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FOOD SCIENCE & TECHNOLOGY | RESEARCH ARTICLE

From poison to food: On the molecular identity and indigenous peoples' utilisation of poisonous "Lab-o" (Wild Yam, Dioscoreaceae) in Bukidnon, Philippines

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Abstract: Like any other country in the tropical and subtropical regions, wild yams are considered an important food by many indigenous peoples in the Philippines and other parts of Southeast Asia. Yet, cases of intoxication and even death have been reported due to wild yam consumption. Unfortunately, some locals cannot differentiate the edible from the poisonous species that require further processing whilst ethnobotanical information remains scarce. In this study, we determined the identity of a poisonous wild yam locally known as "Lab-o" to the Higaonon tribe of Bukidnon province in the Mindanao island, Philippines. Due to highly diverse morphological variations within species and varieties of yams, "Lab-o" was identified through DNA barcoding. Based on the BLASTn analysis, the wild yam in question was identified as *Dioscorea hispida* Dennst. which further confirms the reported toxicity of the yam. To bridge scientific knowledge and indigenous knowledge system, we also explored the traditional processing methods of wild yam detoxification practiced by various indigenous cultures. The DNA barcoding protocol described in this study may serve as a reference to accurately identify the plant in the wild. The poisonous yams may be labelled with appropriate warnings and



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Dave P. Buenavista is a Newton-CHED PhD Scholar at the School of Natural Sciences, Bangor University, Wales, UK. His PhD project focuses on the co-production of knowledge with Indigenous peoples for Global SDGs with emphasis on the ethnobotanical utilisation of food and medicinal plants by the Higaonon tribe in Mindanao Island, Philippines. The fieldwork and laboratory experiment were completed in the Philippines with Nikko Manuel A. Dinopol, Research Assistant at Central Mindanao University, Philippines. The PhD project is supervised by Dr. Eefke Mollee, Lecturer in Agroforestry and International Development at Bangor University, and Professor Morag McDonald, Senior Fellow of the Higher Education Academy; Fellow of the Royal Geographical Society; and Professor of ecology and catchment management at Bangor University. She is a founder and consortium member of the joint European MSc programme in Sustainable Tropical Forestry (SUTROFOR), and the global PhD programme in Forest and Nature for Society (FONASO).

PUBLIC INTEREST STATEMENT

Wild yams are an important staple for many local and indigenous communities, especially in Asia and Africa. However, wild yam consumption is also responsible for several cases of poisoning and even death. Unlike the edible yam, the wild yam locally known as "Lab-o" requires further processing for safe human consumption. The problem lies in the morphological diversity of yams in which distinguishing the edible from the poisonous presents a challenge even for botanists. In this case, the species identification of "Lab-o" as *Dioscorea hispida* was made possible using DNA barcoding technique. The traditional processing of the yam also proved to be useful in removing the toxic components whilst retaining its nutritional properties. With the knowledge of the accurate botanical identity of the poisonous yam coupled with the indigenous knowledge of traditional processing techniques, the safe consumption of *Dioscorea hispida* could be promoted to improve food security in local communities.

preparation guides as preventive information measures to avoid the incidences of poisoning and death. The indigenous knowledge and practices associated with the utilisation of *Dioscorea hispida* Dennst. and other neglected and underutilised plant resources could be likewise considered in addressing food safety and food insecurity in the Asian region.

Subjects: Agriculture and Food; Plant Taxonomy; Food Additives & Ingredients

Keywords: ethnobotany; *Dioscorea*; food safety; DNA barcoding; matK; Mindanao

1. Introduction

“Man ... has discovered the art of making fire, by which hard and stringy roots can be rendered digestible, and poisonous roots or herbs innocuous”
– Charles Darwin, *The Descent of Man* (1871)

