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The Potential of Bean Extract (*Phaseolus vulgaris*) Based on Haemagglutination

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Abstract

Blood typing is a crucial procedure in healthcare,but dependence on expensive an difficult-to-acces imported reagents is a constraint, especially in 3T (underdeveloped, frontier, and othermost) areas. This study aimed to evaluate the potential of bean seed extract (*Phaseolus vulgaris*), as a local natural resource, to be used as an alternative reagent candidate in ABO blood group screening through in vitro haemagglutination testing. The research method involved several stages, namely the extractionof lectin from bean seed using a 0.9% physiological NaCl solution, the preparation of a 5% erythrocytre suspention form donors with blood group A, B, AB, and O and microscope agglutination tests at various extract ratios and dilution titres. Validation was performed by comparing the agglutination results with commercial anti-A and anti-B reagents. Data were analysed qualitatively descriptively and quantitatively using the Kruskal-Wallis and Dunn nonparametric tests. The results showed that the bean lectin extract successfully agglutinated blood group A, B, and AB erythrocytes specifically, while showing no reaction to blood group O. The strongest and most stable agglutination reaction was observed at a mixture ratio of extract and NaCl of 2:4 and 2:6. Statistical analysis showed a significant difference in agglutination scores between blood group O and blood groups A, B, and AB ($p < 0.05$), which confirms the specificity of the lectin reaction. The validation test also showed agglutination patterns consistent with commercial reagents. In conclusion, broad bean seed extract has great potential to be developed as an economical, easily accessible, and effective natural agglutination reagent for ABO blood group screening, thus providing an innovative solution to overcome the limitations of conventional reagents in healthcare facilities.

Keywords: Blood Group; Haemagglutination; *Phaseolus vulgaris*.

Abbreviations: 3T: Underdeveloped, frontier, and outermost, BO: Human blood typing system consisting of types A, B, AB, and O, NaCl: Sodium Chloride (0.9% physiological solution), UDD PMI: Indonesian Red Cross Blood Donor Unit, v/v: Volume per volume (unit of volume comparison), w/v: Weight per volume (weight per volume), rpm: Revolutions per minute (rotations per minute), KPA: Anti-A Positive Control, KPB: Anti-B Positive Control, NC: Negative Control.

INTRODUCTION

In healthcare, blood typing is a basic diagnostic procedure (1). Blood typing is determined through an agglutination reaction between erythrocytes and commercial reagents. Although this method has been widely used, its application has several significant limitations. One of the main obstacles is the dependence on imported reagents based on human or animal serum, which are expensive and difficult to find in 3T (underdeveloped, frontier, and outermost) areas (2). Based on data from the Central Statistics Agency and the Indonesian Red Cross, the total ratio of Indonesian Red Cross Blood Donation Units (UDD PMI) to the number of districts in Indonesia is 0.54% (3,4). In areas that do not have health facilities such as UDD PMI, there are limitations in storage, logistics, and uneven distribution of commercial reagents. The development of alternative methods sourced from local materials is very promising, especially for expanding access to blood group screening in health facilities with limited resources.

One promising innovative approach is the use of natural compounds in the form of lectins. Lectins are carbohydrate-binding proteins that can specifically bind to antigens on erythrocytes (5). Lectins have been used in blood group screening research, such as lectins from Dolichos biflorus (a plant native to India) and lectins from Ulex europaeus (a plant native to Europe) (6). However, exploration of lectin sources from local Indonesian plants is still very limited. In this context, beans (Phaseolus vulgaris), a widely cultivated plant in Indonesia, emerge as a potential candidate. Several previous studies have shown that lectins from Phaseolus vulgaris are capable of agglutinating blood groups through interaction with carbohydrate residues such as galactose. Preliminary in vitro tests on bean extracts

showed fairly high haemagglutination activity, with titres ranging from 1/32 to 1/128 (6), 1/16 to 1/256 (7), and 1/512 (5). However, to date, there has been no further in vitro research in Indonesia that comprehensively evaluates the haemagglutination activity of bean extract, including titre reproducibility tests, ABO blood group screening tests, microscopic observations using a light microscope, and validation tests with commercial reagents as comparators. This research gap indicates a significant opportunity to develop innovations based on local resources that have the potential to replace dependence on imported reagents. Based on this background, this study aims to evaluate the potential of bean extract (Phaseolus vulgaris) through in vitro haemagglutination tests as an innovative candidate in blood group screening.

