Studies on Lectin mediated agglutination reaction on RBC surface antigens using hot and cold water plant extracts

Comment [u1]: First letters of the title should be capitalized and RBC should be in full; Studies on Lectin Mediated Agglutination on Red Blood Cell (RBC) Surface Antigen using Hot and Cold Water Plant Extract

ABSTRACT

Previous research studies on lectins drew a lot of attention because of their various physiological roles in cell agglutination. Based on their carbohydrate-binding properties, plant lectins are widely used for the detection, segregation, and characterization of glycoconjugates. Rhesus (Rh) factor is a protein that is inherited and found on the surface of red blood cells. If the surface protein is present, the RBC is Rh positive; otherwise, it is Rh-negative in nature. In this paper, we use agglutination reactions to investigate the effect of different cold and hot water extracted plants on RBC antigens as an alternative to commercial monoclonal antibodies. Extensive research on the sequence homology and 3-D structure of various plant lectins suggests that they have been conserved throughout evolution and may play important physiological roles that are still unknown.

Comment [u2]: Remove and start with: Lectin has various physiological roles in cell agglutination

Comment [u3]: Rh - positive

Comment [u4]: Your abstract was not properly stated; the abstract should capture the following: title or part of the title, brief on the materials and methods, summary of the results/findings, conclusion(s) and recommendation(s)

Keywords: Rh factor, Agglutination reaction, RBC antigen, Plant Lectins, Antigen, Monoclonal antibody.

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INTRODUCTION

Plant extracts contain lectins, which are carbohydrate-binding proteins found in the seeds of many plants, particularly corals and beans, as well as fungi, bacteria, and animals [1]. Aside from their hemagglutinating ability, they have been assigned a variety of functions and significance in immunohematology is enormous [2]. They are used to detect specific red cell antigens, to activate various types of lymphocytes, and to resolve poly agglutination issues [3]. Lectins are powerful tools for recognizing a diverse range of oligosaccharides, and they have been widely used in many fields of cell biology, biochemistry, and food technology [4-8]. These are glycoprotein domains with highly specific pockets for their counter sugar moieties of polysaccharides, glycolipids, glycoproteins, proteoglycans, and peptidoglycans found on the exterior walls or membranes of both vertebrate and invertebrate cells and microorganisms [9]. Lectins participate in biological recognition phenomena in cell-to-cell contact of all living organisms, such as binding of microorganisms to target tissues, protein sorting, control of morphogenesis, cellular differentiation, fertilisation, adhesion and

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trafficking of leukocytes, metastasis, and inhibition of natural killer cell activity against healthy cells [10-12]. To determine their suitability for consumption, different plant lectins were individually tested for lectin binding activity on the ABO blood group system [13]. 45 percent of the plants tested positive for blood group agglutination against the A, B, AB, and O groups. Blood samples from blood types A+, A-, B+, O+, O-, AB+, and AB- were collected and tested for the effect of different cold and hot water extracted plants on RBC antigens using agglutination reactions as an alternative to commercial monoclonal antibodies [14].

MATERIALS & METHODS

Materials

Leaf samples, distilled water, Eppendorf tubes, tissue grinders, centrifuge, anticoagulant, micro-pipettes, micro tips, glassware, Blood samples of blood types A+, A-, B+, O+, O-, AB+, and AB- from volunteers.

Preparation of extracts

Leaf samples of 35 different plants were collected and made into smoothies [15]. 50 ug of leaf sample, 1ml of hot and 1ml of cold water were added respectively and the extract was prepared using a tissue grinder. The prepared extracts were centrifuged and the supernatant was separated and stored separately.

Agglutination reactions on RBC agents

The blood samples from different individuals having blood groups A+, A-, B+, O+, O-, AB+ and, AB-were collected and stored by adding anticoagulant. The experiment was performed by adding 20ul of blood sample and 20ul of plant extract to the ELISA plates. The ELISA plates were placed in the mixer for 10 minutes and the results were tabulated.

