pi (%)	140 (20.32)	40 (5.10)	14 (1.94)	42 (12.80)
S (%)	61 (8.85)	12 (1.53)	6 (0.83)	33 (10.06)

C: conserve site, V: variable site, Pi: parsimony-informative sites, S singleton sites

Discrimination performance of candidate DNA markers

All pairwise genetic distances were analyzed among the reference sequences, and between each query sequence and each of the reference sequences, using the K2P model of evolution. Our study revealed that ITS exhibited the highest mean variation (0.14; SE 0.02), followed by *trnH-psbA* (0.10; SE 0.01), *matK* (0.03; SE 0.00), and *rbcl* (0.01; SE 0) as shown in Table 15.

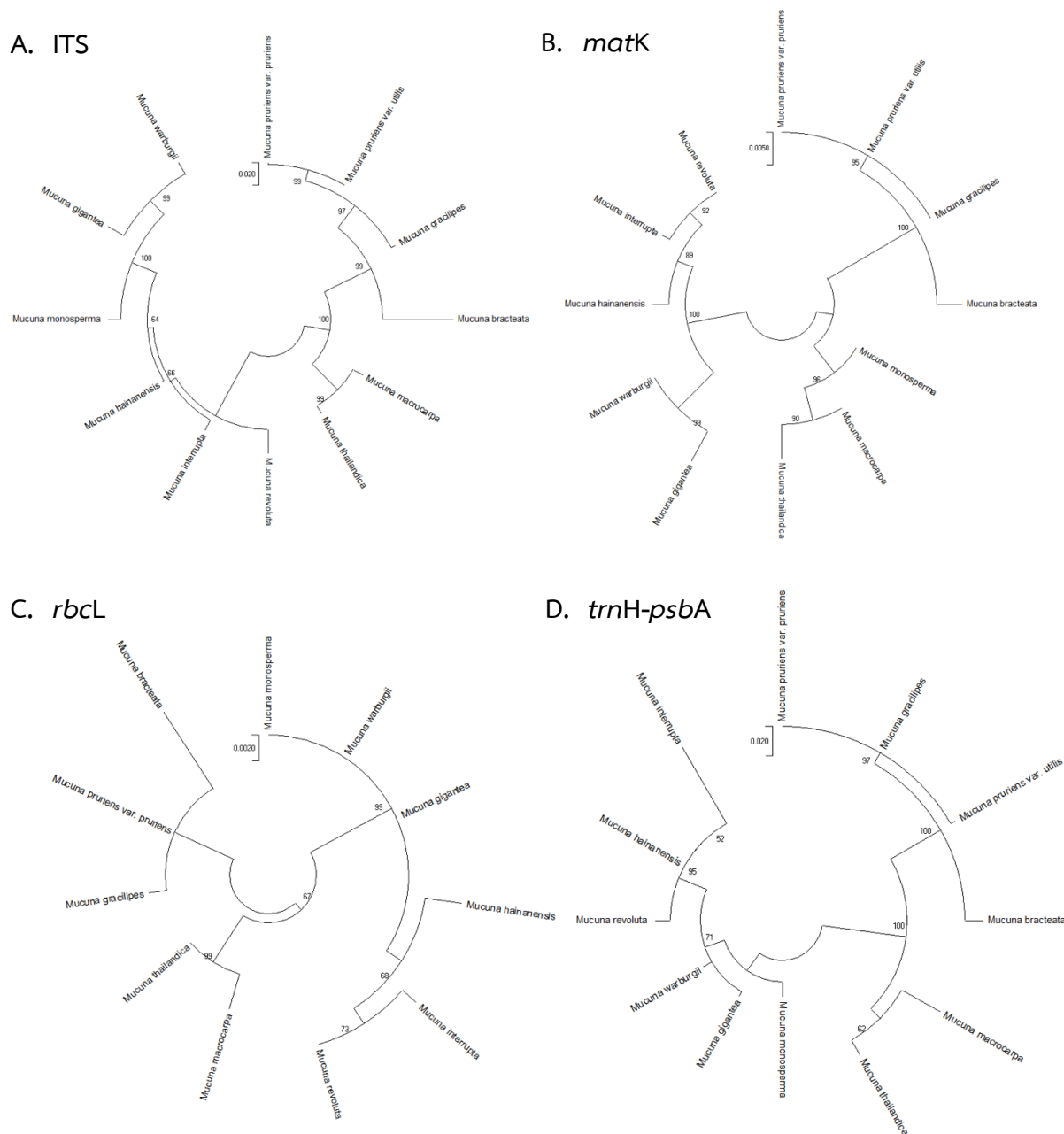
Table 15 Performance and variabilities of different DNA barcode markers

Marker	Mean variation (SE)	Range%
ITS	0.14 (0.02)	0.0013-0.2365
<i>matK</i>	0.03 (0.00)	0-0.0442
<i>rbcl</i>	0.01 (0.00)	0-0.0159
<i>trnH-psbA</i>	0.10 (0.01)	0-0.2202

The data from all candidate DNA markers were utilized to construct phylogenetic trees by the ML method. Only the ML tree constructed on the basis of ITS marker exhibited clear resolution for twelve *Mucuna* species especially *M. pruriens* var. *pruriens* and *M. pruriens* var. *utilis*. The ML tree constructed using the *matK* region could not resolve *M. pruriens* var. *pruriens*, *M. pruriens* var. *utilis*, and *M. gracilipes* because they placed in the same clade. Moreover, the sample of *M. gigantea*, *M. monosperma* and *M. warburgii* also were found in

the same clade of the phylogenetic tree constructed using the *rbcl* region. While, the ML tree constructed by *trnH-psbA* could not discriminate *M. pruriens* var. *pruriens* and *M. gracilipes*.

Fig. 4 Phylogenetic trees constructed by the ML method by MEGA version X software



From the data of variation of nucleotide sequence, sequencing analysis, discrimination performance of four candidate DNA markers, we selected the ITS region for identification and authentication of Ma mui products.

Authentication of Ma mui products used DNA barcode

From the results of discriminatory performance among candidate DNA barcode, the ITS was selected for identification and authentication of Ma mui products claimed as the seed of *M. pruriens*. Ten Ma mui products were purchased from online markets. All products labeled and claimed about aphrodisiac activity for male. The Ma mui products were extracted, amplified, and sequenced the nucleotide sequence in ITS region. We found that the ingredient of all products was only *M. pruriens*. Therefore, we confirmed that the ITS region is suitable for identification and authentication *Mucuna* crude drugs.

Determination of L-DOPA content in *Mucuna* seeds and online Ma mui products

L-DOPA content was analyzed in twenty-one seed samples of nine *Mucuna* species (*M. pruriens* var. *pruriens*, *M. pruriens* var. *utilis*, *M. bracteata*, *M. gigantea*, *M. hainanensis*, *M. interrupta*, *M. macrocarpa*, *M. monosperma*, *M. revoluta*, and *M. thailandica*) collected in Thailand (Fig. 2) including ten Ma mui products, the herbal products claimed as *M. pruriens* as an ingredient for desired aphrodisiac activity purchased from Thai online markets as shown in Fig. 3. The data analyzed from HPLC were compared with the calibration curve of L-DOPA standard. The calibration curve exhibited good linearity with correlation coefficients (R^2) as 1.000 as shown in Fig. 5. On comparing the seed of *Mucuna* species, the content of L-DOPA varied from 2.66 mg/100 mg of extract to 147.42 mg/100 mg of extract (Fig. 6). The highest content of L-DOPA is XS16 of *M. pruriens* var. *pruriens*. On the contrary, *M. monosperma* (XS13) showed the lowest L-DOPA content.

Fig. 5 The calibration curves of L-DOPA standard

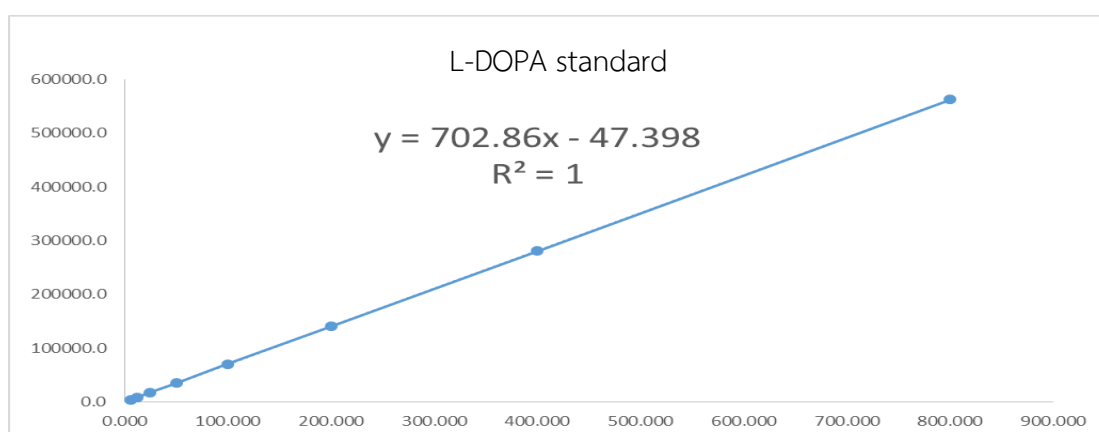
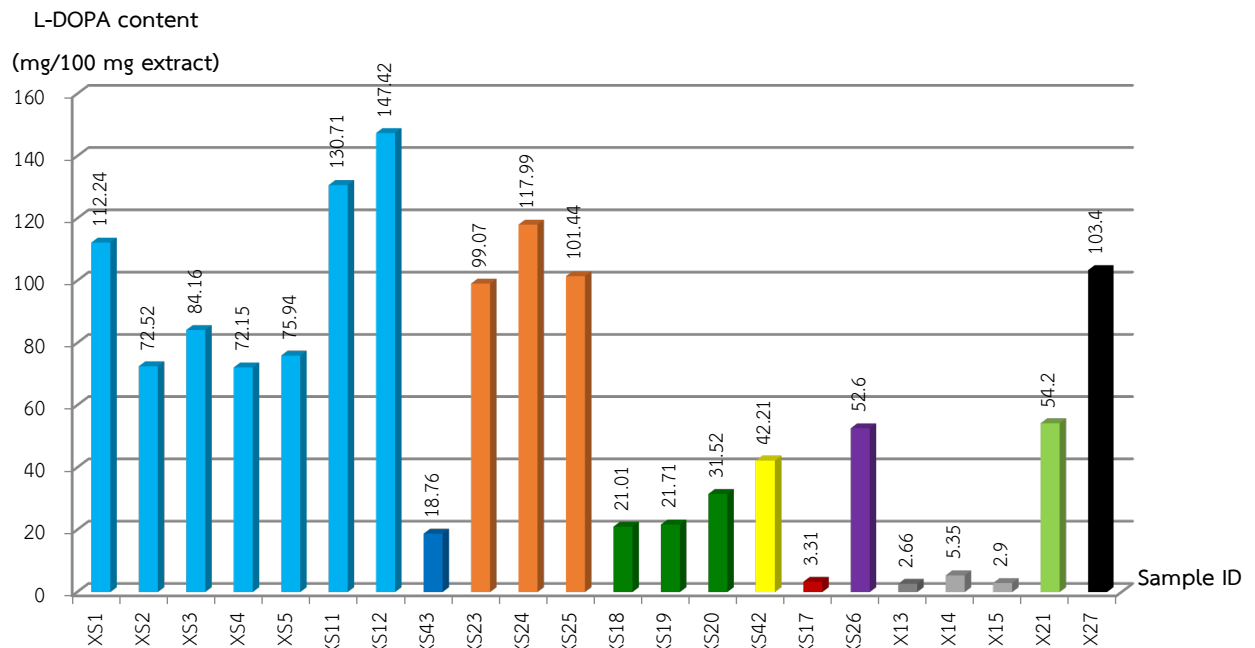
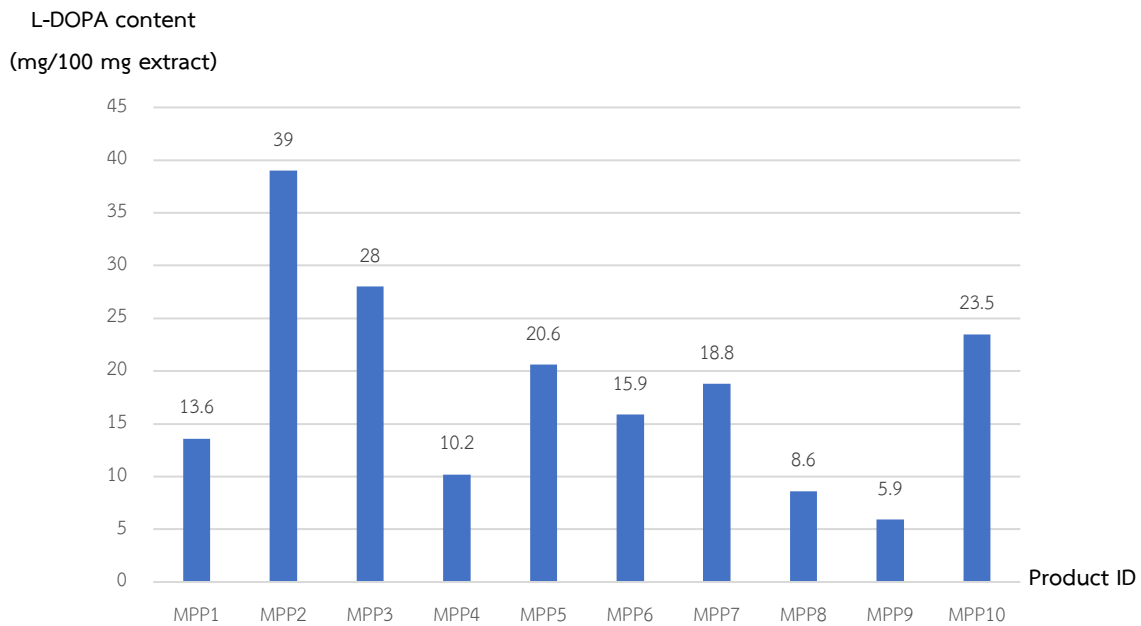


Fig. 6 The quantity of L-DOPA analyzed from *Mucuna* seeds collected in Thailand



M. pruriens var. *pruriens* (blue), *M. pruriens* var. *utilis* (dark blue), *M. bracteata* (orange), *M. gigantea* (dark green), *M. hainanensis* (yellow), *M. interrupta* (red), *M. macrocarpa* (violet), *M. monosperma* (grey), *M. revoluta* (green), and *M. thailandica* (black)

All online Ma mui products were analyzed L-DOPA content for authentication and quality control of products. All products labeled and claimed only *M. pruriens* as ingredients. Our results demonstrated that all products sold in online markets contained L-DOPA. Ten samples showed that the quantity of L-DOPA ranged from 11.86-56.04 mg/100 mg of extract (Fig. 7). The HPLC chromatogram of all Ma mui product exhibited the identical HPLC chromatogram with the seeds of *M. pruriens* samples.

