Abstract

Kratom is a tropical tree indigenous to South East Asian countries and has been traditionally used by natives to increase work efficiency and treat selected illnesses. However, the United Nations Office on Drugs and Crime (UNODC) classified kratom, Mitragyna speciosa, as a plant-based new psychoactive substance (NPS) that must be monitored worldwide, due to increasing reports of abuse. Many countries, including the Philippines, do not put restrictions on the said plant species including its major psychoactive drug, mitragynine. Under this prevailing provision, a research exploration on the said plant species including its major psychoactive drug, mitragynine was carried out to determine the distribution of kratom trees, locally referred to as “mambog”, in the Philippines and authenticate species identity of collected specimens through chemical determination of mitragynine and DNA analysis.

Various samples, specifically leaves, twigs, barks and roots, from claimed kratom species in selected regions of Luzon and the Mindanao Islands of the Philippines were sampled and preserved accordingly before subjecting them to instrumental analysis using Gas Chromatograph-Mass Spectrometer (GC-MS) and DNA barcoding.

During the field exploration, it was well documented that claimed kratom trees are mostly present in wetland areas at low altitudes, and sometimes co-exist with local bangkal (genus Nauclea) trees. Interestingly, while locals identified some of the collected species as kratom through botanical assessment, mitragynine was not detected in some selected sampling sites. Remarkably, among tree parts collected, only leaves and twigs showed evidence of mitragynine, suggesting further disparity among kratom tree parts. On the other hand, DNA barcoding technique was utilized to discern the species level identities of claimed kratom trees in the field, which revealed that acquired plant specimens that were found positive for mitragynine were suggested to be Mitragyna speciosa, the specific kratom species being monitored by the UNODC.

Given that the present laboratory findings confirmed the existence of kratom (Mitragyna speciosa) in the country, it is of great importance for the Philippine government to implement strict monitoring and scientific evaluation on the potential threats of the said plant-based NPS. Preliminary results can also assist law makers to possibly develop regulations in the future, since this paper is a pilot forensic study for kratom found in the Philippines.

Keywords: Forensic Science, Plant-Based New Psychoactive Substances, Kratom, Mitragyna speciosa, Mitragynine.
1. Introduction

*Mitragyna speciosa*, which belongs to the *Rubiaceae* family, is a plant native to numerous tropical countries and commonly referred to as Kratom. This species can grow from 10 to 25 meters in height with straight trunks, smooth barks, clustered round yellow flowers, and oblong shaped fruits. Leaves are dark green and glossy, about 8.5 to 14 centimeters long, and 5 to 10 centimeters wide. Its leaves are smaller at the ends of the branchlets, pointed at the tip, rounded or somewhat heart-shaped at the base [1].

Among different *Mitragyna* species, *Mitragyna speciosa* is the most prominent because of its unique active alkaloid content, mitragynine [Figure-1]. Also, some mitragynine analogues have been isolated from the aforementioned species such as 7-hydroxymitragynine, speciogynine, paynantheine and speciociliatine. However, since mitragynine is the major constituent of Kratom, the said alkaloid is commonly used as the marker compound for the identification and potency determination of Kratom in a variety of products [2].

Mitragynine in Kratom is classified as a psychoactive drug that gives stimulating and analgesic effects depending on the level of consumption [3]. In specific, consuming *Mitragyna speciosa* leaves in small quantities results in stimulating effects, while a higher dosage mimics the effects of opium usage [4]. For instance, the said plant substance is conventionally used by South East Asian farmers, most especially in Thailand and Malaysia, to combat fatigue. It is also used as alternative medication to treat certain illnesses such as muscle pain, diarrhea, hypertension, as well as opioid withdrawal [5]. In the above-mentioned countries, Kratom is normally consumed by chewing the fresh leaves and smoking dried leaves, or even drinking the brewed leaves and barks [6].