Whilst roots have been important food components by early humans, the adoption of food-processing techniques and cooking had shaped human evolution, and societies (Carmody & Wrangham, 2009; Wrangham & Conklin-Brittain, 2003). It is argued that with the advent of the human use of fire, such innovation has been essential in detoxifying poisonous plants, improve the hominid diet, and significantly influence our biological evolution (Wrangham & Conklin-Brittain, 2003). In modern times, the indigenous knowledge and skills of detoxifying poisonous plants for food can still be found in some local and indigenous communities. Amongst the plants used since time immemorial is yam of the genus *Dioscorea* L. (family Dioscoreaceae) which remains an economically important source of food and medicine. In fact, the consumption of yams can be traced back from archaeological records to early agriculture and plant domestication in New Guinea and Southeast Asia (Denham, 2011; Fullagar et al., 2006; Xhaufair et al., 2017). At present, several species of yams are still part of traditional food systems of the local and indigenous communities such as the Sakai tribe of Thailand (Maneenoon et al., 2008), the rural populace of India (S. Kumar et al., 2017), Nepal (Bhandari & Kawabata, 2005; Sharma & Bastakoti, 2009), Indonesia (Trimanto & Hapsari, 2015), and Timor-Leste (Erskine et al., 2014). In the Philippines, many indigenous populations rely on several yam species for subsistence such as the Ati Negrito of Guimaras Island (Ong & Kim, 2017), the Igorots of the Cordillera mountain ranges (Gayao et al., 2018), the Pala'wan and Tagbanua tribes of Palawan Island (Bernadas & Peralta, 2017; Dressler, 2005; Lacuna-Richman, 2004), and the Alangan Mangyan of Mindoro Island (Mandia, 2004).

Apart from food, wild *Dioscorea* species are valuable in the global pharmaceutical industry as the main source of diosgenin—the precursor for the production of steroidal drugs and contraceptives (Price et al., 2016; Shen et al., 2018), and a promising compound against cancer (Corbiere et al., 2004; Jesus et al., 2016; F. Li et al., 2010; Sethi et al., 2018), Alzheimer's disease (Tohda et al., 2012, 2017), and Hepatitis C virus (Wang et al., 2011). This resulted in large market demand and a gradual decline in the wild populations of *Dioscorea*. In India, for example, steroidal drug production is 100% based on diosgenin, which is extracted from tonnes of *Dioscorea* species making them the most significant plant group amongst the medicinal plants (Chaturvedi et al., 2007). The diosgenin production industry involves the harvesting of 550–650 tonnes of plants worldwide with an estimated market value of 500 USD million (Singh & Kaushal, 2007). As such, its ecology and distribution are of particular interest for sustainable utilisation (Shen et al., 2018). However, species identification and classification have impeded in advancing our understanding of the economic potential of this genus (Sun et al., 2012). With approximately 630 species of *Dioscorea* distributed across Southeast Asia, Africa, Central, and South America, and other tropical and subtropical regions, identifying *Dioscorea* species is difficult due to unreliable morphological characters shared within the genus (Xia et al., 2019). Whilst anatomical examinations coupled with HPTLC analyses showed to be advantageous, there is no adequate anatomical

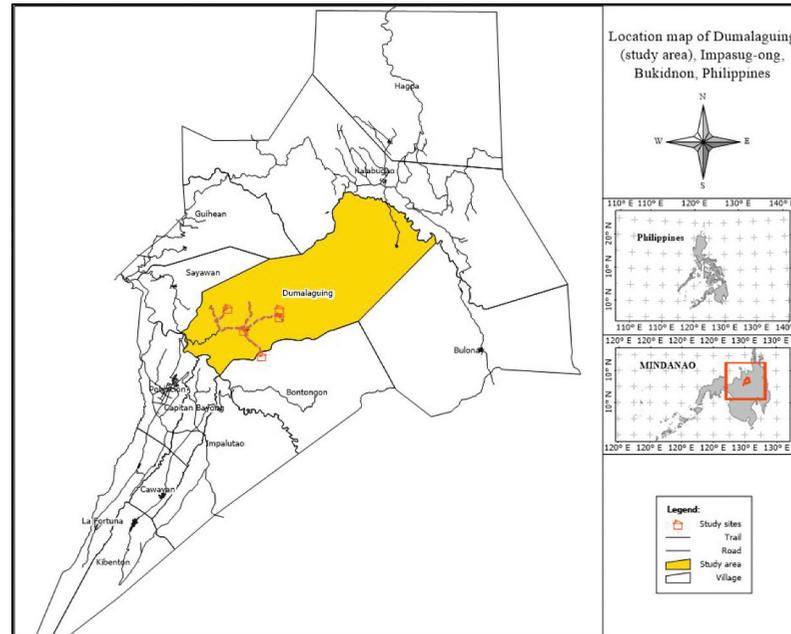
Figure 1. “Lab-o” (Wild Yam) from the forest of Dumalaguing, Bukidnon, Philippines.



