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Der Pharma Chemica, 2013, 5(5):131-138
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Determination of mitragynine for the identification of *mitragyna* species in Kedah (Malaysia) by gas chromatography-mass spectrometry

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ABSTRACT

Analysis of mitragynine in different species of mitragyna, indigenous plant of peninsular Malaysia is an important area of research due to the use of this compound as a drug of abuse. A fast, selective and reproducible ultrasonic assisted extraction (UAE) coupled with gas chromatography-mass spectrometry (GC-MS) method was developed for the determination of mitragynine in different species of mitragyna (mitragynaspeciosa and mitragynarotundifolia). Three samples of mitragyna species, namely KM, KY1 and KY2 were collected from Kedah state of Malaysia and identified as a mitragynine-containing species which are authenticated by botanists. UAE was carried out with chloroform-methanol (1:4-v/v) as extracting solvent at 30℃ and 1.0 h sonication time. The GC-MS analyses of extracted samples confirmed the presence of mitragynine in KM and KY1 samples, while mitragynine was not detected in KY2 samples. Under optimized conditions, the quantitative estimation of mitragynine in KM and KY1 samples were found to be 0.094% and 0.105%, respectively.

Keywords: Mitragynine, *Mitragyna*speciosa, Ultrasonic assisted extraction (UAE), Gas Chromatography Mass Spectrometry (GC-MS).

INTRODUCTION

Mitragynine (9-methoxy-corynantheidine) is an indole alkaloid (Figure 1), commonly present in indigenous plant, *Mitragynaspeciosa* (ketum). The leaves of this plant are known to produce narcotic-like actions when smoked or chewed and have been used traditionally as a stimulant like coca or as a substitute for opium [1]. Its coca-like stimulant has ability to combat fatigue and enhances tolerance to hard work under scoring sun and they are also useful for the treatment of fever, diarrhea and substitute for morphine in treating addicts [2-4]. Moreover, mitragynine has an antinociceptive action through supra spinal opiod receptors, descending noradregenic and serotonergic systems [5,6]. Besides, the crucial role of mitragynine, this plant has been used as drugs of abuse and strictly banned in most of the countries. Therefore, the identification of mitragynine-containing species is essential for forensic toxicological screening and to control the abuse of an indigenous plant. Various species of Mitragyna are present in Kedah state of peninsular Malaysia and the alkaloid content of the same species may vary, based on different geographical origin [1,7-10]. Moreover, most of the species are morphologically similar, causing difficulties to identify the mitragynine containing species and create a great confusion to take the legal action on

offenders. Literature survey indicates that, few reports are available for the determination of mitragynine in *Mitragynaspeciosa* plants of different regions of Malaysia and Thailand by using liquid chromatographic and gas chromatographic methods [10-12].However, no work has been carried out for the determination of mitragynine in different species of mitragyna by using GC-MS method. Extraction of mitragynine is an integral part for the analysis.Various techniques like, ultrasonic-assisted extraction (UAE), microwave-assisted extraction (MAE), supercritical fluid extraction (SFE) and accelerated solvent extraction (ASE) have been developed for the extraction of valuable compounds from plants [13]. Among these techniques ultrasonic-assisted extraction (UAE) has good reputation due to less organic solvent consumption, less time consuming, highly efficient and good percentage of yield [14-16].Therefore, attempts have been made to develop a suitable UAE and GC-MS method for the determination of mitragynine in different species of mitragyna, commonly present in Kedah state of Malaysia and the results are discussed herein.

