

## ศ.ดร.ภญ.จุไรทิพย์ หวังสินทวีกุล

สาขาวิชาเภสัชเวทและเภสัชพฤกษาศาสตร์  
คณะเภสัชศาสตร์  
มหาวิทยาลัยสงขลานครินทร์

Chromatography Exclusive Webinar Ep.2

# KRATOM COMPOUND ANALYSIS

and the importance of reference material

29<sup>th</sup> October 2021 | 1:00 P.M. - 3:00 P.M. | Zoom Platform

### การบรรยายในหัวข้อ

- การเลือกใช้สารมาตรฐานสำหรับงานการวิเคราะห์สารสำคัญในกระต่อม
- การเตรียมตัวอย่าง การสกัดสารสำคัญในพืชกระต่อม และการวิเคราะห์สารสำคัญในพืชกระต่อม



### Speaker

Assoc. Prof. Juraithip Wungsintaweekul  
Faculty of Pharmaceutical Sciences,  
Prince of Songkla University



Scan to  
Register



# พฤกษเคมีของกระท่อม และ วิธีวิเคราะห์

- สายพันธุ์ของกระท่อม
- ลักษณะทาง地貌 และ ลักษณะทางจุลภาค
- สารเคมีที่พบ (primary & secondary metabolites)
- การตรวจกระท่อมด้วยวิธีทางเคมี
  - Thin Layer Chromatography
  - High Performance Liquid Chromatography



สุพัตรา ลิ่มสุวรรณ์วนิชต์ และ จุไรกิพย์ วงศ์สินทวีกุล (2562) การตรวจและการวิเคราะห์สารสำคัญในพืชกระท่อม ในเอกสารวิชาการ พืชกระท่อม: การใช้ประโยชน์จากการค้นคว้าเรื่องนี้และงานวิจัยเกี่ยวกับพืชกระท่อม เพื่อกำหนดนโยบายการแก้ไขปัญหาพืชกระท่อมของประเทศไทย. สถาบันสำรวจและติดตามการปลูกพืชเพื่อการส่งออก สำนักงาน ป.ป.ส.



Pharmaceutical  
Sciences

JW Kratom Rx

29 October 2021

## ***Mitragyna* spp. distribute in India, S.E.Asia and Africa**

- 7 Species found in Asia

*Mitragyna speciosa* (Korth.) Havil.  
*M. tubulosa* (Arn.) Havil.  
*M. parvifolia* (Roxb.) Korth.  
*M. hirsuta* Havil.  
*M. diversifolia* (Wall ex G.Don) Havil.  
*M. rotundifolia* (Roxb.) O. Kuntz.  
*M. javanica* Koord. & Valeton.

- 3 Species found in West Africa

*M. inermis* (Willd.) O. Kuntze  
*M. ciliata* Aubrev. and Pellegr.  
*M. stipulosa* (D.C.) O. Kuntze

- 1 species found in East Africa

*M. rubrostipulata* Havil.



*Mitragyna  
inermis*

Source:

[https://de.wikipedia.org/wiki/  
Mitragyna\\_inermis](https://de.wikipedia.org/wiki/Mitragyna_inermis)



# What Are The Different Types of Kratom?

## What is a strain?

Whereas a species is a genetically unique plant with its own species name, a strain refers to the offspring of a plant **that shares the same genetic makeup but differences in physical or chemical profiles**. All kratom is the same species (*Mitragyna speciosa*), but there are many different *strains* within that species — such as Red Kali, Green Hulu, White Dragon, and many more.

There are three main categories for the different kratom strains based on the **color of the leaf** veins — red, white, and green.

While there's a lot of variability within each category, it serves as a fairly reliable way of cataloging the different effects we can expect from each leaf type.



MAENG DA

BORNEO

VIETNAM

MALAY

RED THAI

RED BALI

INDO

15X EXTRACT



MORE STIMULATING



MORE RELAXING



Red Bali  
Kratom

Stimulating:



Mood Enhancing:



Pain Relieving:



[VIEW PRICES →](#)



Maeng Da  
Kratom

Stimulating:



Mood Enhancing:



Pain Relieving:



[VIEW PRICES →](#)



Red Thai  
Kratom

Stimulating:



Mood Enhancing:



Pain Relieving:



[VIEW PRICES →](#)



Green Malay  
Kratom

Stimulating:



Mood Enhancing:



Pain Relieving:



Ultra E. Indo  
Kratom

Stimulating:



Mood Enhancing:



Pain Relieving:



15X Kratom  
Extract

Stimulating:



Mood Enhancing:



Pain Relieving:



[VIEW PRICES →](#)



Maeng Da Kratom

[VIEW PRICES →](#)



Red Thai Kratom

[VIEW PRICES →](#)



Red Bali Kratom

[VIEW PRICES →](#)



Green Malay Kratom

[VIEW PRICES →](#)



Green Borneo Kratom

[VIEW PRICES →](#)



White Borneo Kratom

[VIEW PRICES →](#)



Premium Indo Kratom

[VIEW PRICES →](#)



Ultra Enhanced Indo

[VIEW PRICES →](#)

KRATOM

SHOP NOW



# Kratom Strains: Red, Green & White Vein Kratom

There are many different types of [kratom](#) — which are referred to as *strains*. Each strain has a different set of effects — some are powerful sedatives and painkillers; others are better for boosting mood or energy.

# Kratom Strains Chart

Source: <http://kratom.org>

Name	Energy	Mood	Pain Relief	Sedation	Anxiety-Relief
Red Thai Kratom	4	2	6	10	8
Green Sumatra Kratom	6	6	5	4	6
Green Thai Kratom	7	6	5	6	5
White Sumatra Kratom	9	9	4	1	6
White Thai Kratom	4	6	4	3	6





Narumon Sengnon

# Kratom *Mitragyna speciosa* (Korth.) Havil.

**Rubiaceae**



Red-veined kratom



Green-veined kratom

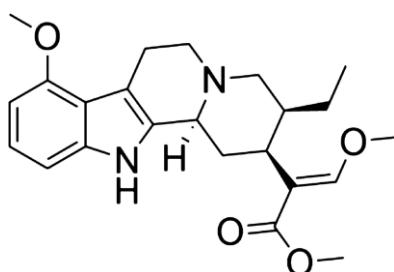


Green-veined kratom:: Serrate apex

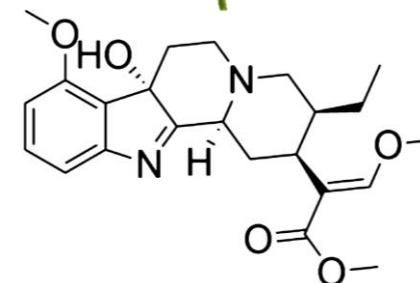
Source: Namphu subdistrict; Bannasarn district; Suratthani

# พืชกระต่อง *Mitragyna speciosa* (Korth.) Havil. [Rubiaceae]

ก้านแดง  
ก้านเขียว  
แตงกว่า  
แมงดา  
ทางกั้ง  
ยักษ์ใหญ่  
เหรียวนกวง<sup>1</sup>  
โพร์กวง<sup>2</sup>  
ขาวปุ่ม



mitragynine



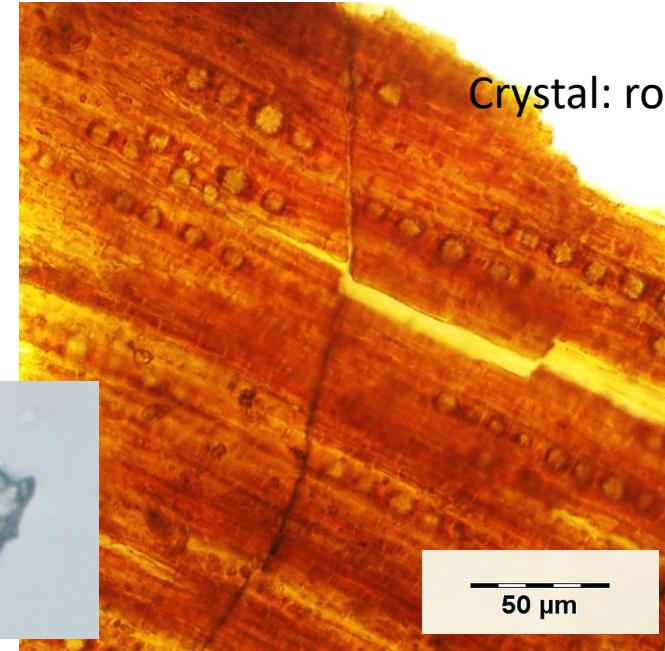
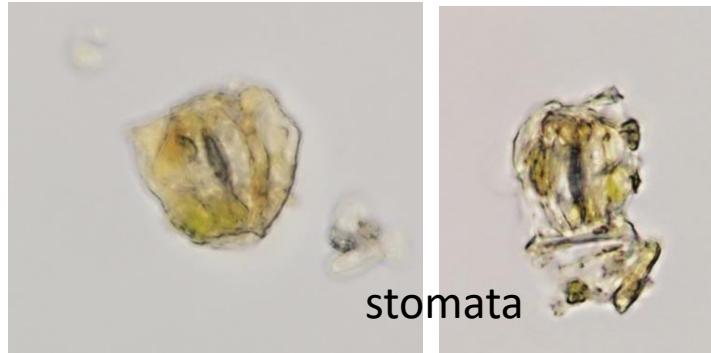
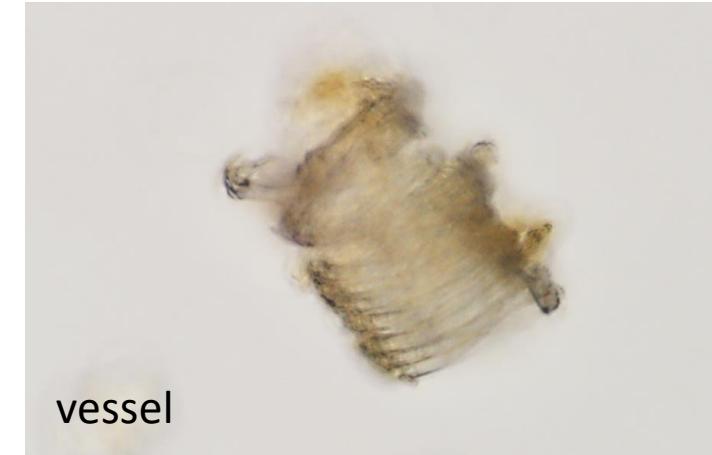
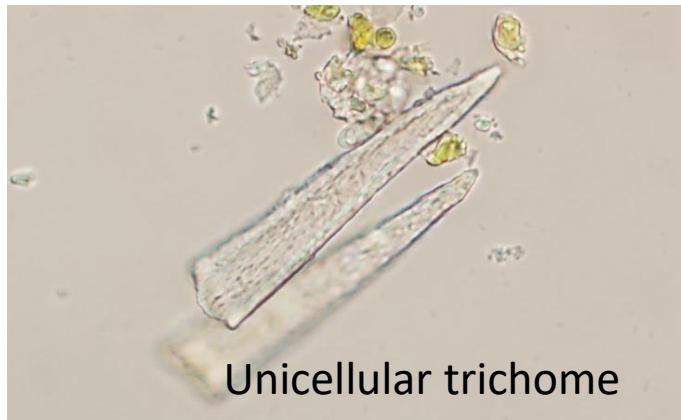
7 $\alpha$ -hydroxy-7H-mitragynine