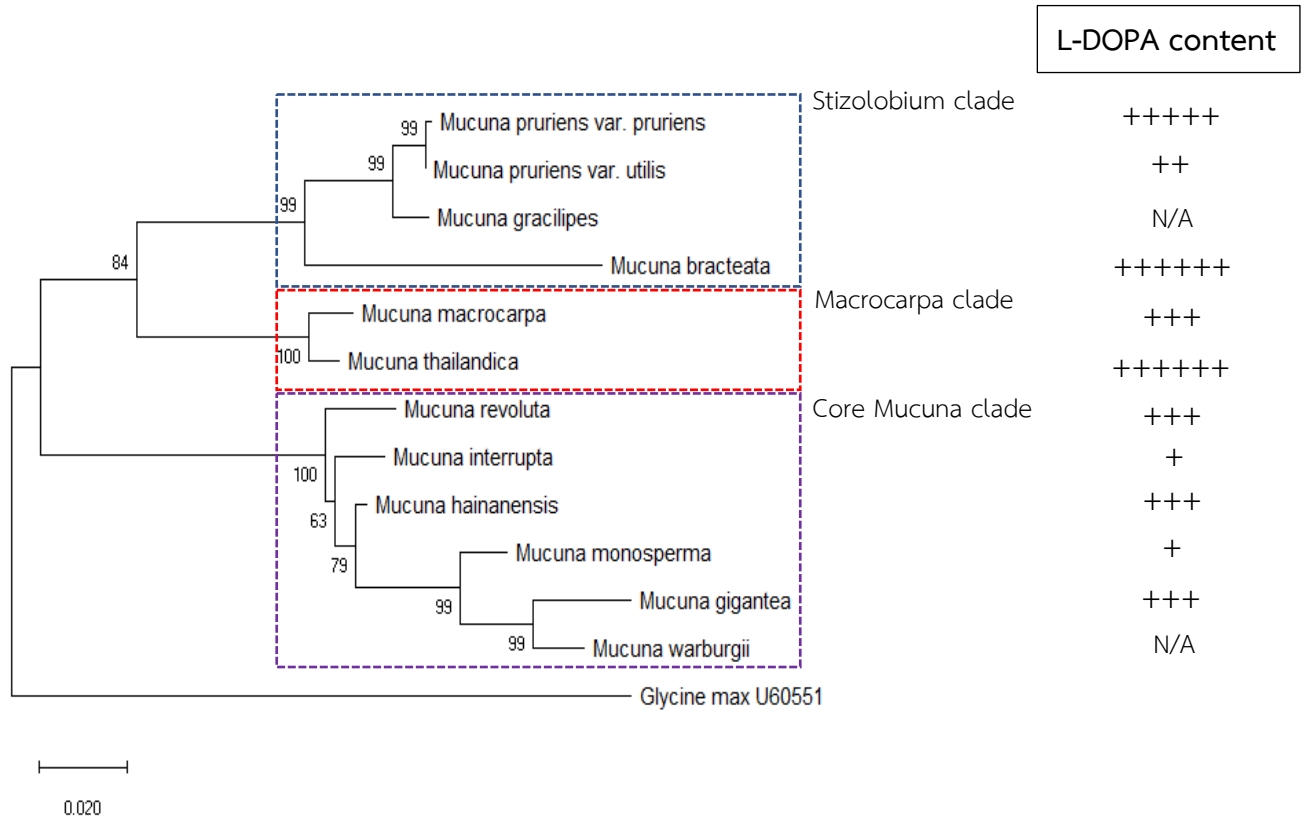
Fig. 7 The quantity of L-DOPA analyzed from *Mucuna* seeds collected in Thailand

Correlation of phylogenetic relationship of Thai *Mucuna* species and L-DOPA contents

The nucleotide sequences in ITS region of *Mucuna* species was constructed the phylogenetic relationship. Neighbor-Joining (NJ) calculation was performed using Kimura two-parameter model with 1000 bootstrap replications. *Glycine max* L. of the same tribe Phaseoleae in Fabaceae family was used as outgroup species for phylogenetic tree rooting. The NJ tree was able to divide to three clades: Stizolobium, Macrocarpa, and core *Mucuna* clade. Stizolobium clade composes of *M. pruriens* var. *pruriens*, *M. pruriens* var. *utilis*, *M. bracteata*, and *M. gracilipes*. Both *M. macrocarpa* and *M. thailandica* placed in Macrocarpa clade. Whereas, the core *Mucuna* clade is comprised of *M. revoluta*, *M. interrupta*, *M. monosperma*, *M. hainanensis*, *M. gigantea*, and *M. warburgii*. The seed of all *Mucuna* plants collected in Thailand except *M. gracilipes*, the rare *Mucuna* species and *M. warburgii*, the exotic *Mucuna* species from Indonesia presenting in Thailand for decoration of garden because its flower is showy and beautiful were analyzed L-DOPA content. Focusing in the correlation of phylogenetic tree and L-DOPA content, the result showed the inclination of the quantity of L-DOPA in clade of Stizolobium and *Mucuna*, which these species placed in both clades are likely contain high L-DOPA content (Fig.8). Therefore, we can conclude that the *Mucuna*

species in Stizolobium and Mucuna clade are suitable for L-DOPA source to develop product in pharmaceutical industry.

Fig. 8 Correlation of phylogenetic relationship of Thai *Mucuna* species and L-DOPA contents



APPENDIX

1. **Intharuksa A.** Chansakaow S., Denduangboripant J., Sukrong S. Multi-locus DNA Barcoding Strengthened with Multiplex PCR Analysis for Authentication of an aphrodisiac *Mucuna* Products in Thailand. Submitted to *Scientific Report* in preparation.
2. **Intharuksa A.,** Yanaso S., phrutivorapongkul A., Chansakaow S., Sukrong S. Comparative analysis of L-dopa contents and its anti-alzheimer's activity in different *Mucuna* species distributed in Thailand. Submitted to *Journal of Ethnopharmacology* in preparation.

ACCESSION LC494390

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ORIGIN

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ACCESSION LC494393

FEATURES Location/Qualifiers

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ACCESSION LC494394

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ACCESSION LC494395

FEATURES Location/Qualifiers

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ORIGIN

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ACCESSION LC494396

FEATURES Location/Qualifiers

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ACCESSION LC494397

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ACCESSION LC494398

FEATURES Location/Qualifiers

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ACCESSION LC494399

FEATURES Location/Qualifiers

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ACCESSION LC494400

FEATURES Location/Qualifiers

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ACCESSION LC494401

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ACCESSION LC494402

FEATURES Location/Qualifiers

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ACCESSION LC494403

FEATURES Location/Qualifiers

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ACCESSION LC494404

FEATURES Location/Qualifiers

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ORIGIN

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ACCESSION LC494405

FEATURES Location/Qualifiers

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ORIGIN

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ACCESSION LC494406

FEATURES Location/Qualifiers

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ORIGIN

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ACCESSION LC494407

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ORIGIN

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ACCESSION LC494408

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ACCESSION LC494409

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ACCESSION LC494410

FEATURES Location/Qualifiers

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ORIGIN

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ACCESSION LC494601

FEATURES Location/Qualifiers

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ACCESSION LC494602

FEATURES Location/Qualifiers

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 2, and 28S ribosomal RNA"

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ACCESSION LC494603

FEATURES Location/Qualifiers

source 1..702
 /collected_by="Aekkhaluck Intharuksa"
 /country="Thailand:Tak"
 /isolate="MGP3"
 /mol_type="genomic DNA"
 /organism="Mucuna gracilipes"

misc_RNA <1..>702
 /note="contains 18S ribosomal RNA, internal transcribed spacer 1, 5.8S ribosomal RNA, internal transcribed spacer 2, and 28S ribosomal RNA"

ORIGIN

1 taacaaggtt tccgtaggtg aacctgcgga aggatcattg tcgtgcctc acaacaagtt
 61 caaccggcga acccggtcat cacaggacaa gctcgatcgg ggctggctcg ggggagatgt
 121 tctgaacac cgaccccgtc ctcccgacc ccgagctggc gagaggcggg cgcgccgcgc
 181 acctctctc gccaaaacac aaaccccggc gttcgtgcg ccaaggaaact cgaaactgtt
 241 cagcgcaatc ttgcggggcc cggagacggc gaccccgcgg accttgccac gacacacaac
 301 atacaaaatg actctcggca acggatatct cggctcttgc atcgatgaag aacgtagcga
 361 aatgcgatac ttggtgtgaa ttgcagaatc ccgtgaacca tcgagtcttt gaacgcaagt
 421 tgcgcccga gccattaggc cgagggcacg cctgcctggg tgtcacacat cgttacccca
 481 aaagcaaacg cctcacgtgc gtgcgcaggg tggatgctga cctcccgcga gcacaccgtc
 541 tcgtggctgg ttgaaaatcg agtccgcggc cgagctcgtc gcgacaaaat ggtggatgag
 601 cgatgctcga gaccagtcgc gcccgaccgc gccaaagtcg gactccccga ccctacacgc
 661 gtccacggac gctcccagcg agacctcagg tcaggcgggg ct

ACCESSION LC494604

FEATURES Location/Qualifiers

source 1..686
 /collected_by="Aekkhaluck Intharuksa"
 /country="Thailand:Chiang Rai"
 /isolate="MBT4"
 /mol_type="genomic DNA"
 /organism="Mucuna bracteata"

misc_RNA <1..>686
 /note="contains 18S ribosomal RNA, internal transcribed
 spacer 1, 5.8S ribosomal RNA, internal transcribed spacer
 2, and 28S ribosomal RNA"

ORIGIN

1 taacaaggtt tccgtaggtg aacctgcgga aggatcattg tcgatgcctc acacaacaag
 61 ttcgaccggc gaacccgttc atcacgcgtg ccggggccag cccgggggcg ccgttccttg
 121 ccgaacaacg accccgtcct ccccgccccg acccggcggg aggcaaaacc tctcgccaaa
 181 acacaaaccc cggcgccacg tgcgccaagg aactcgaaac cgttmagcgc aacctscgcg
 241 ggcccggaga cggcgacccc gcggaacctg ccacgacaca aaacatacaa aacgactctc
 301 ggcaacgat atctcgctc ttgatcgat gaagaacgta gcgaaatgcg atacttggtg
 361 tgaattgcag aatcccgtga accatcgagt ctttgaacgc aagttgcgc cgaagccatt
 421 aggccgaggg cacgcctgcc tgggtgtcac acatcgtcac cccaaaagca aacgcctcga
 481 gcgcgcgcag caggtggac gctggcctcc cgcgagcacc gtctcgtggc tggttgaaaa
 541 ccgagccgcg ggccgagccc gccgcgacaa aatggtggat gagcaacgct cgagaccagt
 601 cgcgcggccc ggccgacgcc ggactccccg aaccacacg cgtccagcac ggacggccgc
 661 agcgagacct caggtcaggc ggggcc

ACCESSION LC494605

FEATURES Location/Qualifiers

source 1..696
 /collected_by="Aekkhaluck Intharuksa"
 /country="Thailand:Chiang Mai"
 /isolate="MMP5"
 /mol_type="genomic DNA"
 /organism="Mucuna macrocarpa"

misc_RNA <1..>696
 /note="contains 18S ribosomal RNA, internal transcribed
 spacer 1, 5.8S ribosomal RNA, internal transcribed spacer
 2, and 28S ribosomal RNA"

ORIGIN

1 taacaaggtt tccgtaggtg aacctgcgga aggatcattg tcgatgcctc gcacaaccag
 61 ttcgaccggc gaacccgttt taccatctac gccaccggg gccggctcgg gggcgccgtt
 121 cggctctgaa cgacgacccc gtccccctc gggggttggc gggaggcggc cggcccgcg
 181 gccccctccc gtcgaacaca aaccccggcg cttcgtgcgc caaggaactc gaaattgttc
 241 ggtgcaatct ccgcggggcc ggagacggcg atcccgcgga cttgtcacg acacacgata
 301 caaatgact ctgcgcaacg gatattctcg ctcttgcac gatgaagaac gtagcgaat
 361 gcgatacttg gtgtgaattg cagaatcccg tgaaccatcg agtctttgaa cgcaagttgc
 421 gcccgaagcc attagggcga gggcacgcct gcctgggtgt cacacatcgt cgccccaaa
 481 gcaaacgcct cacgtgcgtg cgcagggtgg aagctgacct cccgcgggcg acgtcccgcg
 541 gctggtcgaa aatcgagtcc gtggccgggt gcgccgcgat amatggtgga cgagcgacgc
 601 tcgagaccga tcgcgcgcga cccgtccgc atcggactcc ccgacccac acgcgtccgc
 661 ggacgctccg aacgagacct caggtcaggc ggggct

ACCESSION LC494606

FEATURES Location/Qualifiers

source 1..702
 /collected_by="Aekkhaluck Intharuksa"
 /country="Thailand:Tak"
 /isolate="MMM6"
 /mol_type="genomic DNA"
 /organism="Mucuna monosperma"

misc_RNA <1..>702
 /note="contains 18S ribosomal RNA, internal transcribed spacer 1, 5.8S ribosomal RNA, internal transcribed spacer 2, and 28S ribosomal RNA"

ORIGIN

1 taacaaggtt tccgtaggtg aacctgcgga aggatcattg tcgttgctc acacaatcag
 61 ttcgaccggt gaatygttt atcatccacc cctcaccggg gstaggtcgg gggttgttgt
 121 tgttctcaa cacataccty gtccttcctc gggttgggtt ggcgggaggt ggttcctcg
 181 tcctcctcct cccgtcgaam taaaaccccg gcgcttygtg tgccaaggaa ttgaaaaat
 241 gttgggtgca attttcgcgg acccggaac ggtgatctcg cggaccttgc cacgacacac
 301 gatacaaaat gactctcggc aacggatatc tcggctcttg catcgatgaa gaacgtagcg
 361 aaatgcgata cttggtgtga attgcagaat cccgtgaacc atcgagtctt tgaacgcaag
 421 ttgcgccgga agccattagg ttgagggcac gcctgcctgg gtgtcacaca tcgttacct
 481 aaagcaaaac gtctcatgtg cgtgtgcagg gtggaagctg acctcccgtg gggcacgact
 541 ctgcggtg gttgaaaatg gagttcacgg ttgagaatgc cgtgataaaa tgggtgatga
 601 gcgttgctcg agaccaatcg cgtgtactc agttaatgtt ggactcttg acccagatgc
 661 gtctcggac gctcccaacg agacctcagg tcaggcgggg ct

