STUDIES ON LECTINS FROM THAI PLANTS

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ABSTRACT

Plant samples (seeds, pods and fruits) of 178 species in 62 families were examined for lectin activity. Hemagglutination activity was performed with 8 different sources of red blood cells: human (types A, B and O), hamster, mouse, rat, goose and pigeon. Plant lectins were arbitrarily differentiated into 6 groups according to their hemagglutination patterns. Lectins of 107 plant samples were found, of which 19 samples were divalent ion dependent lectins. No blood type specific lectin was found in this study, except lectin of Butea monosperma *seed which showed specificity toward erythrocytes of human but not those of animal origins. The potent lectins which possess more than 100,000 units per gram wet weight were found in seeds of* Artocarpus lakoocha *and* Heliciopsis terminalis *and also in fruit extracts of* Coffea arabica *and* Chrysophyllum cainito.

INTRODUCTION

Lectins are proteins or glycoproteins from plants, bacteria, fungi, viruses, invertebrates, or vertebrates with the ability to interact with carbohydrate structures and to agglutinate cells or precipitate glycoconjugates[1-3]. They can be used as tools for blood typing, diagnosing of microorganisms, mitogenic stimulation of lymphocytes, the discrimination between normal and malignant cells, the purification of glycoconjugates, and as tools to examine cell surface carbohydrates[1-4].

Hundreds of lectins are now well characterized and the number is growing fast. Even most of the lectins which are commercially available and extensively studied are mainly of plant origin, the occurrence of lectins in a number of plant species is fairly well established. In this paper, we report a number of lectins present in tropical plants collected in the north and northeastern Thailand. The specific activity and ion dependence of the screened lectins are also reported.

MATERIALS AND METHODS

Plant samples were collected locally in the north and northeast of Thailand. Identification of the scientific nomenclature was established by expert taxonomists. The questionable samples were confirmed and identified by staff of the Herbarium of the Royal Forest Department, Ministry of Agriculture, Thailand.

Fresh human blood (types A, B and O) and animal blood (hamster, mouse, rat, goose and pigeon) were obtained from the Blood Bank of Srinagarind Hospital and the Animal House of Faculty of Medicine, Khon Kaen University, respectively.

Extraction of lectins. Plant samples were separated into seeds, flesh, husk or pod. In some samples from which the seeds could not be separated, the whole fruits were used for analysis. Plant samples were kept at -20° until use.

The extraction procedures were carried out at 4-10°, unless otherwise stated. Finely ground samples (5g) were suspended in 50 ml of 0.85% sodium chloride (NSS) and homogenized in a Waring blender for 2 min. After removing insoluble debris by filtering through cheese cloth, the filtrate was centrifuged at 10,716 g, for 20 min. The protein was fractionated using 80% saturated ammonium sulphate and centrifuged at 15,381 g for 30 min. The precipitate was dissolved, dialyzed against several changes of NSS. Any precipitate that appeared after dialysis was removed by centrifugation at 15381 g for 30 min and the clear supernatant was kept at -20° for further analysis.

Preparation of red blood cell suspension. Red blood cells were separated from plasma and washed three times in NSS. Finally, the red blood cells were adjusted to 2% (v/v) suspension in NSS.

Hemagglutination test. Two fold serial dilutions of plant extracts (50 µl) with NSS were made in V-bottomed microtitre plates. Then, an equal volume of 2% red blood cell suspension was added to each well and the plates were incubated for 1 hr at room temperature. NSS containing either 50 mM $CaCl_2$, $MgCl_2$ or $MnCl_2$ was used instead of NSS in order to investigate the ion dependence of the lectin. The titre was the highest dilution (in the absence of divalent ion) which caused an agglutination for each cell type tested. The unit of activity was the reciprocal of the titre[5] and the specific agglutination activity is reported as unit/mg protein.

