UNIVERSITY OF MALAYA EXTRACTION OF Mitragyna speciosa

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Abstract

Mitragyna speciosa is a narcotic plant but with specific medicinal importance. The main components of the leaves of *M. speciosa* are indole alkaloids. Previous scientific researches have shown that the alkaloidal extracts possess very potent anti-inflammatory, analgesic and opioid properties. The three most abundant indoles are mitragynine, paynanthine and speciogynine - the first two of which appear to be unique to this species. All these compounds were obtained from classical organic solvent (diethyl acetate, dichloromethane or methanol) extraction. In this research programme, supercritical fluid extraction (SFE) of *M. speciosa* was performed. An optimal condition of supercritical fluid extraction was developed using an analytical-scale SFE system. The conventional extraction methods were conducted in parallel for comparison. CO₂ extract of *M. speciosa* has never been studied yet and CO₂ was chosen as the media of extraction because it is non-toxic and environmentally safe to prepare and use.

Introduction

In view of increasing environmental and health concern about the use of organic solvents in the extraction of natural products, there has been growing interest in using supercritical fluids. The supercritical extraction using carbon dioxide in the supercritical state, i. e. pressure > 73.8 bar and temperature > 31.06 °C, allows extracts to be obtained that are of better quality than those extracted with organic solvents, whose residues, even if present as traces, contaminate the extract. CO₂ is a non-toxic solvent and can be easily and completely removed from products; moreover it possesses unusual properties such as high compressibility, liquid-like density, high diffusivity, low viscosity and low surface tension under supercritical condition. So, supercritical fluid shows a greater ability to diffuse into the ultrafine matrix than the conventional organic solvents, thus improving extraction yield of desired materials from complex matrices.¹

Mitragyna belongs to the family Rubiacea and is found in tropical and subtropical regions of Asia and Africa. *Mitragyna speciosa* is found growing in Thailand and Malaysia. In Malaysia it is found in the northern states of Kedah and Perlis as well as in the East coast states and is commonly known as Ketum or Biak by the local Malays. *Mitragyna speciosa* has many applications in traditional use. There are descriptions of its use as a tonic, cure for fever, treatment for diarrhea and as morphine substitute.^{2, 3} In Thailand, leaf of this plant has been consumed for its opium-like effect and its coca-like stimulant ability to combat fatigue and enhance tolerence to hard work under a scoring sun.^{2, 4, 5}

In the present work we show the results concerning the extraction of crude from the leaves of *M. speciosa*, by means of supercritical carbon dioxide, in a single extraction step. To the best of our knowledge, this is the first report on CO_2 supercritical fluid extract of *M. speciosa*.

Experimental

Fresh leaves and barks of *M. speciosa* were collected from the forest in Sg.Setong, Jelawang, Kelantan on the 5th of November 2006. The voucher specimens KL 5321 were deposited at the Herbarium of the Department of Chemistry, University of Malaya. The dried and ground leaves were accurately weighed, then extracted by the following methods:

SFE method : In this typical experimental study, about 25 g material was placed in 100 ml extraction vessel of the analytical-scale SFT 100 XW SFE system (Fig. 1). CO2 and polar modifier were delivered by SFT-10 Constant Pressure pump and a Series II Isocratic HPLC pump, respectively. The system conditions studied were at 40°C and 5000 psi. Onestep extractions were performed by feeding CO2 (9.8 % purity, SFE grade) into the system until the pressure of 5000 psi was attained and the system temperature was set to 40°C. After 30 minutes of static extraction (no liquid flow), the sample material was subjected to dynamic extraction by flowing liquid CO2 at a rate of 3 ml/min. During the extraction, 2 to 4 crude fractions were collected in the vial. Each fraction took between 4-7 minutes to extract. The extraction of the crude was terminated when it was visually observed that no extract was being collected in the vial and is termed as exhaustive extraction (Table 1). The extracts obtained were concentrated under reduced pressure, yielding dark green residue. Conventional extraction method: Extraction was carried out by cold percolation. The plant material (1 kg) was first sprinkled with 25% ammonia (NH₃) solution and left to soak for an hour. Later it was extracted with ethyl acetate (EA) for four days at room temperature then filtered. Thereafter, the solvent was evaporated to dryness under reduced pressure to afford a dark green coloured sludge.

All crude extracts were sent for ¹H NMR experiment and the composition of individual chromatographic was checked by thin layer chromatographic (TLC, Figure 2).