ACCESSION LC494607

FEATURES Location/Qualifiers

source 1..688
 /collected_by="Aekkhaluck Intharuksa"
 /country="Thailand:Mae Hong Son"
 /isolate="MIT7"
 /mol_type="genomic DNA"
 /organism="Mucuna interrupta"

misc_RNA <1..>688
 /note="contains 18S ribosomal RNA, internal transcribed
 spacer 1, 5.8S ribosomal RNA, internal transcribed spacer
 2, and 28S ribosomal RNA"

ORIGIN

1 taacaaggtt tccgtaggtg aacctgcgga aggatcattg tcgtgcctc acacaatcag
 61 ttcgaccggt gaatctgttt atcatccacc ctaccgggg ccaggtcggg gttgtgttc
 121 tcgaacatcg acccgtcct tctccggtt ggcgggaggt ggtgcctcg tgctgctct
 181 cccgtcgaac aaaaaccccg gcgcttcgtg cgccaaggaa ttgaaattg ttaggtgcaa
 241 tattcgcgga cccggagacg gtgatctgc ggacctgcc acgacacacg atacaaaatg
 301 actctcggca acggatatct cggctcttc atcgatgaag aacgtagcga aatgcgatac
 361 ttggtgtgaa ttgcagaatc ccgtgaacca tcgagctttt gaacgcaagt tgcgcccga
 421 gccattaggt tgagggcacg cctgcctggg tgcacacat cgttaccta aagcaaacgc
 481 ctcatgtgcg tgtgcagggt ggaagctgac ctccgtggg ccacgactcg cggctggtt
 541 aaaatggagt tcatggttga gaatgccgtg ataaaatggt ggatgagcgt tgctcgagac
 601 caatcgctg cgactcggtc aattttggac tcttcgacc aattgcgtc atggacgctc
 661 cgaacgagac ctcaggtcag gcggggcc

ACCESSION LC494608

FEATURES Location/Qualifiers

source 1..690
 /collected_by="Aekkhaluck Intharuksa"
 /country="Thailand:Lampang"
 /isolate="MHS8"
 /mol_type="genomic DNA"
 /organism="Mucuna hainanensis"

misc_RNA <1..>690
 /note="contains 18S ribosomal RNA, internal transcribed
 spacer 1, 5.8S ribosomal RNA, internal transcribed spacer
 2, and 28S ribosomal RNA"

ORIGIN

1 taacaaggtt tccgtaggtg racctgcgga aggatcattg tcgttcctc acacaatcag
 61 ttcgaccggt gaatctgttt atcatccacc ctcaccgggg ccaggcggg gttgtgttc
 121 tcgaacacgc acccgtcct tcctcgggtt ggcgggaggt ggtcgcctcg tgctgcctc
 181 ctccgctcga acaaaaaccc cggcgcttcg tgtgccaagg aatttgaaat tgttcggtgc
 241 aattttcgcg gacccggaga cgtgatctc gcggaccttg ccacgacaca cgatacaaaa
 301 tgactctcgg caacggatat ctcggctctt gcatcgatga agaacgtagc gaaatgcgat
 361 acttggtgtg aattgcagaa tcccgtgaac catcgagtct ttgaacgcaa gttgcgcccg
 421 aagccattag gttgagggca cgctgcctg ggtgtcacac atcgttacc taaagcaaac
 481 gcctcatgtg cgtgtgcagg gtggaagctg acctcccgtg ggccacgact cgcggctggt
 541 tgaaaatgga gttcatggtt gagaatgccg tgataaaatg gtggatgagc gttgctcgag
 601 accaatcgcg tgcgactcgg tcaattttgg actcttcgac ccagttgcgt ccatggacgc
 661 tccaacgag acctcaggtc aggcggggcc

ACCESSION LC494609

FEATURES Location/Qualifiers

source 1..692
 /collected_by="Aekkhaluck Intharuksa"
 /country="Thailand:Chiang Mai"
 /isolate="MTC9"
 /mol_type="genomic DNA"
 /organism="Mucuna thilandica"

misc_RNA <1..>692
 /note="contains 18S ribosomal RNA, internal transcribed
 spacer 1, 5.8S ribosomal RNA, internal transcribed spacer
 2, and 28S ribosomal RNA"

ORIGIN

1 taacaaggtt tccgtaggtg racctgcgga aggatcattg tcgatgcctc gcacaaccag
 61 ttcgaccggc gaacctgttt taccatccac gtcaccggg gccggctcgg gggcgctgtt
 121 cgttcccgaa caacgacccc gtccccccc ggggttgagg ggaggcggc gccccgcgcg
 181 cccctcccg tcgaacacaa acccggcgc ttcgtgcgc aaggaactcg aaattgttcg
 241 gtgcaatctc cgcgggccc gagacggcg tcccgcggac cttgtcacga cacacgatac
 301 aaaatgactc tcggcaacgg atatctcggc tcttgcacg atgaagaac tagcgaaatg
 361 cgatacttgg tgtgaattgc agaatcccgt gaaccatcga gtctttgaac gcaagttgcg
 421 cccgaagcca ttaggccgag ggcacgcctg cctgggtgtc acacatcgtc gccccaaag
 481 caaacgcctc acgtgcgtgc gcagggtgga agctgacctc ccgcgggca cgtcccgcg
 541 ctggtcgaaa atcgggtccg tggccgggtg cgcgcgata aatggtggac gagcgacgct
 601 cgagaccgat cgcgcgcgac ccgtccgcg tcggactccc cgacccaca cgcgtccgcg
 661 gacgctccga acgagacctc aggtcaggcg gc

ACCESSION LC494610

FEATURES Location/Qualifiers

source 1..708

/collected_by="Aekkhaluck Intharuksa"

/country="Thailand:Chiang Mai"

/isolate="MWG10"

/mol_type="genomic DNA"

/organism="Mucuna warburgii"

misc_RNA <1..>708

/note="contains 18S ribosomal RNA, internal transcribed spacer 1, 5.8S ribosomal RNA, internal transcribed spacer 2, and 28S ribosomal RNA"

ORIGIN

```

1 taacaaggtt tccgtaggtg aacctgcgga aggatcattg tcgttgctca acacaatcag
61 ttgaccggt gaactgtttt atcatccacc ctcaccgggg ctaggtcggg gctgttgtt
121 ttctcaaca cctaccccg cttcctcgg gttggcggga ggtggttgc cagctcttg
181 gctatgctcc tctcctgtc gaactaaaac cccggcgctt cgtgtgcaa ggaatctgaa
241 aattgttcg tgcaattttc gcggaccggg acacggtgat ctcgggacc ttgccacacg
301 acacacgata caaatgact ctcggcaacg gatatctcgg ctcttgcatc gatgaagaac
361 gtagcgaaat gcgatacttg gtgtgaattg cagaatcccg tgaaccatcg agtctttgaa
421 cgcaagttgc gcccgaaagg attaggctga gggcacgcct gcctgggtgt cacacatcgt
481 taccctaaag caaacgtccc atgtgcgtgt gcagggtgga agctgacctc ccgcgggcaa
541 gactcgcggc tggttgaaaa tgcagttcat ggttgagaat gccgtgataa aatggtggat
601 gagcattgct cgagaccaat cgcgtgctac tcagctaaat ttgactcct tgaccagat
661 gcatcctccc tcgcatgctc ccaacgagac ctcaggtcag gcggggcc

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ACCESSION LC494611

FEATURES Location/Qualifiers

source 1..686
 /collected_by="Aekkhaluck Intharuksa"
 /country="Thailand:Pattalung"
 /isolate="MGT11"
 /mol_type="genomic DNA"
 /organism="Mucuna gigantea"
 misc_RNA <1..>686
 /note="contains 18S ribosomal RNA, internal transcribed
 spacer 1, 5.8S ribosomal RNA, internal transcribed spacer
 2, and 28S ribosomal RNA"

ORIGIN

1 taacaaggtt tccgtaggtg aacctgcgga aggatcattg tcgttgctca acagtttgac
 61 ctgtgaatct gttatcata caccctcacc ggggctaggt cgtggctgtt gttgttctcc
 121 aacacctacc tcgtccttcc tcgggttggc gggaggtggt tgctctgtgc tcctcctctt
 181 gtcgaactaa aaccccggcg ctctgtgtgc caaggaattt gaaaattgtt cgtgcaatt
 241 ttgcgggacc cggacacggt gatttcgcg accttgccac gacacacgat acaaaatgac
 301 tctcggcaac ggatatctcg gctcttgcac cgatgaagaa cgtagcgaaa tgcgatactt
 361 ggtgtgaatt gcagaatccc gtgaaccatc gactcttga acgcaagttg cgcccgaagc
 421 cattaggttg agggcacgcc tgcctgggtg tcacacatcg ttaccctaaa gtaaactgtt
 481 catgtgcgtt tgcagggtgg aagttgacct cccgtgggcc acaactcgcg gctggttaaa
 541 aatgcagttc atggttgaga atgccgtgat aaaatggtgg atgagcattg ctcgagacca
 601 atcgcgtgct actcagttaa ttttgactc cttgaccag aagcatcctc ggatgctccc
 661 aacgagacct caggtcaggc ggggcc

ACCESSION LC494612

FEATURES Location/Qualifiers

```

source          1..314
                 /collected_by="Aekkhaluck Intharuksa"
                 /country="Thailand:Chiang Mai"
                 /isolate="PPT1"
                 /mol_type="genomic DNA"
                 /organelle="plastid:chloroplast"
                 /organism="Mucuna pruriens var. pruriens"
                 /variety="pruriens"

misc_feature    <1..25
                 /gene="psbA"

misc_feature    26..>314
                 /note="contains psbA-trnH intergenic spacer and tRNA-His
                 (trnH)"

```

ORIGIN

```

1 tcgaagctcc actacaaatg gataaaattt ggatcttaaa gtatacgagt tttgaaagt
61 aaaggagcaa tatcaacttt gttgatatta ctcttttact ttattagta gtctaaatat
121 tgataaatat ttttacatac atttttggtt tattttaaga ttcttttagca ttttttattt
181 cttatgttat gtcgaaaaag tatatatata taaagtatat atataaatgg atgtctctct
241 cttttttatc ctatcctaga agaaaaaaaa gaataataat agaatagaag gggtagaaat
301 tacggtatag atca

```

ACCESSION LC494613

FEATURES Location/Qualifiers

source 1..315

/collected_by="Aekkhaluck Intharuksa"

/country="Thailand:Chiang Mai"

/isolate="PUT2"

/mol_type="genomic DNA"

/organelle="plastid:chloroplast"

/organism="Mucuna pruriens var. utilis"

/variety="utilis"

misc_feature <1..26

/gene="psbA"

misc_feature 27..>315

/note="contains psbA-trnH intergenic spacer and tRNA-His (trnH)"

ORIGIN

```

1 tcgaagctcc atctataaat ggataaaatt tggatcttaa agtatacgag ttttgaaag
61 taaaggagca atatcaactt tgttgatatt actcctttac ttttattagt agtctaaata
121 ttgataaata tttttacata catttttggt ttattttaag attctttagc attttttatt
181 tcttatgtta tgtcgaaaaa gtatatatat ataaagtata tatataaatg gatgtctctc
241 tcttttttat cctatcctag aagaaaaaaa agaataataa tagaatagaa ggggtagaaa
301 ttacgtata gatca

```

ACCESSION LC494614

FEATURES Location/Qualifiers

```

source          1..315
                 /collected_by="Aekkhaluck Intharuksa"
                 /country="Thailand:Tak"
                 /isolate="GPT3"
                 /mol_type="genomic DNA"
                 /organelle="plastid:chloroplast"
                 /organism="Mucuna gracilipes"
misc_feature    <1..26
                 /gene="psbA"
misc_feature    27..>315
                 /note="contains psbA-trnH intergenic spacer and tRNA-His
                 (trnH)"

```

ORIGIN

```

1  tcgaagctcc atctacaaat ggataaaatt tggatcttaa agtatacgag ttttgaaag
61  taaaggagca atatcaactt tgttgatatt actcctttac ttttattagt agtctaaata
121 ttgataaata tttttacata catttttggg ttattttaag attcttttagc attttttatt
181 tcttatgtta tgtcgaaaaa gtatatatat ataaagtata tatataaatg gatgtctctc
241 tcttttttat cctatcctag aagaaaaaaa agaataataa tagaatagaa ggggtagaaa
301 ttacgtata gatca

```

ACCESSION LC494615

FEATURES Location/Qualifiers

source 1..300

/collected_by="Aekkhaluck Intharuksa"

/country="Thailand:Chiang Rai"

/isolate="BTT4"

/mol_type="genomic DNA"

/organelle="plastid:chloroplast"

/organism="Mucuna bracteata"

misc_feature <1..26

/gene="psbA"

misc_feature 27..>300

/note="contains psbA-trnH intergenic spacer and tRNA-His (trnH)"

ORIGIN

```

1 tcgaagctcc atctacaaat ggataaaatt tggatcttaa agtatacgag ttttgaaag
61 taaaggagca atatcaactt tgttgatatt actcctttac ttttattagt agtctaaata
121 ttgataaata tttgtacata catttttggc ttattttaag attccttagc attttttatt
181 tcttatgtta tgtcgaaaaa gtaaagtata tatataaatg gatgtctctc tctttttat
241 cctatcctag gataaaaaaa agaataataa tagaaagggt agaaattccg gtagatgca

```

ACCESSION LC494616

FEATURES Location/Qualifiers

source 1..306

/collected_by="Aekkhaluck Intharuksa"

/country="Thailand:Chiang Mai"

/isolate="MPT5"

/mol_type="genomic DNA"

/organelle="plastid:chloroplast"

/organism="Mucuna macrocarpa"

misc_feature <1..26

/gene="psbA"

misc_feature 27..>306

/note="contains psbA-trnH intergenic spacer and tRNA-His (trnH)"

ORIGIN

```

1 tcgaagctcc atctacaaaa ggaaaaaatt tggatcttta agtatacgag ttttgaagt
61 aaggagcaa tataaacttt gttgatattg ctcctttact ttattatta ttagtagtct
121 acaaatttta aaatatttat acatacattt ttgatttatt ttagattct ttagcatttt
181 ttatttctta tgttatgtcg aaataaaaaa gtatatatat aaatggatgt ctctctcttt
241 tttctttttt tcctatccta gaagaaaaaa aaggataatg aagggtcgaa attcaggtat
301 agatca

```

ACCESSION LC494617

FEATURES Location/Qualifiers

