ใยของเชื้อรา(Dyke and Newton, 1999) การเผาป่าเป็นการกระตุ้นการผลิตดอกของเห็ดมอเรลที่เลวร้าย ไม่ได้ผล มีการรายงานใน Oregon (Arnolds, 1995) สองตัวอย่างดังกล่าวข้างต้นมีความคล้ายคลึงกับวิธีการเก็บเกี่ยวเห็ดเผาะเชิงการค้าในภาคเหนือของประเทศไทย เห็ดเผาะเป็นเห็ดที่ได้รับความนิยมมีเนื้อเยื่อที่อร่อยรสชาติดี สปีชีสนี้ของเห็ดเผาะมีเห็ดเผาะกลุ่มอื่น ๆ ที่มีลักษณะเหมือนกับ *Astraeus* sp.ที่มีขนาดใกล้เคียงกัน รูปร่าง สี แต่มีความแตกต่างทางด้าน เนื้อสัมผัส (ไม่มีความยืดหยุ่น) และไม่มีรสชาติ ซึ่งในประเด็นการเขี่ย / ขุ้ยผิวดินเพื่อหาเห็ดเผาะในต้นฤดูฝนของประชาชนในภาคเหนือ อาจเป็นผลดีต่อประชาชนในการได้รับประทานเห็ดเผาะซึ่งใช้เป็นอาหารในภาคเหนือ และส่งผลกระทบต่อการทำลายเส้นใยของเห็ดสกุลอะมานิทาที่กินได้ และมีพิษซึ่งออกดอกในช่วงเวลาดังกล่าว อาจส่งผลให้การเจริญของเห็ดพิษมีน้อย ดังนั้นจะส่งผลดีต่ออุบัติการณ์การเสียชีวิตจากเห็ดไข่ห่านพิษ หรือ เห็ดพิษสกุลอะมานิทาจะมีน้อยลง

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ภาคผนวก

ข้อเสนอแนะในการศึกษาครั้งต่อไป

a. การศึกษาในเชิงประยุกต์เพื่อหาวิธีการเพาะเลี้ยงเห็ดไข่ห่านพิษ มุ่งเน้นการหาสูตรอาหารที่เหมาะสมในการผลิตดอกเห็ด ส่วนของเส้นใย หาปริมาณสารพิษอะมานิทิน หรือสารฟัลลอยด์ดิน เพื่อสกัด นำสารดังกล่าวมาทำให้บริสุทธิ์เพื่อวัตถุประสงค์ในการผลิตสารมาตรฐานสำหรับอ้างอิงในการตรวจสอบสารพิษอะมานิทิน และสารฟัลลอยด์ดิน ลดการนำเข้าหรือหากมีศักยภาพเพียงพอก็สามารถสกัด หรือพัฒนาวิธีการสังเคราะห์หรือผลิตสารมาตรฐานอะมานิทิน และสารฟัลลอยด์ดิน เพื่อการส่งออกต่อไป
b. การแสวงหาพืช หรือ สัตว์ที่มีคุณสมบัติต้านพิษจากสารพิษอะมานิทิน และสารฟัลลอยด์ดิน ในการป้องกันอันตรายจากพิษของเห็ดไข่ห่าน สะกุลอะมานิทา ที่มีพิษ
c. ข้อจำกัดการศึกษาในครั้งนี้ คือ การทำลายซาก หรือดอกเห็ดพิษจากชาวบ้านที่เก็บของป่า ซึ่งมีผลกระทบต่อการเก็บตัวอย่างเพื่อการศึกษา โดยปกติการเจริญ หรือการออกดอกเห็ดไข่ห่านที่เป็นพิษค่อนข้างหายาก และพบจำนวนน้อยมาก ทำให้ประชาชน หรือนักวิชาการขาดโอกาสที่จะได้รับตัวอย่าง สำหรับการศึกษาในพื้นที่นั้น ๆ
d. พื้นที่ที่ทำการสำรวจบางพื้นที่อยู่ห่างไกล ถนนหนทาง การเข้าถึงลำบาก เนื่องจากการคมนาคมไม่สะดวก

ผลลัพธ์ที่ได้จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ (ระบุชื่อผู้แต่ง ชื่อเรื่อง ชื่อวารสาร ปี เล่มที่ เลขที่ และหน้า) หรือผลงานตามที่คาดไว้ในสัญญาโครงการ
   - อยู่ระหว่างดำเนินการ / manuscript 1
   - อยู่ระหว่างดำเนินการ / manuscript 2

2. การนำผลงานวิจัยไปใช้ประโยชน์

- เชิงพาณิชย์ (มีการนำไปผลิต/ขาย/ก่อให้เกิดรายได้ หรือมีการนำไปประยุกต์ใช้โดยภาคธุรกิจ/บุคคลทั่วไป)

- เชิงนโยบาย (มีการกำหนดนโยบายอิงงานวิจัย/เกิดมาตรการใหม่/เปลี่ยนแปลงระเบียบข้อบังคับหรือวิธีทำงาน)

