Journal of Associated Medical Sciences 2024; 57(2): 107-114

JAMS
Journal of Associated Medical Sciences

Scopus Indexed & Thai-Journal Citation Index Centre (TCI)

Journal of Associated Medical Sciences

Journal homepage: https://www.tci-thaijo.org/index.php/bulletinAMS/index



Analyzing DNA barcoding and identifying toxins caused by neurotoxic mushroom poisoning using liquid chromatography tandem mass spectrometry

Sriprapa Phatsarapongkul[1] Sittiporn Parnmen[1*] Nattakarn Nooron[1] Rungsaeng Chankunasuka[1] Chidkamon Thunkhamrak[1] Unchalee Nitma[1] Nisakorn Palakul[1] Pornpanna Chonnakijkul[1] Sujitra Sikaphan[1] Chutimon Uttawichai[1] Dutsadee Polputpisatkul[1] Archawin Rojanawiwat[2]

[1]Toxicology Center, National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi Province, Thailand.
[2]National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi Province, Thailand.

ARTICLE INFO

Article history:
Received 3 November 2023
Accepted as revised 27 February 2024
Available online 4 March 2024

Keywords:
Ibotenic acid, LC-MS/MS, neurotoxic mushroom, phylogenetic approach

ABSTRACT

Background: Neurotoxic mushroom poisoning often exhibits rapid symptom onset, typically attributed to compounds such as Ibotenic acid, which affect the central nervous system. This study addresses a new case of mushroom-related food poisoning in southern Thailand.

Objective: The objectives are to determine the presence of ibotenic acid in cases of mushroom-related food poisoning utilizing liquid chromatography-tandem mass spectrometry (LC-MS/MS) and to identify toxic *Amanita* species implicated in these cases.

Materials and methods: Remnant mushroom samples obtained from three clinically reported cases were used. Nucleotide similarity was compared against the rRNA/ITS databases using NCBI BLAST search. Phylogenetic analyses were conducted using maximum likelihood (ML) and FastTree approaches. LC-MS/MS was employed to separate of Ibotenic acid, determine its molecular weight and perform precursor ion fragmentation.

Results: Analysis of the rRNA/ITS databases revealed a high nucleotide similarity between suspected mushroom samples and *Amanita digitosa*. Detailed phylogenetic analysis confirmed that mushroom samples from the three poisoning cases clustered with *A. digitosa*. LC-MS/MS analysis showed the presence of ibotenic acid, with precursor ion (*m/z* 159) and product ion (*m/z* 113.1) as the major toxic substances. Clinically, patients poisoned by ibotenic acid-containing mushrooms exhibited a short latent period with symptoms of nausea, vomiting, vertigo, delirium, confusion, and fatigue.

Conclusion: The genus *Amanita* comprises both edible and inedible species that produce several lethal toxins. The report of ibotenic acid in *A. digitosa* is a novel finding, valuable for food safety monitoring and healthcare decision-maker. This is especially notable due to the accuracy and rapidity of the analytical process.

Introduction

A DNA barcode is a short genetic sequence used to identify and classify species.[1] In investigations of mushroom food poisoning cases, DNA barcoding proves to be valuable tool for identifying samples and determining the species, in conjunction with morphological diagnostics.[2–7] Furthermore, this technique can be applied to case involving remnant or incomplete mushroom samples.[2–7] The most commonly utilized DNA barcode region for fungi is the internal transcribed spacer (ITS) region.[8,9] This region contains two variables ITS1 and ITS2 spacer which are relatively conserved

∗ **Corresponding contributor.**
Author's Address: *Toxicology Center, National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi Province, Thailand.*
E-mail address: sittiporn.p@dmsc.mail.go.th
doi: 10.12982/JAMS.2024.032
E-ISSN: 2539-6056

within a species but exhibit significant variations between species.[8,9]

In Thailand, the ingestion of toxic wild mushrooms remains a significant health concern, resulting in morbidity and mortality.[10] The most frequently encountered lethal wild mushrooms are cytotoxic and myotoxic varieties.[2,4,10] Cytotoxic mushroom poisoning, caused by amatoxins, leads to lethal hepatotoxicity, while myotoxic mushroom poisoning, associated with cycloprop-2-ene carboxylic acid, manifests myotoxic symptoms.[10,11] Well-documented examples include *Amanita brunneitoxicaria*, *A. exitialis*, *A. fuliginea*, *A. gleocystidiosa* and *Russula subnigricans*.[2,4,10,12,13] Mushroom poisoning cases typically fall into two primary categories: gastrointestinal irritation and neurotoxicity.[10] Various mushroom genera, including *Agaricus*, *Chlorophyllum*, *Entoloma*, *Hygrocybe*, *Macrocybe*, and others, are recognized sources of gastrointestinal (GI) irritants.[5,7,10] Nevertheless, the specific GI toxins responsible for these symptoms remain inadequately understood.[14,15] In contrast, neurotoxic mushrooms exhibit less genus diversity compared to GI mushrooms.[10] However, cases of poisoning from toxic mushrooms affecting the nervous system are reported annually among patients, necessitating prompt hospital admission due to the rapid progression of symptoms.[6]