study available for most *Dioscorea* species (Raman et al., 2014). Yam taxonomy presents a challenge even to systematists considering the morphological diversity, dioecy, and small flowers of yams (Wilkin et al., 2005). The pacific yam (*Dioscorea nummularia* Lam.), for example, is a highly polymorphic species that is sometimes confused with *D. alata*, *D. glabra*, *D. transversa* and other poorly described *Dioscorea* species (Lebot et al., 2017). Whether ingested as food or medicine, the danger lies in the inability of some locals to differentiate edible from poisonous yam which could lead to intoxication and even life-threatening complications such as acute kidney injury, toxic encephalopathy, and symptomatic hypocalcaemia (Adedoyin et al., 2008; Joob & Wiwanitkit, 2014; K. S. Kang & Taek Heo, 2015; Si, 2009; Sriapha et al., 2015; Yoon et al., 2019). As such, an accurate species identification is of critical importance in ensuring food safety and wellness of consumers.

To resolve taxonomic issues, DNA barcoding proved to be a useful tool in discriminating species of various plant taxa (Hollingsworth et al., 2016; Kress et al., 2005). This approach has been widely applied in identifying and promoting neglected and underutilised species (NUS) (Campanaro et al., 2019), and medicinal plants (J. U. S. Kumar et al., 2018; M. Li et al., 2011). Furthermore, the WHO recommends to barcode various medicinal plants for correct species identification, and verification of purity and concentration of biochemical components (Palhares et al., 2015). This is of particular importance as food and medicinal products of botanical origin have been adulterated and/or substituted, thereby threatening the health of consumers (Han et al., 2016; Peng et al., 2017; Srirama et al., 2017; Tnah et al., 2019). In this study, we examined the identity of a wild yam locally as “Lab-o” (Figure 1) to the Higaonon tribe of Bukidnon in Mindanao Island of the Philippines and explore the indigenous knowledge systems and practices associated with wild yam processing and consumption. The indigenous Higaonon tribe gathers this particular yam from the forests and it serves as a carbohydrate source for the local community in times of food insufficiency or “*tigkabagol*”, particularly, from June to August. Paradoxically, “Lab-o” was reported by the members of the Higaonon tribe to be highly poisonous and inedible if carelessly prepared and consumed. The traditional processing is done by slicing the tuber very thinly and soaking it in brine for at least 3 days then, in flowing water or river for an additional 3 days. After this, the yam chips are boiled and eaten similar to other root crops. The labour-intensive task of gathering and processing the wild yam, from a poisonous to an edible product contributes to the resilience of the Higaonon tribe as it provides an alternative food source. Given the plants’ indispensable value to food security, this study aimed to provide an identification of the botanically unknown yam species through DNA barcoding technique using universal primers for the plastidial *matK* gene and review the traditional use of otherwise considered as neglected and inedible wild plant species.

Figure 2. Map of Barangay Dumalaguig, Impasug-ong, Bukidnon, Philippines.



