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A STUDY OF BLOOD CELL AGGLUTININS IN PLANT EXTRACTS

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Introduction

The fact that certain plant extracts agglutinate blood cells has been known since the last century but the first to demonstrate that some possessed blood group specificity was Renkonen (1948) who tested 99 seed extracts and found 6 which selectively agglutinated some of the blood samples tested. On further study of 4 of the 6 he found that one, *Vicia craccia*, agglutinated A_1 and A_1B cells much more strongly and to a higher titre than the cells of other groups, thus indicating anti-A_1 specificity. The remainder showed anti-H specificity.

Since then, the plant kingdom has yielded a number of blood grouping reagents of which the most useful are an anti-A_1 reagent from *Dolichos biflorus*, anti-H reagents from several species of which *Ulex europeus* is the most important, anti-N reagents from *Vicia graminea* and from several *Bauhinia* species, and an anti-M reagent from *Iberus amara*. Reagents obtained from these species have largely replaced the equivalent anti-sera of animal origin for general use.

The present position of plant agglutinins in blood group serology has been ably presented in two outstanding monographs. Krüpe (1956) covered all aspects of the subject, while Mäkelä (1957) presented the results of his exhaustive studies of 1408 seed samples representing 743 Leguminosae species. Chemical and immunochemical aspects of the subject have been reviewed by Morgan and Watkins (1953) and by Boyd (1960), as well as by Krüpe.

The investigations reported here were undertaken to determine whether any useful blood grouping reagents could be obtained from the extracts of seeds available locally.

Materials and Methods

Since, with a few exceptions, useful lectins have been obtained almost entirely from among the Leguminosae, the present series of tests was confined to that family. The seeds were obtained from the National Herbarium, Melbourne. They were of varying ages and not all of them had been grown in Australia. Many of the species had been tested by other workers overseas but, for reasons to be discussed later, it was decided to test all the specimens supplied.

Preparation of Extracts

After pulverizing the seeds in a pepper mill, 10 times the quantity of 0·85 per cent saline (w/v) was added and, after mixing, the extracts were incubated for two hours at 37°C with occasional shaking. The extract was then centrifuged to obtain a clear supernatant. As far as possible all the initial tests were performed on the day the extract was prepared and, after that, the extract was stored at —20°C.

Testing Routine

As a preliminary survey each extract was tested with cells of A_1, A_2, B, and O groups, as well as cells of known M and N groups. Each extract was tested with these cells using saline, albumen, and papain techniques. Any extract which showed

promising agglutination by one or more of these techniques was tested for specificity with a panel of the appropriate cells using whatever techniques were applicable. Titrations were also carried out against the cell panel.

Saline Agglutinating Tests

The saline agglutinating tests were carried out in tubes using 2 per cent suspensions of the cells in physiological saline. In performing these tests one drop of the extract was placed in the tube and one drop of the cell suspension added. The tube was allowed to stand at room temperature for 30 minutes and then centrifuged at 1500 r.p.m. for one minute. Following this, it was tapped gently with the finger to resuspend the cells and then the result was read.

Albumen Agglutinating Test

The albumen agglutinating tests were performed in tubes in the same manner as the saline agglutinating tests except that a 5 per cent suspension of cells in 30 per cent bovine albumen (Commonwealth Serum Laboratories) was used instead of a 2 per cent suspension of saline.

Papain Test

In performing the papain test the two-stage slide technique of Albrey and Simmons (1960) was used. This is probably the most sensitive of the enzyme methods.

Results

The 150 seed species tested are listed in Table 1. From the preliminary tests on these extracts only two were found to show blood group specificity. *Bauhinia candicans* and *Bauhinia petersiana* both showed anti-N specificity but the former was unstable on storage and there was insufficient available for further testing, so subsequent investigations were restricted to *Bauhinia petersiana*. In Table 2 the consolidated results of a number of quantitative experiments on that extract are presented.

A number of important points emerged from the investigation of *Bauhinia petersiana*. In the preliminary tests the use of cells suspended in albumen appeared to give specific results but, on further investigation, non-specific results were often obtained with cells suspended in this medium. No time of incubation and no particular dilution were found to be reliable in overcoming these non-specific effects. This finding is in accordance with the experience of other laboratories with anti-N reagent derived from *Bauhinia* species.