According to the Department of Disease Control, Ministry of Public Health (http://doe.moph.go.th/surdata/disease.php), the incidence of food poisoning from mushroom consumption in the southern region is relatively low compared to other regions. This poisoning typically arises from confusion in distinguishing between poisonous and edible mushrooms. Furthermore, the samples submitted for laboratory analysis often consist of remnants mushroom sample from the patients' meals, rendering some tests incomplete. Therefore, the integration of DNA barcoding techniques alongside morphological analysis of mushrooms can aid in species identification. Moreover, toxin extraction from mushrooms in this study employed microextraction, utilizing minute sample quantities. The primary objective of this study is to identify suspected cases of neurotoxic mushroom poisoning in *Amanita* samples through DNA barcoding and to ascertain their toxins using liquid chromatography-tandem mass spectrometry (LC-MS/MS) employing multiple reaction monitoring (MRM).

Materials and methods

Mushroom specimens

A mushroom sample obtained from 3 poisoning reported cases was used in this study. All poisoning cases occurred in the southern part of Thailand from April to October 2019. In each case, hospitalization was required. Of the 11 patients examined, there were no death cases.

DNA barcode-based fungal identification

The samples containing 20 mg of fruiting bodies were ground in liquid nitrogen and extracted using the Densitʸ Plant Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions. The entire ITS region was amplified with the primers ITS1F and ITS4.[16,17] PCR amplification and sanger sequencing conditions were as described previously.[2,4] The nuclear ITS sequences were compared for nucleotide similarity against the GenBank database using a nucleotide BLAST search of NCBI.[18] The specimens underwent morphological and microscopic examination using a low-magnification stereomicroscope (HumaScope Stereo) and a compound microscope (Olympus CX31) capable of magnifications ranging from 40 to 1,000. For the phylogenetic analysis, the ITS sequences belonging to *Amanita* were downloaded from GenBank. Phylogenetic analyses and genetic distances were performed using Geneious Prime 2022.0.2 (www.geneious.com). Two phylogenetic methods were used including RAxML version 8.2.7 and FastTree version 2.1.11. Branch support was estimated by using 1000 bootstrap replicates. Only clades that received bootstrap support ≥70% under RAxML and SH-like support value ≥90% under FastTree were considered as strongly supported. Phylogenetic trees were depicted using the program FigTree 1.4.4 (http://tree.bio.ed.ac.uk).

Sample preparation and microextraction

Twenty mg of mushroom samples were blended and extracted with 400 uL of 0.1% formic acid in acetonitrile. The extract was incubated at room temperature overnight, followed by centrifugation at 14,000 rpm for 3 min. The clear supernatant was filtered with VertiPure™PVDF(HL) syringe filters (13 mm, 0.2 μm). The filtrate was submitted for subsequent analysis.

Detection of neurotoxins using LC-MS/MS method

The standard reference material of ibotenic acid and alkaloid muscarine were obtained from Sigma-Aldrich (St. Louis, USA). The separation of the peptide toxins and determination of molecular weight as well as the precursor ion fragmentation were using LC-MS/MS method on Agilent 6495 Triple Quadrupole LC/MS and 1290 infinity LC modules with Agilent MassHunter software (California, USA). In the chromatographic system, a Z0RBAX SB-C18 narrow-bore (2.1 mm x 150 mm, 3.5 μm) columns was used for separation of ibotenic acid. The column temperature was 25°C. The mobile phases were deionized water with 0.5% (v/v) formic acid (mobile phase A) and 0.5% (v/v) formic acid in acetonitrile (mobile phase B). A program was started with 30% mobile phase A and 70% mobile phase B at min 1 and 20:80 at min 10. The total run time for each sample was 15 min. The flow rate was 0.2 mL/min. The injection volume was 5 μL, and the Ibotenic acid concentration was 5 μg/L. The MS conditions were as follows: gas temperature, 200°C; gas flow, 14 L/min; nebulizer, 40 psi; and capillary voltage, 4,000 V. The ESI spray was set for positive ion mode detection. Ibotenic acid was detected using multiple reaction monitoring (MRM) for the following unique parent mass-to-charge(m/z)–product m/z combinations: 159 *m/z*-113.1 *m/z*. For detecting alkaloid muscarine, the extraction and MS conditions was followed as described previously.[6]

