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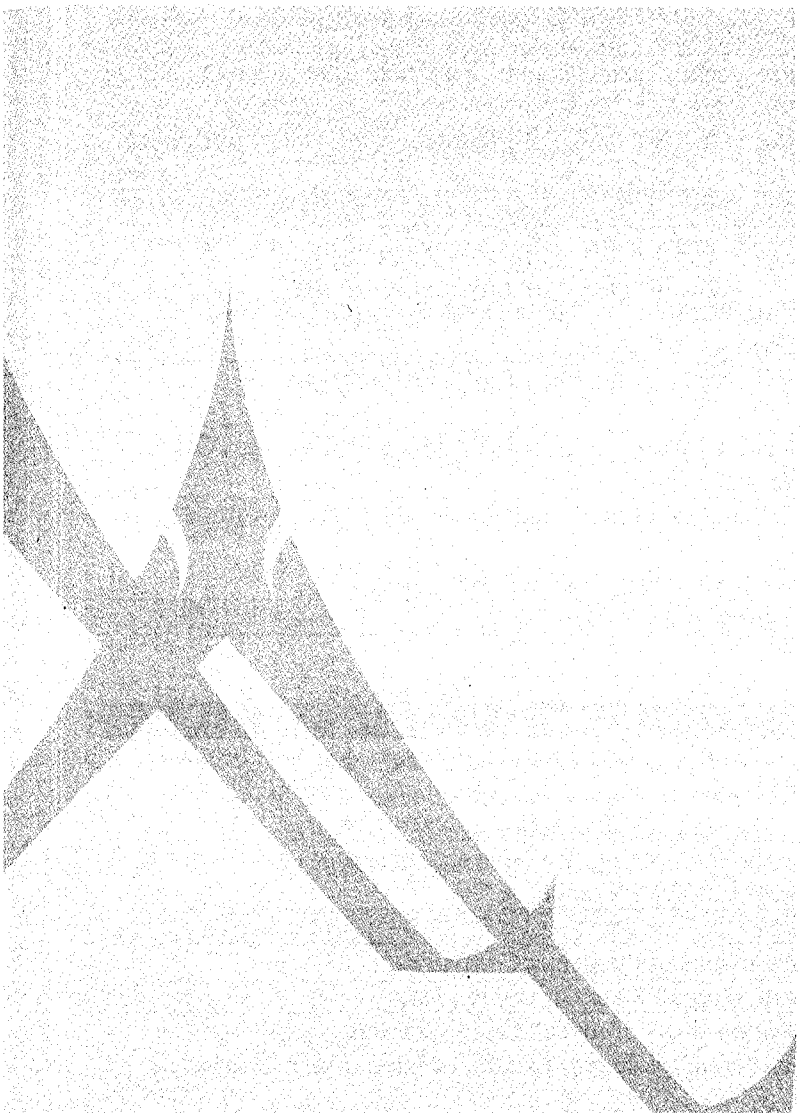

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Screening of Tropical Mushroom Lectins

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Abstract
Lectins, a very heterogeneous set of proteins which are grouped together purely on the basis of their ability to bind saccharides specifically and reversibly, are widely distributed in living organisms including plants, animals, and microorganisms (bacteria, fungi, and viruses). Fungal lectins have been less well studied than plant and animal lectins. A total of 93 mushroom specimens were collected from natural habitats and local markets in the Northeastern, Central, and Western Thailand, particularly in the Northeast region. Accumulations of lectins in crude extracts of these mushroom specimens were detected by hemagglutination assay using human (A, B, and O blood groups) and animal (goose, guinea pig, mouse, rabbit, rat, and sheep) red blood cells. It was found that more than 50% of mushroom extracts predominantly performed hemagglutinating for rat erythrocytes. Some extracts of *Amanitaceae* and *Agaricaceae* specimens rather strongly agglutinated both human and animal (rat, goose, guinea pig, mouse, and sheep) red blood cells, and have been selected for further purification and preliminary characterization of lectins.

Key words: Lectins, tropical mushrooms

Introduction
Lectins are a heterogeneous group of proteins or glycoproteins of non-immune origin that specifically and reversibly bind to carbohydrates of glycoconjugates (Rini, 1995; Lindhorst, 2000). These proteins are ubiquitous in nature, and occur in plants, animals, bacteria, fungi, and viruses (Lis and Sharon, 1998; Mo *et al.*, 2000). Most lectins play a crucial role in diverse biological processes, particularly in host defense mechanisms, inflammation, and metastasis (Imberty *et al.*, 2000). Owing to their binding specificities, lectins are employed in a number of biochemical and clinical research areas (Gilboa-Garber *et al.*, 1997). Many of the plant, animal, and microorganism lectins of known carbohydrate specificities have been engaged in commercialization (Pemberton, 1994). *Agaricus bisporus* lectin is the only one of fungal origin (Wang *et al.*, 1998). The great diversity of mushrooms could provide a source for the lectin exploitation (Doyle and Slifkin, 1994). The extremely high diversity of macrofungi in Thailand, a tropical country, has been reported. At least 90 genera of mushrooms have been recorded in National Forests of Thailand. The common families found belong to *Agaricaceae*, *Polyporaceae*, *Tricholomataceae*, *Russulaceae*, *Hygrophoraceae*, *Lycoperdaceae*, *Ganodermataceae*, *Geastraceae*, *Coriolaceae*, and *Hymenochaetaceae* (Klingesorn *et al.*, 1998; Rodtong *et al.*, 1998; Walting, 1998; Thaithatgoon, 1998; Rodtong and Teaumroong, 2000). In this study, lectins from mushrooms collected from natural habitats and local markets in the Northeastern, Central, and Western Thailand, particularly in the Northeast region, were intensively determined using the hemagglutination assay. The selected

mushroom lectins from this preliminary screening will be purified and characterized to obtain data for future applications.