- เชิงสาธารณะ (มีเครือข่ายความร่วมมือ/สร้างกระแสความสนใจในวงกว้าง)

1. สร้างเครือข่ายการเฝ้าระวังเห็ดพิษในภาคเหนือตอนบน ร่วมกับสาธารณะสุขจังหวัด น่าน ลำปาง ลำพูน แม่ฮ่องสอน เชียงใหม่ และศูนย์วิทยาศาสตร์การแพทย์เชียงใหม่

2. สร้างฐานข้อมูลเชิงพื้นที่ / ผู้ป่วยจากการรับประทานเห็ดพิษ สำหรับวางแผนเตือนภัย ช่วยเหลือ ทั้งการแพทย์ สาธารณสุข สิ่งแวดล้อม

- เชิงวิชาการ (มีการพัฒนาการเรียนการสอน/สร้างนักวิจัยใหม่)

ร่วมเป็นอาจารย์ที่ปรึกษาร่วมวิทยานิพนธ์ ของ น.ส. อัญจนา คุณนะลา หัวเรื่อง การต้านอนุมูลอิสระของซิลิบินินต่อความเป็นพิษของแอลฟา-อะมานิตินในหลอดทดลอง ภาควิชานิติเวชศาสตร์ คระแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่

3. อื่นๆ (เช่น ผลงานตีพิมพ์ในวารสารวิชาการในประเทศ การเสนอผลงานในที่ประชุมวิชาการ หนังสือ การจดสิทธิบัตร)

[Manuscript 1]

α-Amanitin Contents of poisonous *Amanita* Mushrooms in Nan Chiang Mai and Mae Hong Son Community Forests

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Abstract:

The poisonous mushrooms in the Genus *Amanita* were surveyed and collected from June 2008 to August 2009 in Nan, Chiang Mai, and Mae Hong Son community forests. For each study area, based on the morphological characteristics, there were fitty-one species of *Amanita* mushrooms. Of these, only four species were recognised as edible mushrooms by the locals i.e. *Amanita cheapangiana*, *A. princeps*, *A. hemibapha* and *A. caesarea*. For poisonous species, *Amanita phalloides A. virosa A. verna Amanita* sp.1 *Amanita* sp.2 *Amanita* sp.3 และ *A. cokeri,* almost experienced peoplewere know and discriminated them. In Nan has abundant with *Amanita* mushrooms such as *A. bisporigera A. subjunquillea A. citrina A. phalloides* and *A. existalis*, which all of them were rather rare in Chiang Mai and Mae Hong Son. Most of these *Amanita* mushrooms were not known by the local people and were not utilized. Five species of *Amanita* mushrooms i.e. *A. virosa*, *A. verna*, *Amanita* sp.1, *Amanita* sp.*2*, and *Amanita* sp.3 known as poisonous mushrooms and similar in morphology as the edible species of *Amanita* mushrooms. Only the poisonous species of *Amanita* mushrooms in Nan, Chiang mai and Mae Hong Son community forest were subsequently examined for the presence of α - amanitin by thin layer chromatography and confirmed by high performance liquid chromatography. It was found that the presence of α-amanitin positive in paper or thin layer chromatography. It was found that *Amanita cokeri, A. phalloides, Amanita* sp.1, *Amanita* sp.2, *Amanita* sp.3, *A. verna,* and *A. virosa* were α-amanitin positive of 0.12-0.16, 0.30-0.46, 0.12 - 0.34, 0.12 - 0.45, 0.17 - 0.80, 1.39-2.86 and 0.59 – 1.81 mg/g of dry tissue, respectively.

Keywords : amanitin, *Amanita,* Nan, Chiang Mai, Mae Hong Son, community, forests

Introduction

In Thailand, wild mushrooms are common only in rainy season, which occurs from June to October. Although the edible wild species command higher prices than cultivated mushrooms, people prefer to consume them due to their flavour and texture (Dell *et al.*, 2000). Some poisonous wild mushrooms are often indistinguishable from edible species. The high risks of illness and death rate from consuming the poisonous wild mushrooms were reported to increase every year.

Amatoxins interfere with DNA transcription by inhibiting RNA polymerase II(RNA polymerase B), synthesis of messenger RNA and subsequent protein synthesis is interrupted[12]. Cells with high rates of protein synthesis (e.g. those of the gastrointestinal tract, the liver and the kidney) are particularly sensitive to injury. Depending on the development stage, the concentration of amatoxin is higher in old than in young mushrooms[10]. α - Amanitin (a member of amatoxin) are frequently lethal, and are responsible for 90 % of

fatal human mushroom poisonings worldwide[1]. The human LD_{50} is 0.1 mg / kg body weight. This approximately 7 mg toxin for an adult male.

Chaiear reported a case study relating to the death of Thai people who consumed the poisonous mushrooms which mainly belong to genus *Amanita*. However, there is little information available about the α - amanitin in *Amanita* spp. in Thailand. In this study, *Amanita* mushrooms were subsequently examined for the presence of α - amanitin by Thin Layer Chromatography and confirmed by High Performance Liquid Chromatography that has been proven sensitve enough to detect toxins [6].

