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

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

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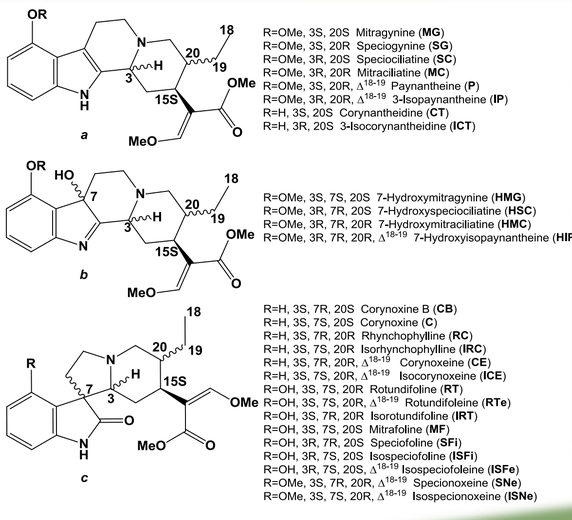
Mitragyna speciosa (K.) H. (*Rubiaceae*), is a tropical tree that is indigenous to Southeast Asia and Indochina. Also known as Kratom, it has been widely used, for hundreds of years, for its ***stimulant and opioid-like analgesic effects*** [1]. The principal pharmacologically active alkaloids in kratom 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_2O 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_2O mixture or acidic H_2O (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_2O 1:1 mixture or in EtOH under reflux (exhaustive). In some cases, purified alkaloids were isolated by precipitation (NH_4OH). 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.

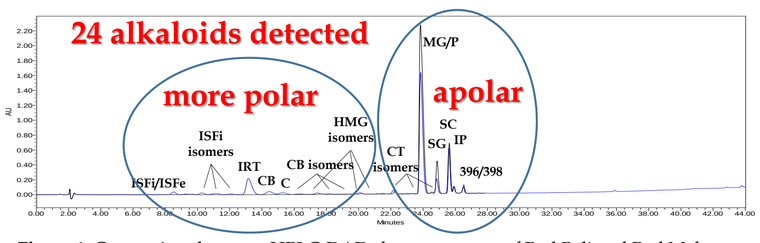


Figure 1: Comparison between HPLC-DAD chromatograms of Red Bali and Red Malay

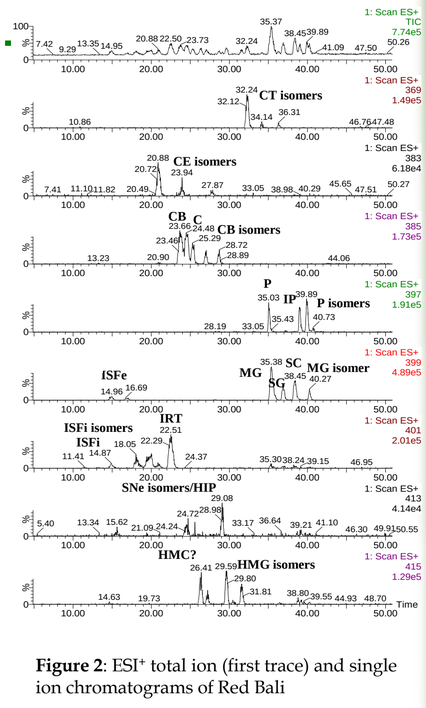


Figure 2: ESI+ total ion (first trace) and single ion chromatograms of Red Bali

Instrumentation

MW

MicroSYNTH Milestone 2450 MHz 1000 W

HC

Pilot scale reactor Rotocav® 3000 rpm, 3 kW

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

US

Titanium horn 21 kHz, 250 W

Extraction procedure

PRETREATMENT

Phosphate buffer (pH 7.5)

Conv., US-assisted, HC

EXTRACTION

EtOH, MeOH/H_2O 1:1, EtOH/H_2O 7:3, H_2O (pH 3)

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

WORK UP

Filtration, evaporation

Precipitation of alkaloids with NH_4OH and filtration or extraction with CH_2Cl_2

