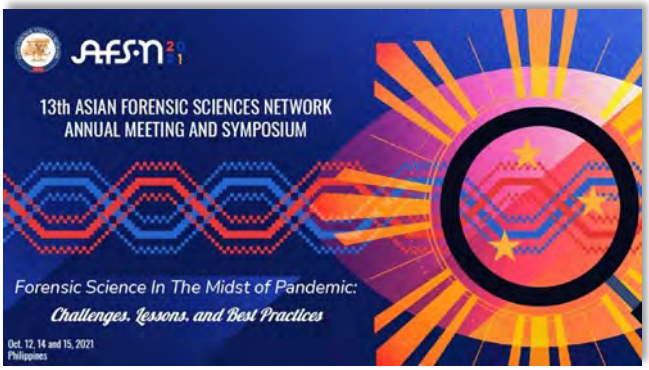
ForensicAsia
THE ASIAN FORENSIC SCIENCES NETWORK NEWSLETTER | ISSUE 12 | OCTOBER 2022

AFSN President's Address

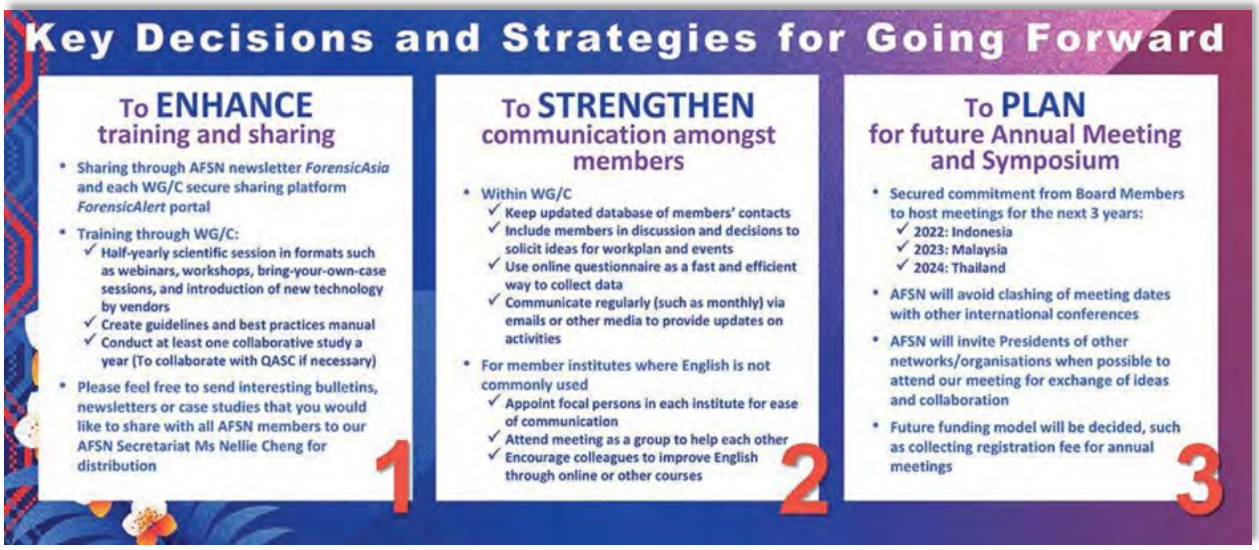
Dear colleagues and friends,

Greetings to all colleagues in Asia and around the world!



It was such a pleasure to virtually meet so many of you during the **13th AFSN Annual Meeting and Symposium** hosted by the Philippines National Police Forensic Group (formerly called Philippines National Police Crime Laboratory) on 14-15 October, 2021 and pre-conference events from 11-13 October. The event, a first-ever fully virtual conference for AFSN, had attracted more than 1,500 participants from 35 countries, and there were 10 parallel scientific sessions for the workgroups and committee.

During the annual meeting in 2021, the Board held fruitful discussions with the workgroups/committee steering committee members where the following strategies were put forward:



2022 has been a re-normalising year, where the world is slowly returning to pre-pandemic norms, with some regions resuming earlier than others. AFSN has had a busy calendar for the year, where the Board met twice in March and July, and also had communication sessions with the workgroups/committee steering committees on the activities and events for the year. I am very pleased to share that a new technical workgroup – **Forensic Medicine Workgroup (FMWG)**, Chaired by Dr Joseph Palmero, was officially formed on 2 March this year with the scope of the workgroup as "Examination and interpretation of physical injuries; post-mortem examination". I hope that FMWG will become the platform for forensic pathologists within our member institutes to share knowledge, and also to collaborate with other sister networks such as APMLA and INPALMS.

Within the ten AFSN technical workgroups and committee, we have had many webinars, scientific sharing, collaborative exercises and discussions held during the year. I am also heartened to see workgroups and committee collaborating to hold joint events. In order to promote identity within each workgroup/committee, the Board will launch **WG/C logos** for all WG/C during the Jakarta meeting. I would like to acknowledge the Philippines National Police Forensic Group for designing the beautiful logos.

The Board also hopes to leverage on the new **"AFSN Board Seminar Series"** to share the Board Members' experience and best practices in the various aspects of management, leadership, training, collaboration, and technological advancements. The first and second of this online event will be held respectively by the Philippines National Police Forensic Group, Philippines and the Health Sciences Authority, Singapore. Stay tuned for more details.

To encourage more research and scientific sharing of knowledge within our member institutes, the Board, together with the ForensicAsia Editorial Team, has launched the **"Best Paper of the Year"** award since the last issue of ForensicAsia. We hope that this will create a very lively research and knowledge sharing culture in AFSN.

In the international front, AFSN has been collaborating and contributing to the development of the **International Forensic Strategic Alliance (IFSA) Minimum Requirement Documents (MRDs)** in various forensic disciplines. Apart from participating in the review of the draft MRDs by many WGs, the Department of Chemistry (KIMIA), Malaysia has also taken the lead to draft the MRD for Toxicology, and this has been further reviewed by AFSN TXWG as well as various IFSA members. I am also delighted to see that there has been great interest in using the IFSA MRDs in local languages, and the Institute of Forensic Science, China, has translated the MRDs in Drugs, CSI and DNA to Chinese, and KIMIA has similarly translated the Drugs MRD to Bahasa Malay. I am very pleased to inform that we have renewed the **IFSA Multilateral Partnership Agreement** with all partners for another 5 years to continue with the excellent collaboration.

Indeed, although the past three years have been a very challenging time for the entire Board, we had forged strong bonds as we journeyed ahead with a united commitment – to serve the forensic laboratories in Asia and enhance the quality of forensic services. I would like to sincerely thank all **Board Members** who had given me their utmost support in the past three years in so many different areas, and they had unreservedly stepped forward to take up responsibilities even though they faced many challenges back in their home front. I would like to thank Ms Nellie Cheng, who had served as **AFSN Secretariat** for the past three years, for doing a wonderful secretariat work in coordinating different meetings and events, as well as providing effective communication with all members. Appreciation goes to all **WG/C Steering Committee Members**, for all your hard work in organising activities for the WG/C. Indeed, as Helen Keller once said *"Alone we can do so little; together we can do so much."*

As I step down as the President at the coming Board Election at the **AFSN Annual General Meeting** in Jakarta, I would like to give my assurance that I will continue to provide strong support to AFSN Board. Lastly, I would also like to express my gratitude to all colleagues and member institutes who have supported AFSN activities and have grown with us – **I hope we will continue to collaborate and achieve more for AFSN together!**

A big kudos to Dr Lui Chi Pang, Editor of ForensicAsia, and his team for yet another excellent issue of **ForensicAsia**. I hope you will enjoy reading the articles in this issue.

Dr Angeline Yap
AFSN President
Health Sciences Authority
Singapore

Editor's Address

Dear colleagues and members of AFSN,

As the Covid-19 is becoming endemic, I am glad to see that many countries have started to open up their borders, allowing the increase of international travel in 2022. To our AFSN colleagues, this is a good news to be able to meet face to face in our upcoming 14th AFSN Annual Meeting and Symposium in Jakarta, Indonesia, in November this year. At the same time, I am also glad to see that we have received a number of articles for publication in our ForensicAsia.

In this Issue, we have a total of 3 technical articles and 3 case studies. It is surprising to see that 5 of the articles are in the illicit drugs discipline (with 3 of them on NPS encountered in our region), and one in forensic biology discipline. We are also glad to have received one article under the international scene from Mr Henry Maynard, Chair, American Society of Crime Laboratory Directors (ASCLD) Forensic Research Committee (FRC), introducing the work of FRC and to invite us to join their campaign to advance forensic science globally in a number of ways.

I would like to take this opportunity to thank those who have supported ForensicAsia by contributing your valuable research and studies, our guest editors in reviewing the articles, as well as our editorial assistants who have helped in the administrative matters and the artwork design for the online publication of this new Issue.

Happy reading.

Dr Lui Chi Pang
Editor

Editorial Committee

For enquiries, feedback or contribution of articles, please email to asianforensic@outlook.com.
For contribution of articles, please refer to the guidelines at www.asianforensic.net.

Content

ASCLD FRC Initiatives

International Scene

Mr. Henry Maynard
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Over the last few years, the American Society of Crime Laboratory Directors (ASCLD) Forensic Research Committee (FRC) has created and launched tools to help advance forensic science research and further research collaborations within the forensic science community. We'd like to highlight three FRC initiatives and ask you to consider participating. With your assistance and through sharing this information with your network we will take a big step forward in advancing forensic science research globally.

The Laboratories and Educators Alliance Program (LEAP)

The goal of LEAP is to facilitate collaborative research between operational and academic forensic science laboratories and institutions. The program provides a platform for laboratories, researchers, and students to seek projects aligning with their mutual research capabilities. Currently, there are over 100 LEAP partners with representation from the United States, Canada, Australia, and Saudi Arabia. With your help, we can increase these numbers and help improve global forensic science collaboration.

LEAP Partner Map:
https://www.zeemaps.com/view?group=2593428&x=-100.076364&y=39.142359&z=13

University Researcher Sign-up Form:
https://www.ascld.org/wp-content/uploads/2020/06/LEAP-Lab-Sign-up-Form-2020.pdf

Operational Laboratory Sign-up Form:
https://www.ascld.org/wp-content/uploads/2020/06/LEAP-Lab-Sign-up-Form-2020.pdf

The FRC Collaboration Hub

This FRC initiative allows researchers to highlight research projects that require forensic practitioner support (as a subject matter expert, as a collaborator, as a beta-tester, as a participant, etc.). Any researcher can request support by providing basic information about their project and what assistance they need. Once posted online, forensic practitioners can review the list of active research projects, determine if they want to participate and if so, obtain the researcher's contact information needed to discuss coordination. This helps make it easier for researchers to solicit input from forensic practitioners while also allowing practitioners a centralized list of active research projects that they may want to support. Current projects focus on decision-making while triaging evidence at crime scenes, implementing and increasing blind proficiency testing, designing and prototyping EviHunter (a suite of automated tools to analyze and discover Android-app-generated evidential data), developing tools to aid handwriting and document examiners, and more. ASCLD hopes that this program will further communication and cooperation between forensic researchers and practitioners.

Sign-up Form:
https://www.ascld.org/researchers-seeking-practitioners-study-form/

FRC Collaboration Hub:
https://www.ascld.org/researcher-practitioner-collaboration-directory/

The Validation/Evaluation Repository

This repository allows forensic science labs the opportunity to share information about their validations and evaluations. Labs can submit validation summaries or complete validations. By sharing information, we can learn from each other's studies and make our future studies that much better. This would also help advance the collective understanding of the robustness of forensic methods, technologies, and tools. Submissions to date include validations for antiepileptics/acidic drug confirmation and quantitation, hemp analysis, drug-facilitated crime analysis, THC-related compounds, benzodiazepines analysis, ProFlex Thermalcycler with GlobalFiler, STRmix with Globalfiler, and more. In the future, this could lead to coordinated large-scale, inter-laboratory studies.