Materials and Methods

Mushroom Specimens

Fresh fruit bodies of mushrooms were collected from natural habitats and local markets in the Northeastern (Chaiyaphum and Nakhon Ratchasima Provinces), Central (Nakhon Pathom Province), and Western (Kanchanaburi Province) Thailand. These mushroom specimens were identified and classified using their macroscopic and microscopic characteristics, then dried at 40°C for 2 to 3 days depending on their structures. The dried specimens were stored in desiccators for lectin extraction.

Lectin Extraction

Dried mushroom specimens were ground into powders. Lectins were then extracted from mushroom powders by homogenizing the powders with 10 times (w/v) of 0.01 M phosphate buffer saline (PBS, pH 7.2) containing 0.02 M sodium bisulphite at 4°C. Filtrates of the homogenates were collected and applied for the detection of lectin accumulations using hemagglutination assay.

Hemagglutination Assay

Accumulations of lectins in crude extracts of these mushroom specimens were detected by testing for hemagglutination activity against human (A, B, and O blood groups) and animal (goose, guinea pig, mouse, rabbit, rat, and sheep) red blood cells. Blood collected from both human and animal bodies was maintained in 4% of sodium citrate. Then red blood cells were washed three times with 0.01 M PBS (pH 7.4), and resuspended in the same buffer solution to give a 5% cell suspension. The hemagglutination assay was performed at room temperature using two-fold serial dilution of mushroom filtrate (crude extract) (Wright, 1998). The hemagglutination titer, which is defined as the reciprocal of the highest dilution exhibiting hemagglutination, was recorded.

Total Protein Estimation

The total protein concentration in crude extract of mushroom was estimated by spectrophotometrical measurement of the extract filtrate at A_{280} (Bollag and Edelstein, 1996).

Results and Discussion

Ninety-three mushroom specimens (64, 13, 12, and 4 specimens from Nakhon Ratchasima, Chaiyaphum, Nakhon Pathom, and Kanchanaburi Provinces respectively) were collected, identified and classified, and preliminary screened for the presence of hemagglutination activity against human and animal red blood cells. The collected mushroom specimens could be basically identified to be the members of 12 families: *Agaricaceae*, *Amanitaceae*, *Auriculariaceae*, *Boletaceae*, *Cantharellaceae*, *Lycoperdaceae*, *Pleurotaceae*, *Pluteaceae*, *Polyporaceae*, *Russulaceae*, *Schizophyllaceae*, and *Tricholomataceae*.

For the detection of lectins, crude extracts of these 93 mushroom specimens were tested for hemagglutination activity. Forty eight extracts were found to exhibit their specific agglutinating activity against red blood cells of human blood groups A, B, and O; and five animal species: goose, guinea pig, mouse, rat, and sheep (Figure 1;

Table 1, for example). Only the hemolytic activity of some mushroom extracts against rabbit erythrocytes was detected. Forty-two (around 87%) out of 48 extracts having positive hemagglutination tests were observed to be predominantly agglutinating for rat erythrocytes (Figure 1). Fifty five to sixty percents of the 48 extracts gave the positive agglutinating activity against red blood cells of human blood groups A, B, and O; and four animal species: goose, guinea pig, mouse, and sheep. The hemolytic activity and partial hemolysis against human and animal red blood cells could also be observed at the proportion of less than 30% (Figure 1). This might be caused by impurities in the crude extracts. To overcome this problem, steps of lectin purification (Colowick and Kaplan, 1955) and protease inhibition should be performed in our further study.

The distinctive record of mushroom lectin titre could be summarized in Table 1. The high lectin titres were detected in mushroom specimens belonging to genera *Amanita, Macrocybe*, *Macrolepiota*, *Lycoperdon*, *Termitomyces*, and *Volvariella*. A crude extract of *Amanita* sp., which was collected from the grape orchard in Nakhon Ratchasima Province, presented the highest lectin titre at 1024 when tested against rat red blood cells. The extract also gave the positive agglutination activity against human erythrocytes of blood groups A, B, and O, at titres of 512, 256, and 256 respectively. From this study, some mushroom specimens, which were identified as belonging to the same genus, e.g. *Macrolepiota* (Table 1), were observed to produce different results of hemagglutination test. Crude extracts of these *Macrolepiota* and *Amanita* specimens also gave very rapid and strong hemagglutination reaction. An extract of a species of *Lycoperdon* had its specific activity only against rat red blood cells, and gave the lectin titre of 256. The hapten inhibition test will be carried out to clarify these mushroom lectin specificity.

