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Potential toxicity of wild *Ipomoea* ingested by schoolchildren in remote Northeastern Thailand

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ABSTRACT

Background: Natural plant toxins can cause food poisoning upon intentional or unintentional consumption of wild plants. Some toxic wild plants can be mistaken for edible species because of their morphological resemblance. This study examined a poisoning case report of schoolchildren who consumed a steamed tuberous root of wild *Ipomoea*, misidentified as *I. mauritiana*, and experienced gastrointestinal toxicity.

Objectives: This study aimed to identify the tuberous root of wild *Ipomoea* using the internal transcribed spacer (ITS) region as a DNA barcode and characterize compounds obtained using gas chromatography-mass spectrometry (GC-MS).

Materials and methods: DNA was extracted from fresh and cooked samples of the storage root. PCR amplification and DNA sequencing of the entire ITS region were performed. FastTree and maximum likelihood analyses were used to obtain phylogenetic trees of the *Ipomoea* species. Root extracts were prepared for GC-MS analysis, and potentially harmful phytochemicals responsible for poisonous plant exposure were predicted based on a well-established plant toxin database.

Results: ITS phylogeny showed a close relationship between wild toxic *Ipomoea* and edible *I. mauritiana*. The chemometric profile obtained from GC-MS analysis of the root extracts revealed the presence of 31 phytochemicals. Among them, two putatively toxic compounds identified were β-amyrin and coumarin.

Conclusion: Misidentification of the wild poisonous plant reported herein resulted in toxic plant ingestion. Although most poisonous plant exposures are not life threatening, measures should be taken to ensure the safety of the general public.

Introduction

Wild plant foraging is crucial for survival in many parts of the world. Vulnerable communities living in remote areas may rely on wild edible plants that are naturally grown or reproduced for consumption and medicinal use. All parts of the plant, including leaves, stems, flowers, fruits, roots, and tubers, can be used.[1-3] Tuberous roots of many plants play a vital role in the human diet as a source of carbohydrates, and they are a staple food for some indigenous people.[4] Sweet potato which belongs to the genus *Ipomoea* (family *Convolvulaceae*) can be gradually harvested as a food crop over a long period of time.[5] Within this large pantropical genus of approximately 800 species, mostly having wild origins, more than 70 species have tuberous roots, of which only 24 are edible.[6-8] For some species, the tuberous roots are morphologically indistinguishable and misidentification of palatable and poisonous plants can occur.

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Plants contain several non-nutrient phytochemicals that are synthesized as secondary metabolites. Compounds such as phenolics, alkaloids, and terpenes are important for plants to cope with biotic and abiotic stresses but may exhibit phytotoxic activity as a result of their defensive properties.[9,10] Plant toxins can be classified into four groups based on their resultant toxidromes: cardiotoxic, neurotoxic, cytotoxic, and gastrointestinal/hepatotoxic.[11] In Thailand, a 10-year retrospective analysis of plant poisoning cases revealed that the gastrointestinal toxidrome was most frequently encountered. In a total of 2,901 poisonous plant exposure cases, 69.8% involved children aged under 13 years. Most cases were caused by *Jatropha curcas*, and *Manihot esculenta* was the most common cause of death.[12] The present study involved a case of unintentional ingestion of the storage root of an unknown species of *Ipomoea*, which consequently led to food poisoning in schoolchildren. The morphology of this wild tuberous root resembled that of edible *I. mauritiana*. Hence, the objectives of this study were to (i) identify the tuberous root of wild *Ipomoea* using the internal transcribed spacer (ITS) region as a DNA barcode to reconstruct phylogenetic trees; and (ii) characterize compounds obtained using gas chromatography-mass spectrometry (GC-MS) and identify putative phytotoxins present in the storage root of wild *Ipomoea* based on a well-established plant toxin database.

Materials and methods

Clinical plant samples

A fresh tuberous root sample and remaining portions of cooked sample obtained from a clinically reported case were delivered to the Toxicology Center, National Institute of Health (voucher specimens: DMSC24649 and DMSC24650). The storage root was collected from a rural area in Sisaket Province, Northeastern Thailand. It was initially identified as giant potato, a common name for *I. mauritiana*, which is palatable and used in traditional medicine. After dividing the root into halves, one half was further cut into cubes and used to prepare a steamed dish for the schoolchildren.