JW Kratom Rx

PSU<sup>®</sup>  
Pharmaceutical Sciences

# ลักษณะทางจุลภาค: ตรวจสอบลักษณะเซลล์ภายในตัวกล้องจุลทรรศน์

## ก้านแดง



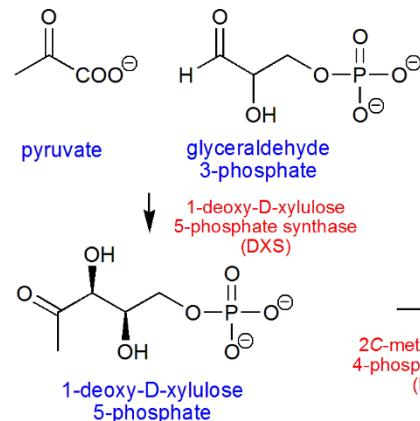
JW Kratom Rx

**PSU** Pharmaceutical Sciences

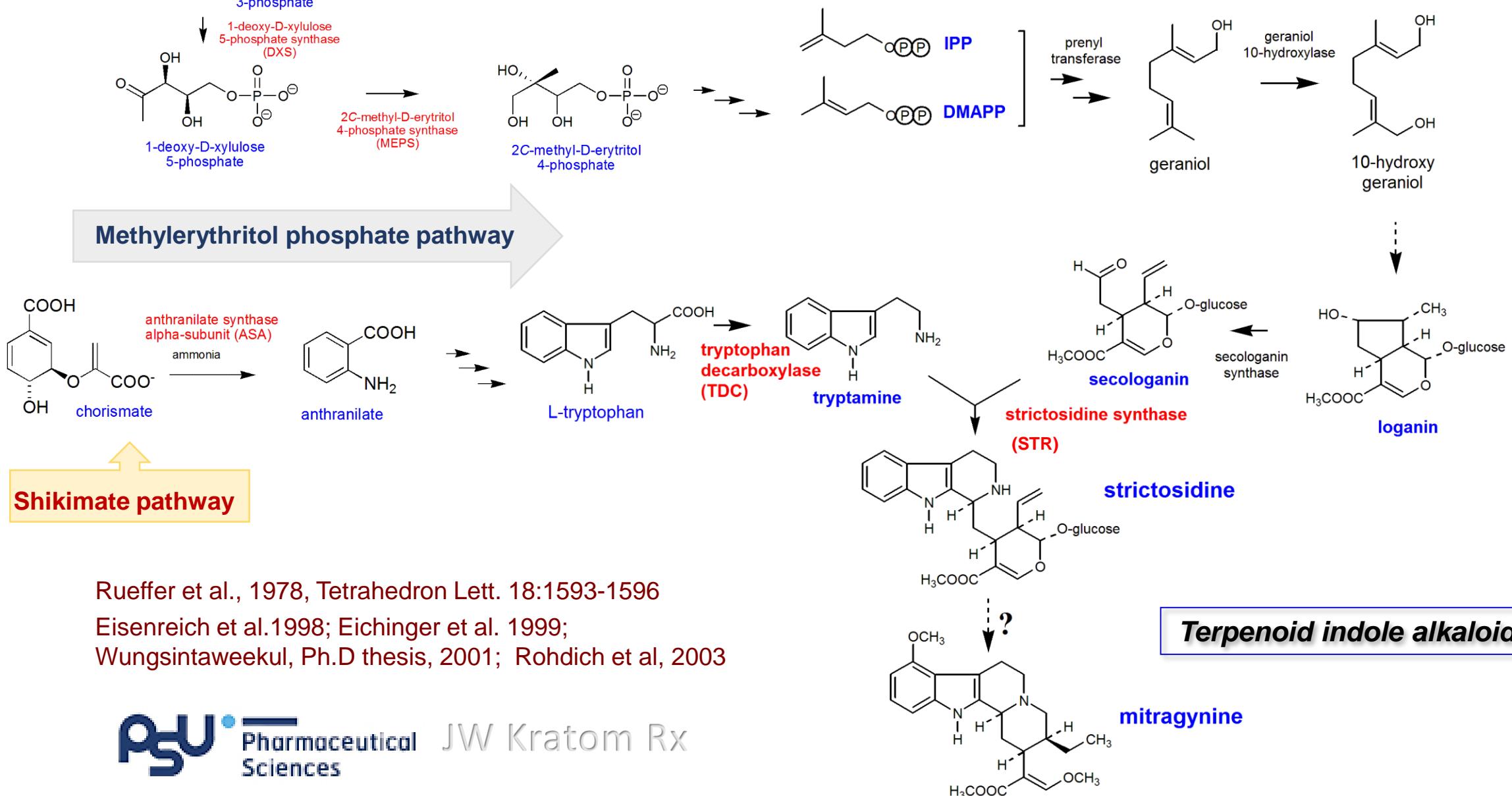
Credit: Dr. Tidarat PUANGPAIROTE

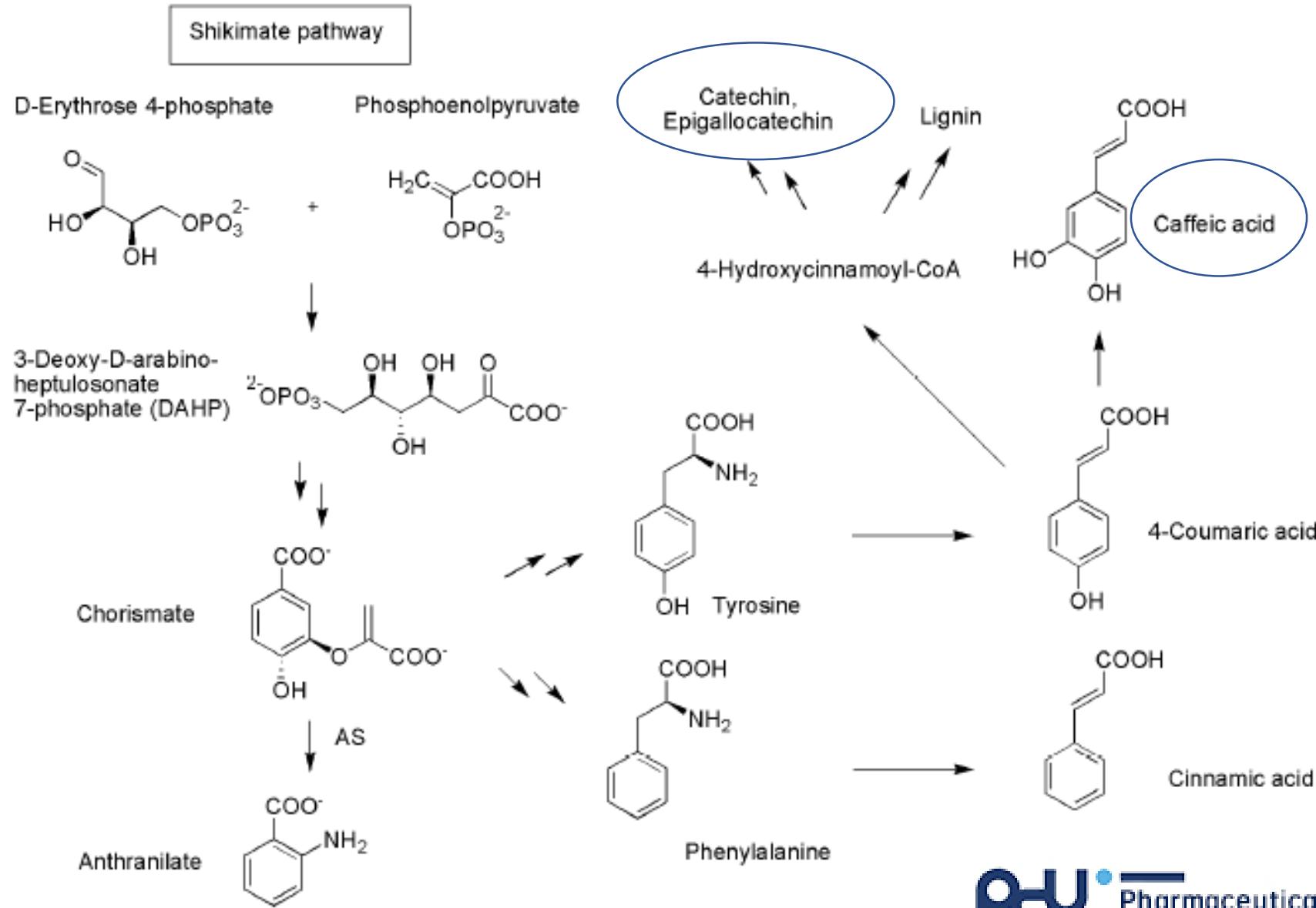
## องค์ประกอบทางเคมีของกระท่อม



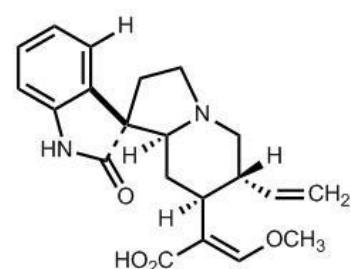
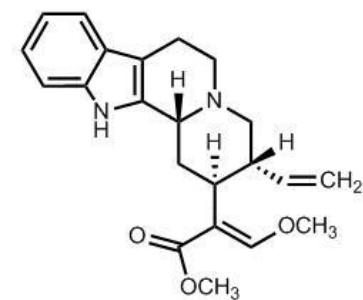
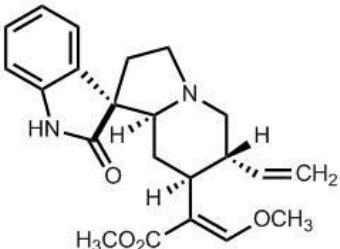
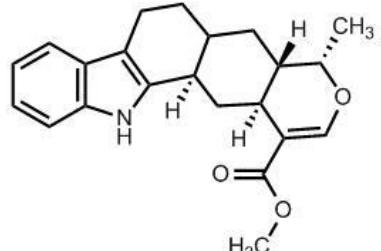
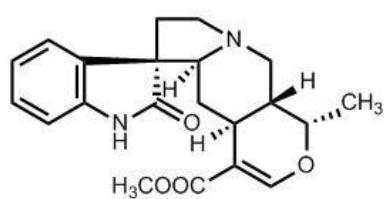
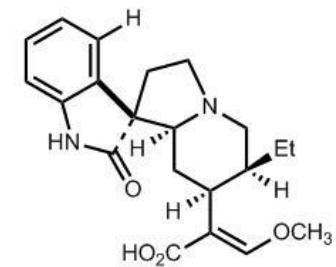
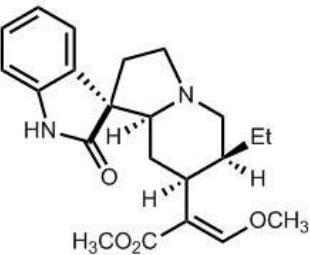
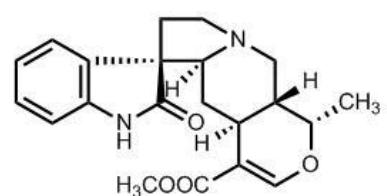
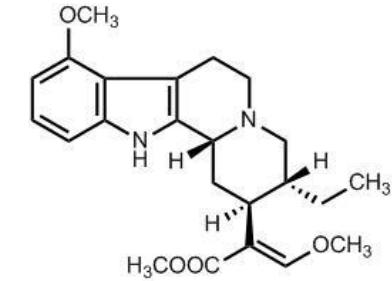
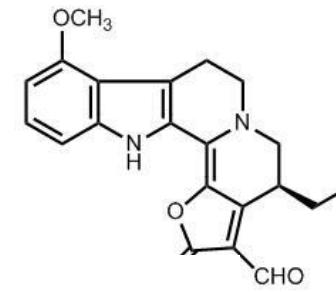
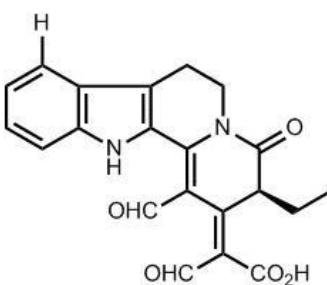
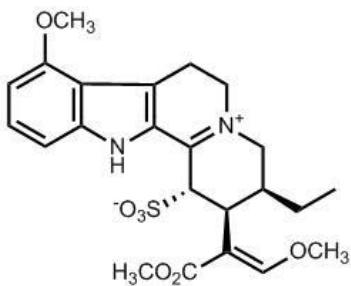
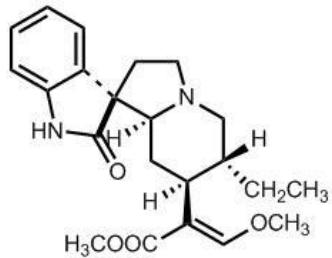
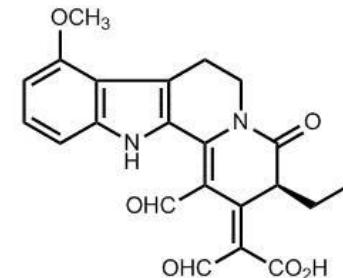
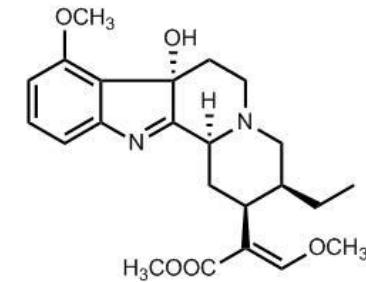
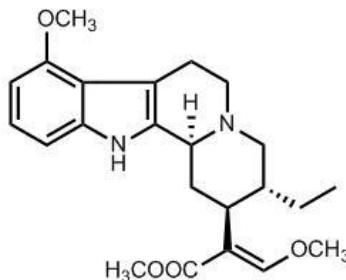
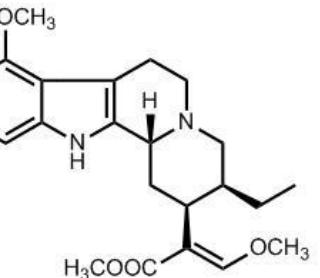
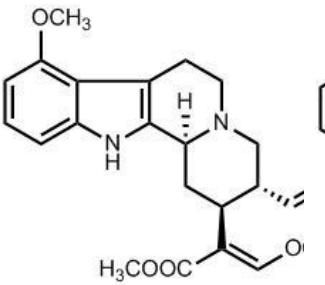
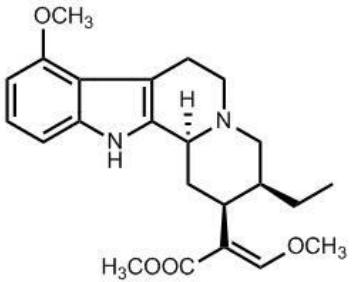