2. Materials and methods

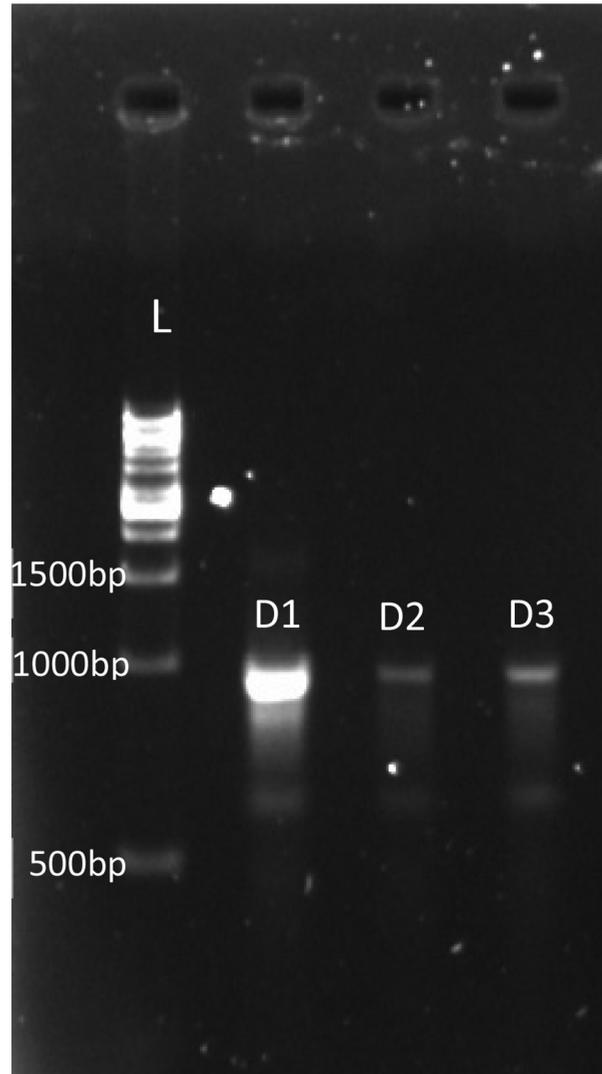
2.1. Collection of plant materials

With the assistance of the local guides from the Higaonon tribe, the fresh leaves and tubers of the wild yam (*Dioscorea* sp.) locally known as “Lab-o” (Figure 1) were collected from the forest of Barangay Dumalaguig (N 08.34529°; E125.05598°), Province of Bukidnon, Republic of the Philippines (Figure 2). Due to military restrictions within the forested areas, the plant materials were limited from one sampling site and three plant individuals. All plants were sterile and cannot be taxonomically identified based on morphology. The excised leaves were placed in labelled polyethylene bags and immediately transported to the Tuklas Lunas Development Centre at Central Mindanao University, University Town, Musuan, Bukidnon, Philippines. The leaf samples were stored temporarily in the biofreezer (Thermo Scientific Revco UxF) at -82°C before gDNA extraction.

2.2. Genomic DNA extraction

The genomic DNA (gDNA) was extracted from the *Dioscorea* leaves using the DNeasy® Plant Mini Kit (Qiagen Biotech Co., Hilden, Germany) with modifications in the protocol. The gDNA extraction was accomplished by using 100 mg of leaf samples milled at 30 Hz for 10 min. The milled plant material was mixed with 400 μL of buffer (AP1) and 4 μL RNase A, vortexed, then incubated for 65°C for 2 h. The second buffer (P3) was then added to the mixture followed by 5-min incubation on ice. The resulting lysate was centrifuged for 5 min at $10\,000 \times g$, pipetted into the QIAshredder Mini spin column, and centrifuged for 2 min at $20\,000 \times g$. The flow-through was transferred in a new tube and 1.5 volumes of buffer (AW1) added to dissolve the precipitate. Then, 650 μL of the mixture was transferred to a DNeasy Mini spin column to be centrifuged for 1 min at $6\,000 \times g$. The resulting flow-through was discarded and the sample in the spin column washed with 500 μL buffer (AW2), centrifuged for 2 min at $20\,000 \times g$ to dry the membrane. The column was transferred to a 2 mL centrifuge tube for the elution process. Lastly, 50 μL of buffer AE was added followed by 5 min of incubation at room temperature (15°C – 25°C) and then centrifuged at $6\,000 \times g$ for 1 min. The final step was performed twice to give 100 μL eluted DNA.

Figure 3. Genomic DNA isolated from three samples of “Lab-o” amplified with primers for *matK* (D1, D2, D3; L- DNA Ladder).



