Hemagglutinating Potential of Hyacinth *(Lablab purpureus)* Bean Extract

Alyson Y. Alcaide[1], Trisha Felicitty S. Calindas[2], Maria Isabel Rima O. Llobrera[3], Marijoh M. Morales[4], Alyssa Nicole M. Tadifa[5], and Angelica B. Torres[6]

[1]Department of Medical Laboratory Science. alyson.alcaide@lorma.edu
[2]Department of Medical Laboratory Science. trishafelicitty.calindas@lorma.edu
[3]Department of Medical Laboratory Science. mariaisabelrima.llobrera@lorma.edu
[4]Department of Medical Laboratory Science. marijoh.morales@lorma.edu
[5]Department of Medical Laboratory Science. alyssanicole.tadifa@lorma.edu
[6]Department of Medical Laboratory Science. angelica.torres@lorma.edu

Abstract

Among the many blood group systems, the most medically relevant when it comes to carrying out blood transfusions and organ transplantations is the ABO blood group system. Blood typing, in general, plays a very important role in clinical diagnosis, transfusion medicine, and immunohematology. These experiments were centralized on the hemagglutinating prospects of the said Hyacinth Bean (Lablab purpureus) extract. Seeds had been obtained in Barangay Dasay, Miraan, locos Sur, and were authenticated at Don Mariano Marcos Memorial State University. The phytochemicals assay was conducted to ascertain the presence of biologically active compounds, more specifically lectins with a carbohydrate-binding affinity and an agglutination-inducing properties. The beans were subjected to standard extraction, precipitation, and buffer preparation protocols to obtain crude extract from which the crude lectin-containing extract was derived. Different treatment concentrations of the extract were prepared for testing hemagglutination. Macroscopic grading of agglutination reactions was employed to compare against commercial antisera (positive control). Subsequently, the experiment yielded proof of hemagglutinating activity found in the extract across ABO group with some concentration demonstrating the agglutinating power equivalent to commercial antisera particularly those of types A and B. This study reinforces the paradigm concerning the use of sustainable plant-based diagnostic resources, and furthermore encourages investigations that will optimize extraction methods of the active lectin components, as well as standardization of the treatment protocols for consistent and accurate diagnostic use.

Keywords: Hyacinth (Lablab purpureus) Bean; hemagglutination; blood typing; ABO blood system; lectin; polyagglutination

1. Introduction

The classification of human blood group system is the foundation of transfusion medicine and immunohematology. Typing of blood, especially in ABO and the Rh system, remains crucial to the delivery of safe blood transfusions and minimizing adverse immunologic responses. The ABO system, which was discovered by Karl Landsteiner in 1901, categorizes blood as possessing or not possessing antigens A and B on the red blood cells' surface (Harmening, 2019). The classification remains not only crucial in transfusion medicine but in organ transplantation and maternal-fetal medicine (Gajic et.al, 2021).

Lectins, plant carbohydrate-binding proteins, have been gained interest since they are able to bind to glycoproteins present on the surface of erythrocytes specifically, leading to macroscopic agglutination. Hyacinth bean (Lablab purpureus), which is widely cultivated in Asia and Africa, has been identified as a valuable source of hemagglutinating active plant lectins. These bioactive proteins have long been traditionally known to be of nutritional and medicinal significance, and recent research has identified their red blood cell agglutinating property (Zhou et al., 2024; Nabavi, 2020).

Legume-derived lectins have been extensively documented to possess high binding affinity to certain blood group antigens (Katoch et al., 2021). For example, Dolichos biflorus lectin has exhibited selective agglutination with blood group A1 erythrocytes (Pacha-Gupta et al., 2016). Like Crotalaria spectabilis and other legumes, research proved hemagglutinating activity against ABO blood groups (De Oliveira et al., 2017). These results validate the postulation that hyacinth bean lectins would display similar activity and specificity.

Additionally, previous research has recorded lectin-containing plants like Phaseolus vulgaris and Artocarpus heterophyllus as having an important hemagglutination activity, justifying the employment of plant-based reagents in serology (Sultana, 2019; Dzulkipli et al., 2023). Interestingly, a study by Liu et al. (2020) identified a lectin from Lablab purpureus—designated as FRIL—that inactivated viral particles, supporting the biomedical potential of this plant.

