PROGRESS IN APPLIED SCIENCE AND TECHNOLOGY
Faculty of Science and Technology
Rajamangala University of Technology Thanyaburi
Prog Appl Sci Tech. 2024; 14(3):22-30
ISSN (Print): 2730-3012
ISSN (Online): 2730-3020
https://ph02.tci-thaijo.org/index.php/past

Research Article

Received: October 16, 2024
Revised: November 13, 2024
Accepted: December 02, 2024

DOI: 10.60101/past.2024.256342

Thai Vegetable Extracts Affecting Human Red Blood Cell Surface Antigens in the ABO Blood Group System

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Abstract

Plant proteins capable of agglutinating human red blood cells (RBCs) extend beyond traditional lectins to include ribosome-inactivating proteins (RIPs), antimicrobial peptides (AMPs), and various defense proteins. While these proteins have been extensively studied in legumes, their presence and activity in common vegetables remain largely unexplored. Understanding their distribution and specificity could reveal novel tools for glycobiology and human ABO blood typing applications. This study aimed to evaluate the agglutination activity of nine Thai vegetable extracts against human red blood cells, determine their ABO blood group specificity, and assess the correlation between protein content and agglutination strength. Additionally, we sought to identify potential plant proteins contributing to the observed agglutination patterns. In this study, crude protein extracts were prepared from nine different vegetables using phosphate-buffered saline (PBS) with pH 7.4. Total protein concentration was quantified using the Biuret method. Agglutination assays were performed using standardized human RBC suspensions from different blood groups. The specificity and strength of agglutination were evaluated using serial dilutions under controlled conditions. Among the nine extracts tested, five demonstrated significant agglutinating activity with varying specificities. Three extracts (*L. polychrous* mushroom, mulberry leaf, and winged bean) showed non-specific agglutination across all blood groups, suggesting the presence of broadly reactive proteins. Notably, *D. biflorus* seed and lima bean extracts exhibited specific agglutination against blood group A, indicating potential blood-type-specific proteins. Four extracts (*C. serratum* leaf, cucumber, *L. leucocephala* seed and long bean) showed no detectable agglutination activity. This study reveals previously unreported agglutinating activities in common vegetable extracts, with some showing blood group specificity. The diverse agglutination patterns observed suggest the presence of both lectin and non-lectin proteins with distinct binding properties. While these findings indicate potential applications in ABO blood typing and glycobiology research, further protein characterization and improved quantification methods are needed. Future studies should focus on isolating and identifying the specific proteins or lectin responsible for the observed agglutination patterns.

Keywords: Plant Proteins, Agglutination, Blood Group, Vegetable Extracts.

1. Introduction

Thai vegetables are a rich source of nutrients and bioactive compounds that offer various health benefits. Beyond their role as staple ingredients in daily diets, some vegetables possess the potential to influence physiological systems, including the blood and immune systems (1). One particularly intriguing property is the ability of certain plant extracts to interact with red blood cells via the carbohydrate structures on the surface of these cells, specifically within the ABO blood group system. This system, crucial for blood transfusion and immune reactions, categorizes blood into types A, B, O and AB based on the presence or absence of antigens (A and B) on the surface of red blood cells (2). Individuals with type O blood have red blood cells (RBCs) that lack both A and B antigens. In contrast, individuals with type AB blood have RBCs that display both A and B antigens. N-acetylgalactosamine is the immunodominant sugar responsible for A specificity, while D-galactose is the corresponding sugar for B specificity. The genetic control of A and B antigens is located on chromosome 9. The A allele produces N-acetylgalactosaminyl -transferase (A-transferase), which transfers N-acetylgalactosamine to an oligosaccharide chain (3). Meanwhile, the B allele produces D-galactosyltransferase (B-transferase), which transfers D-galactose to an oligosaccharide chain.

Recent discoveries have shown that some plant extracts contain proteins capable of inducing red blood cell agglutination. These extracts vary in their properties, with some plants exhibiting antioxidant, anti-inflammatory, or immune-modulating effects, which may influence the clustering of antigens on red blood cell surfaces. Such characteristics hold promise for medical research and potential applications in the prevention and treatment of blood and immune-related disorders (4).

