

**APPLICATION OF LECTINS IN FORENSIC SEROLOGY:
A Simple Practical Method to Establish Source of Blood.**

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INTRODUCTION

The knowledge that extracts of some plants agglutinate the erythrocytes of human and other animal species selectively, goes back to almost a century ago (Stillmark 1888). However it was not until Bird (1954) suggested that lectins might serve to distinguish the erythrocytes of different animal species, that their utility in forensic examination of blood was recognized. Using extracts from four plant species (Ricinus communis, Dolichos lab lab, Vicia faba, and Phaseolus lunatus), Bird was also able to demonstrate that ten animal species could be clustered under only four groups. The pioneering efforts of Bird and other early workers provided useful leads in establishing the identity of blood, on the basis of differential agglutination reactions with lectins. A number of studies have been reported during the last three decades, reporting lectins which can be useful in distinguishing the blood of different animal species (Makela 1957; Sathe et. al., 1970; Arora and Sengupta 1976; Bhalla et al. 1979; Mastana 1983; Roy and Bhalla 1981).

The purpose of this paper is to present an overview of our investigations of serological activity of plant extracts with the red cells of a number of vertebrate species, and to demonstrate the use of plant agglutinins in establishing the source species of blood.

MATERIAL AND METHODS

In the present study 120 floral extracts of different plant parts such as fruits, stemtubers, leaves and roots have been investigated against red cell suspension of 10 mammalian species viz., man, monkey, rabbit, rat, guinea pig, horse, cow, buffalo, sheep and goat. Plant extracts were prepared in isotonic phosphate buffer saline, pH 7.4, by method described by Moore et. al., (1972). Sodium azide (0.01) was added as preservative and the extracts were stored in deep freezer at -20 C. The agglutinating activity of the extracts was tested within one month of the preparation with a large number of red cell samples from each species. The red cells from different sources were obtained from animal houses and hospitals. The hemagglutinating assay was carried out with saline suspended red cells and papain treated red cells in phosphate buffer saline using standard serological techniques.

RESULTS AND DISCUSSION

In all 29 lectins showed non-specific or species specific or mono specific serological activity with the blood samples of 10 mammalian species (Table I). As evident from the results in Table I. the origin of blood of man, rabbit, guinea pig, rat, cow and sheep can be achieved by employing species specific hemagglutinins. Red cells of monkey, buffalo, horse and goat can be differentiated by using a combination of lectins which react in an assortative manner, i.e. using one or two lectins to differentiate a species (Table II).

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Table I. Serological Activity of Plant Extracts against Mammalian Red Cells