Results

DNA barcode-based fungal identification

Five remnant mushroom samples obtained from three poisoning cases were analyzed. Results of BLAST search revealed the highest pairwise identity for all samples tested with scores ranging from 95.61 % to 97.01% identity to *Amanita digitosa* (94.98 to 97.01%) and followed by scores ranging from 91.15 to 92.14% identity to a group of *A. ibotengutake* (Table 1). Phylogenetic analyses were used to confirm the relationship of clinical mushroom samples. A matrix of 669 unambiguously aligned nucleotide characters was constructed. All clinical mushroom samples are clustered with *A. digitosa* (100/99) with 95 to 100% identical at the nucleotide level (Figure 1 and 2). Based on the remnant mushroom samples, its general features include pileus brown dark grey at centre towards to haft of greyish at margin, convex to plane; lamellae free, cylindrical stipe, hallow, white to pale grey; bulb subglobose with saccate volva; and subglobose basidiospores, smooth, hyaline, inamyloid (Figure 3).

Table 1 Nucleotide similarity using ITS region.

Sample numbers	Location	GenBank accession numbers	BLAST identification	Nucleotide similarity (%)
D456	Yala province	OR725114	*A. digitosa* KT213722	95.61
			A. ibotengutake AB080987	92.08
D457	Yala province	OR725115	*A. digitosa* KT213722	95.61
			A. ibotengutake AB080987	92.07
D480	Trang province	OR725116	*A. digitosa* KT213722	94.98
			A. ibotengutake AB080990	92.13
D481	Trang province	OR725117	*A. digitosa* KT213722	94.98
			A. ibotengutake AB080990	92.14
D520	Songkhla province	OR725118	*A. digitosa* KT213722	97.01
			A. ibotengutake AB080987	91.15

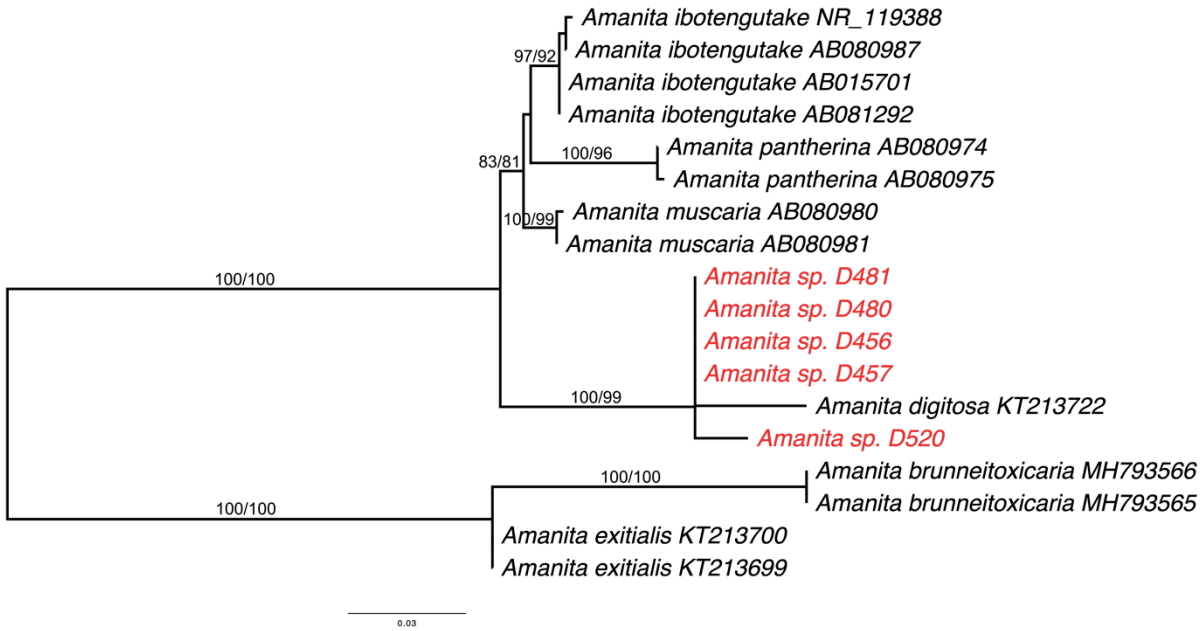


Figure 1 *Phylogenetic tree depicting relationships of the Amanita based on analysis of the ITS region. Mushroom obtained from poisoning cases are written in red. Bootstrap support ≥70% under RAxML and SH-like support value ≥90% under FastTree are indicates above branches.*