The Validation/Evaluation Repository:
https://www.ascld.org/validation-evaluation-repository/

Please consider joining us in this campaign to advance forensic science globally by signing up for LEAP, adding research projects to the FRC Collaboration Hub, and submitting evaluations and validations to the repository. For more information about these initiatives or other ASCLD FRC Initiatives please review the ASCLD FRC Website:

https://www.ascld.org/forensic-research-committee/

Comparison of three DNA extraction methods from soft tissues soaked in a septic tank

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Abstract

STR profiling for the personal identification of soft tissues is one of the important identification methods in the forensic sciences. Many DNA extraction methods have already been developed aiming at extracting high amounts of amplifiable DNA. However, few studies have been conducted to recommend the best DNA extraction method from soft tissues soaked in a septic tank. In this study, we compared the success rate of STR markers for the detection of soft tissues (skin and subcutaneous soft tissue, scalp soft tissue, etc.) soaked in a septic-tank with three DNA extraction methods including Chelex-100 method, automatic DNA extraction workstation method, and Chelex-100 method combined with automatic DNA extraction workstation method. Our results indicated that human soft tissues soaked in a septic tank are available for subjected DNA typing, and Chelex-100 combined with automatic DNA extraction workstation was the optimal method.

Introduction

Human soft tissues contain a large number of nucleated cells and high-quality DNA. If human soft tissues were soaked in a septic tank for about 20 days, the genomic DNA would be polluted by putrid bacteria and inorganic salts. However, there are few studies on the success rate of STR detection. DNA extraction is one of the most basic and essential techniques in the study that allow huge advances in the forensic community. This study aims to select an optimal DNA extraction method for contaminated soft tissues in a septic tank by analyzing the STR success rate with three kinds of DNA extraction methods.

Materials and Methods

Sample preparation and DNA extraction

Human soft tissues (N=48; including 2 samples from muscle soft tissue; 4 samples from visceral soft tissue; 42 samples from skin and subcutaneous soft tissue, scalp soft tissue, etc.) were soaked in a septic tank for about 20 days at temperature of 35°C. Each tissue was washed with distilled H_2O to remove the surface contaminants. A size of about 0.5cm×0.5cm×0.5cm was taken from each tissue and divided into 3 parts on average. A total of 144 samples were extracted by three different DNA extraction methods, separately.

Method 1: Chelex-100 method. All tissue samples were added with 200μL of 5% Chelex-100, 20μL proteinase K (10mg/mL), and 10μL of 1M DTT. The mixtures were incubated at 56°C with a Thermomixer comfort (Eppendorf, Germany) until they were completely dissolved. This step was followed by a second incubation step of 8 min at 100°C. Finally, all samples were centrifugated at 13000 rpm for 3 min and stored at -20°C.

Method 2: Automatic DNA extraction workstation method. DNA extraction from all 48 samples was performed using the BK-Magnetic beads Based Trace DNA Extraction Kit (24-Channel Automatic Dedicated) on a 24-Channel Automatic DNA Extraction Workstation (BOKUN BIOTECH, China). The procedures were completed according to the manufacturers' instructions with a final elution volume of 40μL.

Method 3: Method 1 combined with Method 2. After 48 samples were extracted by Method 1, the supernatant of each sample was purified using Method 2.

Amplification and electrophoresis

The DNA amplification was performed in a 10μL of total volume using the GlobalFiler™ Express PCR Amplification kit (Life Technologies, USA). Each reaction contained 2μL PCR mix, 2μL primer mix, in addition to 6μL of template DNA. The reaction was conducted on a Veriti™ 96-Well Thermal Cycler (Life Technologies, USA) according to the following cycling conditions: initial denaturation at 95°C for 1 min; 29 cycles of (denaturation at 94°C for 10 sec, annealing and extension at 59°C for 90 sec) followed by a final extension step at 60°C for 10 min.

PCR products were prepared for capillary electrophoresis with 1μL PCR products, 0.4μL LIZ600 size standard, and 9μL Hi-Di™ Formamide per well. Separation and detection of amplified products were performed using the 3500xL Genetic Analyzer (Life Technologies, USA). The collected data were analyzed using GeneMapper®ID-X version 1.5 software. According to the results, 200 relative fluorescence unit (RFU) was set as the peak detection threshold for STR allele calls. Peaks lower than 15% of the parent peak were removed as a stutter.

Results

STR success rate varied among the different methods and STR profile of more than 15 loci was set as the success threshold. Table 1 shows the success rate comparison of different DNA extraction methods. Among the three DNA extraction methods, the peak heights of each locus were relatively balanced, and the surrounding noise was low.

Method 3 showed the highest success rate, the complete STR profiles were generated in 57.14% of 42 samples. 35.71% of 42 samples yielded partial profile, and 7.15% failed to generate any reportable alleles. When using Method 2, the ratio of complete and partial STR profiles was reduced to 45.24%, and 26.19% respectively, and the ratio of failed samples was increased to 28.57%. Method 1 showed the lowest STR detection rate with an average of 45.24%, the peak height decreased as the amplicon size increased and significant allele drop-in and drop-out were observed.

The main difference among the three methods was the purity and concentration of DNA. Method 3 showed the highest purity and concentration, which produced the best electropherogram and the highest STR detection rate (Fig.1). Figure 2 indicated that the STR profile obtained by methods 1 and 2 was not detected in large segments of loci.

The STR success rate was also affected by the different kinds of tissue types. Muscle and visceral soft tissues had no PCR products using all three DNA extraction methods. Other soft tissues, including skin, subcutaneous soft tissue, and scalp soft tissue, etc. showed 45.24% STR profiles with Method 1, 71.43% STR profiles with Method 2, and 92.86% STR profiles with Method 3, respectively.

Table 1: Comparison of success rate of three different DNA extraction methods

Type of samples	Number of samples	Method 1		Method 2		Method 3	
		Number of success	Success rate	Number of success	Success rate	Number of success	Success rate
Muscle	2	0	0%	0	0%	0	0%
Viscera	4	0	0%	0	0%	0	0%
Skin, etc.	42	19	45.24%	30	71.43%	39	92.86%

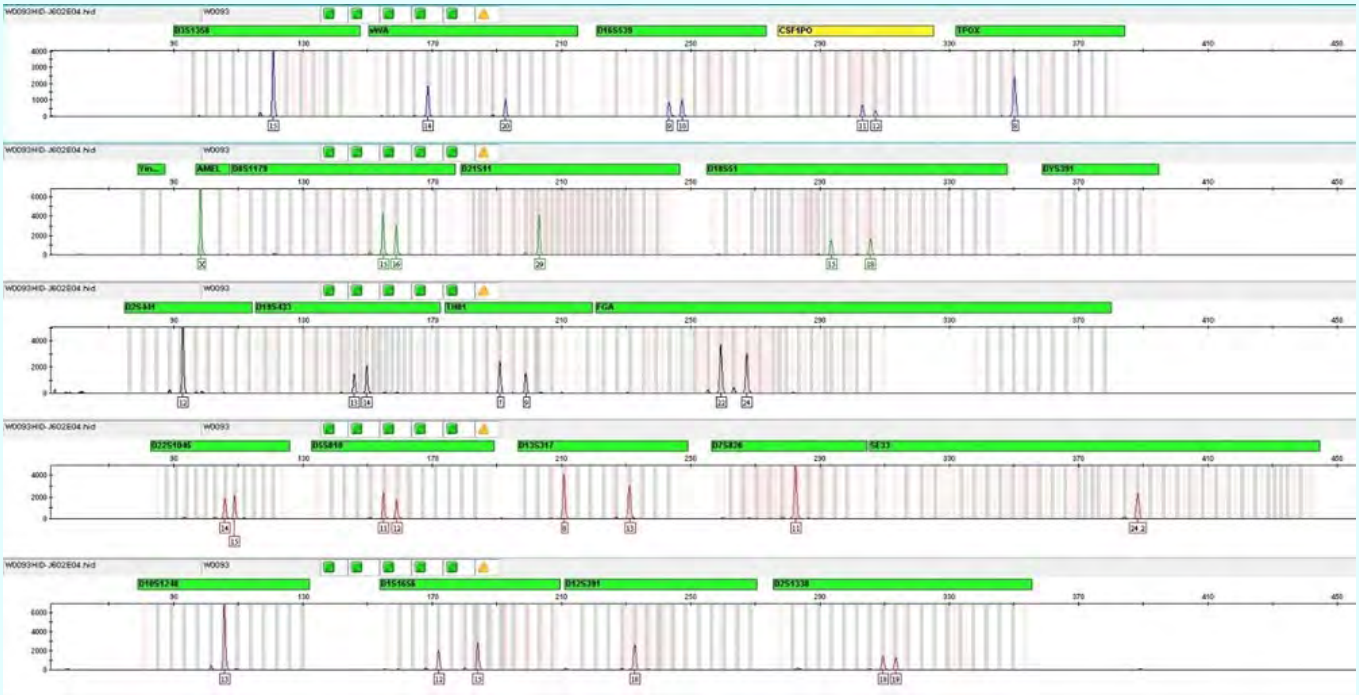

Figure 1. The STR profile of a skin sample with the GlobalFiler™ Express PCR Amplification kit using method 3

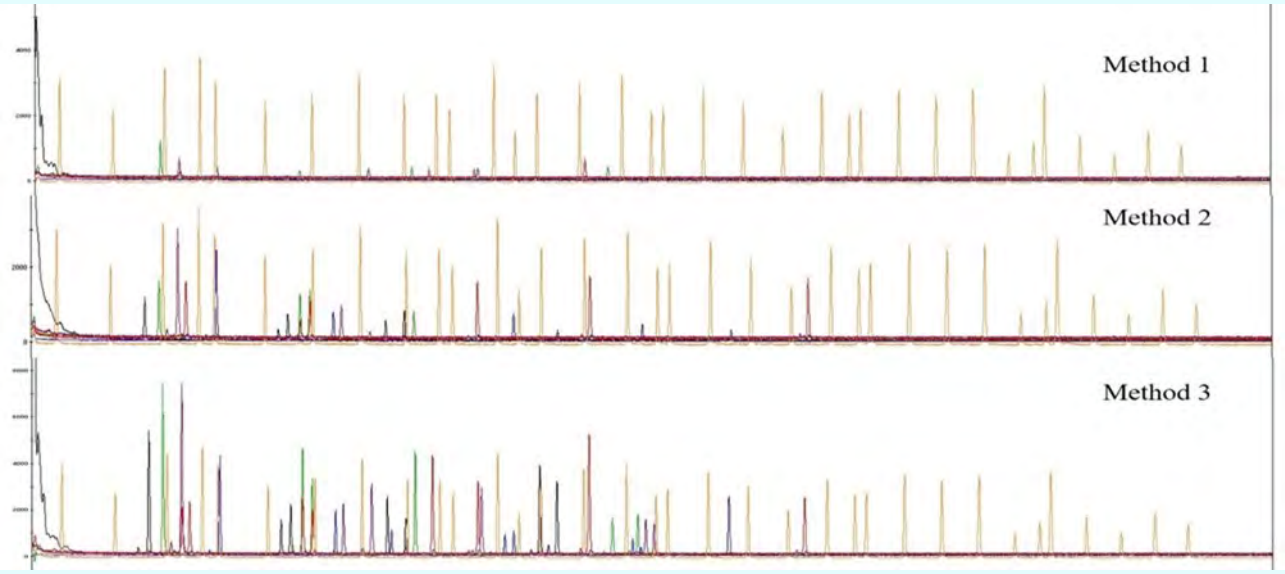

Figure 2. Comparison of electrophoretogram of three different DNA extraction methods with the same sample

Discussion

Soft tissue block is a kind of biological material that is often used in the identification of the deceased in cases of dissections or large disasters. There are many reports on DNA extraction from formalin-fixed tissues in forensic studies[1-2], DNA extraction from these samples and genetic profile analysis are known to be a great challenging task due to the influence of formaldehyde. However, there are few detailed studies for DNA extraction from soft tissues soaked in a septic tank. Septic tank mainly through precipitation and filtration to remove most of the non-dissolved pollutants, and then through microbial fermentation to degrade the dissolved pollutants, finally achieve the reduction of pollution indicators[3]. Septic tank contains a variety of anaerobic bacteria, which be used to decompose solid solids, and contains a variety of corrosive toxic gases, such as hydrogen sulfide, methane, cyanide, and so on[4].In this study, three different DNA extraction methods were used to evaluate the STR success rate for soft tissues soaked in a septic tank.

