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# Green extraction protocols of Mitragyna speciosa leaves leading to a possible large scale production

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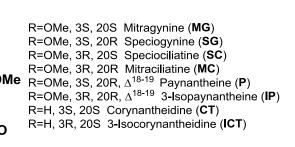
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*Mitragyna speciosa* (K.) H. (*Rubiaceae*), is a tropical tree that is indigenous to Southeast Asia and Indochina. Also known as Kratom, it has been widely used, for hundreds of years, for its stimulant and opioid-like analgesic effects [1]. The principal pharmacologically active alkaloids in kratom leaves include mitragynine (MG), 7-hydroxymitragynine (HMG), speciociliatine (SC), speciogynine (SG) and paynantheine (P) [2]. In recent decades, extractions of *M. speciosa* alkaloids have been performed in various different ways, using either organic solvents or water [3]. The most common methods are maceration in methanol [4] and soxhlet extraction. In a previous work, dried M. speciosa leaves were extracted using ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE) and supercritical carbon dioxide extraction (SFE), using methanol,

ethanol, water and binary mixtures [5]. Of the several methods tested, MAE in a closed vessel (110°C, 60 W, MeOH/H<sub>2</sub>O 1:1) gave the highest alkaloid fraction amount, while UAE with an immersion horn (25°C, 21.4 kHz, 50 W, MeOH) showed the best yield for MG+P. The present study aims to design a green protocol for *alkaloids extraction*, in particular MG, from the leaves using green techniques and solvents both in pretreatment and in extraction steps. For this purpose, we compared several non-conventional techniques (ultrasound, microwave, hydrodynamic cavitation) with classic methods. Dried *M. speciosa* leaves belonging to a *red vein variety from* **Bali** were in some cases pretreated with a phosphate buffer (pH = 7.5) and then extracted with EtOH, EtOH/H<sub>2</sub>O mixture or acidic H<sub>2</sub>O (pH = 3), using UAE, MAE. Moreover, hydrodynamic cavitation (HC) was also used for the scaling-up of the processes, using a pilot scale reactor (Rotocav<sup>®</sup>). Conventional extractions were carried out at rt in a MeOH/H<sub>2</sub>O 1:1 mixture or in EtOH under reflux (exhaustive). In some cases, purified alkaloids were isolated by precipitation (NH<sub>4</sub>OH). All the samples were analyzed using HPLC-DAD for the quantification of the principal alkaloids present





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R=OMe. 3S. 7S. 20S 7-Hvdroxymitragynine (HMC R=OMe, 3R, 7R, 20S 7-Hydroxyspeciociliatine (HSC R=OMe, 3R, 7R, 20R 7-Hydroxymitraciliatine (HMC R=OMe, 3R, 7R, 20R,  $\Delta^{18-19}$  7-Hvdroxvisopavnantheine (HIP

=H, 3S, 7R, 20S Corynoxine B (CB) R=H, 3S, 7S, 20S Corynoxine (**C**) R=H, 3S, 7R, 20R Rhynchophylline (RC R=H. 3S. 7S. 20R Isorhynchophylline (IRC) R=H, 3S, 7R, 20R, ∆<sup>18-19</sup> Corynoxeine (**CÉ** R=H. 3S, 7S, 20R,  $\Delta^{18-19}$  Isocorynoxeine (ICE R=OH. 3S. 7S. 20R Rotundifoline (RT) R=OH, 3S, 7S, 20R,  $\Delta^{18-19}$  Rotundifoleine (**RTe**) R=OH. 3S. 7R. 20R Isorotundifoline (IRT) R=OH, 3S, 7S, 20S Mitrafoline (**MF**) R=OH. 3R. 7R. 20S Speciofoline (SFi R=OH, 3R, 7S, 20S Isospeciofoline (**ISFi** R=OH, 3R, 7S, 20S,  $\Delta^{18-19}$  Isospeciofoleine (**ISFe**) R=OMe, 3S, 7R, 20R,  $\Delta^{18-19}$  Specionoxeine (**SNe**) 7S, 20R,  $\Delta^{18-19}$  Isospecionoxeine (ISN