Eight different sources of red blood cells from human (types A, B, and O), hamster, mouse, rat, goose and pigeon were used to screen the lectin activity in the plant samples. The lectins were grouped according to their hemagglutinating patterns into 6 groups as indicated in Table 1. Group "A" are lectins which possessed positive hemagglutination to all cell types tested, whereas group "N" are samples which showed non-reactivity with all red blood cell employed. Since a number of plant lectins exhibited agglutination only in the presence of divalent ions, therefore plant samples which showed negative hemagglutination were further subjected to hemagglutination test in the presence of divalent ions either

$CaCl_2$, $MgCl_2$ or $MnCl_2$. Plant samples which exhibited lectin activity in this condition were grouped as "N+". Lectins which have reactivity only toward human red blood cells are grouped as "H" while those which preferentially agglutinate animal red blood cells are grouped as "C". Sample which possessed hemagglutination toward both human and rodent erythrocytes are grouped as "B" whereas lectins which agglutinated only rodent's red blood cells are grouped as "D". In addition, samples which exhibited agglutinating activity only to rat's erythrocytes are grouped as "R".

RESULTS AND DISCUSSION

The carbohydrate specificities of many lectins have been grouped by the ability of monosaccharides or their glycosides to inhibit lectin-induced hemagglutination[6]. However, lectins of the same apparent monosaccharide specificity were found to demonstrate different reactivities toward different oligosaccharide chains, and differential affinities to animal cells and glycoproteins, which implies that they have their own binding specificity extending beyond the monosaccharide unit[6,7]. Therefore, a panel of 8 different sources of red blood cells was used to screen the lectin activity in this study, ensuring the maximum types of lectin detected. Genetic polymorphism and post-translational modification of the lectins, which probably occur in plants of the same species but grow in different geographical areas, have been shown to play an important role in the discrepancy of properties of lectins extracted from the same species[8-10]. Therefore, eventhough some plant lectins reported in this study, have been reported elsewhere, they are probably new lectins which exhibit different properties and are thus included in this report.

Using a battery of erythrocytes, 54 lectins from 450 species of Indian plants were revealed by their hemagglutinating activities[11]. In the present study, plant lectins were arbitrarily grouped according to their hemagglutination patterns into 6 groups (Table 1). According to the screening procedure, as many as 88 plant samples of 77 species were found to possess hemagglutination activity against one or more types of erythrocytes in the absence of divalent ion. The largest number of lectins were contributed by the family Leguminosae (28 species), mostly belonging to the genera *Cassia* and *Bauhinia*. The overall prevalence of phytolectins is summarized in Table 1. In our screening program, 178 plant species of 62 families were thoroughly examined for lectin activity. About 50% of the overall plant sample examined possessed lectin activity (Table 2). According to their biological hemagglutinating spectra, group A lectins which are major lectins (61/88), proved to be non specific, agglutinating all types of erythrocytes tested. In addition, 119 plant samples were found to be nonreactive to hemagglutination (group N), of which, 19 samples showed their activities in the presence of divalent ions (group N+).

The activity presented as unit/g wet weight and unit/mg protein are shown in Table 2. No human blood type specific lectin was found in this screening program. Only lectin of *Butea monosperma* turned out to be specific for all types of human erythrocytes but did not react with those of animal origins (group H). Moreover, 4 lectins which showed specificity to rat, 9 to rodent and 12 to all animal erythrocyte tested, are also demonstrated. The erythrocyte most prone to agglutination with manifest sensitivity was rat erythrocyte

TABLE 1 Prevalence of lectins in Thai plants as determined by hemagglutination activity in the absence of divalent ions.

Group	No. plant sample	Hemagglutination activity							
		A*	B*	O*	H*	M*	R*	G*	P*
A	61	+	+	+	+	+	+	+	+
B	1	+	+	+	+	+	+	-	-
C	12	-	-	-	+	+	+	+	+
D	9	-	-	-	+	+	+	-	-
H	1	+	+	+	-	-	-	-	-
R	4	-	-	-	-	-	+	-	-
N	119	-	-	-	-	-	-	-	-

* A,B,O,H,M,R,G, and P are red blood cells of human types A, B, O and those of hamster, mouse, rat, goose, and pigeon, respectively.

