HEMAGGLUTINATION REACTION OF THE PLANT SHOOT SYSTEM OF *Moringa oleifera* (MALUNGGAY), *Momordica charantia* (BITTER GOURD), AND *Phaseolus vulgaris* (BLACK BEANS) EXTRACTS FOR ABO BLOOD GROUP SYSTEM

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ABSTRACT

In the pursuit of affordable, ethical alternatives to commercial blood typing reagents, this study explored the hemagglutination potential of crude plant extracts from the shoot systems of *Moringa oleifera* (malunggay), *Momordica charantia* (bitter gourd), and *Phaseolus vulgaris* (black beans) in ABO blood grouping. The main objective was to determine whether these plant extracts could induce specific agglutination reactions and serve as alternative reagents for blood typing. An experimental research design was employed, using both slide and tube hemagglutination assays. Crude extracts from the seeds, stems, leaves, and seed pods of the selected plants were prepared and tested against blood types A, B, AB, and O. In terms of percentage yield, bitter gourd seed pods yielded the highest, followed by malunggay leaves, indicating higher concentrations of soluble bioactive compounds. For the agglutination reactions and grading, malunggay leaves exhibited strong agglutination with type A blood 4+ grade, bitter gourd seeds and seed pods reacted strongly with all blood types 4+, and bitter gourd stem showed specific agglutination with type B 4+. In contrast, black bean extracts showed no agglutination activity. These findings validated the agglutinating potential of malunggay and bitter gourd shoot systems and highlighted their viability as inexpensive, plant-based reagents for ABO blood typing. Further research is recommended to isolate and purify the active lectins for standardization and broader diagnostic use, particularly in low-resource clinical and field settings.

Keywords: agglutination, antisera, blood coagulation, lectins, plant-based reagents

INTRODUCTION

Background of the Study

In healthcare, hemagglutination is used to determine an individual's blood type by using antibodies that bind to specific antigens on the surface of red blood cells (RBCs) (Dutta, 2021). Antisera are commonly used for blood typing and medical transfusion practices, but they are expensive. As Charles River Laboratories stated in 2019, producing high-quality antisera involves complex processes, including immunization of biological materials and meticulous purification, which significantly increase costs. These plant-based alternatives could offer a more affordable, widely accessible option. They may also mitigate ethical and practical issues associated with the use of animal-derived reagents, offering safer alternatives for blood typing while addressing ethical concerns related to animal products.

Conventional blood typing reagents are commonly derived from animal plasma exposed to specific antigens, a process that incurs high production costs due to labor-intensive procedures, specialized equipment, and strict regulations. This reliance on costly, resource-intensive methods creates barriers in resource-constrained settings, particularly in low-income countries where access to these reagents is often unreliable (Kumar et al., 2018).

Given the high demand for affordable, accessible blood typing solutions, exploring plant-based alternatives offers an opportunity to address these challenges. Certain plants, such as *Moringa oleifera* (malunggay), *Momordica charantia* (bitter gourd), and *Phaseolus vulgaris*

(black beans), exhibit hemagglutination properties that could make them viable options for crude extracts used in ABO blood group testing. These plants are widely available, easy to cultivate, and contain bioactive compounds that induce hemagglutination without requiring extensive refinement.

Despite their potential, the application of plant-derived crude extracts, particularly from the shoot systems of these plants, in blood typing remains underexplored. Utilizing the shoot systems offers a significant advantage, as they are renewable resources that can be harvested without destroying the entire plant. This approach not only capitalizes on an abundant and sustainable botanical resource but also aligns with global efforts to develop environmentally sustainable healthcare solutions.

Developing plant-based substitutes for antisera could improve access to blood typing diagnostics, especially in low-resource areas. Extracts from plants such as malunggay, bitter gourd, and black beans may contain lectins that specifically bind to ABO blood group antigens, offering a safe, affordable, and eco-friendly alternative to traditional animal-derived antibodies. Because these plants are easy to grow and sustainable, their use could reduce costs, ethical concerns, and environmental impact, making blood typing more accessible in rural clinics, blood banks, and educational laboratories while supporting safer transfusions and better healthcare outcomes.

Statement of the Objectives

The study determined the hemagglutination reactions of the shoot systems of malunggay, bitter gourd, and black bean extracts using the ABO blood group system. The study was conducted from January to April 2025. Specifically, it sought to:

1. Determine the percent yield of the crude extract derived from the shoot system of the following plants.
    a. *Moringa oleifera*
    b. *Momordica charantia*
    c. *Phaseolus vulgaris*

2. Determine the agglutination reactions and corresponding reaction grades of the crude extracts derived from the shoot systems of the selected plants.