DNA extraction, PCR amplification and DNA sequencing

This case was reported as unintentional toxic plant ingestion due to misidentification of the wild tuberous root of *Ipomoea* (Figure 1). Based solely on its appearance without other diagnostic characters, such as leaves and flowers, the morphology of this tuberous root resembled that of edible *I. mauritiana*. Thus, molecular approaches were employed to confirm the taxonomic entity of the root.

Twenty milligrams of each fresh and cooked samples were ground to a fine powder under liquid nitrogen. DNA was extracted using the DNeasy™ Plant Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions. DNA samples were quantified using a NanoDrop UV-Vis spectrophotometer (Thermo Fisher Scientific, USA), and diluted to a final concentration of 30 ng/µL. PCR amplification and DNA sequencing of the entire ITS region were performed using ITS1 forward primer and ITS4 reverse primer.[13] Each PCR reaction of 25 µL contained 9.5 µL of OnePCR™ master mix with fluorescence dye (GeneDireX®, Taiwan), 2.5 µL of 10 µM of each primer, 1 µL of DNA template, and 9.5 µL of nuclease-free water. The amplification was performed using Mastercycler Gradient 5331 (Eppendorf, Germany) under the following conditions: initial denaturation at 95°C for 2 min, followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 5 min. PCR products were examined using 2% (w/v) agarose gel electrophoresis, and cleaned using the QIAquick PCR Purification Kit (QIAGEN, Germany). Sanger sequencing of the purified amplicons was performed at the Toxicology Center, National Institute of Health, Ministry of Public Health, Thailand.

Figure 1. *Fresh tuberous root sample of wild Ipomoea collected from a rural area in Sisaket Province, Northeastern Thailand.*

Phylogenetic analyses

The ITS sequences generated from both fresh and cooked plant samples were aligned with other derived sequences of *Ipomoea* species containing storage roots using Geneious Prime 2021.2.2 (https://www.geneious.com).[7] FastTree 2.1.11 was also performed in Geneious Prime to generate an approximately-maximum-likelihood phylogenetic tree using the GTR model and to compute local support values with the Shimodaira-Hasegawa (SH) test.[14] SH-like support was estimated using 1,000 resamples. Maximum likelihood (ML) analysis on the other hand, was conducted on the CIPRES Science Gateway portal using RAxML 8.2.12 with the GTRGAMMA model.[15] Branch support was estimated using 1,000 bootstrap replicates. The phylogenetic trees were depicted using FigTree 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree), and clades that received SH-like support ≥0.90 and bootstrap support ≥70% were considered strongly supported.

Preparation of root extracts for GC-MS analysis

Twenty-five grams each of fresh and cooked tuberous root samples were washed thoroughly with running water and left to dry at 25°C. The samples were ground and extracted with water and dichloromethane (1:1), followed by addition of anhydrous sodium sulphate. In addition to the neutral fraction obtained, acidic and basic fractions were prepared. The acidic fraction (pH ~3-4) was obtained by acidifying with 6N HCl, whereas the basic fraction (pH ~9-11) was obtained by basifying with NH_4OH. All three fractions from both fresh and cooked sample extracts were filtered, and the filtrates were evaporated to dryness under a nitrogen stream. The residues were dissolved in ethyl

acetate and filtered using syringe filters (13 mm diameter) with hydrophilic PVDF membranes (0.2 µm pore size) (VertiPure™ PVDF-HL, Thailand). Six separate filtrates were subjected to GC-MS analysis.

GC-MS analysis and identification of putative plant toxins

The filtrates containing secondary metabolites from the root extracts of wild *Ipomoea* were analysed using an Agilent 7890A/5975A GC-MS (Agilent Technologies, Santa Clara, CA, USA). Separation of the compounds was performed using an analytical HP-5MS column (30 m × 0.25 mm, 0.25 µm film thickness) coated with 5% phenyl-methylpolysiloxane (Agilent Technologies). The column temperature was programmed as follows: 70°C for 0.5 min, rising to 150°C at a rate of 10°C/min for 10 min, and 310°C at a rate of 25°C/min for 10 min. Helium was used as the carrier gas at a flow rate of 1 mL/min. The sample injection volume was 1 µL. The temperatures of the injection and detector were adjusted to 250°C and 280°C, respectively. The MS operating conditions included electron ionisation mode of 70 eV and ion source temperature of 250°C. Data processing and acquisition were performed using Agilent G1701EA MSD Productivity ChemStation software (Agilent Technologies). GC-MS profiling of the secondary metabolites was performed using the NIST 17 mass spectral library (National Institute of Standards and Technology, USA). To ensure that the tuberous root samples were not contaminated with organic chemicals such as pesticides, the Agilent RTL pesticide and endocrine disruptor MS library (RTLPEST3.L, Agilent Technologies) was also employed. All detected compounds were compared against the Toxic Plants-PhytoToxins database, which is a compilation of 1,586 phytotoxins obtained from 844 plant species.[16]