In some of the experiments the saline suspension medium used was Rous and Turner solution, a glucose-citrate solution extensively employed in blood group serology, but the results when it was used in this series were unreliable. Although N cells gave satisfactory agglutination in titres of from 32 to 128, the agglutinations with MN cells were unsatisfactory and the results were not clear cut. When, however, the cells were suspended in sodium chloride solution satisfactory, clear cut agglutinations were obtained with both MN and N cells in titres from 32 to 128. It is probable that it is the presence of either the glucose or the citrate in the Rous and Turner solution that has the inhibitory effect on the reaction. As a practical point, if a specimen of blood is received already in Rous and Turner solution, satisfactory results are obtained if the cells are centrifuged and the supernatant Rous and Turner solution removed and replaced by sodium chloride solution. The concentration of sodium chloride is not critical because cells suspended in sodium chloride solutions of from 0·85 to 1·5 gave practically identical titration scores. The thermal range

had little effect on reactions for the scores showed no significant variation when the cells were tested at 0°C, 20°C, and 37°C.

Several of the seed extracts in the series gave pan-agglutination with a number of cell samples of Groups A_1, A_2, B, and O suspended in saline. In Table 3, the results of titrating these seed extracts with the various cell suspensions have been set out. It can be seen that none of them gave any significant variation in their titre score according to the cell suspension used, indicating that they had no blood group specificity.

As found in other series, notably that of Mäkelä (1957), *Crotolaria striata* showed A + B specificity. That is to say it agglutinated groups containing both the A and the B antigen.

A number of extracts gave pan-agglutination when tested with papain treated cells of Groups A_1, A_2, B, and O. Many of these reactions were weak or doubtful and, on the whole, one gained the impression that the use of papain by this technique was not of great practical assistance in these studies. The results of the tests on these papain phytagglutinins are set out in Table 4.

Summary

Extracts from seeds of 150 Leguminosae species obtained from the National Herbarium, Melbourne, were tested against a panel of human erythrocytes in an attempt to discover seeds available locally with blood group specificity.

A number of extracts agglutinated saline suspensions of all cells tested, while others agglutinated all papain-treated cells.

Bauhinia petersiana gave strong agglutination with N and MN cells suspended in saline but failed to agglutinate M cells. Thus it showed anti-N specificity.