# Proposed mitragynine biosynthesis in Kratom

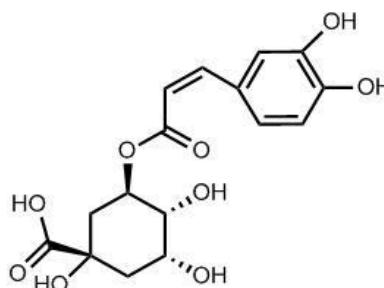
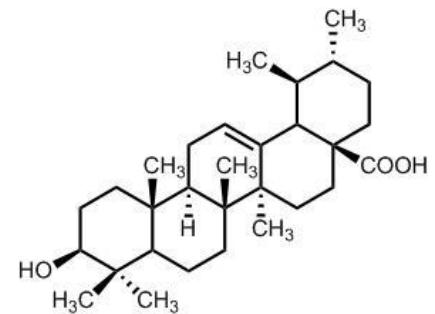
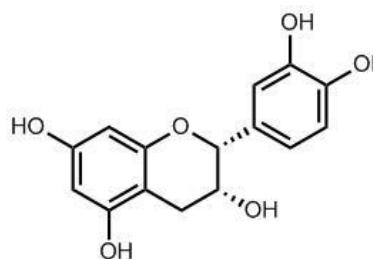
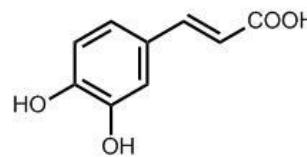
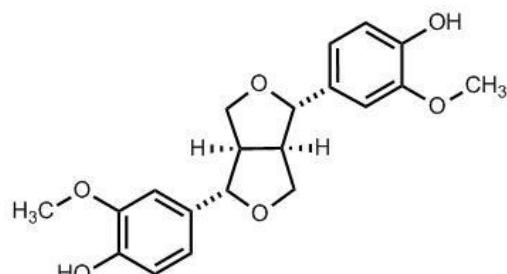
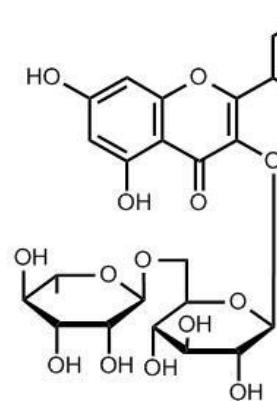
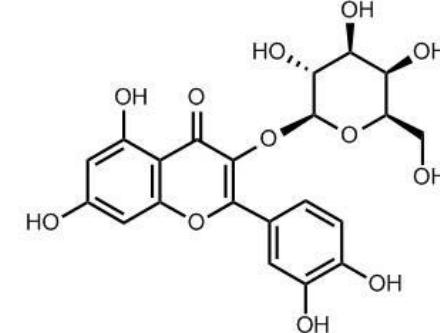
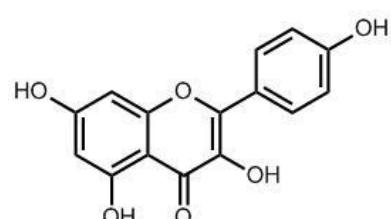
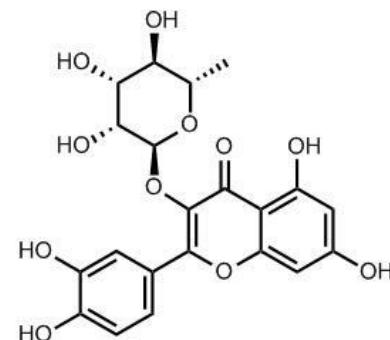
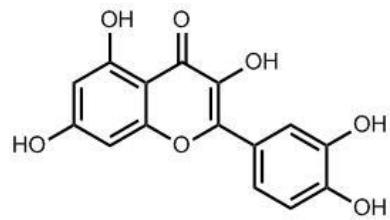
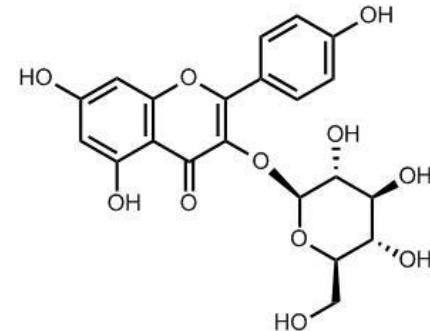
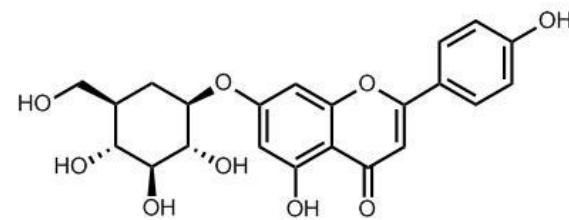




Charoornratana et al. (2013) Z. Naturforsch 68C: 394-405



## Alkaloids found in Kratom leaf and bark



JW Kratom Rx

 **Pharmaceutical Sciences**

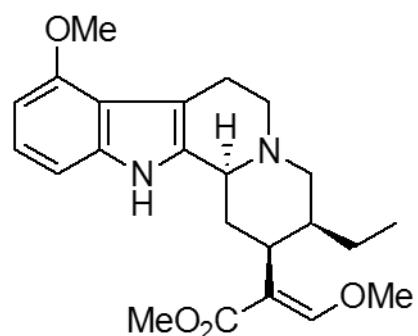
**Flavonoids, Tannins,  
Lignans, Terpenoids  
in Kratom leaf**

ส่วนของพืช	ชื่อสารเคมี
ใบ	<p>กลุ่มสาร: แอลคาโลยด์</p> <p>ajmalicine; akuammigine; angustine; corynantheidine; corynantheidaline; corynantheidalinic acid; corynoxeine; corynoxine; corynoxine B; hirsutine; hirsuteine; isocorynoxeine; isomitraphylline; isomitrafoline; isospeciofoline; isorhynchophylline; isocorynantheidine; javaphylline; mitraciliatine; mitrafoline; mitragynalinic acid; mitragynine oxindole mitrajavine; mitraphylline; mitrasulgynine; mitragynaline; mitragynine; mitralactonal; paynantheine; speciociliatine; speciofoline; speciogynine; 3-isoajmalicine; 3,4,5,6-tetradehydromitragynine; <math>7\alpha</math>-hydroxy-7H-mitragynine; 3-dehydro-mitragynine<sup>4-12</sup></p> <p>กลุ่มสาร: ฟลาโวนอยด์</p> <p>apigenin; apigenin-7-O- rhamnoglucoside; astragalin; cosmoisin; hyperoside; kaempferol; quercetin; quercitrin; quercetin-3-galactoside-7-rhamnoside; rutin; (-)-epicatechin<sup>9,13</sup></p> <p>กลุ่มสาร: เพนิลโพรพาโนยด์ caffeic acid; chlorogenic acid<sup>13</sup></p> <p>กลุ่มสาร: ลิกแนน (+)-pinoresinol<sup>14</sup></p> <p>กลุ่มสาร: ไตรเทอร์ปีนอยด์ ursolic acid; oleanolic acid<sup>15</sup></p>
เปลือกต้น	<p>กลุ่มสาร: แอลคาโลยด์</p> <p>ciliaphylline; isomitraphylline; isorhynchophylline; isospecionoxine; javaphylline; mitraciliatine; mitragynine oxindole A; mitragynine oxindole B; mitraphylline; rhynchociline; rhynchophylline; speciogynine; speciociliatine; specionoxine<sup>7,8</sup></p>
เปลือกราก	<p>กลุ่มสาร: แอลคาโลยด์</p> <p>ciliaphylline; corynoxeine; isocorynoxeine; isomitraphylline; isorhynchophylline; isospecionoxine; mitraciliatine; mitraphylline; rhynchociline; rhynchophylline; speciociliatine; speciogynine; specionoxine<sup>8,16</sup></p>
ผล	กลุ่มสาร: แอลคาโลยด์ $7\alpha$ -hydroxyspeciociliatine <sup>17</sup>
หมักด้วยแบคทีเรีย	<p>กลุ่มสาร: แอลคาโลยด์</p> <p>mitragynine pseudoindoxyl; hydroxylmitragynine pseudoindoxyl<sup>18</sup></p>

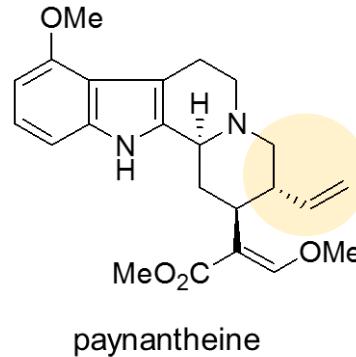
# Alkaloid profile of *Mitragyna speciosa* Korth. The percentage is the estimated content in the alkaloid extracts.

Alkaloid	Percentage (%)
Mitragynine	66
Paynantheine	9
Speciogynine	7
7-hydroxymitragynine	2
Speciociliatine	1
Mitriphylline	<1
Isomitriphylline	<1
Speciophylline	<1
Rhynchophylline	<1
Isorhynchophylline	<1
Ajmalicine	<1
Corynantheidine	<1
Corynoxine A	<1
Corynoxine B	<1

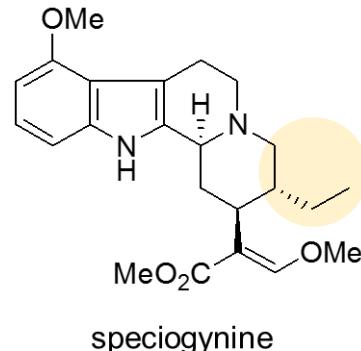
Hassan et al. (2013) Neurosci. Biobehav. Rev. 37(2): 138-151.



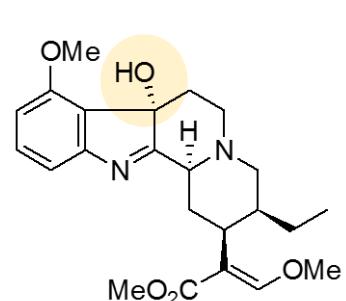
mitragynine



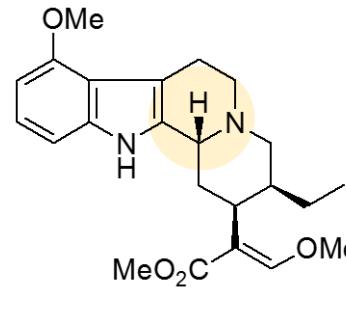
paynantheine



speciogynine



7 $\alpha$ -hydroxy-7H-mitragynine



speciociliatine

# IN-HOUSE isolation of mitragynine and other alkaloids

Kratom leaves powder (1.1 kg DW)

↓ macerated with basified MeOH (pH 9)

Crude MeOH extract (198 g)

↓ acid-base extraction

Crude alkaloid extract (7.62 g, 0.7%)

↓ SiO<sub>2</sub> column  
Hexane: CHCl<sub>3</sub>, CHCl<sub>3</sub>, CHCl<sub>3</sub>: MeOH

F-1

Sephadex® LH-20

Mitragynine  
(2.21 g, 29% dry wt)

F-2

SiO<sub>2</sub> column  
Hexane: EtOAc  
Semi-preparative  
HPLC

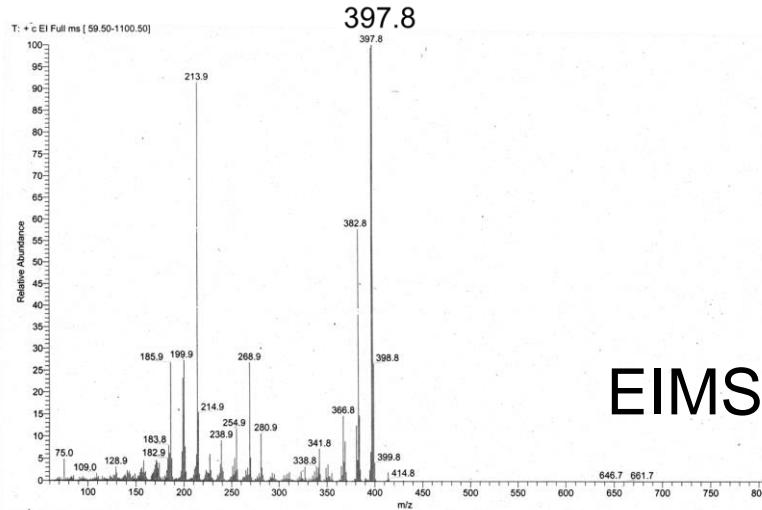
Paynantheine  
(20.4 mg, 0.27% dry wt)