Results and discussion

Poisoning symptoms

Nine schoolchildren (9-10 years of age) consumed portions of the tuberous root of wild *Ipomoea* after steaming. Within 30 min to 4 hrs, they experienced symptoms of poisoning, including nausea, vomiting, abdominal pain, and dizziness. The symptoms were similar to those reported in Sri Lankan villagers who misidentified toxic *I. asarifolia* as the leafy vegetable *I. aquatica*.[17] Other cases of poisoning from ingestion of *Ipomoea* have occurred in livestock, with specific phytotoxic substances, such as ergoline alkaloids in the leaves of *I. asarifolia*, ipomeamarone in the storage roots of *I. batatas*, and swainsonine in *I. carnea*.[18–20]

Phylogenetic analyses

In this study, two ITS sequences of fresh and cooked tuberous root samples were generated from their respective PCR products and submitted to GenBank (accession numbers OM030216 and OM030217). An aligned matrix of 656 characters was constructed using 65 other sequences of *Ipomoea* species retrieved from GenBank and a sequence for *Solanum tuberosum* as outgroup. Both FastTree and ML analyses produced a congruent tree topology. Thus, only the ML tree (*lnL* = -6842.6) with SH-like support ≥0.90, bootstrap support (BS) ≥70%, and edibility status of the *Ipomoea* members is shown. Phylogenetic placement of both fresh and cooked samples of wild *Ipomoea* revealed a close relationship with edible *I. mauritiana* (SH-like =0.95; BS =97%) (Figure 2).

Several species of *Ipomoea* have been reported to possess health benefits and are cultivated as food plants.[21,22] Of the 36 *Ipomoea* species currently known from Thailand, nine species (with tuberous roots) are edible.[23-25] The large storage root of wild *Ipomoea* obtained in this study was morphologically similar to that of *I. mauritiana* and was, therefore, misidentified as edible. Misidentification of poisonous plants as common edible plants or indigenous medicinal herbs was one of the main causes of poisoning, as previously noted to occur with the schoolchildren at a remote primary school in the northern part of the country.[26]

GC-MS analysis

GC-MS analysis of fresh and cooked root extracts of wild *Ipomoea* revealed chromatograms of the acidic, basic, and neutral fractions (Figure 3). In all fractions, the corresponding chemical constituents were identified based on their peak retention time, peak area (%), and quality matching of the compounds (>90%) to those of known compounds described in the NIST library. A total of 31 distinct compounds were detected (Table 1). Notably, based on the chemometric profiles, a diterpenoid and triterpenoids were found only in the fresh sample extract, whereas a coumarin, an n-alkane, and lipid-soluble compounds were present in the cooked sample extract. Other phytochemicals, including a flavonoid, a fatty amide, a fatty alcohol, phytosterols, and fatty acids, were present in both extracts. These results support the findings of a previous study by Viji and Paulsamy, who obtained 27 bioactive compounds from the acetone extract of the tuberous roots of *I. mauritiana* using GC-MS.[27] In the present study, no compound was matched with those present in the Agilent RTL pesticide and endocrine disruptor MS library, indicating the absence of residual pesticides and other organic chemical contaminants from the environment.

Identification of putative plant toxins

All 31 compounds detected via GC-MS were compared against the Toxic Plants-PhytoToxins database.[16] The results revealed two chemical compounds in the wild *Ipomoea* samples that could potentially exhibit toxicological properties. These were triterpenoid (β-amyrin), present in the fresh sample extract, and coumarin (scopoletin), which was found in the cooked sample extract. Quantitatively, the GC-MS profile revealed the highest peak area for scopoletin (12.11%) in the neutral fraction of the cooked sample extract (Table 1).