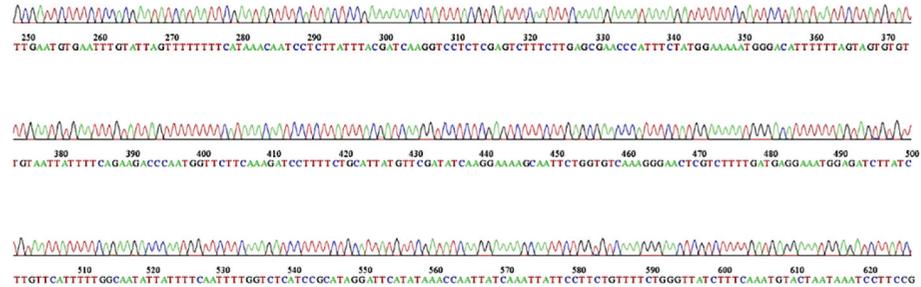
2.3. PCR amplification

A set of primers, *matK-390 f* (5'-CGATCTATTCATTCAATATTC-3') and *matK-1326 r* (5'-TCTAGCACACGAAAGTCGAAGT-3') were used for amplification of *matK* gene. The selection of *matK* sequences over other DNA barcodes (i.e. *rbcl*, *psbA-trnH*, *trnL-F*) was based on the previous findings showing that this plastid encoded gene shows a higher discrimination rate for *Dioscorea* species when compared to other barcodes (Sun et al., 2012; Xia et al., 2019). With the use of gDNA as a template, *matK* was amplified using a PCR thermocycler (Applied Biosystems™ Veriti™, 96-Well Thermal Cycler, Thermo Fisher Scientific). The polymerase chain reaction was performed in a 20 μ L mixture containing the following: ultrapure water, 1 x KAPA *Taq* Buffer B with Mg^{+} , 2.5 mM $MgCl_2$, 0.12 mM dNTPs, 0.3 μ M forward primer, 0.3 μ M reverse primer, 1 U/ μ L Kapa *Taq* DNA polymerase and 1 μ L of gDNA. The amplification was carried out in the following conditions: initial denaturation at 94°C for 45 s, annealing at 55°C for 30 s, and extension at 72°C for 90 s.

2.4. Visualisation

Both the gDNA and PCR products were visualised through gel electrophoresis using 1% agarose in Tris-Boric Acid-EDTA (TBE) stained with Biotium GelRed (10% vol/vol). Gels were loaded with 2 μ L of

Figure 4. Electropherogram section from “Lab-o” *matK* sequence.



gDNA samples or 5 μ L of the PCR product mixed with 2 μ L of 2x loading dye (Vivantis NM0410) loaded into the wells of the agarose gel. One μ L of 1 Kb plus Ladder (Invitrogen, Inc.) was loaded on the first well of the gel. The gel was run at 100 V for 30 min using the Mupid-One Electrophoresis system and viewed using Gel Doc EZ Imager (Bio-Rad Laboratories, California, U.S.A). The PCR bands on the gel were used to determine the success of the gDNA extraction method.

2.5. Sequencing and BLASTn analysis

The bidirectional DNA sequencing of the PCR products was carried out by Macrogen Inc. (Seoul, South Korea). All sequences of the gDNA were edited and aligned using the default parameters in BioEdit Sequence Alignment Editor. To minimize the positional dissimilarity, all missing data and gaps within the sequences were removed. Basic Local Alignment Search Tool (BLAST) analysis was done for homology determination to available sequences in the National Centre for Biotechnology Information (NCBI) nucleotide database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

3. Results and discussion

Using the leaf samples of “Lab-o”, gDNA extraction of high molecular weight was successfully isolated and amplified from three DNA plant samples (designated D1, D2, D3). In the PCR amplification step, *matK* yielded a single band with an amplicon length of 1000 bp suggesting a 100% success rate in the amplification and sequencing efficiency (Figure 3). The results of the sequenced PCR products were of high quality and were confirmed in the electropherogram. The peaks in the electropherogram were non-overlapping and broad, with minimal background noise (Figure 4).

The final sequence with 1154 bp was then compared against the NCBI GenBank database for identity match using the BLASTn analysis (downloaded 8 November 2019). The top “hits” (best matches) in the database with 99 to 100 sequence identity and E-value ≤ 0.001 were considered a match (Arulandhu et al., 2019; Haldar, 2019). The BLASTn algorithm is based on the estimation of the similarity scores between the query sequence and the sequences of the different species of organisms available in the database; hence, the “hits” are listed from top to bottom with decreasing similarity (Haldar, 2019). The Expect value (E), on the other hand, is a parameter that determines the number of expected “hits” obtained by chance in the NCBI database for a fixed length of nucleotide. As such, the lower the E-value, or closer to zero, the more “significant” the match is (Haldar, 2019). The result of the BLASTn analysis identifies the leaf samples of “Lab-o” belonging to *Dioscorea hispida* Dennst. of family Dioscoreaceae.