F-3

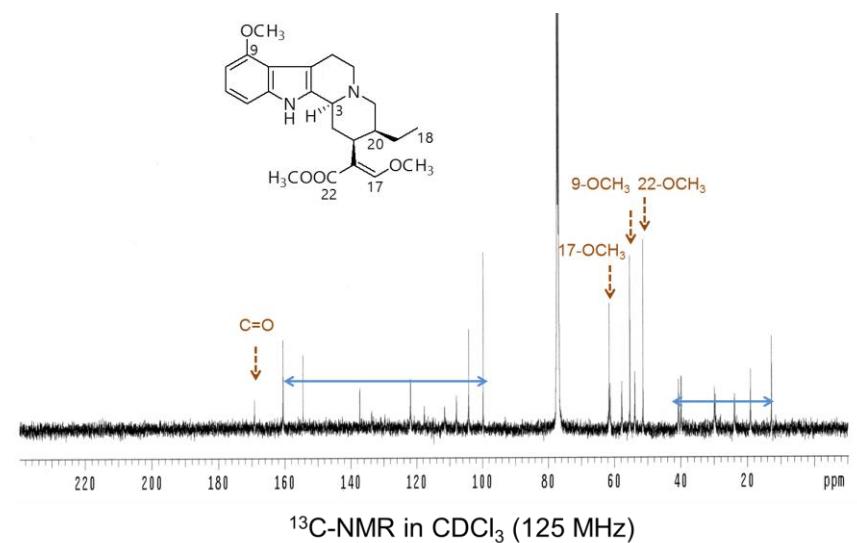
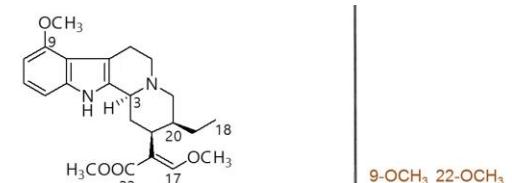
SiO<sub>2</sub> column  
Hexane: EtOAc,  
EtOAc  
EtOAc: MeOH

Speciogynine  
(47.9 mg, 0.63% dry wt)

Keawpradub N (1990); Limsuwanchote S (2014)



EIMS



# Quantification methods for mitragynine, its metabolites and related alkaloids

## Identification of kratom and kratom products 'mitragynine'

- Botanical approach
- Microscopic examination
- Chemical reaction
- Thin layer chromatography
- HPLC/HPLC-DAD/HPLC-UV
- GC/GC-FID/GC-MS
- DNA fingerprint



## Determination of mitragynine and related metabolites 'plasma and urine'

- HPLC-UV
- UHPLC-MS-DAD
- LC-MS
- LC-MS-MS
- GC-MS

## Thin Layer Chromatography: การตรวจสอปสารมิตรากี้นบ

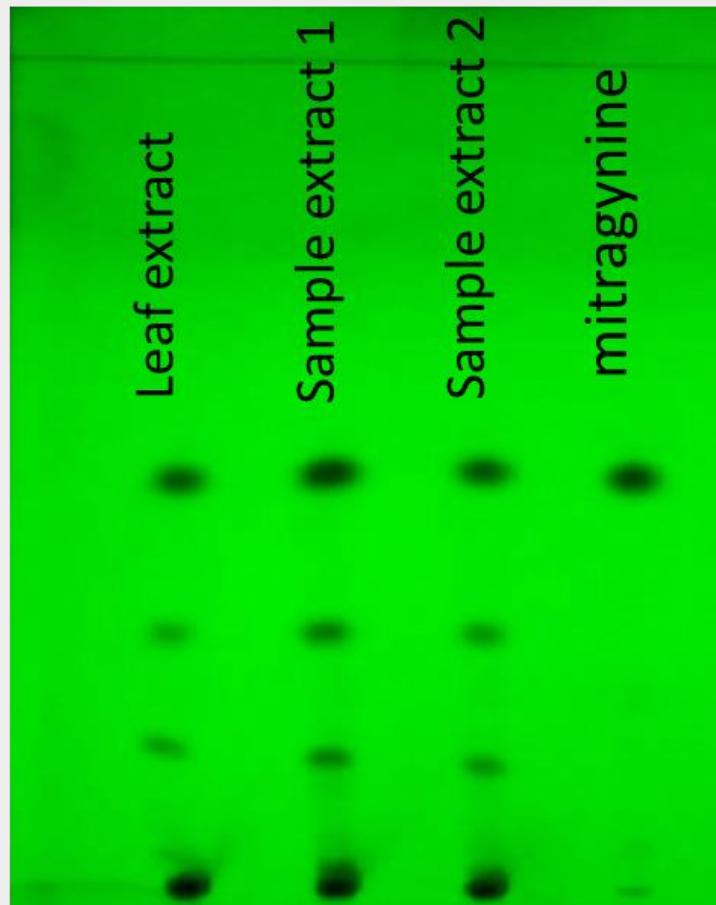
<b>Stationary phase</b>	<b>Mobile phase</b>	<b>Ratio</b>	<b>Spraying reagent</b>	<b>Rf-value</b>	<b>References</b>
Silica gel 60 F254 TLC	CHCl <sub>3</sub> : MeOH	9:1	Iodoplatinate	0.91	Scott et al. 2014
Silica gel HPTLC	EtOAc: hexane	4:1	Dragendorff- H <sub>2</sub> SO <sub>4</sub>	0.92	Parthasarathy et al. 2009
Silica gel 60 G254 HPTLC	EtOAc: hexane	2:8	Iodine, Dragendorff	0.82	Beng et al. 2011
Silica gel 60 F254 TLC	EtOAc: hexane: 25% NH <sub>3</sub>	15:30:1	-	0.49	Kowalcuk et al. 2013
Silica gel 60 F254 TLC	EtOAc: CHCl <sub>3</sub>	1:1	Fast Black k salt	0.67	วราพงษ์ เสนะวีระกุล และคณะ, 2018
	EtOAc: hexane	4:6	Fast Black k salt	0.59	

# การตรวจสารมัตราชัยบีนในพืชกระถ่อม

ตรวจในพืช



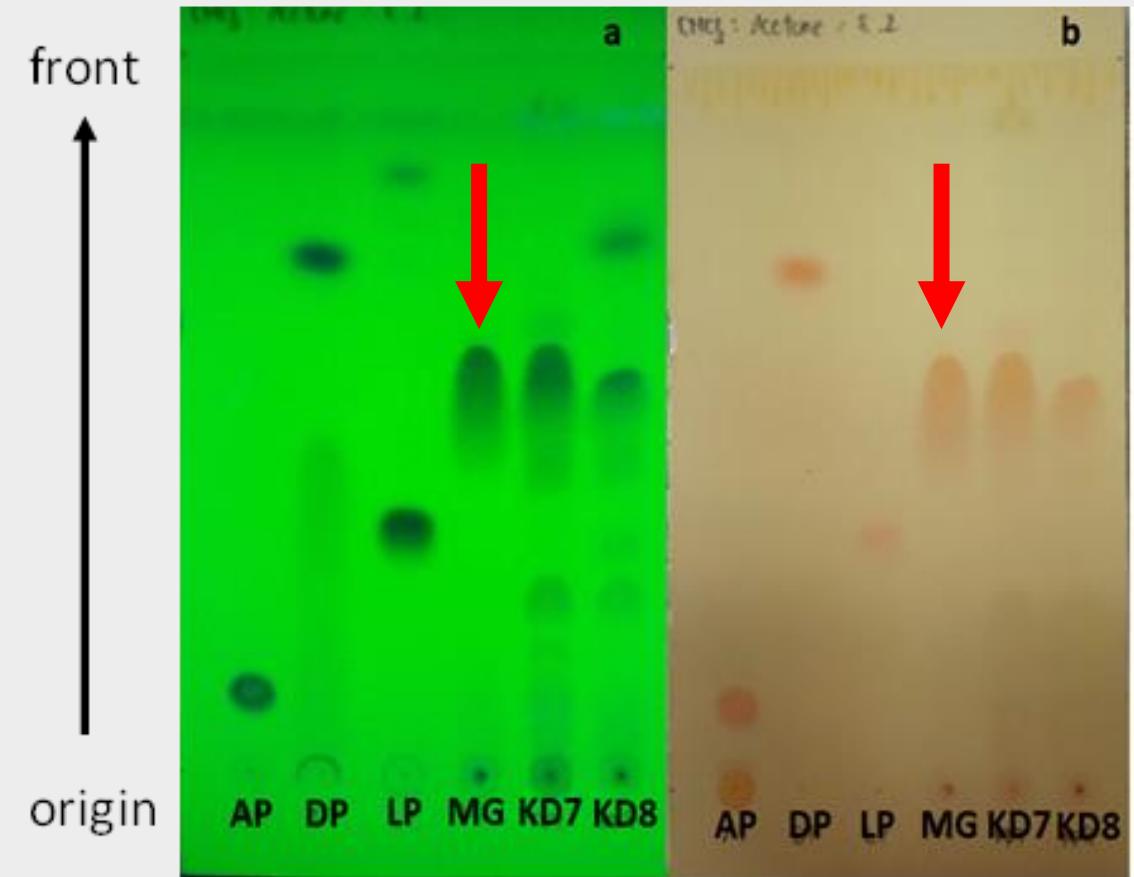
TLC plate: Silica gel GF254



แผนกรองคเลบผิวบาง

นำต้ม 4 x 100

mobile phase:  $\text{CHCl}_3$ : acetone; 8:2



Unpublished result



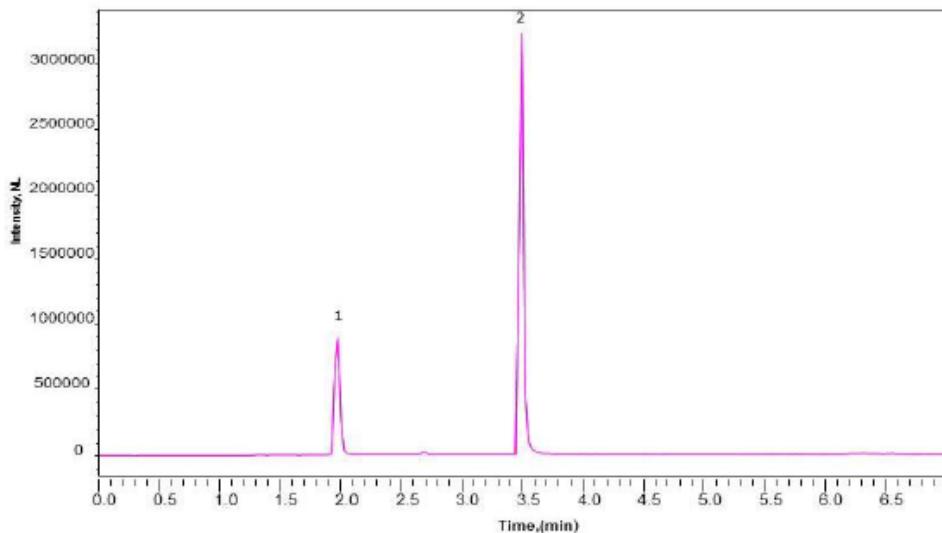
# Quantification of kratom alkaloids using chromatographic methods

Technique	Target alkaloid	Sample/Specimen	Sensitivity	References
HPLC-UV	mitragynine	rat serum	LOD: 0.03 µg/mL LOQ: 0.1 µg/mL	Janchawee et al., 2007
	mitragynine	rat plasma	LOD: 0.025 µg/mL LOQ: 0.050 µg/mL	Parthasarathy et al., 2010
HPLC-DAD	mitragynine	kratom cocktail	LOD: 1.0 µg/mL LOQ: 3.0 µg/mL	Chittrakarn et al., 2012
	mitragynine	kratom plant	LOD: 0.25 µg/mL LOQ: 0.50 µg/mL	Parthasarathy et al., 2013
LC-MS	mitragynine,	kratom plant	Linearity: 1.0-10.0 µg/mL	Kikura-Hanajiri et al., 2009
	7-hydroxymitragynine	kratom commercial products	Linearity: 0.01-1 µg/mL	
LC-MS-MS	mitragynine	human urine	LOD: 0.02 ng/mL LOQ: 0.1 ng/mL	Lu et al., 2009
	mitragynine	rat plasma	LOQ: 0.2 ng/mL	de Moraes et al., 2009
GC-MS	mitragynine	human urine	LOD: 2 ng/mL LOQ: 50 ng/mL	Arndt et al., 2011
	mitragynine	kratom plant	-	Chan et al., 2005
ELISA	mitragynine, paynantheine, speciogynine, speciociliatine	rat plasma and urine	LOD: 100 ng/mL	Philipp et al., 2011
	mitragynine	Kratom plant	LOD: 32.5 µg/mL LOQ: 32.9 µg/mL	Limsuwanchote et al., 2014