Based on this background, this study formulates several main questions, namely: (1) Does bean extract (Phaseolus vulgaris) show consistent haemagglutination activity against ABO blood groups? (2) Can bean extract identify blood groups with results comparable to commercial Anti A and Anti-B reagents? and (3) How does the variation in the ratio of bean extract to NaCl affect the strength of the erythrocyte agglutination reaction? Therefore, this study aims to (1) determine the optimal ratio between bean extract and physiological NaCl that produces the strongest agglutination reaction, (2) compare the pattern and intensity of agglutination reactions between pea extract treatments and commercial reagents, and (3) assess the consistency of agglutination results from pea extract through test replication (titre replication). Overall, this study is expected to provide strong scientific data on the potential of lectin from beans as an innovative candidate for blood group screening reagents that are local, affordable, and replicable, thereby contributing to improved access and efficiency of health services, especially in resource-limited areas.

MATERIALS AND METHODS

Time and Place of Research This research was conducted over a period of four months, from July to October 2025, at the Molecular Biology and Haematology Laboratory, Faculty of Health Sciences, Dr. Soetomo University.**Materials and Equipment** UsedThe main materials were green beans (Phaseolus vulgaris) from traditional markets and supermarkets. Supporting materials included whole blood groups A, B, AB, and O, 0.9% physiological NaCl solution, biuret solution, and commercial Anti-A and Anti-B reagents. The equipment used included a moisture meter, mortar, 50 and 80 mesh sieves, Erlenmeyer flasks,test tubes, Pasteur pipettes, magnetic stirrer, 4°C refrigerator, centrifuge, U-bottom plate test,microscope slides, cover slips, light microscope, digital camera, and image processing software (MScopes).**Research Variables** The variables in this study were classified into independent, control, and dependent variables. The independent variable studied was the concentration of bean extract (Phaseolus vulgaris),which was defined as the amount of extract containing natural lectin, determined through two parameters: (a) variation in the ratio of extract mixture with physiological NaCl (with categories 2:2, 2:4, 2:6, and 2:8 v/v) and (b) lectin dilution titre (1/256, 1/128, 1/64, and 1/32). These variables were measured using a ratio and titre scale, which were tested through direct agglutination tests on ABO system erythrocytes. The control variables used were commercial Anti-A and Anti-B reagents, which served as standard reagents for comparing the agglutination reaction results of the bean extract. The measurement was carried out through a direct agglutination test on ABO system erythrocytes and categorised nominally (Anti-A and Anti-B). Meanwhile, the dependent variable in this study was the degree of erythrocyte agglutination, which is the level of erythrocyte clumping due to the interaction of agglutinins in bean extract with ABO antigens. This degree of agglutination was measured through macroscopic observation on a microtiter U-plate and assessed using a haemagglutination scale of 0 to 4 (ordinal scale), with categories 0 (none), 1 (very weak), 2 (weak), 3 (moderate), and 4 (strong)(8). **Research Stages Collection and Preparation of Materials** The research began with the collection of bean seeds from two sources, namely traditional markets and supermarkets. All seeds underwent sorting, cleaning, washing, weighing, and moisture content measurement using a moisture meter. Drying was carried out naturally under the sun until the moisture content fell below 10%. After drying, the beans were weighed againto ensure moisture content stability (9) **Process of Extracting Lectin from Bean Seeds** Dried bean seeds were ground using a mortar until they became a fine powder and tested for protein content using the Biuret test. A positive result was indicated by a colour change to purple, signifying the presence of protein indicating lectin (10). The powder, which has been standardised in size using a 50-mesh sieve, was extracted using a 0.9% physiological NaCl solution at a ratio of 1:10 (weight/volume). The selection of 0.9% NaCl was based on its isotonic properties towards red blood cells and its ability to maintain the stability and activity of proteins, including lectins, during the extraction process (11). The extraction process was carried out by stirring using a magnetic stirrer for 3 hours at room temperature (25–27°C) until a homogeneous solution was formed. The filtrate from the extraction was filtered using an 80-mesh sieve and incubated at 4°C for 24–48 hours to facilitate natural precipitation, then centrifuged three times at 1500 rpm for 2 minutes. The supernatant obtained was collected and stored at 4°C as a crude lectin fraction (7) **Blood Sample Preparation** Blood samples from groups A, B, AB, and O were used in this study, each from one healthy donor who had provided written informed