RESULTS AND DISCUSSIONS:

Statistical Analysis

The results of the studies in table 1, were expressed as (P) if the sample shows an agglutination reaction and as (N) if the sample shows no agglutination reaction.

Table 1:

S.No	Blood type	A+	A-	B+	AB+	AB-	O+	O-

Comment [u7]: Add: There are about 500 species of plants where the hemagglutinating lectins have been documented. Of the total number of plants available in the plant kingdom, it can be considered that lectins are present in different plants as an exception rather than the rule. The hemagglutinating activity of lectins is inhibited by simple sugars (monosaccharide), which represent the binding site for the lectin on the cell surface [16]. Certain plant extracts contain more than one form of lectin [17]. Lectins can be inactivated by procedures which denature or break down proteins such as heating, extremes of pH, and treatment with proteolytic enzymes such as papain or trypsin [18].

Comment [u8]: Remove and add to the abstract starting with; different plant lectins were.....

Comment [u9]: State the aim and the objective(s) of the study at the end of the introduction

Comment [u10]: E

Comment [u11]: State the location(s) were they were collected

Comment [u12]: A new heading: Sample Collection which comes first before Preparation of Extract

Comment [u13]: Start with words (Fifty) as the beginning of statement of Preparation of Extracts

Comment [u14]: 1m each of hot and cold water....

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Comment [u18]: 20ul each of blood sample and plant extract...

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Comment [u22]: State the title of the result/table

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	Original Name	CE	HE	CE	HE	CE	HE	CE	HE	CE	HE	CE	HE	CE	HE
1	Banana	N	P	P	N	P	N	P	P	N	N	N	P	P	N
2	Drumstick	P	P	N	N	P	N	P	P	N	P	P	N	N	N
3	black gram	N	P	P	N	P	N	N	P	P	N	N	P	P	N
4	Henna	P	P	P	N	N	P	P	N	P	P	N	P	N	P
5	Night flowering	P	P	N	N	P	N	P	P	N	N	P	P	P	N
6	jasmine	N	P	N	N	P	P	N	N	N	P	P	N	P	N
7	Bottle guard	P	N	P	P	N	N	P	N	N	N	P	P	N	P
8	Holy basil	P	N	P	P	P	N	P	P	P	N	N	P	N	P
9	Basil	P	P	N	N	P	P	N	P	N	P	P	N	P	N
10	Yellow cucumber	N	P	P	P	P	N	N	P	N	P	N	P	P	N
11	Madonna lily	P	N	N	N	N	P	P	N	N	P	P	P	N	P
12	Marigold leaf	N	P	P	P	P	N	P	P	N	P	N	N	P	P
13	Tomato	P	P	N	N	P	P	N	P	P	N	P	N	P	N
14	Snake gourd	P	N	P	P	N	N	P	P	N	P	P	N	P	P
15	Lesser mallow	N	P	P	N	N	P	P	P	P	P	P	N	N	P
16	Ridge gourd	P	P	N	P	N	P	P	N	P	P	N	P	P	N
17	Brinjal	P	P	N	P	N	P	N	N	N	P	P	N	P	N
18	Bitter gourd	P	N	N	P	P	N	P	P	P	N	N	P	P	P
19	Periwinkle	P	P	P	P	N	P	N	N	P	P	N	P	N	P
20	winter jasmine	N	P	P	P	N	N	N	P	P	N	N	P	P	N
21	Aloe Vera	P	N	N	N	P	P	P	N	P	N	N	P	N	N
22	Sodom apple	N	P	P	N	N	P	P	N	P	P	N	N	N	P
23	Guava	P	P	N	P	N	P	N	P	P	P	N	P	P	P
24	Hibiscus	N	P	P	N	N	P	P	P	P	N	P	N	P	N
25	Pomegranate	N	P	P	P	N	P	N	P	P	N	P	P	N	N
26	Neem	P	P	N	N	P	P	N	P	N	P	N	N	P	N
27	Money plant	P	P	N	P	N	P	P	P	P	P	P	N	N	P
28	Soap nut	P	N	N	P	P	N	P	P	N	N	P	P	P	P
29	Rudraksha	P	N	N	N	P	P	N	P	P	N	P	N	P	P
30	Jackfruit	N	P	P	N	N	P	P	N	P	N	P	N	P	P
31	Canon ball flower	N	P	N	P	P	N	N	P	N	N	P	N	P	N