Scopoletin, a thermally stable phenolic compound with a low molecular weight, has been found in different plant families. This compound plays an important role in traditional medicine in Africa, Asia, and Europe.[28] Scopoletin is biosynthesized from the phenylpropanoid pathway, and its synthesis can be induced in response to plant exposure to biotic and abiotic stresses such as pathogen infection, tissue damage, drought, heat, and cold.[29,30] It can accumulate in the roots, especially under iron-deficient conditions.[30,31] In addition to plant defense, scopoletin and other coumarins are also reported to have insecticidal and acaricidal effects.[32-34] Such biopesticide activities could result in potential negative health impacts on animals. Although without previous

reports of human evidence, it is known that swallowing the chemical product of scopoletin can lead to gastrointestinal disorders involving nausea and vomiting.[35] However, the degree of toxicity may depend on the quantity of the substance and individual sensitivity. Further investigations involving toxicity assessments using bioassays are required.

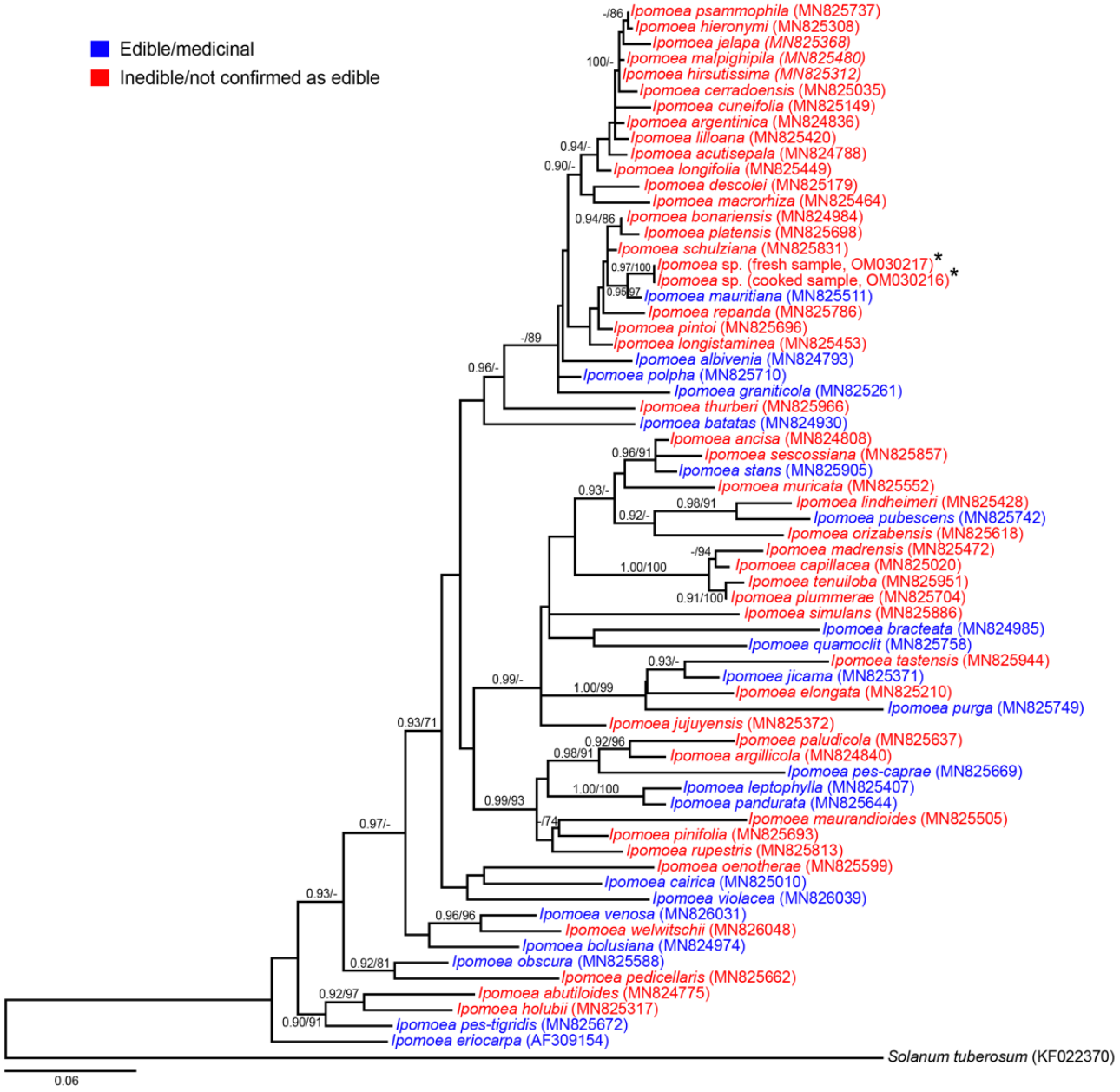

Figure 2. *ITS phylogeny based on ML analysis reveals relationships of Ipomoea species with tuberous roots. SH-like support (≥0.90) and bootstrap support (≥70%) are shown above the branches. GenBank accession numbers are provided in parentheses. *plant samples used in this study.*

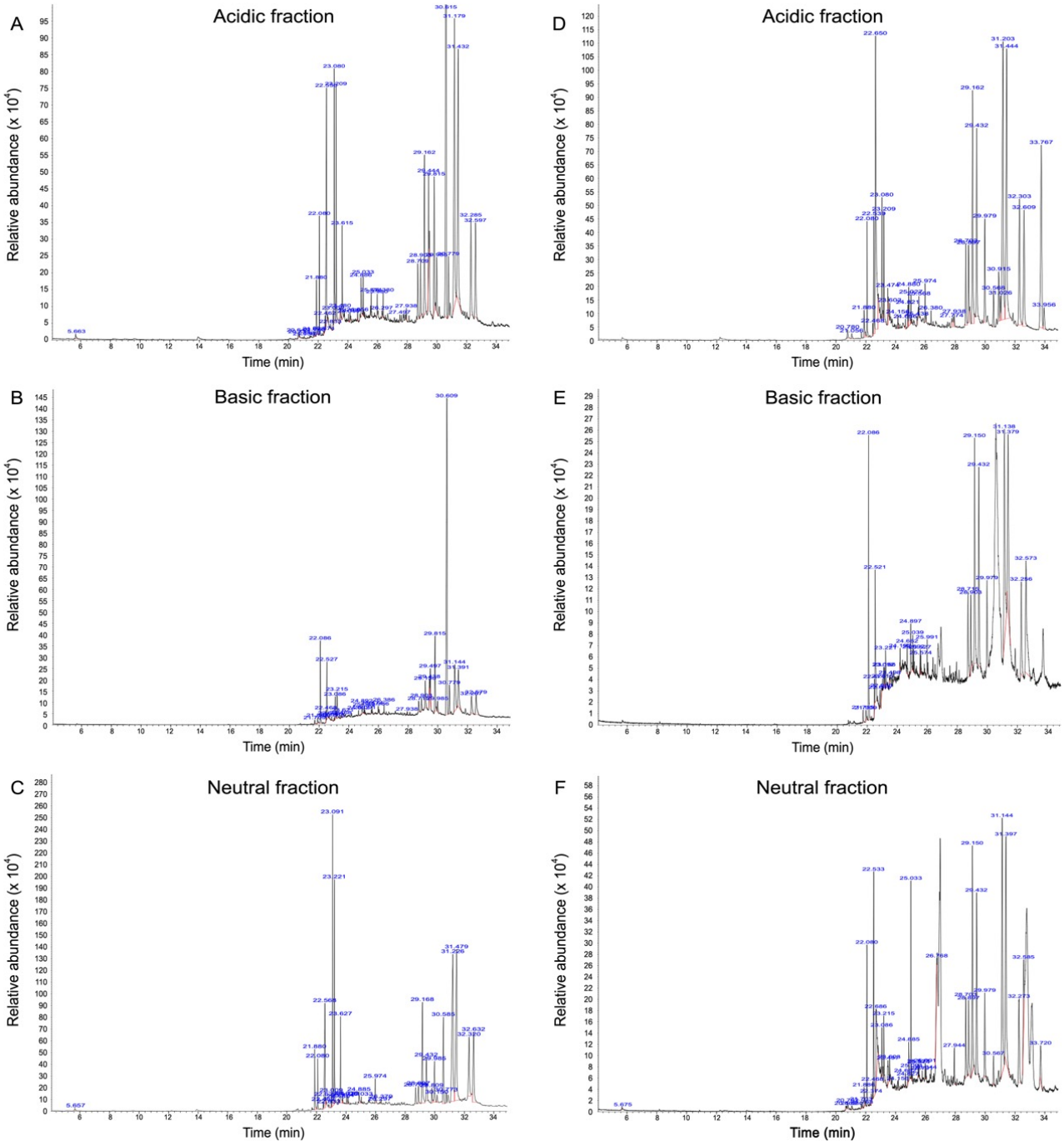

Figure 3. *GC-MS chromatograms of wild Ipomoea obtained from root extracts of fresh (A-C) and cooked samples (D-F). In both types of sample extracts, chemical compounds were detected in acidic, basic, and neutral fractions.*