MATERIALS AND METHODS

Collection of mushrooms

Fruiting bodies of *Amanita* spp. were collected from June 2008 to August 2009 in Nan Mae Hong Son and Chiang Mai community forests.The harvested fruiting bodies were dried at 80 °C. for 2 days and powdered in a Moulinex blender.

Standard, reference compounds

Standards of α-amanitin were purchased from Sigma. Stock solutions of 100 ng/μl were prepared in methanol (HPLC grade) and stored, protected from light, at – 20 °C. Methanol and acetonitrile (HPLC grade) were purchased from Merck. The HPLC buffers were filtered through 0.45 micrometre membrane (Sartorius, Gottingen, Germany).

Amanitin extraction

About 2 g of powdered samples (single fruiting bodies) were defatted with 30 ml of light petroleum for 3 h in an Soxhlet extractor. The insoluble residue were dried in an oven and extracted with 30 ml of boiling methanol for 3 h in an Soxhlet extractor. The resulting extracts were evaporated to dryness using a rotary evaporator and redissolved in 2 ml of methanol and centrifuged for 30 min using a bench centrifuge. The supernatant were used as a methanolic extract.

Qualitative test for the presence of α-amanitin

This will be modified from the methods of Block[2], Wieland[12], and Yocum[13]. α -Amanitin standard solutions will be prepared by dissolving 1 mg of α-amanitin (standard, Sigma-Singapore) in 10 ml of methanol. Portions (30 μl) of the methanolic extracts from the samples, amanitin standard solution, will be loaded on to a Whatman No.1 chromatographic paper, which will be developed for 40 min , using butanone-acetone-water (30:3:5, v/v) as running solvent. The dried chromatogram will be sprayed with a 1 % solution of trans-cinnamaldehyde in methanol and immediately exposed to the vapour of fuming concentrated hydrochloric acid (violet spots indicate α-amanitin ; while orange, yellow, brown and other spots are of no significance).

High-performance liquid chromatography (HPLC) analysis of α-amanitin

1. Apparatus and chromatographic conditions

Chromatographic conditions will be carried out by gradient elution. The HPLC apparatus will be composed of the following units: a solvent – deliver module, a sample injection valve with a 10 μl loop and a variable-wavelength UV diode-array detector. This detector allows the monitoring of the eluate at two wavelengths simultaneously and the recording of absorbance spectra at definite time intervals (from 1 spectrum per 2 s to 16 spectra/s) In this work, the system will be set at 1 spectrum/s.

2. Separations will be performed at ambient temperature on a reversed-phase 5-μm ultrasphere ODS column (250x4.6 mm I.D.). The mobile phase will be a mixture of two solvents: solvent A will be 0.02 M aqueous ammonium acetate-acetonitrile (90:10, v/v) and solvent B will be 0.02 M aqueous ammonium acetate-acetonitrile (76:24, v/v). The pH of mixtures A and B will be adjusted to 5 with filtered glacial acetic acid. The gradient profile will be as follows: 100 % A for 4 min, then 57 % B for 16 min, then 100 % B for 10 min and

finally 100 % A. The mobile phase flow-rate will be 1 ml/min. The absorbance of the eluate will be monitored simultaneously at 207 and 305 nm.

3 Quality control parameters of the method

A calibration graph will be prepared in the extraction medium with increasing amounts of the α-amanitin yielding concentrations of 1, 5, 10, 25, 50 and 100 ng/μl. The limit of detection will be defined as the lowest concentration of α-amanitin. The accuracy of the method will be investigated for α-amanitin at six concentrations (1, 5, 10, 25, 50 and 100 ng/μl) by comparing the amount of α-amanitin added to the extraction medium with that actually measured.

4. Preparation of sample extracts for HPLC injection

Dried samples from qualitative test of α-amanitin producing species will be evaluated for the types and quantity of the toxin using a modification of the method of Enjalbert[6]. Each samples about 2 g of chopped sample will be placed in a cartridge of a freezer-mill, crushed for 1-2 minutes and extracted with 3 ml of extraction medium [methanol-water-0.01 M hydrochloric acid (5:4:1, v/v/v)]. Overall, 30 ml of extraction medium will be used. The mixed extracts will be incubated overnight at 4 °C, then centrifuged at 6,000 g for 10 minutes. The supernatant will be collected and preserved at 4 ° C and the pellet will be mixed again with 8 ml of the extraction medium and incubated for other 12 h at 4 ° C. The mixture will be centrifuged again at 6,000 g for 10 minutes and the supernatant will be pooled with the others. A 10 μl aliquot of the combined supernatants will be used for the separation and determination of the α-amanitin.

Results

Qualitative test for the presence of α-amanitin

The presene of α-amanitin in *Amanita* mushrooms in Nan Chiang Mai and Mae Hong Son community forests (table 1)were found in dried samples of All of them gave violet spots on a dried chromatogram, which indicates the presence of α-amanitin. The other species gave negative results. A dried samples confirmed the presence of α-amanitin by using high-performance liquid chromatography (HPLC) analysis of α-amanitin.