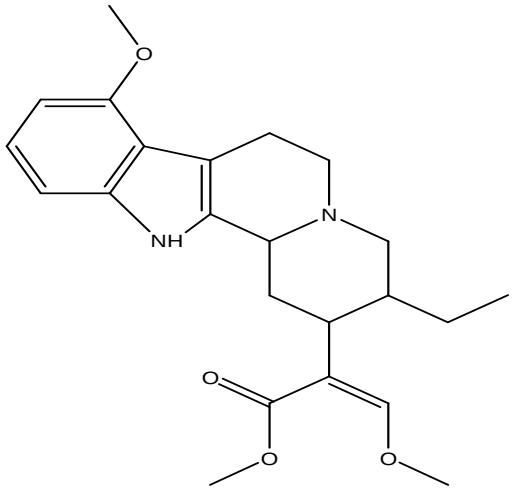

Figure 1.Chemical structure of mitragynine

MATERIALS AND METHODS

Materials, Chemicals and Instruments

Fresh leaves of mitragyna species (*Mitragynaspeciosa* and *Mitragynarotundifolia*) were collected from Kedah, Malaysia. Samples were marked as KM, KY1 and KY2. These samples were authenticated by botanist from Department of Botany, Faculty of Forestry, Universiti Putra Malaysia (UPM), Serdang, Malaysia. Standard of Mitragynine was purchased from ChromaDex (USA), Chloroform (AR grade) of Merck (Darmstadt, Germany) and methanol (HPLC grade) was purchased from QReC (Auckland, New Zealand). Standard solution of mitragynine (1000 µg/mL) was prepared in methanol and stored in freezer at -4°C. Working standard solutions of lower concentration were prepared by dilution with methanol. Ultrasonic-assisted extractions of the samples were carried out in an ultrasonic cleaning bath equipped with digital timer and temperature controller (model- JAC-2010) of Kodo Technical Research Co. Ltd, South Korea and Savant speedvac concentrator (model-SPD2010) of Waltham MA, USA.Whatman 0.45 µm Nylon membrane filter were purchased from(Maidstone, England). GC-MS analyses were carried out, using Perkin Elmer (Clarus 600) GC-MS system in combination with (PE Clarus 600S) Mass Spectrometer detector and an autosampler. PE Elite-5MS capillary column (30 m × 0.25 mm i.e., and 0.25 µm film thicknesses) and helium as a carrier gas were used for the analysis.

Ultrasonic assisted extraction of mitragyna species

The extraction of mitragynine from the leaves of different species of mitragyna was performed with standard procedure [10, 17-23]. For the extraction of leaves of mitragynaspeciosa (KM), 4.0 g of dried leaves were crushed and soaked in 50.0 mL conical flask followed by the addition of 25.0 mL of extracting solvent (chloroform-methanol, 1:4-v/v) and conical flask was covered with aluminium foil to prevent the loss of solvent by evaporation. The conical flask was allowed to ultrasonicate at 30^{o}C for 1.0 h and kept overnight at room temperature. Final solution was filtered throughWhatman 0.45 µm Nylon membrane filter and transferred to a clean glass tube before evaporated to dryness under vacuum at 30^{o}C for 45.0 min using savant concentrator. Similarly, the extraction of

mitragynaspeciosa (KY1) and mitragynarotundifolia (KY2) species were also carried out in the same way. The final prepared solution of each species (1.0 µL) was used for GC-MS analysis.

Gas Chromatographic Mass Spectrometric Analysis

All qualitative and quantitative gas chromatographic analyses of standard sample and all extracted samples were carried out on GC-MS system described above. Aliquots of 1.0 µL of each sample species were injected into the system, separately and respectively. Helium gas was used as a carrier gas with a flow rate of 1.0 mL/min. All the experiments were carried out with oven temperature, programmed from 70°C (initial time: 2.0 min) to 280°C (increasing rate : 7.5°C/min) and then held at 280°C for 15.0 min. Mass spectra were collected in scan mode in the range of m/z 40-400, with the electron energy set to 70 eV. The spectra were searched against a NIST08 library for structural identifications and confirmations.