Name of the Plant	Man	Monkey	G.Pig	Rat	Rabbit	Sheep	Goat	Horse	Buffalo	Cow	Total
<i>Lycoperscium esculentum</i>	4+	4+	4+	4+	4+	3+	3+	4+	4+	4+	10
<i>Solanum tuberosum</i>	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	10
<i>Coccinia indica</i>	3+	3+	4+	3+	3+	3+	3+	3+	3+	3+	10
<i>Brassica oleracea</i>	ws+	1+	1+	-	1+	1+	1+	-	-	1+	7
<i>Citrus aurantifolia</i>	2+	H	2+	2+	H	2+	H	-	2+	2+	6
<i>Citrus festuosus</i>	2+	-	2+	-	2+	2+	2+	2+	-	-	6
<i>Antigonon leptopus</i>	-	-	1+	1+	-	-	H	1+	1+	1+	5
<i>Tamarindus indicum</i>	-(1+)	-(1+)	-	1+	H	-(1+)	-(1+)	-	-	-	4
<i>Lagerstomia indica</i>	2+	-(1+)	1+	1+	H	-	-	-	-	-	4
<i>Achras sapota</i>	2+	1+	1+	1+	-	-	-	-	-	-	4
<i>Delinia indica</i>	1+	H	1+	1+	-	-	-	-	-	-	3
<i>Crotolaria medicaginia</i>	ws+	ws+	1+	H	H	-	H	H	-	-	3
<i>Adhotoda vesica</i>	2+	-	2+	2+	-	-	-	-	-	-	3
<i>Floscopa scans</i>	-	-	-	-	-	-	-	2+	2+	2+	3
<i>Tinospora cardifolia</i>	-	-	-	1+	1+	-	-	-	-	-	2
<i>Pterosperium acerifolium</i>	4+	4+	-	-	-	-	-	-	-	-	2
<i>Emblia officinalis</i>	1+	H	-	1+	-	-	-	-	-	-	2
<i>Rauwolfia caulescens</i>	-	-	-	2+	-	-	-	-	-	2+	2
<i>Catharanthus roseus</i>	-	1+	-	-	1+	-	-	-	-	-	2
<i>Jacaranda mimosaefolia</i>	1+	-	-	-	-	-	-	-	-	-	1
<i>Arisaema specicosum</i>	-	-	-	H	1+	-	-	-	-	-	1
<i>Anthocephalus indicus</i>	-	-	-	3+	-	-	-	-	-	-	1
<i>Alocasia indicum</i>	-	-	-	-	1+	-	-	-	-	-	1
<i>Allium sativum</i>	-	-	-	-	1+	-	-	-	-	-	1
<i>Lantana indica</i>	-	-	2+	-	-	-	-	-	-	-	1
<i>Luffa acutangula</i>	-	-	-	-	1+	-	-	-	-	-	1
<i>Sechium edule</i>	1+	-	-	-	-	-	-	-	-	-	1
<i>Raphanus sativus</i>	-	-	-	-	-	(1+)	-	-	-	-	1
<i>Mimosa juliflora</i>	-	-	-	-	-	-	-	-	-	-	1
Total Number of Lectins Showing positive reaction	16	10	13	13	11	8	6	6	6	9	

+ indicates haemagglutination in Saline;
(+) indicates activity in papain medium;
H indicates haemolysis in saline.
Strength of reactions
2+ - 4+ Strong reaction
w+ - 1+ weak reaction
ws+ Variable strength weak to strong.

Table II. Lectins reacting in assortative manner.

Name of the Plant	Man	Monkey	Horse	Buffalo	Goat
<i>Ptersperimum acerifolium</i>	+	+	-	-	-
<i>Citruslus festulosus</i>	+	-	+	-	+
<i>Citrus aurantifolia</i>	+	-	-	+	-
<i>Brassica oleracea</i>	+	+	-	-	+
<i>Antigonon leptopus</i>	-	-	+	+	-

Table III. Serological activities of Lectins against Human Cord Red Cells.

Name of the Plant	Cord Red Cells	Adult Human Red Cells
<i>Lycopersicum esculentum</i>	1+	4+
<i>Delinia indica</i>	3+	1+
<i>Jacaranda mimosaeefolia</i>	-	1+
<i>Anthocephalus indicus</i>	3+	-
<i>Emblica officinalis</i>	-	1+
<i>Sechium edule</i>	H	1+

Table III depicts the comparative hemagglutinating activity of 19 floral extracts against human adult and cord red blood cells. The fruit pulp extract of Anthocephalus indicus showed positive specific hemagglutination reaction with cord red cells i.e. it gave a strong positive reaction with cord red cells and failed to react with human red cells. Three lectins Jacaranda mimosaeifolia, Crotolaria mediciginea and Embllica officinalis showed negative specific reactions with cord red cells with respect to human red cells.

The results of the present study suggest that all the 10 mammalian red cells tested in the present study can be distinguished using either exclusive species specific hemagglutinins or a combination of hemagglutinins which react in an assortative manner. The fact that these lectins show species specific reactions with some species and not with the others tested does not rule out the possibility that they will also not react with the red cells of species not tested. The present study also envisages that there are some differences as observed by hemagglutination by lectins on the red cell membrane between human adult and cord red cells. This informatin can be utilized for differentiation of human foetal blood from the adult blood in forensic serology. It goes without saying that a prior standardization of lectins specificities is essential before these could be of practical value in forensic examination of blood. A better understanding of the use of lectins in such investigations can be achieved through extensive screening studies carried out under conditions in which test materials are generally found at the site of crime.

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