However, excessive consumption of these vegetables can sometimes cause adverse effects, such as bloating, nausea, diarrhea and vomiting. It has been suggested that cases of presumed bacterial food poisoning might, in fact, be due to the ingestion of certain plant compounds (5). Previous studies have focused the direct impact of specific plant extracts on red blood cells, either by inhibiting or inducing agglutination, depending on the blood type. These effects are particularly noted in legumes and raw grains (6-8). Additionally, some common plants in Thailand have been studied for their plant proteins that can cause cell agglutination (9). This observation led the researchers to explore nine vegetable species found in Nakhon Ratchasima province, Thailand, to determine if their extracts contain proteins that affect the agglutination of human red blood cells across all ABO blood types. Preliminary studies on these extracts have not yet identified their hemagglutination properties, despite the vegetables being commonly available in local markets. This study aimed to evaluate the agglutination activity of nine Thai vegetable extracts against human red blood cells, determine their ABO blood group specificity, and assess the correlation between protein content and agglutination strength, offering potential applications in medicine and future business development.

2. Materials and methods

2.1 Vegetable samples

The samples consisted of 9 types of vegetables commonly found in Nakhon Ratchasima, Northeastern Thailand and other regions of the country, including: *Clerodendrum serratum* leaf, cucumber (*Cucumis sativus*) fruit, *Dolichos biflorus* seed, *Lentinus polychrous* mushroom, *Leucaena leucocephala* seed, lima bean (*Phaseolus lunatus*), long bean (*Vigna unguiculata*), mulberry (*Morus alba*) leaf and winged bean (*Psophocarpus tetragonolobus*) were collected freshly from markets in Nakhon Ratchasima, Thailand.

2.2 Preparation of vegetable extracts

Each of the nine vegetable samples was carefully cleaned and air-dried. To prepare the extracts, specific parts of each vegetable known to contain potential bioactive compounds were selected, rather than using the whole plant, to control for variations in secondary metabolite levels that could influence the results. Each selected sample part including seeds of *C. serratum*, *D. biflorus*, *L. leucocephala*, lima bean, long bean and winged bean, leaves of mulberry, fruits of cucumber, and fruiting bodies of *L. polychrous* mushroom were ground individually, adding phosphate-buffered saline

(PBS) in a 1:1 ratio (10 g of vegetable to 10 mL PBS). Filter the ground vegetable through a cloth or fine sieve to remove solid residues. Dilute the filtrate by adding PBS 1X (working solution) in a 1:1 ratio. Centrifuge the diluted solution at 4000 rpm for 10 minutes to remove fine debris. Centrifuge the supernatant again at 4000 rpm for 20 minutes, then store the clarified extract at 4°C for further use.

2.3 Standard red blood cells

Commercially prepared red blood cells (A-cells, B-cells, and O-cells) were obtained from the Thai Red Cross Society for use in this study. These standard red blood cells, with known ABO antigen profiles, were utilized to assess the hemagglutination properties of Thai vegetable extracts. The use of these standardized cells ensures consistency and reliability in determining whether the extracts exhibit specific or nonspecific reactivity toward particular blood group antigens, or if no reaction occurs. By employing high-quality, pre-characterized cells from an accredited source, we minimized variability and ensured that the experimental outcomes were robust and reproducible for advanced research applications.

2.4 Testing the agglutination reaction

Preliminary agglutination test to determine whether the vegetable extracts induce agglutination in human red blood cells: Prepare three test tubes (12x75 mm) for each blood type (A, B and O) for a total of 27 tubes. Label the tubes according to the blood type and vegetable extract. Perform the agglutination test using two drops of each vegetable extract and one drop of 3% standard red blood cell (RBC) suspension were obtained from the Thai Red Cross Society. Centrifuge at 3000 rpm for 15 seconds, then read and record the results. Record the results of the vegetable extracts that induce agglutination for further testing.