High-performance liquid chromatography (HPLC) analysis of α-amanitin

Quality control parameters, high-performance liquid chromatography (HPLC) analysis of α-amanitin, were investigated by preparing the extraction medium with increasing amounts of α-amanitin yielding concentrations of 1, 5, 10, 25, 50 and 100 ng/μl. The limit of detection is defined as the lowest concentration of α-amanitin. Quantification of toxins, based on peak areas, was linear between 1 to 100 ng/μl (correlation coefficient 0.99). This concentration ranges were included the concentration of toxins were usually found in dry tissue of mushroom extracts.

A 10 μl aliquot solution of the sample was used for determination of the α-amanitin contents of each *Amanita* sample. The results of the analyses are shown in Table 1. It was found that *Amanita phalloides, A. verna,* and *A. virosa* in section *Phalloidae* hadα-amanitin. Only *Amanita cokeri* in section *Lepidella* had α-amanitin. The other species of sections *Validae*, *Amanita*, and *Vaginatae* had no α-amanitin.

In Nan, it was found that the quantity of α-amanitin in *Amanita cokeri, A. phalloides, Amanita* sp.1, *Amanita* sp.2, *Amanita* sp.3, *A. verna,* and *A. virosa* were found to be 0.12, 0.46, 0.23, 0.12, 0.17, and 0.167 mg/g of dry tissue, respectively.

In Chiang Mai, it was found that the quantity of α-amanitin in *Amanita cokeri, A. phalloides, Amanita* sp.1, *Amanita* sp.2, *Amanita* sp.3, *A. verna,* and *A. virosa* were found to be 0.16, 0.30, 0.34, 0.45, 0.80, 2.86, and 0.59 mg/g of dry tissue, respectively

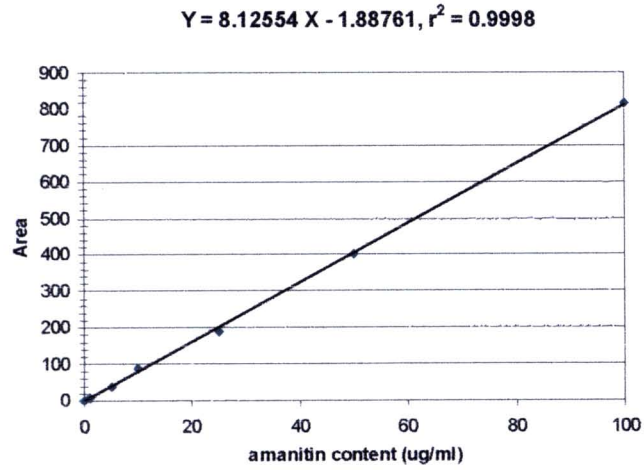

Figure 1 Linearity of α-amanitin determination (y = 0.812554 x –1.88761)

In Mae Hong Son, it was found that the quantity of α-amanitin in *Amanita cokeri, A. phalloides, Amanita* sp.1, *Amanita* sp.2, *Amanita* sp.3, *A. verna,* and *A. virosa* were found to be 0.13, 0.43, 0.12, 0.21, 0.23, 1.39, and 1.59 mg/g of dry tissue, respectively. Each of these species showed HPLC peaks that agreed with the standard of α - amanitin. Other amanitins or other toxins, for which standards were not available, may be present.

Table 1 Qualitative test for the presence and HPLC analysis of α-amanitin content of *Amanita* mushroom

section	species	Presence α-amanitin Test [1]	α-amanitin content mg/ g.dry wt.[2]		
			N	CM	MHS
Lepidella	*Amanita cokeri*	+ (Rf.=0.35)	0.12	0.16	0.13
Phalloideae	*Amanita arocheae*	-	-	-	-
	Amanita phalloides	+ (Rf.=0.35)	0.46	0.30	0.43
	Amanita pseudoporphyria	-	-	-	-
	Amanita subjunquillea	-	-	-	-
	Amanita sp. 1	+ (Rf.=0.35)	0.23	0.34	0.12
	Amanita sp. 2	+ (Rf.=0.35)	0.12	0.45	0.21
	Amanita sp. 3	+ (Rf.=0.35)	0.17	0.80	0.23
	Amanita verna	+ (Rf.=0.35)	1.67	2.86	1.39
	Amanita virosa	+ (Rf.=0.35)	1.81	0.59	1.59

Note; N= Nan, CM=Chiang Mai, MHS=Mae Hong Son community forest
1 = Qualitative test for α-amanitin
2 = weight (g) of dry tissue

Discussion

The qualitative tests for the presence of α-amanitin of *Amanita* mushroom extracts revealed the presence of α-amanitin from *Amanita* sp.1, *Amanita* sp.2, *Amanita* sp.3, *A. verna,* and *A. virosa.* All of them showed violet spots on the dried chromatogram which indicates the presence of α-amanitin. This results agreed with Yocum and Simons[13] who investigated *Amanita phalloides, A. bisporegera, A. verna, A. virosa* and *A. rubescens* and detected toxins in all except *A. rubescens.*