```

source          1..302
                 /collected_by="Aekkhaluck Intharuksa"
                 /country="Thailand:Chiang Mai"
                 /isolate="TCT6"
                 /mol_type="genomic DNA"
                 /organelle="plastid:chloroplast"
                 /organism="Mucuna thilandica"
misc_feature    <1..25
                 /gene="psbA"
misc_feature    26..>302
                 /note="contains psbA-trnH intergenic spacer and tRNA-His
                 (trnH)"

```

ORIGIN

```

1  tcgaagctcc actacaaatg gataaaatta ggatcttaaa gtatacgagt ttttgaaagt
61  aaggagcaa tatcaacttt gttgatattg ctcctttact tttagtagta gtctacaaat
121 ttaaaaatat ttatacatat atttttgatt tctttttaga ttcttttagca ttttttattt
181 cttatgttat gtcgaaataa aaaagtatat atataaatgg atgtctctct ctttttctt
241 tttttcctat cctagaagaa aaaaaaatga taatgaaagg gtagaaattc aggtatagat
301 ca

```

ACCESSION LC494618

FEATURES Location/Qualifiers

source 1..297

/collected_by="Aekkhaluck Intharuksa"

/country="Thailand:Tak"

/isolate="MMT7"

/mol_type="genomic DNA"

/organelle="plastid:chloroplast"

/organism="Mucuna monosperma"

misc_feature <1..26

/gene="psbA"

misc_feature 27..>297

/note="contains psbA-trnH intergenic spacer and tRNA-His (trnH)"

ORIGIN

```

1 tcgaagctcc atctacaaat ggataaaatt gggatcttac agtatacgac ttttgaaag
61 taaagtagca atatccactt tgttgatatt gctactttac ttttattagt agtcgaaaaa
121 tgaaaaaata ttaatacata catTTTTTat ttcagattct ttagcatttt tgatttctta
181 tgttatgtcg aaataaaaaa gtatatatat ggatgtatct ctcttttttc tttttattc
241 ctatcctaga agaataaaag aatgataatg aaagggtaga aattcaggta taaatcg

```

ACCESSION LC494619

FEATURES Location/Qualifiers

source 1..296

/collected_by="Aekkhaluck Intharuksa"

/country="Thailand:Mae Hong Son"

/isolate="ITT8"

/mol_type="genomic DNA"

/organelle="plastid:chloroplast"

/organism="Mucuna interrupta"

misc_feature <1..28

/gene="psbA"

misc_feature 29..>296

/note="contains psbA-trnH intergenic spacer and tRNA-His (trnH)"

ORIGIN

```

1  tcgacgcgcc tcctgcagac gggcctaacc gggatcttac agtatacgac ttttgaagt
61  caagtagcaa tatcaacaaa gtggatattg ctacttgact tttagtagta gtctccaaat
121  tattaaatac taatacatac attttttatt taagattctt tagcatTTTT gatttcttat
181  gttatgtcga aataaaaaag tatatatatg gatgtatctc tctttttct tttttattcc
241  tatcctagaa gaataaaaga atgatactga aagggtagaa attcaggtat aaatca

```

ACCESSION LC494620

FEATURES Location/Qualifiers

source 1..297

/collected_by="Aekkhaluck Intharuksa"

/country="Thailand:Lampang"

/isolate="HST9"

/mol_type="genomic DNA"

/organelle="plastid:chloroplast"

/organism="Mucuna hainanensis"

misc_feature <1..26

/gene="psbA"

misc_feature 27..>297

/note="contains psbA-trnH intergenic spacer and tRNA-His (trnH)"

ORIGIN