Even with increasing evidence, few studies have been specifically directed towards hyacinth bean lectins for blood typing purposes. In this regard, the present study endeavors to examine the hemagglutinating activity of hyacinth bean extract on the ABO blood group system. It also endeavors to find out if plant lectins can be effective, low-cost substitutes for commercial antisera in the determination of blood types. Through it, this book aims to add not just to diagnostic innovation but also to increasing the application of natural biocompounds in clinical and biomedical sciences.

2. Objectives

General Objective:

To assess the hemagglutinating potential of Lablab purpureus (hyacinth bean) extract on various human red blood cells of various ABO blood types compared with that of a known positive control via macroscopic agglutination reaction grading.

Specific Objectives:

To measure the hemagglutinating potential of Lablab purpureus extract throughout ABO blood types relative to a known positive control as ascertained using macroscopic agglutination grading.

To determine which treatment (blood type and condition) has the highest hemagglutinating response to Lablab purpureus extract and compare it with the positive control reaction.

To statistically compare if there is a significant difference between the hemagglutinating activity of the hyacinth bean extract and the positive control for different ABO blood types.

3. Materials and Methods

Research Design

This study employed an experimental research design. Experimental research is a scientific method in performing research where independent variables were applied to dependent variables to observe their effect on the later

Plant Collection and Identification

Newly harvested matured hyacinth bean (Lablab purpureus) samples were gathered from Barangay Dasay, Narvacan, Ilocos Sur. Botanical authentication and identification were done by the Don Mariano Marcos Memorial State University-North La Union Campus Agriculture Department to confirm that the right species were utilized. Only mature seed pods were used in the analysis.

Phytochemical Screening

Phytochemical analysis was conducted at Lorma Colleges, San Fernando, La Union, by the College of Pharmacy. The process used for extraction was maceration using ethanol, resulting in a yellowish extract with an aromatic smell. Mayer's, Hager's, Molisch, Benedict's, Lead Acetate, Ferric Chloride, Froth, Gelatin, and Filter Paper tests were conducted. Positive results showed the presence of carbohydrates, alkaloids, saponins, polyphenolic compounds, reducing sugars, flavonoids, tannins, and essential oils.

Hyacinth Bean Extract Preparation

Hyacinth bean seeds (100g) were oven-dried at 55°C for one hour with intervals of five minutes, thereafter coarsely ground in a blender. A quarter (25g) was soaked in 100 mL distilled water in a conical flask, stirred at 10-minute intervals for one hour, filtered with mesh grit, and allowed to stand at room temperature for 12 hours. The supernatant was harvested for further processing.

Lectin Buffer Preparation

Lectin buffer was made by dissolving 6.057g of tri-sodium citrate dihydrate, 8.70g sodium chloride, 0.203g magnesium chloride, and 0.11g calcium chloride in water distilled. pH was adjusted from 9.8 to 7.20 with concentrated hydrochloric acid, and the final volume was brought up to 1000 mL in a volumetric flask.

Protein Precipitation

Crude protein of the extract was precipitated with a 10% w/v ammonium sulfate solution (10g/100 mL of supernatant). The mixture was left overnight for complete precipitation. The precipitated protein was used for the treatment preparation.

Treatment Preparation

Three treatment solutions were prepared as follows:

- **Treatment 1**: 50 µL hyacinth bean extract + 25 µL buffer
- **Treatment 2**: 100 µL hyacinth bean extract + 25 µL buffer
- **Treatment 3**: 200 µL hyacinth bean extract + 25 µL buffer

Blood Sample Collection and Blood Typing

Following McPherson et al. (2021), 5 mL of blood was drawn from each of 15 qualified donors and collected in EDTA vacutainer tubes. Forward blood typing was

performed using the tube method with commercial antisera (Anti-A, Anti-B, Anti-D) per Harmening (2019), to confirm ABO and Rh status of the donors.

Red Blood Cell Suspension Preparation

Red blood cells were centrifuged, washed three times with normal saline solution (NSS) and resuspended to prepare a 3% RBC suspension. To provide equal distribution for hemagglutination testing.