### Instrumentation

#### based on literature data [6].

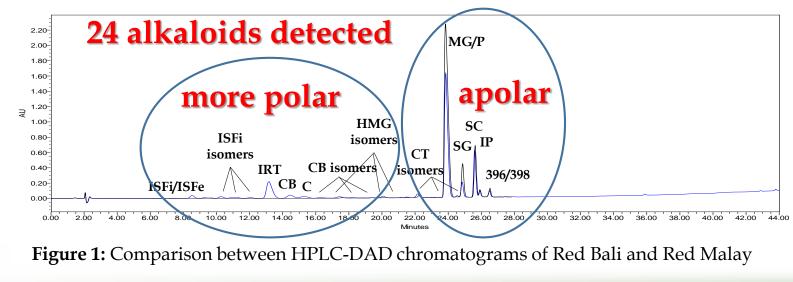
## Red Vein Bali Kratom

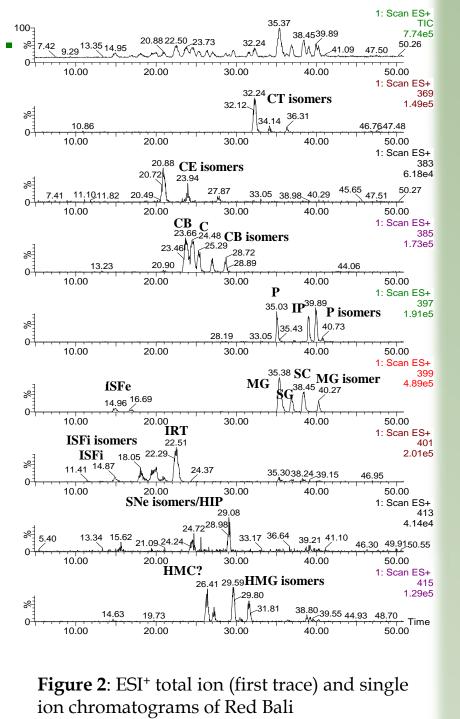
#### HPLC-DAD analyses

- ◆ Instrument: Waters 1525 Binary HPLC pump equipped with 2998 PDA.
- Column: XTerra MS C8 column (4.6 x 150 mm, 5 μm, Waters).
- ♦ Mobile phase: Water with 0.1% TFA (A), and acetonitrile with 0.1% TFA (B) (1 ml/min)
- ✤ Gradient (time, B%): 0.01, 20; 7.5, 20; 15, 30; 26, 60; 39.5, 100; 44, 100.
- Monitoring wavelengths: 222 nm

#### HPLC-MS-MS analyses

- ◆ Instrument: UPLC Acquity Waters system equipped with a Binary Solvent Manager, Sample Manager, Column Manager, a PDA and Micromass Quattro microTM API (triple quadrupole) detectors
- Solvents and column as described before (0.5 ml/min)
- ✤ Gradient (time, B%): 0.01, 20; 15, 20; 30, 30; 52, 60; 68, 100; 80, 100.





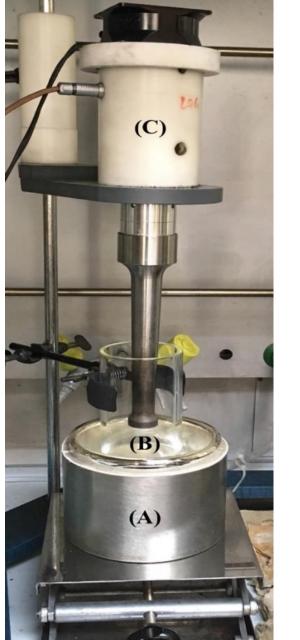
MW

**MicroSYNTH** Milestone **2450 MHz 1000** W



US

### Cup-horn PEX1, R.E.U.S. 25 kHz, 200 W



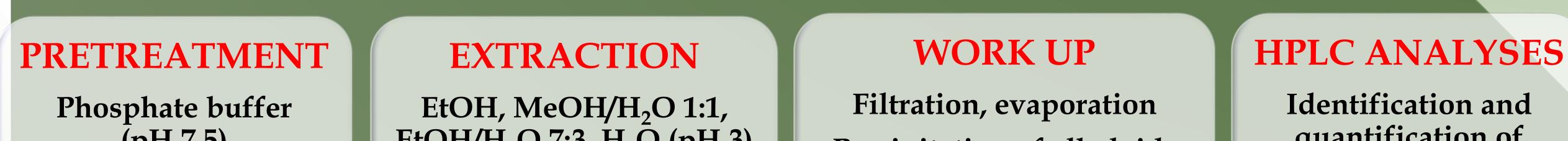
Titanium horn

21 kHz, 250 W



**Pilot scale reactor Rotocav**® 3000 rpm, 3 kW

### **Extraction procedure**



### (pH 7.5) Conv., US-assisted, HC

EtOH/H<sub>2</sub>O 7:3, H<sub>2</sub>O (pH 3) Conv., US-assisted, HC, **MW-assisted** 