The limit of detection of α-amanitin standard was less than 1 ng/μl. Linearity was determined using extracts of negative samples spiked with α - amanitin ranging from 1 ng/μl to 100 ng/μl . The corelation coefficient (r) of 0.9962 indicated that the linearity of this method was sufficient for this research. The linearity was described by the equation y = 0.812554 x –1.88761(Figure 2), was the concentration of α-amanitin in dried mushroom extracts (ng/μl) and y was the peak area. The recovery of α-amanitin was determined using the extracts spiked with α-amanitin at concentrations of 100 ng/μl, ranging from 93.9 – 100.0 %. The acuracy was determined by spiking extracts samples with different amounts of α - amanitin (1, 5, 10, 25, 50 and 100 ng/μl) and comparing the theoretical with measured concentrations.

The presence of α-amanitin tests are showed in paper chromatogram (Figures 1, Table 1). It was found that *Amanita* sp.1, *Amanita* sp.2, *Amanita* sp.3, *A. verna A. virosa* and *A. cokeri*, were presence α-amanitin content. These species and other species were confirmed by HPLC analysis compared with the standard α-amanitin, it was found that the species with α-amanitin show peaks the same as the peak of standard α-amanitin, or same as retention time.

The α-amanitin content of *Amanita cokeri, A. phalloides, Amanita* sp.1, *Amanita* sp.2, *Amanita* sp.3, *A. verna* and *A. virosa* which collected from Nan, Chiang Mai and Mae Hong Son community forest were found to be 0.12-0.16, 0.30-0.46, 0.12-0.34, 0.12-0.45, 0.17-0.80, 1.39-2.86 and 0.59-1.81 mg/g of dry tissue respectively. Bresinsky and Besl[3] reported that α-amanitin content of *A. phalloides*, *A. verna* and *A. virosa* varied from 1.0-2.5, 1.5-4.5 , and 1-3 mg/g dry tissue, respecitively. Enjalbert[6] reported that the content of toxic peptides in a single sample of *A. phalloides* was 0.07 % of the fresh tissue (123 μg /g) or 2.26 mg. Although, the α-amanitin contents of all species found in Nan, Chiang Mai, and Mae Hon Son community forests which had α-amanitin were less than the α-amanitin content of *Amanita verna* and *A. virosa* reported by Bresinsky and Besl[3]. These two species are very dangerous to Thai people who eat them more than four fruiting bodies.

The edible species, *Amanita cheapangiana, A. caesarea, A. hamibapha,* and *A. princeps* in this study had no α-amanitin. The methods in this study were analysed only α amanitin, so that other amanitin or other toxins, notably ibotenic acid or muscimol, may be present remained in the other species. Tests for other fungal toxins on need to be performed and more specimens need to be examined, before any species are considered safe to eat. The distribution of α-amanitin in *Amanita* has long been a subject of controversy. The presence of trace quantities of amatoxins in all species tested, including the common edible species *Agaricus bisporus* (Lange) Pilát, using radioimmunoassay (RIA)[9].

The determination of the α-amanitin content of *Amanita* species was done by HPLC. The α-amanitin contents was not previously reported in Thailand. The α-amanitin contents of the *Amanita species* studied were less than the α-amanitin content of *A. phalloides* [6], *A. verna and A. virosa*[3]. This may be due to differences in climate, location, soil type and other environmetal factors which may effect the toxin content of *A. phalloides* carpophores[8]. The entire fruiting body of all *Amanita* species studied for toxin extraction. The total α - amanitin content was determined. The time of collecting samples also affects the toxin concentrations which differed from the early season (May to June) to the late rainy season (August to October) was not similar for all poisonous *Amanita* species.

Conclusion

Ten species of poisonous *Amanita* mushrooms in Nan, Chiang mai and Mae Hong Son community forest were subsequently examined for the presence of α - amanitin by thin

layer chromatography and confirmed by high performance liquid chromatography. It was found that the presence of α-amanitin positive in paper or thin layer chromatography. It was found that *Amanita cokeri, A. phalloides, Amanita* sp.1, *Amanita* sp.2, *Amanita* sp.3, *A. verna,* and *A. virosa* were α-amanitin positive of 0.12-0.16, 0.30-0.46, 0.12 - 0.34, 0.12 - 0.45, 0.17 - 0.80, 1.39-2.86 and 0.59 – 1.81 mg/g of dry tissue, respectively.