RESULTS AND DISCUSSION

Ultrasonic assisted extraction (UAE)

The extraction conditions play a very crucial role for extraction efficiency and recovery of mitragynine alkaloid. The mixture of 25.0 mL (chloroform-methanol, 1:4-v/v) was used as an extracting solvent. Methanol penetrate into the epidermis of the leaf resulting in the release of chlorophylls and other compounds from the cellular tissues while chloroform was used as an unreactive, miscible and conveniently volatile solvent for the extraction of mitragynine [10, 24]. As a result of extensive experimentation, the optimized ultrasonic assisted extraction (UAE) conditions were developed and reported herein. The effect of temperature was studied in the range from 25-50°C and the best results were achieved at 30°C. To exploit the maximum extraction, time of sonication was also studied and it has been observed that 1.0 h has resulted in greater performance.

Gas Chromatography Mass Spectrometry (GC-MS)

Standard solution containingmitragynine was resolved on GC-MS system with optimized chromatographic conditions described above and the total ion chromatogram and mass spectrum of mitragynine (1000 µg/mL) (Figure 2). The peak at 36.10 min retention time was identified as mitragynine peak with the value of m/z =398, based on its mass spectrum as shown.The calibration curve was linear for mitragynine in the concentration range of 50.0-1000.0 µg/mL, with correlation of determination (R^2) of 0.9914. The peak of extracted mitragynine from three samples KM, KY1 and KY2 were determined by comparing their retention time and mass spectrum with standard of mitragynine, respectively.