Although several studies strongly confirm Kratom’s essential usages, the addictive and abuse capability of Kratom mainly associated to its narcotic component, mitragynine, has gained attention among international drug law enforcement agencies [7]. In fact, the UNODC categorized Kratom *Mitragyna speciosa* as plant-based NPS because of major global reports of abuse among recreational users taking advantage of Kratom’s non-inclusion to any schedules of United Nations Drug Conventions, including its mitragynine drug [8]. Furthermore, Kratom related health and even death reports have been significantly noted both when consumed solely and mixed with other substances [9]. In view of the aforesaid circumstances, various countries already employ regulation strategies to combat the emerging threats of Kratom because of the increasing popularity of certain Kratom products in the illicit market such as Kratom Cocktail, Captain Kratom Gold XL, O.P.M.S. Kratom and Krypton [10, 11]. In fact, the Philippines being one of the few countries that do not put restrictions on Kratom or its alkaloid, mitragynine, already have existing reports of online selling of Kratom products which are being marketed as vitamins and supplements [12]. Additionally, Kratom’s botanical, taxonomical and chemical data are well studied in various countries; however, in the Philippines, there is a scarcity of scientific records.

Because of these underlying conditions, a Kratom pilot research exploration was conducted in various geographical locations in the Philippines as there are confirmed reports suggesting that Kratom trees are abundant in that country [13]. In specific, this study aims to obtain extensive information on the distribution of local *Mitragyna speciosa* existing in the country and determine the possible presence of mitragynine in different tree parts among claimed Kratom trees. Even though detection of mitragynine can be a major investigarive tool in identifying potential *Mitragyna*...
speciosa in collected alleged Kratom samples, this method can still not be used absolutely to authenticate claims of Mitragyna speciosa in the field. Thus, DNA barcoding was utilized in this research since this approach is well established at species level identification, and is independent of plant age and environmental parameters that might affect chemical detection of mitragynine in suspected samples [14]. It is also worth noting that even Filipino taxonomists concentrating on the plant morphology of Mitragyna species are also lacking.

The expected scientific data will be vital in assessing important factors such as health risks and abuse potential of existing Philippine Kratom (Mitragyna speciosa) needed by anti-drug policy makers in the Philippines for crafting prospective regulations in the future.

2. Materials and Methods

2.1 Sample Collection and Survey

Since Kratom, locally known as Mambog, was previously reported to be present in several provinces in the Philippines [15], the researchers coordinated with the Department of Environment and Natural Resources-Philippines to discern prospective sampling sites and to acquire pertinent permits for sample collection of suspected Kratom Mitragyna species.

The research team, guided by government foresters and other local guides, collected allowable amounts, not exceeding 1 kilogram per sampling site of fresh samples from suspected local Kratom trees in multiple provinces in Luzon and the Mindanao Islands of the Philippines, as shown in Table-1 and Figure-2. These included leaves, twigs, barks and roots. Importantly, photographs with appearances of the sampled trees and their habitats [Figure-3], and coordinates of all sampling areas were documented using a GPS map camera application software. Partial ecological conditions wherein suspected Kratom trees are situated were also noted.

Consequently, samples were immediately placed inside properly labeled self-sealing plastic bags before being transported to the laboratory for analysis. Leaf samples for DNA analyses were separated and were preserved using silica gel. In all sampling sites, collective surveys were conducted on locals to verify their knowledge about Mambog’s usages, to initially assess potential abuse of the said plant-based NPS in their respective regions.