Following the ranking, the top “hits” for species identification was consistent with identity percentage of 98.87% (Accession No. AY957589.1), 98.88% (Accession No. KJ922806.1, KJ922835.1, KY710780.1, KJ922792.1), and 99.33% (Accession No. AY957589.1). The

forementioned accession numbers had a significant E-Value of 0. For the purpose of plant species identification, above 98% sequence identity was considered a match in interpreting the results of BLASTn analysis (Arulandhu et al., 2019). *Dioscorea hispida* Dennst. is one of the 17 known species of *Dioscorea* in the Philippines (Pelser et al., 2011). It occurs throughout the country and native to tropical Asia (Burkill, 1951). It can be found naturally growing in tropical rainforest, thicket forest understory and forest margins, at low to medium altitudes up to 1500 m (Lim, 2016). This species is taxonomically distinct from other *Dioscorea* species through its sessile (without a stalk) male flowers (Burkill, 1951). As such, a sterile specimen would be impossible to be taxonomically ascertained. In this study, we demonstrate the advantage of the DNA barcoding technique in identifying problematic and cryptic taxa. The precise identification could further provide fundamental information on the origin, distribution, and authentication of raw materials such as leaves and seeds (Campanaro et al., 2019). As predicted, the use of *matK* as a barcode was found to be efficient in discriminating against the aforementioned species of Dioscoreaceae.

The accurate identification of this species is very important since it is considered to be the most poisonous yam and it is often found in the wild together with other species of *Dioscorea* (Burkill, 1951; Lim, 2016; Sharma & Bastakoti, 2009). *Dioscorea hispida* Dennst. accounted for 252 cases of poisoning and three deaths in Thailand (Sriapha et al., 2015) and four incidences of poisoning and another cause of death in the Philippines (Azanza, 2006; Azanza et al., 2019). Its toxicity is mainly attributed to the presence of an alkaloid dioscorine, and cyanogenic glycosides that make the yam poisonous when directly consumed or incorrectly processed (Burkill, 1951; Kumoro et al., 2011; Lim, 2016). Dioscorine is a neurotoxic isoquinuclidine alkaloid that affects the nervous system and can cause acute kidney injury, toxic encephalopathy, and even death (Kang & Taek Heo, 2015; Sasiwatpaisit et al., 2014; Sriapha et al., 2015). Also, as a cyanogenic plant, it could cause amblyopia, paralysis, neuropathy, and metabolic disorders (Jones, 1998). Following cyanide ingestion, symptoms occur in minutes to hours depending on the dose: low-dose exposures frequently cause headache, dizziness, mild confusion, abdominal cramping, nausea, and vomiting; large-dose exposures eventually lead to dyspnea, respiratory depression, apnea, hypotension, arrhythmias, coma, and seizure (Hendry-Hofer et al., 2019).

The molecular identification of “Lab-o” further confirms the Higaonon tribes’ report on the inedibility of the yam when incorrectly processed before consumption. The Higaonon tribes’ traditional processing of the tuber in brine for at least 3 days helps remove the cyanide content of the plant. Recent studies showed that the cyanide levels in *Dioscorea hispida* Dennst. immersed in a salt solution (5% NaCl) significantly decreases the cyanide concentration up to 99.70% making it safe for human consumption (Kresnadipayana & Waty, 2019). The practical application of salt in the technological processing of the wild yam is currently being explored as part of improved and safer food preparation in Indonesia (Kresnadipayana & Waty, 2019). The process of detoxification appeared to vary from region and indigenous groups in Asia. In Nepal, the sliced tubers of *Dioscorea hispida* Dennst. are mixed with *Dioscorea kamoonsensis* Kunth. when boiled to reduce the toxicity, then dipped in running water for 24 h before it is eaten (Sharma & Bastakoti, 2009). In Java (Indonesia), the tuber slices are coated with ashes, soaked in seawater, washed, and then sun-dried whereas, in Odisha (India), it is detoxified by boiling together with *Antidesma acuminata* Wight, or tamarind (Lim, 2016). In Malaysia, it is boiled, roasted, soaked in water, and made into flour for pancakes and porridge (Ashri et al., 2014). By soaking and cooking the wild yam tubers, a lot of hydrogen cyanide (a known inhibitor of cellular respiration chain at the cytochrome oxidase level) is lost as well as other water-soluble anti-nutritional components like phenolics and tannins, thereby reducing the danger of toxicity (Shanthakumari et al., 2008). The traditional processing of boiling the yam tubers allows greater retention of nutrients, moderate loss of minerals, as well as significant removal of anti-nutritional components (Panda et al., 2020).