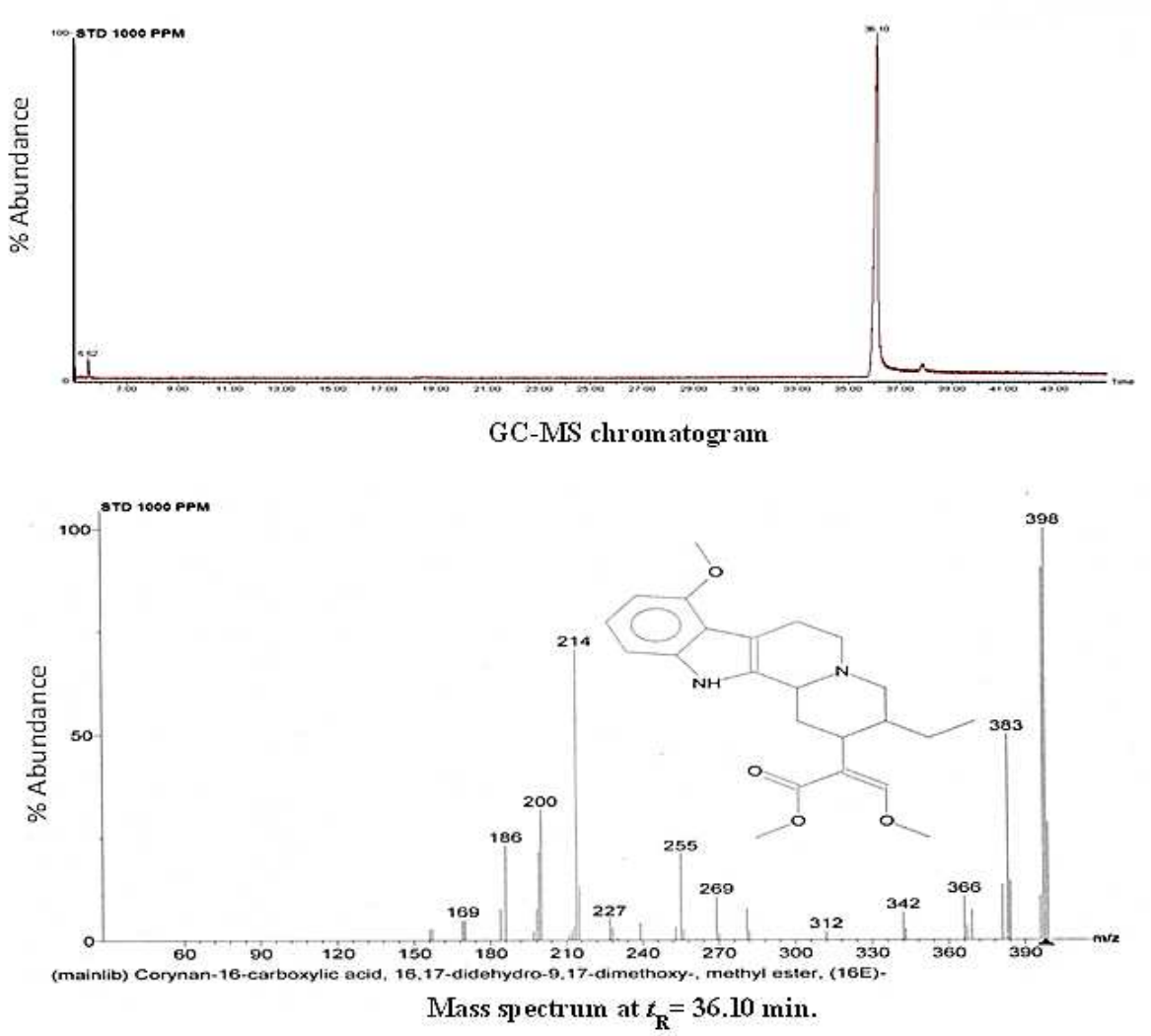

Figure 2.GC-MS chromatogram (a) and mass spectrum (b) of standard solution of mitragynine. Experimental conditions: injection volume-1.0 µL of 1000 µg/mL mitragynine, 1.0 mL/min flow rate with helium as carrier gas and column temperature 70°C and increased by 7.5°C/minup to 280°C

Determination of Mitragynine in KM, KY1 and KY2 samples

The analyses of mitragynine extracted from three samples KM, KY1 and KY2 were carried out by optimized and developed UAE and GC-MS method, respectively. The ion chromatograms of these samples are shown in Figures 3-5. Mitragynine base peak was identified at m/z = 398 and some of its fragments ion peaks were observed at m/z = 383, 255, 214, 200 and 186. Figure 3 clearly indicate the peak of mitragynine at 35.88 min and confirmed with the mass spectrum of KM sample.Similarly Figure 4 shows the presence of mitragynine at 35.77 min in KY1 sample but in case of KY2 sample no peak of mitragynine was detected in the chromatogram (Figure 5). These results clearly indicate that mitragynine is present in *Mitragynaspeciosa* species marked as KM and KY1and not present or may be present in undetectable amount in *Mitrgynarotundifolia* species marked as KY2 and authenticated by botanist. The quantitative estimation of mitragynine in both mitragynaspeciosa species (KM, KY1) were also carried out and found to be 0.094% and 0.105%, respectively.