consent. Blood sampling is performed aseptically by trained analysts in accordance with laboratory safety procedures. The total volume of blood used is approximately 5 drops per blood group. The process begins with washing the whole blood using 0.9% physiological NaCl to remove plasma and antibodies that could interfere with the agglutination test. Washing is performed three times with centrifugation at 3500 rpm for two minutes until pure erythrocytes are obtained. A 5% suspension is then made by mixing one drop of erythrocytes with 19 drops of saline solution. All processes are performed aseptically to maintain the purity of the results (12) **Macroscopic In Vitro Agglutination Test** The agglutination test was conducted to assess the ability of bean extract to induce agglutination in human erythrocytes. The lectin extract obtained in the previous stage was used as an agglutination test reagent to assess its biological activity. Each well of the microtiter plate was filled with a total volume of 100 µL, consisting of a mixture of bean extract and 5% erythrocyte suspension with varying drop ratios (2:2, 2:4, 2:6, and 2:8), where one drop was equivalent to ±25 µL. Thus, these ratios represent variations in the proportion of extract: physiological NaCl to determine the optimum concentration of natural agglutinin. In addition, variations in the dilution titre of the extract (1/256, 1/128, 1/64, and 1/32) were also carried out to test the stability of agglutination activity against dilution. A fixed volume of 25 µL of erythrocyte suspension was added to each well. A 5% erythrocyte suspension was added to each treatment, then observed macroscopically for a maximum of 10 minutes. Each test was performed three times to ensure reproducibility of the results. The agglutination reaction was assessed using a standard visual haemagglutination score of 0-4 (8). **Validation Test of Results with Commercial Reagents** Validation was performed to ensure that the agglutination reaction that occurred was specific to the ABO blood group antigen (Oktari et al., 2016). A comparative test was performed using commercial Anti-A and Anti-B reagents as positive controls and untreated columns as negative controls. Treatment with bean extract was performed at specific concentrations and titres. The reaction results were compared macroscopically with positive and negative controls to assess the suitability of the agglutination pattern (13).

Data analysis

The data obtained from the agglutination observations were analysed qualitatively using descriptive-comparative methods and quantitatively using the JASP statistical application version 0.95.3. Quantitative analysis was performed using the Kruskal Wallis nonparametric test to determine differences between ratios, followed by the Dunn test. The nonparametric test was chosen based on the ordinal scale characteristics of the data and the fact that it did not meet the assumptions of normal distribution. The significance level was set at $\alpha = 0.05$. The results of the analysis are presented in the form of a table of mean rank values and p-values to illustrate the significance between treatment groups.

RESULTS AND DISCUSSION

Overview of Research Result

This study aims to evaluate the potential of lectin extract from *Phaseolus vulgaris* (green bean) seeds as an alternative agglutinin in identifying ABO blood groups through in vitro haemagglutination tests. The results of the study show that all stages of the research, from material preparation, extraction process, macroscopic haemagglutination test, to titer validation and replication tests, have achieved measurable indicators with an average success rate of 90–100% against the research targets. In general, the bean lectin extract showed stable and specific agglutination ability towards blood group A and B erythrocytes, while blood group O did not show any clumping reaction. Overall, the results of this research indicate that bean extract has the potential to be used as a natural, environmentally friendly, and economical agglutination reagent base material.