Comment [u24]: B

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32	Black plum	N	P	P	N	N	P	P	N	P	N	P	N	P	N
33	Mango	P	P	N	N	N	P	P	N	P	N	P	P	N	P
34	Custard apple	N	P	P	N	N	P	P	P	N	P	P	N	P	P
35	Butterfly pea	N	N	P	P	N	N	P	P	N	N	P	P	N	P

Comment [u28]: Key: CE –
HE –

Comment [u29]: The table should be on one page

Figure 1. Henna (Lawsonia inermis)

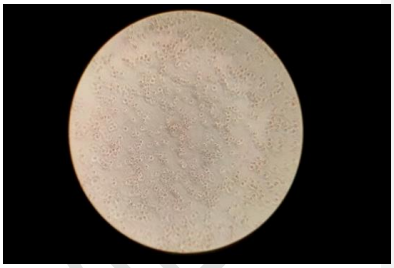

Figure 2. Neem (*Azadirachta indica*)

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Comment [u32]: Plate II

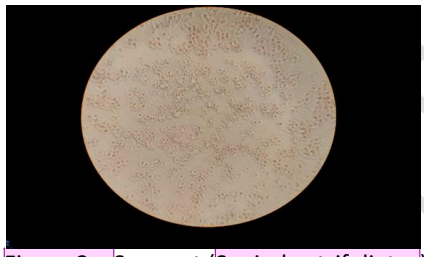

Figure 3. Soapnut (Sapindus trifoliatus)

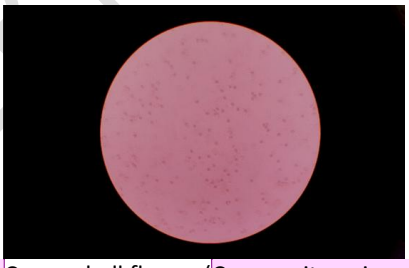

Figure 4. Cannonball flower (Couroupita guianensis)

Comment [u33]: Plate III

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Comment [u35]: Plate IV

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Blood cross-matching was done to verify our results figure 1 shows, Henna (*Lawsonia inermis*) for B+ve blood sample with a cold water sample and O+ve serum sample, are compatible. Figure 2 shows, Neem (*Azadirachta indica*) for AB-ve blood sample with a cold water sample and O+ve serum sample, they are compatible. Figure 3 shows, Soapnut (*Sapindus trifoliatus*)

Comment [u37]: the results.

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Comment [u39]: Plate I

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for the A-ve blood sample with a cold water sample and O+ve serum sample, are incompatible. Figure 4 shows, Cannonball flower (*Couroupita guianensis*) for the B+ve blood sample and the O+ve serum sample, they are compatible.

Comment [u42]: Plate IV

Comment [u43]: You did not properly discuss your results/findings. Why having positives and negatives on the different blood types (reasons) and back up your reasons with other author's findings. State also the reasons of having compatible and incompatible and back up with other people's work. You need to work more on your results and discussions as it is scanty and insignificant. You need to properly interpret your result and discuss it accordingly. Follow these stpes:
-Interpret your results properly
- Discuss it by stating why it is so (according to your interpretation) - Reasons
- Relate your reasons with other author's work

CONCLUSION

It is concluded that most of the plant varieties have shown a positive result for the B+ blood group (31 positive results). Followed by AB+ blood group (27 positive results), 0+ and 0- blood groups (26 positive results), AB- blood group (23 positive results), A- blood group (20 positive results), and A+ blood group (16 positive results).

Comment [u44]: What of the aspect of compatibility and incompatibility?

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Comment [u47]: Were added after the addition of a paragraph in the introduction