2.5 Agglutination strength test

To evaluate the agglutination strength of vegetable extracts that demonstrated positive reactions, a two-fold serial dilution method was employed. For each vegetable extract and blood type (A, B, and O), 10 test tubes (12 × 75 mm) were prepared and appropriately labeled. Serial dilutions of the vegetable extracts were conducted in these tubes. A 3% red blood cell (RBC) suspension was prepared for each blood type. One drop of the suspension was added to each tube containing the diluted extracts. The tubes were centrifuged at 3000 rpm for 15 seconds to promote agglutination. The agglutination strength was then recorded and graded according to macroscopic observations. The interpretation of agglutination reactions is summarized in Figure 1 and Table 1, which detail the grading criteria used in the study.

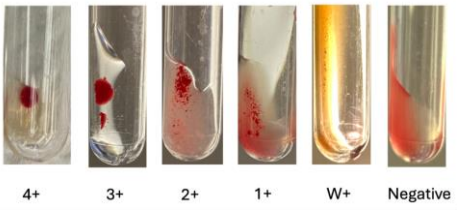

Figure 1 Interpretation of agglutination reaction

Table 1 Description of interpretation of agglutination reaction

Macroscopically findings	Description
One solid agglutinate	4+
Several large agglutinate	3+
Medium-size agglutinate, clear background	2+
Small agglutinate, turbid background	1+
Barely visible agglutination	w+ or +/-
No agglutination	0

2.6 Protein concentrations

The protein concentrations of the vegetable extracts were determined using the Biuret method, a reliable assay for quantifying protein levels. A total of 10 test tubes (12 × 75 mm) were prepared and labeled according to the corresponding vegetable extracts. To each tube, 1000 µL of Biuret reagent was added, followed by the addition of 10 µL of the respective vegetable extract. One tube was designated as the blank control, to which no extract was added. The contents of each tube were thoroughly mixed using a vortex mixer and incubated at room temperature for 5 minutes to allow for complete reaction. The absorbance of the solutions was measured at 550 nm using a spectrophotometer within 5 hours of preparation to ensure accuracy. The recorded absorbance values were used to calculate protein concentrations based on a standard curve prepared with known protein concentrations.

2.7 Data analysis

Data were collected through a Standard tube test to determine the strength of the agglutination reaction, along with protein measurements using the Biuret method. The results were analyzed using IBM SPSS Statistics version 26.

3. Results

3.1 Vegetable extracts and their interaction with human red blood cells

Among nine vegetable extracts, five extracts including *D. biflorus* seed, *L. polychrous* mushroom, lima bean (*P. lunatus*), mulberry (*M. alba*) leaf and winged bean (*P. tetragonolobus*) showed positive reactions with human red blood cells, while four extracts including *C. serratum* seed, cucumber (*C. sativus*) fruit, *L. leucocephala* seed and long bean (*V. unguiculata*) did not induce any agglutination. Three of the five extracts resulted in agglutination including *L. polychrous* mushroom, mulberry (*M. alba*) leaf and winged bean (*P. tetragonolobus*) exhibited non-specific agglutination across all blood types, while two extracts including *D. biflorus* seed and lima bean (*P. lunatus*) showed specific agglutination for blood type A, as shown in Table 1.

3.2 Agglutination strength of the vegetable extracts

The five vegetable extracts that showed positive results in the Standard tube test were further tested for agglutination strength against blood types A, B and O using two-fold dilutions as shown in Table 2.

Table 2 Effects of vegetable extracts on red blood cell agglutination.

Vegetable extracts	Blood groups		
	A	B	O
C. serratum leaf	-	-	-
C. sativus fruit	-	-	-
D. biflorus seed	+	-	-
L. polychrous mushroom	+	+	+
L. leucocephala seed	-	-	-
M. alba leaf	+	+	+
P. lunatus seed	+	-	-
P. tetragonolobus seed	+	+	+
V. unguiculata seed	-	-	-

Winged bean (*P. tetragonolobus*) extract exhibited the highest agglutination strength at a dilution of 1:32 for blood types A, B and O, with a reaction of weak agglutination for blood types A and O, and a 3+ reaction for blood type B as shown in Table 3.

Mulberry (*M. alba*) leaf extract showed the highest strength at a dilution of 1:16 for all blood types, with weak agglutination for blood type A, 1+ for blood type B and 2+ for blood type O as shown in Table 4.

