HEMAGGLUTINATION EVALUATION OF PLANT EXTRACTS FROM TARO (*Colocasia esculenta*), PANDAN (*Pandanus amaryllifolius*), AND TOBACCO (*Nicotiana tabacum*) IN ABO BLOOD GROUP

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ABSTRACT

Lectins are carbohydrate-binding proteins widely found in plants, recognized for their capacity to agglutinate red blood cells by binding to surface antigens. This study evaluated the hemagglutination activity of plant extracts containing lectins from taro (*Colocasia esculenta*), pandan (*Pandanus amaryllifolius*), and tobacco (*Nicotiana tabacum*) in relation to ABO blood group typing. Aqueous extraction followed by dialysis was used to purify lectins in the plant extracts. Percent yield analysis showed that tobacco produced the highest yield, followed by taro and pandan. Hemagglutination activity was then evaluated through macroscopic reaction grading using slide tests and tube methods. Despite its high yield, tobacco extract showed no visible agglutination. In contrast, taro extract exhibited moderate agglutination with blood types B and AB, while pandan extract exhibited moderate agglutination with types A and AB. Specificity testing using antibody titration confirmed that both taro and pandan extracts induced agglutination at lower concentrations, indicating strong and specific lectin activity. Statistical analysis using one-way ANOVA revealed a significant difference in hemagglutination activity among the plant extracts, confirming that the type of extract influences reactivity. The findings indicate that taro and pandan extracts containing lectins possess strong, specific hemagglutination properties, supporting their potential use as natural reagents in ABO blood typing. Their ability to induce agglutination even at lower concentrations highlights their promise as eco-friendly, cost-effective alternatives to synthetic reagents in clinical diagnostics.

Keywords: Antibody titer, dialysis purification, plant extract containing lectins, slide test, tube method

INTRODUCTION

Background of the Study

Blood typing techniques rely on specific antisera antibodies to detect blood group antigens. These antibodies bind to antigens on red blood cells, causing hemagglutination (Lab Tests Guide, 2023). Clumping identifies blood types by which antisera react—for instance, agglutination with anti-A but not anti-B antibodies indicates type A blood (Hernandez, 2023). This process is crucial for transfusion and transplantation compatibility as well as other diagnostic applications.

Antiserum used for humans is often produced from immunized animals (European Medicines Agency, 2016), making it costly—about ₱1,200 per 10 mL in the Philippine medical supply (Philippine Medical Supply, 2024). Its production raises ethical issues concerning animal use. Alternatively, lectins—proteins found in plants, animals, and microbes—bind to cell-surface sugars and induce agglutination (Butnariu & Butu, 2021). This property allows lectins to serve as substitutes for traditional antisera in blood typing.

The shift toward plant-based reagents is also supported by ethical and scientific concerns regarding animal-derived antibodies, which show variability, low specificity, and

reproducibility issues (Gray *et al*., 2020). The European Union's Directive 2010/63/EU promotes non-animal methods, aligning with global efforts to improve research practices. By leveraging the Philippines' biodiversity, plant lectins offer a feasible alternative that aligns with these ethical and scientific standards.

Hemagglutination, the clumping of red blood cells through antigen–antibody or lectin interactions, is a well-established diagnostic principle (LibreTexts, 2023). Its applications include blood typing, bacterial strain identification, and viral assays (Dutta, 2021). Discoveries by Landsteiner and subsequent studies on erythrocyte agglutination laid the foundation for transfusion safety and remain central in modern immunodiagnostics (Coico & Sunshine, 2015).

While numerous studies have investigated lectins from plants for antimicrobial, antifungal, and anticancer uses, fewer have examined their direct applications in diagnostics, such as ABO typing (Pereira *et al*., 2014; Pereira *et al*., 2018). Limited research exists on locally available plants such as taro, pandan, and tobacco, despite their rich phytochemical composition and documented hemagglutination properties (Ahmed *et al*., 2020; Ooi *et al*., 2004; Diyana *et al*., 2021; Delporte *et al*., 2015). This gap highlights the opportunity to develop affordable, plant-based blood typing reagents. Although the plants are well studied, hemagglutination has not been explored in depth, particularly in the context of blood typing.