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. *A. digitosa* KT213722		95	95	95	95	96	87	87	87	86	85	85	85	85
2. *Amanita* sp. D456	95		100	99	99	99	89	89	90	89	87	88	87	87
3. *Amanita* sp. D457	95	100		99	99	99	89	89	90	89	87	88	87	87
4. *Amanita* sp. D480	95	99	99		100	98	90	90	90	89	87	88	87	87
5. *Amanita* sp. D481	95	99	99	100		98	90	90	90	89	87	88	87	87
6. *Amanita* sp. D520	96	99	99	98	98		89	89	90	89	87	87	86	86
7. *A. ibotengutake* AB080987	87	89	89	90	90	89		100	100	100	94	94	91	91
8. *A. ibotengutake* AB015701	87	89	89	90	90	89	100		100	100	94	94	91	91
9. *A. ibotengutake* AB081292	87	90	90	90	90	90	100	100		99	94	94	92	91
10. *A. ibotengutake* NR_119388	86	89	89	89	89	89	100	100	99		94	94	91	91
11. *A. muscaria* AB080980	85	87	87	87	87	87	94	94	94	94		100	93	93
12. *A. muscaria* AB080981	85	88	88	88	88	87	94	94	94	94	100		93	93
13. *A. pantherina* AB080974	85	87	87	87	87	86	91	91	92	91	93	93		100
14. *A. pantherina* AB080975	85	87	87	87	87	86	91	91	91	91	93	93	100	

Figure 2 *Genetic distance matrix of Amanita. Nucleotide sequence percent (%) identity are shown in the table. Orange color indicates percent identify between A. digitosa and mushroom sample obtained from poisoning cases.*

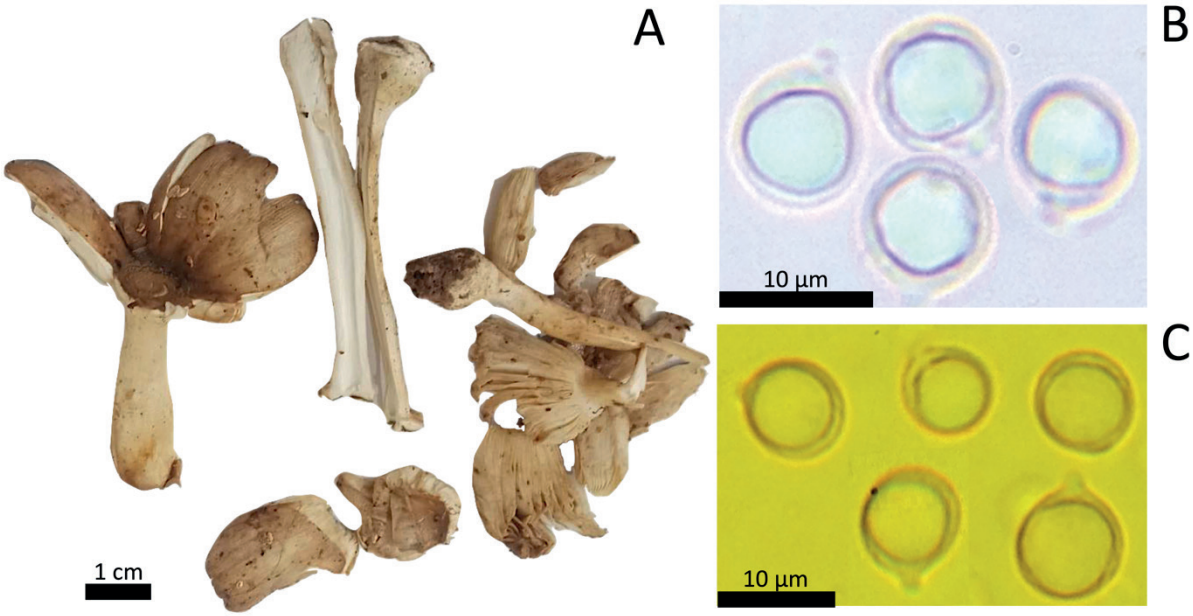


Figure 3 *The remnant mushroom samples. A: Amanita sp. (D480), B: Hyaline subglobose basidiospores, C: Inamyloid subglobose basidiospores.*

Detection of neurotoxin using LC-MS/MS

The purified mushroom extract was analyzed with MS/MS spectra based on the fragmentation of the precursor ion. The LC-MS/MS determination revealed the presence of ibotenic acid with ionic transitions of precursor ion (*m/z* 159) and product ion (*m/z* 113.1) in all *Amanita* samples (Figure 4). All samples did not detect alkaloid muscarine (Figure 5).

