

Psychoactive plant abuse: the identification of mitragynine in ketum and in ketum preparations

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ABSTRACT

Recently, the abuse of ketum, an indigenous psychoactive plant, has received a lot of attention in Malaysia. To help national law enforcement agencies control its abuse, the laboratory of the Forensic Division has developed a procedure for its positive identification. Botanical identification may not be practical or conclusive, owing to the wide range of ketum materials available on the market, including dry macerated leaves, powdered leaves and drinks. In order to confirm that a substance is, in fact, ketum or that a preparation is derived from ketum, gas chromatography–mass spectrometry is used to definitively identify the presence of the psychoactive principle mitragynine.

Keywords: ketum; mitragynine; identification by gas chromatography–mass spectrometry

Introduction

Mitragyna speciosa Korth. (Rubiaceae) is a tropical plant indigenous to Thailand and peninsular Malaysia. In Thailand, the leaves of the plant are known as “kratom”, while in Malaysia, they are commonly called “ketum” or “biak”. Traditionally, ketum leaves have been used by local populations for their opium-like effect and their coca-like stimulative ability to combat fatigue and enhance tolerance to hard work under the scorching sun. In 1907, Wray [1] described how the leaves were processed and used as a substitute for opium in peninsular Malaysia. It is reported in the local media that traditional healers use ketum to wean addicts off heroin addiction, to deworm, to cure diarrhoea, to improve blood circulation and even to treat diabetes. However, a study conducted in Thailand in 1975 [2] showed that ketum users became addicted. Typical withdrawal symptoms include hostility, aggression, excessive tearing, inability to

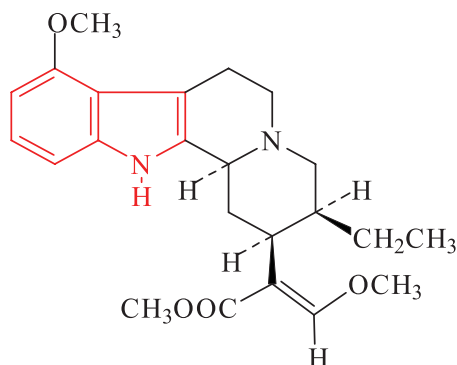
*The authors would like to thank the Pharmaceutical Services Division and the Institute of Medical Research of the Ministry of Health of Malaysia for providing the mitragynine reference standard. The authors would also like to thank Ikram M. Said of the National University of Malaysia for providing some of the bibliographical references.

work, aching of muscles and bones and the jerky movement of limbs. It was also reported in the study that anorexia, weight loss and insomnia were common among long-term ketum addicts. The ketum plant has also been banned in Thailand since 1943 and has also been banned in Australia and Myanmar.

In Malaysia, the use of most indigenous plant-based drugs (with the exception of cannabis) is not commonplace. From 1978 to 2003 there were only a handful of cases involving the use of *Datura stramonium*—which contains the tropane alkaloids scopolamine and hyoscyamine—and ketum leaves. Recently, there has been a growing trend among drug addicts to use the bitter-tasting ketum leaves to get high when they are unable to get their regular supply of cannabis or heroin. In early 2004, stalls selling ketum drinks and teas had mushroomed in several towns around the country, and youths were reported to be drawn to the concoctions. That upsurge in ketum abuse has caused considerable concern among the public and law enforcement authorities, and there is a perception that the consumption of ketum leads to the abuse of other drugs such as cannabis and heroin. The ready availability and very low price of ketum compared with other controlled drugs has contributed to its popularity. Fresh and powdered leaves for making drinks are available for 4 ringgit (RM) (about 1 United States dollar) and RM 25 per kilogram, respectively, while small packet drinks are sold at RM 1, according to information from the media and law enforcement authorities.