Preparation and Selection of Materials

Bean seeds were obtained from two sources, namely traditional markets and supermarkets. Seeds from supermarkets were more uniform in size and had a more stable moisture content after natural drying below 10%. These conditions met the criteria for raw material homogeneity for the protein extraction process. Thus, the material preparation stage was considered completely successful (100%) according to the raw material quality indicators.

Table 1. Sources and characteristics of the bean seeds used.

Source	Intial Weight (grams)	Initial Moisture Content (%)	Final Weight (grams)	Final Moisture Content (%)
Traditional Market	70	39,1	29	6,3
Supermarket Trial (1)	150	41,5	34,5	Lo
Supermarket Tria (2)	106,2	26,7	28,2	5,5

Lectin Extraction Process

The Biuret test showed a positive reaction (purple colour), indicating the presence of protein compounds (lectin) in the bean powder. After the stirring and gradual filtration process, the resulting filtrate appeared clear and yellowish without coarse sediment. Repeated centrifugation strengthened the clarity of the extract, indicating the successful separation of soluble protein fractions, including lectin. These results show that the extraction method using a 0.9% physiological NaCl solution was able to produce a stable and homogeneous active protein fraction. The achievement indicator of "formation of a clear extract without sediment" was fully achieved (100%). The results of the lectin extraction process from bean seeds using a 0.9% physiological NaCl solution are shown in **Figure 1**.

Figure 1. Process of lectin extraction from bean seeds using 0.9% physiological NaCl solution. Clear yellowish filtrate without sediment indicates successful separation of soluble protein fractions.

Preparation of Erythrocyte Suspension

Erythrocytes of blood groups A, B, AB, and O were washed three times using physiological NaCl solution to remove residual plasma and free proteins. The clear supernatant produced indicated the success of the washing process. The resulting 5% erythrocyte suspension is stable and homogeneous, with no signs of haemolysis. This result ensures the validity of the agglutination test and meets the preparation stage achievement indicator (100%). The washing process and preparation of the 5% erythrocyte suspension can be seen in **Figure 2.**

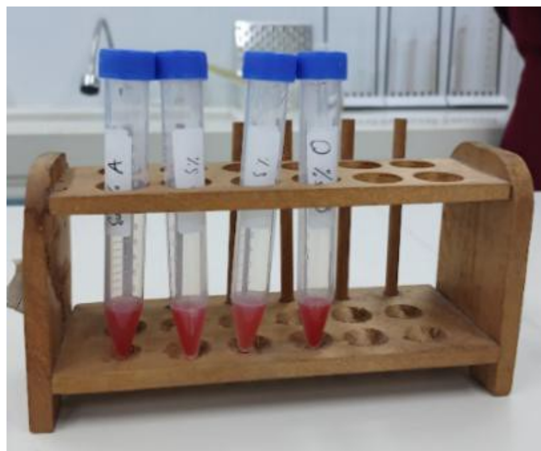

Figure 2. Whole blood washing to obtain pure erythrocytes and preparation of a 5% erythrocyte suspension. The clear supernatant indicates successful removal of plasma and free proteins.