Chelex-100 method is the simplest and most convenient DNA extraction method. In the process of DNA extraction by using this method, the digestion time of tissue blocks was still significantly prolonged even if the amounts of protein K and DTT were added. In general, similar to formalin-fixed tissues, STR typing showed a typical degraded DNA pattern: left to right decrease in allelic peak heights and drop-in or drop-out of the larger alleles[5]. There were several reasons for the failure of method 1, for example, due to the presence of inorganic salts in a septic tank, the generation of cross-links between nucleic acids and proteins resulting in the difficulty of DNA release, and a large number of remnants or substances that inhibit the PCR amplification reaction. In the process of DNA extraction using method 2, it has been found that there were still some tissues that cannot be completely digested, although DNA was purified using the magnetic beads method to a certain degree, the amount of DNA yielded was not enough for PCR amplification, the overall peak heights of STR profile was low, and some alleles were not detected at some loci. Compared to the previous two methods, the success rate of STR profiling from soft tissues soaked in a septic tank using method 3 was the highest, and significant improvements were observed in PCR amplification efficiency. The advantages were that sufficient DNA concentrations were yielded by completely dissolving tissue blocks and the quality of DNA could largely be improved during purification.

The results in the present study also showed that there were no PCR products of DNA extraction from 2 cases of muscle soft tissues and 4 cases of visceral soft tissues by all three methods. However, full or partial STR profiles were obtained from the majority of skin soft tissues using method 3. The detection rate of DNA typing in formalin-fixed lung tissue was higher than that in the other tissues[6], which was relatively similar to this study. The alveolar cells, epidermis, and dermis cells belong to epithelial cells and have stronger tolerance to spoilage bacteria and inorganic salts. Beni Lew showed that when the reaction temperature increased from 10°C to 28°C, the solubility of non-dissolved pollutants increased by 2.2 times, the biodegradation rate of dissolved pollutants increased by 1.5 times[7]. therefore, tissue cells, such as muscle and viscera, are more likely to be polluted at temperature of 35°C by external contamination and surroundings resulting in the breakdown of DNA chain and small fragments of DNA that are not suitable for STR typing in forensic testing.

Conclusion

In this study, three kinds of extraction methods were used to evaluate the success rate of STR markers for soft tissues soaked in a septic tank. The results suggest that Chelex-100 combined with automatic magnetic beads DNA extraction workstation method is the optimal method based on adequate digestion and purification. The human soft tissues (skin and subcutaneous soft tissue, scalp soft tissue, etc.) are preferred to select for the subsequent DNA typing. Muscle and viscera soft tissues are suggested to detect mitochondrial DNA or SNP using next-generation sequencing (NGS) due to the degradation.

References

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Hierarchical Cluster Analysis Potential Application on Suspected Plant-based New Psychoactive Substances (NPS) Found in the Philippines using FT-IR/ATR Spectral Data

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Abstract

Due to the crackdown on traditional drugs, there has been significant abuse reports on plant-based NPS, especially in countries wherein they are naturally available, have social importance, and are legal to abuse. In the Philippines, there are existing plant-based NPS namely kratom and angel's trumpet. Therefore, an efficient method to discern such substances is vital. This study aims to explore the potential of hierarchical cluster analysis using FT-IR/ATR spectral data, as a statistical grouping model to discriminate authenticated kratom and angel's trumpet plants. It further highlights important factors to interpret grouping results such as extracting solvent and soaking period, FT-IR/ATR fingerprint region range, and geographical location. Results revealed that samples soaked in methanol-chloroform (10:1) for 12 hours, and using either FT-IR/ATR spectral data range of 650-1500cm^{-1} or 650-2000cm^{-1}, appeared to be the most suitable conditions for the explored statistical method. Importantly, angel's trumpet *Brugmansia candida* samples from Tagaytay, Cavite and Los Baños, Laguna which are geographically close clustered significantly hence, further indicated that plant location might be an additional key factor that could affect grouping results. The proposed protocol indeed has the potential to create grouping models, that can be used to quickly assess the species level identities of claimed kratom and angel's trumpet, in a simple and cost-effective manner.

Introduction

New psychoactive substances (NPS) have been a persistent challenge not only to drug law operatives, but most particularly to forensic laboratories because of their complexity. Alarmingly, some NPS drugs seem to be alternative products of syndicates in recent years, because majority of them are still not controlled. Meanwhile, not all NPS are synthetically made, as some can be readily abused by drug dependents without laboratory preparations. The said substances are referred to as plant-based NPS[1]. In the Philippines, there are existing plant-based NPS namely kratom and angel's trumpet that are gaining attention among authorities as they are still not controlled thus, prone to be abused (Figure 1).

Figure 1. Plant-based NPS found in the Philippines

Some established methodologies for plant identification are time consuming and sometimes impractical to perform by forensic laboratories[2-3]. In fact, the Philippine drug law only gives analyst limited period to release reports needed for prosecution[4]. Moreover, traditional plant identification schemes are often affected by different factors, and seems not cost-effective in certain situations, as it utilizes various consumables and reagents. Therefore, developing efficient identification techniques for plant-based NPS, without the need for extensive sample preparation, expensive materials, and lengthy analysis times, are indeed necessary.

The main objective of this study is to investigate whether multivariate hierarchical cluster analysis using the data from fourier transform infrared spectrometer (FT-IR) with attenuated total reflectance (ATR) attachment, can potentially create a statistical grouping model to identify and discriminate kratom and angel's trumpet species. It further explores important factors that may affect statistical groupings specifically the crude sample's extracting solvent and soaking period, FT-IR fingerprint region that will be used for statistical analysis, and the geographical location of the authenticated samples.

Materials and Methods

Sample Collection and Preparation

Samples of suspected kratom were collected in Baco, Oriental Mindoro (K-OM), Talacogon, Agusan del Sur (K-ADS), and Nabunturan, Compostela Valley (K-CV). Meanwhile, claimed to be angel's trumpet plants were sampled in Santa Fe, Nueva Vizcaya (T-NV), Los Baños, Laguna (T-L), and Tagaytay, Cavite (T-T) (Figure 2).

For K-OM and K-ADS, a total of two (2) plants were collected and were coded as "A"-first plant and "B"-second plant. For K-CV, T-NV, T-L, and T-T, only one (1) plant per area was acquired. Moreover, all individual plants were sampled in triplicates, and numerical codes from "1-3", were further assigned.

Figure 2. Sampling sites

Leaf samples were placed in self-sealing plastic bags. Certified reference materials of marker psychoactive drugs of kratom and angel's trumpet namely mitragynine and scopolamine respectively, were purchased through authorized suppliers. All leaf samples were washed with water and air dried before preparing samples for analysis. To around 4 grams of sample acquired separately from kratom and angel's trumpet, 25ml of methanol-chloroform (10:1) were added before subjecting to ultrasonication for 1 hour. After soaking, crude extracts were filtered and 2ml of resulting filtrates were dried under nitrogen gas, and were reconstituted with 4 ml of methanol. The sample extracts prepared were then subjected to gas chromatograph-mass spectrometer (GC-MS) analysis for the detection of marker drugs.

Detection of Marker Drugs

Samples were analyzed using Agilent GC-MS with a 7890A GC system and a 5975C mass selective detector, with a split injection of 50:1. High purity helium was used as carrier gas with a flow rate of 1.2 ml/min. Moreover, DB-5MS column (30 m x 250 µm x 0.25 µm) was used following an oven program: initial temperature of 50^{o}C held for 1 minute, with a ramp rate of 20^{o}C/min, up to final temperature of 300^{o}C held for 5 minutes. The MS scan range applied was 40 to 600 amu.

The presence of mitragynine and scopolamine in the sample extracts analyzed were identified by comparing the chromatograms and mass spectra obtained from the sample extracts, against that of their respective reference standards. The GC-MS results of samples and standards were further examined against the scientific working group for the analysis of seized drugs (SWGDRUG) spectral library, to validate the presence of the said marker drugs.

Plant Species Authentication

DNA analysis was performed at the University of the Philippines-Philippine Genome Center (UP-PGC), to determine the species level identities of suspected kratom and angel's trumpet plants. DNA samples were extracted from plant leaves using Isolate II DNA kit, following the manufacturer's protocol. DNA amplification was carried out by polymerase chain reaction (PCR), and amplicons were purified using a clean-up kit. The generated sequences from the mat-KIM and matK-MALP markers, were aligned to produce consensus sequences using BioEdit software. Data interpretation of the resulting sequences were performed by matching with existing sequence profiles, from the barcode of life database (BOLD) through the basic local alignment search tool (BLAST). The top three (3) BLAST search results were considered for the plant species evaluation process.

FT-IR/ATR Data Acquisition

A Shimadzu FT-IR instrument, IR Prestige-21 equipped with ZnSe ATR attachment (PIKE Technologies), was utilized to analyze all authenticated plant-based NPS samples and generate multivariate data. Leaf samples were subjected to similar sample preparation procedures stated in the previous section, with the exclusion of the methanol solvent reconstitution step. Two (2) different solvent systems specifically methanol-chloroform (10:1) and ethyl acetate were separately used for the extraction of both authenticated kratom and angel's trumpet plants. Furthermore, all samples were soaked in the said solvents for 12 hours and 24 hours.

The FTIR/ATR spectra were obtained using 30 scans within the wavenumber region of 650–4000 cm^{-1} at 4 cm^{-1} resolution. The corresponding corrected intensity values of spectral peaks generated in the fingerprint region's 650-1500cm^{-1} and 650-2000cm^{-1}, were processed for statistical analysis. A licensed Statgraphics Centurion-18 was used as a statistical tool to perform hierarchical cluster analysis utilizing ward's method and squared euclidean distance.

Data Comparison and Evaluation

Dendrograms generated using the Statgraphics Centurion-18 software were evaluated in terms of their grouping patterns, considering important factors specifically (1) extracting solvent (methanol-chloroform (10:1) Vs. ethyl acetate), (2) sample soaking period in solvent (12hrs Vs. 24hrs), and (3) FT-IR/ATR fingerprint region range (650-1500 cm^{-1} Vs. 650 to 2000 cm^{-1}). Different geographical locations of all collected plant-based NPS were also reviewed, to verify if it could be an important parameter to consider during sample clustering evaluation.

The optimal conditions were identified based on how authenticated samples statistically clustered. The selection criteria included the (1) ability for triplicate samplings taken from the same plant to form group, (2) ability for similar plant species collected from the same area to cluster, (3) ability to discriminate between different plant species, i.e. different plants of the same species collected from different geographical locations should cluster.

Limitations of the study

Unfortunately, laboratory restrictions due to the declaration of COVID-19 pandemic at the time of this study, hindered the analysis of all K-OM samples soaked in ethyl acetate for 24 hours. Moreover, only mat-KIM DNA marker was used for its DNA barcoding.