Ultimately, exploring *C. esculenta*, *P. amaryllifolius*, and *N. tabacum* for hemagglutination activity provides a sustainable and ethical alternative to animal-derived antisera. Addressing challenges of extraction variability and ensuring reliable agglutination testing could enable the development of diagnostic kits that are not only scientifically robust but also practical in low-resource settings (Muhibi *et al*., 2024). Such innovation contributes to affordable and accessible healthcare solutions in the Philippines and beyond.

Statement of the Objectives

This study was conducted to evaluate the hemagglutination activity of lectin-containing plant extracts from *C. esculenta, P. amaryllifolius,* and *N. tabacum* for their potential use as alternative antisera in ABO blood group typing. Conducted from January to April 2025, the study specifically aimed to answer the following questions:

1. To determine the percent yield of the extract from the following plants:
    a. *Colocasia esculenta*
    b. *Pandanus amaryllifolius*
    c. *Nicotiana tabacum*

2. To evaluate the hemagglutination activity of the plant lectins in terms of the following:
    a. Hemagglutination reaction grading through macroscopic observation
    b. Specificity of ABO blood groups based on antibody titer

METHODOLOGY

Research Design

The study used a comparative-experimental design. Hemagglutination reactions were tested using commercial antisera as the control to evaluate the potential of plant lectin extracts as alternative reagents for ABO blood typing. Different plant extracts were assessed for their strength and specificity, and the results were compared with standard blood typing sera to determine accuracy.

Study Site and Collection

The research was conducted at the Center for Natural Sciences laboratories of Saint Mary's University, Bayombong, Nueva Vizcaya, using facilities equipped to evaluate the hemagglutination activity of plant extracts and assess their potential application as natural alternative antisera for blood typing. Plant specimens were collected from Brgy. Pogonsino, Bagabag, Nueva Vizcaya, and Roxas, Isabela.

Plant Certification

The *Colocasia esculenta*, *Pandanus amaryllifolius*, and *Nicotiana tabacum* were identified and certified for their botanical or taxonomic identity by the College of Forestry, Environment and Resources Management at Nueva Vizcaya State University, Bayombong campus.

Blood Donors

Blood samples were collected from four volunteer donors at Saint Mary's University, each representing one of the four ABO blood groups (A, B, AB, and O). The donors met the inclusion criteria and provided informed consent prior to participation. These samples were used to evaluate the hemagglutination activity of the plant extracts.

Data Gathering Procedure

Plant Collection and Extraction

The plant samples were washed, air-dried for 24 hours in a shaded area, and homogenized into a coarse or powdered form. About 25 grams of each sample were mixed with 50 mL of normal saline solution (NSS), stirred, and filtered through a mesh to separate the residues. The filtrate was left to settle at room temperature, after which the supernatant was collected for analysis, following the same standardized procedure for all samples with minor adjustments as needed.

Percent Yield Computed

Percent yield was calculated to determine the amount of usable lectin-containing extract recovered after processing. Plant materials were air-dried for 24 hours, homogenized with 50 mL of NSS, and filtered to obtain the soluble components. The yield was expressed as the ratio of the collected extract volume to the initial homogenized mixture, multiplied by 100.

Precipitation of Proteins

Proteins were precipitated by adding an 80% w/v ammonium sulfate solution, prepared by dissolving 80 g of ammonium sulfate in 100 mL of Normal Saline Solution (NSS), to the plant extracts. Each mixture was stirred thoroughly and left to stand overnight to allow complete protein precipitation. The following day, the mixtures were centrifuged at 3,400 RPM for 15 minutes. After centrifugation, the supernatants were discarded, and the protein precipitates were collected for the next step.

Dialysis Purification

Dialysis was performed for further protein purification using tubing with a molecular weight cutoff of 8,000–14,000 Da and a 25 mm diameter. The tubing was first hydrated in Normal Saline Solution (NSS), then filled with the protein precipitates using a pipette, and

sealed at both ends with clamps and knots to prevent leakage. The sealed tubing was submerged in NSS and stored in a refrigerator for 24 hours. After dialysis, the tubing was cut open, and the purified protein samples were transferred into clean test tubes.