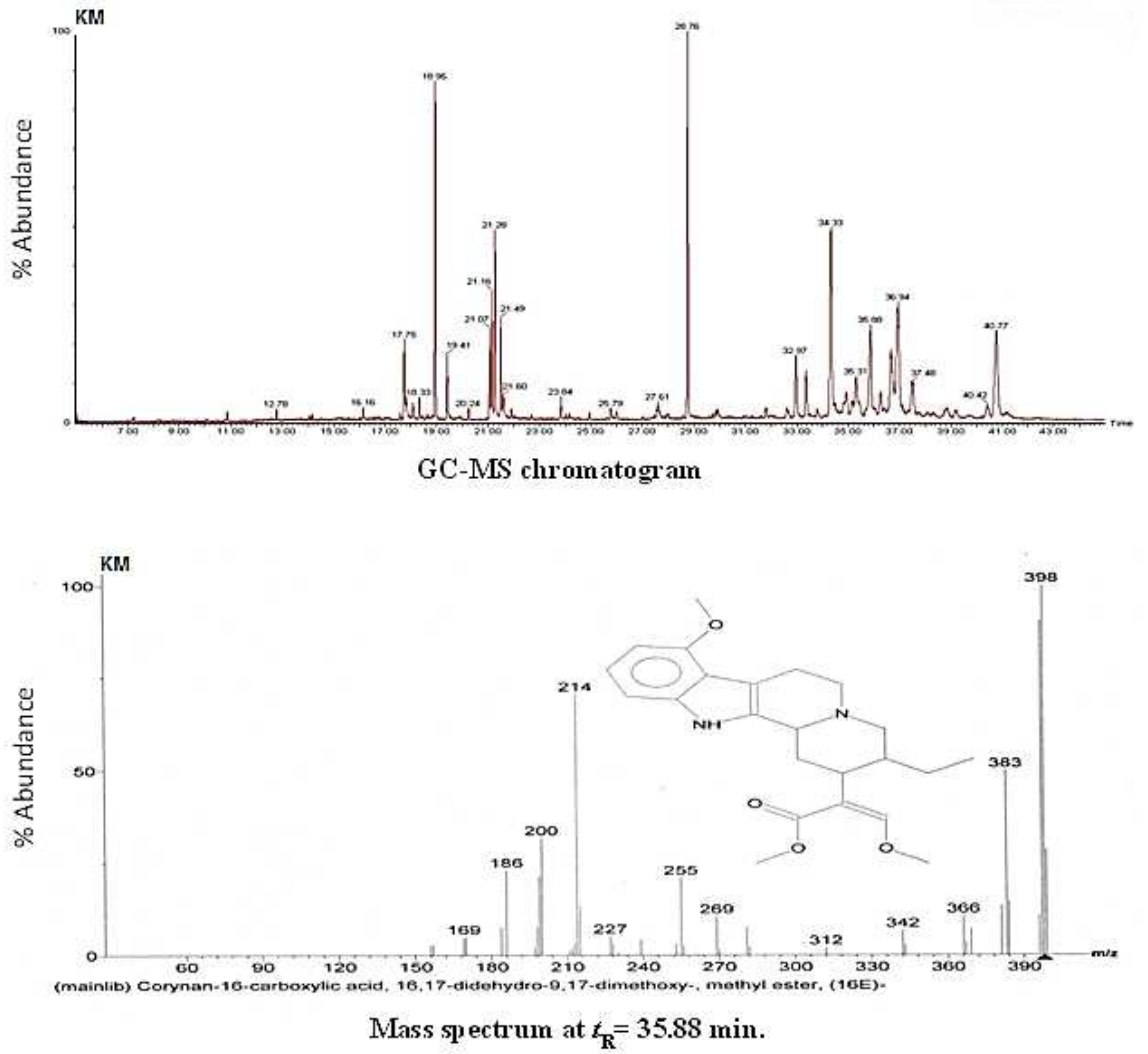

Figure 3. GC-MS chromatogram (a) and mass spectrum (b) of the extract of *Mitragynaspeciosa*species marked as KM sample. Experimental conditions are as given in Figure 2