Results and Discussion

The collected leaf samples claimed to be kratom and angel's trumpet both coincided with the general botanical literature descriptions[5-6]. GC-MS analysis confirmed the presence of marker drugs in samples, except from sampled plants in Talacogon, Agusan del Sur where mitragynine was not detected. Importantly, the DNA match results further suggested that it was likely a different species. Meanwhile, although only one (1) DNA marker was used to authenticate samples from Baco, Oriental Mindoro, GC-MS result indicated that they were indeed Mitragyna speciosa, since mitragynine is unique in the said species [7]. Similarly, Nabunturan, Compostela Valley samples were confirmed to be Mitragyna speciosa. On the other hand, all suspected angel's trumpet plants were authenticated as Brugmansia candida (Table 1).

Table 1. Plant sample characterization and species authentication results

Sampling Areas and Sample Codes	Marker Psychoactive Drug Detected	DNA Barcoding Results	
		DNA Marker 1 (mat-KIM)	DNA Marker 2 (matK-MALP)
Suspected Angel's Trumpet **LOS BAÑOS, LAGUNA (TL)**	Scopolamine	*Brugmansia candida*	*Brugmansia candida*
		Datura stramonium	*Datura stramonium*
		Brugmansia suaveolens	*Brugmansia suaveolens*
Suspected Angel's Trumpet **TAGAYTAY, CAVITE (TT)**	Scopolamine	*Brugmansia candida*	*Brugmansia candida*
		Datura stramonium	*Datura stramonium*
		Brugmansia suaveolens	*Brugmansia suaveolens*
Suspected Angel's Trumpet **SANTA FE, NUEVA VIZCAYA (TNV)**	Scopolamine	*Brugmansia candida*	*Brugmansia candida*
		Brugmansia suaveolens	*Datura stramonium*
		Brugmansia sanguinea	*Datura stramonium*
Suspected Kratom **TALACOGON, AGUSAN DEL SUR (KADS)**	None	*Mitragyna speciosa*	*Mitragyna hirsuta*
		Mitragyna speciosa	*Mitragyna rotundifolia*
		Mitragyna hirsuta	*Mitragyna diversifolia*
Suspected Kratom **NABUNTURAN, COMPOSTELA VALLEY (KCV)**	Mitragynine	*Mitragyna speciosa*	*Mitragyna speciosa*
		Mitragyna speciosa	*Mitragyna speciosa*
		Mitragyna hirsuta	*Mitragyna hirsuta*
Suspected Kratom **BACO, ORIENTAL MINDORO (KOM)**	Mitragynine	*Mitragyna speciosa*	Not performed due to the declaration of COVID-19 pandemic.
		Mitragyna speciosa	
		Mitragyna speciosa	

The fingerprint region data of FT-IR/ATR were utilized in this statistical grouping study, as the absorption pattern in this area provides the most characteristic information about the sample. Therefore, it is useful for sample discrimination studies[8]. The hierarchical cluster analysis revealed that samples soaked in methanol-chloroform (10:1) for 12 hours, using either FT-IR spectral range of 650-1500cm^{-1} or 650-2000cm^{-1}, appeared to have provided more grouping consistency, in terms of the criteria set for the selection of optimal conditions (Figure 3).

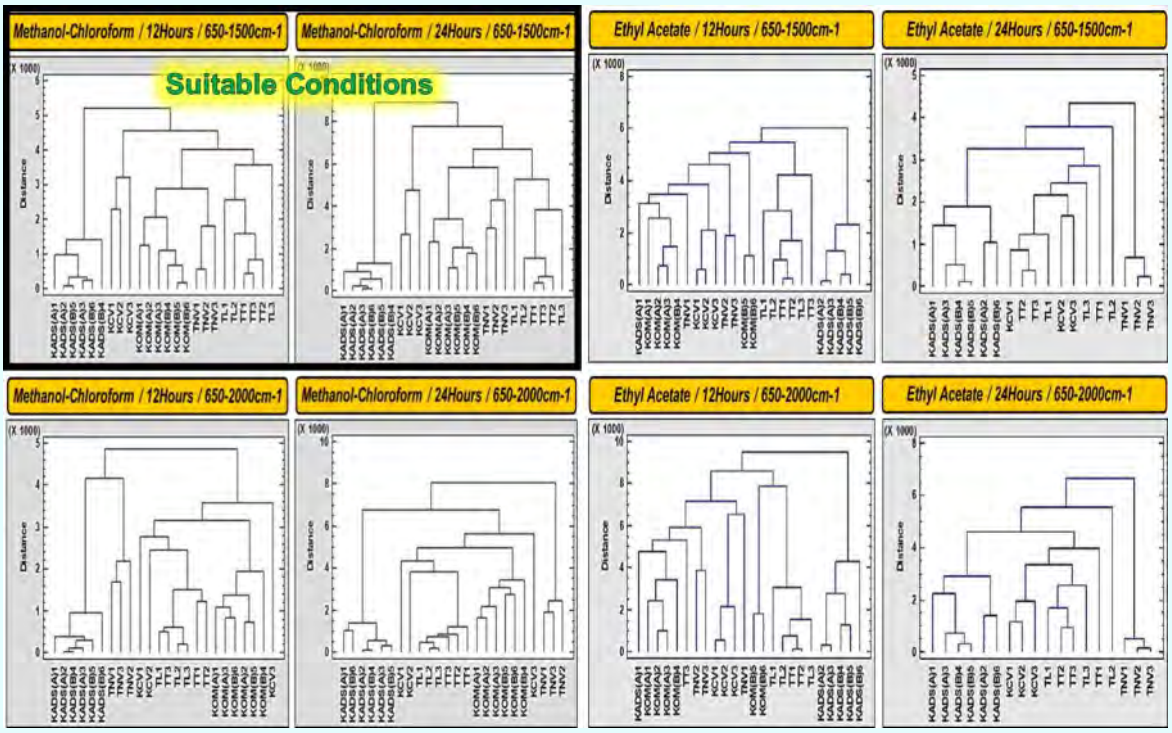

Figure 3. Hierarchical cluster analysis results using FT-IR/ATR spectral data

Using the selected optimal conditions, dendrograms generated showed good clustering among samples collected from same plant (triplicate sampling) and same geographical location (A and B) which also served as control. However, it was observed that although K-OM and K-CV were both confirmed Kratom *Mitragyna speciosa*, they did not group with each other. This can be attributed to their geographical location being far away from each other. Suspected kratom (K-ADS) samples that appeared to be of different species, were also discriminated from the said samples.

It is worth mentioning that angel's trumpet *Brugmansia candida* samples from Tagaytay, Cavite (T-T) and Los Baños, Laguna (T-L) which are geographically close, appeared to cluster significantly using the optimal conditions selected. In fact, other dendrograms with different parameters showed similar grouping trend as well. Noteworthily, similar species from a distant province of Santa Fe, Nueva Vizcaya (T-NV), did not group with the said sites. Hence, the above-said findings further suggested that geographical location could be a significant parameter, to consider for this type of statistical investigations.

Conclusion and Recommendations

The results of this study showed that sample clustering using FT-IR/ATR data can be affected by the type of solvent used and its soaking period, but independent of the selection of FT-IR spectral fingerprint region. In addition, the geographical location where the samples originate may also affect the clustering results. However, to validate the said observation, it is recommended to collect more samples from different geographical regions in the Philippines. The use of other plant parts and multivariate techniques can also be investigated in the future.

This exploratory study can be used as a reference to future studies dealing with plant-based NPS identification and discrimination, with the use of inexpensive statistical tools. Lastly, this kind of undertaking is beneficial in the standpoint of drug-policy-making in the country, as it can provide quick and reliable reports about dominant plant-based NPS species being abused in the Philippines.

Acknowledgement

The researchers are grateful to Mr. Derrick Arnold C. Carreon (Former Director, PDEA Laboratory Service), Ms. Angela D. Salvador (Acting Deputy Director, PDEA Laboratory Service) and Ms. Kresta Muluken R. David (Former Chairperson, Chemistry Department, Adamson University-Manila), for their warm support in making this PDEA-Adamson University scientific research collaboration possible.

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New Psychotropic Substance (NPS) encountered in Sri Lanka

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Abstract

Until the early 1980s drug addiction was not regarded as a serious social problem in Sri Lanka. It has now become a major issue because of its impact on public health, drug related crimes, drug induced diseases, poverty and family life destruction. Main categories of seized drugs in Sri Lanka are Heroin, cannabis and methamphetamine (Ice).[1] Illicit drugs pose the greatest threat to the country's urban areas and major cities, with urban areas having a higher level of drug prevalence than rural areas. [2]

NPS are synthetic substances that are created by modifying the chemical structures of the active ingredients found in traditional drugs. Chemists can create new analogues that can be legally traded or provided, but they are not suitable for human consumption. The main categories of NPS are synthetic stimulants, synthetic cannabinoids, synthetic hallucinogens and synthetic depressants. In Sri Lanka, NPS drugs include cathinones, ketamine, phenethylamines, synthetic cannabinoids and pharmaceutical drugs which are misused. For example, methylphenidate, a pharmaceutical drug which is used to treat narcolepsy and attention deficit hyperactivity disorder (ADHD), is misused or abused for its stimulant properties. Misuse or abuse of methylphenidate at high dosage can cause serious heart and blood pressure problems.[3]

Despite the fact that there are several main categories of major drugs used in Sri Lanka, there have been 171 users arrested in 2019, which are counted under the category of psychoactive substances. It accounts for 0.19 percent of total arrested cases, which is higher than the percentages of cocaine and Hashish arrested cases in 2019. NPS drug seizures has a significant increase in recent years according to the drug related statistics.[4]

The Government Analyst's Department is the accredited government laboratory in Sri Lanka which performs the analysis of illicit drugs received to the country as a requirement of judicial investigation. There is a remarkable increase in the number of drug cases received by the narcotic section in the past few years. In Sri Lanka the most prevalent NPS drugs are phenethylamines under the category of 2C drugs. Ring substituted phenethylamines or 2Cs are designer drugs such as 2C-B (4-bromo- 2,5- dimethoxyphenethylamine, 2C-E (4-ethyl-2,5-dimethoxyphenethylamine and 2C-C (4-chloro-2,5-dimethoxyphenethylamine) having hallucinogenic and stimulant effects.[5] 2C refers to the group of newly substituted designer hallucinogens with methoxy groups at the 2 and 5 positions on the ring.[6]

Ketamine is an anesthetic drug with hallucinogenic effects.[7] In 2020 the Sri Lankan Navy confiscated the largest ever drug seizure from a foreign vessel at sea comprising 605 kg of crystal methamphetamine and 579 kg of ketamine. Khat or Catha edulis which contains the psychoactive ingredients cathinone and cathine is smuggled to the country, mainly via courier and postal cargo. Most of the khat cases were disguised as Chinese tea.

Seized drug samples are submitted to the laboratory from enforcement agencies such as the Police, Police Narcotic Bureau and Excise Department for routine analysis. NPS drugs received by the laboratory between 2018 to 2021 were analyzed using Thin Layer Chromatography (TLC), Gas Chromatography Mass Spectrometry (GCMS), Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography with Flame Ionization Detector (GC-FID).

Table I shows the breakdown of the various types of NPS drugs examined between 2018 to 2021. According to the above data exhibits containing 2C-I were identified only in 2018. 2C-E were identified in the last three years. 2C-C were identified in 2019 and 2020. 2C-B were identified in 2020 and 2021. The popularity in 2C series can be mainly observed in 2019. Higher number of ketamine and khat cases were analysed in 2020. There is a significant drop in number of cases in 2021 due to the lock down imposed in the country during Covid-19 pandemic season.