Macroscopic In Vitro Agglutination Test

Macroscopic agglutination reactions were successfully observed in several combinations of concentrations and titers of the extract. Agglutination was most clearly seen in blood group A with a NaCl and bean extract ratio of 2:4 and a titre of 1/256, blood group B with a NaCl and bean extract ratio of 2:4 and a titre of 1/64, and blood group AB with a NaCl and bean extract ratio of 2:6 and a titre of 1/64. Meanwhile, no agglutination occurred in blood group O, indicating no interaction between the active components of the extract and the A or B antigens on the surface of the erythrocytes of that blood group. These results indicate that the bean seed extract contains agglutinins (lectins) that specifically interact with the A and B antigens on the surface of erythrocytes. The indicator "clear erythrocyte agglutination" was achieved at 100%, with slight variations in the repeat results. Table 2 shows the results of the macroscopic agglutination test using the U-bottom plate test method. A red spot in the centre of the well indicates a positive agglutination reaction, while clear liquid indicates no clumping.

Table 2. Results of Macroscopic Agglutination Test at Various Ratios of Bean Extract and NaCl Mixtures and Variations in Dilution Titres.

Blood Group	Nacl Ratio: Bean Extract	Titre	First Experiment	Second Experiment	Third Experiment	Visual Results
A	2:2	1/256	0	0	0	
		1/128	0	0	0	
		1/64	0	0	0	
		1/32	0	0	0	
	2:4	1/256	4	4	4	
		1/128	0	0	0	
		1/64	0	0	0	
		1/32	0	0	0	
	2:6	1/256	2	2	2	
		1/128	2	2	2	
		1/64	2	2	2	
		1/32	2	2	2	
	2:8	1/256	4	4	4	
		1/128	4	4	4	
		1/64	4	4	4	
		1/32	4	4	4	
B	2:2	1/256	0	0	0	
		1/128	0	0	0	
		1/64	0	0	0	
		1/32	0	0	0	
	2:4	1/256	4	4	4	
		1/128	4	4	4	
		1/64	4	4	4	
		1/32	0	0	0	
	2:6	1/256	1	1	1	
		1/128	1	1	1	
		1/64	1	1	1	
		1/32	0	0	0	
	2:8	1/256	4	4	4	
		1/128	4	4	4	
		1/64	4	4	4	
		1/32	4	4	4	
AB	2:2	1/256	0	0	0	
		1/128	0	0	0	
		1/64	0	0	0	
		1/32	0	0	0	
	2:4	1/256	0	0	0	
		1/128	0	0	0	
		1/64	0	0	0	
		1/32	0	0	0	
	2:6	1/256	3	3	3	
		1/128	3	3	3	
		1/64	3	3	3	
		1/32	0	0	0	
	2:8	1/256	4	4	4	
		1/128	4	4	4	
		1/64	4	4	4	
		1/32	4	4	4	
O	2:2	1/256	0	0	0	
		1/128	0	0	0	
		1/64	0	0	0	
		1/32	0	0	0	
	2:4	1/256	0	0	0	
		1/128	0	0	0	
		1/64	0	0	0	
		1/32	0	0	0	
	2:6	1/256	0	0	0	
		1/128	0	0	0	
		1/64	0	0	0	
		1/32	0	0	0	
	2:8	1/256	2	2	2	
		1/128	2	2	2	
		1/64	2	2	2	
		1/32	2	2	2	

Agglutination test results show that a mixture ratio of bean extract:NaCl of 2:4 produces the strongest agglutination in blood groups A and B. Physiochemically, this condition is thought to be influenced by the balance between the concentration of active lectin protein and the ionic strength of the solution. At a ratio of 2:2, the amount of protein is relatively high, but the viscosity of the solution increases, limiting lectin–antigen interaction. The 2:4 and 2:6 ratios are the optimum points where the lectin protein concentration is sufficient to form erythrocyte aggregation without interfering with particle mobility. Meanwhile, at a ratio of 2:8, although the agglutination strength is high, the reaction tends to be unstable and there is slight variation between repetitions due to a decrease in the concentration of active protein in the final phase of the reaction. Therefore, the ratios of 2:4 and 2:6 are considered to provide the best balance between intensity, consistency, and clarity of visual results, so they are maintained as the optimum points in subsequent analyses. Strong positive agglutination reactions in blood groups A, B, and AB indicate that the lectin in the bean extract is capable of recognising specific carbohydrate residues on antigens A and B, whereas in blood group O (which lacks these antigens) no reaction occurs (14).