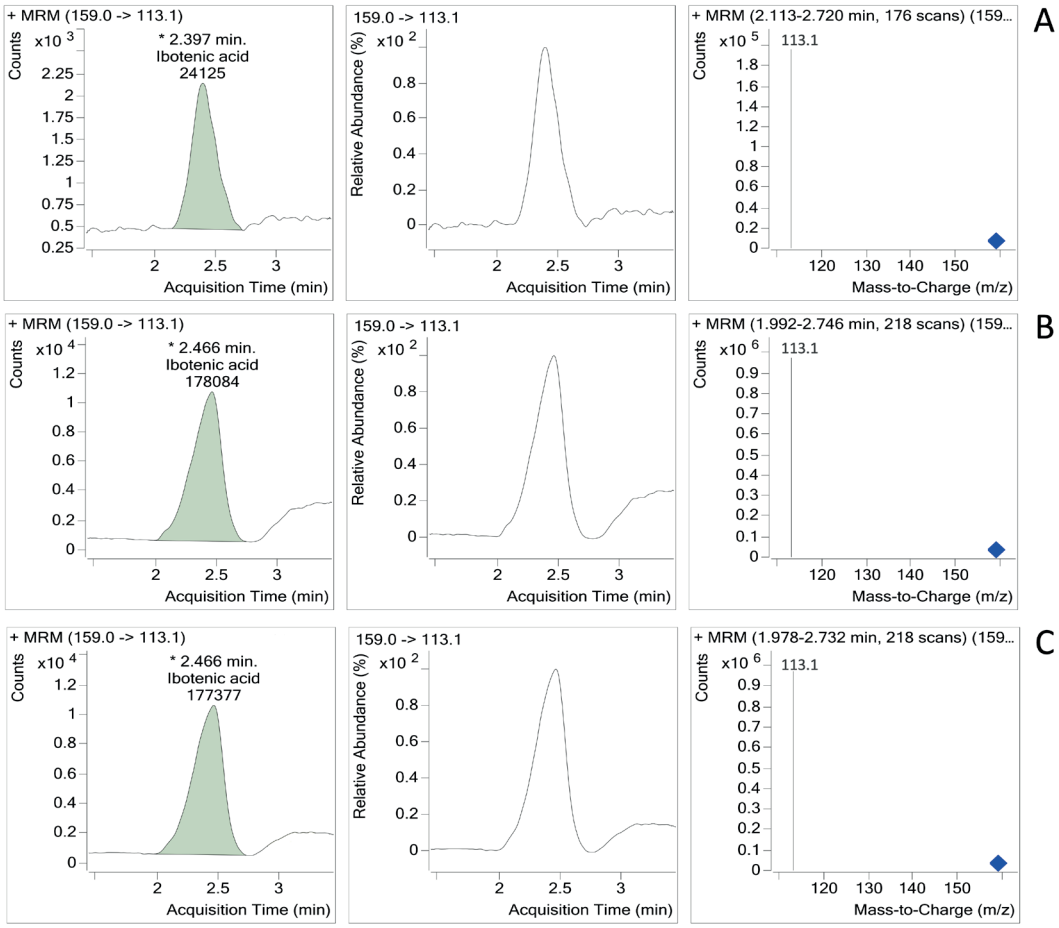


Figure 4. *Liquid chromatography-tandem mass spectrometric MRM chromatograms of Ibotenic acid neurotoxicant obtained from remnant mushroom samples (A) standard Ibotenic acid, (B) Amanita sp. (D480) and (C) Amanita sp. (D481). The diamond-shape represents the molecular ion of ibotenic acid (m/z 159).*