TABLE 2 Screening of lectins from Thai plants

		SAMPLE	GROUP	ION	U/g wet wt	U/mg protein
	Anacardiaceae					
1	*Bouea macrophylla* Griff	seed	A	-	4,800	4,078
	Annonaceae					
2	*Anomianthus dulcis* Sinel.	fruit	N+	(Ca,Mn)A	-	-
	Burseraceae					
3	*Canarium kerrii* Craib	flesh	A	-	44	25
4	*Garuga pinnata* Roxb.	husk	A	(Mg,Mn)	640	144
5	*Protium serratum* Engler	seed	R	-	80	158
	Capparidaceae					
6	*Capparis flavicans* Kurz	fruit	C	-	56	10
	Capparis flavicans Kurz	seed	C	-	28,672	4,366
7	*Capparis lanceolaris* DC.	seed	A	-	41,647	3,685
	Caprifoliaceae					
8	*Sambucus eberhardtii* P. Danguy	fruit	C	-	320	145
	Combretaceae					
9	*Combretum quadrangulare* Zurz.	seed	A	-	768	62
10	*Terminalia alata* Heyne er Roth	pod	C	-	14849	17066
11	*Terminalia chebula* Retz.	seed	A	-	144	55
	Connaraceae					
12	*Ellipanthus tomentosus* Kurz	fruit	A	-	160	41
	Cucurbitaceae					
13	*Neoalsomitra sarcophylla* Hutch.	husk	N+	(Mn)D	-	-
14	*Sechium edule* Sw.	husk	D	(Ca,Mg,Mn)	512	1,361
	Sechium edule Sw.	flesh	D	(Ca,Mg,Mn)	3,072	7,013
15	*Trichosanthes* sp.	seed, fruit	A	-	5,120	217
	Dipterocarpaceae					
16	*Dipterocarpus obtusifolius* Teysm ex Mig	seed	A	-	1,024	238
	Ebenaceae					
17	*Diospyros glandulosa* Lace	seed	C	-	44,329	51,200
	Elaeagnaceae					
18	*Elaeagnus latifolia* L.	seed	N+	(Ca,Mn)A	-	-
	Euphorbiaceae					
19	*Bridelia tomentosa* Bl.	fruit	A	-	170	108
20	*Jatropha curcas* L.	husk	D	(Ca,Mg,Mn)	12,288	43,574
21	*Phyllanthus amarus* Schum & Thonn	whole plant	A	-	24	5
22	*Phyllanthus emblica* L.	seed	A	-	12,902	1,020
23	*Ricinus communis* L.	seed	A	-	49,152	3,151
	Fagaceae					
24	*Quercus helferiana* A.DC.	seed	A	(Mg,Mn)	80	82

		SAMPLE	GROUP	ION	U/g wet wt	U/mg protein
	Flacourtiaceae					
25	*Hydnocarpus antheliminthicus* Pierre	seed	N+	(Ca)A	-	-
	Guttiferae					
26	*Calophyllum inophyllum* L.	husk	C	(Ca,Mg,Mn)	8,192	43,115
	Ixonanthaceae					
27	*Irvingia malayana* Oliv.ex A Benn	seed	A	-	20	4
	Labiatae					
28	*Perilla frutescens* Britt.	seed	D	-	16,384	10,666
	Leguminosae					
29	*Bauhinia acuminata* L.	pod	C	(Ca,Mg,Mn)	7168	1163
30	*Bauhinia malabarica* Roxb.	pod	C	(Mn)	1,280	3,764
31	*Bauhinia variegata* L.	seed	A	-	448	25
32	*Butea monosperma* Ktze.	seed	H	(Mn)	80	27
33	*Cassia bakeriana* Craib.	pod	A	(Mn)	1,728	141
	Cassia bakeriana Craib.	seed	A	-	44	31
34	*Cassia garretiana* Craib.	seed	N+	(Ca,Mn)A	-	-
35	*Cassia javanica*	flesh	A	(Ca,Mg,Mn)	52	12
	Cassia javanica	seed	A	(Ca,Mg,Mn)	56	35
36	*Cassia surattensis* Burm.f. subsp. *glauca* (Lam) K & SS Larsen	seed	N+	(Ca,Mn)D	-	-
37	*Dalbergia fusca* Pierre	seed	A	(Mg)	160	11
38	*Dalbergia oliveri* Gamble	seed, pod	N+	(Mn)A	-	-
39	*Delonix regia* Rafin.	pod	A	(Ca,Mg,Mn)	20	108
40	*Dolichos lablab* L.	seed	A	-	20,480	804
41	*Gliricidia sepium* Steud	seed	A	-	24	10
42	*Glucine max* Merr.	seed	A	-	320	17
43	*Leucaena glauca* Benth	seed	D	-	512	744
44	*Leucaena* sp.	seed	N+	(Mn)A	-	-
45	*Millettia brandisiana* Kurz	pod	A	(Ca,Mg,Mn)	112	78
	Millettia brandisiana Kurz	seed	A	(Ca,Mg,Mn)	14	23
46	*Millettia leucantha* Kurz	seed	H, E	-	640	33
47	*Phaseolus lunatus* L.	seed	A	(Ca,Mg,Mn)	32	3
48	*Phaseolus mungo* L.	seed	N+	(Mn)A	-	-
49	*Phaseolus* sp.	seed	A	-	11,264	835
50	*Pisum sativum* L.	seed	A	(Mn)	1,664	88
51	*Pterocarpus indicus* willd	husk	R	(Ca,Mn)	14,336	1,477
	Pterocarpus indicus Willd	seed	C	-	448	125
52	*Sindora siamensis* Teijsm ex Miq	seed	N+	(Mn)C	-	-
53	*Viagna radiata* Wilczek	seed	N+	(Mn)A	-	-
54	*Vicia faba* L.	seed	B	(Mn)	176	6
55	*Vigna* sp.	seed	N+	(Mn)A	-	-
56	*Vincia len* Linn.	seed	A	(Mn)	20,480	699
	Lythraceae					
57	*Lagerstroemia macrocarpa* Wall.	seed	A	-	3584	362