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
Exaust.	-	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 plant/buffer 1:10	EtOH, magn. stirr., 2 h plant/solv. 1:10	Filtration, evaporation	5.6	7.3/16.4	4.1/9.2
3	**US horn**, <25°C, 15 min plant/buffer 1:10	EtOH/H_2O 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 plant/solv. 1:20	EtOH, magn. stirr., 2 h plant/solv. 1:10	Filtration, evaporation	3.5	**11.4/25.6**	4.0/9.1
5	**HC**, 20÷50°C, 10 min plant/solv. 1:20	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 plant/solv. 1:20	EtOH/H_2O 7:3, **US horn**, <25°C 15 min, plant/solv. 1:10	Filtration, evaporation	3.8	**10.8/24.2**	4.1/9.3

Exaust. = 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_2O 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_2O
- MW using acidic H_2O 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_2O (pH 3) afforded alkaloid amounts quite far from the purified sample obtained with MeOH/H_2O 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_2O 1:1 plant/solv. 1:10	Ppt with NH_4OH/ filtration	**1.2**	**37.7+4.8/81.7**	**5.11/9.83**
1	Rt, magn. stirr., 15 min plant/buffer 1:15	H_2O (pH 3), 50°C, ag. magn., 2 h plant/solv. 1:15	Ppt with NH_4OH/ extraction with CH_2Cl_2	**0.41**	0.04/44.5	0.002/1.83
2	Rt, magn. stirr., 15 min plant/buffer 1:15	H_2O (pH 3), MW rfx, 30 min plant/solv. 1:15	Ppt with NH_4OH/ extraction with CH_2Cl_2	**0.214**	9.75/43.6	0.21/0.93
3	US horn, <25°C, 15 min plant/buffer 1:15	H_2O (pH 3), US horn, <25°C, 15 min plant/solv. 1:15	Ppt with NH_4OH/ extraction with CH_2Cl_2	**0.178**	24.2/46,9	0.43/0.83
4	US horn, <25°C, 15 min plant/buffer 1:15	H_2O (pH 3), US horn, <25°C, 30 min, plant/solv. 1:15	Ppt with NH_4OH/ filtration	**0.156**	26.5/50.0	0.41/0.78
5	US horn, <25°C, 15 min plant/buffer 1:15	H_2O (pH 3), US horn, <25°C, 30 min, plant/solv. 1:15	Extraction with CH_2Cl_2 aqueous phase sample **4**	**0.210**	10.5/47.9	0.22/1.01
6	US horn, <25°C, 15 min plant/buffer 1:15	H_2O (pH 3), US horn, <25°C, 60 min plant/solv. 1:15	Ppt with NH_4OH/ filtration	**0.067**	26.1/46.0	0.17/0.31
7	US horn, <25°C, 15 min plant/buffer 1:15	H_2O (pH 3), US horn, <25°C, 60 min plant/solv. 1:15	Extraction with CH_2Cl_2 aqueous phase sample **6**	**0.145**	14.0/47.8	0.20/0.70
8	US Reus, <25°C, 15 min plant/buffer 1:15	H_2O (pH 3), US Reus, 20÷50°C, 15 min plant/solv. 1:15	Ppt with NH_4OH/ filtration	**0.116**	39.3/69.4	0.46/0.80
9	**US Reus**, <25°C, 15 min plant/buffer 1:15	H_2O (pH 3), **US Reus**, 20÷50°C, 30 min plant/solv. 1:15	Ppt with NH_4OH/ filtration	**0.307**	11.9/46.5	0.37/1.43
10	-	H_2O (pH 3), **US Reus**, 20÷50°C, 15min plant/solv. 1:15	Ppt with NH_4OH/ filtration	0.284	23.0/41.1	0.65/1.17
11	US horn, <25°C, 15 min plant/buffer 1:15	H_2O (pH 3), US horn, 20÷50°C, 30 min plant/solv. 1:15	Ppt with NH_4OH/ filtration	**0.264**	18.5/52.8	0.49/1.39
12	-	H_2O (pH 3), **US horn**, 20÷50°C, 30 min plant/solv. 1:15	Ppt with NH_4OH/ filtration	0.410	34.9/59.2	1.43/2.42
13	-	H_2O (pH 3), **US horn**, 20÷50°C, 30 min plant/solv. 1:20	Ppt with NH_4OH/ filtration	0.426	35.1/61.0	1.49/2.60
14	-	H_2O (pH 3), HC, 20÷45°C, 10 min plant/solv. 1:20	Ppt with NH_4OH/ extraction with CH_2Cl_2	1.2	3.25/8.7	0.39/1.12

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

Conclusions

- **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 PRETRATMENT**
- **POSSIBLE EASY SCALE-UP OF UAE USING THE HC REACTOR**

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