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[Manuscript 2]
Amanita mushroom diversity in Nan, Chiang Mai and Mae Hong Son Community Forest

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Abstract

Mushrooms in the Genus *Amanita* were surveyed and collected from June 2008 to August 2009 in Nan Chiang Mai and Mae Hong Son, community forests. For each study area, based on the morphological characteristics, there were fitty-one species of *Amanita* mushrooms. Of these, only four species were recognised as edible mushrooms by the locals i.e. *Amanita cheapangiana*, *A. princeps*, *A. hemibapha* and *A. caesarea*. For poisonous species, *Amanita phalloides A. virosa A. verna Amanita* sp.1 *Amanita* sp.2 *Amanita* sp.3 และ *A. cokeri*, almost experienced peoplewere know and discriminated them. In Nan has abundant with *Amanita* mushrooms such as *A. bisporigera A. subjunquillea A. citrina A. phalloides* and *A. existalis*, which all of them were rather rare in Chiang Mai and Mae Hong Son. Most of these *Amanita* mushrooms were not known by the local people and were not utilized. Five species of *Amanita* mushrooms i.e. *A. virosa*, *A. verna*, *Amanita* sp.1, *Amanita* sp.2, and *Amanita* sp.3 known as poisonous mushrooms and similar in morphology as the edible species of *Amanita* mushrooms, were compared in terms of specific morphological characteristics. Moreover, databases of *Amanita* and other mushrooms in Nan, Mae Hong Son, and Chiang Mai community forests were created and developed to be as source of knowledge for the public.

Keywords: Amanita mushrooms, Nan, Chiang Mai, Mae Hong Son, community forest, database

Introduction

Amanita Pers. is a large genus that is common in Thailand, composed of edible and poisonous species. Edible species, *Amanita caesarea, A. hemibapha, A. princeps,* and *A. cheapangiana,* are used as food for Thai people. Shepherd and Totterdell (1988) indicated that *Amanita* is one of the most simply to identify of all the agaric genera. However, species identification is regularly difficult without the use of a microscopic characteristics. Since the invention and spread of the PCR, different DNA fingerprinting methods have been introduced for the assessment the genetic relationships among taxonomically related organisms (Kårén *et al.*, 1997). Vicente *et al.*(2002) used Amplified Ribosomal DNA Restriction Analysis (ARDRA) for the analysis of the phenetic relationship relationships among several Spanish Amanita species. This technique is based on the comparison of the electrophoretic profiles obtained after the digestion of the ITS1-5.8S-ITS2 region with different restriction endonucleases. Nuclear rDNA genes have been widely employed for inferring taxonomic and phylogenetic relationships on a wide range of organisms, recently including genus Amanita (Yang and Oberwinkler, 1998; Drehmel et al., 1999). Although rDNA sequence analysis or Amplified Ribosomal DNA Restriction

Analysis (ARDRA) has been widely applied for the molecular typing of fungi (including Amanita species) isolated in the field study (Kårén et al., 1997). It is conceivable but still more expensive sequencing technologies. The morphological classification can be very useful as the primary method of mycorrhizal classification, when used in conjunction with molecular techniques (Sakakibara et al.,2002)

However, in cases of *Amanita* mushroom poisoning that the physicians must be rescued and recovered the patient within the few hours. They need to know the poisonous species for decision and therapy the patient. Some species of this genus are poisonous mushrooms and they are looklike edilbe species that causes Thai people dead every year. There is a need to accurately define edible, poisonous and other mushrooms in Thailand where wild mushrooms are traditionally collected from forests for consuming. This reseach focused on the distribution of edible and poisonous species of *Amanita* mushrooms in Nan, Chiang Mai, Mae Hong Son community forests for comparing the differences of morphological characteristics of *Amanita* mushrooms.

The aims of this research were :

To investigate the diversity of *Amanita* mushrooms in Nan, Chiang Mai and Mae Hong Son community forests

Materials and Methods

Nan, Chiang Mai, and Mae Hong Son community forests were selected as study areas (Table 1) according to the types of forest (mixed deciduous, dry dipterocarp, moist evergreen, hill evergreen, and dry evergreen/pine forest) and the sites recommended by the experienced mushroom collectors in each community. Each study site was investigated at least three times per month. Mature fruiting bodies of mushrooms were collected. The following information was recorded, *i.e.* ,species names of mushrooms found in each site, macroscopic and microscopic characteristics of fruiting body of each species, collected date, substratum, habitat, relative air humidity, soil moisture, air temperature, altitude, location, soil pH and light intensity at the ground level (Pegler, 1998). The collected specimens were identified and the classified by conventional morphological methods (Singer, 1986).

Results:

Diversity of *Amanita* mushrooms in Nan, Chiang Mai and Mae Hong Son community forests

Fiffy one species of *Amanita* were found in Nan, Chiang Mai, and Mae Hong Son community forest were identified by using conventional taxomomic keys (Bas, 1979). Some species were collected in different locations. *Amanita* includes two subgenera, *Lepidella* and *Amanita*. Subgenus *Lepidella* has sections *Amidella, Lepidella, Phalloideae,* and *Validae*. Subgenus *Amanita* has section *Amanita* and *Vaginatae*.

Subgenus *Lepidella*, section *Amidella* (*Amanita avellaneosquamosa*) was collected in Chiang mai community fores, rarelyt. Section *Lepidella* (*A. castanopsis, A. cokeri, A. gymnopus, A. hongoi, Amanita* sp., *A. thiersii, A. virginea* and *A. virgineoides*) were commonly found in all study areas. *A. gymnopus* was found abundant in Chiang Mai community forest. *A. thiersii* is rare in Chiang mai community forest *A. arocheae A. phalloides, A. verna, A. virosa, A. subjunquillea,* and *A. pseudoporphyria* are in section *Phalloides.* All of them are rare and scattered in the study areas.