Donor Screening and Blood Collection

Blood donors of types A, B, AB, and O were screened through a brief health interview to ensure eligibility. For venipuncture, standard procedures were followed: the donor was identified and prepared, hands were sanitized, gloves were worn, and the site was disinfected with 70% isopropyl alcohol. A tourniquet was applied, a suitable vein was selected, and 5 mL of blood was drawn into an EDTA tube. After collection, gauze was applied, the needle's safety feature was activated, and the used materials were properly discarded. Tubes were labeled with the donor's full name, age, time, and date of collection.

Red Blood Cell Suspension

Freshly drawn EDTA-anticoagulated blood was placed in a labeled 10-mL screw-capped conical tube and diluted to the 10-mL mark with normal saline solution (NSS). The mixture was gently inverted and centrifuged at 3,400 rpm for 2–3 minutes, after which the supernatant was removed with a Pasteur pipette. The red cells were washed three times by resuspending in NSS, mixing, and centrifuging to eliminate plasma and other non-red cell components. The final red cell suspension was then prepared in a Wasserman tube at the required concentration.

Hemagglutination Assay

The hemagglutination assay was conducted to test the agglutination activity of plant extracts containing lectins against ABO-typed red blood cells. Three approaches—slide, tube, and serial dilution—were used to observe and measure agglutination.

Control Procedures

Positive controls were prepared by mixing red blood cells with anti-A and anti-B antibodies to confirm test accuracy, while negative controls were used with saline solution to rule out false positives and verify reaction specificity.

Hemagglutination Assay Methods

In the slide method, plant extracts were combined with red cell suspensions on glass slides to observe visible clumping; positives were confirmed by the tube method through centrifugation and grading of agglutination. The serial dilution method further determined the minimum lectin concentration required for agglutination by performing two-fold dilutions, with the endpoint recorded at the last visible reaction.

Treatment of Data

The hemagglutination activity of *Colocasia esculenta*, *Pandanus amaryllifolius*, and *Nicotiana tabacum* extracts across different ABO blood types was statistically analyzed using one-way ANOVA. The ability of plant lectin extracts to hemagglutinate red blood cells was expressed as hemagglutination titer and compared among blood groups A, B, AB, and O.

Ethical Considerations

The study was approved by the Saint Mary's University Research Ethics Board (SMUREB) with address and information at 2nd Floor, Rev. John Van Bauwell Hall, SMU Main

Campus, Ponce Street, DMM, Bayombong, Nueva Vizcaya, Philippines, with a cellphone number: 09177053041 and email: reb@smu.edu.ph.

RESULTS AND DISCUSSIONS

Section 1. Percent Yield

Table 1

Percent Yield of Colocasia esculenta, Pandanus amaryllifolius and Nicotiana tabacum extracts

Plant Lectin Extracts	Percent Yield
Taro	40 %
Pandan	40 %
Tobacco	66.67 %

The table shows the percentage yield of the three plant extracts, with *N. tabacum* having the highest yield at 66.67%, while *C. esculenta* and *P. amaryllifolius* yielded the same amounts, but lower. This suggests that more extractable material was obtained from N. tabacum, though functional testing, such as hemagglutination, is still needed to confirm effectiveness.

Percent yield serves as a measure of extraction efficiency and helps optimize conditions such as solvent, temperature, and duration. A high yield may suggest efficiency but does not always reflect biological activity, as it can also be affected by impurities or errors (Patra *et al*., 2022; Steen, 2022).

In this study, *N. tabacum* had the highest yield but showed no agglutination, demonstrating that yield does not guarantee activity. In contrast, *C. esculenta* and *P. amaryllifolius* had lower yields, but both produced positive hemagglutination, confirming the presence of active bioactive compounds.