METHODOLOGY

Research Design

The study used an experimental approach to determine the hemagglutination reaction of crude extracts from the shoot systems of malunggay, bitter gourd, and black beans using the ABO blood group system by blood typing. Different laboratory procedures were conducted to assess the effectiveness of these plant extracts in agglutinating different blood types. Various plant extracts with agglutination activity were prepared and compared with a control sample, which served as a baseline to assess the possibility of using natural plant extracts as substitutes for solutions or reagents crucial to ABO blood typing.

Study Site and Sample Collection

The shoot systems of malunggay, bitter gourd, and black beans were collected from farms and gardens. Malunggay and bitter gourd were collected in Mabasa, Dupax del Norte, and black beans in San Pablo, Diadi. These locations were free of pollutants because the gardens were situated far from highways, and the soil was rich, ensuring the plants grew properly and abundantly without exposure to contaminants.

The study was conducted at the SMU–Center for Natural Science Research Laboratory, a controlled facility equipped with the necessary apparatus for accurate testing. This setting ensured reliable measurement of the hemagglutination activity of the plant extracts and supported a valid evaluation of the reactions among the different plant shoot systems.

Plant Identification

The taxonomic identification of the plants used in the experiment was certified by the Office of the Research Extension and Training at Nueva Vizcaya State University, Bayombong Campus, Bayombong, Nueva Vizcaya.

Blood Donors

Randomly selected Saint Mary's University students who consented to donate blood participated in the study. Four donors with blood types A, B, AB, and O were selected, and the selection process was repeated three times across three trials. Only ABO blood groups were considered, excluding factors like gender or age. For donors uncertain of their blood type, blood typing was performed under the supervision of a registered medical technologist before experimentation.

Preparation of Crude Extracts from the Plants

The shoot systems of malunggay, bitter gourd, and black beans were collected from farms and gardens in Dupax del Norte. The plants were washed, patted dry, and separated into parts before being stored in a temperature-controlled area. After air-drying for several days, they were oven-dried in the laboratory to ensure complete dehydration and prevent decay during the experiment.

Seeds and Leaves of the Plants

The procedure for deriving crude extracts from the leaves and seeds of the raw materials was adapted from the studies of Elmahboob et al. (2015) and Saha et al. (2022). The crude extracts were obtained from malunggay, bitter gourd, and black beans using chemical extraction and several centrifugation steps, and were then stored in a cool, dry place after drying to remove any moisture.

Measured amounts of malunggay, bitter gourd, and black bean plant parts were ground into fine powder, and 5 grams of each were extracted using 80 ml of phosphate-buffered saline. The mixtures were shaken for five hours at room temperature, refrigerated overnight at 4°C, and then filtered through mesh grit and Whatman No. 2 paper to obtain the liquid crude extracts. The crude extract solution from the leaves and seeds of malunggay, bitter gourd, and black bean was first centrifuged at 6000 rpm for 15 minutes at room temperature, following the method by Elmahboob et al. (2015). The liquid portion (supernatant) was then treated with saturated ammonium sulfate at 30%, 60%, and 80% to separate proteins. Afterward, the precipitated proteins were collected by a second centrifugation at 6000 rpm for 10 minutes.

The three protein pellets, each obtained at a different ammonium sulfate concentration, were combined in a single tube, dissolved in 5 mL of 0.85% NaCl, and subjected to a final centrifugation. The resulting supernatant was then dialyzed overnight against 0.85% NaCl.

Seed Pods and Stem of the Plants

Crude extracts from the stems and seed pods of malunggay, bitter gourd, and black beans were prepared following Saha et al. (2014). Plant materials were ground into a powder, extracted with 0.15 M NaCl, and filtered. The filtrates were centrifuged, and proteins were fractionally precipitated with ammonium sulfate, combined, dissolved in saline, centrifuged again, and dialyzed overnight. The resulting lectin-rich solutions were used for hemagglutination assays to test red blood cell agglutination for ABO blood typing. Biomass yield

was measured before and after extraction to calculate crude extract percentages, ensuring sufficient lectin concentration for effective agglutination.