Acknowledgement

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TABLE 1

List of species tested for Lectins

1 *Acacia accuminata*
2 *A. acinacea*
3 *A. aneura*
4 *A. arabica*
5 *A. armata*
6 *A. baileyana*
7 *A. bidentata*
8 *A. brachybotrya*
9 *A. bouritii*
10 *A. buxifolia*
11 *A. cognata*
12 *A. cyclopis*
13 *A. decurrens*
14 *A. denticulosa*
15 *A. dietrichiana*
16 *A. difformis*
17 *A. diffusa*
18 *A. drummondii*
19 *A. dunnii*
20 *A. elata*
21 *A. elongata*
22 *A. falciformis*
23 *A. farnesiana*
24 *A. fimbriata*
25 *A. glaucescens*
26 *A. gilberti*
27 *A. horrida*
28 *A. implexa*
29 *A. iteaphylla*
30 *A. juniperina*
31 *A. lasiocalyx*
32 *A. ligulata*
33 *A. lincata*
34 *A. longifolia*
35 *A. longissima*
36 *A. macrodenia*
37 *A. maidenii*
38 *A. meissnerii*
39 *A. melanoxylon*
40 *A. microbotria*
41 *A. myrtifolia*
42 *A. normalis*
43 *A. notabilis*
44 *A. obtusa*
45 *A. oxycedrus*
46 *A. oswaldii*
47 *A. pendula*
48 *A. pentadenia*
49 *A. podalyrialfolia*
50 *A. pruinosa*
51 *A. pubescens*
52 *A. pycnantha*
53 *A. restiacea*
54 *A. rhetinodes*
55 *A. rigens*
56 *A. rivalis*
57 *A. rossei*
58 *A. rubida*
59 *A. rupicola*
60 *A. saligna*
61 *A. suaveolens*
62 *A. scarpioides*
63 *A. signata*
64 *A. sophorae*
65 *A. spectabilis*
66 *A. stricta*
67 *A. subporosa*
68 *A. terminalis*
69 *A. trineura*
70 *A. verniciflua*
71 *A. verticillata*
72 *A. vestita*
73 *A. victoriae*
74 *A. visco*
75 *A. wattsiana*
76 *Albizzia fastigiata*
77 *A. lophantha*
78 *A. odoratissima*
79 *Amorpha fructicosa*
80 *Baphia racemosa*
81 *Baptisia australis*
82 *Bauhinia candicans*
83 *B. petersiana*
84 *Bonjeania hirsuta*
85 *Bossiaea linophylla*
86 *Burtonia scabra*
87 *B. villosa*
88 *Brachysema lanceolatum*
89 *B. subcordatum*
90 *Caesalpinia gillicsii*
91 *Cassia artemisioides*
92 *C. costata*
93 *C. eremophila*
94 *C. floribunda*
95 *C. laevigata*
96 *C. marykandica*
97 *C. mexicana*
98 *C. nodosa*
99 *C. occidentalis*
100 *C. pleurocarpa*
101 *Carmichaelia aligera*
102 *C. sylvatica*
103 *Cercis siliquastrum*
104 *Chorisema cordata*
105 *Clianthus puniceus*
106 *C. puniceus* var. *albus*
107 *Colutea arborescens*
108 *C. cilicia*
109 *C. gracilis*
110 *C. istrea*
111 *Coronilla coronata*
112 *Cytisus everestianus*
113 *C. laburnum*
114 *C. sessiliflorus*
115 *C. supinus*
116 *C. triflorus*
117 *Crotolaria striata*
118 *Dorycnium rectum*
119 *Erythrina acanthocarpa*
120 *E. arborescens*
121 *E. caffra*
122 *E. crista-galli*
123 *E. humeana*
124 *Genista aethnensis*
125 *Gleditschia caspica*
126 *G. inermis*
127 *G. sinensis*
128 *Hardenbergia comptonia*
129 *H. monophylla*
130 *Indigofera cytisoides*
131 *Kennedya prostrata*
132 *Laburnum anagyroides* var. *alschingeri*
133 *Lathyrus venosus*
134 *Lupinus polyphyllus*
135 *Mimosa acanthocarpa*
136 *M. bahamensis*
137 *Phaseolus aureus*
138 *Priestlya hirsuta*
139 *Psoralea dentata*
140 *Robinia pseudoacacia* var. *unifolia*
141 *R. holdtii*
142 *Sesbania emerus*
143 *S. grandiflora*
144 *S. tripetii*
145 *Sophora occidentalis*
146 *S. prostrata*
147 *Swainsonia galegifolia* var. *violacea*
148 *Parkinsonia aculeata*
149 *Virgilia divaricata*
150 *Thermopsis montana*

TABLE 2

Reactions of Bauhinia petersiana

Cell suspension	Titre								
	4	8	16	32	64	128	256	512	1024
M cells (M.V.) in saline	—	—	—	—	—	—	—	—	—
MN cells (G.H.) in saline	4	4	3	3	2	—	—	—	—
MN cells in Rous & Turner solution	1	1	—	—	—	—	—	—	—
N cells (U.T.) in saline**	4	4	3	3	2	2	—	—	—
N cells in Rous & Turner solution	2	2	2	1	—	—	—	—	—
N cells in saline after removal of Rous & Turner solution	4	4	4	2	2	—	—	—	—

**Essentially the same results were obtained when N cells were suspended in 0·85%, 1·0%, 1·2%, or 1·5% saline, and when 0·85% saline was tested at 37°C, 20°C, or 4°C.