Section 2. Evaluation Of Hemagglutination Activity of the Plant Lectins

Hemagglutination Reaction Grading through Macroscopic Observation

Table 2

Hemagglutination Results via Slide Method

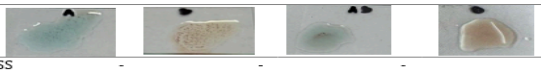
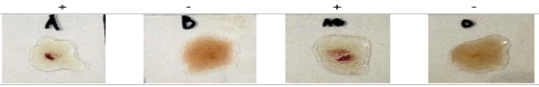
Treatment Groups		Blood Group A	Blood Group B	Blood Group AB	Blood Group O
Positive Control	Anti-A	+	-	+	-
	Anti-B	-	+	+	-
					
Negative Control	NSS	-	-	-	-
					
Taro Extract		-	+	+	-
					
Pandan Extract		+	-	+	-
					
Tobacco Extract		-	-	-	-
					

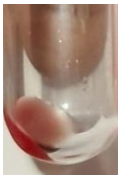
Table 2 shows the hemagglutination results using the slide method, where extracts from *C. esculenta*, *P. amaryllifolius*, and *N. tabacum* were tested against blood types A, B, AB, and O. Taro extract agglutinated B and AB, indicating specificity for A and B antigens, while pandan extract reacted with A and AB. No agglutination was observed with tobacco extract or blood group O, and results were confirmed with positive and negative controls.

Although the slide method is convenient, it is prone to false positives or negatives due to drying and weak interactions (Dayyal, 2017), consistent with the inconsistent results of tobacco extract. The specificity of taro and pandan lectins for A and B antigens supports previous findings, as lectins from *C. esculenta* and *P. amaryllifolius* do not bind to the H antigen in group O, unlike *Ulex europaeus* lectin (Kumari, 2023). This explains the absence of agglutination in O blood and reinforces the antigen selectivity of these antibodies.

The results confirm antigen-specific binding of taro to B antigens and pandan to A antigens, while excluding O due to lack of targets. Despite its high yield, *N. tabacum* showed no activity, highlighting that yield alone does not indicate diagnostic utility. Proper controls and consistent specificity support the potential of *C. esculenta* and *P. amaryllifolius* as plant-based alternatives for ABO blood grouping.

Table 3

Hemagglutination Results via Tube Method (Grading)

Treatment Groups		**Blood Group**				**Description**
		A	**B**	**AB**	**O**	
Positive Control	Anti-A	4+	-	4+	-	One solid aggregate of cells in blood types A and AB
	Anti-B	-	4+	4+	-	One solid aggregate of cells in blood types B and AB
						
Negative Control	NSS	-	-	-	-	No cell aggregates
						
Taro extract		-	4+	4+	-	One solid aggregate of cells in blood types B and AB; no cell

					aggregates in blood types A and O
Pandan extract	4+ 	- 	4+ 	- 	One solid aggregate of cells in blood types A and AB; no cell aggregates in blood types B and O
Tobacco extract	- 	- 	- 	- 	No cell aggregates in all blood types

The table presents the graded hemagglutination reactions observed through the tube method using extracts of *C. esculenta, P. amaryllifolius, and N. tabacum* on blood types A, B, AB, and O. Strong agglutination (4+) was seen with pandan in groups A and AB, and with taro in groups B and AB, confirming antigen specificity. In contrast, tobacco extract did not show agglutination. Positive controls (Anti-A and Anti-B sera) reacted as expected, while the saline negative control showed no reaction.

The tube method offered a more controlled environment than the slide test, reducing interference and enhancing reaction clarity (Mujahid *et al*., 2015). Proper controls validated that observed agglutination resulted from true lectin–antigen interactions (Harmening, 2019).

Overall, pandan and taro confirmed selective binding to A and B antigens, while tobacco extract lacked activity despite its higher yield. This highlights that functional effectiveness depends on lectin specificity rather than extract quantity.

Specificity of ABO Blood Groups Based on Antibody Titer

Table 4

Controls for the Antibody Titer Identification for Serial Dilution

Treatment Groups		**Dilution**								**Description**
		1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128-1:256	
Positive Control	Anti-A	+	+	+	+	+	+	+	-	low to high concentration of antibodies present; endpoint titer is 1:64

	Anti-B	+	+	+	+	+	+	+	-	low to high concentration of antibodies present; endpoint titer is 1:64
Negative Control	NSS	-	-	-	-	-	-	-	-	no visible agglutination

Table 4 presents the results of positive and negative controls in the serial dilution method. Antisera A and B (positive control) produced agglutination from 1:1 up to 1:64, confirming an antibody titer of 1:64, while the negative control (NSS) showed no agglutination across all dilutions. This verified that the assay functioned properly and no false positives occurred.