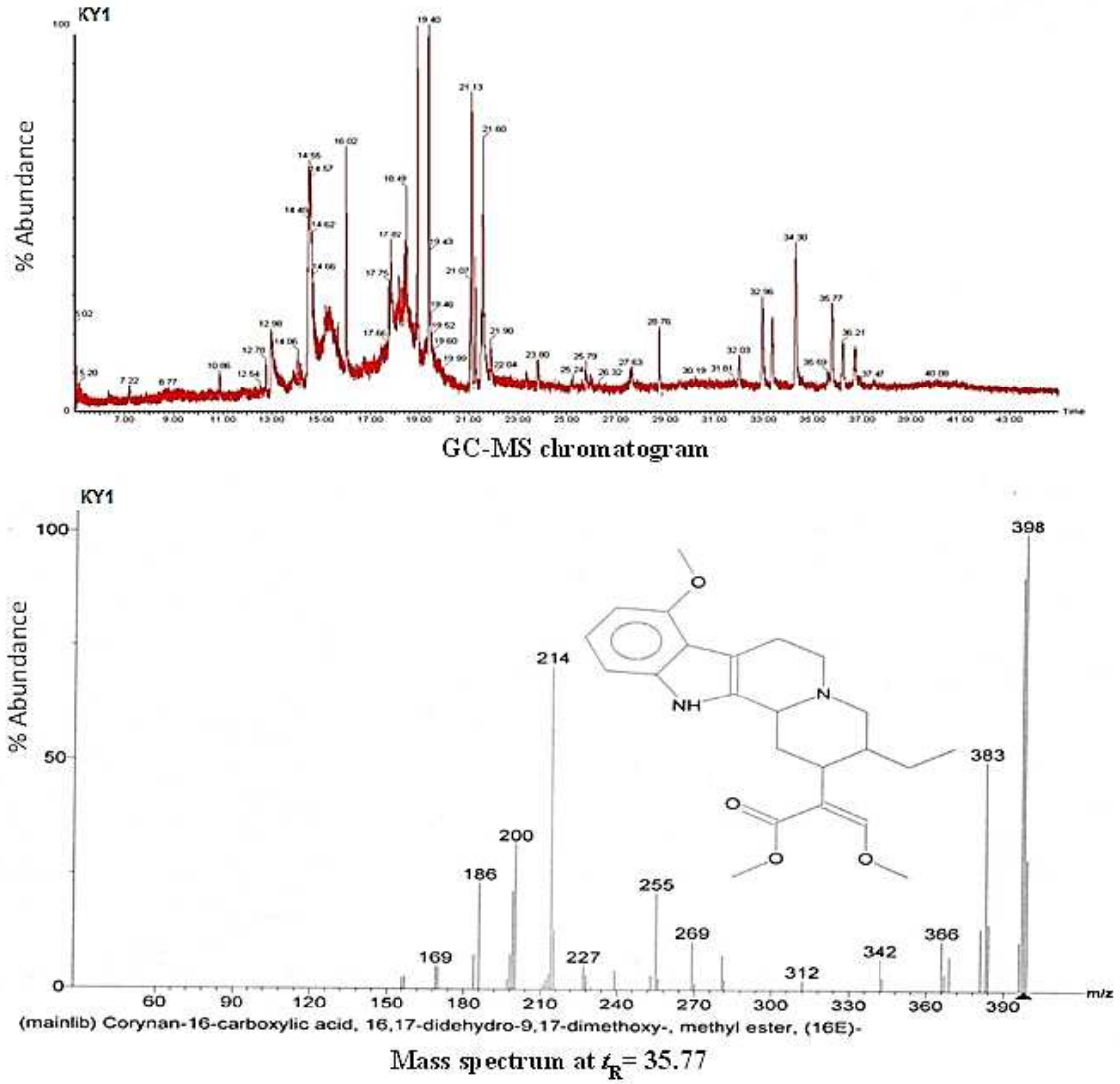

Figure 4. GC-MS chromatogram and mass spectrum of the extract of*Mitragynaspeciosa*species marked as KY1 sample. Experimental conditions are as given in Figure 2

