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

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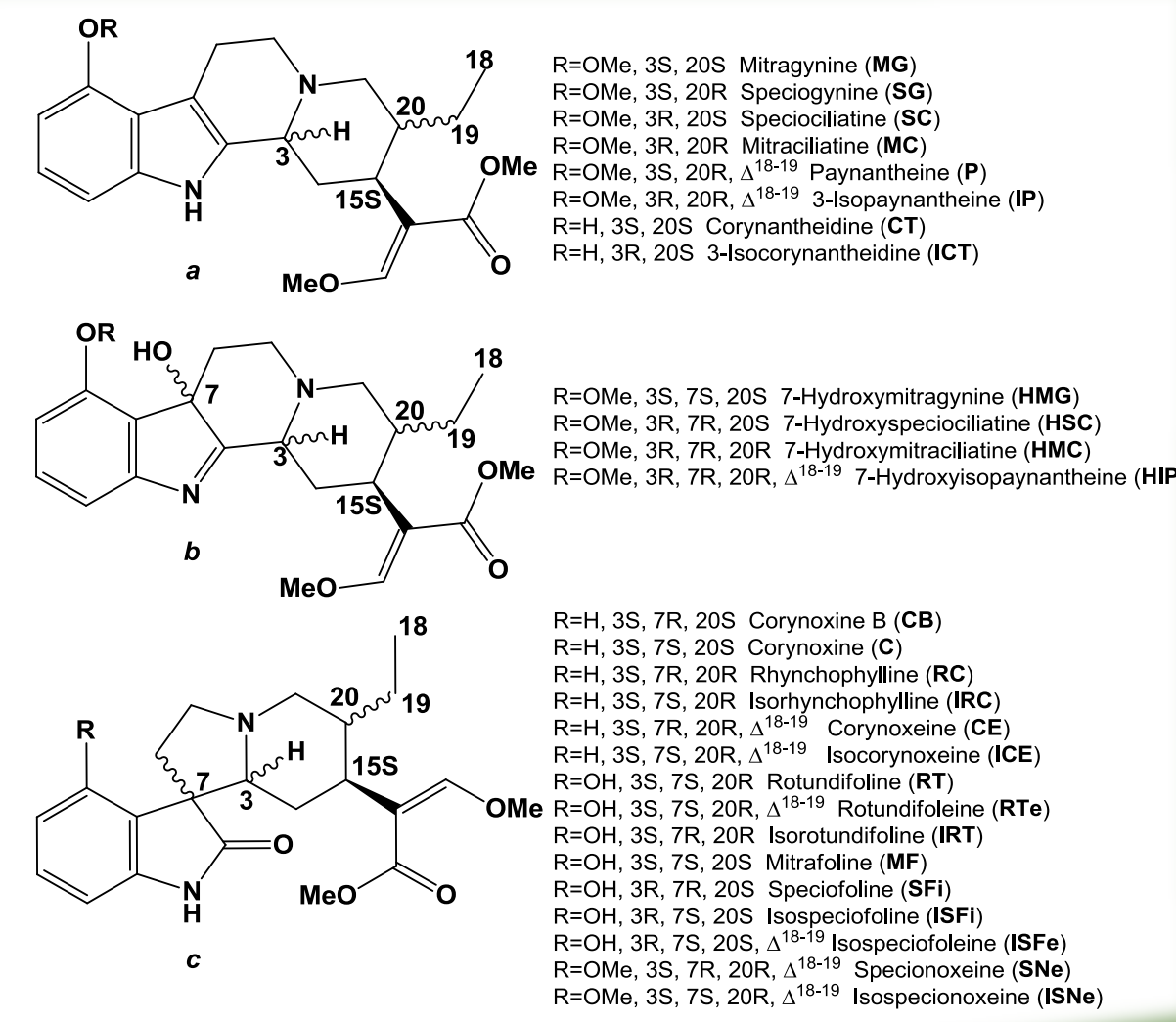
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*Mitragyna speciosa* (K.) H. (Rubiaceae), is a tropical tree that is indigenous to Southeast Asia and Indochina. Also known as Kratom, it has been widely used, for hundreds of years, for its **stimulant and opioid-like analgesic effects** [1]. The 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®). 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 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.