Controls were essential for reliability: positive controls confirmed reagent activity, while negative controls detected non-specific effects (Foldesi, 2021). Their inclusion validated both the sensitivity and specificity of the hemagglutination test.

Overall, the positive control confirmed accurate antigen–antibody reactions, and the negative control ensured the absence of background interference, strengthening confidence in the experimental results.

Table 5

Antibody Titer Specificity of Plant Extracts from Colocasia esculenta

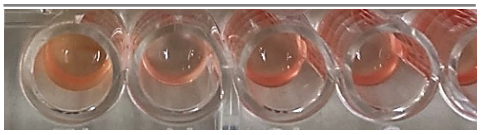
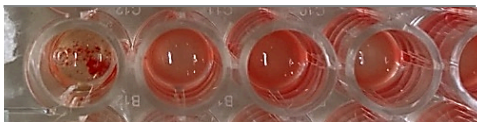
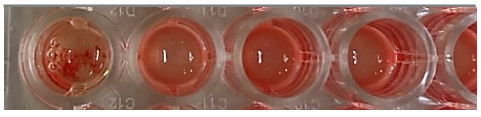
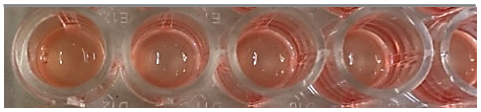
Blood Groups	Dilution			Description
	1:1	1:2	1:4 - 256	
A	-	-	-	no visible agglutination
				
B	+	+	-	moderate concentration of antibodies present; endpoint titer is 1:2
				
	f			
AB	+	+	-	moderate concentration of antibodies present; endpoint titer is 1:2
				
O	-	-	-	no visible agglutination
				

Table 5 shows the results of the serial dilution assay for *C. esculenta* extract, which agglutinated blood groups B and AB at dilutions 1:1 and 1:2. The endpoint titer was 1:2, confirming specificity for B-type antigens. No agglutination occurred with blood groups A and O, showing that activity was antigen-dependent and not due to non-specific interactions.

The taro extract containing lectin, particularly tarin, exhibited strong affinity for galactose-containing glycoconjugates abundant in B antigens, explaining the reactivity with blood types B and AB (Pereira *et al*., 2018).

These findings confirmed that the C. esculenta extract specifically targeted B antigens, with clear reactivity only in B and AB groups, further supporting its antigen-dependent lectin activity.

Table 6

Antibody Titer Specificity of Plant Extracts from Pandanus amaryllifolius

Blood Groups	Dilution			Description
	1:1	1:2	1:4 - 256	
A	+	+	-	moderate concentration of antibodies present; endpoint titer is 1:2
B	-	-	-	no visible agglutination
AB	+	+	-	moderate concentration of antibodies present; endpoint titer is 1:2
O	-	-	-	no visible agglutination

The results in Table 6 show the hemagglutination titers of the P. amaryllifolius extract obtained using the serial dilution method. Agglutination was observed in blood groups A and AB at dilutions 1:1 and 1:2, with no reaction in groups B and O, confirming strong specificity for A-type antigens. This highlighted the potential of pandan extract as a selective binder for identifying blood group A.

P. amaryllifolius contained lectins such as Pandanin, which specifically bind to N-acetylgalactosamine on A antigens. This explained the agglutination in groups A and AB, while no activity was seen in groups B and O. The difference between A and B antigens stems from glycosyltransferases: enzyme A adds N-acetylgalactosamine, while enzyme B adds D-galactose to the H-substance. The $NHCOCH_3$ group at the C2 position of N-acetylgalactosamine enables Pandanin to recognize and bind selectively (Gylmiyarova *et al*., 2018).

These findings confirmed pandan's specificity for A antigens, with consistent reactivity in A and AB blood groups. Its antigen selectivity emphasized its value as a plant-based reagent for low-cost or preliminary blood typing.