Hemagglutination Testing

Hemagglutination testing were performed in 180 test tubes (12 per donor). In each set of donors there were:

- 3 tubes for Treatment 1
- 3 tubes for Treatment 2
- 3 tubes for Treatment 3
- 3 tubes for positive control (commercial antisera)

Two drops of 3% RBC suspension were added to each tube, followed by the appropriate treatment or antisera. The samples were centrifuged for 1000 rpm in 60 seconds and observed for agglutination using the tilt-and-wiggle method.

Agglutination Grading

Macroscopic agglutination was graded based on the standard system outlined by Harmening (2019):

Table 1. Agglutination Grading System

Grade	Description
5+	The red cell button is solid agglutinate; clear background
4+	Several large agglutinates; clear background
3+	Many medium-sized agglutinates; reddish background
2+	Small-sized agglutinates; the background is turbid with many free red cells
1	No agglutinated red cells are visible; red cells are observed flowing off the red cell button during the process of grading (homogeneous mixture; negative)

[1]If no agglutination is observed in the sample, the macroscopic procedure must be followed to confirm if there is agglutination microscopically.

This grading system was used to assess the hemagglutinating activity of each treatment in comparison to the positive control across all ABO blood types.

3. Results

Hemagglutinating Activity of Hyacinth Bean Extract and Positive Control across the ABO system

The first objective of this study was to determine the hemagglutinating activity of the hyacinth bean extract combined with a buffer solution and the commercial antisera.

Table 2. Averaged Grading of the Hemagglutination Activity

Treatments & control	Average Result	Macroscopic Grading
Treatment 1	3.90	4+
Treatment 2	4.16	4+
Treatment 3	4.24	5+
Control	4.47	5+

Of all the three treatments, Treatment 3 recorded the highest mean hemagglutination grade of 4.24. This is evidence of a stronger interaction between lectin and erythrocyte surface antigens compared to Treatment 1 (3.90) and Treatment 2 (4.16). The control, which was the commercial standard, recorded a mean grade of 4.47. This finding confirms that Treatment 3 had higher hemagglutinating activity compared to the other treatments.

To determine whether there was a significant difference between the treatments and the control group, a one-way ANOVA test was performed. The result, shown in Table 2, indicates a difference ($p = 0.0079 < 0.01$).

Table 3. Significant Difference Between and Among the Different Treatments and the Control Group

Source	*SS*	*df*	*MS*	*F*	*p-value*
Treatment 1	2.43	3	0.81	4.36	0.0079
Error	10.39	56	0.19		
Total	12.82	59			

ANOVA, the post hoc t-tests were conducted to determine specific differences between treatments. As shown in Table 3, a significant difference was observed only between Treatment 1 and the control ($p = 0.0007$), while other comparisons were not significant.

Table 4. Post hoc analysis using p-values for pairwise t-tests

	Treatment 1	Treatment 2	Treatment 3	Control
Treatment 1				
Treatment 2	.1149			
Treatment 3	.0385	.6064		
Control	.0007 *p=<0.01	.0528	.1499	

5. Discussion

The first objective of this study was to determine whether Lablab purpureus extract exhibits hemagglutinating activity when tested on human red blood cells (RBCs) of various ABO blood groups. The results shown demonstrate that all three treatments using the extract induced observable hemagglutination across A, B, AB, and O Rh(D) positive and negative samples. The averaged hemagglutination grades were approximately 4.0 for Treatment 1, 4.16 for Treatment 2, and 4.24 for Treatment 3, compared to 4.47 for the positive control. These grades reflect consistent formation of visible agglutinate clusters, suggesting that Lablab purpureus contains lectins capable of binding to RBC surface antigens and inducing agglutination. The close similarity between the mean grade of Treatment 3 and the control underscores the strong hemagglutination potential of this extract under optimal conditions.

As noted by Ouattar (2022), phytolectins are known for their affinity to carbohydrates on red blood cell membranes, often resulting in hemagglutination. Lablab purpureus has been reported by Rai et al. (2022) to contain a high concentration of lectins, which likely account for the activity observed in this study.