This finding reinforces the assumption that the active component in bean extract functions as a natural agglutinin that is selective towards the antigenic structure on the surface of erythrocytes. This finding is consistent with several previous studies reporting that lectins from *Phaseolus vulgaris* are capable of agglutinating human erythrocytes through specific binding to carbohydrate residues, particularly galactose and N-acetylgalactosamine, which are part of the A and B antigen structures (5). Conversely, blood group O erythrocytes do not have these terminal residues, so they cannot bind to bean lectin and do not cause agglutination (15). This supports the hypothesis that bean lectin exhibits specificity towards the A and B antigens in the ABO blood group system. Thus, these test results reinforce the hypothesis that bean extract contains natural agglutinins that can be used as an alternative to commercial reagents.

Validation Test with Commercial Reagents

The validation test showed consistent results, namely agglutination only occurred in the treatment group and positive control (Anti-A, Anti-B), while the negative control showed no reaction. This confirms that the agglutination that occurred was specific and not the result of a cross-reaction. Stable test results in three repetitions prove that bean extract has high reproducibility and meets the validation stage success indicator (100%). The results of the agglutination validation test of bean extract with commercial reagents are shown in Figure 3. The red spots or deposits visible in the wells indicate the agglutination of erythrocytes, i.e. clumping due to the interaction between agglutinin (lectin) in the bean extract and specific antigens on the surface of erythrocytes. A positive agglutination reaction is characterised by a distinct red spot in the centre of the well, while the surrounding fluid appears clear or slightly cloudy. In the first image, it can be seen that treatment with bean extract (1/256;2:4, 1/64;2:4, and 1/64;2:6) showed agglutination patterns similar to the positive controls, commercial reagents Anti-A (KPA) and Anti-B (KPB), indicating the presence of haemagglutination activity specific to blood groups A, B, and AB.

Conversely, wells without red spots or with a homogeneous pink suspension indicated no agglutination (negative reaction), as seen in the negative control (NC) and in the test with blood group O. This phenomenon reinforces that the lectin in the bean extract is able to selectively recognise the A and B antigens, while not reacting to blood group O erythrocytes that do not have these antigens.

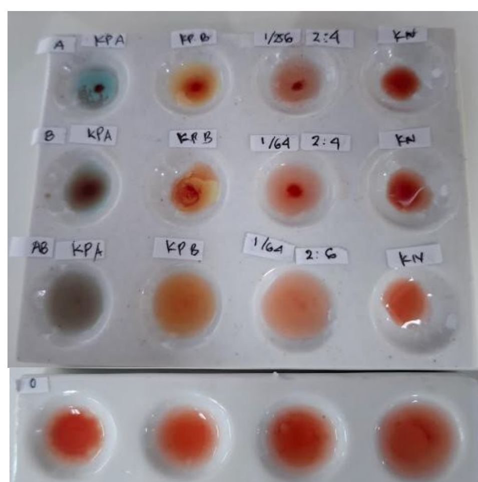

Figure 3. Results of Agglutination Validation Test of Bean Extract with Commercial Anti-A and Anti-B Reagents.

Agglutination Titer Reproducibility

The consistency of the results was tested through three replicates at each dilution. The agglutination titre was stable in the range of 1/64 to 1/256, indicating that the reaction power did not decrease significantly between replicates. Small variations that appeared in the three repetitions at several concentrations were due to differences in the visual intensity of agglutination in some tests, where the erythrocyte clumps appeared finer or less clear, resulting in a slightly weaker positive interpretation compared to the previous repetition.

Another factor contributing to this variation was the lighting during visual observation, which could cause differences in perception when assessing the strength of agglutination. Therefore, the achievement of the "consistency of replication results" indicator was set at 90%, reflecting that most of the results showed similar

reaction patterns with minor deviations that were still within reasonable limits for visual observation-based tests. Despite minor variations, the replication results still showed a consistent reaction trend and did not change the main conclusions of the study, so that the validity of the results was maintained and scientifically accountable.