When the total protein concentration in crude extract of mushroom was estimated, there was no significant difference of protein concentrations between the extracts containing lectins and the extracts exhibiting negative hemagglutination activity. Approximate protein concentrations ranged from 8 to 16 mg/ml.

Conclusions

This preliminary screening of mushroom lectins shows clearly that a wide variety of tropical mushrooms in Thailand accumulates lectins in their fruit bodies. The lectins could be simply extracted and basically tested for their activity. More than 50% of mushroom extracts were found to predominantly perform hemagglutinating for rat red blood cells. The high incidence of lectin accumulations was observed in mushroom specimens belonging to genera *Amanita, Macrocybe*, *Macrolepiota*, *Lycoperdon*, *Termitomyces*, and *Volvariella*. Some crude extracts of *Amanita* sp. and *Macrolepiota* sp. rather strongly agglutinated both human and animal (rat, goose, guinea pig, mouse, and sheep) red blood cells, and have been selected for further purification and preliminary characterization of lectins.

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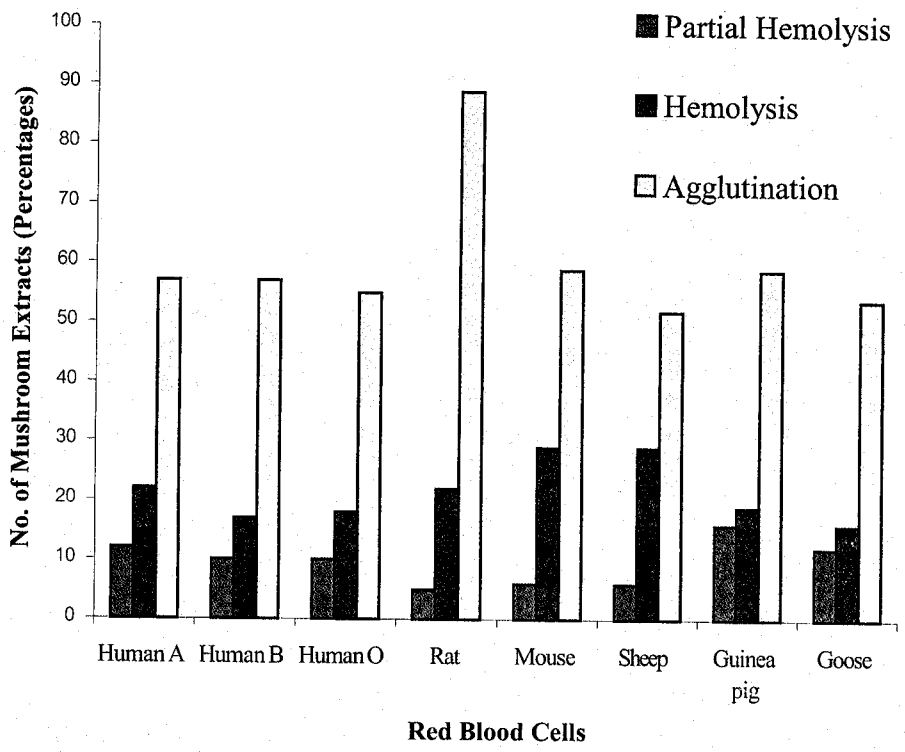

Figure 1. Proportion of positive hemagglutination test and hemolytic reaction of 48 tropical mushroom extracts when tested against human and animal red blood cells.

Table 1. Hemagglutination activity of crude extracts of 14 mushroom specimens.

Mushroom *Species*	Source[1]	Activity against erythrocytes (Titer)								
		Human			Animal					
		A	B	O	Rabbit	Rat	Goose	Mouse	Sheep	Guinea pig
Amanita sp.	NR	512	256	256	0	1024	32	0	64	128
Lentinus edodes	NR	H	PH	H	0	64	64	24	H	H
Lentinus sp.	NR	PH	0	PH	0	64	0	16	2	0
Lycoperdon sp.	NR	0	0	0	0	256	0	0	0	0
Macrocybe sp.	NR	PH	PH	PH	H	128	0	H	PH	PH
Macrolepiota sp.(1)	NR	16	16	64	0	64	32	64	32	64
Macrolepiota sp.(2)	NR	H	H	H	H	96	0	PH	0	0
Macrolepiota sp.(3)	NR	32	64	64	0	64	64	16	8	24
Macrolepiota sp.(4)	NR	8	16	16	0	32	512	0	2	32
Macrolepiota sp.(5)	NR	8	16	8	0	96	8	0	4	64
Pycnoporus sp.	NR	16	64	64	H	H	H	16	PH	96
Termitomyces sp.(1)	KB	0	0	0	0	96	0	0	0	0
Termitomyces sp.(2)	NR	PH	PH	0	0	160	0	H	H	H
Volvariella sp.	NR	0	0	0	H	64	0	H	H	0

H: Hemolysis, PH: Partial hemolysis

[1] Source: NR = Nakhon Ratchasima Province; KB = Kanchanaburi Province