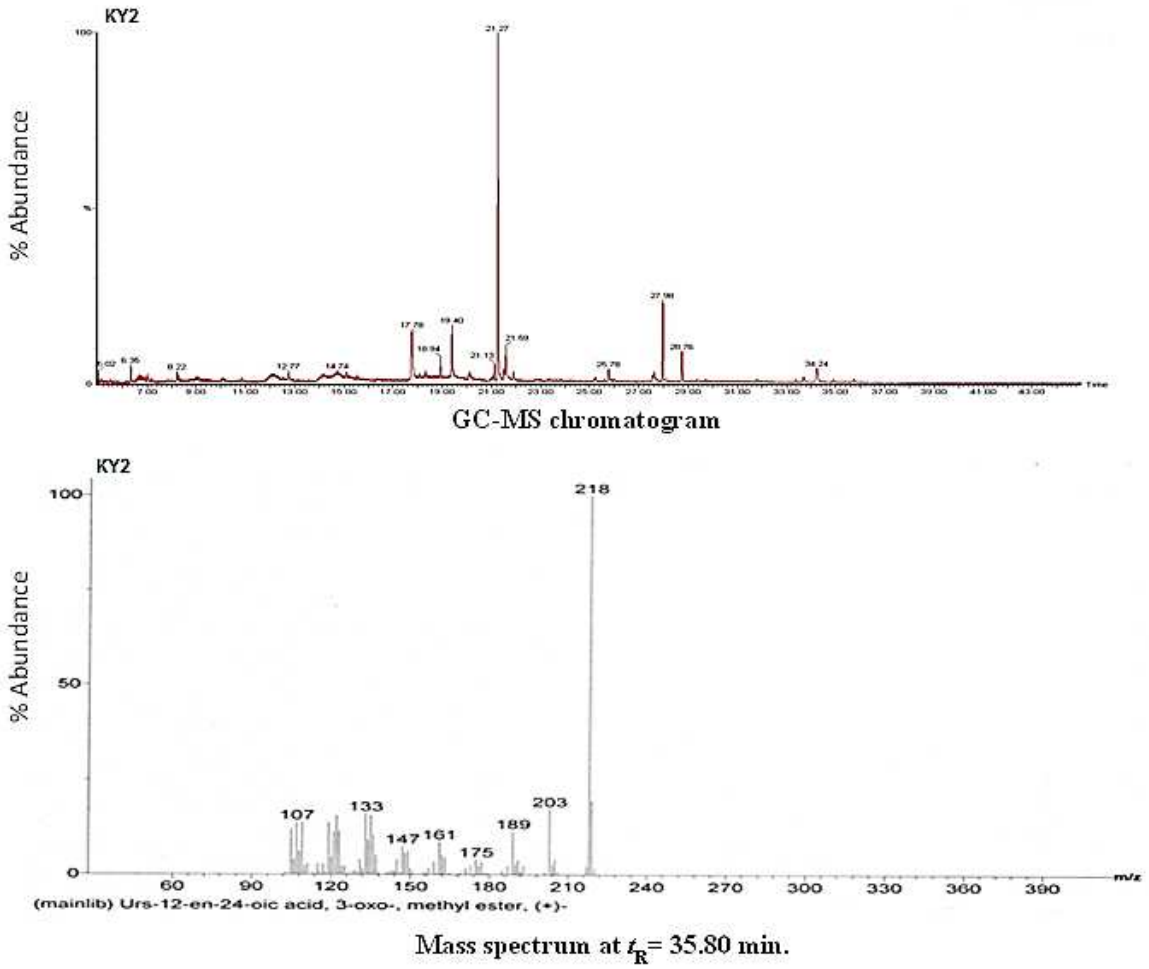

Figure 5. GC-MS chromatogram (a) and mass spectrum (b) of the extract of*Mitragynarotundifolia* species marked as KY2 sample. Experimental conditions are as given in Figure 2

CONCLUSION

From the results presented herein, it may be concluded that the reported UAE and GC-MS method is fast, efficient and selective for the determination and identification of mitragynine in Kedah, Malaysia. Briefly, it is noteworthy that findings from this study could provide a great deal of valuable information for relevant law enforcement authorities in order to trail the origin of the plant thus disclose the illegal activities such as drug trafficking and smuggling.

Acknowledgement
The authors would like to thank UniversitiTeknologi Malaysia (UTM) and Department of Chemistry Malaysia (Johor Branch) for facilitations and the Ministry of Higher Education Malaysia (MOHE) for financial supports through Research Grant Vote Numbers Q.J130000.2526.02H29 and Q.J130000.2426.00G04.

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