L. polychrous mushroom extract showed the highest agglutination strength at 1:64 for blood types A and B, both with a 1+ reaction, and 1:32 for blood type O, with a 1+ reaction as shown in Table 5.

Lima bean extract showed the highest agglutination strength at 1:512 for blood type A with a 1+ reaction, while no agglutination was observed for blood types B and O as shown in Table 6.

D. biflorus seed extract exhibited the highest strength at 1:64 for blood type A with a 2+ reaction, and no agglutination for blood types B and O as shown in Table 7.

3.3 Correlation between agglutination strength and protein levels in vegetable extracts

Protein levels among these extracts varied, as presented in Table 8. Notably, extracts from *L. leucocephala* seeds, long beans (*V. unguiculata*), *C. serratum* leaf, and cucumber (*C. sativus*) exhibited protein levels comparable to those of five other extracts that induce agglutination.

The correlation analysis revealed that extracts with higher protein concentrations tended to exhibit stronger agglutination reactions. Specifically, *P. lunatus* (lima bean) and *D. biflorus* seed extracts, with protein concentrations of 2.94 g/dL and 1.78 g/dL, respectively, demonstrated the highest agglutination titers at 1:512 and 1:128 as shown in Table 9. This positive correlation is depicted in Figure 2, where the X-axis represents agglutination strength (dilution titers from 1:16 to 1:512), and the Y-axis denotes protein concentration (g/dL). Each data point (labeled 1–5) corresponds to one of the five vegetable extracts tested, and the trendline illustrates the overall relationship.

Table 3 Agglutination strength of winged bean (*P. tetragonolobus*) extract on red blood cells.

Blood groups	Two-fold dilution									
	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
A	4+	4+	3+	1+	W	N	N	N	N	N
B	4+	4+	4+	4+	3+	N	N	N	N	N
O	4+	4+	3+	3+	W	N	N	N	N	N

W: Weakly reaction N: No agglutination

Table 4 Agglutination strength of mulberry (*M. alba*) leaf extract on red blood cells.

Blood groups	Two-fold dilution									
	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
A	3+	2+	W	W	N	N	N	N	N	N
B	4+	4+	3+	1+	N	N	N	N	N	N
O	4+	4+	3+	2+	N	N	N	N	N	N

W: Weakly reaction N: No agglutination

Table 5 Agglutination strength of *L. polychrous* mushroom extract on red blood cells.

Blood groups	Two-fold dilution									
	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
A	4+	4+	4+	4+	3+	1+	N	N	N	N
B	4+	4+	4+	4+	2+	1+	N	N	N	N
O	4+	4+	4+	4+	1+	N	N	N	N	N

W: Weakly reaction N: No agglutination

Table 6 Agglutination strength of lima bean (*P. lunatus*) extract on red blood cells.

Blood groups	Two-fold dilution									
	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
A	4+	4+	4+	4+	4+	4+	3+	2+	1+	N
B	N	N	N	N	N	N	N	N	N	N
O	N	N	N	N	N	N	N	N	N	N

W: Weakly reaction N: No agglutination

Table 7 Agglutination strength of *D. biflorus* seed extract on red blood cells

Blood groups	Two-fold dilution									
	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
A	4+	4+	4+	4+	4+	2+	N	N	N	N
B	N	N	N	N	N	N	N	N	N	N
O	N	N	N	N	N	N	N	N	N	N

W: Weakly reaction N: No agglutination

A moderate positive correlation ($R^2 = 0.646$) was observed between protein levels and agglutination strength, suggesting that while higher protein concentrations generally corresponded to stronger agglutination reactions, some variability existed among the samples. This variability indicates that factors other than protein concentration may influence the agglutination process, warranting further investigation into the specific proteins or other bioactive compounds responsible for these effects.

Table 8 Protein levels in vegetable extracts.

Vegetable extracts	Protein level (g/dL)
P. lunatus seed	2.94
V. unguiculata seed	1.98
D. biflorus seed	1.78
M. alba leaf	1.70
C. sativus fruit	1.56
L. leucocephala seed	1.52
P. tetragonolobus seed	1.48
L. polychrous mushroom	1.31
C. serratum leaf	0.85

Table 9 Maximum agglutination strength and protein levels in vegetable extracts.