LOD: limit of detection; LOQ: limit of quantification



## UHPLC/MS Analysis of Kratom and its Metabolite on Ascentis® Express C18, 2 µm



Peak Number	Compound	Concentration µg/mL
1	7-OH Mitragynine	5
2	Mitragynine	5

### Conditions:

**column:** Ascentis® Express C18, 5 cm x 2.1 mm I.D., 2 µm

**mobile phase:** [A] Water (0.1% (v/v) Formic Acid) ; [B] Acetonitrile (0.1% (v/v) Formic Acid)

**gradient:** 10% B to 95% B in 4 min; hold at 95% for 1 min.; 95% B to 10% B in 0.1 min; hold at 10% B for 2 min

**flow rate:** 0.4 mL/min

**column temp.:** 25 °C

**detector:** MSD, ESI-(+)

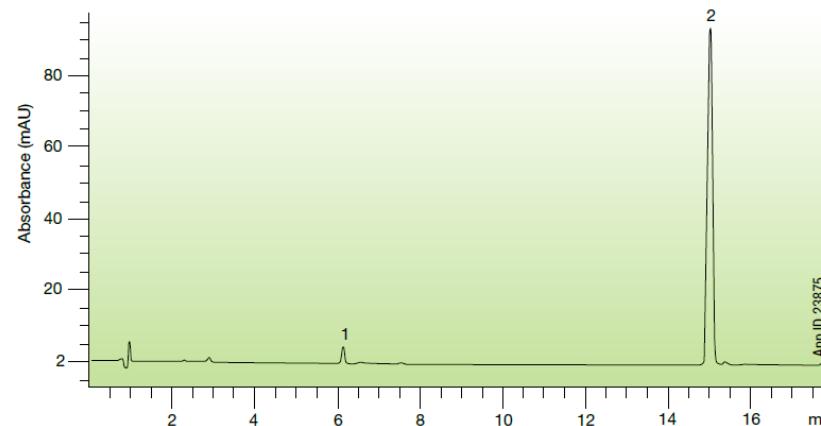
**injection:** 2 µL

**sample:** Kratom compounds, varied concentration, 95:5 Acetonitrile:Water

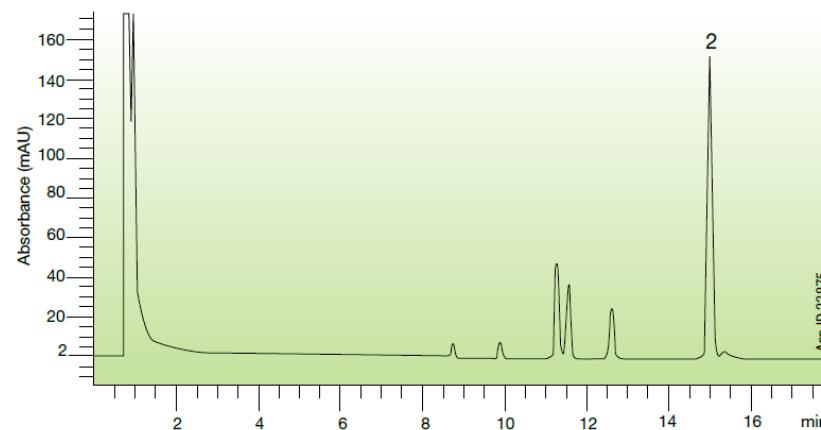
Determination of Mitragynine in *Mitragyna speciosa* Raw Materials and Finished Products by Liquid Chromatography with UV Detection: Single-Laboratory Validation  
Elizabeth M. Mudge and Paula N. Brown

JOURNAL OF AOAC INTERNATIONAL VOL. 100, NO. 1, 2017

Mitragynine and 7-OH mitragynine alkaloids in a standard mixture<sup>1</sup>.



Mitragynine alkaloids in raw materials<sup>1</sup>.



Agilent 1200 Series Liquid Chromatograph

*HPLC Conditions*

- (a) *Analytical column.*—Kinetex 5 µm EVO C18 100Å (Phenomenex, Torrance, CA), 150×4.6 mm, 5 µm particle size.
- (b) *Column temperature.*—25°C.
- (c) *Detector conditions.*—Monitor at 226 nm (4 nm bandwidth), no reference.
- (d) *Flow rate.*—1.5 mL/min.
- (e) *Run time.*—18 min, plus 4 min column re-equilibration time.
- (f) *Injection volume.*—5 µL.
- (g) *Gradient conditions.*—See Table 1.

# Kratom compounds analysis: leaves and products

Kikura-Hanajiri et al. (2009) Simultaneous analysis of mitragynine, 7-hydroxymitragynine, and other alkaloids in the psychotropic plant “kratom” (*Mitragyna speciosa*) by LC-ESI-MS. Forensic Toxicology 27:67–74.

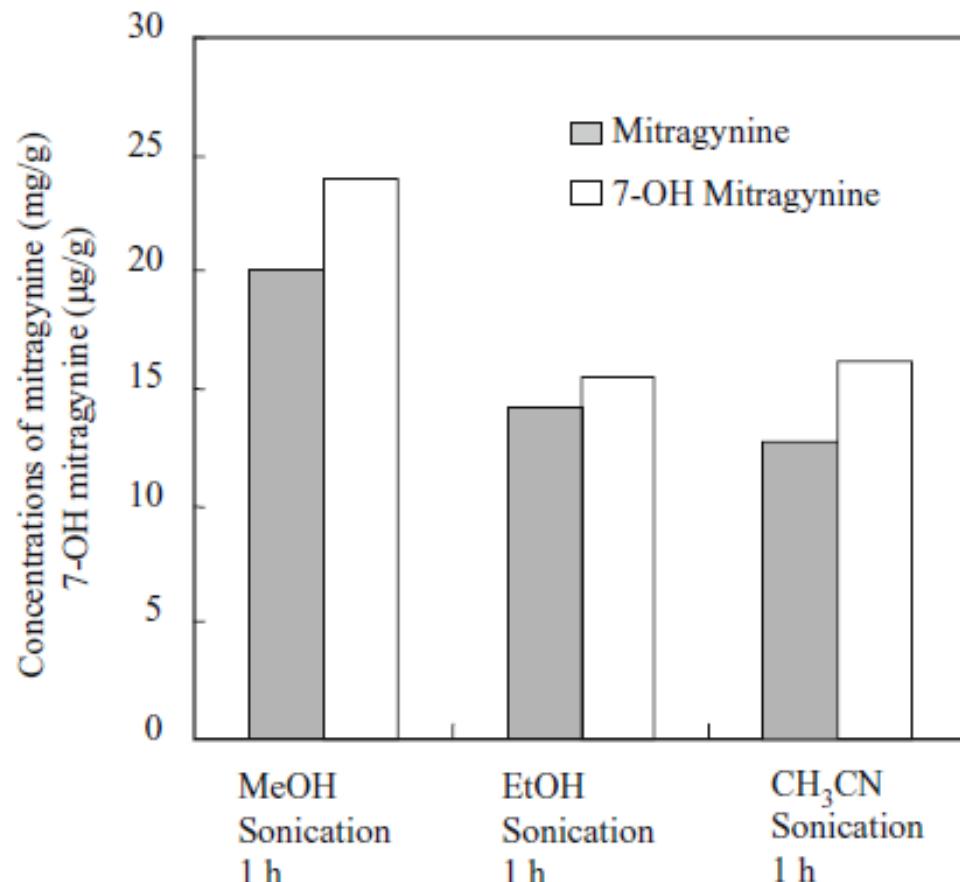


Fig. 3 Concentrations of mitragynine and 7-OH-mitragynine from product No. 4 using different organic solvents for extraction

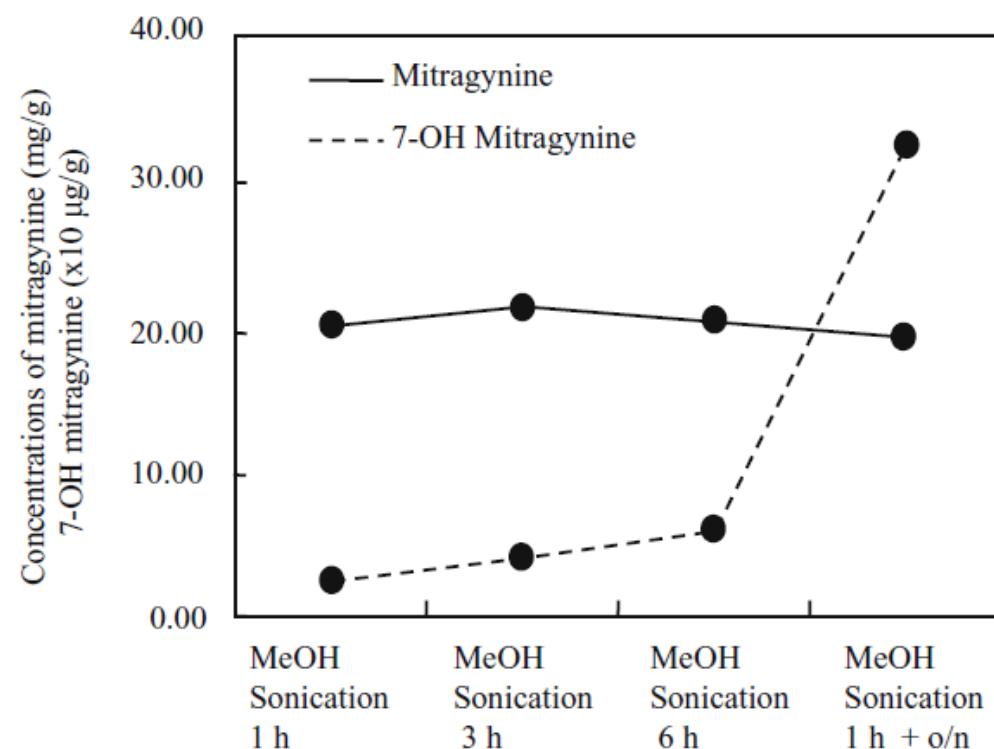
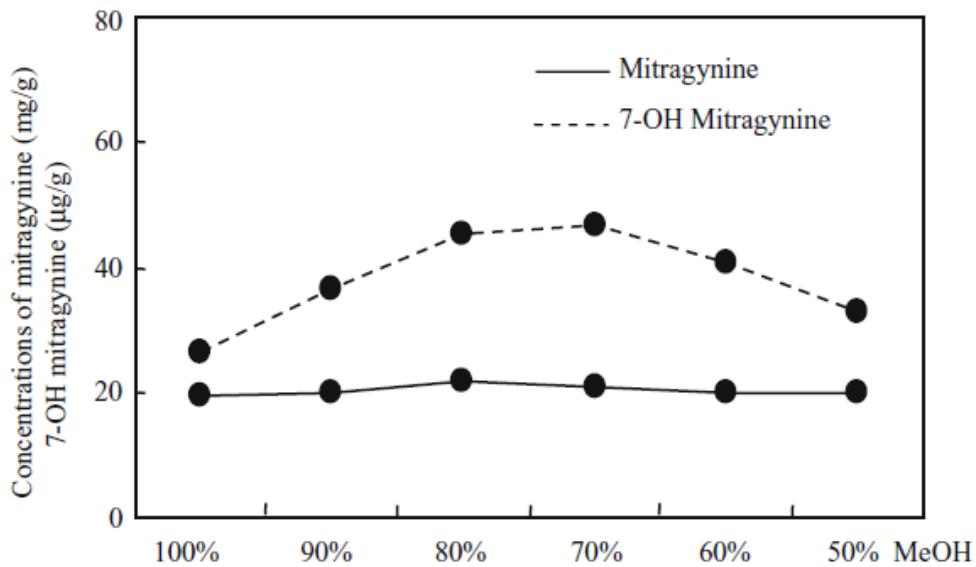
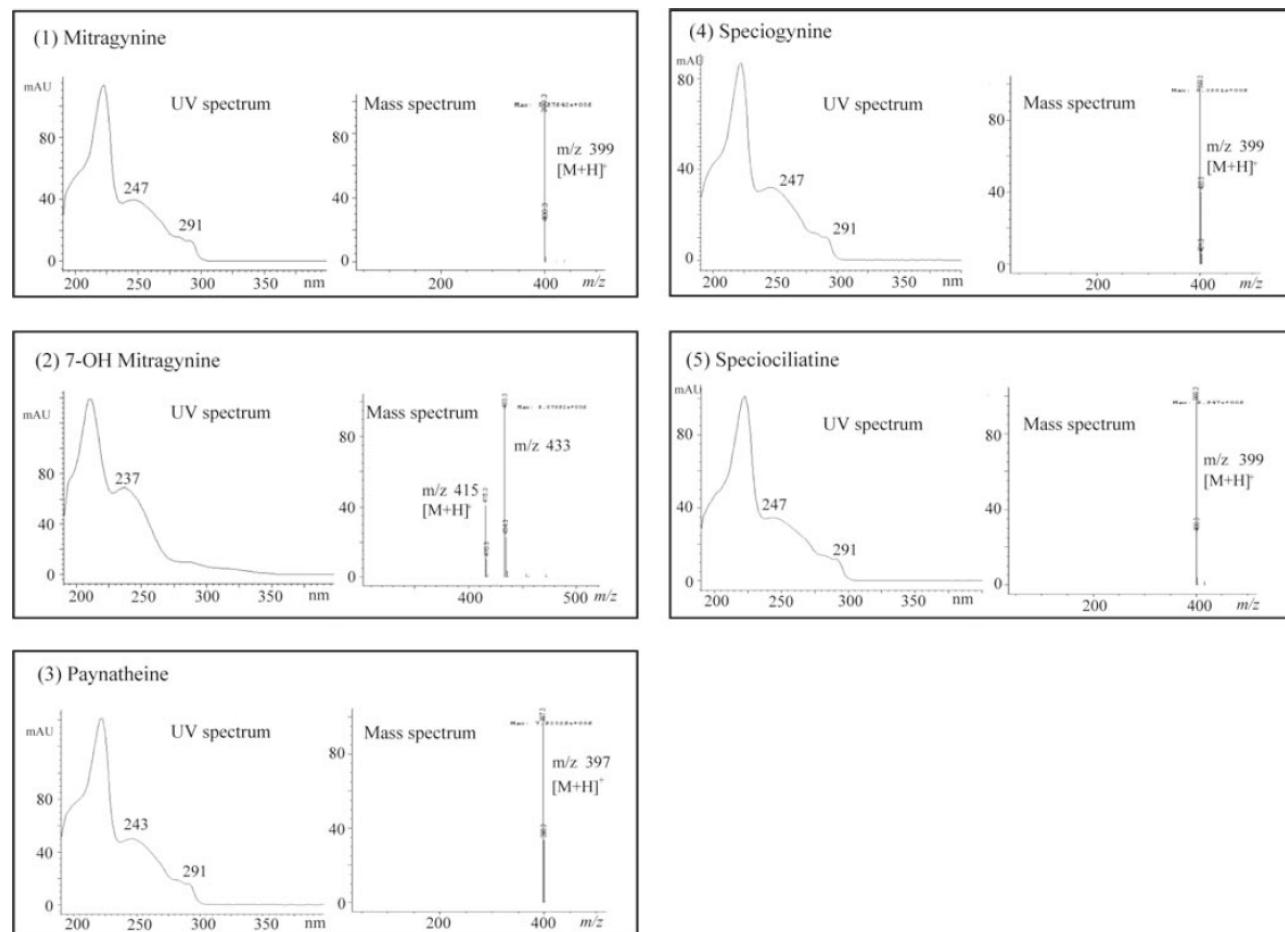