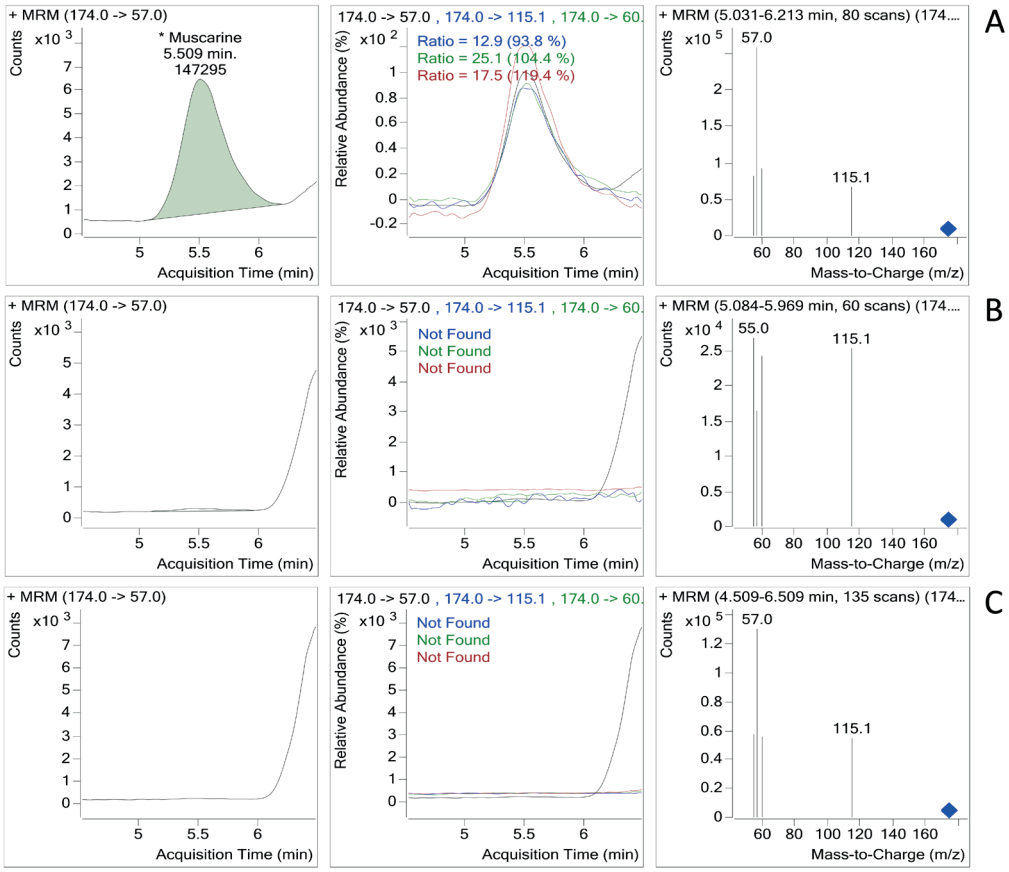


Figure 5. *Liquid chromatography-tandem mass spectrometric MRM chromatograms of alkaloid muscarine obtained from remnant mushroom samples (A) standard alkaloid muscarine, (B) Amanita sp. (D480) and (C) Amanita sp. (D481). The diamond-shape represents the molecular ion of alkaloid muscarine (m/z 174).*

Discussion

Epidemiological data has shown increasing incidence rates of mushroom poisoning cases in Thailand.[19] More than 80% of poisoning cases occur during the rainy season. Mushroom poisoning cases are reported in the north, northeast, east, and west regions from May to October, while in the southern part of the country, they occur from October to April. Food mushroom poisoning, as classified by White *et al*., is divided into six types based on clinical symptoms including group 1 cytotoxic mushroom poisoning, group 2 neurotoxic mushroom poisoning, group 3 myotoxic mushroom poisoning, group 4 metabolic/endocrine toxicity mushroom poisoning, group 5 gastrointestinal irritant mushroom poisoning and group 6 miscellaneous adverse reactions to mushrooms.[11] The most rapid onset of poisoning occurs in group 2, which is associated with neurotoxic mushrooms and manifests within 15-30 minutes. Group 2 includes four subgroups: hallucinogenic mushrooms (2A), autonomic toxicity mushrooms (2B), central nervous system toxicity mushrooms (2C), and morel neurologic syndrome (2D).[11] In Thailand, hallucinogenic mushrooms that produce psilocybin and psilocin are classified as 'category 5' narcotics under the Thai Narcotics Act B.E. 2522 (1979).[20] Mushroom food poisoning caused by neurotoxins has been reported, including cases involving *Inosperma* and *Pseudosperma*.[6] Both genera produce the alkaloid muscarine, which falls under the category of autonomic toxicity mushrooms (group 2B). Patients who ingest these mushrooms typically experience a short latent period of 15 to 30 minutes, with symptoms such as perspiration, hypersalivation, lachrymation, bradycardia, diarrhea, and fatigue.[6]

In this study, we examined mushroom samples obtained from suspected cases of neurotoxic mushroom poisoning. These samples consisted of remnant wild mushrooms harvested by patients and the Surveillance & Rapid Response Team (SRRT) from the Department of Disease Control. The study encompassed three poisoning cases: one in April 2019 from Yala province with one patient, one in August 2019 from Trang province with two patients, and the last cases in October 2019 from Songkhla province with eight patients. All mushroom poisoning cases underwent routine screening for lethal toxic substances, including alpha-amanitin, beta-amanitin, and the neurotoxic substance muscarine. The samples did not detect toxic substances from the above poisoning case. Based on their morphology, the samples were initially identified as belonging to the genus *Amanita*. A BLAST search of the ITS barcode confirmed that all mushroom samples closely matched A. digitosa, followed by *A. ibotengutake*.[12,21] Phylogenetic analyses based on ITS sequences also confirmed that all mushroom samples formed a robust clade with A. digitosa (100/99). While *A.*