To curb and control ketum abuse in Malaysia, its major alkaloidal constituent, mitragynine (see figure I), was listed in the First Schedule and the Third Schedule (psychotropic substances) of the Poisons Act 1952 of January 2003. Under the Act, the planting of the tree is not an offence, and enforcement agencies have no authority to fell the trees. The maximum penalty for possessing or selling ketum leaves or other ketum preparations such as drinks and teas containing mitragynine is a fine of RM 10,000, a four-year jail sentence or both. Since the alkaloid is exclusive to *Mitragyna speciosa*, the Act has had the effect of controlling ketum abuse without making the trees illegal or requiring them to be cut down. In general, law enforcement agencies in the country are calling for all ketum trees nationwide to be cut down, while ketum proponents point to its potential medicinal value and warn of the irreparable loss to the country's biodiversity should the indigenous tree be eradicated.

The present article provides information on the identification of mitragynine in ketum and ketum preparations seized by law enforcement agencies for prosecution purposes. The samples submitted to the laboratory for analysis ranged from small packet drinks to several kilograms of fresh leaves, dry leaves and finely ground powder. It would be quite impossible to identify ketum herbal products and preparations on the basis of general morphological features, especially when the materials examined are in the form of drinks, powdered leaves, macerated dry leaves or decaying leaves. But identification can be made on the basis of the presence of mitragynine. Mitragynine is identified by comparing samples against a reference standard using a gas chromatography-flame ionization detector (GC-FID) and gas chromatography/mass spectrometry (GC/MS).

Figure 1. Chemical structure of mitragynine

Mitragynine C₂₃H₃₀N₂O₄
MW : 398.5

Analytical procedure

Reagents

Mitragynine reference standard was obtained from the Institute of Medical Research of the Ministry of Health of Malaysia. Methanol and chloroform were purchased from Fisher Scientific.

Extraction

Leaves and powdered leaves

Two grams of powder or crushed leaves were ultrasonicated in 25 ml of CHCl₃/methanol (1:4) for 10 minutes. The solution was allowed to settle, and an aliquot was taken for analysis.

Liquid samples (drinks and teas)

Twenty ml of the liquid sample were acidified with a few drops of concentrated HCl and extracted with 20 ml of diethyl ether. The ether layer was discarded, and the aqueous portion was basified with an NaOH solution and checked with pH paper. The solution was extracted with 20 ml of chloroform twice, and the combined extracts were washed with distilled water. The chloroform extract was filtered through anhydrous sodium sulphate and left to dry in the fume cupboard. The dry extract was taken up in 1 ml of methanol for analysis.

Gas chromatography-flame ionization detector

A Shimadzu gas chromatograph GC-17A with a flame ionization detector and fitted with a 30 m × 0.25 mm id, 0.25 μm film thickness HP-5 capillary column was utilized. The temperature programme was 200° C, held for 2 minutes, then increased at 10° C per minute to 300° C and held for 20 minutes. The injector

and detector temperature were set at 280° C. One μ l of the standard (approximately 0.10 mg/ml in methanol) and each sample solution were injected using an auto-sampler.

Gas chromatography-mass spectrometry

A Shimadzu GCMS-QP5050 mass spectrometer interfaced with a Shimadzu GC-17 gas chromatograph and equipped with a 30 m \times 0.25 mm id, 0.1 μ m film thickness DB-5 capillary column was utilized. The temperature programme described above was used. One μ l of the standard (approximately 0.10 mg/ml in methanol) and each sample solution were injected using an auto-sampler.

Results and discussion

The GC/FID spectra of mitragynine reference standard and leaf extract are shown in figure II and III, respectively. The peak at 16.5 minutes in figure III was tentatively identified as mitragynine. The mitragynine peak in the various ketum preparations was definitively identified by comparing its retention time and mass spectrum with the mitragynine reference standard (see figures IV-VII).