Fig. 4 Concentrations of mitragynine and 7-OH mitragynine from product No. 4 as a function of extraction time using methanol. *o/n*, Overnight storage at room temperature

Powdered sample (10–50 mg) was extracted with 10 ml of 80% methanol aqueous by ultrasonication for 1 h.



**Fig. 5** Concentrations of mitragynine and 7-OH-mitragynine from product No. 4 as a function of different concentrations of methanol for extraction



**Fig. 2** Ultraviolet (UV) and mass spectra of the standard compounds of five alkaloids in *M. speciosa*. The retention times of mitragynine, 7-OH-mitragynine, paynathine, speciogynine, and speciociliatine were 19.9, 21.5, 20.5, 19.9, and 23.3 min, respectively

## Agilent 1100 Series HPLC 1100 Series

LC/MSD SL

Column: Atlantis dC18 column at 40°C.

## Mobile phase:

- A. 10 mM ammonium formate, pH 3.5
  - B. methanol

## Elution: gradient mode;

Total run time 35 min

Injection volume: 1  $\mu$ L  
PDA detector [190-400 nm; 254 nm]

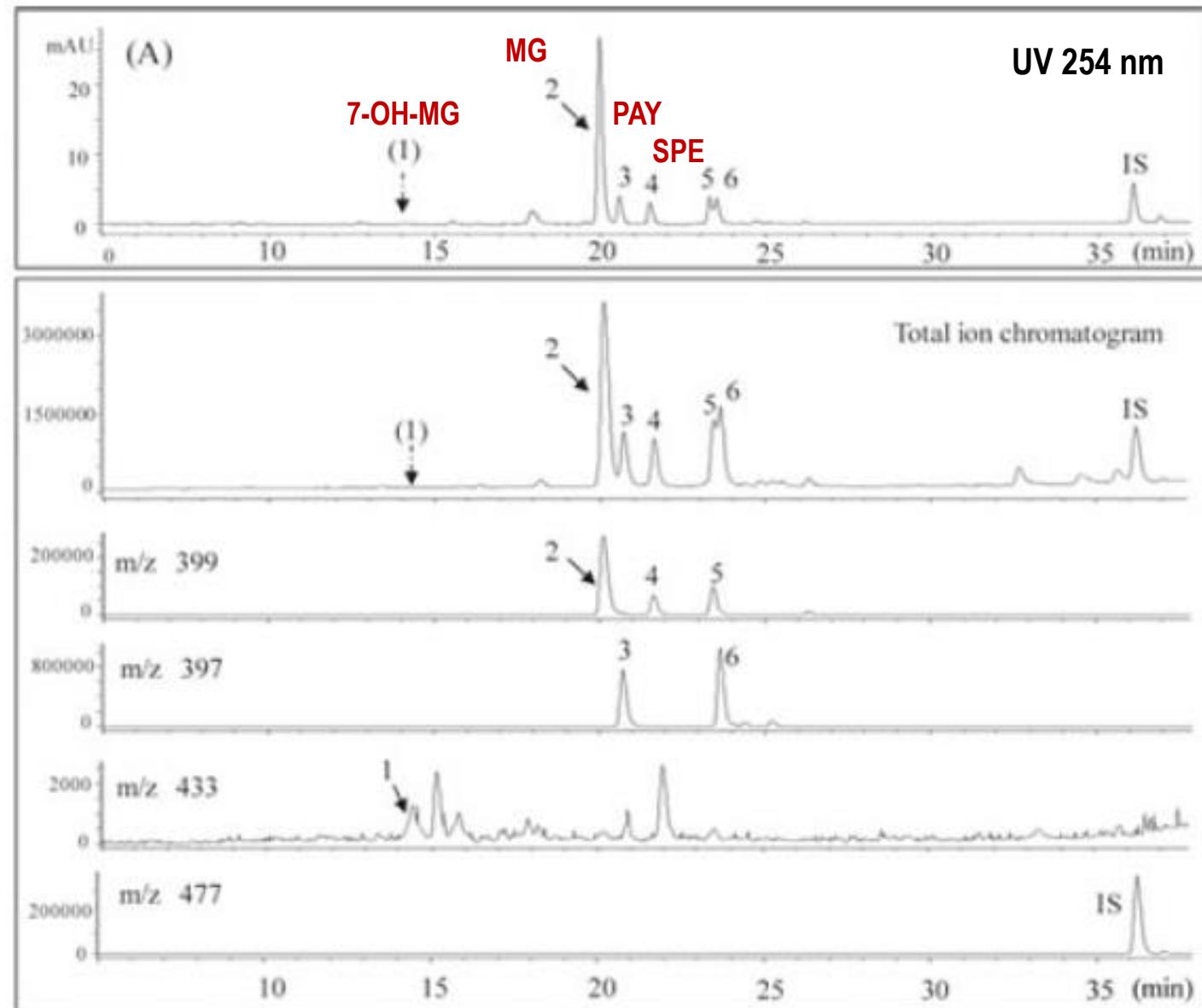
Fig. 6A UV chromatograms, total ion chromatograms, and mass chromatograms of the extracts from

1: 7-OH-mitragynine; 2: mitragynine;

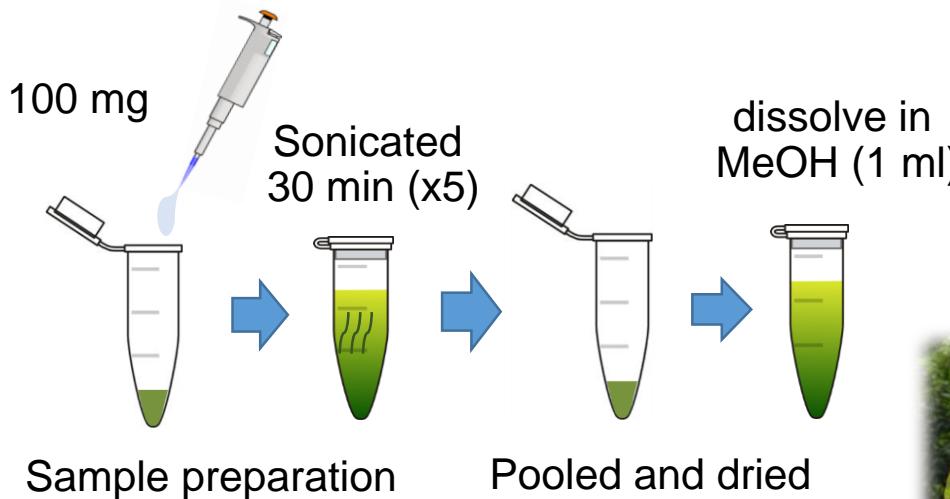
3: paynantheine; 4: speciogynine;

5: speciociliatine; 6: unidentified

## Raw material of *M. speciosa* (big leaves)



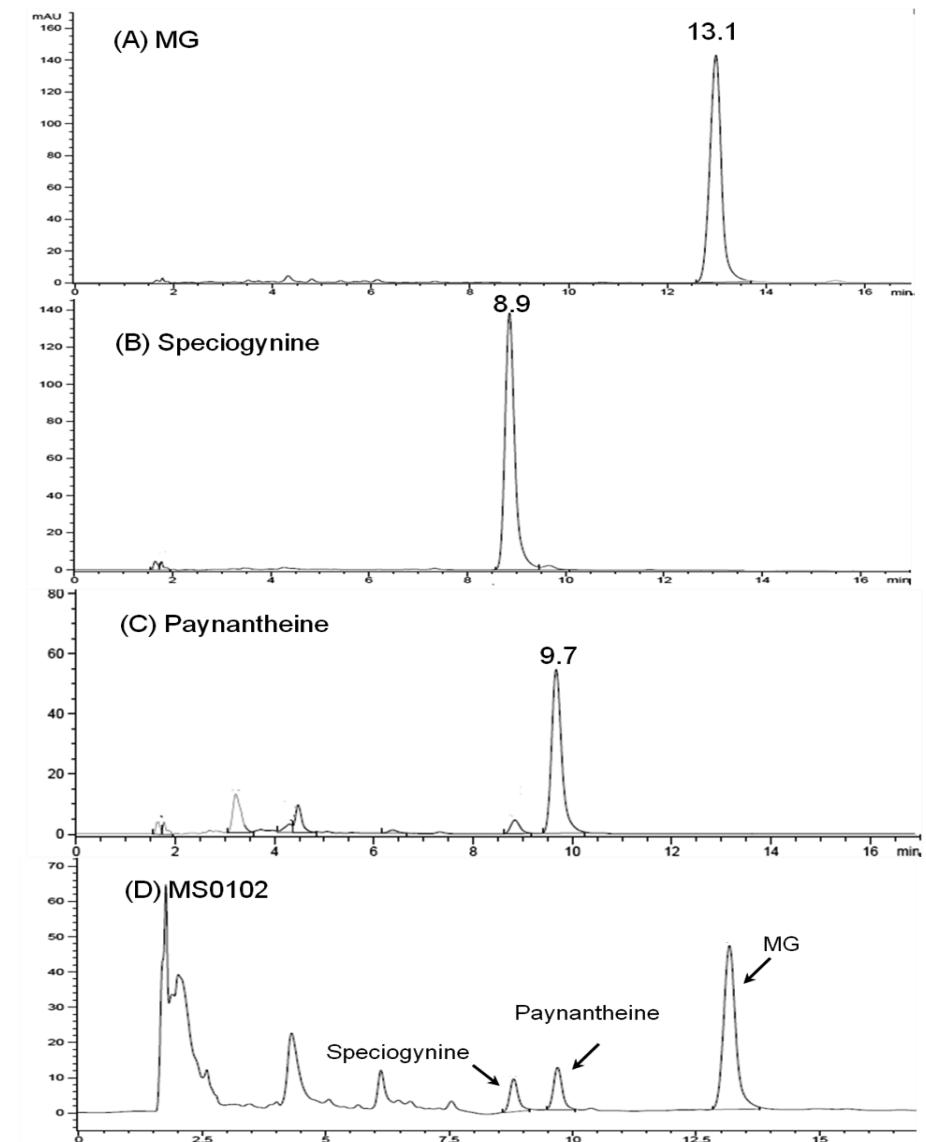
# Quantification of MG in kratom leave samples