TABLE 3

Titration of Saline Pan-agglutinins

Species	Cells	Titre								
		4	8	16	32	64	128	256	512	1024
Baphia racemosa	A_1	3	1	1	—	—	—	—	—	—
	A_2	3	1	1	—	—	—	—	—	—
	B	3	1	—	—	—	—	—	—	—
	O	3	2	—	—	—	—	—	—	—
Cytisus triflorus	A_1	3	1	±	—	—	—	—	—	—
	A_2	3	3	±	±	—	—	—	—	—
	B	3	2	2	—	—	—	—	—	—
	O	3	3	—	—	—	—	—	—	—
Cytisus laburnum	A_1	3	3	—	—	—	—	—	—	—
	A_2	3	2	—	—	—	—	—	—	—
	B	3	3	—	—	—	—	—	—	—
	O	4	2	—	—	—	—	—	—	—
Erythrina acanthocarpa	A_1	4	4	4	3	—	—	—	—	—
	A_2	4	4	4	4	3	—	—	—	—
	B	4	4	4	4	2	—	—	—	—
	O	4	4	4	±	—	—	—	—	—
Erythrina arborescens	A_1	3	3	3	—	—	—	—	—	—
	A_2	3	3	1	—	—	—	—	—	—
	B	3	3	3	—	—	—	—	—	—
	O	4	3	3	—	—	—	—	—	—
Erythrina caffra	A_1	4	3	3	3	3	—	—	—	—
	A_2	4	4	4	4	3	3	1	1	—
	B	4	4	4	4	3	—	—	—	—
	O	4	4	3	3	3	3	—	—	—
Erythrina crista-galli	A_1	3	3	±	—	—	—	—	—	—
	A_2	4	4	2	—	—	—	—	—	—
	B	4	3	2	—	—	—	—	—	—
	O	4	3	3	—	—	—	—	—	—
Erythrina humeana	A_1	4	3	3	3	3	—	—	—	—
	A_2	4	4	3	3	2	2	—	—	—
	B	4	4	3	3	2	2	—	—	—
	O	4	4	4	4	3	3	—	—	—
Genista aethnensis	A_1	4	4	4	3	2	±	—	—	—
	A_2	4	4	4	3	1	±	—	—	—
	B	4	4	4	3	3	±	—	—	—
	O	4	4	4	3	3	—	—	—	—
Lathyrus venosus	A_1	4	4	3	2	—	—	—	—	—
	A_2	4	4	3	—	—	—	—	—	—
	B	4	4	3	1	—	—	—	—	—
	O	4	4	3	2	—	—	—	—	—
Phaseolus aureus	A_1	4	4	1	—	—	—	—	—	—
	A_2	4	4	1	—	—	—	—	—	—
	B	4	3	1	—	—	—	—	—	—
	O	4	3	—	—	—	—	—	—	—
Robinia pseudoacacia	A_1	4	4	2	1	—	—	—	—	—
	A_2	4	4	1	—	—	—	—	—	—
	B	4	4	2	1	—	—	—	—	—
	O	4	4	1	—	—	—	—	—	—
Robinia holdtii	A_1	4	4	4	3	2	2	—	—	—
	A_2	4	4	4	3	2	1	—	—	—
	B	4	4	4	3	2	2	—	—	—
	O	4	4	4	3	3	1	—	—	—

TABLE 4

Titration of Papian Pan-agglutinins

Species	Cells	Titre							
		4	8	16	32	64	128	256	512
Acacia cognata	A_1	+	+	+	+	—	—	—	—
	A_2	+	+	+	+	—	—	—	—
	B	+	+	+	+	—	—	—	—
	O	+	+	+	+	—	—	—	—
Cassia eremophila	A_1	+	+	—	—	—	—	—	—
	A_2	+	+	—	—	—	—	—	—
	B	+	+	—	—	—	—	—	—
	O	+	+	+	—	—	—	—	—
Cassia nodosa	A_1	+	+	—	—	—	—	—	—
	A_2	+	+	—	—	—	—	—	—
	B	+	+	—	—	—	—	—	—
	O	+	+	+	+	—	—	—	—
Cercis siliquastrum	A_1	+	+	+	+	—	—	—	—
	A_2	+	+	+	+	—	—	—	—
	B	+	+	+	+	—	—	—	—
	O	+	+	+	+	—	—	—	—
Dorycnium rectum	A_1	+	+	+	+	+	—	—	—
	A_2	+	+	+	+	+	—	—	—
	B	+	+	+	+	+	—	—	—
	O	+	+	+	+	+	—	—	—
Gleditschia sinensis	A_1	+	—	—	—	—	—	—	—
	A_2	+	+	—	—	—	—	—	—
	B	+	—	—	—	—	—	—	—
	O	+	+	+	—	—	—	—	—
Virgilia divaricata	A_1	+	—	—	—	—	—	—	—
	A_2	±	—	—	—	—	—	—	—
	B	±	—	—	—	—	—	—	—
	O	+	—	—	—	—	—	—	—