Data Analysis

The data from the agglutination test observations were analysed quantitatively and qualitatively. Quantitatively, each well on the microplate was given a visual agglutination score of 0–4 based on the intensity and pattern of the clumps, with the categories being 0 (negative), 1 (weak), 2 (moderate), 3 (strong), and 4 (very strong). The scores from three replicates were recorded in an observation table and the median or mean was calculated for each treatment. This ordinal-scale quantitative data was analysed using nonparametric tests with the JASP application. The Kruskal Wallis test was used to determine differences in agglutination levels between NaCl:bean extract mixture ratios and between blood groups. If there were significant differences, the analysis was continued with Dunn's test to see the differences between treatment pairs, including comparisons between bean extract and positive controls (commercial Anti-A and Anti-B reagents) and negative controls (no treatment).

In addition, clotting time (minutes) was also recorded as a supporting parameter for agglutination reactivity, where commercial reagents showed a rapid reaction (<1 minute) while bean extract showed a clotting time in the range of 8–9 minutes. Qualitatively, the observation results were described based on the agglutination pattern formed, the clarity of the liquid, and the consistency of the reaction between repetitions. The agglutination pattern of the bean extract was then compared with the positive and negative control results to assess the specificity, equivalence, and validity of the reaction. All observation results were supplemented with documentary photographs, agglutination score tables, JASP analysis results, and supporting narrative descriptions to reinforce visual and statistical interpretations. Table 3 shows the results of the Kruskal Wallis test and Table 4 shows the results of the Dunn test.

Table 3. Results of the Kruskal Wallis Test on the Agglutination Scores of Bean Extracts Based on Blood Type.

Variable	H	df	p-value	Description
Agglutination Score (A, B, AB, O)	8.242	**3**	0.041	Significant

Table 4. Dunn's Test Results on Bean Extract Agglutination Scores Based on Blood Type.

Comparison	Z	p-value	Description
A - AB	-0.985	0.325	Insignificant
A - B	-0.744	0.457	Insignificant
A - O	1.969	0.049	Significant
AB - B	0.311	0.755	Insignificant
AB - O	2.558	0.011	Significant
B - O	2.491	0.013	Significant

Discussion

Thorough discussion represents the causal effect mainly explains for why and how the results of the research were taken place, and do not only re-express the mentioned results in the form of sentences, not repeat them. Concluding sentence should be given at the end of the discussion.

CONCLUSIONS

Pea seed extract (*Phaseolus vulgaris*) has been shown to have significant potential as an alternative agglutination reagent for ABO blood group screening. This study shows that the lectin contained in pea extract is capable of specifically agglutinating blood group A, B, and AB erythrocytes, while showing no reaction to blood group O. Macroscopic testing and statistical analysis confirmed significant differences in reaction between blood group O and blood groups A, B, and AB, proving the specificity of lectin to antigens A and B. The optimal ratio of extract and physiological NaCl for producing strong and stable agglutination was 2:4 and 2:6. Furthermore, validation test results showed agglutination patterns consistent with commercial Anti-A and Anti-B reagents, reinforcing the validity of this extract as a candidate reagent. Thus, this study successfully demonstrated that bean extract is an effective, stable, and selective natural agglutinin, with the potential to be further developed into an economical and accessible phytodiagnostic reagen

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Authors' Contributions: Muhammad Zelizar Yarta: methodology design, data analysis, and writing of results and discussion. Jessy Gre Dini Kurnia: data collection, agglutination test, and preparation of introduction/literature review. Shafy Dwi Febrianto: lectin extraction, documentation of results, and statistical analysis. Treticha Kaa Lera Mau: validation of findings

and compilation of tables/data visualisation. Cityta Putri Kwarta: final editing of the manuscript and formatting.

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