2.2 Chemicals and Reference Standard

Mitragynine Certified Reference Standard (Cerilliant) with a concentration of 100µg/mL in 1mL methanol, methanol (RCI Labscan HPLC Grade), and chloroform (J.T. Baker AR Grade) were purchased through authorized local dealers.
Table 1 - Sampling sites and corresponding habitats.

| Sampling Site Code | Sampling Location                  | Coordinates                           | Ecological Position | Altitude |
|--------------------|------------------------------------|---------------------------------------|---------------------|----------|
| P-1                | Baco, Oriental Mindoro             | 13° 21' 57" N 121° 6' 20" E          | Swampy              | Low      |
| P-2                | Victoria, Oriental Mindoro         | 13° 11' 39" N 121° 14' 45"E          | Near Wetland        | Low      |
| P-3                | San Miguel, Surigao del Sur        | 8° 53' 41" N 126° 1' 8" E            | Plain Land          | Low      |
| P-4/N-9            | San Francisco, Agusan del Sur      | 8° 23' 29" N 125° 54' 49" E          | Marshy              | Low      |
| P-5/N-10           | Bunawan, Agusan del Sur            | 8° 12' 51" N 125° 55' 35" E          | Marshy              | Low      |
| P-6                | Nabunturan, Compostela Valley      | 7° 36' 44" N 125° 59' 4" E           | Plain Land          | Low      |
| N-1                | Ilagan, Isabela                    | 17° 10' 03" N 121° 58' 10" E         | Mountainous         | High     |
| N-2                | Ternate, Cavite                    | 14° 16' 6" N 120° 38' 33" E          | Mountainous         | High     |
| N-3                | Paluan, Occidental Mindoro         | 13° 17' 46" N 120° 30' 44" E         | Plain Land          | Low      |
| N-4                | Mamburao, Occidental Mindoro       | 13° 13' 55" N 120° 34' 19" E         | Plain Land          | Low      |
| N-5                | Bulalacao, Oriental Mindoro        | 12° 20' 48" N 121° 19' 42" E         | Mountainous         | High     |
| N-6                | Mansalay, Oriental Mindoro         | 12° 29' 47" N 121° 25' 31" E         | Mountainous         | High     |
| N-7                | Talacogon, Agusan del Sur          | 8° 23' 40" N 125° 49' 0" E           | Marshy              | Low      |
| N-8                | Loreto, Agusan del Sur             | 8° 7' 14" N 125° 52' 29" E           | Marshy              | Low      |

2.3 Mitragynine Extraction

All gathered samples were stored at room temperature and were washed with water and air dried before being subjected to chemical analysis. Appropriate amounts of representative samples around 4 grams were weighed, blended and ground with mortar and pestle before soaking in 25 mL of chloroform/methanol extracting solvent (1:4) for 24 hours. After overnight soaking, specimens were ultrasonicated for 1 hour. The resulting extracts were then filtered using a Whatman 0.45 μm Nylon membrane filter, and corresponding filtrates were dried under oven at 70 °C. The extracts were then reconstituted with methanol, and diluted aliquots were taken for instrumental analysis using GC-MS.

2.4 GC-MS Analysis

All samples for qualitative determination of mitragynine were analyzed using an Agilent GC-MS with a 7890A GC System and a 5975C mass selective detector. The instrument was equipped with a G4513A Injector and a 7693 Series Autosampler. Amounts of 1μL aliquot of individual sample extracts were injected in triplicate into the above mentioned system with injection port temperature of 280°C. Helium gas (Linde Ultra High Purity) was used as carrier gas with a flow rate of 1mL/min. DB-5MS (30m x 250μm x 0.25μm) was utilized and carried out with a column oven program as follows: initial temperature of 140 °C held for 3 minutes with a ramp rate of 20 °C/min up to final temperature of 300 °C with a hold time of 16 minutes,
resulting in a total run time of 27 minutes. A split injection was used with a split ratio of 20:1. Meanwhile, mass spectra of all samples were acquired in scan mode in the range of 40 to 550 amu, with interface temperature of 250 °C, and MS ion source temperature of 230 °C, with electron energy at 70eV.

Chromatograms of extracted samples tentatively identified as mitragynine, including its fragmentation patterns, were examined by comparing it with the GC-MS results of mitragynine Certified Reference Standard. MS spectra results of samples and standard were examined against a Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) forensic spectral library.