However, one critical observation is the agglutination seen across all tested blood groups, regardless of ABO or Rh status. This indicates that Lablab purpureus lectins may induce polyagglutination, a phenomenon in which RBCs agglutinate with multiple antisera due to non-specific lectin binding. As defined by Harmening (2019), polyagglutination is an undesirable trait in precise blood typing reagents. Therefore, while Lablab purpureus lectins are clearly bioactive, their broad reactivity implies a lack of antigen specificity, limiting their utility in differential blood typing applications.

The second objective was to determine which treatment condition of the Lablab purpureus extract produced the highest hemagglutinating activity. Among the three treatment groups, Treatment 3 showed the highest average grade (4.24), suggesting a stronger lectin concentration or enhanced stability of the active component. This was followed closely by Treatment 2 (4.16) and Treatment 1 (3.90), while the positive control yielded the highest value (4.47).

The strong hemagglutination observed with Treatment 3 implies that its preparation method preserved a more bioactive form of the lectins. According to Karandikar (2022), higher grades of agglutination reflect stronger antigen-lectin binding, which could be due to optimal valency or affinity. Therefore, it is plausible that Treatment 3 retained a greater proportion of multivalent lectins capable of cross-linking RBCs effectively.

This finding also suggests that Lablab purpureus lectins may hold potential in broad agglutination patterns, such as preliminary blood grouping or lectin screening. However, further characterization is necessary to identify and potentially refine the lectin specificity.

The third objective was to assess whether the differences observed among the treatments were statistically significant. A one-way ANOVA (Table 4) revealed a significant difference between and among the treatment groups and the control ($F = 4.36$,

p = 0.0079). This indicates that at least one treatment differed significantly in its agglutinating activity.

Post hoc analysis using pairwise t-tests found that only the difference between Treatment 1 and the Control was statistically significant ($p = 0.0007$) at the $\alpha = 0.01$ level. No other comparisons between treatment pairs or between treatments and the control were significant. This suggests that while the overall extract demonstrates hemagglutination potential, only Treatment 1 was meaningfully weaker than the control, while Treatments 2 and 3 performed similarly.

The consistency in agglutination across ABO groups, combined with literature such as American (2014) and Vadivel (2018), supports the conclusion that Lablab purpureus lectins are broadly reactive. Vadivel's findings, in particular, that lectin activity in Lablab purpureus varies among blood types but is generally high.

6. Conclusion

Major Findings

The study found that Hyacinth bean extract exhibits polyagglutinative hemagglutinating activity since the ABO system agglutinated. Treatment #3 (200Ml) showed the strongest activity (graded as 5), while Treatment #1 (50Ml extract) had the weakest (graded as 3), indicating a positive correlation between the extract concentration and hemagglutinating strength. Furthermore, ANOVA analysis statistically confirmed that there are significant differences among the treatments which demonstrated that all three treatments are effective, but varying in strength of all three are effective, but varying in strength based on concentration.

Conclusion

The study concludes that hyacinth bean extract lectin is polyagglutinative, showing non-specific binding to all blood types due to its affinity for common glycoproteins or carbohydrates. This makes it suitable for general hemagglutination tests where blood type specificity is not required. Treatment 3 showed the strongest hemagglutinating activity exhibiting almost the same activity to the positive control likely due to its higher lectin concentration. In contrast, Treatment 1 showed the weakest activity, confirming a direct correlation between concentration and hemagglutination strength. Statistical analysis (ANOVA) confirmed significant differences among treatments, verifying the effectiveness of the extract compared to the control.

Recommendation

The researchers recommend that future studies focus on the isolation, purification, and structural characterization of hyacinth bean lectins to better understand their molecular binding patterns, stability, safety, and polyagglutinative behavior. Techniques such as electrophoresis, lectin profiling, and mass spectrometry are suggested to link structure with function. Further exploration of Treatment 3 is encouraged, as it consistently demonstrated the highest hemagglutination activity, indicating its potential for advanced applications. Additionally, researchers should consider using ethanolic extraction methods to improve accuracy and reduce contamination risks associated with lengthy procedures. Expanding the sample size and including a broader range of blood types—especially rare and Rh-negative types—would provide a more comprehensive understanding of the extract's agglutination profile and enhance its potential utility in diagnostics and clinical research.