Table 1 Details of Community forests: Nan, Chiang Mai and Mae Hong Son

Commnity forest	sea level	details of forest
1. Nan (Tha Wang Pha, Cheremphakeit, Bokrea, Mae Jarim, Pua, Na Mun, and Chiang Klang)	350-400 m asl.	It is a dry *dipterocarp* forest consisting of *Shorea obtuse, S. siamensis, Diperocapus obtusifolius, D. tuberculatus and Imparata cylindrical*
2. Chiang Mai		
Mae Wang	350-400 m asl.	It is a dry *dipterocarp* forest consisting of *Shorea obtuse, S. siamensis, Diperocapus obtusifolius, D. tuberculatus and Imparata cylindrical*
San Kam Phang	400 m asl.	It is a dry *dipterocarp* forest consisting of *Shorea obtuse, S. siamensis, Diperocapus obtusifolius, D. tuberculatus and Imparata cylindrical*
Muang (Sutep-Pui)	1000-1300 m asl.	It is a primary *evergreen* forest consisting of *Castanopsis acuminatissima C. armata, c. diversifolia C. ferox, C. tribuloides, Lithocarpus tenuinervis, Schima wallichii, Magnolia lifer Michelia ballonii, Semecarpus cochinchinensis*
Chiang Dao (Phang pan)	500-600 m asl.	It is a *deciduous* forest consisting of *dipterocarp-oak Dipterocarpus obtusifolius, D. turbinatus, Shorea siamensis, S. obtuse, Tectona grandis, Terminalia glaucilfolia Mangifera caloneura and Bambusa spp.*
3. Mae Hong Son		
Doi pu ya	350-400 m asl.	It is a dry *dipterocarp* forest consisting of *Shorea obtuse, S. siamensis, Diperocapus obtusifolius, D. tuberculatus and Imparata cylindrical*
Doi Kum Phar	350-400 m asl.	It is a dry *dipterocarp* forest consisting of *Shorea obtuse, S. siamensis, Diperocapus obtusifolius, D. tuberculatus and Imparata cylindrical*

Section *validae* (*A. xanthella, A. brunnescens, A. fritillaria* and *A. spissacea*) are rare and scattered in some study areas. Subgenus *Amanita*, sections *Amanita* and *Vaginatae*, are mophologiclly related, sharing features such as the whitsh, yellowish, gleyish or cream colour of the fruiting body, spore shapes and non-amyloid spores. Only 6 species in section *Amanita* (*A. obsita, A. sychnopyramis, A. farnosa, A. siamensis, A. cecilliae* and *A. concentrica*) were found in some study areas. *A. farinosa* and *A. concentrica* are rare in Chiang Mai community forest. Section *Vaginatae*, viz. *A. angustilamellata, A. battarae, A. caesarea, A. calopus, A. chepangiana, A. fuligineodisca, A.fulva, A. griseofolia, A. hemibapha, A. huijsmanii, A. longistriata, A. ovalispora, A. princeps, A. spreta, Amanita* sp.1, and *A. vaginata,* were commonly in all study areas.

Table 2 *Amanita* mushroom in Nan, Chiang Mai, and Mae Hong Son community forests

Subgenus/ Section	Species	Study areas*		
		Nan	*Mae Hong Son*	*Chiang Mai*
Lepidella				
Amidella	Amanita avellaneosquamosa	+	+	+
Lepidella	*Amanita castanopsis*	+	+	+
	Amanita cokeri	+	++	++
	Amanita gymnopus	++	++	++
	Amanita hongoi	++	++	++
	Amanita sp.	++	+	+
	Amanita thiersii Bas	++	+	+
	Amanita virginea	+	++	++
	Amanita virgineoides	+	++	++
Phalloideae	*Amanita arocheae*	+	+	+
	Amanita phalloides # 1	++	+	+
	Amanita phalloides # 2	++	+	+
	Amanita phalloides # 3	+	+	+
	Amanita phalloides # 4	+	+	+
	Amanita phalloides # 5	+	+	+
	Amanita verna # 1	++	++	++
	Amanita verna # 2	++	++	++
	Amanita verna # 3	++	++	++
	Amanita virosa # 1	++	++	++
	Amanita virosa # 2	++	++	++
	Amanita subjunquillea	++	+	++
	Amanita pseudoporphyria	+	+	+
Lepidella				
Validae	*Amanita xanthella*	+	+	+
	Amanita brunnescens	+	+	+
	Amanita fritillaria	+	+	+
	Amanita spissacea	+	+	+
Amanita				
Amanita	*Amanita obsita*	+	+	+
	Amanita sychnopyramis	+	+	+
	Amanita farinosa	+	+	+
	Amanita siamensis	-	-	+
	Amanita cecilliae	+	+	+
	Amanita concentrica	+	+	+
Vaginatae				
	Amanita angustilamellata	+	+	+
	Amanita battarae	+	-	+
	Amanita caesarea	+	+	+
	Amanita calopus	+	+	+
	Amanita chepangiana # 1	++	++	++
	Amanita chepangiana # 2	++	++	++
	Amanita chepangiana # 3	++	++	++
	Amanita chepangiana # 4	++	++	++
	Amanita fuligineodisca	+	+	+
	Amanita fulva	+	+	+