2.5 DNA Analysis

Leaf specimens previously confirmed to contain mitragynine by GC-MS analysis were subjected to DNA barcoding method using chloroplast rbcL markers. Genomic DNA were extracted from ground samples using a DNeasy Plant Mini Kit (Qiagen, Germany) following the manufacturer’s protocol. All extracted DNA was kept at -20 °C prior to the use. Pre-identified DNA primers, specifically chloroplast markers rbcL-Mit1 and rbcL-Mit2, were used for PCR amplification. Moreover, all extracted samples for sequencing were sent to Macrogen in Korea. Data interpretation of the resulting sequences were performed by matching with existing sequences from the online Barcode of Life Database (BOLD) through the Basic Local Alignment Search Tool (BLAST). On another note, DNA barcoding of samples which gave negative results for the presence of mitragynine are not within the scope of this present study.

3. Results and Discussion

It was noted that the majority of the suspected Kratom trees in the sampled sites coincide with the general botanical features of Kratom previously discussed. Importantly,
Interestingly, mitragynine was not detected in samples collected in N-1 to N-10, although locals identified our tagged trees in the area as Mambo (Kratom local name), because physical appearances coincide with that of a Kratom tree. Therefore, it is of great worth to discern the species level identity of the afore mentioned trees for scientific verification using DNA barcoding. In view of the foregoing, DNA analysis results revealed that all the samples containing mitragynine are suggested to be *Mitragyna speciosa*, which further support multiple reports that mitragynine is distinctive to *Mitragyna speciosa* among 10 pre-identified *Mitragyna* species [16].

Moreover, aside from leaves, only twigs from the alleged Kratom trees, specifically in sites P-1 and P-2, showed evidence of mitragynine, which implies potential mitragynine content variation among Kratom tree parts. It is noteworthy to mention the disparity in leaf and trunk appearances from all collected trees, of which leaf and trunk that contain mitragynine from sites P-1 to P-6 are somehow far from the botanical appearances of negative samples from sites N-1 to N-6. However, except for sampled trees in sites N-7 to N-10 all from Agusan del Sur, all claimed Kratom species within the Agusan Marsh are almost identical in terms of physical appearance but yielded different results with respect to mitragynine detection. A related outcome was also observed with samples collected from Mindoro Island wherein samples also gave dissimilar findings, despite the fact that they are situated on one particular island. This can be associated with the difference in the species identities of the plants tested, and the earlier hypothesis that mitragynine chemical detection can be potentially affected by some environmental factors [17].

In terms of ecological conditions, confirmed *Mitragyna speciosa* in this study were all located in close proximity to wetlands, which agrees with preceding literature that *Mitragyna*’s habitat are normally surveyed in tropical swampy forests and marshes. In fact, one of the major sampling sites is within a marsh in Agusan del Sur, and confirmed Kratom species are actually outside peat land which is known to

Table-2 shows the summarized mitragynine analysis results of leaves collected from alleged Kratom species in all sampling sites. Significantly, mitragynine was detected in leaf specimens analyzed in sites P-1 to P-6.

Moreover, the results from the developed GC-MS method revealed that the confirmed samples with mitragynine have similar retention times and exhibit all the major and confirmatory ions matching with that of mitragynine standard result patterns. In particular, retention time of samples with detected mitragynine were around 15 minutes (Figure-4), and corresponding fragmentation patterns exhibited a high quality match in MS spectral library used, which were also comparable with the results of the mitragynine reference standard received from Cerilliant (Figure-5).

Figure 5- Representative MS spectra of reference standard, leaf sample, and twig sample that contains mitragynine.

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have an acidic environment [18]. Hence, this further suggests that local Kratom trees in the said location are naturally grown in watery areas and non-acidic environments [19]. Meanwhile, the present findings further suggest that aside from the said optimal habitat of Kratom, the said species can also be seen in plain land areas, as revealed by samples analyzed in Surigao del Sur and Compostela Valley. In addition to the foregoing findings, the majority of the samples that gave negative results for mitragynine were sampled in mountainous area, which further implies that Mitragyna seems to inhabit only in low altitude areas.