Table 1 List of compounds identified by GC-MS analysis in fresh and cooked samples of wild *Ipomoea* tuberous root.

Sample	Fraction	Compound detected	Formula	m/z	Retention time (min)	Peak area (%)	Quality matching (%)	Nature of compound
Fresh tuberous root	Acidic	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	20.645	0.17	96	Fatty acid
		Pentadecanoic acid	$C_{15}H_{30}O_2$	242	21.75	0.11	95	Fatty acid
		(E)-5-Octadecene	$C_{18}H_{36}$	252	21.88	1.08	91	Fatty acid
		7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276	22.08	1.67	99	Flavonoid
		Palmitic acid	$C_{16}H_{32}O_2$	256	22.556	6	98	Fatty acid
		n-Nonadecanol-1	$C_{19}H_{40}O$	284	23.009	0.25	93	Fatty alcohol
		9-Octadecen-1-ol, (Z)-	$C_{18}H_{36}O$	268	23.08	4.04	99	Fatty acid
		Linoleic acid	$C_{18}H_{32}O_2$	280	23.48	0.1	98	Fatty acid
		Stearic acid	$C_{18}H_{36}O_2$	284	23.615	1.86	99	Fatty acid
		Erucamide	$C_{22}H_{43}NO$	337	25.98	0.38	91	Fatty amide
		Campesterol	$C_{28}H_{48}O$	400	28.709	1.7	98	Phytosterol
		γ-Sitosterol	$C_{29}H_{50}O$	414	29.444	2.65	99	Phytosterol
		Olean-12-en-3-ol, acetate, (3.beta.)-	$C_{32}H_{52}O_2$	468	30.779	2.01	99	Triterpenoid
	Basic	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276	22.086	4.38	95	Flavonoid
		Palmitic acid	$C_{16}H_{32}O_2$	256	22.527	4.01	94	Fatty acid
		1,13-Tetradecadiene	$C_{14}H_{26}$	194	23.086	1.32	93	Fatty acid
		Stearic acid	$C_{18}H_{36}O_2$	284	23.621	0.21	96	Fatty acid
		Campesterol	$C_{28}H_{48}O$	400	28.715	1.37	95	Phytosterol
		Stigmasterol	$C_{29}H_{48}O$	412	28.903	1.31	90	Phytosterol
		γ-Sitosterol	$C_{29}H_{50}O$	414	29.438	1.39	91	Phytosterol
		β-Amyrin	$C_{30}H_{50}O$	426	30.779	3.06	93	Triterpenoid
	Neutral	n-Heptadecanol-1	$C_{17}H_{36}O$	256	21.88	2.2	91	Fatty alcohol
		7,9-Di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276	22.08	1.47	98	Flavonoid
		Palmitic acid	$C_{16}H_{32}O_2$	256	22.568	5.68	99	Fatty acid
		Cyclohexadecane	$C_{16}H_{32}$	224	23.009	0.42	99	Fatty acid
		1,9-Tetradecadiene	$C_{14}H_{26}$	194	23.091	8.26	99	Fatty acid
		Docosanoic acid	$C_{22}H_{44}O_2$	340	23.221	6.39	91	Fatty acid
		10(E),12(Z)-Conjugated linoleic acid	$C_{18}H_{32}O_2$	280	23.48	0.2	95	Fatty acid
		Stearic acid	$C_{18}H_{36}O_2$	284	23.627	3.25	99	Fatty acid
		E,E,Z-1,3,12-Nonadeca-triene-5,14-diol	$C_{19}H_{34}O_2$	294	24.038	0.27	91	Fatty acid
		1,2-Diethylcyclohexadecane	$C_{20}H_{40}$	280	24.133	0.28	94	Fatty acid
		Erucamide	$C_{22}H_{43}NO$	337	25.974	0.92	95	Fatty amide
		Campesterol	$C_{28}H_{48}O$	400	28.709	0.96	98	Phytosterol
		Stigmasterol	$C_{29}H_{48}O$	412	28.897	0.97	99	Phytosterol
		γ-Sitosterol	$C_{29}H_{50}O$	414	29.432	3.08	99	Phytosterol
		2,6-Phenanthrenediol, 1,2,3,4,4a,9,10,10a-octahydro-1,1,4a-trimethyl-7-(1-methylethyl)-, monomethyl ether,[2S-(2. alpha.,4a.alpha.,10a.beta.)]-	$C_{21}H_{32}O_2$	316	29.809	0.91	90	Diterpenoid