**Precipitation of alkaloids** with NH<sub>4</sub>OH and filtration or extraction with CH<sub>2</sub>Cl<sub>2</sub>

### quantification of *MG*+*P* and *total* alkaloids at 222 nm

## **Conventional** solvents

Sample	Pretreatment	Extraction conditions	Work up	Extr. Yield	MG+P/TAlk	MG+P/TAlk
				<b>w/w</b> %	w/w % Ext	mg/g plant
Exaust.	-	EtOH rfx, 2 h	Filtration,	36	3.52/7.9	12.7/28.5
		plant/solv. 1:170	evaporation			
1	-	EtOH, magn. stirr., 2 h	Filtration,	5.4	6.4/14.4	3.5/7.85
		plant/solv. 1:10	evaporation			
2	Rt, magn. stirr., 2 h	EtOH, magn. stirr., 2 h	Filtration,	5.6	7.3/16.4	4.1/9.2
	plant/buffer 1:10	plant/solv. 1:10	evaporation			
3	<b>US horn</b> , <25°C, 15 min	EtOH/H <sub>2</sub> O 7:3, <b>US horn</b> , <25°C	Filtration,	13.0	4.0/8.98	5.2/11.7
	plant/buffer 1:10	15 min, plant/solv. 1:10	evaporation			
4	HC, 20÷50°C, 10 min	EtOH, magn. stirr., 2 h	Filtration,	3.5	11.4/25.6	4.0/9.1
	plant/solv. 1:20	plant/solv. 1:10	evaporation			
5	HC, 20÷50°C, 10 min	EtOH, <mark>US horn</mark> , <25°C	Filtration,	3.7	10.5/23.6	3.9/8.9
	plant/solv. 1:20	15 min, plant/solv. 1:10	evaporation			
6	HC, 20÷50°C, 10 min	EtOH/H <sub>2</sub> O 7:3 <b>, US horn</b> , <25°C	Filtration,	3.8	10.8/24.2	4.1/9.3
	plant/solv. 1:20	15 min, plant/solv. 1:10	evaporation			

Exhaust. = exhaustive extraction. MG+P/Talk = mitragynine and paynanteine amount on total alkaloids

- Best extraction yields and highest MG+P mg/g plant amounts were observed with UAE (with pretreatment using EtOH, without it using acidic  $H_2O$ )
- ◆Highest MG+P w/w % in the extract were obtained using EtOH or EtOH/H<sub>2</sub>O 7:3 mixture (UAE) with HC-assisted pretreatment

## Non conventional solvents

Sample	Pretreatment	Extraction conditions	Work up	Extr. Yield w/w %	MG+P/TAlk w/w % Ext	MG+P/TAlk mg/g plant
Pur. Alk.	-	MeOH/H <sub>2</sub> O 1:1	Ppt with NH <sub>4</sub> OH/	1.2	37.7+4.8/81.7	5.11/9.83
		plant/solv. 1:10	filtration			
1	Rt, magn. stirr., 15 min	H <sub>2</sub> O (pH 3), 50°C, ag. magn., 2 h	Ppt with NH <sub>4</sub> OH/	0.41	0.04/44.5	0.002/1.83
	plant/buffer 1:15	plant/solv. 1:15	extraction with CH <sub>2</sub> Cl <sub>2</sub>			
2	Rt, magn. stirr., 15 min	H <sub>2</sub> O (pH 3), MW rfx, 30 min	Ppt with NH <sub>4</sub> OH/	0.214	9.75/43.6	0.21/0.93
	plant/buffer 1:15	plant/solv. 1:15	extraction with $CH_2Cl_2$			
3	US horn, <25°C, 15 min	H <sub>2</sub> O (pH 3), US horn, <25°C, 15 min	Ppt with NH <sub>4</sub> OH/	0.178	24.2/46,9	0.43/0.83
	plant/buffer 1:15	plant/solv. 1:15	extraction with CH <sub>2</sub> Cl <sub>2</sub>			
4	US horn, <25°C, 15 min	H <sub>2</sub> O (pH 3), US horn, <25°C, 30 min,	Ppt with NH <sub>4</sub> OH/	0.156	26.5/50.0	0.41/0.78
	plant/buffer 1:15	plant/solv. 1:15	filtration			
5	US horn, <25°C, 15 min	H <sub>2</sub> O (pH 3), US horn, <25°C, 30 min,	Extraction with CH <sub>2</sub> Cl <sub>2</sub>	0.210	10.5/47.9	0.22/1.01
	plant/buffer 1:15	plant/solv. 1:15	aqueous phase sample <b>4</b>			
6	US horn, <25°C, 15 min	H <sub>2</sub> O (pH 3), US horn, <25°C, 60 min	Ppt with NH <sub>4</sub> OH/	0.067	26.1/46.0	0.17/0.31
	plant/buffer 1:15	plant/solv. 1:15	filtration			
7	US horn, <25°C, 15 min	H <sub>2</sub> O (pH 3), US horn, <25°C, 60 min	Extraction with CH <sub>2</sub> Cl <sub>2</sub>	0.145	14.0/47.8	0.20/0.70
	plant/buffer 1:15	plant/solv. 1:15	aqueous phase sample <b>6</b>			
8	US Reus, <25°C, 15 min	H <sub>2</sub> O (pH 3), US Reus, 20÷50°C, 15 min	Ppt with NH <sub>4</sub> OH/	0.116	39.3/69.4	0.46/0.80
	plant/buffer 1:15	plant/solv. 1:15	filtration			
9	<b>US Reus</b> , <25°C, 15 min	H <sub>2</sub> O (pH 3), <b>US Reus</b> , 20÷50°C, 30 min	Ppt with NH <sub>4</sub> OH/	0.307	11.9/46.5	0.37/1.43
	plant/buffer 1:15	plant/solv. 1:15	filtration			
10	-	H <sub>2</sub> O (pH 3), <b>US Reus</b> , 20÷50°C, 15min	Ppt with NH <sub>4</sub> OH/	0.284	23.0/41.1	0.65/1.17
		plant/solv. 1:15	filtration			
11	US horn, <25°C, 15 min	H <sub>2</sub> O (pH 3), US horn, 20÷50°C, 30 min	Ppt with NH <sub>4</sub> OH/	0.264	18.5/52.8	0.49/1.39
	plant/buffer 1:15	plant/solv. 1:15	filtration			
12	-	H <sub>2</sub> O (pH 3), <b>US horn</b> , 20÷50°C, 30 min	Ppt with NH <sub>4</sub> OH/	0.410	34.9/59.2	1.43/2.42
		plant/solv. 1:15	filtration			
13	-	H <sub>2</sub> O (pH 3), <b>US horn</b> , 20÷50°C, 30 min	Ppt with NH <sub>4</sub> OH/	0.426	35.1/61.0	1.49/2.60
		plant/solv. 1:20	filtration			
14	-	H <sub>2</sub> O (pH 3), HC, 20÷45°C, 10 min	Ppt with NH <sub>4</sub> OH/	1.2	3.25/8.7	0.39/1.12
		plant/solv. 1:20	extraction with CH <sub>2</sub> Cl <sub>2</sub>			