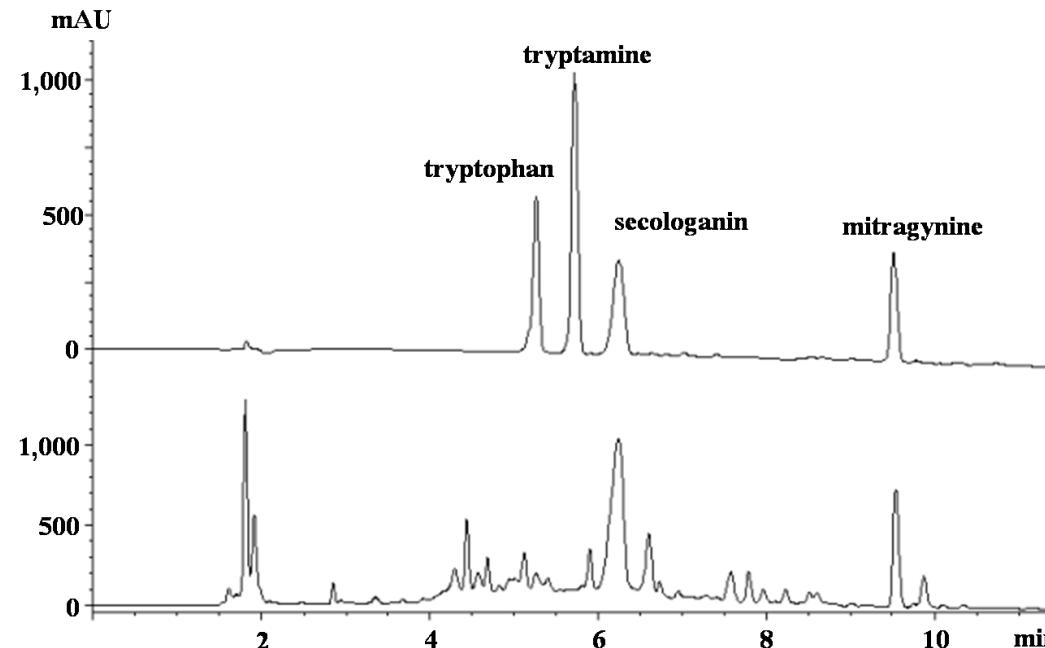
## HPLC Analysis

Model : Agilent 1100 series  
Detector : DAD-detector, 225 nm  
Column : Verticep™ UPS C<sub>18</sub> (4.6 x 250 mm, 5 µm)  
Mobile phase: 20 mM ammonium acetate (pH 6.0) : ACN (35:65)  
Injection volume: 20 µl  
Flow rate: 1.0 ml/min

Wungsintawekul et al. (2012) Biotechnology Letters 34:1945–1950



# HPLC - based metabolites analysis



50 mg of lyophilized powder

- reflux with methanol at 70 °C for 1 hour.
- wash pigment out by partition with petroleum ether (2x)
- pooled methanol fractions; evaporated
- HPLC analysis

Agilent 1100 series HPLC-DAD-FLD

Column: VertiSep™ UPS C18 (250 mm x 4.6 mm i.d., 5 µM)  
Mobile phase: 100 mM H<sub>3</sub>PO<sub>4</sub> pH 2.4/H<sub>2</sub>O: acetonitrile;  
(10:90 → 90:10 ; 15 min)  
Detector: UV 225; 238 nm for mitragynine, secologanin  
fluorescence excite 270 nm; emission 370 nm for  
tryptophan, tryptamine

Condition

HPLC (Agilent 1260)

Column : Kinetex 2.6  $\mu$ m. EVO C18 (250 x 4.6 mm)

Mobile phase : 20 mM Ammonium acetate pH 6 : acetonitrile

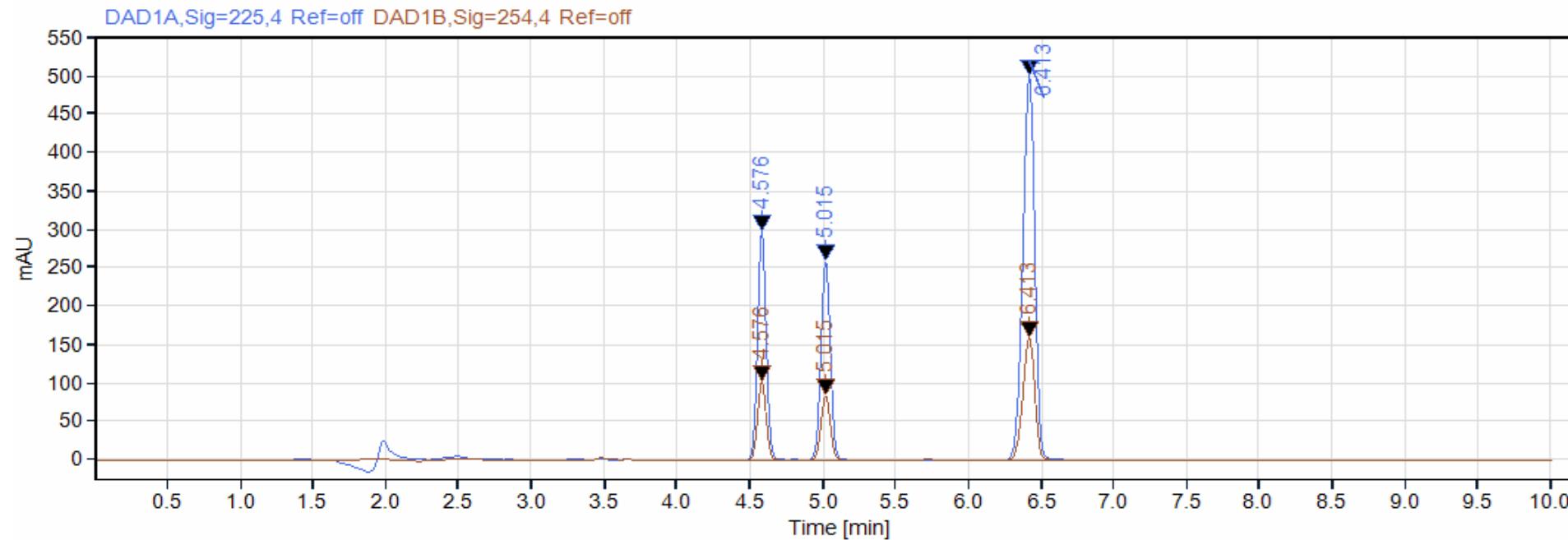
Isocratic elution: 40:60

Flow rate : 1 mL/min

Inject volume : 10  $\mu$ L

Run time : 10 min

UV-PDA detector



Limsuwanchote et al. (2014) Forensic Science International 244: 70-77.

**Plant material:** The leaves of three red vein strains of *M. speciosa* (K.) H., from Thailand (Red Thai), Malaysia (Red Malay) and Bali (Red Bali), a white vein strain from Borneo (White Borneo) and a green vein strain from Malaysia (Green Malay) were all provided by Herba Invest S.R.O. (Bratislava, Slovakia).

**HPLC-DAD analyses:** MG standard and alkaloid extract solutions were prepared via dissolution in ethanol and 1 to 10 dilution in a mixture of  $\text{H}_2\text{O}/\text{CH}_3\text{CN}$  2:1 (TFA 0.1%). The solutions were filtered through a 0.45  $\mu\text{m}$  syringe filter and analyzed by HPLC on an XTerra MS C8 column (4.6 x 150 mm, 5  $\mu\text{m}$ , Waters). The PDA detector was. The analyses were carried out on a Waters 1525 Binary HPLC pump equipped with 2998 PDA, using a flow rate of 1 mL/min and an injected volume of 20  $\mu\text{L}$ . The quantification of the MG standard and all the alkaloids in the extracts was performed at 222 nm. 0.1% TFA aqueous solution (A), and acetonitrile, with 0.1% TFA (B), were used as the mobile phases accordingly to the following gradient profile: (time, B%) 0.01, 20; 7.5, 20; 15, 30; 26, 60; 39.5, 100; 44, 100.

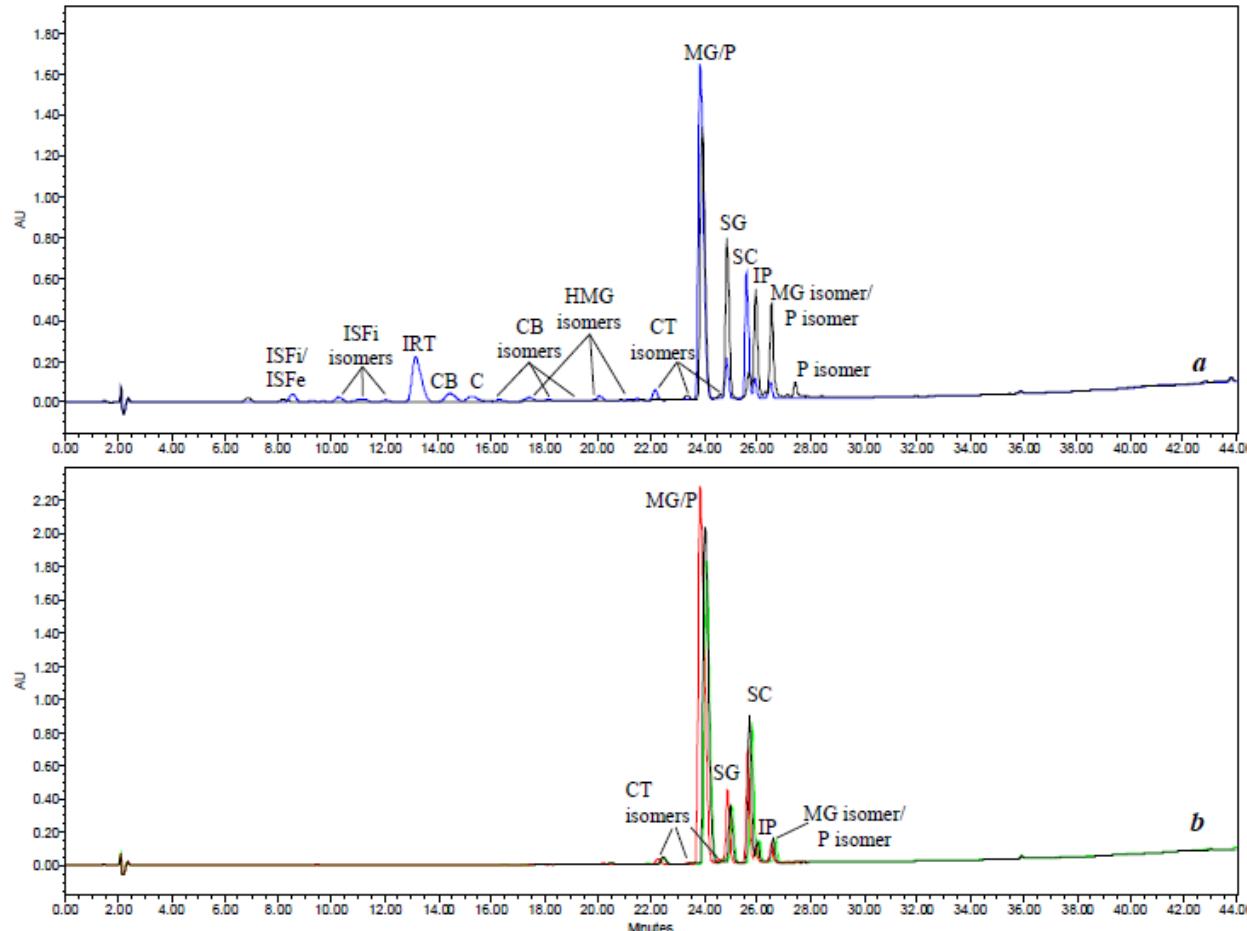
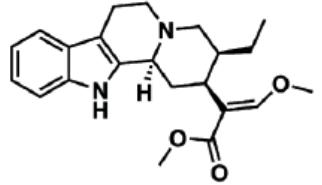
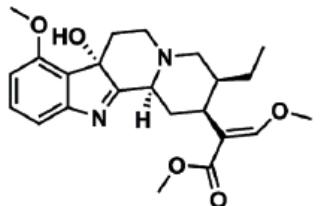


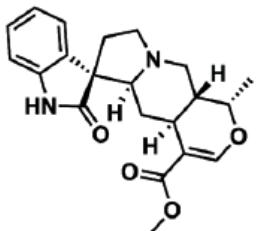
Figure 2: Comparison between HPLC-DAD chromatograms of Red Bali and Red Malay (a) and Red Thai/White Borneo/Green Malay (b).