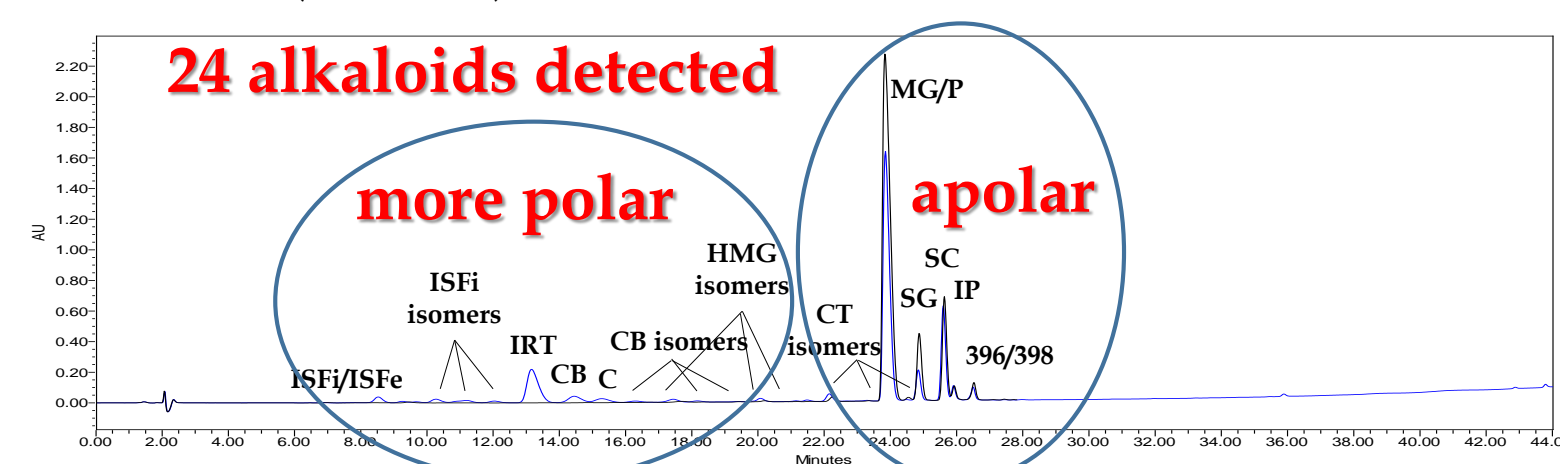


Figure 1: Comparison between HPLC-DAD chromatograms of Red Bali and Red Malay

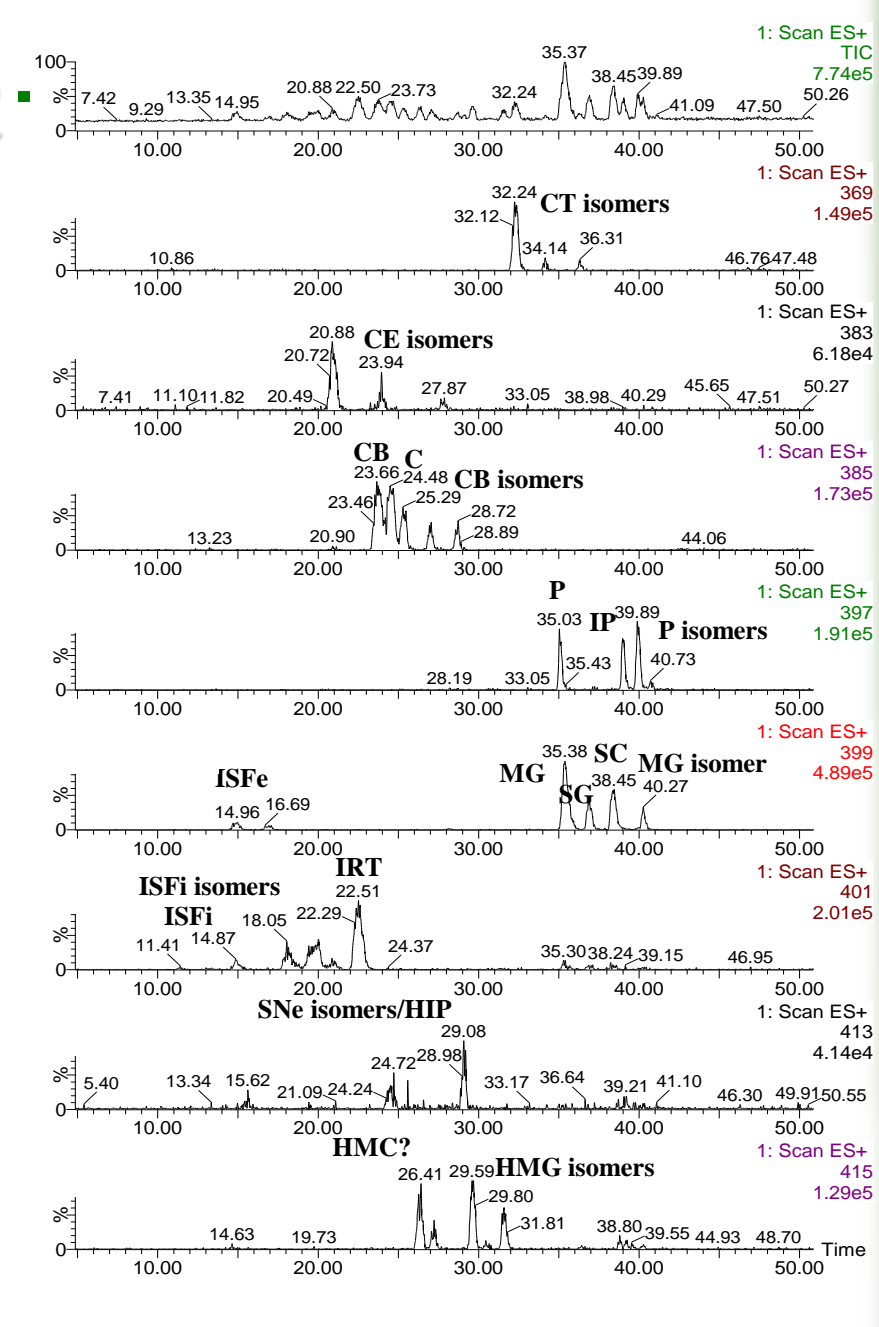


Figure 2: ESI+ total ion (first trace) and single ion chromatograms of Red Bali

**MW**  
MicroSYNTH  
Milestone  
2450 MHz  
1000 W



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

**US**



**Titanium horn**  
21 kHz, 250 W

## Instrumentation



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

## Extraction procedure

### PRETREATMENT

Phosphate buffer  
(pH 7.5)

Conv., US-assisted,  
HC

### EXTRACTION

EtOH, MeOH/H<sub>2</sub>O 1:1,  
EtOH/H<sub>2</sub>O 7:3, H<sub>2</sub>O (pH 3)

Conv., US-assisted, HC,  
MW-assisted

### WORK UP

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

### HPLC ANALYSES

Identification and  
quantification of  
MG+P and total  
alkaloids at 222 nm

## Conventional solvents

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

Exhaust. = exhaustive extraction. MG+P/Talk = mitragynine and paynantheine 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<sub>2</sub>O)
- 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
- 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 exhaustive 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