Vegetable extracts	Maximum agglutination strength	Protein level (g/dL)
P. lunatus	1:512	2.94
D. biflorus	1:128	1.78
M. alba	1:16	1.70
P. tetragonolobus	1:32	1.48
L. polychrous	1:64	1.31

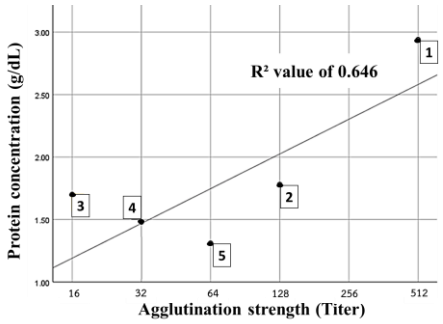

Figure 2 Correlation between protein levels in vegetable extracts and maximum agglutination strength on red blood cells (1 = P. lunatus seed, 2 = D. biflorus seed, 3 = M. alba leaf, 4 = P. tetragonolobus seed, 5 = L. polychrous mushroom)

4. Discussions and Conclusions

The results revealed that five out of nine vegetable extracts induced agglutination reactions, while three extracts (cucumber, *L. leucocephala* seeds and long bean) did not show any interaction with RBCs. The positive agglutination reactions observed with *D. biflorus* seed, *L. polychrous* mushroom, lima bean (*P. lunatus*), mulberry (*M. alba*) leaf and winged bean (*P. tetragonolobus*) indicate a strong potential for these vegetable extracts to influence RBC antigen interactions. Interestingly, only two extracts (lima bean and *D. biflorus* seed) showed specific agglutination for blood type A, while the other three extracts exhibited non-specific agglutination across all blood types. A study of Sharon et al. (10) showed extracts from *D. biflorus* and lima beans demonstrated a strong affinity for blood type A antigens, indicating the specific agglutination properties of plant protein to immunodominant blood group A (N-acetylgalactosamine), particularly in legumes. In contrast, other extracts, such as those from mulberry leaf and winged bean, tend to cause non-specific agglutination across all blood types. These findings support the observed results in the present study, confirming both specific and non-specific agglutination patterns among the tested vegetable extracts.

The complex interaction between plant extracts and red blood cell (RBC) antigens is evident through varying agglutination strengths across different blood types, as indicated by the present study on Thai vegetable extracts. For instance, the winged bean (*P. tetragonolobus*) extract showed the highest agglutination strength at a 1:32 dilution, affecting all blood types, while mulberry leaf extract exhibited a strength of 1:16 with notable variations in agglutination intensity across blood types A, B and O. This variation in plant extract activity aligns with findings from Zubcevic et al. (11), where plant lectins were shown to induce RBC agglutination with varying specificities depending on the blood group system. Their research emphasized the diverse binding affinities of lectins from different plants, reinforcing the idea that both the type of lectin and its concentration significantly influence the agglutination reaction. Together, these studies indicate the potential for plant lectins to be used in blood typing and other medical applications due to their specificity and interaction with RBC antigens.

The study by Wu et al. (12) delves into the recognition factors of *D. biflorus* agglutinin (DBA), a lectin known for its specific affinity towards the A antigen in human blood. This research further supports the findings in the present study, where *D.*

biflorus extract demonstrated strong agglutination activity specifically for blood type A at a dilution of 1:64. The molecular interactions focused by Wu et al. suggest that DBA's high specificity for A antigens is due to its ability to accommodate unique structural features present in blood group A. Similarly, lima bean extract exhibited the highest agglutination strength at a dilution of 1:512, indicating its specific affinity for blood type A. These findings emphasize the potential application of *D. biflorus* and lima bean lectins in blood typing and as bioactive agents that target blood group-specific antigens, further emphasizing the relevance of these plant extracts in clinical diagnostics and therapeutics.

The study by Sultana (13) on the preparation of an affinity chromatographic matrix to purify mulberry seed lectin demonstrates a significant method for isolating plant-based lectins, which can strongly agglutinate with human red blood cells (RBCs). This research parallels the findings in the present study, where mulberry leaf extracts showed varying agglutination strengths across different blood types.