The process of drying the tuber starch also reduces some toxins (Kresnadipayana & Waty, 2019). This lengthy and labour-intensive preparation (i.e., chopping, drying, washing) is similarly practiced by the indigenous Amami people of Japan in processing their poisonous famine food “sotetsu” from *Cycas revoluta* (Hayward & Kuwahara, 2013). In the case of highly toxic *Aconitum carmichaelii* Debeaux traditionally eaten by the locals of Qinling mountains (China), detoxification of the root vegetable requires drying, and long hours of boiling up to 8–10 h (Y. Kang et al., 2012). The adoption of cooking to detoxify and improve the nutritional quality of plant foods is considered a key feature of human evolutionary adaptation (Wrangham & Conklin-Brittain, 2003). These exemplify the invaluable contribution of indigenous knowledge systems and practices in the valorisation of the wild plants as well as in the improvement of the human diet.

Though some species are poisonous, wild yams also play an important role in times of food shortage and famine in Southeast Asia. In the Philippines, for example, typhoons and other natural calamities often result in the displacement of the rural populace and severe agricultural loss (Bamforth, 2015; Bohra-Mishra et al., 2017; Chandra et al., 2017). As a result of domestic food shortage, the country imports 10% of its rice annually in the past 25 years, and in 2008, it became the world’s largest importer of rice—the staple and main source of sustenance for the majority of the Filipinos (Dawe et al., 2008). Yet, for the country’s indigenous population, this problem is mitigated by processing and consuming different species of wild yams as a rice substitute (Gayao et al., 2018; Warren, 2018). Similarly, in some parts of Indonesia and Timor-Leste, wild yam serves as a substitute for maize and rice during the dry season and food deficit years (Erskine et al., 2014; Miyagawa, 2002; Trimanto & Hapsari, 2015). Depending on the varieties, *Dioscorea hispida* Dennst. tuber contains 15.8–37.8% water, 1.13–6.20% crude protein, 1.99–9.36% crude lipid and 0.29–1.24% ash (Saleha et al., 2018). Its total carbohydrate (58.3–71.9%) is higher when compared with other wild edible yam species such as *D. bulbifera*, *D. deltoidea*, *D. versicolor*, and *D. triphylla* which only in the range of 17.4–25.9% (Bhandari & Kawabata, 2005). Moreover, *Dioscorea hispida* Dennst. may provide better nutrition for diabetics due to its hypoglycemic effects (Harijono et al., 2013; Hashimoto et al., 2009).

Beyond serving as a traditional staple, *Dioscorea hispida* Dennst. is cultivated by farmers in East Java to be processed into chips for local trade (Fauziah & Mas’udah, 2015; Trimanto & Hapsari, 2015). In contrast, indigenous peoples in the Philippines and other Asian regions still depend on the wild populations of *Dioscorea hispida* Dennst. However, due to the incidences of poisoning and death attributed to ingesting the plant, correct identification of the species must be taken into consideration. The DNA barcoding strategy could be used to accurately identify the plant in the wild which could be labelled with appropriate warnings together with preparation guides to avoid incidences of plant food poisoning. The promotion of the indigenous knowledge and practices associated with the traditional utilisation of *Dioscorea hispida* Dennst. and other neglected and underutilised plant resources could be likewise considered in addressing food insecurities in the Asian region.

4. Conclusion

Despite the long and tedious preparation involved in detoxifying famine foods, wild yams play an important role in the food and nutritional security of the Higaonon tribe and other indigenous peoples in the Asian region. Yet, harvesting *Dioscorea* species in the wild entails a serious health risk due to the taxonomic difficulty of differentiating the non-poisonous and poisonous yam relatives. This problem could be resolved through the DNA barcoding technique which proved to be useful in the accurate and correct identification of *Dioscorea hispida* Dennst. Considering the benefits accrued by Indonesian farmers in cultivating *Dioscorea hispida* Dennst., the economic potential of “Lab-o” could be further explored as an additional source of livelihood for the locals. This could likewise change the negative connotations on the use of famine food similar to the “sotetsu” of the Amami people in Japan. Finally, the molecular identification of food plant resources is invaluable for ensuring food safety. This

study also demonstrates the importance of integrating local and indigenous knowledge in the process of knowledge production particularly on the potential economic uses of local plant resources.

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Competing interest

The authors declare no competing interest.

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