		SAMPLE	GROUP	ION	U/g wet wt	U/mg protein
58	*Lagerstroemia* sp.	seed	A	-	42,091	2,002
59	*Lagerstroemia speciosa* (L.) Pers.	seed	A	-	14,336	2,295
	Magnoliaceae					
60	*Magnolia henryi* Dunn.	fruit	N+	(Mn)C	-	-
	Malvaceae					
61	*Abelmoschus esculentus* Moenah	fruit	A	-	34	69
	Abelmoschus esculentus Moenah	seed	A	-	36,352	6,400
	Moraceae					
62	*Artocarpus lakoocha* Roxb.	seed	A	-	174,000	2,819
63	*Ficus bengalensis* L.	husk	A	(Ca,Mg,Mn)	28	114
64	*Ficus callosa* Willd.	flesh w seed	A	-	15,086	2,534
65	*Ficus glaberrima* Bl.	fruit	A	-	4,352	695
66	*Ficus hispida* L.f.	flesh w seed	N+	(Ca,Mg,Mn)A	-	-
67	*Ficus racemosa* L.	flesh w seed	A	-	29	242
	Ficus racemosa L.	husk	A	-	192	5,818
68	*Streblus asper* Lour	stem	A	(Ca)	512	185
	Myrtaceae					
69	*Eugenia malaccensis* L.	seed	A	-	102	44
70	*Eugenia paniala*	husk	N+	(Ca,Mg,Mn)H	-	-
	Eugenia paniala	seed	A	(Ca,Mg,Mn)	960	1,358
71	*Eucalyptus* sp.	fruit	A	-	1,536	337
	Palmae					
72	*Arenga pinnata* Merr.	seed	D	-	48	484
73	*Caryota mitis* Lour.	fruit	A	(Mn)	768	87
	Caryota mitis Lour.	seed	A	-	160	234
	Passifloraceae					
74	*Passiflora foetida* L.	seed	N+	(Ca)A	-	-
	Piperaceae					
75	*Piper nigrum* L.	fruit	D	(Mn)	96	4,571
	Proteaceae					
76	*Heliciopsis terminalis* Sleumer	husk	A	-	42,325	4,222
	Heliciopsis terminalis Sleumer	seed	A	-	137,625	7,634
77	*Macadamia tetraphylla* L.	husk	A	-	529	1,333
	Rhamnaceae					
78	*Zizyphus oenoplia* Mill	fruit	D	-	320	1,939
79	*Zizyphus* sp.	fruit	C	-	80	761
	Rosaceae					
80	*Pyrus communis* L.	fruit	A	-	3,091	920
81	*Pyrus malus* L.	fruit	A	-	80	571
	Rubiaceae					
82	*Coffea arabica* L.	fruit	R	(Ca,Mn)	131,072	21,195
83	*Mitragyna javanica* Koord	fruit	R	(Mn)	160	260
84	*Morinda tomentosa* Heyne ex Roth	fruit	N+	(Ca,Mg,Mn)H	-	-
85	*Randia* sp.	fruit	D	-	448	7,529