The extraction process, adapted from Saha et al. (2014), involved grinding black bean stems, malunggay seed pods (1000 g), bitter gourd seed pods (1500 g), and black bean seed pods (250 g) into fine powder. Each 5 g sample was mixed with 80 mL of 0.15 M NaCl (1:8 w/v) and stored at 4°C for 48 hours. After filtration and centrifugation, the supernatants were precipitated with ammonium sulfate (40–70%), and the resulting pellets were dissolved in saline, re-centrifuged, and dialyzed overnight against 0.85% NaCl. The final extracts were used in hemagglutination assays for blood typing, with biomass yields and extract volumes measured to determine crude extract percentages and lectin activity.

Blood Sample Collection

Trained researchers, assisted by a Registered Medical Technologist, collected 5 mL of blood from each participant using an open-system method with EDTA as an anticoagulant (McPherson & Pincus, 2017). The procedure followed strict biosafety protocols, including the use of PPE, sterile materials, disinfected workspaces, and proper biohazard disposal. Participants' identities and medical histories were verified, informed consent was obtained, and post-procedure care was provided. All samples were securely stored, and any incidents were documented to ensure safe, ethical, and compliant blood collection.

Hemagglutination Assay

The hemagglutination assay utilized both slide and tube methods. The slide method served as a preliminary test to identify specific blood types that reacted with the plant extracts and to grade the strength of visible agglutination. Once specificity was confirmed, the tube method quantified the degree of agglutination (graded 4+ to 0) to evaluate the extract's potency. Before testing, blood samples were prepared into a 3% red cell suspension by repeatedly washing with normal saline and centrifuging, ensuring clean, uniform red blood cells for accurate hemagglutination assessment.

Slide method

A drop of 3% red blood cells (RBCs) from each blood type was mixed 1:1 with plant-extract antisera from malunggay, bitter gourd, and black beans on a glass slide. The researchers observed whether the RBCs clumped and graded the reaction to determine which blood types reacted to each plant extract. Positive and negative controls were included: commercial antisera served as the positive control, and plant extract with distilled water as the negative. The commercial antisera results were used to validate the plant-extract antisera outcomes.

Tube method

For the tube method, red blood cell suspensions were mixed with plant extracts in labeled test tubes, gently swirled, and centrifuged at 3400 rpm for 15 seconds. Agglutination was then observed, and the results were graded according to the degree of clumping.

Ethical Considerations

The study underwent an ethics review by the Institutional Biosafety Committee (IBC 2025-0877) of Saint Mary's University Research Ethics Board (SMU-REB) in Bayombong, Nueva Vizcaya, for approval and monitoring. The board was located on the 2nd floor of Rev. Fr. John Van Bauwel Hall, Saint Mary's University Main Campus, Ponce Street, Don Mariano Marcos,

Bayombong, Nueva Vizcaya. It can be reached via email at reb@smu.edu.ph or by calling 09177053041.

RESULTS AND DISCUSSIONS

Section 1. Percent Yield of the Crude Extract

Table 1

Percent Yield of Malunggay, Bitter Gourd, Black Beans

Plant Sample	Biomass Yield	Percent Yield
A. Malunggay		
1. Seeds	10g	21.93%
2. Stem	10g	30.49%
3. Leaves	10g	50%
4. Seedpods	10g	33.56%
B. Bitter Gourd		
1. Seeds	10g	19.84%
2. Stem	10g	27.86%
3. Leaves	10g	20.41%
4. Seedpods	10g	64.52%
C. Black Beans		
1. Stem	10g	35.84%
2. Leaves	10g	36.10%
3. Seedpods	10g	25.97%

The results showed that bitter gourd seed pods (64.52%) and malunggay leaves (50%) had the highest percent yields, indicating greater amounts of extractable soluble compounds. In contrast, bitter gourd and malunggay seeds yielded lower percentages (19.84% and 21.93%), while black bean leaves and stems had moderate yields (~36%). These findings suggest that bitter gourd seed pods and malunggay leaves are the most efficient sources for further hemagglutination analysis.

The solubility and retained bioactivity of these compounds support the use of dried crude extracts in assays, as demonstrated in studies such as those by Sousa et al. (2020) for malunggay and Mitra et al. (2018) for bitter gourd. These references reinforce the relevance of biomass yield in evaluating the potential of plant shoot systems as sources of lectins for hemagglutination testing. Likewise, Kubola and Siriamornpun (2014) demonstrated that bitter gourd's aerial parts, including seed pods, have high levels of bioactive compounds. Luz et al. (2014) also reported successful extraction yields from malunggay seeds using salt-based precipitation techniques, reinforcing the practicality of these methods for maximizing recovery.