Table 1

	2CE	2C-B	2C-C	2CI	KETAMINE	CATHINONE IN KHAT	METHYL PHENIDATE	4-METHYLMETHCATHINONE	AMITRYPTYLINE	BENZHEXOLE	CATHINE
2018				2		5				2	1
2019	7		6			3	4		1		1
2020	2	1	1		12	7		1			
2021	1	3			1	1					

The challenges faced by the lab in NPS analysis are lack of some reference materials of novel drugs and lack of necessary high resolution instruments such as Liquid Chromatography Mass Spectrometry (LC-MS/MS).

In Sri Lanka, Psychotropic substances are controlled by “Convention Against Illicit Traffic in Narcotic Drugs and Psychotropic Substances Act No:1 of 2008.” The manufacture, possession, sale, purchase, transport, import and export of any narcotic drug or psychotropic substance listed, is an offence under this Act. But the problem is all NPS varieties seized in the country are not included in this Act. Therefore, it is timely to review and amend the Act to include the newly emerged NPS drugs that have become available both in the country and internationally to curb their proliferance.

In conclusion, the narcotic laboratory needs to be upgraded with modern laboratory equipment and enhance its technical capacity to overcome the challenges faced in the analysis of novel drugs and to ensure timely detection of NPS. Further, it is vital to strengthen the capacities of law enforcement agencies to control the problem of abuse and trafficking of NPS drugs and to improve the implementation of law in the country.

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Investigation of Illicit Medicine Manufacturer Using Handheld Raman Spectrometer: A Case Study of Pharmaceutical Crime

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Introduction

Illicit pharmaceutical industry poses a significant threat to public health. Data indicate that increasing amounts of falsified medicines are illicitly manufactured in Southeast Asia as illicit pharmaceutical producers in India and China have shifted their manufacturing process to other Southeast Asian countries in order to avoid regulation and enforcement [1]. An extensive variety of illicitly manufactured medicines have been detected in Southeast Asian countries in recent years including falsified vaccines, anabolic steroids, anti-cancer medication, sleeping pills and also drugs for weight loss [1]. The emergence of unauthorised or unlicensed online pharmacies has opened the door for the sales of these falsified or counterfeit medicines of unknown origin and safety. Forensic laboratories can play an important role in investigating pharmaceutical crimes.

Case History

In December 2020, a clandestine facility manufacturing medicines was shut down in Penang state, Malaysia. During the raid operation, the clandestine facility was in the process of manufacturing green capsules as shown in Figure 1 and Figure 2. Besides that, large amounts of tablets and capsules were found in the facility as shown in Figure 3 and 4.

Figure 1. Manual capsule filling machine

Figure 2. Semi-automatic capsule filling machine

Figure 3. Brown tablets in plastic bag

Figure 4. Red capsules in scene

An on-site investigation was conducted by chemists from the Department of Chemistry by using a Rigaku handheld 1064nm Raman spectrometer. Based on the investigation by Raman spectrometer, several active pharmaceutical ingredients and chemicals were identified as below:

- Active Pharmaceutical Ingredient (API): Acetaminophen, prednisolone, aspirin, dexamethasone as shown in Figure 5
- Cutting agent: Wheat starch, lactose monohydrate
- Excipient: Polyvinylpyrrolidone cross linked
- Other: Pyridoxine hydrochloride (Vitamin B6), palmitic acid

In addition, there were several bags of unknown brown coloured powder suspected to be plant materials that could not be analyzed by Raman spectrometer. Eventually, five different types of samples were submitted to the laboratory for further analysis.

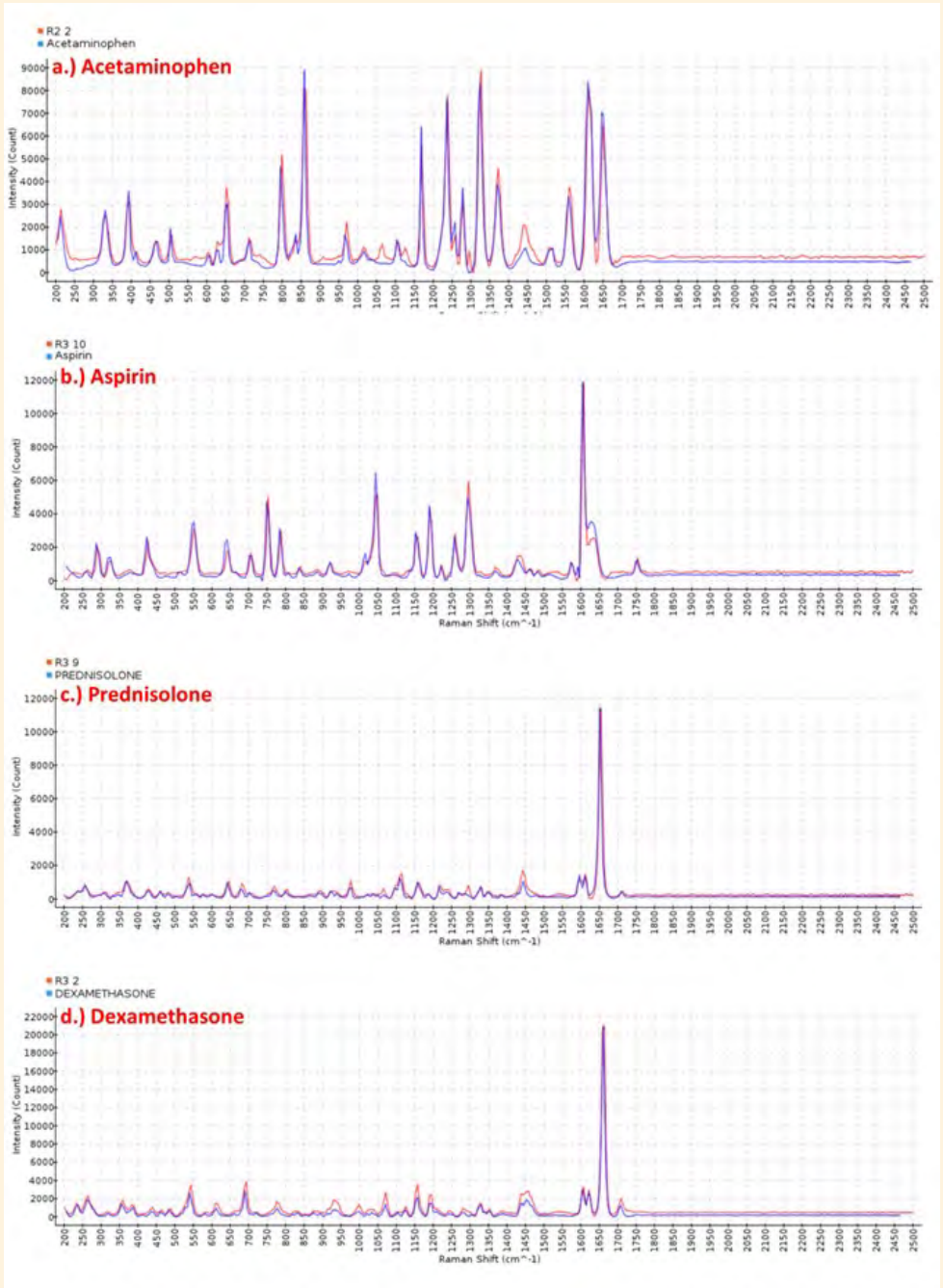

Figure 5. Raman spectra of: (a) Acetaminophen, (b) Aspirin, (c) Prednisolone, and (d) Dexamethasone, over the 200-2500cm^{-1} range

Material and Method

Sample Preparation

The tablets and content of capsules were pulverised and approximately 100mg to 150mg were dissolved in 10.0 ml of chloroform/methanol mixture (1:1, v/v). The mixture was sonicated for 10 mins and then left to settle for 10 mins. The extract was filtered and then subjected to GC-MS analysis.

Gas Chromatography Mass Spectroscopy (GC-MS) Condition

The samples were analyzed using an Agilent 7890B gas chromatograph equipped with an Agilent 5977B mass selective detector with parameters as shown in Table 1. The mass spectra of the samples were compared with NIST 2014 and SWGDRUG MS Version 3.4 libraries.

Table 1 GC-MS(EI) Parameter for: (A)Green capsules and black tablets, (B) Red capsules and brown tablets

Method	A [2]	B
Column	DB-5MS capillary column (30 m × 0.25 mm × 0.25 µm)	
Oven Temperature	150°C (2 min), 30°C/min to 275°C (0 min), 20°C/min to 310°C (10 min)	80°C (2 min), 20°C/min to 150°C (2 min), 10°C/min to 280°C (5 min) 20°C/min to 300°C (14 min)
Injector Temperature	270°C	
Injection Mode	Split Mode (20:1)	Split Mode (50:1)
Injection Volume	1 µL	1 µL
Column Flow rate	1.0 mL/min (Helium)	
Transfer Line	280°C	
Scan Range	35–400 m/z	30–500 m/z
Run Time	16.9 min	40.5 min

Results and Discussion

Five different types of samples found at the clandestine laboratory were subsequently analyzed by GC-MS in this investigation. As the result of GC-MS analysis, the green capsules and black tablet were found to contain dexamethasone (retention time of 7.823min). The retention time and mass spectra of dexamethasone in the samples also match with the high purity dexamethasone powder seized at the scene, as shown in Figure 6. Dexamethasone is a corticosteroid that is usually used to relieve inflammation and treat certain forms of arthritis.

The brown tablets and red capsules were found to contain acetaminophen (retention time of 11.557min) and chlorpheniramine (retention time of 15.117min) respectively. Acetaminophen (pain reliever) and chlorpheniramine (antihistamine) are commonly available medicines that are sold over-the-counter (OTC) without prescription. The total ion chromatograms and mass spectra of the samples are shown in Figure 6.