Also, the presence of bangkal Nauclea tree in some of the sampling sites where alleged Kratom species are situated are of great interest, recommending its potential to be an indicator species for Kratom Mitragyna. Meanwhile, an earlier study reported the considerable presence of Mitragyna rotundifolia in Agusan Marsh [20]; however, the present study further add that aside from Mitragyna rotundifolia, Mitragyna speciosa also co-exists with the said species within Agusan marsh as revealed by our chemical and DNA analysis results.

Additionally, a preliminary survey from respondents of selected sampling sites showed that Mambog is well known in their respective localities. However, the majority of the respondents revealed that the main usage of Mambog for them is for house making and furniture utilization. Also, about 18% of the respondents utilize Mambog as firewood. When asked if they tried consuming Mambog, around 67% answered no, while 27% gave no response. Importantly, others told about Mambog’s medicinal usage as an alternative herbal medicine to treat diarrhea and wounds, which is congruent with former literature [21]. Remarkably, a few elder respondents reported its recreational use giving effects similar to Marijuana. Overall, the preliminary survey suggests that Kratom’s recreational use in the Philippines is not popular at present (Figure-6).

4. Conclusion and Recommendations

With the aid of mitragynine detection analysis and DNA barcoding, the concluded Kratom research exploration successfully discerned some distribution of Mitragyna speciosa in the Philippines, specifically in Oriental Mindoro, Agusan del Sur, Surigao del Sur, and Compostela Valley.

This study further implies that it is difficult to rely on botanical identification of Kratom, which is prone to error since most of the claimed local Mambog trees are morphologically similar. This practice will certainly cause difficulties in identifying mitragynine containing species, as manifested by the recent findings that showed that not all samples claimed to be Kratom species by locals contained mitragynine. This concern can be significantly associated with the factual evidence that there is a dearth of literature on Kratom’s morphology in the country.

Therefore, it is suggested that Kratom is best verified by detecting the presence of mitragynine, which is unique to Mitragyna speciosa, and authenticate its species identity using DNA barcoding technique to address prevailing uncertainty on Kratom’s botanical identification. Never-
However, combining the aforesaid chemical and molecular techniques will provide a great deal of vital information for law enforcement authorities to create monitoring programs on potential offenders in the future, if ever Kratom and its psychoactive constituent mitragynine will be regulated in the Philippines. In specific, the suggested protocol will be beneficial in monitoring and inspecting suspected Kratom products potentially being imported now in the country or locally cultivated for illicit usage.

On the other hand, it is of great importance to conduct a follow-up research on local Kratom to check the concentration of mitragynine and if it varies among *Mitragyna speciosa* species found from different geographical locations. It is also important to study some factors influencing the concentration of mitragynine in Kratom such as environmental and ecosystem conditions. As Luzon and Mindanao were the only major islands of the Philippines extensively covered in this study, it is suggested to explore the possibility that Kratom might be found in certain provinces in the Visayas Islands of the Philippines. In terms of Kratom tree parts, since mitragynine was only detected in leaves and twigs, it is vital to additionally analyze other parts such as fruits and flowers. Thus, the sampling period must be during the flowering season of Kratom to obtain significant amount of such samples.

Lastly, substantial efforts must be made to report the initial findings of this pilot study on local Kratom to Philippine policy makers, as well as concerned international organizations such as the UNODC and the International Narcotics Control Board (INCB) who advocates in identifying drug substances with addictive properties, health hazards, and that are liable to be abused. This preliminary output will contribute to the drug forensic field by showing support that the Philippine government through the Philippine Drug Enforcement Agency, the lead agency for anti-drug law enforcement, is actively participating in research ventures to combat serious worldwide threats of plant-based NPS.

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**Conflict of Interest**

None

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None

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