## 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 plant/solv. 1:10	Ppt with NH <sub>4</sub> OH/ filtration	1.2	37.7+4.8/81.7	5.11/9.83
1	Rt, magn. stirr., 15 min	H <sub>2</sub> O (pH 3), 50°C, ag. magn., 2 h plant/buffer 1:15	Ppt with NH <sub>4</sub> OH/ extraction with CH <sub>2</sub> Cl <sub>2</sub>	0.41	0.04/44.5	0.002/1.83
2	Rt, magn. stirr., 15 min	H <sub>2</sub> O (pH 3), MW rfx, 30 min plant/buffer 1:15	Ppt with NH <sub>4</sub> OH/ extraction with CH <sub>2</sub> Cl <sub>2</sub>	0.214	9.75/43.6	0.21/0.93
3	US horn, <25°C, 15 min	H <sub>2</sub> O (pH 3), US horn, <25°C, 15 min plant/buffer 1:15	Ppt with NH <sub>4</sub> OH/ extraction with CH <sub>2</sub> Cl <sub>2</sub>	0.178	24.2/46.9	0.43/0.83
4	US horn, <25°C, 15 min	H <sub>2</sub> O (pH 3), US horn, <25°C, 30 min, plant/buffer 1:15	Ppt with NH <sub>4</sub> OH/ filtration	0.156	26.5/50.0	0.41/0.78
5	US horn, <25°C, 15 min	H <sub>2</sub> O (pH 3), US horn, <25°C, 30 min, plant/solv. 1:15	Extraction with CH <sub>2</sub> Cl <sub>2</sub> aqueous phase sample 4	0.210	10.5/47.9	0.22/1.01
6	US horn, <25°C, 15 min	H <sub>2</sub> O (pH 3), US horn, <25°C, 60 min plant/buffer 1:15	Ppt with NH <sub>4</sub> OH/ filtration	0.067	26.1/46.0	0.17/0.31
7	US horn, <25°C, 15 min	H <sub>2</sub> O (pH 3), US horn, <25°C, 60 min plant/buffer 1:15	Extraction with CH <sub>2</sub> Cl <sub>2</sub> aqueous phase sample 6	0.145	14.0/47.8	0.20/0.70
8	US Reus, <25°C, 15 min	H <sub>2</sub> O (pH 3), US Reus, 20±50°C, 15 min plant/buffer 1:15	Ppt with NH <sub>4</sub> OH/ filtration	0.116	39.3/69.4	0.46/0.80
9	US Reus, <25°C, 15 min	H <sub>2</sub> O (pH 3), US Reus, 20±50°C, 30 min plant/buffer 1:15	Ppt with NH <sub>4</sub> OH/ filtration	0.307	11.9/46.5	0.37/1.43
10	-	H <sub>2</sub> O (pH 3), US Reus, 20±50°C, 15 min plant/solv. 1:15	Ppt with NH <sub>4</sub> OH/ filtration	0.284	23.0/41.1	0.65/1.17
11	US horn, <25°C, 15 min	H <sub>2</sub> O (pH 3), US horn, 20±50°C, 30 min plant/buffer 1:15	Ppt with NH <sub>4</sub> OH/ filtration	0.264	18.5/52.8	0.49/1.39
12	-	H <sub>2</sub> O (pH 3), US horn, 20±50°C, 30 min plant/solv. 1:15	Ppt with NH <sub>4</sub> OH/ filtration	0.410	34.9/59.2	1.43/2.42
13	-	H <sub>2</sub> O (pH 3), US horn, 20±50°C, 30 min plant/solv. 1:20	Ppt with NH <sub>4</sub> OH/ filtration	0.426	35.1/61.0	1.49/2.60
14	-	H <sub>2</sub> O (pH 3), HC, 20±45°C, 10 min plant/solv. 1:20	Ppt with NH <sub>4</sub> OH/ extraction with CH <sub>2</sub> Cl <sub>2</sub>	1.2	3.25/8.7	0.39/1.12

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

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

## Conclusions

## References

## Acknowledgements

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