```

1 tcgaagctcc atctacaaat ggataaaatt gggatcttac agtatacgac ttttgaaag
61 taaagtagca atatcaacaa agtggatatt gctactttac ttttattagt agtctccaaa
121 ttattaaata ttaatacata cattttttat ttaagattct ttagcatttt tgatttctta
181 tgttatgtcg aaataaaaaa gtatatatat ggatgtatct ctcttttttc tttttattc
241 ctatcctaga agaataaaag aatgataatg aaagggtaga aattcaggta taaatca

```

ACCESSION LC494621

FEATURES Location/Qualifiers

source 1..297

/collected_by="Aekkhaluck Intharuksa"

/country="Thailand:Chiang Mai"

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/organelle="plastid:chloroplast"

/organism="Mucuna warburgii"

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/gene="psbA"

misc_feature 27..>297

/note="contains psbA-trnH intergenic spacer and tRNA-His (trnH)"

ORIGIN

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61 taaagtagca atatcaacaa agtggatatt gctactttac ttttattagt agtctaaaaa
121 tgaaaaaata ttaatacata catTTTTTat ttaagattct ttagcatttt ggatttctta
181 tgttatgtcg aaataaaaaa gtatatatat ggatctatct ctcttttttc tttttattc
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ACCESSION LC494622

FEATURES Location/Qualifiers

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/organelle="plastid:chloroplast"

/organism="Mucuna gigantea"

misc_feature <1..26

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misc_feature 27..>297

/note="contains psbA-trnH intergenic spacer and tRNA-His (trnH)"

ORIGIN

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121 tgaaaaaata ttaatacata catTTTTTat ttaagattct ttagcatttt tgatttctta
181 tgttatgtcg aaataaaaaa gtatatatat ggatgtatct ctcttttttc tttttattc
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ACCESSION LC495170