7. Acknowledgement

The researchers would like to extend their heartfelt gratitude and appreciation to the people who have taken a great part in the accomplishment and success of this study. The success of this research wouldn't be possible without their support and researchers express their sincerest gratitude to all of them:

Aldrin Patrick G. Estocapio, RMT, the researchers' adviser for his unwavering guidance and counsel, his proficiency in the area of Medical Laboratory Science, and the full extent of time he devoted to the completion of this study;

Mr. Jerome P. Vera, Mr. Mark Ericson B. Baladad members of the CMLS-Research Technical Panel, and Mrs. Josephine C. Milan, RMT, MSMT, Chairperson of the CMLS-RTP and

Dean of the CMLS, for their insightful criticisms, valuable inputs, and suggestions in the improvement of the study;

Mr. Jose Enrico M. Sumaya, and Mr. Mark Ericson B. Baladad instructors of the researchers, for their patience, unrelenting encouragement, and support during the completion and success of the research.

To Dean Ellen Mae P. Abiqui, RPh, MSPharm, CPT, and Mr. Jerald B. Macapagal, LPT, Laboratory Custodian, College of Pharmacy, LORMA Colleges, for their technical assistance and expertise in phytochemical analysis.

To Dr. Junifer Rey E. Tabafunda, Chancellor, and Dr. Analyn V. Sagun, Dean of College of Agriculture, Faculty, Crop Science Department of Don Mariano Marcos Memorial State University – North La Union Campus (DMMMSUNLUC), for their humble assistance in identifying the species and certifying the plant specimen;

To the researchers' beloved families, parents, and friends who backed them emotionally and financially and provided motivation, love, and prayers whom they, the researchers, used as a source of strength and inspiration for the accomplishment of the study;

Most importantly, Omnipotent God, from the beginning to the end, granted the wisdom, understanding, and knowledge the researchers required and never ceased to watch over them during the achievement and victory of this modest work owing to His kingdom and glory.

8. References

American. (2014, November 28). *Antibacterial and antioxidant activities of a food lectin isolated from the seeds of Lablab purpureous*. Academia.edu. https://www.academia.edu/9546756/Antibacterial_and_Antioxidant_Activities_of_a_Food_Lectin_Isolated_from_the_Seeds_of_Lablab_purpureous

De Oliveira, W. R., Rego, E. J. L., Von Buettner Ristow, P. C. L., da Silva Velozo, E., de Carvalho Carneiro, D., Bastos, B. L., & da Cunha Lima, S. T. (2018). Isolation, characterization and analysis of the agglutinative activity of a lectin from *Crotalaria spectabilis*. *Journal of Plant Biochemistry and Biotechnology*. https://doi.org/10.1007/s13562-018-0446-x

Dr V. Vadivel. (2018, November 29). *Nutritional and chemical evaluation of raw seeds of five varieties of Lablab purpureus (L.) Sweet*. Academia.edu. https://www.academia.edu/37876122/Nutritional_and_chemical_evaluation_of_raw_seeds_of_five_varieties_of_Lablab_purpureus_L_Sweet

Dzulkipli, N. A., Haida, Z., Anuar, T. S., & Kamarudin, E. (2023). Effect of lectins from selected Malaysian local fruits on human blood group antigens. *Journal of Sustainability Science and Management*. https://www.semanticscholar.org/paper/5df288869da6ee193d7226929d0be0a853bbc13b

Gajic-Veljanoski, O., Li, C., Schaink, A. K., Guo, J., Higgins, C., Shehata, N., Okun, N., De Vrijer, B., Pechlivanoglou, P., Ng, V., & Sikich, N. (2021). Noninvasive fetal RHD blood group genotyping: A systematic review of economic evaluations. *Journal of Obstetrics and Gynaecology Canada, 43*(12), 1416–1425.e5. https://doi.org/10.1016/j.jogc.2021.07.014

Harmening, D. (2019). *Modern blood banking & transfusion practices*. F.A. Davis Company.