Figure II. Gas chromatogram of mitragynine reference standard

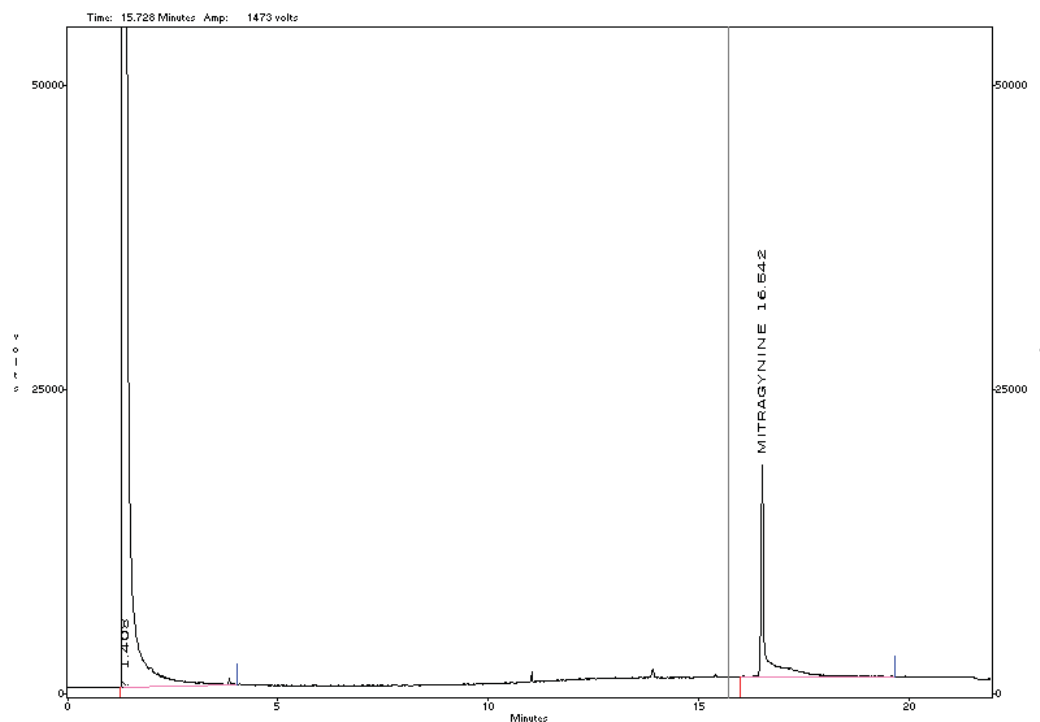


Figure III. Gas chromatogram of leaf extract

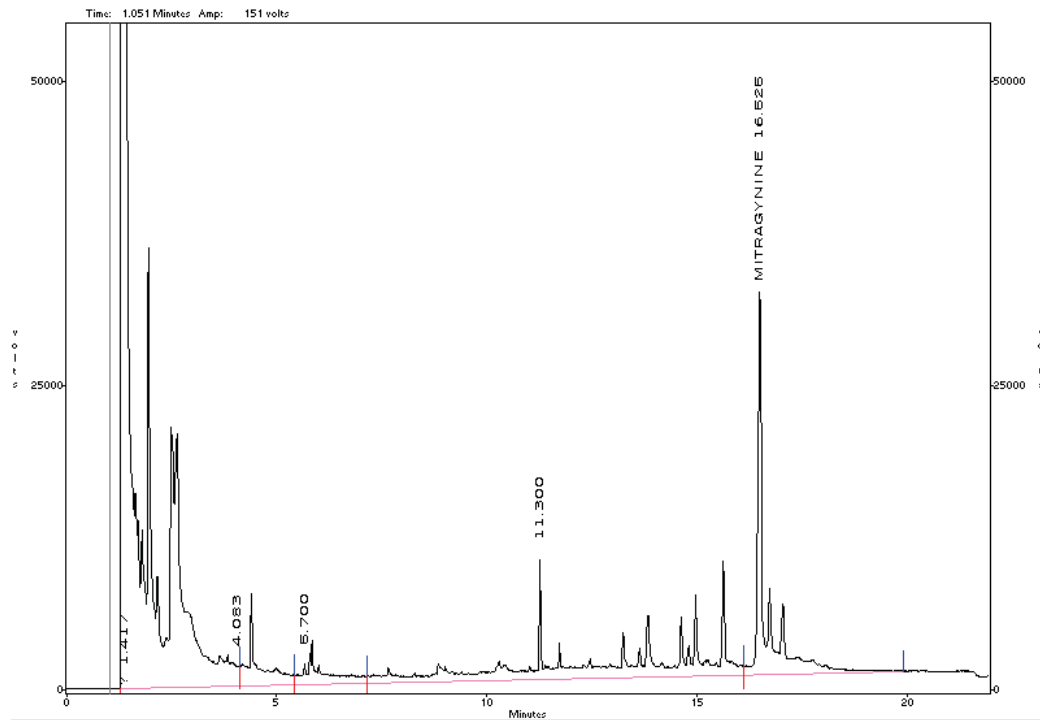


Figure IV. Total ion chromatogram of mitragynine reference standard (ca. 0.10 mg/ml)