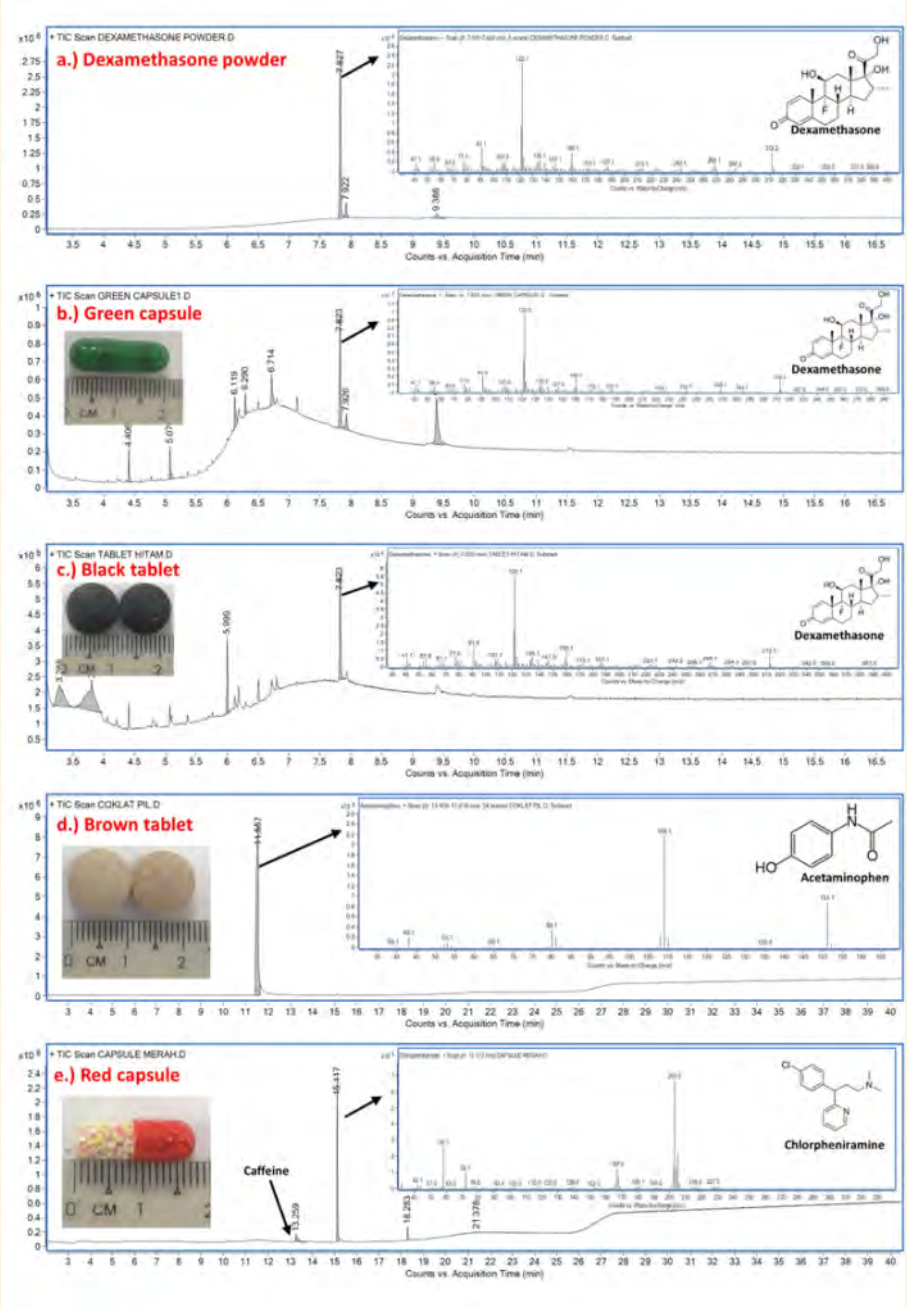

Figure 6. GC-MS total ion chromatogram (TIC) and electron ionization (EI) mass spectra of: (a) Dexamethasone powder, and content of (b) green capsules, (c) black tablets, d) brown tablets and (e) red capsules.

Conclusion

In summary, forensic laboratories can provide effective support to law enforcement including in the investigation of pharmaceutical crimes. As part of that the application of Raman spectroscopy in clandestine laboratory investigation is highly effective, as it provides the non-destructive and rapid identification of unknown materials at the scene.

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Case Study

Philippine Angel's Trumpet: Exploring a Non-Regulated Psychoactive Plant

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Introduction

Angel's trumpet plants are native to South America but became naturalized in some tropical areas in Asia [1]. In the Philippines, angel's trumpet is referred to as "talampunay" or "katsubong" depending on the geographical location. Locally, it is commonly cultivated for ornamental purposes because of its attractive trumpet-shaped flowers as shown in Figure 1. The said plant substance is also considered by several indigenous cultures as essential traditional medicine, and is used during religious ceremonies and rituals [2]. However, there is an impending threat on this introduced species since recreational usage reports, alongside with poisoning incidents among Filipinos, are significantly observed through the years. This is mainly attributed to its psychoactive contents namely scopolamine and hyoscyamine [3]. An exploratory study to acquire preliminary data on existing angel's trumpet plants in the Philippines was carried out, in order to have an overview about its metabolite profile and species level identities. This undertaking is beneficial to drug policy makers, as angel's trumpet including its psychoactive contents are still not locally controlled.

Figure 1. Angel's trumpet plants found in the Philippines

Materials and Methods

Suspected angel's trumpets were collected in selected regions of the Philippines as shown in Figure 2. Plant samples specifically leaves, flowers, and twigs needed for chemical analysis, were prepared and analyzed using gas chromatograph-mass spectrometer (GC-MS) using the parameters indicated in Table 1. Meanwhile, DNA analysis was performed at the University of the Philippines-Philippine Genome Center (UP-PGC), to determine the species level identities of claimed to be angel's trumpet plants collected in the wild. The DNA were extracted from leaf samples using extraction kit, following its manufacturer's protocol. The top three (3) basic local alignment search tool (BLAST) results for the sequenced DNA samples were considered for the plant species identification process.

Figure 2. Sampling sites

Table 1. GC-MS parameters

GC-MS Parameters	
Column	DB-5MS
Column Oven Program	50°C for 1 min then ramp at 20°C/ min to 300°C then hold for 5 min
Injection Temperature	250°C
Injection Mode	Split
Split Ratio	50:01:00
Column Flow	1.2 mL/min
Ion Source Temperature	250°C
Interface Temperature	230°C
Acquisition Mode	Scan
Scan Range	45 to 400 amu
Spectral Libraries	SWGDRUG and MPW

Summary of Results

a. Results revealed that samples collected in Apayao, Benguet, Nueva Vizcaya, Bataan, Cavite, Laguna, and South Cotabato, were confirmed to be trumpet plants based on botanical and chemical examination.

b. On the other note, laboratory findings of samples from Rizal and Negros Occidental yielded negative indications that they are were trumpet plants.

c. All confirmed angel's trumpet plants were sampled in high altitude areas.

d. While scopolamine was detected in all confirmed trumpet plant samples, detection of hyoscyamine however varied among samples as it was only observed in Benguet twig specimens.

e. Importantly, variation in scopolamine contents seemed to differ in terms of plant parts examined.

f. Other plant metabolites such as essential fatty acids and vitamin E, were detected in the said samples.

g. Samples subjected to DNA barcoding appeared to be *Brugmansia hybrid* species.

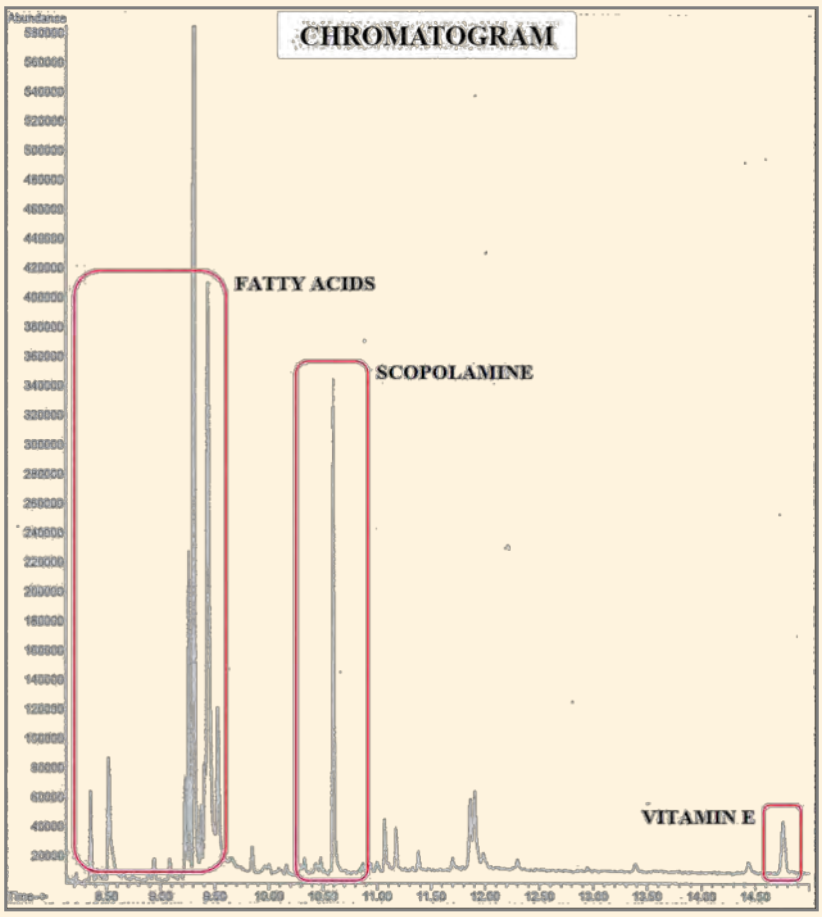

Figure 3. Representative sample chromatogram of confirmed angel's trumpet

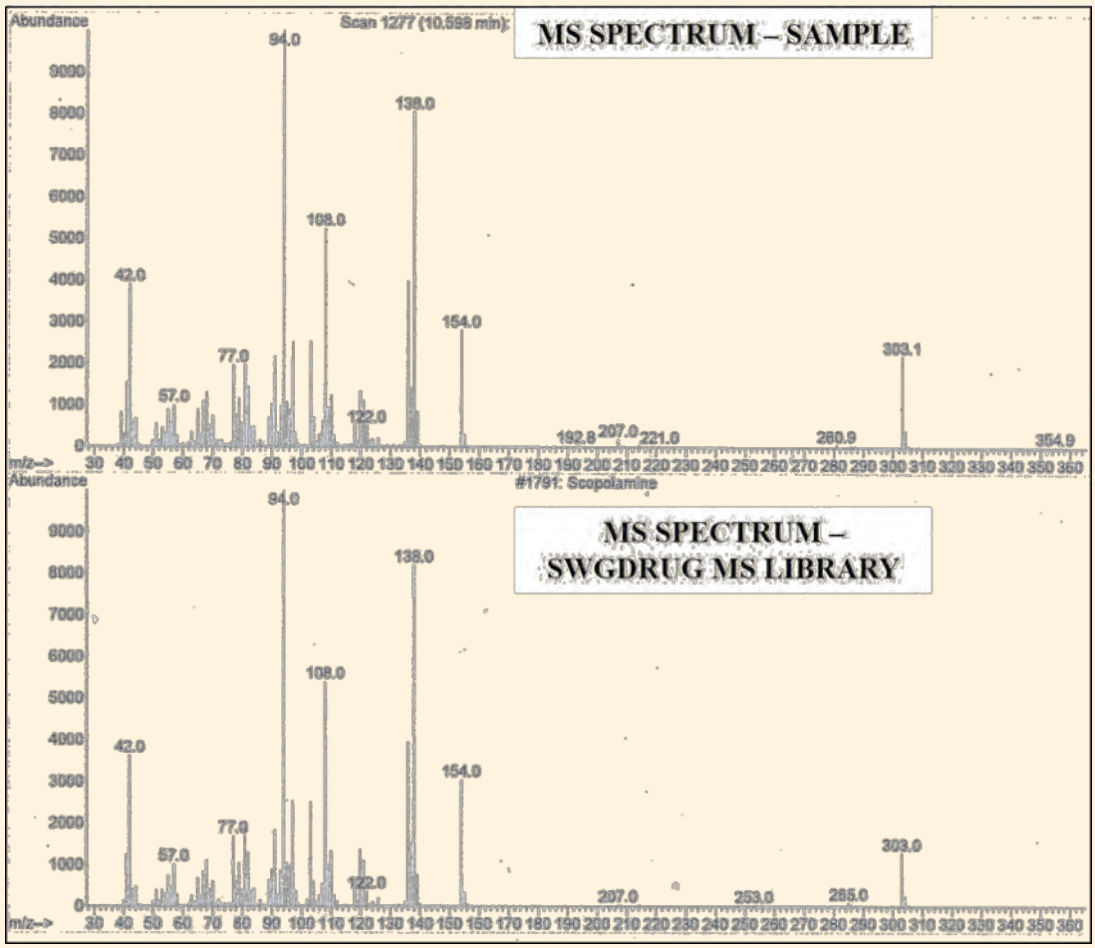

Figure 4. Representative scopolamine MS spectral match of confirmed angel's trumpet