Karandikar, M., Khuje, P., & Sable, B. (2022). Comparative scores of ABO and Rh hemagglutination reactions. *Medical Journal of Dr. D.Y. Patil Vidyapeeth, 0*(0), 0. https://doi.org/10.4103/mjdrdypu.mjdrdypu_574_20

Katoch, R., & Tripathi, A. (2021). Research advances and prospects of legume lectins. *Journal of Biosciences, 46*(4). https://doi.org/10.1007/s12038-021-00225-8

Liu, Y., Shahed-Al-Mahmud, M., Chen, X., Chen, T., Liao, K., Lo, J. M., Wu, Y., Ho, M., Wu, C., Wong, C., Jan, J., & Ma, C. (2020). A carbohydrate-binding protein from the edible lablab beans effectively blocks the infections of influenza viruses and SARS-CoV-2. *Cell Reports, 32*(6), 108016. https://doi.org/10.1016/j.celrep.2020.108016

McPherson, R. A., & Pincus, M. R. (2021). *Henry's clinical diagnosis and management by laboratory methods* (24th ed.). Elsevier.

Nabavi, Z. S., Baniardalani, M., & Basseri, H. (2020). Purification and partial characterization of agglutinin lectin from hemolymph of German cockroach, *Blattella germanica*. *Journal of Arthropod-Borne Diseases*.

https://www.semanticscholar.org/paper/f73bbe6abce5ec53aa1174995e3f5d26f8487c43

Ouattar, H., Zouirech, O., Kara, M., Assouguem, A., Almutairi, S. M., Al-Hemaid, F. M., Rasheed, R. A., Ullah, R., Abbasi, A. M., Aouane, M., & Mikou, K. (2022). In Vitro Study of the Phytochemical Composition and Antioxidant, Immunostimulant, and Hemolytic Activities of Nigella sativa (Ranunculaceae) and Lepidium sativum Seeds. *Molecules*, *27*(18), 5946. https://doi.org/10.3390/molecules27185946

Pacha-Gupta, R., Sundarrajan, P., Desouza, F., Nagrath, K., Tauro, L., Krishnakumar, P., Srivastava, R., & Sinha, T. (2016). Isolation and partial characterization of lectins from Indian varieties of leguminous plants. *International Journal of Advanced Research, 4*(5). https://www.journalijar.com/uploads/486_IJAR-10088.pdf

Rai, K. K., Rai, N., & Pandey-Rai, S. (2021). Unlocking Pharmacological and Therapeutic Potential of Hyacinth Bean (Lablab purpureus L.): Role of OMICS Based Biology, Biotic and Abiotic Elicitors. In *IntechOpen eBooks*. https://doi.org/10.5772/intechopen.99345

Sultana, M., Ahmed, F. R. S., & Alam, M. (2019). Identification of lectins from the seeds of Bangladeshi plants *Sesbania bispinosa* and *Senna occidentalis* by hemagglutination assay. *Asian Journal of Green Chemistry*. https://www.semanticscholar.org/paper/62c63990823daf65c9c34f8a45d4dd358fba0567

Zhou, J., Wang, W., Zhang, Z., Zhu, G., Qiao, J., Guo, S., Bai, Y., Zhao, C., Teng, C., Qin, P., Zhang, L., & Ren, G. (2024). An underutilized bean: Hyacinth bean [*Lablab purpureus* (L.) sweet]: Bioactive compounds, functional activity, and future food prospect and applications. *Journal of the Science of Food and Agriculture*. https://doi.org/10.1002/jsfa.13708

9. Appendices

APPENDIX A

Raw Data

Average hemagglutinating grade of Treatment 1

	Treatment 1			
Patient	**Evaluator 1**	**Evaluator 2**	**Evaluator 3**	**Average**
1. A, Rh(D) +	4.33	4.00	3.33	3.89
2. B, Rh(D) +	4.00	3.00	4.00	3.67
3. O, Rh(D) +	4.00	3.00	4.33	3.78
4. A, Rh(D) +	4.33	3.33	4.00	3.89
5. B, Rh(D) +	4.67	4.00	5.00	4.56
6. O, Rh(D) +	4.00	3.67	4.00	3.89
7. AB, Rh(D) +	4.33	4.33	4.33	4.33
8. B, Rh(D) -	4.00	2.33	2.33	2.89
9. B, Rh(D) +	4.00	3.33	4.00	3.78
10. AB, Rh(D) +	4.33	4.00	4.67	4.33
11. AB, Rh(D) +	4.67	3.33	4.00	4.00
12. A, Rh(D) +	3.33	2.33	3.00	2.89
13. O, Rh(D) +	5.00	4.67	5.00	4.89
14. A, Rh(D) +	5.00	2.67	3.33	3.67
15. B, Rh(D) +	4.00	3.67	4.67	4.11
				3.90

