Comparison of the mitragynine content of aqueous extracts from *Mitragynaspeciosa* Korth leaves from two different geographical areas in Malaysia

T.B. Goh, M.N. Mordi, K.H. Khoo and S.M. Mansor

The Drug Research Centre, Universiti Sains Malaysia, Penang 11800 Malaysia.

Email: gohteikbeng@yahoo.com

Abstract

It was interesting to find that the concentration of mitragynine in aqueous extracts of *Mitragynaspeciosa* Korth leaves (ketum leaves) from both Penang and Kedah States of Malaysia was merely 0.10 µg/ml and 0.09 µg/ml respectively. There was significant difference observed between the freeze dried yields obtained and the aqueous extracts from Kedah and Penang, 124.0 mg/60 ml and 262.5 mg/60 ml respectively. Both the aqueous extracts shared the same chemical profile under GCMS chromatogram and contained at least two isomers with 9-methoxy group.

Keywords: ketum, kratom, Penang, Kedah, chemical profile, narcotic, traditional medicine, Thailand.

Introduction

Thai and Malay natives or opium addicts used *Mitragynaspeciosa* Korth leaves (Ketum leaves) as an opium substitute when opium itself was unavailable and the effect would last for several hours [1,2]. It was in 1897, when the leaves and the bark of this plant were reported by Ridley [3] as a cure for opium habit and was further quoted by Hooper in 1907 [4]. In the same year, Holmes also referred to its leaves as an opium substitute [5]. Jansen and Prast [6] mentioned in their report that Burkill (1930) recorded other uses of Kratom as a wound poultice, cure for fever and as a suppressor of the opiate withdrawal syndrome.

People in southern Thailand use the leaves as traditional medicine for common illness such as cough, diarrhea, muscle pain and hypertension [7]. It was also used to decrease tiredness and increase heat tolerance while working under the sun as well as work efficiency of the farmers, rubber tree gardeners and labourers [6,8,9]. It is interesting to note that the low dose effects from chewing of whole leaves are described to be stimulating while high dose effects of the extracts are more akin to a narcotic analgesia [5].

Early all kratom use in Thailand was by chewing fresh leaves, smoking the dry leaves or drinking as a tea suspension [6,9], or even eating it in the form of resin, for stimulant effects to overcome the burden of hard work. The leaves were consumed either in powder form or having them boiled in water. The boost in energy and strength was experienced within 5-20 minutes after consumption.
This plant has unique dual opioid properties which exert a stimulant effect at low doses and sedative and analgesic effects at the higher doses in humans [8,10]. These effects have also been observed in animal models as reported by Macko[11].

The aim of this research is to study the differences in physical and chemical properties of the aqueous extracts from leaves of MitragynaspeciosaKorth from two locations in Malaysia: Penang and Kedah. This study includes investigating their freeze dried yield, chemical profile and mitragynine concentration.

**Materials and Methods**

**Samples**

Two samples of aqueous ketum extracts were purchased from Penang and Kedah. The colour and appearance of these two samples were recorded and the pH of these two samples were also measured.

**Sample treatment**

60 mL of each aqueous ketum extract was accurately measured and freeze dried for 72 hours using freeze drying machine Labconco model 7753037 with vacuum strength at 0.120 mbar and collecting temperature at -50°C. The appearance and weight of the dried samples were again recorded.

**Preparation of freeze dried ketum extracts**

20 mg of freeze dried ketum extracts from Penang and Kedah were weighed and accurately transferred into 10 mL volumetric flasks respectively. These samples were sonicated in 5 mL methanol until dissolved before topping up with methanol to 10 mL and were hand shaken for 5 minutes to mix homogeneously. The samples was filtered with 0.45 um syringe filter and 1 uL was injected into the GC-MS respectively.

**Analytical methods**

A Hewlett-Packard HP 6890 gas chromatograph equipped with 7673B autosampler was used to quantitatively calculate mitragynine in these two samples from the standard curve. The mitragynine standard curve was prepared using pure mitragynine supplied by Institute of Medical Research (IMR). The detector used was set at 280°C. A HP-g5MS with fused silica capillary column (30 m x 0.25 mm x 0.25 um) was used. Injector temperature was 280°C with initial oven column oven temperature of 180°C and at a programmed rate of 10°C min^-1 ramp upto 280°C. The carrier gas, helium, was used at a flow rate of 1.00 ml min^-1 at 0.45 psi constant pressure. 1 uL of filtered sample was injected into the GC-MS at split ratio of 10:1 under the scan mode. The mitragynine calibration curve was linear with the equation of y= 105.7x – 8.2 over the range of 0.05 to 50 µgml^-1 (n=5, r² = 0.9994±0.002). The mean coefficients of variation for within day assay precision varied from 2.3 to 5.6% at a concentration of 0.05 to 50 µgml^-1. For the day to day variation, the mean coefficient of variation was 4.90±0.60%. The detection limit of assay for mitragynine was 0.50 ng.