FEATURES Location/Qualifiers

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 /organism="Mucuna revoluta"

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 LNL SVVRSQMLQNSFLIQIVMKKLDTIVPIIPLIRSLAKEKFCNGLGHPISKPVWANL
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ORIGIN

1 gttttttat tactattgta attggaatag tctttttact ccaaaaaaat cgatttctac
 61 tttttttca aaaagtaatc caagattatt ctgttccta tataatttat atgtatggga
 121 atacgaatct atctttcttt ttcttcgtaa caaatcctct cagttacgat tcaaatattt
 181 tcgcgttttt ttgaacgaa tttttttcta tgaaaaata gaacatcttg tagaaatatt
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 301 atatcgagga aaatcgattc tggtttcaaa gaatacgctt cttttgataa ataaatggaa
 361 atactatttt atctatatat ggcaatgtca ttttaatttt tggctcaac caggaacgat
 421 tgatataaag caattatctc agcattcatt tcacctttg ggtatttttt taagtattcg
 481 gctaaatctt tcagtggtag gaagtcaaat gttgcaaat tcatttctaa ttcaaattgt
 541 tatgaaaaag cttgatacaa tagttccaat tttccttta attagatcat tggctaaaga
 601 aaaattttgt aatggattgg gtcacccat tagtaagccg gtttgggcca atttatctga
 661 ttttgatatt atttaccgat tttgcgaat atgcagaaat tttgctcatt attacaaagg
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ACCESSION LC495171

FEATURES Location/Qualifiers

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 /organism="Mucuna revoluta"

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ORIGIN

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121 ttctgaaca ccgaccccg cttcctcgg gttggcggga ggtggtggcc tcgtgctgcc
181 ccgtcccgtc gaacaaaaac cccggcgctt cgtgcgcaa ggaattgaa attgttgggt
241 gcaattttcg cggacccgga gacggtgatc tcgcggacct tgccacgaca cacgatacaa