Average hemagglutinating grade of Treatment 2

	Treatment 2			
Patient	**Evaluator 1**	**Evaluator 2**	**Evaluator 3**	**Average**
1. A, Rh(D) +	4.67	4.67	3.67	4.33
2. B, Rh(D) +	4.33	3.67	4.00	4.00
3. O, Rh(D) +	4.67	3.67	3.67	4.00
4. A, Rh(D) +	5.00	4.33	4.67	4.67
5. B, Rh(D) +	5.00	4.00	4.33	4.44
6. O, Rh(D) +	5.00	4.00	4.67	4.56
7. AB, Rh(D) +	4.67	4.00	4.67	4.44
8. B, Rh(D) -	3.67	3.00	3.33	3.33
9. B, Rh(D) +	4.67	3.67	4.67	4.33
10. AB, Rh(D)+	5.00	4.33	4.33	4.56
11. AB, Rh(D) +	5.00	4.33	4.67	4.67
12. A, Rh(D) +	4.00	2.33	2.67	3.00
13. O, Rh(D) +	4.33	3.00	4.00	3.78
14. A, Rh(D) +	5.00	4.00	3.33	4.11
15. B, Rh(D) +	4.33	3.67	4.33	4.11
				4.16

Average hemagglutinating grade of Treatment 3

	Treatment 3			
Patient	**Evaluator 1**	**Evaluator 2**	**Evaluator 3**	**Average**
1.A, Rh(D) +	4.67	4.00	3.67	4.11
2. B, Rh(D) +	5.00	4.00	4.33	4.44
3. O, Rh(D) +	5.00	4.00	4.33	4.44
4. A, Rh(D) +	4.67	4.33	4.33	4.44
5. B, Rh(D) +	4.67	4.33	4.33	4.44
6. O, Rh(D) +	4.67	3.67	4.00	4.11
7. AB, Rh(D) +	5.00	4.00	4.67	4.56
8. B, Rh(D) -	4.67	2.33	3.00	3.33
9. B, Rh(D) +	4.33	3.00	4.00	3.78
10. AB, Rh(D) +	5.00	3.67	4.00	4.22
11. AB, Rh(D) +	5.00	4.00	4.00	4.33
12. A, Rh(D) +	4.33	3.00	4.00	3.78
13. O, Rh(D) +	4.67	3.67	4.33	4.22
14. A, Rh(D) +	4.67	4.00	4.67	4.44
15. B, Rh(D) +	5.00	4.67	5.00	4.89
				4.24

Average hemagglutinating grade of the control

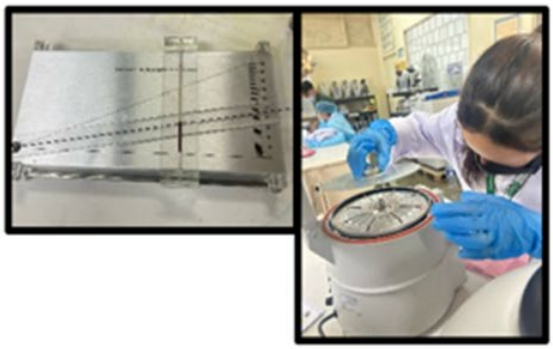
	Control			
Patient	Evaluator 1	Evaluator 2	Evaluator 3	Average
1- A, Rh(D) +	4.50	4.50	4.50	4.50
2- B, Rh(D) +	4.50	4.50	4.50	4.50
3- O, Rh(D) +	4.00	4.00	4.00	4.00
4- A, Rh(D) +	4.50	4.50	4.50	4.50
5- B, Rh(D) +	4.50	4.50	4.50	4.50
6- O, Rh(D) +	4.00	4.00	4.00	4.00
7- AB, Rh(D) +	4.67	4.67	4.67	4.67
8- B, Rh(D) -	5.00	5.00	5.00	5.00
9- B, Rh(D) +	4.50	4.50	4.50	4.50
10- AB, Rh(D) +	4.67	4.67	4.67	4.67
11- AB, Rh(D) +	4.67	4.67	4.67	4.67
12- A, Rh(D) +	4.50	4.50	4.50	4.50
13- O, Rh(D) +	4.00	4.00	4.00	4.00
14- A, Rh(D) +	4.50	4.50	4.50	4.50
15- B, Rh(D) +	4.50	4.50	4.50	4.50
				4.47