- ◆UAE, both with titanium horn or cup horn, increased dramatically the solubility of apolar alkaloids (in particular, MG+P) in acidic H<sub>2</sub>O ★MW using acidic H<sub>2</sub>O did not affect positively extraction yields and alkaloids amounts in the extract
- Total alkaloids content (w/w % in the extract or mg/g plant) obtained in ethanolic UAE were comparable to exhausive ethanolic conventional protocol, while H<sub>2</sub>O (pH 3) afforded alkaloid amounts quite far from the purified sample obtained with MeOH/H<sub>2</sub>O

conventional extraction

Pur. Alk. = purified alkaloids. MG+P/Talk = mitragynine and paynanteine amount on total alkaloids

**COMPARABLE YIELDS FOR UAE TO CONVENTIONAL PROTOCOLS \***LOWER AMOUNTS OF SOLVENTS USED, LOWER EXTRACTION TIMES **AND TEMPERATURES** Conclusions **GENERALLY INCREASED PURITY OF FINAL EXTRACT WITH THE** PHOSPHATE BUFFER PRETRATMENT **\* POSSIBLE EASY SCALE-UP OF UAE USING THE HC REACTOR** 

### References

#### [1] Brown, P.N.; Lund, J.A.; Murch, S.J. A botanical, phytochemical and ethnomedicinal review of the genus Mitragyna Korth: Implications for products sold as kratom. Journal of Ethnopharmacology, 2017, 202, 302–325. [2] Barceloux D.G. (2012) Kratom [Mitragyna speciosa (Korth.) Havil.]. In Medical Toxicology of Drug Abuse: Synthesized Chemicals and Psychoactive Plants. John Wiley & Sons Inc. (Ed.). Hoboken, NJ, 880-885. [3] Kumarnsit E.; Keawpradub N.; Nuankaew W. Effect of Mitragyna speciosa aqueous extract on ethanol withdrawal symptoms in mice, Fitoterapia, 2007, 78, 182–185. [4] Kumarnsit E.; Keawpradub N.; Nuankaew W. Acute and long-term effects of alkaloid extract of *Mitragyna speciosa* on food and water intake and body weight in rats, *Fitoterapia*, 2006, 77, 339–345. [5] Orio L.; Alexandru L.; Cravotto G.; Mantegna S.; Barge A. UAE, MAE, SFE-CO<sub>2</sub> and classical methods for the extraction of Mitragyna speciosa leaves. Ultrasonics Sonochemistry, 2012, 19, 591–595. [6] Boffa, L.; Ghe, C.; Barge, A.; Muccioli, G.; Cravotto, G. Alkaloid profiles and activity in different Mitragyna speciosa strains. Natural Product Communications, 2018, 13, 9, 1111-1116.

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