301 aatgactctc ggcaacggat atctcggctc ttgcatgat gaagaacgta gcgaaatgcg
361 atacttggtg tgaattgcag aatcccgta accatcgagt cttgaacgc aagttgcgcc
421 cgaagccatt aggttgaggg cagcctgcc tgggtgtcac acatcgttac ctaaagcaa
481 acgcctcatg cgcgtgtgca ggtggaagc tgacctccg tgggccacga ctgcggctg
541 gttgaaaatg gattcatgg ttgagaatgc cgtgataaaa tggatgatga gtgttgcctg
601 agaccaatcg cgtgcgactc ggtcaatgtt ggactcatcg acccaaatcg cgtccacgga
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ACCESSION LC495172

FEATURES Location/Qualifiers

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/country="Thailand:Tak"

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/mol_type="genomic DNA"

/organelle="plastid:chloroplast"

/organism="Mucuna revoluta"

misc_feature <1..27

/gene="psbA"

misc_feature 28..>298

/note="contains psbA-trnH intergenic spacer and tRNA-His (trnH)"

ORIGIN

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121 attattaaat attaatacat acatttttta ttttaagattc ttttcatth ttgattctt
181 atgttatgtc gaaataaaaa agtatatata tggatgtatc tctctttttt cttttttatt
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ACCESSION LC495173

FEATURES Location/Qualifiers

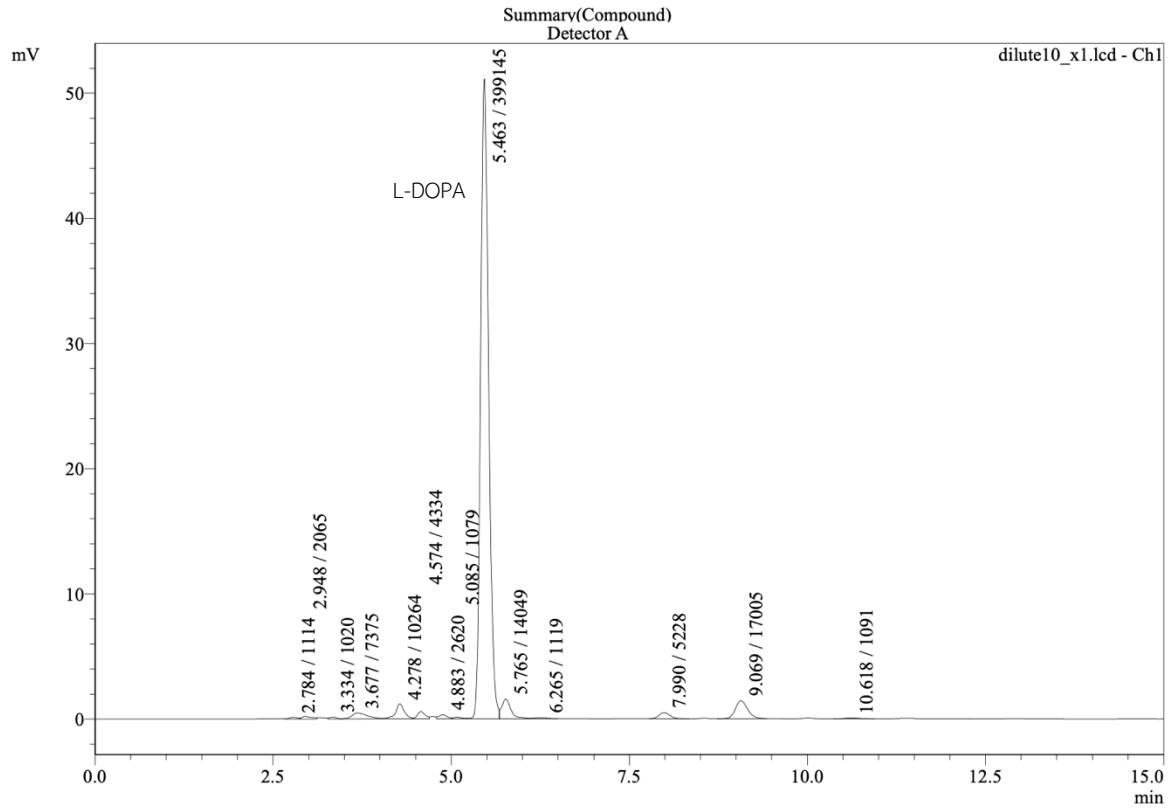
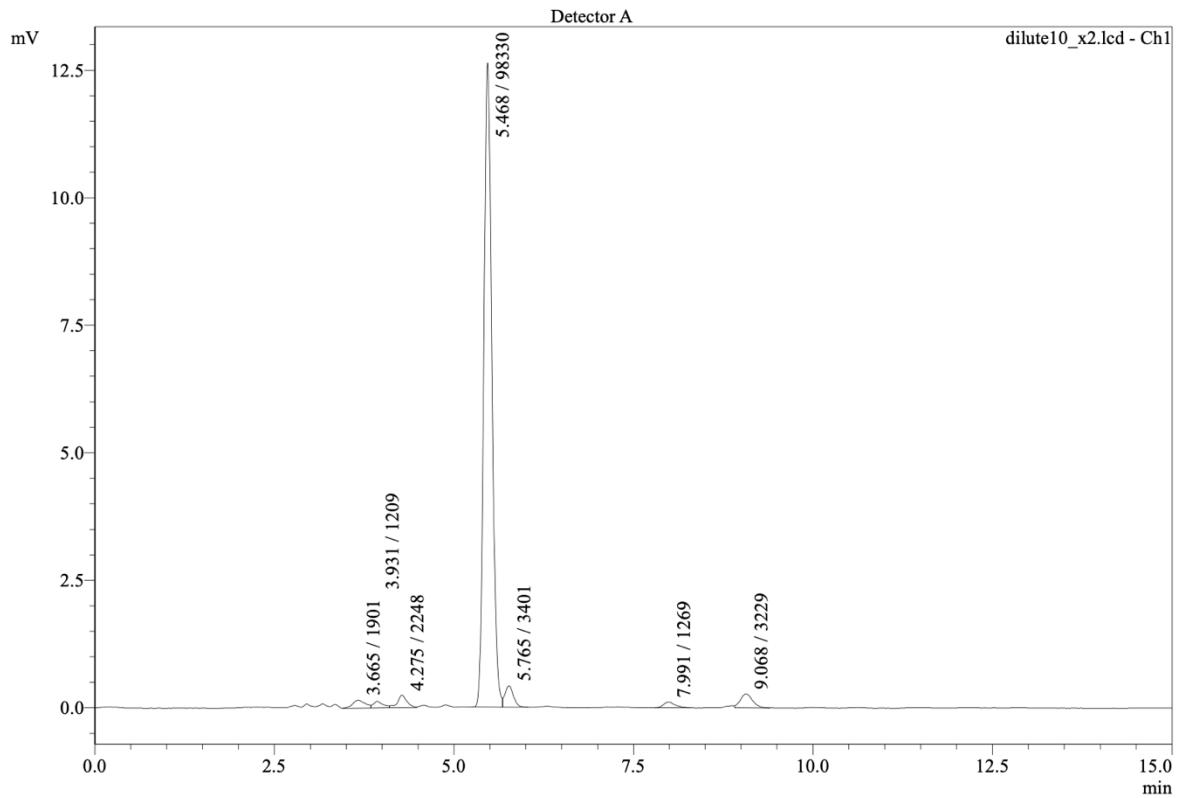
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 /isolate="Rev"
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 /organelle="plastid:chloroplast"
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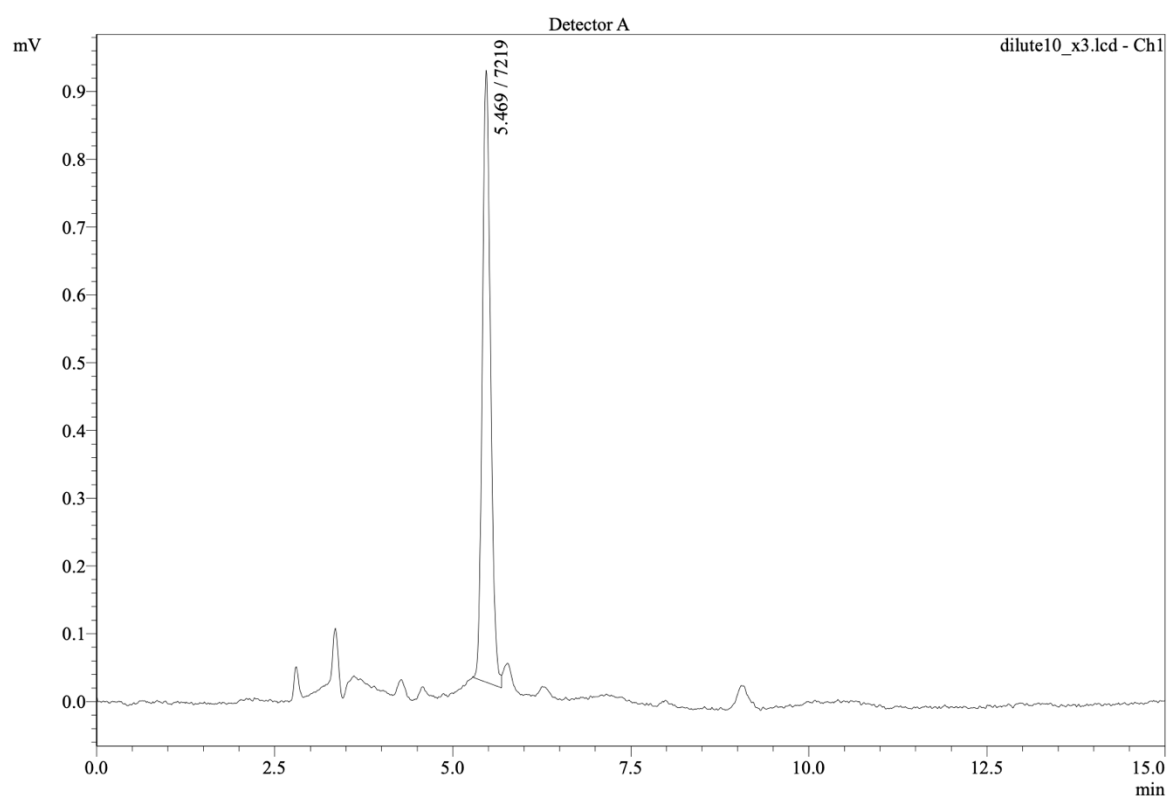
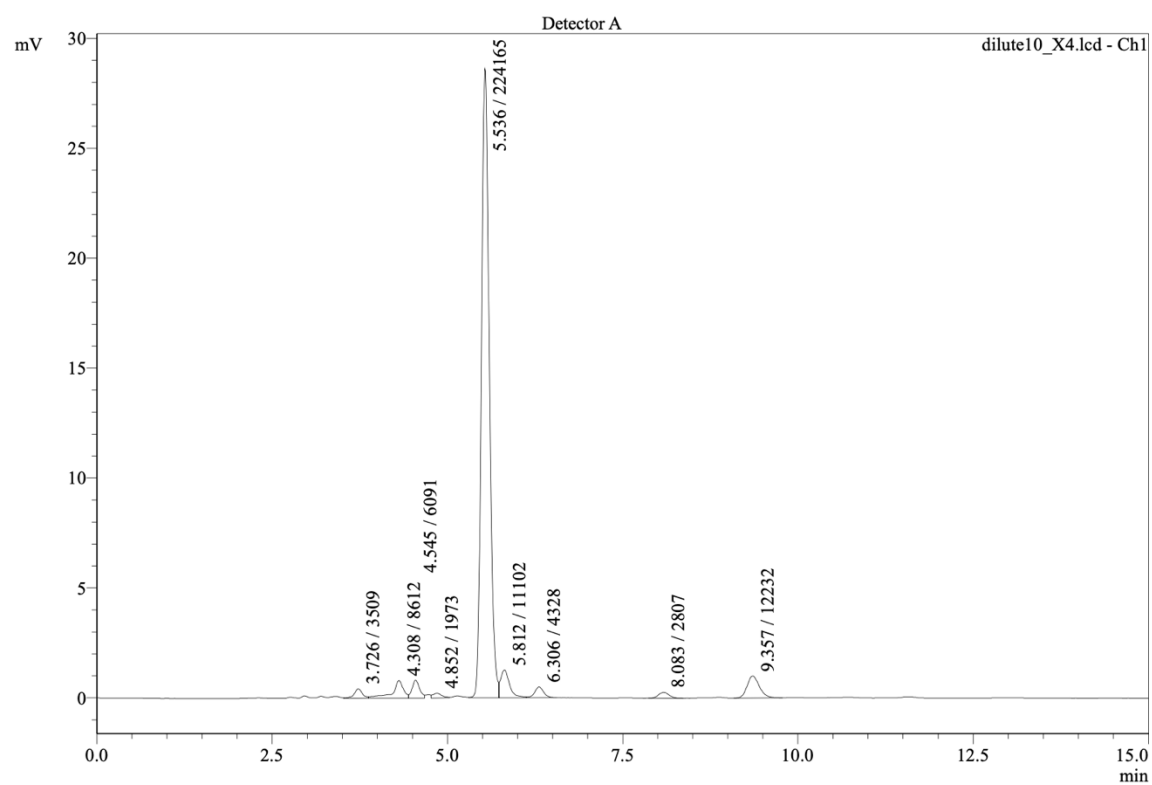
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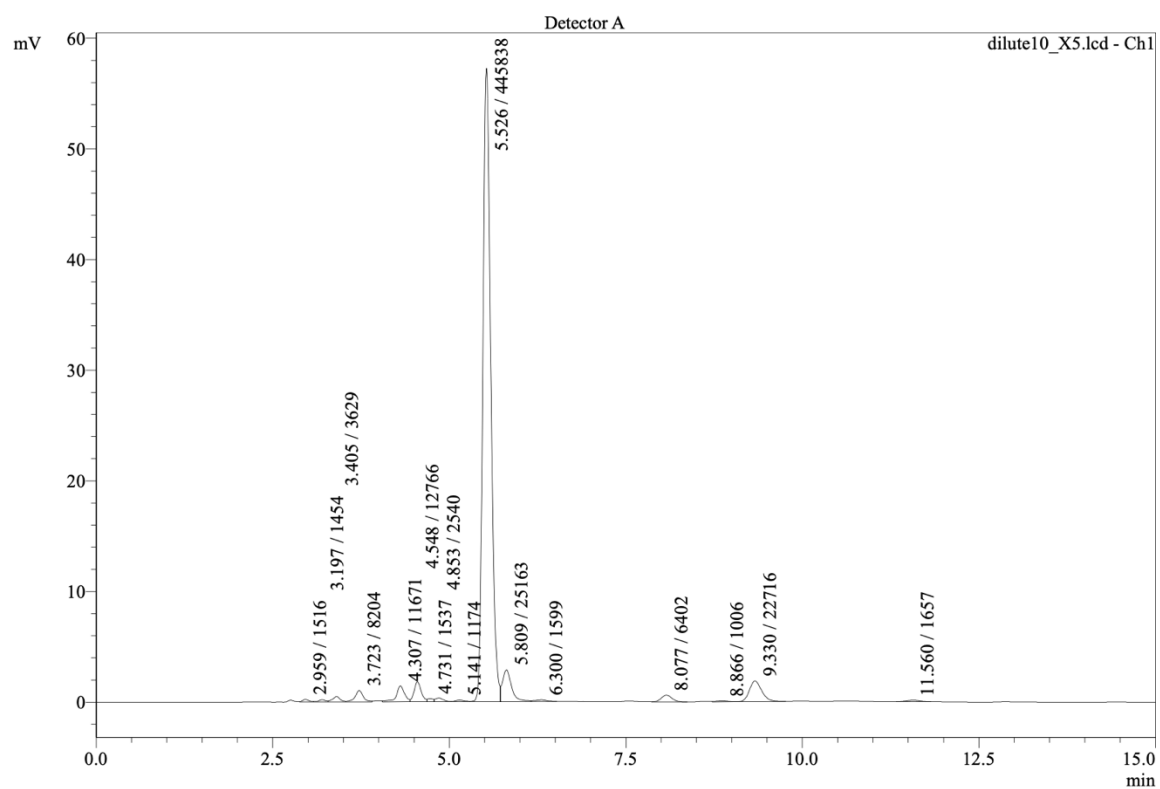
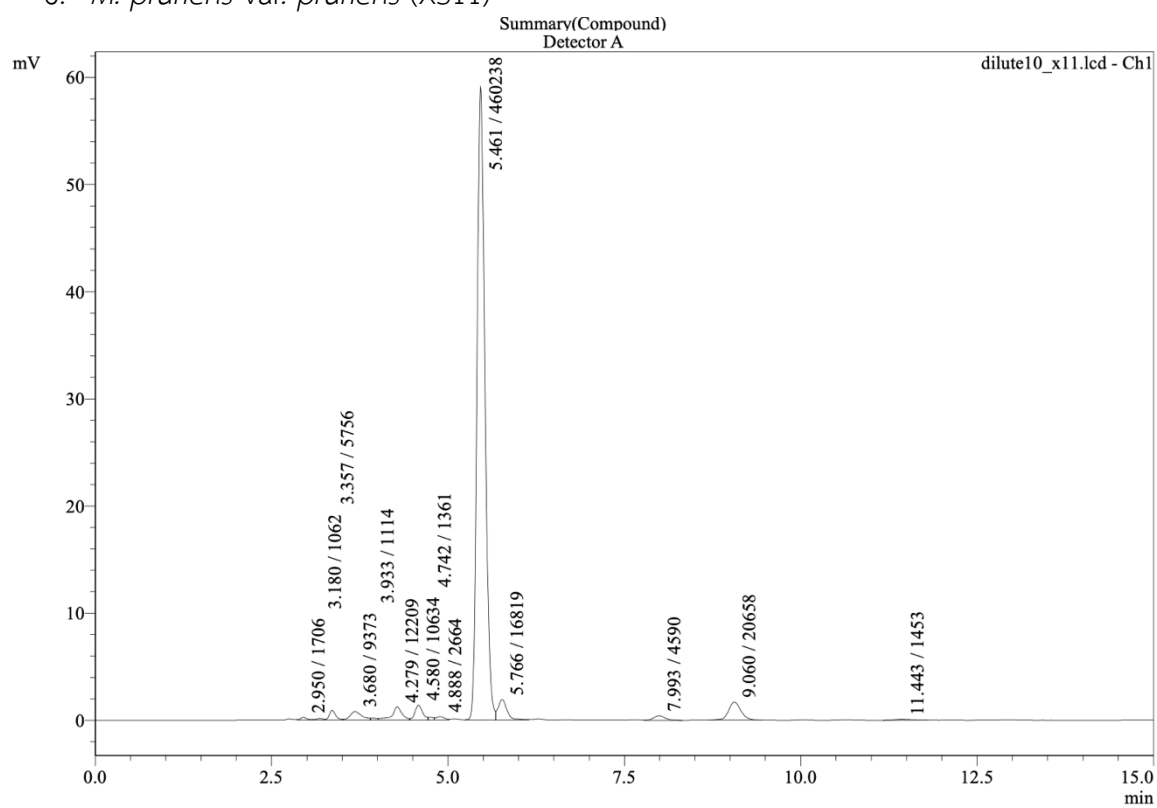