APPENDIX B

Documentation

Blood Collection.

Centrifugation Process.

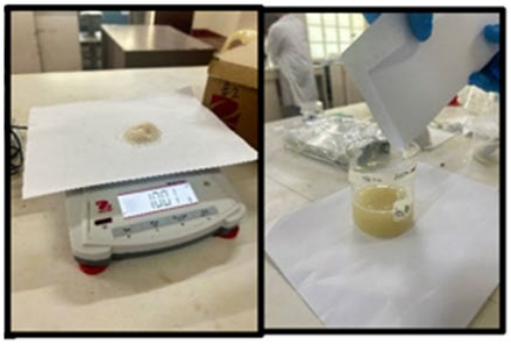


Hematocrit Reading.

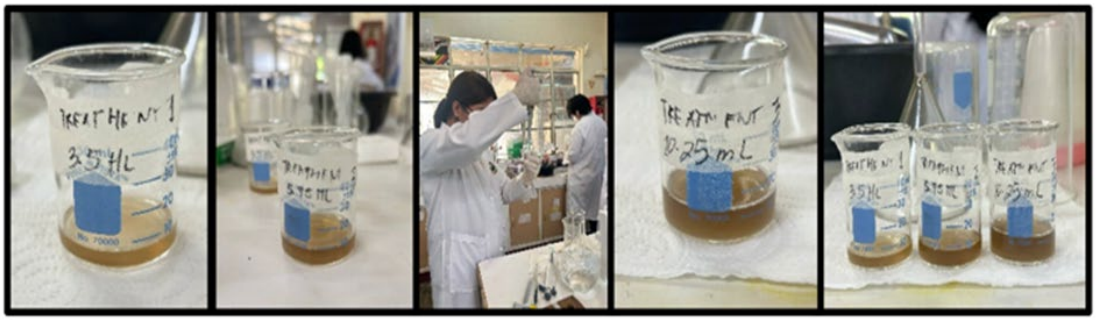
Red Blood Cell Suspension

Hyacinth Bean Extract Preparation.

Lectin Buffer Preparation.



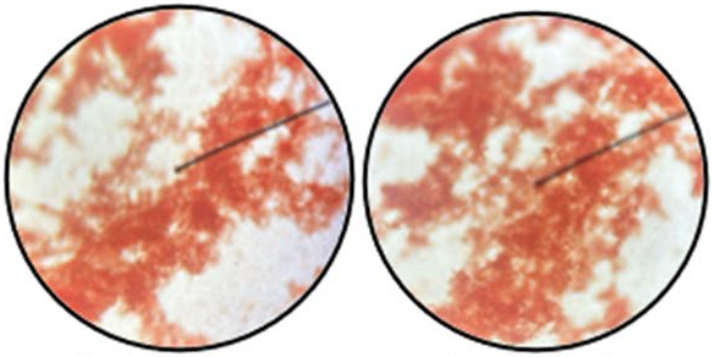
Precipitation of Proteins.



Treatment Preparation.

Hemagglutination testing process.

Macroscopic Observation.



Microscopic View of Red Blood Cells Showing Agglutination.

10. Author(s) Biodata

The authors are third-year Bachelor of Science in Medical Laboratory Science students at Lorma Colleges. Their academic interests include clinical diagnostics, immunohematology, and plant-based biomedical research. This study reflects their commitment to exploring natural, cost-effective alternatives that support innovation and accessibility in laboratory science and healthcare.