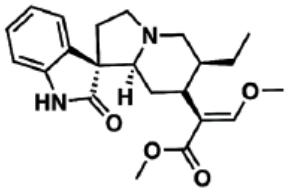
### **Corynantheidine**



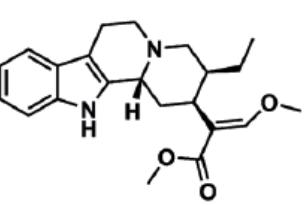
## 7-Hydroxymitragynine



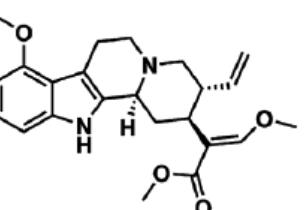
### **Mitraphylline**



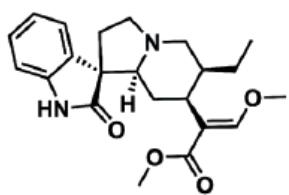
## Corynoxine



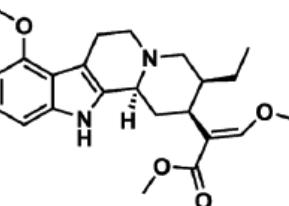
### **Isocorynantheidine**



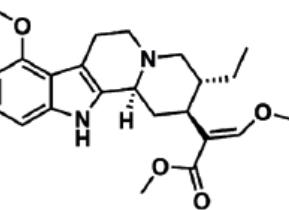
Paynantheine



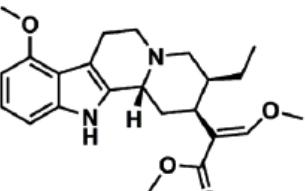
## Corynoxine B



## Mitragynine



## **Speciogynine**

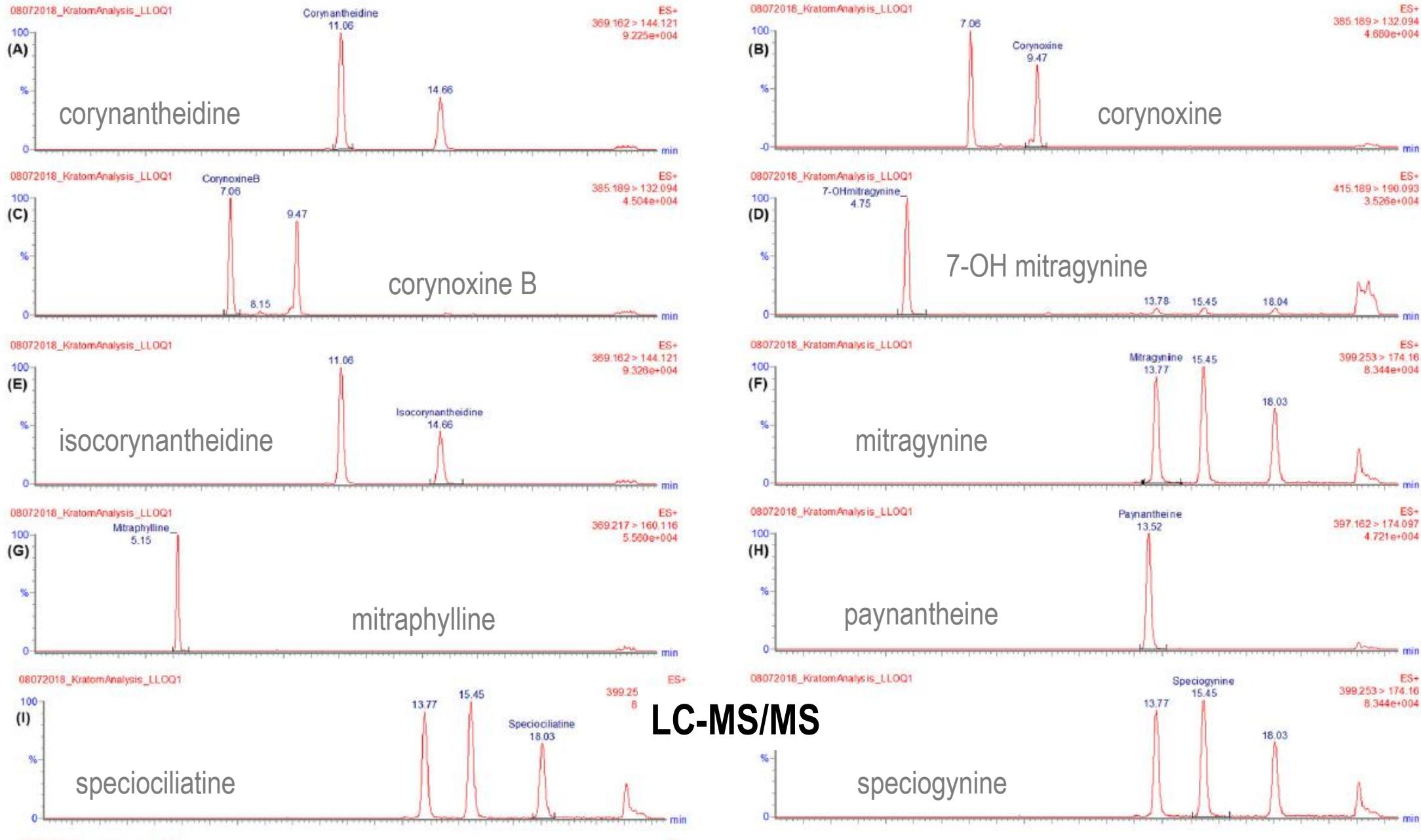


## **Speciociliatine**

# Simultaneous quantification of ten key Kratom alkaloids in *Mitragyna speciosa* leaf extracts and commercial products by ultra-performance liquid chromatography–tandem mass spectrometry

Sharma et al (2019) Drug Testing and Analysis 1-10

- A Waters Acquity Class I UPLC coupled with an XevoTQ-S Micro triple quadrupole mass spectrometer (Milford, MA, USA) was used for the quantitative analysis of kratom alkaloids.
  - Chromatographic separation of kratom alkaloids sharing monoisotopic masses ( $M + H^+$ ,  $(m/z)$ ) was achieved on a Waters Acquity BEH C18 column ( $1.7 \mu\text{m}$ ,  $2.1 \times 100 \text{ mm}$ ) with a slow gradient using a mobile phase consisting of aqueous ammonium acetate buffer ( $10 \text{ mM}$ , pH 3.5; A) and acetonitrile (B).
  - The flow rate of the mobile phase was held at  $0.35 \text{ mL/min}$ .



# Kratom cocktail or fatal 4 x 100 formula



1. Boiled kratom leaves

2. cola drink

3. cough syrup

4. ice cube/mosquito coil/  
tramadol/sedative drugs



Pre-treatment of kratom cocktail

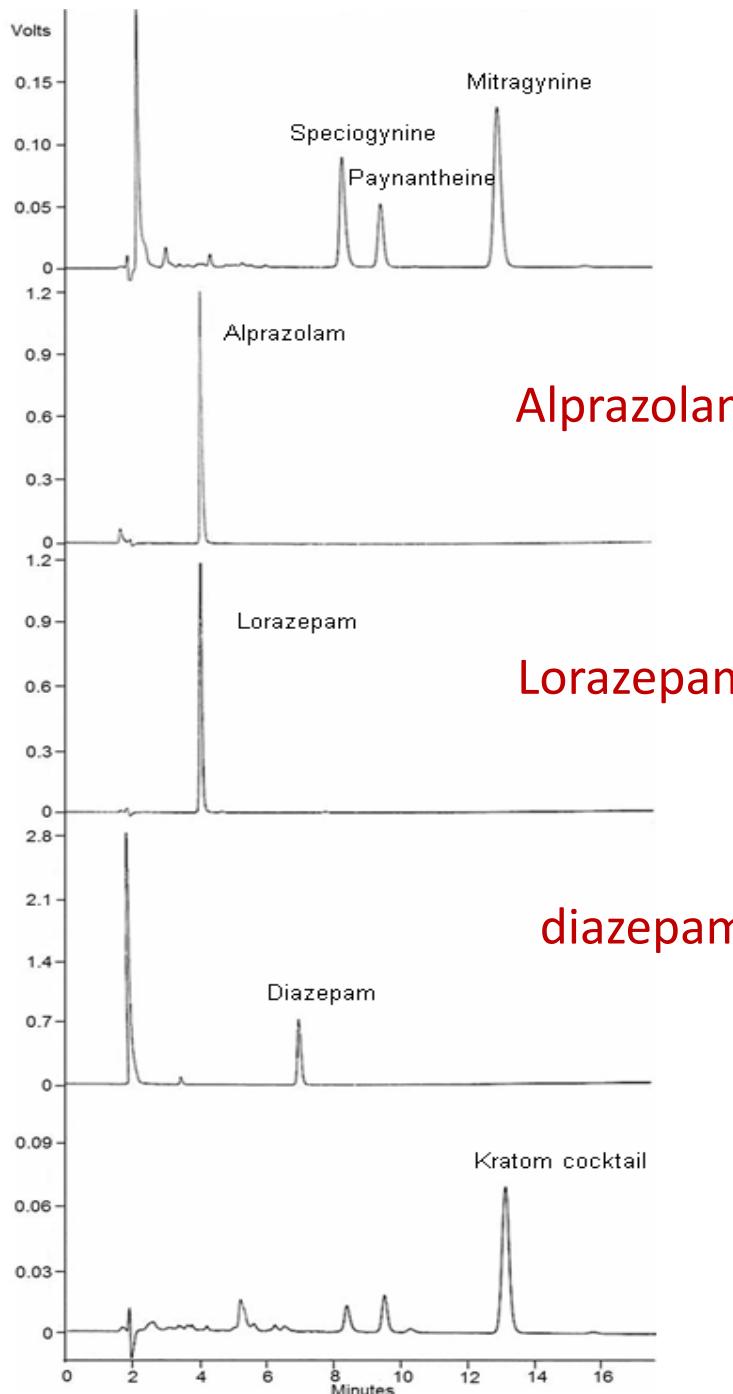
10 ml → Adjust to pH 9 using NH<sub>4</sub>OH (28%)



Partitioned with Chloroform



Pooled and evaporated → HPLC



## Quantification of MG in kratom cocktail

### HPLC analysis

The HPLC system consisted of a Shimadzu model 10 ADVP equipped with an LC-10 ADVP pump, DGU-14A degasser, SIL-10ADVP autosampler, and SPD-10AVP UV-VIS detector (Shimadzu, Kyoto, Japan). The chromatographic separation was obtained on a Verticep™ UPS C<sub>18</sub> column (250 × 4.6 mm i.d., particle size 5 µm) (Nonthaburi, Thailand) using an isocratic elution of acetonitrile/20 mM ammonium acetate (pH 6) (65: 35, v/v) at flow rate 1.0 mL/min. The injection volume was 20 µL. The detection wavelength was 225 nm. Under separation conditions, the LOQs of MG, paynantheine, and speciogynine were 0.649, 0.266, and 0.236 mg/L, respectively.

Limsuwanchote et al. (2017) Forensic Toxicology 35: 167-172



# MEDICAL KRATOM

Stimulant

Combat Fatigue

Pain Relief

Drugs Substitution

AS FOOD



AS DRUG

## FOOD & DRUG INDUSTRY

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การทดสอบความเป็นพิษเฉียบพลัน-  
ก่อเจียบพลัน-เรื้อรัง

การแปรรูปยาในร่างกาย

- อาหาร
- อาหารฟังก์ชัน
- ผลิตภัณฑ์สุขภาพ
- ยาสมุนไพร
- ยาแผนโบราณ
- ยาพัฒนาจากสมุนไพร

ศบก.: ห้องปฏิบัติการควบคุมคุณภาพสมุนไพร

แนวทางปฏิบัติทางเกษตรที่ดี (GAP)

แนวทางปฏิบัติการเก็บเกี่ยวที่ดี (GHP)

แนวทางปฏิบัติการผลิตที่ดี (GMP)

ผ่านการรับรองจาก อย. สธ.

ขึ้นทะเบียน

พรีคลินิก

คลินิก เพส 1

คลินิก เพส 2

คลินิกเพส 3-4

การทดสอบทางคลินิก ในมนุษย์



**ศูนย์วิจัยและพัฒนาผลิตภัณฑ์จากพืชกระท่อม**  
**คณะเภสัชศาสตร์**  
**มหาวิทยาลัยสังฆภานุวัติบูร์**