Table 7

Antibody Titer Specificity of Plant Extracts from Nicotiana tabacum

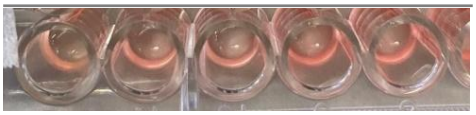
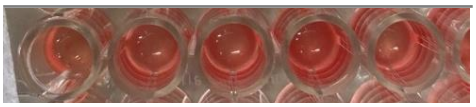
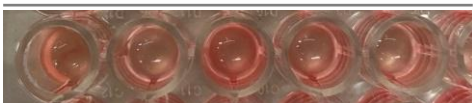
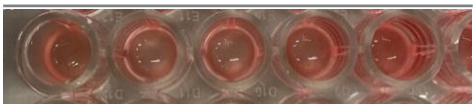
Blood Groups	Dilution			Description
	1:1	1:2	1:4 - 256	
A	-	-	-	no visible agglutination
				
B	-	-	-	no visible agglutination
				
AB	-	-	-	no visible agglutination
				
O	-	-	-	no visible agglutination
				

Table 7 illustrates the endpoint titers of N. tabacum extract obtained through serial dilution. No agglutination was observed in any blood group across all dilutions, indicating that the tobacco lectins were either inactive or unable to bind to ABO antigens under the test conditions.

This absence of activity may be explained by the lectin Nictaba, which is expressed only under stress stimuli such as cold, herbivory, or methyl jasmonate treatment (Chen *et al*., 2002; Delporte *et al*., 2025). Without induction, Nictaba remains inactive, accounting for the lack of hemagglutination. In contrast, taro and pandan extracts demonstrated expected lectin-antigen binding, confirming their functional specificity for B and A antigens, respectively. Serial dilution and titer analysis, as emphasized by Rodak *et al*. (2020), remain essential in immunohematology for determining antibody strength and ensuring reliable clinical interpretation.

These results showed that while pandan and taro extracts exhibited clear lectin activity, tobacco extract did not, underscoring that the diagnostic potential of plant-based reagents depends on both the source and the activation of their bioactive compounds.

Table 8

Descriptive Statistics of Hemagglutination Grades and One-Way ANOVA by Treatment Group

Treatment Group	n	Mean	SD	F	p
Taro extract	4	2.00	2.31		
Pandan extract	4	2.00	2.31		
Tobacco extract	4	0.00	0.00		
Anti-A (Positive)	4	2.00	2.31		
Anti-B (Positive)	4	2.00	2.31		
NSS (Negative)	4	0.00	0.00		
ANOVA Summary				6.40	.001*

significant at α=0.001

The results in Table 8 present the mean hemagglutination grades, their standard deviations, and the one-way ANOVA outcomes for each treatment group. Taro extract, pandan extract, and commercial antisera (anti-A and anti-B) all showed similar mean grades of 2.00 with high variability (SD = 2.31), while tobacco extract and the negative control (NSS) showed

no activity (mean = 0.00, SD = 0.00). These results indicated that taro and pandan extracts produced hemagglutination activity comparable to that of standard antisera, whereas tobacco showed no detectable activity.

The ANOVA confirmed statistically significant differences among groups ($F = 6.40$, $p = .001$), leading to rejection of the null hypothesis and establishing that at least one treatment induced a distinct hemagglutination response. These findings validated the biological activity of taro and pandan extracts and highlighted their potential as plant-based alternatives in diagnostic or therapeutic applications.

CONCLUSION AND RECOMMENDATIONS

Conclusion

This study evaluated the hemagglutination activity of plant extracts containing lectins from *Colocasia esculenta, Pandanus amaryllifolius*, and *Nicotiana tabacum* for ABO blood group typing. Tobacco extract had the highest yield but showed no agglutination, indicating that lectins were inactive under non-stress conditions. In contrast, taro extract agglutinated blood types B and AB, while pandan extract agglutinated A and AB, confirming their specificity for galactose- and N-acetylgalactosamine-containing antigens. Both extracts maintained activity at lower concentrations, highlighting their potential as natural alternatives to synthetic reagents in blood typing.

Recommendations

1. Isolate and purify lectins from plant extracts using techniques such as affinity chromatography and gel electrophoresis to reduce interference and improve potency and specificity.
2. Develop strategies to extend the shelf life of plant extracts for enhanced stability and long-term usability.
3. Utilize automated or semi-automated procedures to improve the accuracy and reliability of ABO blood typing assays with plant-based reagents.

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