Table 2. Plant sample characterization and species authentication results

Sampling Site	Altitude	Flower Color	Position of Flower	Plant Part	GC-MS Analysis		DNA Analysis	
					Scopolamine	Hyoscyamine	DNA Marker 1: matk-MALP	DNA Marker 2: matk-KIM
La Trinidad, Benguet	High	Pink	Downward	Leaves	+	-	1. ***Datura stramonium*** 2. *Brugmansia candida* 3. *Brugmansia suaveolens*	1. ***Brugmansia candida*** 2. *Brugmansia suaveolens* 3. *Brugmansia sanguinea*
				Flowers	+	-		
				Twigs	+	+		
Baguio, Benguet	High	Yellow	Downward	Leaves	+	-	1. ***Brugmansia candida*** 2. *Datura stramonium* 3. *Brugmansia arborea*	1. ***Brugmansia candida*** 2. *Brugmansia suaveolens* 3. *Brugmansia sanguinea*
				Flowers	+	-		
				Twigs	+	+		
Santa Fe, Nueva Vizcaya	High	Orange	Downward	Leaves	+	-	1. ***Brugmansia candida*** 2. *Datura stramonium* 3. *Datura stramonium*	1. ***Brugmansia candida*** 2. *Brugmansia suaveolens* 3. *Brugmansia sanguinea*
				Flowers	+	-		
				Twigs	+	-		
Conner, Apayao	High	Yellow	Downward	Leaves	+	-	1. ***Brugmansia candida*** 2. *Datura stramonium* 3. *Brugmansia suaveolens*	1. ***Brugmansia candida*** 2. *Brugmansia suaveolens* 3. *Brugmansia sanguinea*
				Flowers	+	-		
				Twigs	+	-		
Tagaytay, Cavite	High	Orange	Downward	Leaves	+	-	1. ***Brugmansia candida*** 2. *Datura stramonium* 3. *Brugmansia suaveolens*	1. ***Brugmansia candida*** 2. *Datura stramonium* 3. *Brugmansia suaveolens*
				Flowers	Not performed			
				Twigs				
Antipolo, Rizal	High	N/A	N/A	Leaves	-	-	1. ***Solanum mauritianum*** 2. *Solanum violaceimarmoratum* 3. *Solanum violaceimarmoratum*	1. ***Brugmansia candida*** 2. *Brugmansia sanguinea* 3. *Brugmansia suaveolens*
				Flowers	Not performed			
				Twigs				
Los Baños, Laguna	High	Orange	Downward	Leaves	+	-	1. ***Brugmansia candida*** 2. *Datura stramonium* 3. *Brugmansia suaveolens*	1. ***Brugmansia candida*** 2. *Datura stramonium* 3. *Brugmansia suaveolens*
				Flowers	+	-		
				Twigs	Not performed			
Lake Sebu, South Cotabato	High	Orange	Downward	Leaves	+	-	Not performed	
				Flowers	Not performed			
				Twigs				
Polomolok, South Cotabato	High	Orange	Downward	Leaves	+	-	Not performed	
				Flowers	+	-		
				Twigs	Not performed			
Talisay, Negros Occidental	High	Orange	Downward	Leaves	-	-	Not performed	
				Flowers	-	-		
				Twigs	-	-		
Bagac, Bataan	High	Orange	Downward	Leaves	+	-	Not performed	
				Flowers	+	-		
				Twigs	+	-		

Table 3. Other detected plant metabolites with important applications

Other Detected Metabolites	Important Applications [4]
Palmitic acid	Disinfectant and soap production
Linolenic acid	Cosmetics and lubricant additive
Linoleic acid	Cosmetics, food additive, and treatment of preeclampsia
Stearic acid	Food additive and personal care products
Methly linolenate	Fragrance component, air refreshener, and flavoring agent
Vitamin E	Skin antioxidant and fragrance component

Conclusion and Recommendations

Based on this initial study, angel's trumpet appeared to be widely distributed in the wild, specifically in high altitude areas of the Philippines. Although organized survey regarding the plant's usages was not included in the exploration, it was evident that locals were aware about its worrying threats. Thus, it is also important to document local perceptions on angel's trumpet, as this will give holistic data and guide policy makers in their potential regulatory evaluation in the future.

Importantly, preliminary data indicated that there were other important natural products that can be isolated in the said psychoactive plant, which can be utilized in various applications. Therefore, non-traditional policies that will allow isolation of other plant metabolites from both illicit and non-regulated substances that can be used for other important applications, can be reviewed. If this will be pursued, investigating whether some environmental factors play a vital role in the concentration of psychoactive alkaloids and other plant metabolites of angel's trumpet, will be of great significance to be researched as well.

Acknowledgement

The researchers are grateful to all the involved regional offices of the Philippine Drug Enforcement Agency and the Department of Environment and Natural Resources, for their warm assistance during the period of sample collection and field survey.

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Case Study

Clandestine Erimin-5 Laboratory in Penang State, Malaysia

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Introduction

Erimin® a proprietary product of Sumitomo corporation in Japan, which contained nimetazepam, has been discontinued since 2015 [1]. Following its discontinuation, falsified Erimin-5 tablets continue to appear on drug markets and may contain nimetazepam or other type of benzodiazepine. Based on a report released by United Nations Office on Drugs and Crime (UNODC) in 2021, a total of five clandestine laboratories manufacturing benzodiazepine-type substances were dismantled in Malaysia from 2015 to 2020 [1].

Case History

In August 2018, a clandestine laboratory was discovered and dismantled by Royal Malaysia Police in Penang state, Malaysia. Large amounts of Erimin-5 tablets, pre-tableting powders, methamphetamine hydrochloride, chemicals and processing equipment were seized. Based on the chemical and equipment at the site, it was concluded that this clandestine laboratory was producing falsified Erimin-5 tablets. Figure 1 to 3 shown the chemical and machine seized at clandestine laboratory.

Figure 1. Rotary tablet press machine

Figure 2. V-Shape powder mixer shaker

Figure 3. Tablet packaging machine

Using a 1064nm Rigaku handheld Raman spectrometer, forensic chemists from Department of Chemistry had identified menthol, corn starch and mannitol found in the site, while Erimin-5 tablets and pre-tableting powders were submitted to laboratory for analysis. The Erimin-5 tablets and powder were subsequently found be to contain either nitrazepam and clozapine by GCMS. Among the drugs and chemical seized were:

- 26 Kg of Methamphetamine hydrochloride
- 5 Kg of tablets contained 4-methoxymethamphetamine
- 101000 pieces of aluminium foil packaging with barcode “A5000K” and “A5000AA” containing approximately 183 Kg of Erimin-5 tablets
- Approximately 103 Kg of Erimin-5 tablets (loose form)
- Approximately 390 Kg of pre-tableting powders contained clozapine
- Approximately 78 Kg of powders contained 4-methoxymethamphetamine
- Other chemicals such as dextrin, corn starch, 2-pyrrolidone, menthol, mannitol, ethanol

Material and Method

Sample Preparation

Each Erimin-5 tablet was crushed into homogenized powder. Approximately 50mg of homogenized powder was placed in 5 ml volumetric flask, and 5.0 ml of chloroform/methanol mixture (1:1, v/v) was added. The mixture was sonicated for 10 min then left 10 mins to settle. The extract was filtered and then subjected to GC-MS analysis.

Gas chromatography Mass Spectroscopy (GC/MS) Condition

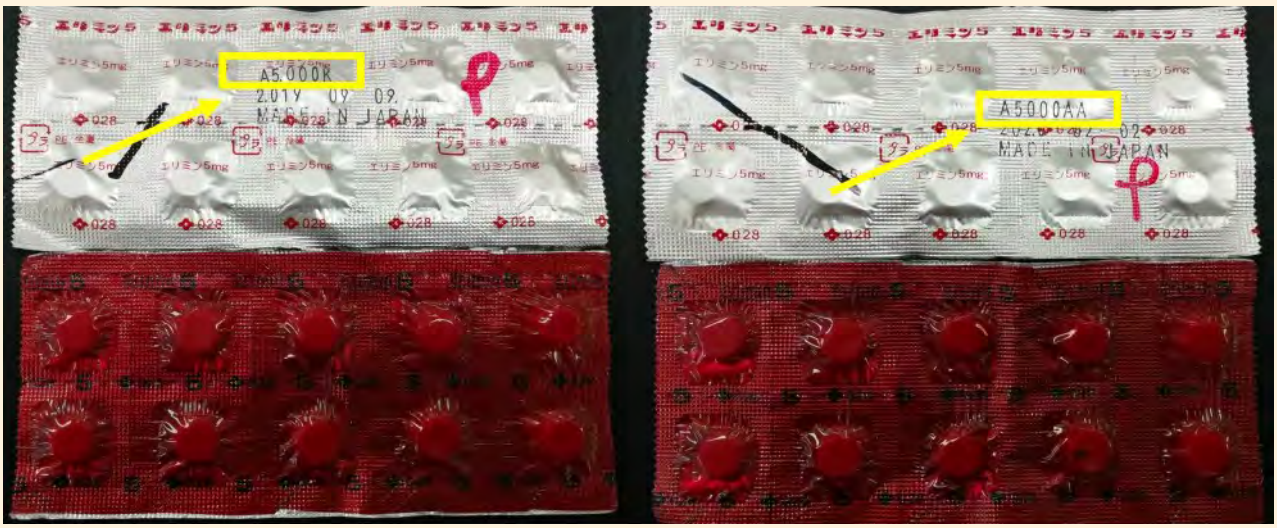
The Erimin-5 tablets were analysed using an Agilent 7890B gas chromatograph equipped with an Agilent 5977B mass selective detector with parameters as shown in Table 1. The mass spectra of samples were compared with NIST 2014 library.

Table 1. GC-MS(EI) Condition

Column	DB-5MS capillary column (30 m × 0.25 mm × 0.25 µm)
Oven temperature	220°C (2 mins) with 20°C/min to 300°C (6 mins)
Injector Temperature	270°C
Injection Mode	Split Mode (50:1)
Injection Volume	1 µL
Column Flow rate	1.0 mL/min (Helium)
Transfer Line	280°C
Scan Range	50–500 m/z
Run Time	12 mins

Result and Discussion

There were two different types of imprints imprinted on Erimin-5 packaging found at the site, namely "A5000K" and "A5000AA (Figure 4). As the result of GC-MS analysis, the Erimin-5 tablet in all aluminium foil "A5000K" was found to contain nitrazepam. On the other hand, the Erimin-5 tablets in all aluminium foil "A5000AA" was found to contain clozapine. Figure 5 shows the GC-MS total ion chromatogram (TIC) of "A5000K" Erimin-5 tablet containing nitrazepam at retention time of 6.475 minute. The base ion peak is observed at *m/z* 280, with other major ions at *m/z* 253, 234, 206. Figure 6 shows the GC-MS total ion chromatogram (TIC) of "A5000AA" Erimin-5 tablet containing clozapine at retention time of 6.916 minute. The base ion peak is observed at *m/z* 243, with other major ions at *m/z* 256, 192 and 227.



a) Aluminium foil with imprint "A5000K" *b) Aluminium foil with imprint "A5000AA"*

Figure 4. Aluminium foil packaging of Erimin-5 tablets: (a) A5000K and (b) A5000AA