digitosa is native to Thailand, *A. ibotengutake* was originally described in Japan. Morphologically, these mushroom samples exhibited greater similarity to *A. digitosa* than *A. ibotengutake*, with similar macroscopic morphology and basidiospore shape.[12,21] *A. ibotengutake* has been reported to contain ibotenic acid and muscimol.[21] These toxins have also been found in species closely related to *A. ibotengutake*, such as *A. muscaria* and *A. pantherina.*[15,22,23] However, the toxic substance of *A. digitosa* remained unknown at the time.[2,12] There have been reports of gastrointestinal irritant symptoms in patients who ingested *A. digitosa*.[2] All the mentioned species belong to the section *Amanita.*[2,12] In Thailand, some species within this section have been identified as causing mushroom food poisoning, with adverse effects ranging from mild gastrointestinal symptoms to severe cytotoxic effects.[2,10,12]

Based on the LC-MS/MS assay, all three poisoning cases involving the ingestion of *A. digitosa* detected the presence of ibotenic acid. Ibotenic acid serves as the main compound, functioning as a prodrug that converts to muscimol through decarboxylation or photochemical reactions.[15,23] Naturally, the ibotenic acid in toxic Amanitas can be converted to muscimol through drying, cooking, or ingestion.[15] Once absorbed into the body, ibotenic acid undergoes decarboxylation, transforming into muscimol. Both ibotenic acid and muscimol readily cross the blood-brain barrier and stimulate glutamate and gamma-aminobutyric acid (GABA) receptors.[15] According to poisoning reports, ingestion of mushrooms containing ibotenic acid and muscimol leads to neuropsychiatric, gastrointestinal irritation, and muscular symptoms.[15,23,24] These toxins are excreted in their original form through the urinary system within six hours.[24] In the reported poisoning cases, patients had ingested what they believed to be an edible species of termite mushroom in the genus *Termitomyces* (Lyophyllaceae). Most patients who ingested *A. digitosa* experienced rapid onset symptoms within five minutes, including nausea, vomiting, vertigo, delirium, confusion, and fatigue. Some patients required intubation in the intensive care unit, but all cases had no reported fatalities.

Conclusions

In summary, this study marks the first documented presence of ibotenic acid in *A. digitosa*. Safeguarding food safety, particularly in cases of mushroom consumption, is of utmost importance. To mitigate the risk of potentially life-threatening incidents, it is crucial to exercise vigilance in mushroom identification, prioritize education, and adhere to safe foraging practices.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgements

This work was financially supported by the Thailand Science Research and Innovation (Grant No. 164021/2022 and 183769/2023).

References

[1] Ratnasingham S, Hebert PDN. bold: The Barcode of Life Data System (http://www.barcodinglife.org). Mol Ecol Notes. 2007; 7(3): 355-64. doi.org/10.1111/j.1471-8286.2007.01678.x.

[2] Parnmen S, Sikaphan S, Leudang S, Boonpratuang T, Rangsiruji A, Naksuwankul K. Molecular identification of poisonous mushrooms using nuclear ITS region and peptide toxins: a retrospective study on fatal cases in Thailand. J Toxicol Sci. 2016; 41(1): 65-76.

[3] Parnmen S, Nooron N, Leudang S, Sikaphan S, Polputpisatkul D, Rangsiruji A. Phylogenetic evidence revealed *Cantharocybe virosa* (Agaricales, Hygrophoraceae) as a new clinical record for gastrointestinal mushroom poisoning in Thailand. Toxicol Res. 2020; 36(3): 239-48. doi.org/10.1007/s43188-019-00024-2.

[4] Ramchiun S, Sikaphan S, Leudang S, Polputpisatkul D, Nantachaiphong N, Khaentaw T, et al. Molecular characterization and liquid chromatography-mass spectrometric multiple reaction monitoring-based detection in case of suspected phalloides syndrome poisoning. J Assoc Med Sci. 2019; 52(1): 48-55. doi: 10.14456/jams.2019.9.

[5] Leudang S, Sikaphan S, Parnmen S, Nantachaiphong N, Polputpisatkul D, Ramchiun S, et al. DNA-based identification of gastrointestinal irritant mushrooms in the genus *Chlorophyllum*: A food poisoning case in Thailand. J Heal Res. 2017; 31(1): 41-9. doi: 10.14456/jhr.2017.6.

[6] Parnmen S, Nooron N, Leudang S, Sikaphan S, Polputpisatkul D, Pringsulaka O, et al. Foodborne illness caused by muscarine-containing mushrooms and identification of mushroom remnants using phylogenetics and LC-MS/MS. Food Control. 2021; 128: 108182. doi.org/10.1016/j.foodcont.2021.108182.