Table 1 List of compounds identified by GC-MS analysis in fresh and cooked samples of wild *Ipomoea* tuberous root. (continue)

Sample	Fraction	Compound detected	Formula	m/z	Retention time (min)	Peak area (%)	Quality matching (%)	Nature of compound
Cooked tuberous root	Acidic	1-Hexadecanol	$C_{16}H_{34}O$	242	21.886	0.61	91	Fatty alcohol
		7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276	22.08	3.4	95	Flavonoid
		Palmitic acid	$C_{16}H_{32}O_2$	256	22.533	6.45	98	Fatty acid
		Scopoletin	$C_{10}H_8O_4$	192	22.686	5.47	97	Coumarin
		1,9-Tetradecadiene	$C_{14}H_{26}$	194	23.086	1.06	95	Fatty acid
		Cyclopentadecane	$C_{15}H_{30}$	210	23.215	1.59	97	Fatty acid
		Campesterol	$C_{28}H_{48}O$	400	28.703	2.63	99	Phytosterol
		Stigmasterol	$C_{29}H_{48}O$	412	28.897	2.83	97	Phytosterol
		γ-Sitosterol	$C_{29}H_{50}O$	414	29.432	6.78	99	Phytosterol
	Basic	(Z)-7-Hexadecene	$C_{16}H_{32}$	224	21.909	0.4	91	Fatty acid
		7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276	22.086	6.59	98	Flavonoid
		Palmitic acid	$C_{16}H_{32}O_2$	256	22.521	5.27	99	Fatty acid
		Scopoletin	$C_{10}H_8O_4$	192	22.95	0.34	96	Coumarin
		Campesterol	$C_{28}H_{48}O$	400	28.715	3.1	95	Phytosterol
		Stigmasterol	$C_{29}H_{48}O$	412	28.903	2.92	96	Phytosterol
		γ-Sitosterol	$C_{29}H_{50}O$	414	29.432	7.28	99	Phytosterol
	Neutral	Palmitic acid	$C_{16}H_{32}O_2$	256	22.539	2.4	99	Fatty acid
		Scopoletin	$C_{10}H_8O_4$	192	22.65	12.11	97	Coumarin
		1,9-Tetradecadiene	$C_{14}H_{26}$	194	23.08	1.65	98	Fatty acid
		Linoleic acid	$C_{18}H_{32}O_2$	280	23.474	0.58	98	Fatty acid
		Stearic acid	$C_{18}H_{36}O_2$	284	23.603	0.24	99	Fatty acid
		1-Hexacosene	$C_{26}H5_2$	364	24.88	1.14	90	Fatty acid
		Docosane	$C_{22}H_{46}$	310	25.568	0.28	97	n-Alkane
		Erucamide	$C_{22}H_{43}NO$	337	25.974	0.68	98	Fatty amide
		Vitamin E	$C_{29}H_{50}O_2$	430	27.774	0.42	93	Lipid-soluble compound
		Campesterol	$C_{28}H_{48}O$	400	28.703	2.4	99	Phytosterol
		Stigmasterol	$C_{29}H_{48}O$	412	28.897	2.14	99	Phytosterol
		γ-Sitosterol	$C_{29}H_{50}O$	414	29.432	5.69	99	Phytosterol

Conclusion

Foraging for wild edible plants is common among the indigenous people of developing countries. However, misidentification of poisonous plants for edible species can occur because of a lack of knowledge and experience. The present study showed that the ingestion of wild toxic Ipomoea, mistaken for edible *I. mauritiana*, resulted in food poisoning in the schoolchildren living in the rural area of Northeastern Thailand. Awareness of plant food safety is important to prevent food poisoning from wild plants.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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