(Continue)

Subgenus/ Section	Species	Study areas*		
		Nan	*Mae Hong Son*	*Chiang Mai*
	Amanita griseofolia	+	+	+
	Amanita hemibapha	+	+	+
	Amanita huijsmanii	+	+	+
Vaginatae	*Amanita longistriata*	+	+	+
	Amanita ovalispora	++	++	++
	Amanita princeps	++	++	++
	Amanita spreta	++	++	++
	Amanita sp.1	++	++	++
	Amanita vaginata	++	++	++
Total		50	48	51

Note: N= Nan MH = Mae Hong Son CM = Chiang Mai
(- / + = absent / presence) + = rare / ++ = little
+++ = more / ++++ = abundant

Most of the edible species of *Amanita* were named locally by the local mushroom collectors such as Hed Kai Kao (*A. chepangiana*), Hed Kai Laung (*A. hemibapha*), and Hed Kai Kao (*A. princeps*). Some of the poisonous species such as Hed Kai Han Theen Dhum (*A. phalloides*) , Hed Kai Han Theen Dhum (*A. verna*), and Hed Kai Han Theen Dhum (*A. virosa*) has general local names (the names are not specify to each species) in each study area. It found that 86 % of *Amanita* species (47 species) had no local name were not eaten and unutilized in the study area. Only species in section *Vaginatae*, viz. *A. angustilamellata, A. caesarea, A. chepangina, A. fulva, A. hemibapha, A. princeps, A. vaginata* had known local names while *A. caesarea, A. chepangiana, A. hemibapha,* and *A. princeps* were eaten. All poisonous species were in section *Phalloideae,* viz. *A. phalloides, A. verna,* and *A. virosa.* All have the same " Hed Kai Han Dteen Dton, Hed Dteen Dum". The other species in section *Phalloidae*, the local people did not interest and had no local name.

Discussion

Fiffy one species of *Amanita* mushrooms in Nan, Chiang Mai, and Mae Hong Son community forests were identified to 2 subgenera *Lepidella* and *Amanita.* Subgenus *Leppidella* has 4 sections such as section *Amidella* (*Amanita avellaneosquamosa*), section *Lepidella* (*A. castanopsis, A. cokeri, A. gymnopus, A. hongoi, Amanita* sp., *A. thiersii, A. virginea,* and *A. virgineoides*), section *Phalloides* (*A. arocheae, A. phalloides, A. verna, A. virosa, A. subjunquillea,* and *A. pseudoporphyria*), and section *Validae* (*A. xanthella, A. brunnescens, A. fritillaria,* and *A. spissacea*). Subgenus *Amanita* has two sections such as section *Amanita* (*A. obsita, A. sychnopyramis, A. farinosa, A. siamensis, A. cecilliae,* and *A. concentrica*), and section *Vaginatae A. angustilamellata, A. battarae, A. caesarea, A. calopus, A. chepangiana, A. fuligineodisca, A.fulva, A. griseofolia, A. hemibapha, A. huijsmanii, A. longistriata, A. ovalispora, A. princeps, A. spreta, Amanita* sp.1, and *A. vaginata*).

Only, 4 species of the edible species of *Amanita* were named locally by the local mushroom collectors such as Hed Kai Kao (*A. chepangiana*), Hed Kai Laung (*A. hemibapha*), and Hed Kai Kao (*A. princeps*). The other 47 species had no local name

and were not eaten. Three poisonous species, *A. phalloides, A. verna,* and *A. virosa,* had the same local name. More than 40 species were found and still unidentified (data not shown), some of them look like the poisonous species in section *Phalloideae,* e.g. *A. phalloides, A. subjunquillea, A. verna,* and *A. virosa.* There are some deadly species in China that are macroscopically similar to *A. verna, A. exitialis* Zhu,Yang and Li (Yang *et al.*, 2001), *A. oberwinklerrana* Zhu, (Yang and Doi, 1999), and *A. subjunquillea* var. alba Zhu, (Yang , 1997). As mentioned by Yang *et al.*(2000), more than 50 species of *Amanita* were found in China. The distribution of *Amanita* is world wide and with more than 560 species (Tulloss, 2009).

Although some *Amanita* species are edible, a number are very poisonous. In spite of these poisonings, *Amanita* remains a popular mushroom for local people in Thailand. *Amanita caesarea, A. chepangiana, A. hemibapha,* and *A. princeps* are very common in local Thai markets during the rainy season. The well known deadly species, *A. phalloides, A. verna,* and *A. virosa,* were found in this study.Inquiries from local people who prefer to eat *Amanita* mushrooms, indicate that they will not stop eating various species in this genus so there is a need for more public information on the identification of edible and poisonous species and preventing.

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