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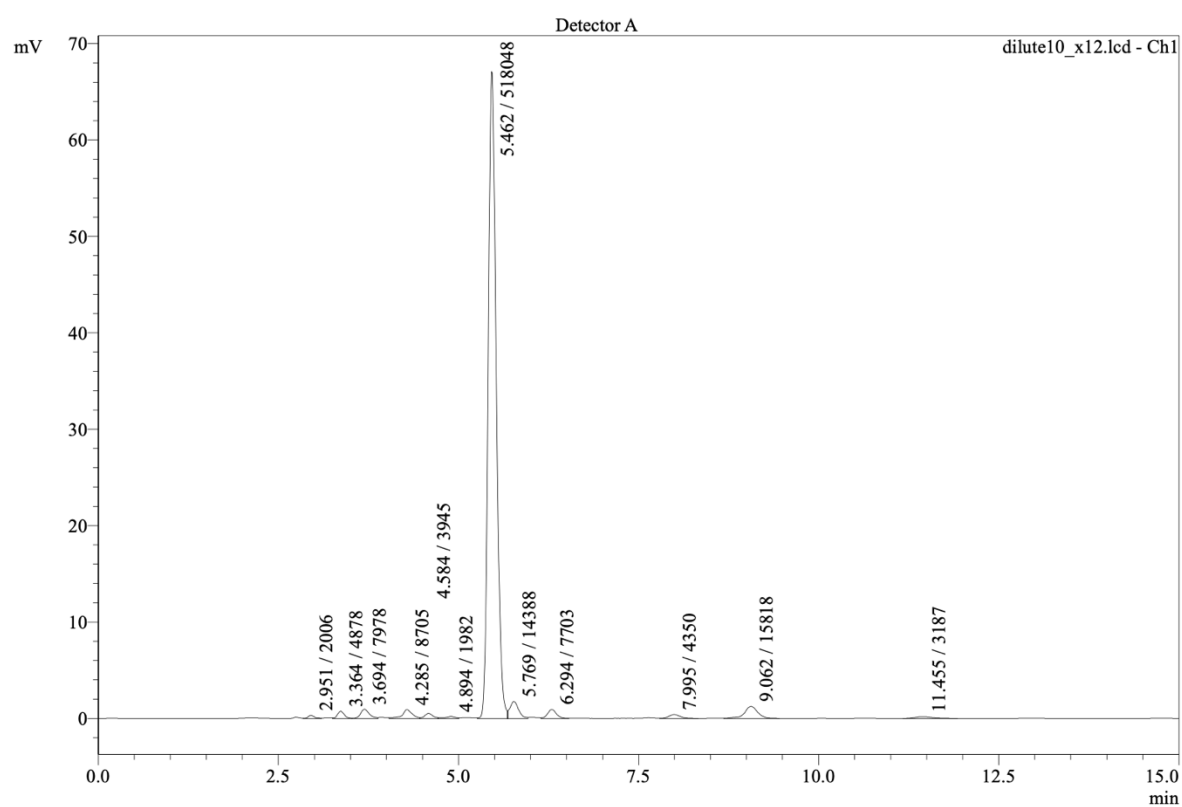
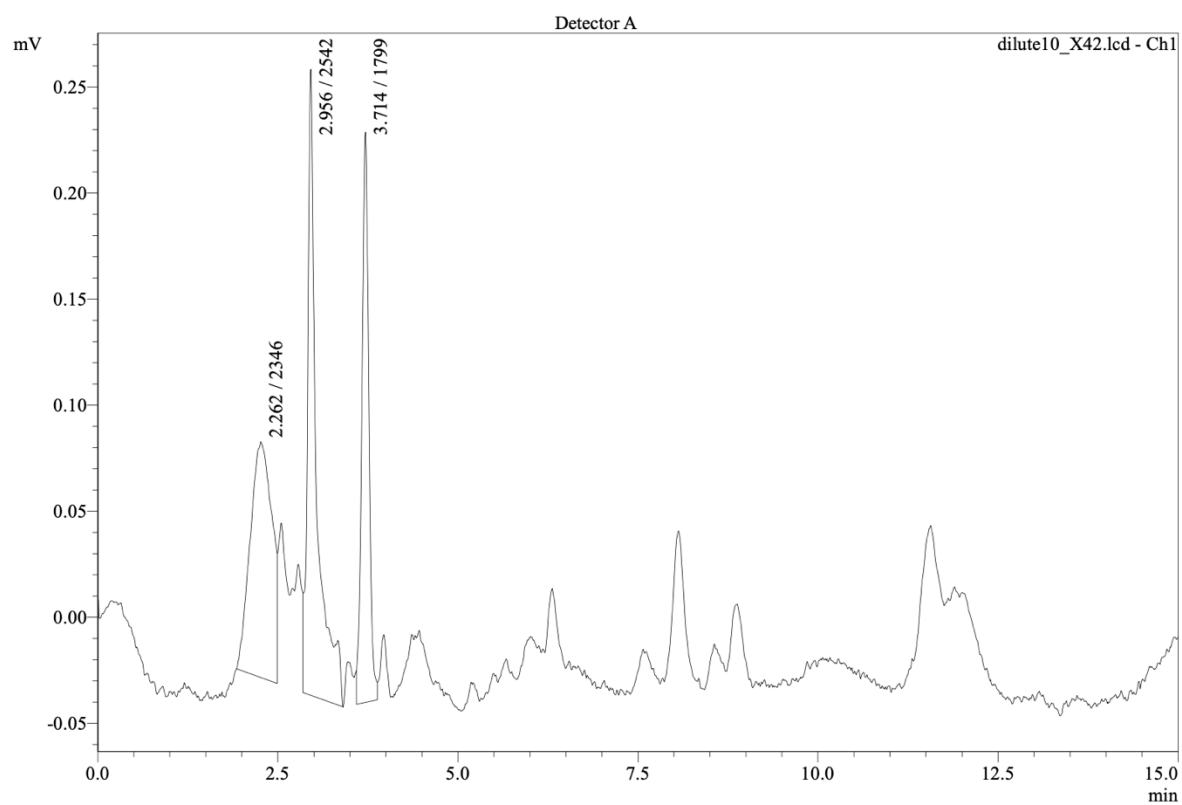
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 121 caaccggag ttccgcctga agaagcaggt gctgccgtag ctgccgaatc ttctactggt
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 541 tacggtagag cagtttatga atgtcttcgc gggggacttg attttaccaa agatgatgaa
 601 aatgtgaatt cccaaccttt tatgcgttgg agagaccgtt tcttattttg tgccgaagct
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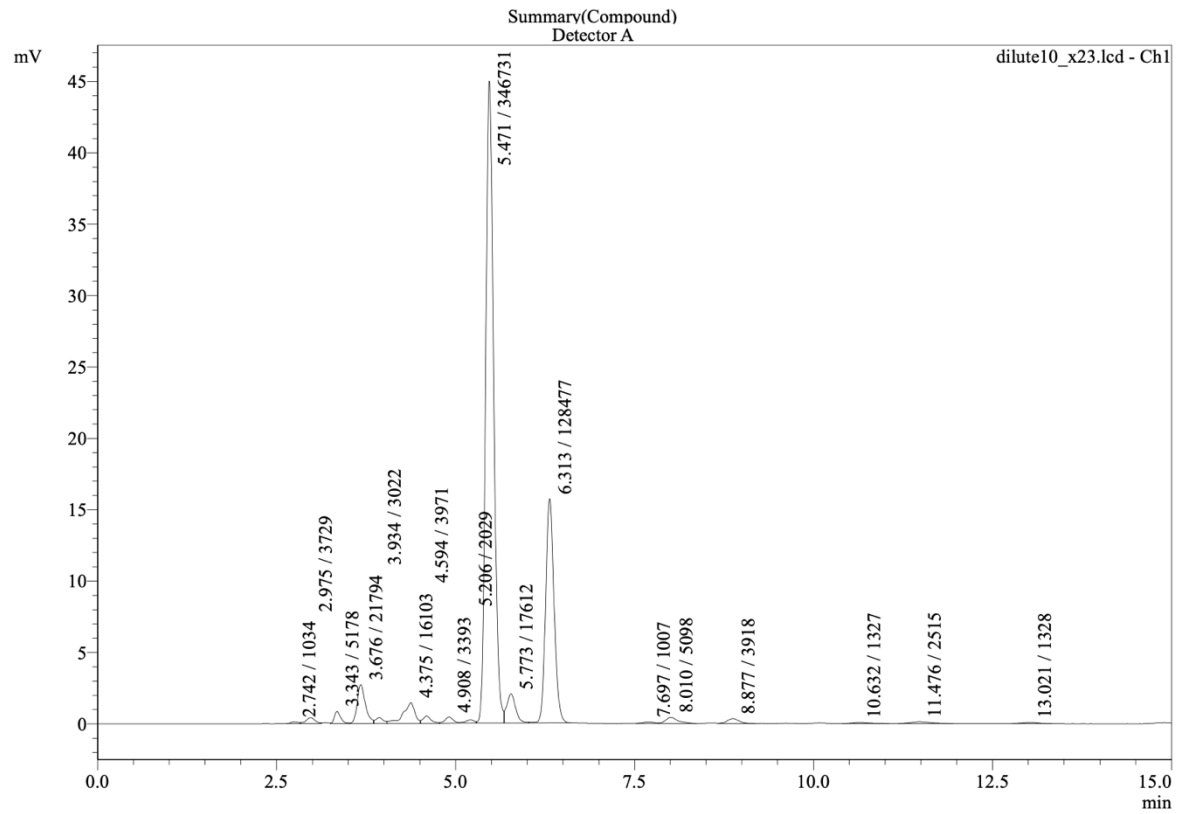
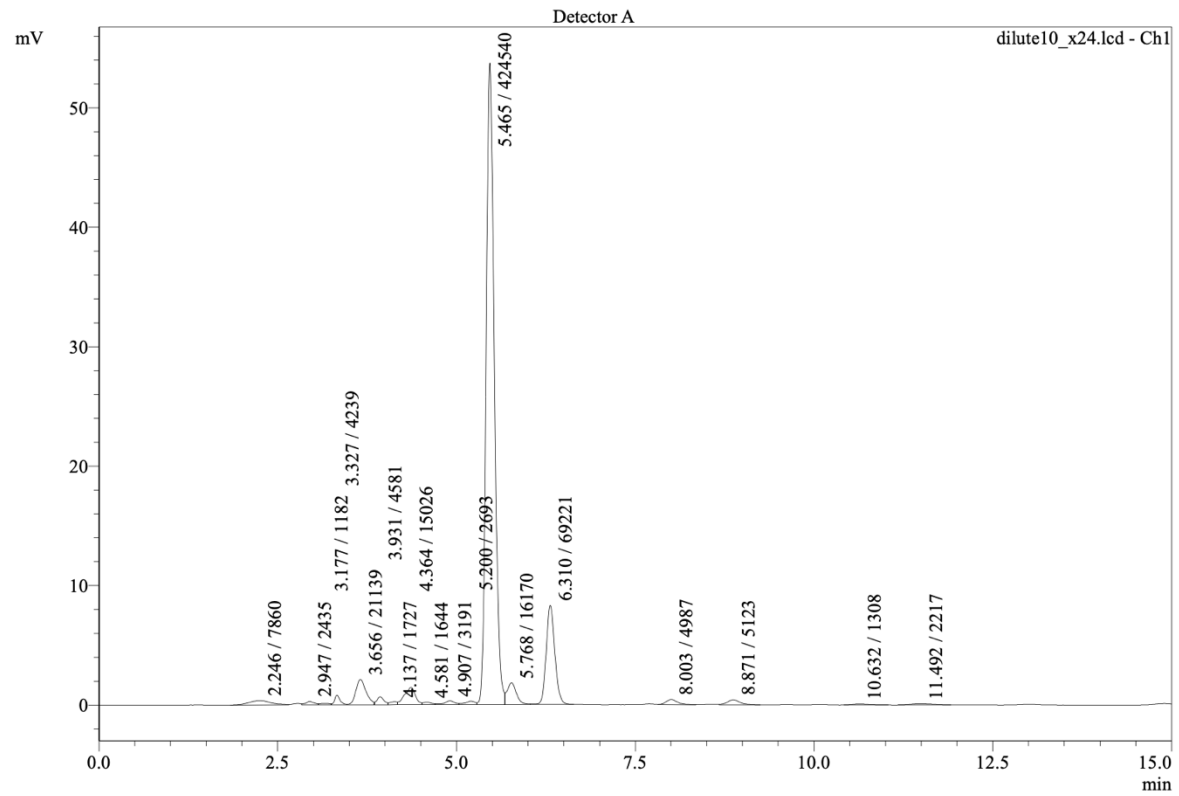
HPLC chromatogram

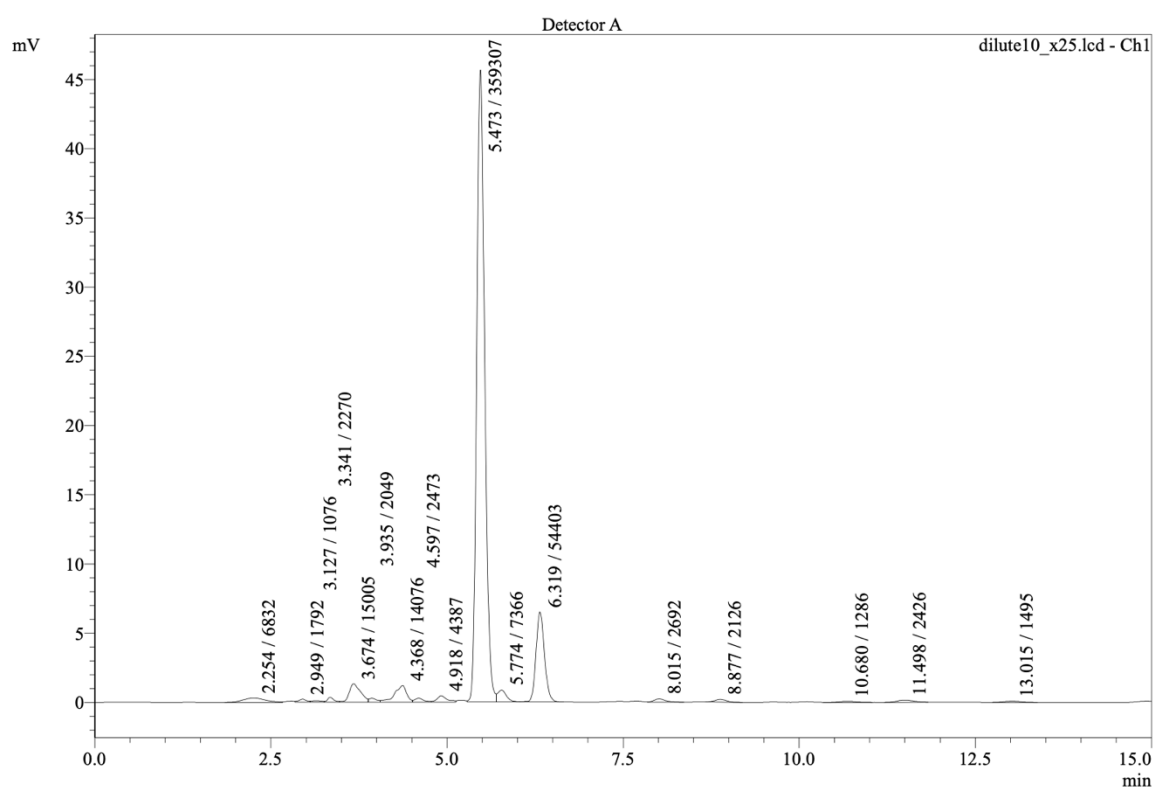
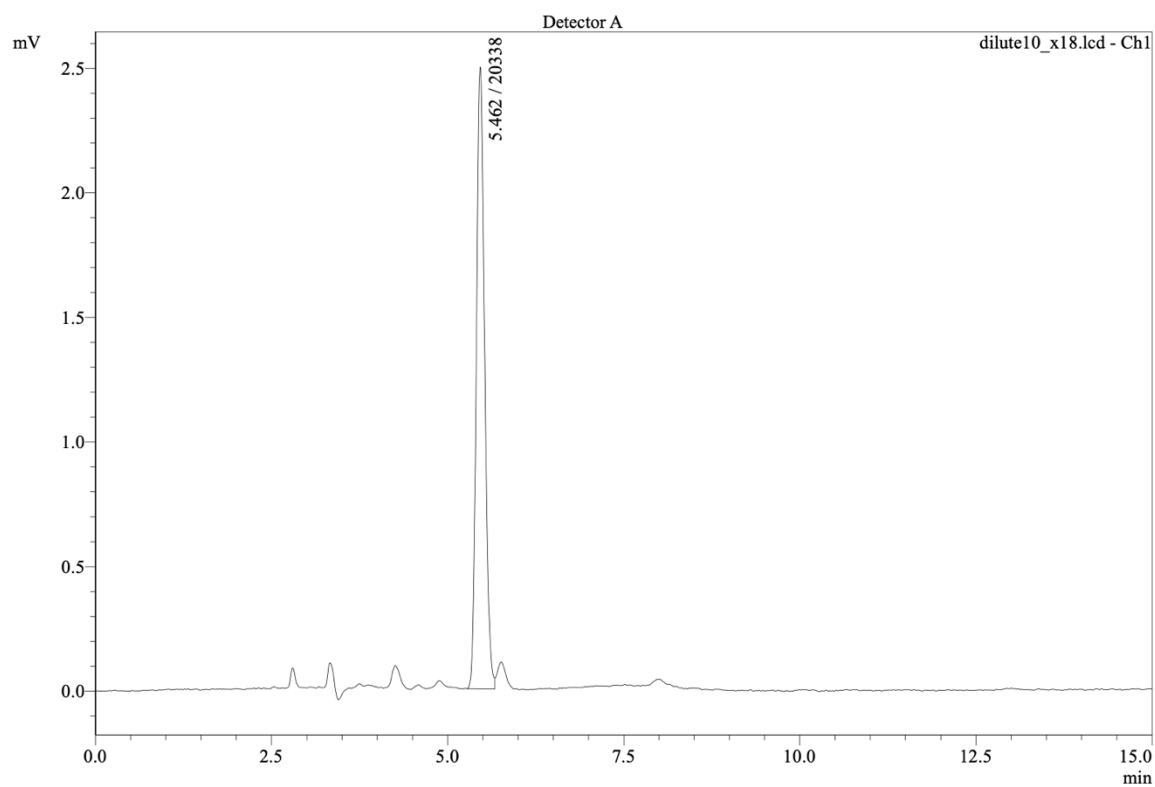
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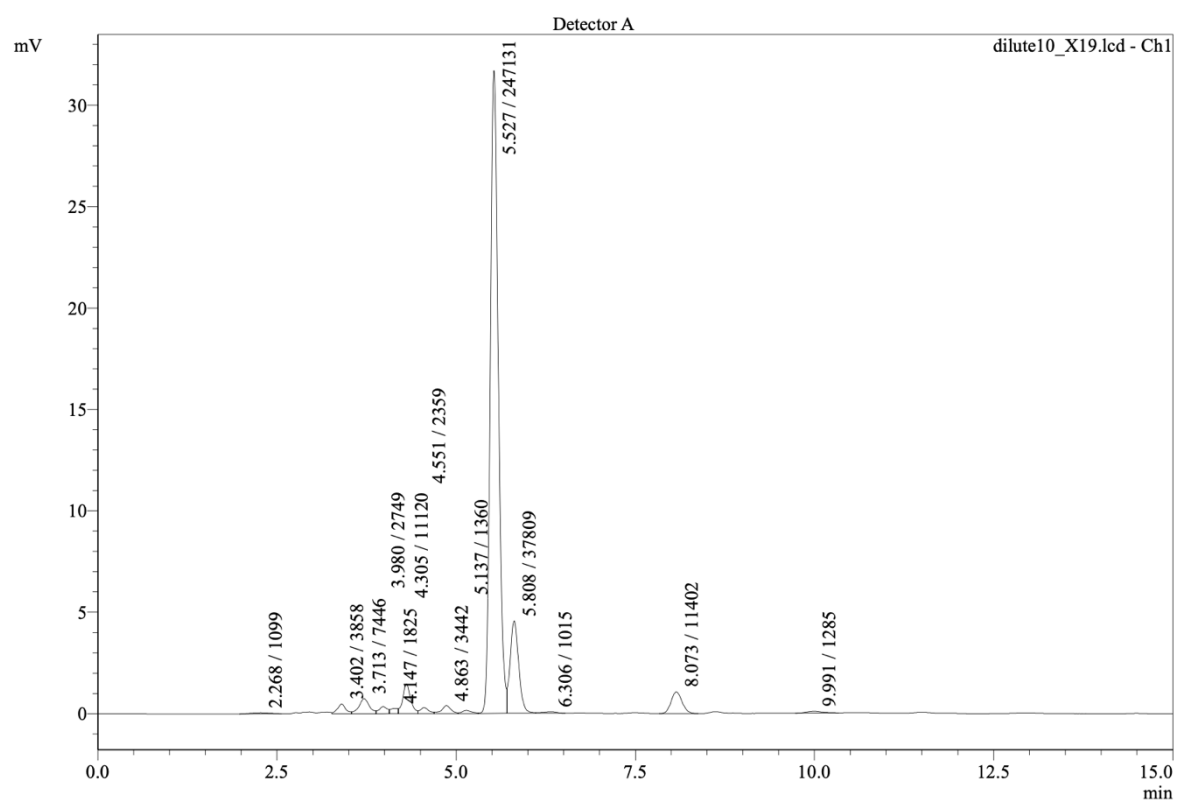
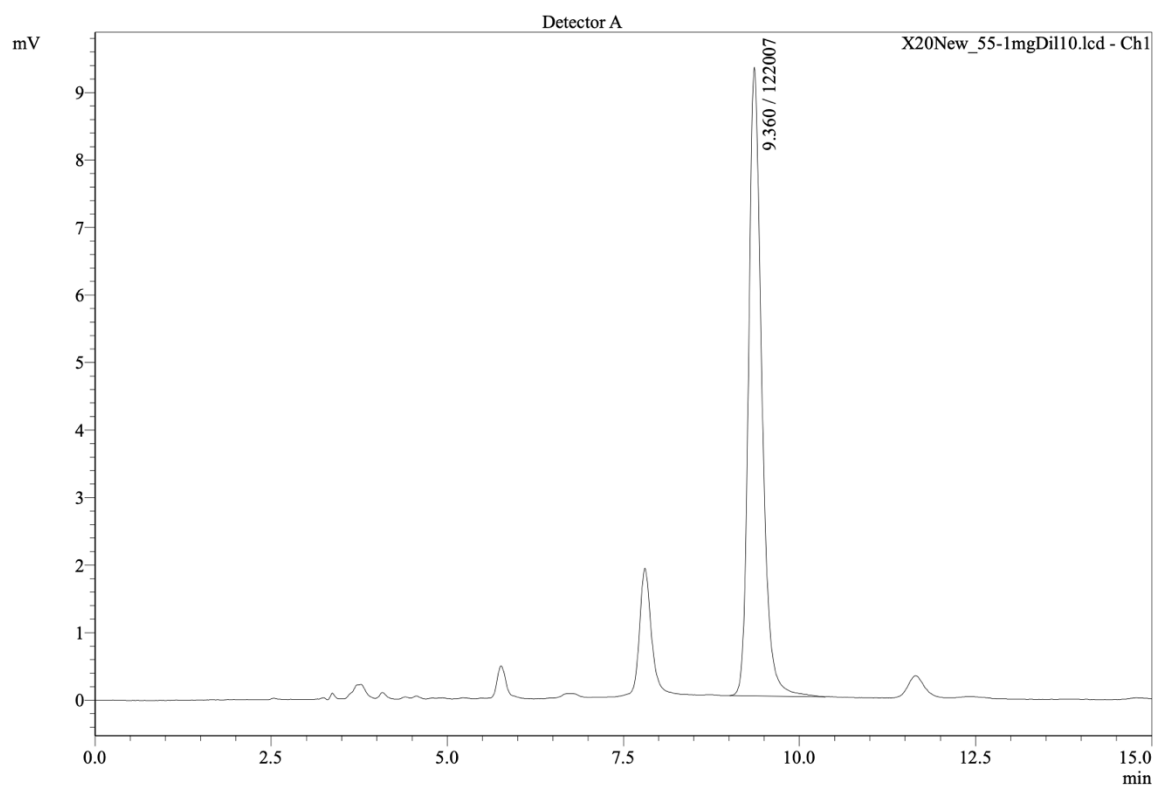
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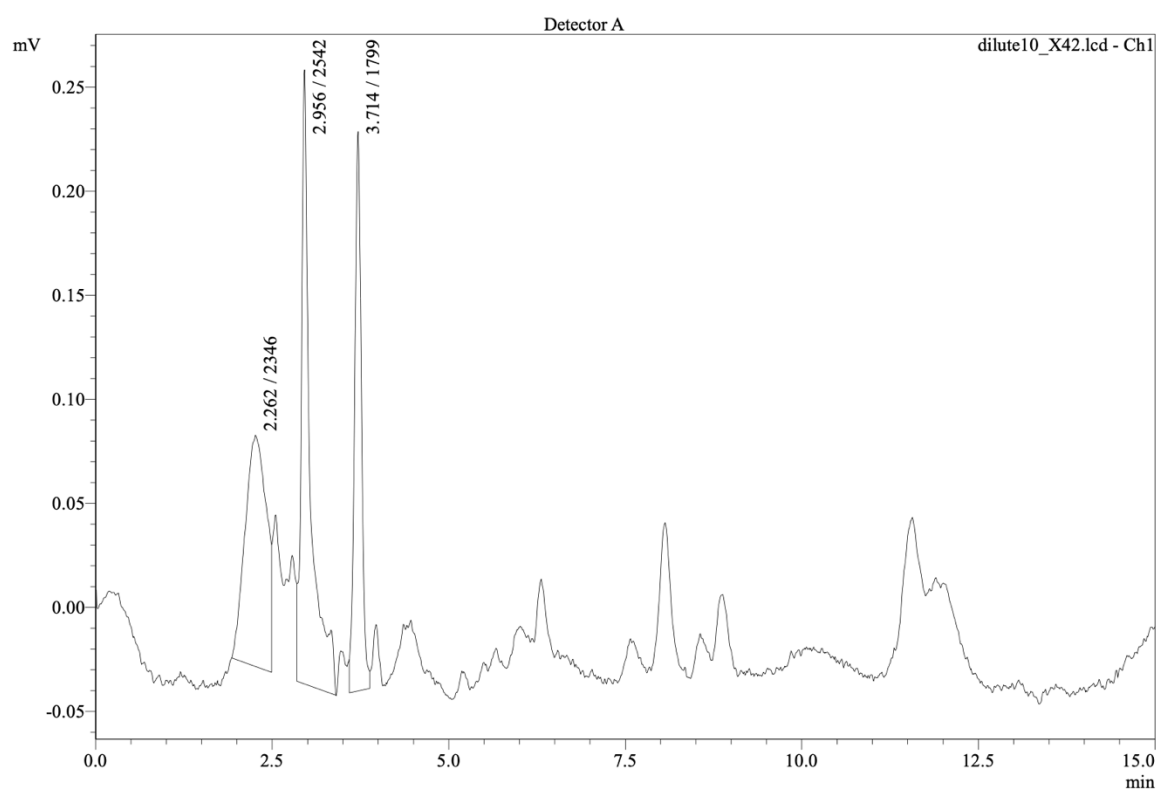
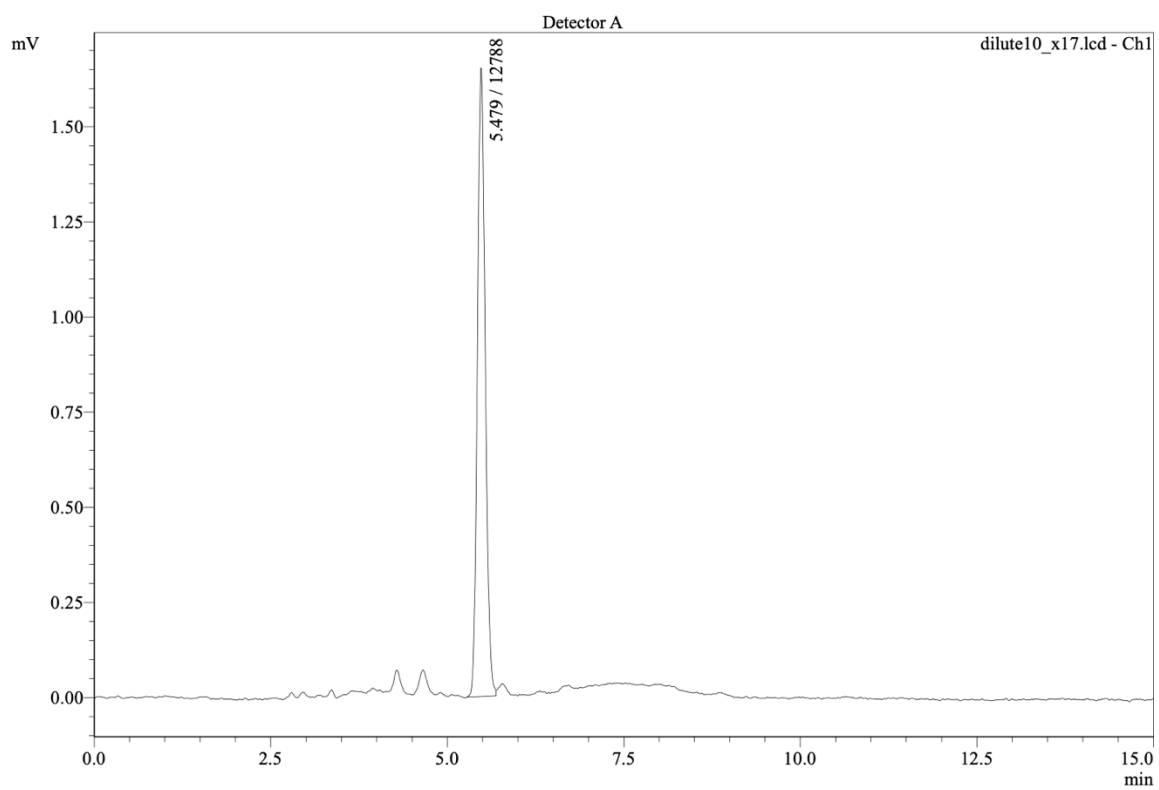
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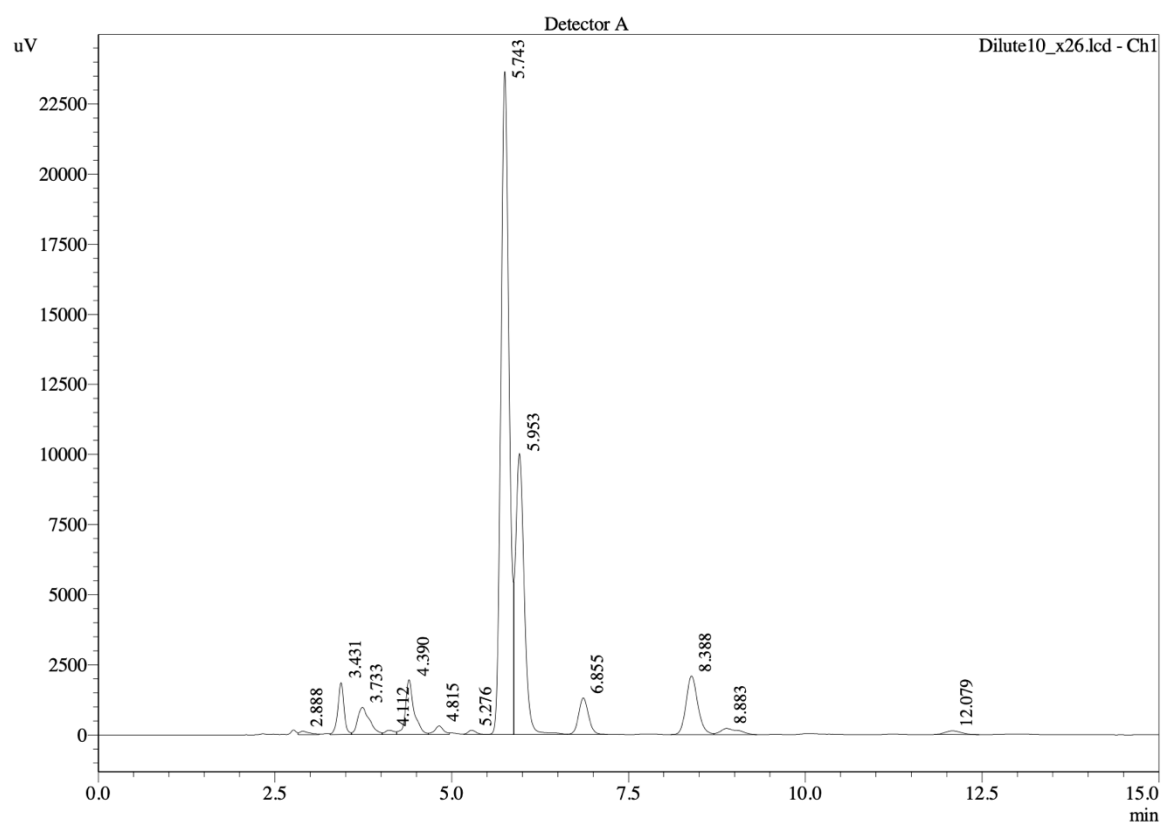
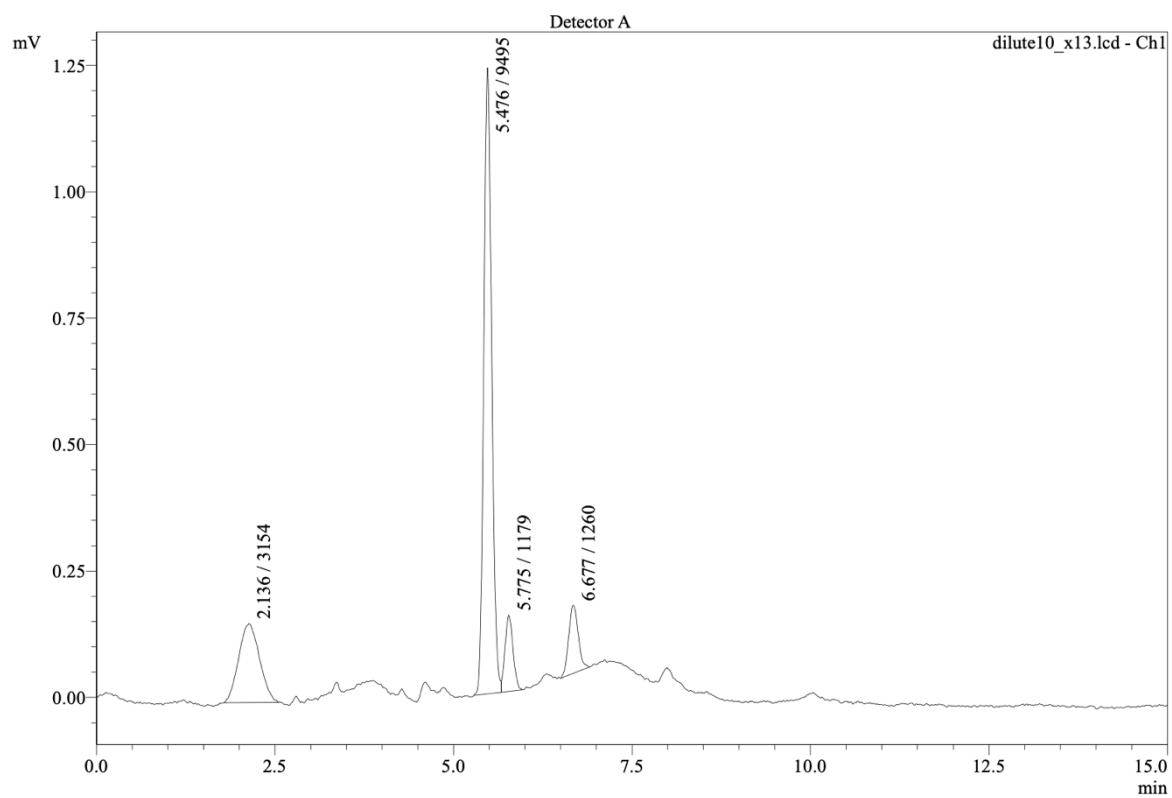
7. *M. pruriens* var. *pruriens* (XS12)8. *M. pruriens* var. *utilis* (XS43)

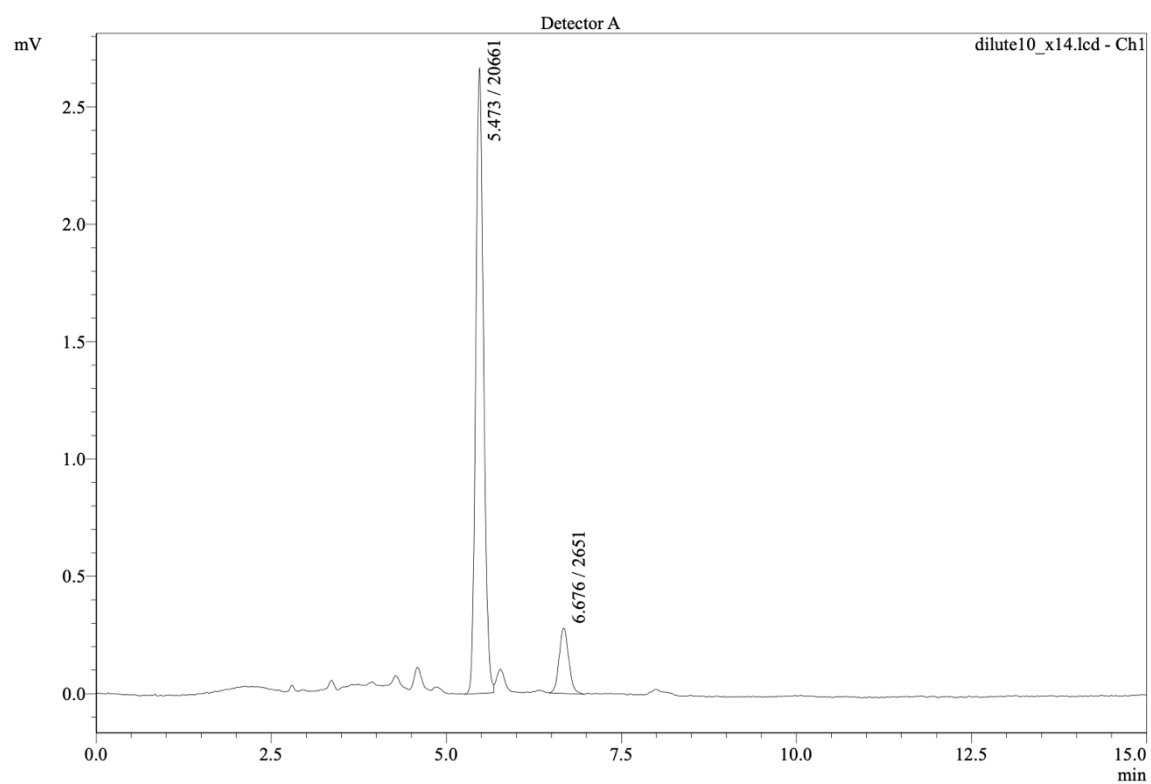
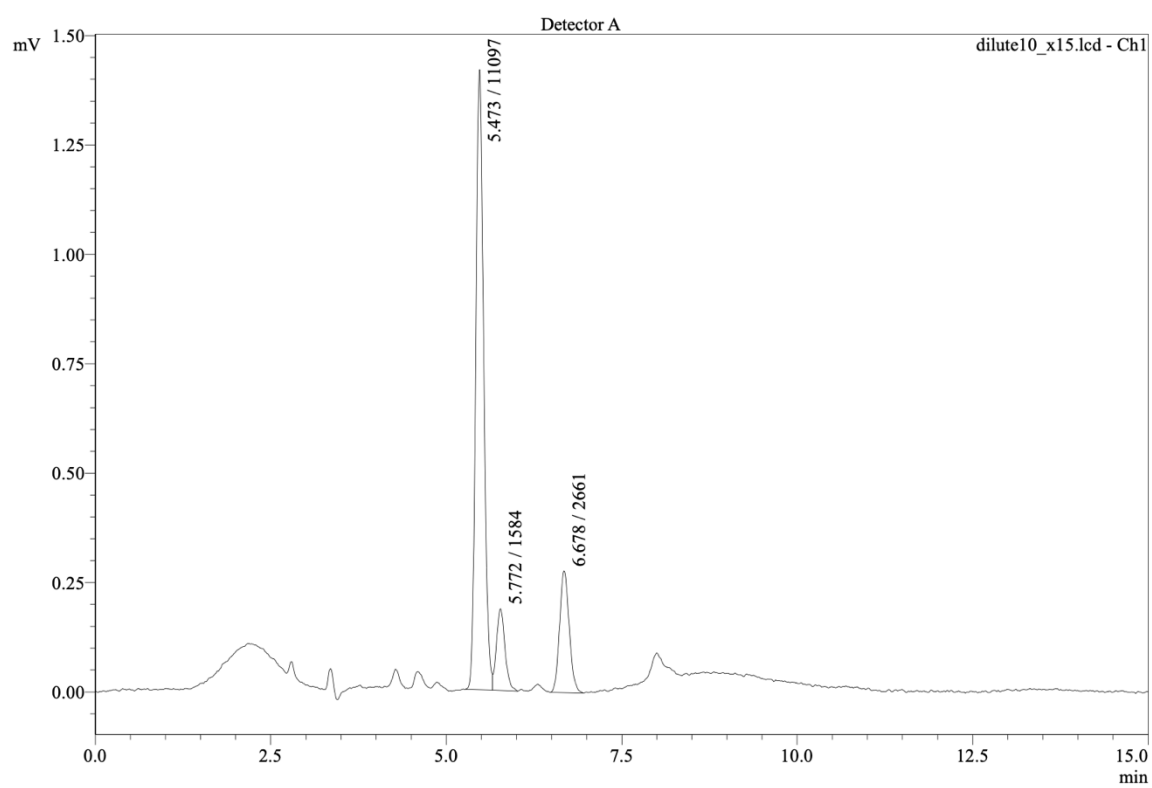
9. *M. bracteata* (XS23)10. *M. bracteata* (XS24)

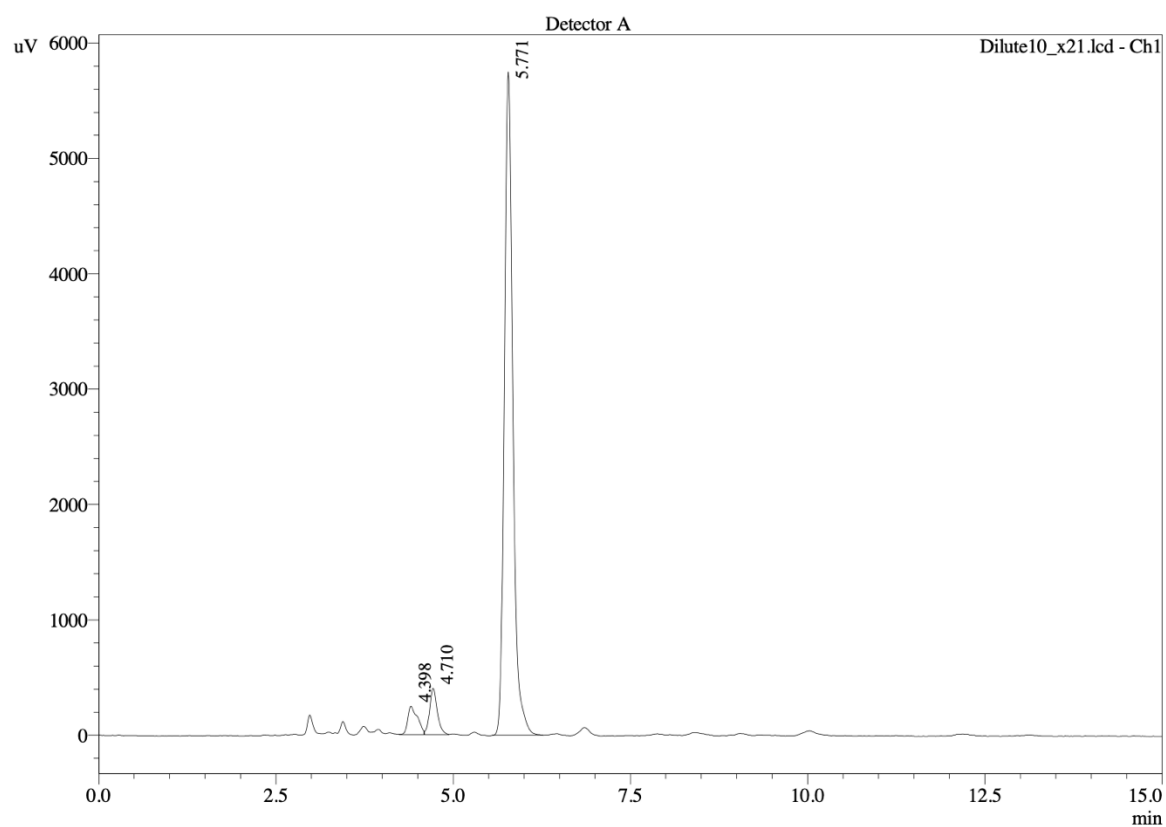
11. *M. bracteata* (XS25)12. *M. gigantea* (XS18)

13. *M. gigantea* (XS19)14. *M. gigantea* (XS20)

15. *M. hainanensis* (XS42)16. *M. interrupta* (XS17)

17. *M. macrocarpa* (XS26)18. *M. monosperma* (XS13)

19. *M. monosperma* (XS14)20. *M. monosperma* (XS15)

21. *M. revoluta* (XS21)22. *M. thailandica* (XS27)