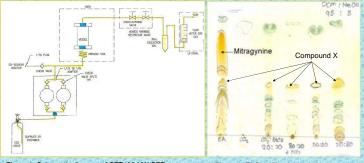


Figure 1. Schematic diagram of SFT100 XW SFE system

Figure 2. TLC profile of the crude extracts

Results and discussions

Preliminary experiments were carried out in order to select relevant factors as well as their experimental domain to obtain the highest crude extraction recovery. Because the yield extracted by pure supercritical carbon dioxide was not satisfactory, it was thought that increasing the polarity of the extraction solvent might overcome the low yield. Ethanol (95%) was chosen as modifier in this study and the concentrations used were 20, 50 and 80% (v/v), in liquid carbon dioxide. The higher the percentages of modifier, the higher was the extraction yield achieved. The amounts of crude extracts obtained by SFE method using various ratio of modifier and by percolation were listed in Table 1.

Table 1. Percentage of yields by different methods and conditions of extraction

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	Factors				
Method	Pressure (psi)	Temperature (°C)	Static extraction (minute)	Modifier (ethanol %) ^a	Yield (%) ^b
SFE (25.0977 g dried material)	5000	40	30	0	0.17
SFE (25.2715 g dried material)	5000	40	30	20	1.06
SFE (25.1647 g material saturated with NH ₃	5000	40	30	20	4.05
SFE (25.0209 g dried material)	5000	40	30	50	3.54
SFE (25.0287 g dried material)	5000	40	30	80	8.92
Conventional extraction (1000 g material percolated with ethyl acetate	-	-	-	-	6.63
	SFE (25.0977 g dried material) SFE (25.2715 g dried material) SFE (25.1647 g material saturated with NH ₃ SFE (25.0209 g dried material) SFE (25.0287 g dried material) Conventional extraction (1000 g material percolated	SFE 5000 (25.0977 g dried material) 5000 SFE 5000 (25.2715 g dried material) 5000 SFE 5000 (25.1647 g material saturated with NH ₃ 5000 SFE 5000 (25.0209 g dried material) 5000 SFE 5000 (25.0209 g dried material) 5000 SFE 5000 (25.0287 g dried material) 5000 Conventional extraction 0 (1000 g material percolated -	Fressule (Jsf)Freingle autre (°C)SFE (25.0977 g dried material)500040SFE (25.1647 g material saturated with NH3500040SFE (25.0209 g dried material)500040SFE (25.0209 g dried material)500040SFE (25.0287 g dried material)500040SFE (25.0287 g dried material)500040Conventional extraction (1000 g material percolated	MethodPressure (psi)Temperature (°C)Static extraction (minute)SFE50004030(25.0977 g dried material)50004030SFE50004030(25.1647 g material saturated with NH350004030SFE50004030(25.0209 g dried material)50004030SFE50004030(25.0209 g dried material)50004030SFE50004030(25.0287 g dried material)50004030Conventional extraction (1000 g material percolated	MethodPressure (psi)Temperature (°C)Static extraction (minute)Modifier (ethanol %)aSFE (25.0977 g dried material)50004003000SFE (25.2715 g dried material)500040030020SFE (25.1647 g material saturated with NH4, SFE500040030020SFE (25.0209 g dried material)500040030020SFE (25.0209 g dried material)500040030050SFE (25.0287 g dried material)500040030080Conventional extraction (1000 g material percolated)

^a Modifier (ethanol %) = volume of added ethanol (ml)/volume of extraction vessel (100 ml) ^b Extraction yield (%) = (the amount of crude extract/sample mass) x 100

Alkaloid X with the R_f value of 0.44 was detectable in each extract with TLC (developed in dichloromethane-methanol, 95:5, v/v) except in the SFE crude using only CO₂ (Figure 2). Using ethanol as modifier, the TLC patterns indicated the presence of similar compounds and the concentration of the alkaloidal zone was proportional to the concentration of the modifier. Only alkaloidal bands were significantly shown by the crude from material that has been saturated with NH₃ where as from the percolation extract, different type of alkaloids and other compounds have been extracted out which were not detected from the TLC of SFE crude. In this study, all crude extracts showed the similar patterns of NMR spectrum.

Conclusions

Comparison of the TLC of SFE extracts with that of the EA extract gave the followings :

- No alkaloid was extracted out with 100% CO₂.
- Alkaloids were extracted with eluent system using EtOH as co-solvent; 80% CO₂: 20% EtOH, 50% CO₂: 50% EtOH and 20% CO₂: 80% EtOH.
- Interestingly the major alkaloid found in the EA extract, mitragynine, was not extracted in all the SFE extracts.

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