[7] Nooron N, Parnmen S, Chonnakijkul P, Sikaphan S, Chankunasuka R, Phatsarapongkul S, et al. Use of nuclear ITS region as DNA barcode marker for the species identification of mushroom in the genus *Macrocybe* causing foodborne illness. Thai J Toxicol. 2023; 38(1): 55-67. Available from: https://li01.tci-thaijo.org/index.php/ThaiJToxicol/article/view/258482.

[8] Xu J. Fungal DNA barcoding. Genome. 2016; 59(11): 913-32. doi: 10.1139/gen-2016-0046.

[9] Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, et al. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc Natl Acad Sci USA. 2012; 109(16): 6241-6. doi:10.1073/pnas.1117018109.

[10] Nooron N, Parnmen S, Sikaphana S, Leudang S, Uttawichai C, Polputpisatkul D. The situation of mushrooms food poisoning in Thailand: symptoms and common species list. Thai J Toxicol. 2020; 35(2): 58-69. Available from: https://li01.tci-thaijo.org/index.php/ ThaiJToxicol/article/view/248088.

[11] White J, Weinstein SA, De Haro L, Bédry R, Schaper A, Rumack BH, et al. Mushroom poisoning: A proposed new clinical classification. Toxicon. 2019; 157: 53-65.

doi: 10.1016/j.toxicon.2018.11.007.

[12] Li GJ, Hyde KD, Zhao RL, Hongsanan S, Abdel-Aziz FA, Abdel-Wahab MA, et al. Fungal diversity notes 253--366: taxonomic and phylogenetic contributions to fungal taxa. Fungal Divers. 2016; 1-237. doi.org/10.1007/s13225-016-0366-9.

[13] Trakulsrichai S, Jeeratheepatanont P, Sriapha C, Tongpoo A, Wananukul W. Myotoxic mushroom poisoning in Thailand: clinical characteristics and outcomes. Int J Gen Med. 2020; 13: 1139-46. doi: 10.2147/IJGM.S271914.

[14] He M-Q, Wang M-Q, Chen Z-H, Deng W-Q, Li T-H, Vizzini A, et al. Potential benefits and harms: a review of poisonous mushrooms in the world. Fungal Biol Rev. 2022; 42: 56-68.

[15] Duffy TJ. Toxic Fungi of Western North America. Vol. 2008, Group. MykoWeb; 2008. 166 p. Available from: www.mykoweb.com

[16] Gardes M, Bruns TD. ITS primers with enhanced specificity for basidiomycetes--application to the identification of mycorrhizae and rusts. Mol Ecol. 1993; 2(2): 113-8. doi.org/10.1111/j.1365-294X.1993.tb00005.x.

[17] White TJ, Bruns TD, Lee SB, Taylor JW. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White T., editor. PCR Protocols: a guide to methods and applications. New York: Academic Press; 1990. p. 315-22.

[18] Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990; 215(3): 403-10. doi.org/10.1016/S0022-2836(05)80360-2.

[19] Department of Disease Control. Mushroom poisoning. National Disease Surveillance (Report 506). 2023. Available from: http://doe.moph.go.th/surdata/index.php

[20] Saingam D, Assanangkornchai S, Geater AF, Balthip Q. Pattern and consequences of krathom (*Mitragyna speciosa* Korth.) use among male villagers in southern Thailand: a qualitative study. Int J Drug Policy. 2013; 24(4): 351-8. doi: 10.1016/ j.drugpo.2012.09.004.

[21] Oda T, Yamazaki T, Tanaka C, Terashita T, Taniguchi N, Tsuda M. *Amanita ibotengutake* sp. nov., a poisonous fungus from Japan. Mycol Prog. 2002; 1(4): 355-65. doi.org/10.1007/s11557-006-0032-9.

[22] Tsujikawa K, Kuwayama K, Miyaguchi H, Kanamori T, Iwata Y, Inoue H, et al. Determination of muscimol and ibotenic acid in *Amanita* mushrooms by high-performance liquid chromatography and liquid chromatography-tandem mass spectrometry. J Chromatogr B, Anal Technol Biomed life Sci. 2007; 852(1-2): 430-5. doi: 10.1016/j.jchromb.2007.01.046.

[23] Bresinsky A, Besl H. A colour atlas of poisonous fungi: A handbook for pharmacists, doctors, and biologists. London: Wolfe Publishing Ltd; 1990. P.1-295.

[24] Hiroshima Y, Nakae H, Gommori K. *Amanita ibotengutake* intoxication treated with plasma exchange. Ther Apher Dial. 2010; 14(5): 483-4. doi: 10.1111/j.1744-9987.2010.00862.x.