**Results and Discussion**

The antinociceptive effect of pure mitragynine, a major constituent (about 66% of crude base extract) from the leaves of MitragyninespeciosaKorth, was less potent than the crude aqueous extract of M. speciosa in vivo experiments[12]. This finding indicates that other minor mitragynine analogues might have a very potent antinociceptive effect. This prompted further investigation of the chemical profile and concentration of mitragynine in aqueous extract.
Table 1. Comparison of the properties for aqueous extracts of ketum leaves from two different geographical locations in Malaysia.

<table>
<thead>
<tr>
<th>Testing Parameter</th>
<th>Location</th>
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<tbody>
<tr>
<td></td>
<td>Penang</td>
</tr>
<tr>
<td>Appearance of solution</td>
<td>Greyish Blue</td>
</tr>
<tr>
<td>pH</td>
<td>4.66±0.02</td>
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<tr>
<td>Weight of freeze dried extracts, mg</td>
<td>262.5±0.1</td>
</tr>
<tr>
<td>Mitragynine, ug/ml-1</td>
<td>0.10±0.005</td>
</tr>
</tbody>
</table>

Results based on three replicates, n=3, Mean±SD

From Table 1, the appearance of the aqueous solution from Penang was greyish blue compared to transparent brown from Kedah. The pH of aqueous extract from Penang was 4.663, whereas the aqueous extract from Kedah was 5.537. The appearance of freeze dried product from Penang is darker in colour compared to freeze dried product from Kedah which is lighter in colour, although both extracts shared the same chemical profile qualitatively under the GCMS chromatogram as shown in Figures 1 and 2.

Figure 1. Chemical profile for aqueous extracts from Penang.
Figure 2. Chemical profile for aqueous extracts from Kedah.

The chemical profile indicated both the aqueous extracts contained two isomers at retention time *ca.* 15.79 min and *ca.* 16.30 min respectively, in addition to mitragynine at retention time *ca.* 15.30 min.

The weight of extracts (yields) obtained after freeze drying from 60 ml of the aqueous extract of Penang was 262.5 mg, twice the quantity compared to 124 mg from the aqueous extract of Kedah. The freeze dried sample is shown in Figure 3. The differences observed and shown might be due to the differences in species of ketum and geographical region between Penang and Kedah states in Malaysia.

Figure 3. Freeze dried aqueous extract samples from Penang and Kedah.

It was interesting to find that the mitragynine concentration of aqueous extracts from Penang and Kedah was merely 0.10µg/ml and 0.09µg/ml respectively (from Table 1). The mitragynine peak was shown at the retention time of *ca.* 15.3 min under the condition mentioned above. The percentage of mitragynine was *ca.* 19.54% for aqueous extract from Penang but approximately twice
this amount for aqueous extract from Kedah, although concentration of mitragynine is nearly comparable. This might mean the aqueous extracts from Penang contained more impurities than aqueous extract from Kedah.

The recorded MS patterns (M/Z species) of mitragynine and its isomers for both aqueous extracts, as shown in Figures 4-6, were 186, 200, 214, 255, 269 and 398, all these species were 30 units (equivalent to a methoxy group) higher than the demethoxy group [13]. These findings suggested that mitragynine and its two isomers contained 9-methoxy group which was essential for its biologically active properties [14].
From Figure 4 it was noted that the intensity of m/z species of 255 was lower than 269 and intensity of m/z species of 225 was lower than 239, this suggested an allosteric structure for mitragynine [13]. From Figures 5-6, the intensity of m/z species of 255 was higher than 269 for both Isomer 1 and Isomer 2. This suggested either a pseudo or a normal in stereostructure for both isomers [13].

**Conclusion**

Although there are differences and variations observed in terms of freeze dried yields and some physical properties, both aqueous extracts from Penang and Kedah share the same chemical profile and contain two main mitragynine isomers. The mitragynine contents are low for both aqueous extracts.

**Acknowledgments**

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**References**