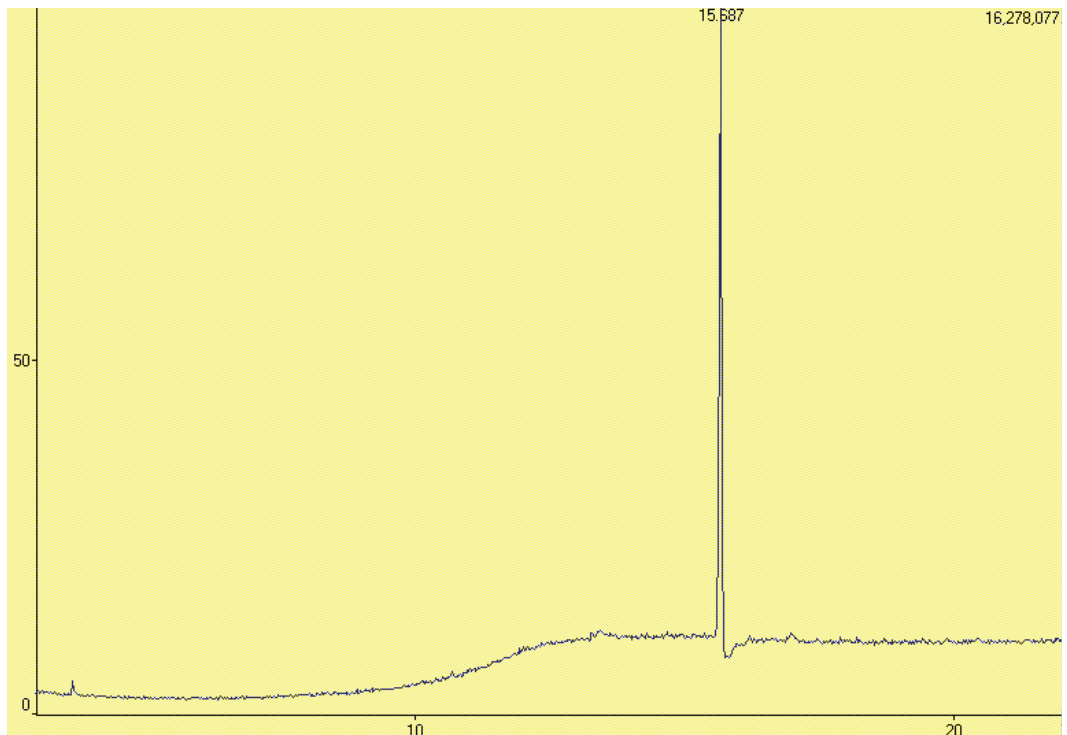


Figure V. Mass spectrum of mitragynine reference standard

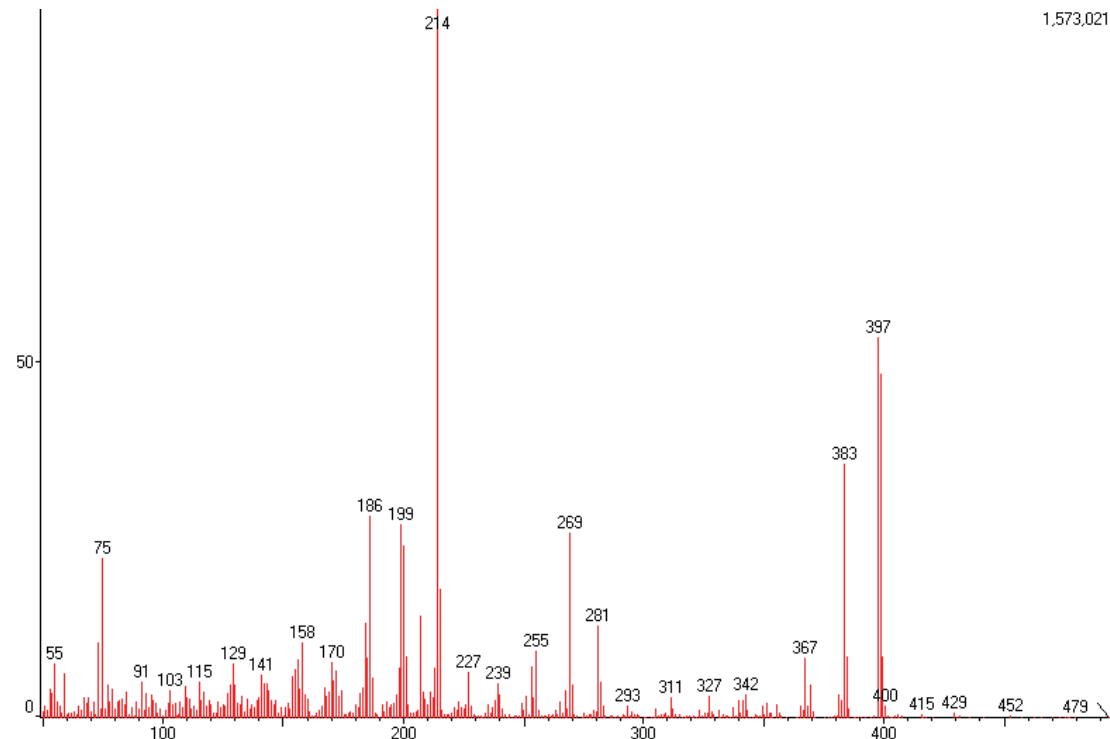


Figure VI. Total ion chromatogram of leaves extract

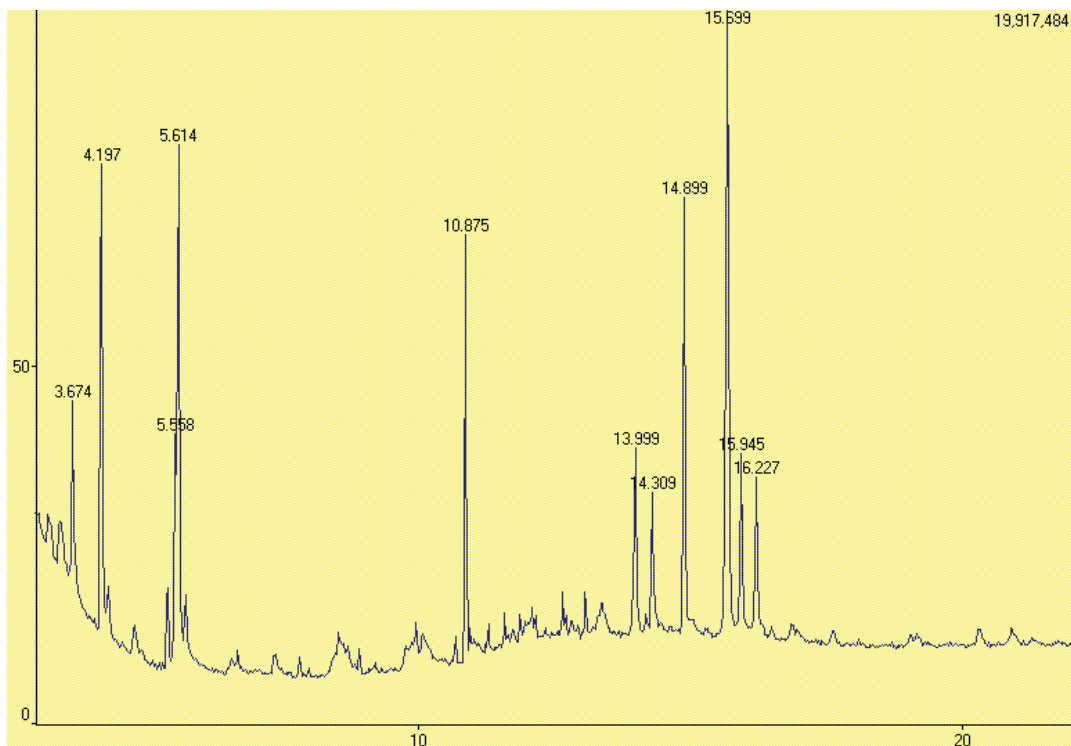
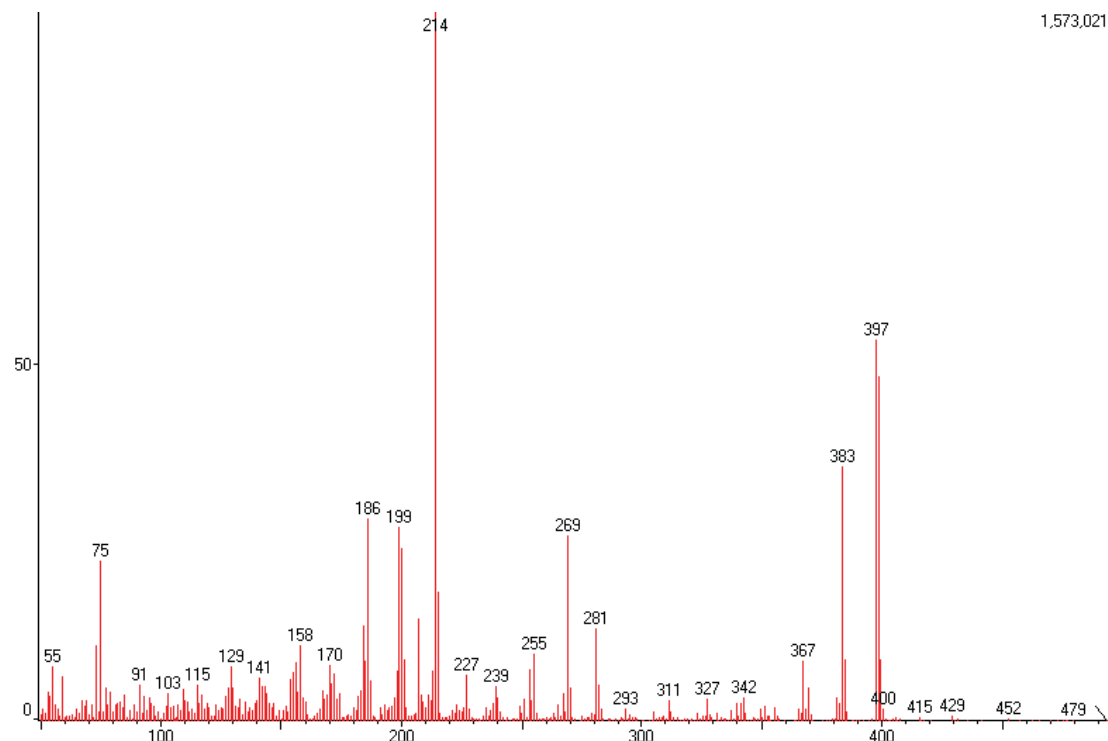


Figure VII. Total ion chromatogram of drink extract

Methanolic extracts of the leaves and powder were studied initially, but the resulting gas chromatograms were not satisfactory. Extraction with a mixture of methanol and chloroform gave much better results.

Owing to the limitation of the thin-layer chromatography technique, which is not a definitive technique, and the scarcity of the mitragynine reference standard, identification by that technique was not attempted. The screening of dry macerated leaves and ketum powdered leaves using the Duquenois-Levine test for cannabis produced a dull blackish green colour that was not extractable into the chloroform layer. Beyond the differentiation from cannabis herb, other colour tests, for example, the van Urk, Ehrlich and Wasicky reagents, which probably react with mitragynine's indole moiety, were not performed as part of the present work.

The laboratory has reprised its main function of providing crucial drug-testing services to the national law enforcement agencies in their effort to control the abuse of an indigenous psychoactive plant and preparations derived from it. With the provision of that testing, the relevant agencies can now confidently monitor, control and prevent the abuse of the plant in all its forms.

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