The findings of Schertz et al. (14) on the inheritance of anti-A1 hemagglutinating activity in lima bean (*P. lunatus*) provide data that complement the present study's results. Their research demonstrated the genetic basis of hemagglutinating properties in lima bean, showing strong specificity towards A-type blood antigens. This aligns with the current study's observation that lima bean extracts exhibited the highest agglutination strength at a 1:512 dilution, with a particular affinity for blood type A. The specificity of lima bean lectins for A antigens, as described in both studies, indicates their potential application in blood typing and diagnostics.

The relationship between protein concentration and biological activity, such as agglutination strength, is a significant focus in plant extract studies. Sarkar et al. (15) quantified protein content in various edible plant leaves, demonstrating that protein concentration plays a crucial role in determining the functional properties of plant extracts. This observation parallels the findings of the present study, where higher protein levels in lima beans and ***D. biflorus*** extracts (2.94 g/dL and 1.78 g/dL, respectively) were linked to stronger agglutination reactions in red blood cells. The Biuret method, used in both studies, consistently reveals that protein-rich extracts tend to exhibit more pronounced biological interactions, including agglutination. The moderate positive correlation ($R^2 = 0.646$) observed in the current study suggests that while protein concentration is a key factor in agglutination, other elements may also influence this outcome, as also discussed by Sarkar et al. when comparing protein content and functional effects across different plant species. Additionally, the correlation between plant lectins and their ability to interact with red blood cell (RBC) antigens, particularly in relation to the ABO blood group system. This aligns well with the findings from the study by Rudrappan and Veeran (16), which explored the role of plant-based lectins in identifying rare blood groups, including the Bombay blood group. These reports emphasize the importance of protein quantification in understanding plant-based bioactivities.

The application of standard A, B, or O cells for testing was deemed appropriate for this study as it focused primarily on the differentiation of ABO blood group antigens. However, we acknowledge that other blood group systems, such as Rh and MNS, may influence the agglutination response and warrant further investigation. Future studies could expand this approach to detect specificity for Rh and MNS antigens, particularly due to the reliance of the current methodology on the specificity of proteins in the plant extracts for glycoproteins or proteins with antigenic properties on the red blood cell (RBC) surface. Such studies would require new experimental conditions with enhanced detection sensitivity, given the relatively lower abundance of Rh and MNS antigens compared to ABO antigens. Thus, advanced detection techniques will be essential to accurately monitor and characterize protein interactions during agglutination in these blood group systems.

The anti-A test will give positive results for both A1 and A2 antigens, while the *D. biforous* plant extract will give positive results for A1 only and negative results for A2 antigens. Therefore, it is used in medicine to separate blood group subgroups. When known, it can be used to prevent blood transfusions that are safe, which will prevent people with the subgroup from creating

clinically important antibodies later. This will make it more difficult to give blood to people in the subgroup that create antibodies to the main antigen. (For example, subgroup A2 creates anti-A1 and cannot receive A1 blood. Only A2 cells can be given for safety.) In addition, the exploration of plant-based reagents could lead to the discovery of bioactive compounds with broader medical applications, thereby opening new avenues for innovation in diagnostics and therapeutics.

In conclusion, the results of this study demonstrate that several Thai vegetable extracts have the potential to induce agglutination of human red blood cells, with some extracts showing specificity for blood type A. The correlation between protein levels and agglutination strength provides understanding into the role of plant extracts in these interactions. These findings open up avenues for further research into the potential applications of vegetable extracts in medical and biotechnological fields, particularly in relation to blood group antigens and immune modulation. Future studies should explore what are bioactive compounds in these vegetables, chemical properties of the compounds, molecular mechanisms underlying the specificity of certain extracts and evaluate their potential therapeutic or diagnostic uses.

Acknowledgements

The authors would like to express their sincere gratitude to Regional Blood Centre V Nakhonratchasima, Thai Redcross Society, Thailand for their invaluable guidance and advice. Additionally, we extend our appreciation to the Faculty of Allied Health Sciences, Nakhonratchasima College, for their generous support and contributions to the success of this research project.

Declaration of Conflicting Interests

The authors declare they have no conflicts of interest for this article, and they alone are responsible for the content.

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