		SAMPLE	GROUP	ION	U/g wet wt	U/mg protein
	Sapindaceae					
86	*Nephelium longana* Camb.	seed	A	-	-	-
87	*Nephelium hypoleucum* Kurz	seed	A	-	40	174
88	*Pisum sativa* L.	seed	A	-	1,280	45
	Sapotaceae					
89	*Chrysophyllum cainito* L.	fruit	A	-	145,480	8,143
	Simarubaceae					
90	*Harrisonia perforata* Merri.	fruit	C	-	24,576	33,436
	Staphyleaceae					
91	*Turpinia pomifera* DC.	fruit	A	-	1,536	269
	Theaceae					
92	*Schima wallichii* Korth.	fruit	A	(Mn)	96	507
93	*Schima wallichii* Korth.	seed	N+	(Mn)A	-	-
94	*Thea sinensis* L.	fruit	A	(Ca,Mg,Mn)	112	164
	Thea sinensis L.	seed	C	(Ca,Mn)	7,168	10,138
	Vitidaceae					
95	*Cissus cornosa* Roxb.	fruit	N+	(Ca,Mg,Mn)A	-	-
96	*Muntingia calabura* L.	fruit	A	-	20	71

*Lectins were grouped as described in Materials and Methods which showed positive agglutination to 87 plant samples as compared to human erythrocytes which were agglutinated by 63 different lectins. The present study brings out the occurrence of potent lectin (>100,000 unit/g wet weight) in as many as 4 species, including *Artocarpus lakoocha*, *Heliciopsis terminalis*, *Coffea arabica* and *Chrysophyllum cainito*. In at least two of these species, that is *C. arabica* and *C. cainito*, the lectin activity was not present in the seed but was found in the fruit extract. All of these lectins except *C. arabica* were belong to group A which agglutinated all 8 types of erythrocytes employed in the assay system, whereas *C. arabica* was belongs to R group.

Since plant lectins reported here were characterized upon their hemagglutination activities, it is possible that some plant samples which were inactive on hemagglutination and reported as negative in this study, may contain lectin activity when assayed using other types of cells, such as lymphocytes or spermatozoa. Three isoagglutinins from tubers of *Colocasia esculenta* were demonstrated according to their agglutination activities toward human spermatozoa but not toward erythrocytes[12]. Even agglutination of a variety of erythrocytes is a cheap, simple and convenient method for lectin screening, a disadvantage or pitfall of this method is that it is limited only to the samples which do not cause hemolysis. In the present study, 20 plant species were excluded from the screening program because of their hemolytic activities. However, even the latex agglutination test has been developed to overcome the problem by conjugating various glycoproteins and sugars individually to the latex particles[13], the technique is highly expensive and a limited number of glycoproteins and sugars can be used for screening in comparison to the erythrocyte screening procedure.

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บทคัดย่อ

จากการศึกษาเลกตินจากตัวอย่างพืชส่วนเมล็ด ฝักและผล ในพืช 178 ชนิด 62 วงศ์ สามารถจำแนกเลกตินจากพืชได้เป็น 6 กลุ่มตามคุณสมบัติการทำให้เกิดการเกาะกลุ่มของเม็ดเลือดแดงชนิดต่างๆ (เม็ดเลือดแดงของมนุษย์กลุ่มเอ, บีและโอ, หนูแฮมสเตอร์, หนูถีบจักร, หนูขาว ห่านและนกพิราบ) พบเลกตินในพืช 107 ตัวอย่าง ในจำนวนนี้ 19 ตัวอย่างเป็นเลกตินที่แสดงคุณสมบัติเฉพาะเมื่อมีอิออนชนิด divalent ร่วมในปฏิกิริยา การศึกษานี้ไม่พบเลกตินที่มีความจำเพาะต่อชนิดของเม็ดเลือดแดงชนิดใดชนิดหนึ่งโดยเฉพาะ ยกเว้นเลกตินจากเมล็ดทองกวาว (*Butea monosperma*) ซึ่งแสดงความจำเพาะต่อเม็ดเลือดแดงของมนุษย์เท่านั้น นอกจากนี้ ได้พบพืชที่มีเลกตินในปริมาณสูงมากกว่า 100,000 หน่วยต่อน้ำหนักสด 1 กรัมถึง 4 ชนิด คือ ส่วนเมล็ดของมะหาด (*Artocarpus lakoocha*) และเหมือดคน (*Heliciopsis terminalis*), และในส่วนผลของกาแฟ (*Coffea arabica*) และ star apple (*Chrysophyllum cainito*)