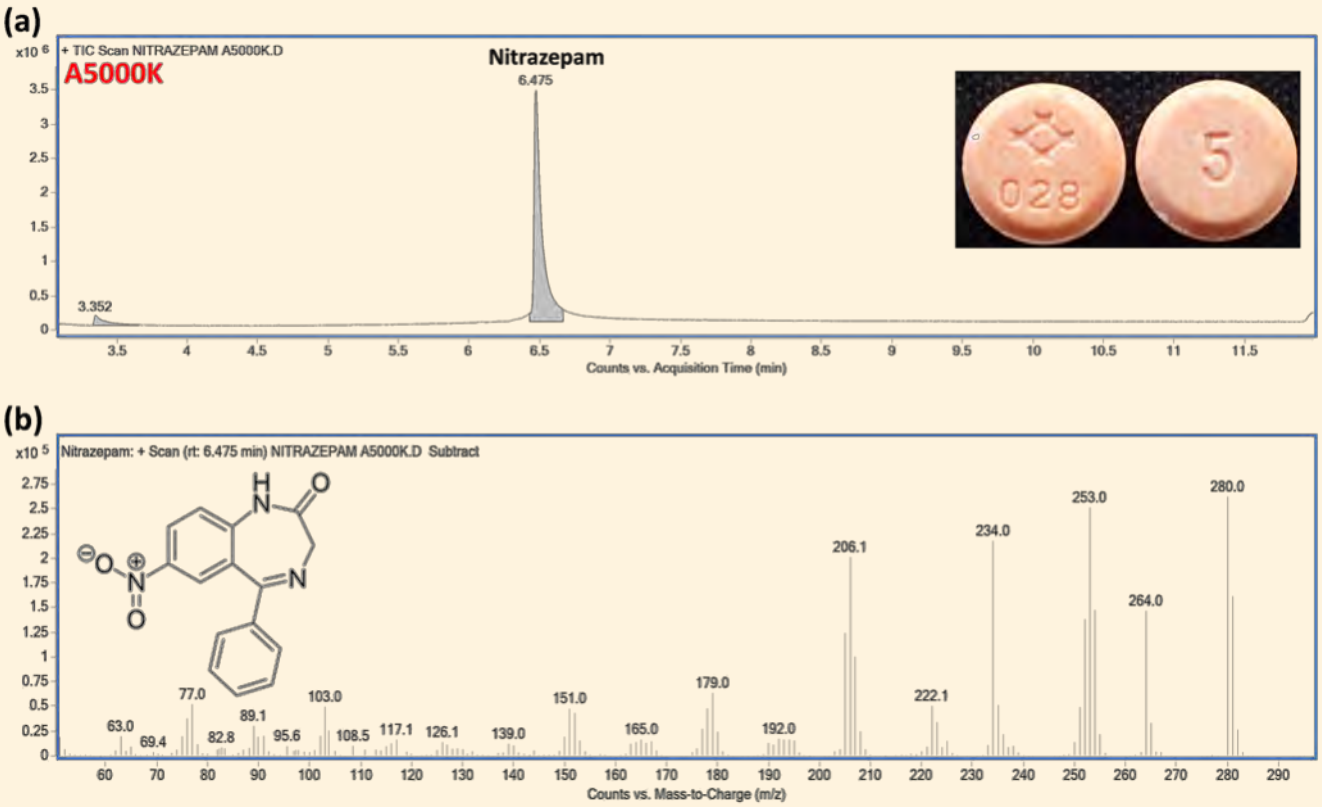


Figure 5. (a) GC-MS total ion chromatogram of Erimin-5 tablet (A5000k) containing Nitrazepam (RT= 6.475 min); (b) Mass spectrum of Nitrazepam

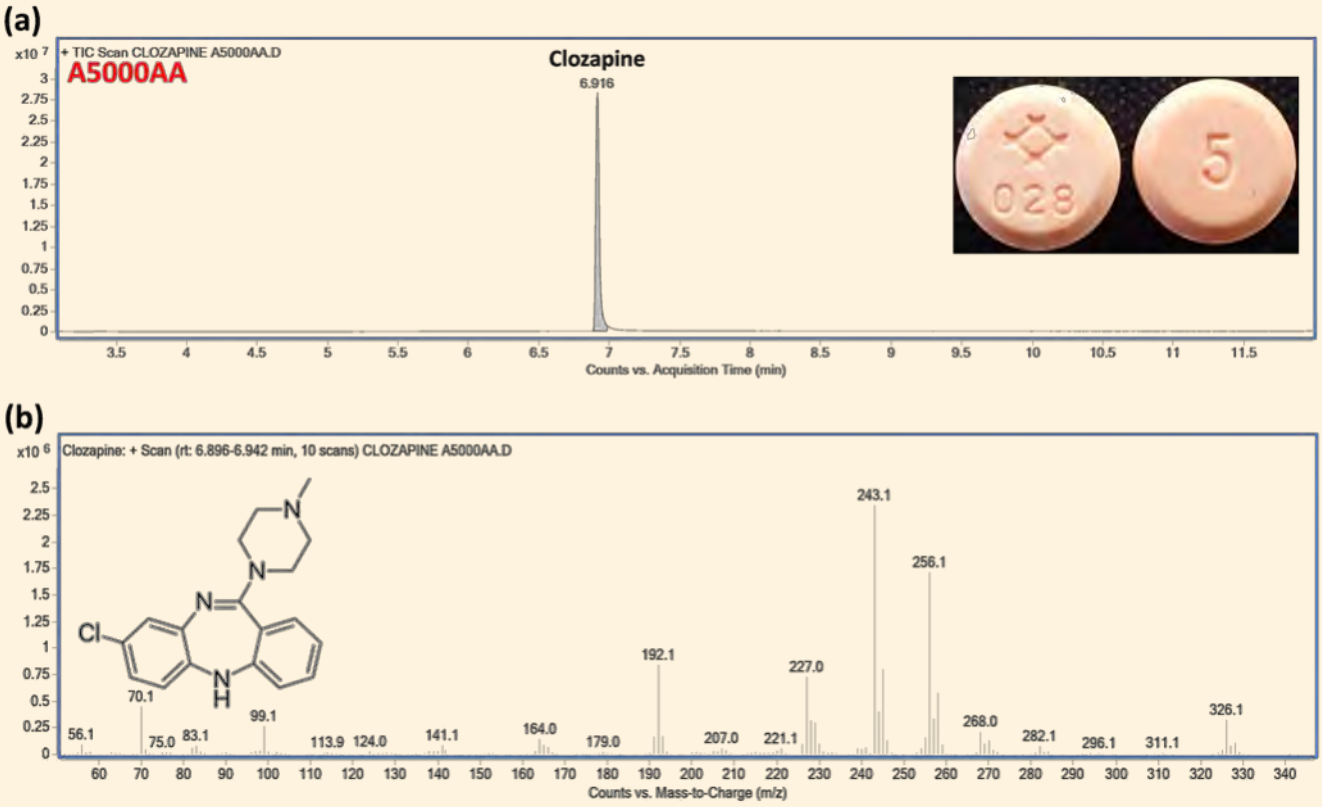


Figure 6. (a) GC-MS total ion chromatogram of Erimin-5 tablet (A5000AA) containing Clozapine (RT= 6.916 min); (b) Mass spectrum of Clozapine

Based on the investigation, it can conclude that the clandestine laboratory was in the process of manufacturing falsified Erimin-5 tablets with clozapine. It is interesting to note that the drug syndicate using imprints ‘A5000K” and “A5000AA” on aluminium foil packaging to distinguish Erimin-5 with nitrazepam and clozapine respectively.

Clozapine is a legal medicine that is used as anti-psychotic, mainly used to treat schizophrenia patients. The finding of clozapine in illicit Erimin-5 tablets shows that the drug syndicates had shifted their modus operandi by using legal medicine in illicit Erimin-5 production. In Malaysia, clozapine was listed under First Schedule of Poison Act 1952, while nitrazepam was classified as 1,4-benzodiazepines derivatives under First Schedule of Poison Act 1952.

Conclusion

This paper presents an illicit Erimin-5 clandestine laboratory had been dismantled in Malaysia at 2018. This is the first report of a clandestine laboratory utilizing the legal medicine clozapine as active ingredient in falsified Erimin-5 tablets. There is a growing concern that the drug dealers may use non-controlled substances to avoid any criminal prosecution.

Acknowledgment

I would like to thank to the Royal Malaysia Police for their contribution of picture at clandestine laboratory.

Reference

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Country/Region	*No.*	*Name of Member Institute (as at October 2022)*
Bangladesh	1	National Forensic DNA Profiling Laboratory
Brunei Darussalam	2	Department of Scientific Services
India	3	Centre for DNA Fingerprinting and Diagnostics
	4	Directorate of Forensic Science, Himachal Pradesh
Indonesia	5	Department of Police Medicine of the Indonesian National Police
	6	Eijkman Institute for Molecular Biology
	7	Forensic Laboratory Centre of Indonesian National Police Headquarters
	8	Indonesian Association of Forensic Pathologist
	9	Laboratory of National Narcotics Board
	10	Master Program of Forensic Science, Postgraduate School, Universitas Airlangga
Lao PDR	11	Food and Drug Quality Control Center
Malaysia	12	CyberSecurity Malaysia
	13	Department of Chemistry
	14	Malaysian Communications and Multimedia Commission
	15	Royal Malaysia Police Forensic Laboratory
Mongolia	16	Mongolian National Institute of Forensic Science
People's Republic of China	17	Beijing Forensic Science Institute
	18	Forensic Science Center of Guangdong Provincial Public Security Department
	19	Forensic Science Division, Department of Fujian Provincial Public Security
	20	Gansu University of Political Science and Law, Key Laboratory of Evidence Science Techniques Research and Application
	21	Guangzhou Forensic Science Institute
	22	Institute of Forensic Science, Ministry of Public Security
	23	Institute of Forensic Science, Dezhou Public Security Bureau
	24	Institute of Forensic Science, Hangzhou Public Security Department
	25	Institute of Forensic Science, Shandong Public Security Department
	26	Institute of Forensic Science, Suzhou Public Security Bureau
	27	Institute of Forensic Science, Tianjin Public Security Bureau
	28	The Institute of Evidence Law and Forensic Science, China University of Political Science and Law
	29	Forensic Science Division of the Government Laboratory, Hong Kong Special Administrative Region
	30	Forensic Science Department of Judiciary Police, Macau Special Administrative Region
Philippines	31	Laboratory Service, Philippine Drug Enforcement Agency
	32	National Bureau of Investigation
	33	National Reference Laboratory for Environmental and Occupational Health, Toxicology and Micronutrient Assay, East Avenue Medical Center, Department of Health
	34	Natural Sciences Research Institute, University of the Philippines Diliman Quezon City
	35	Philippine National Police
Republic of Kazakhstan	36	Forensic Examinations Centre of the Ministry of Justice

Country/Region	*No.*	*Name of Member Institute (as at December 2020)*
Republic of Korea	37	Daejeon Health Institute of Technology, Daejeon Health Sciences University
	38	Graduate School of Forensic Science, Soon Chun Hyang University
	39	Korea Coast Guard Research Institute
	40	National Digital Forensic Center of Supreme Prosecutors' Office
	41	National Forensic Service
	42	Scientific Investigation Center of Korean National Police Agency
	43	Scientific Investigation Laboratory, Ministry of National Defense
Singapore	44	Corrupt Practices Investigation Bureau
	45	Health Sciences Authority
	46	Ministry of Home Affairs
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Thailand	49	Central Institute of Forensic Science
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	51	Department of Forensic Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University
	52	Department of Medical Sciences
	53	Department of Forensic Medicine, Thammasat University Hospital
	54	Faculty of Medicine, Chiang Mai University
	55	Human Genetics Unit, Department of Pathology, Faculty of Medicine, Ramathibodi Hospital
	56	Institute of Forensic Medicine, Police General Hospital, The Royal Thai Police
	57	Office of Narcotics Control Board
The Republic of the Union of Myanmar	58	Defence Services Medical Research Centre
Timor-Leste	59	POLÍCIA CIENTÍFICA DE INVESTIGAÇÃO CRIMINAL - LABORATÓRIO DE POLÍCIA CIENTÍFICA
Vietnam	60	Forensic Medicine Center of Ho Chi Minh City
	61	National Institute of Forensic Medicine
	62	Forensic Science Institute Vietnam