The data presented highlights the variability in extract yields among different plant parts, with bitter gourd seed pods and malunggay leaves demonstrating the highest percent yields. These results indicate that these specific parts possess a higher concentration, making them efficient sources of crude extract. This is significant in the context of producing alternative antisera, as higher extract yields can provide more material for further processing and analysis.

Section 2. Hemagglutination Reaction and Grading

Table 2

Hemagglutination Reaction and Grading of Malunggay

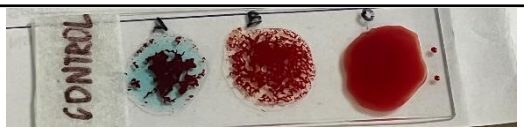
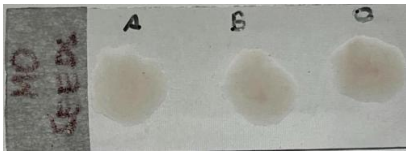
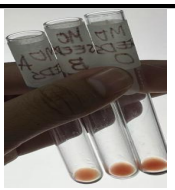
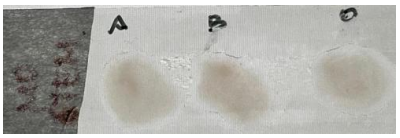
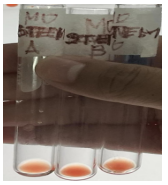
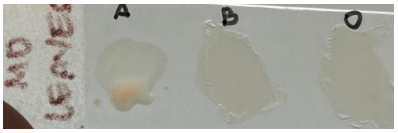
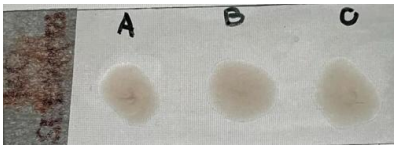
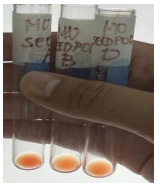
		Agglutination Reaction				Grading		
	Results	A	B	O	Results	A	B	O
Controls	 slide method				 tube method			
a. Seeds		-	-	-		0	0	0
b. Stem		-	-	-		0	0	0
c. Leaves		+	-	-		4+	0	0
d. Seed pods		-	-	-		0	0	0

Table 2 shows the hemagglutination reaction and grading of Malunggay using the ABO blood group system, with 4+ as the highest grade and 0 indicating a negative reaction. The hemagglutination tests showed that among malunggay (*M. oleifera*) shoot system extracts, only the leaves induced agglutination with blood group A, showing a positive reaction in the slide method and a 4+ grade in the tube method. Extracts from the seeds, seed pods, and stems did not produce agglutination in either method, yielding negative reactions and a 0 grade.

Malunggay contains various bioactive compounds, including proteins, peptides, and polysaccharides, that can affect cell agglutination. Its seeds contain peptides and glycoproteins with hemagglutination activity, similar to those of lectin-rich plants. They also contain cationic peptides that act as natural flocculants (Sousa et al., 2020) and are rich in health-promoting peptides (Gopalkrishna, 2016). In addition, malunggay seeds contain edible oils and coagulant proteins, both useful for water purification and dyeing. Lectins extracted from the seeds show both coagulant and anticoagulant properties in human blood (Luz et al., 2014).

Hence, the result shows that among the malunggay shoot parts tested, only the leaves exhibited a strong hemagglutination reaction specifically with blood type A, while the seeds, seed pods, and stems showed no agglutination across all blood types, because they contain more of the active compounds needed to trigger the reaction than the other plant parts.

Table 3

Hemagglutination Reaction and Grading of Bitter gourd

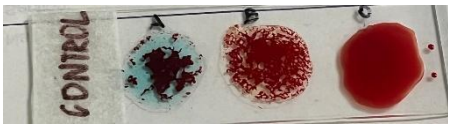
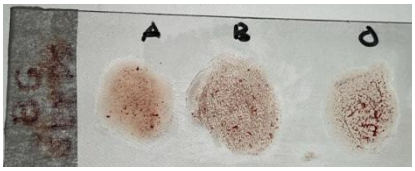
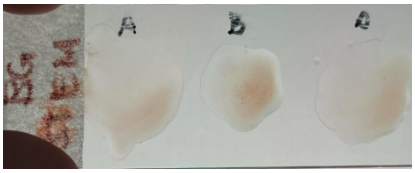
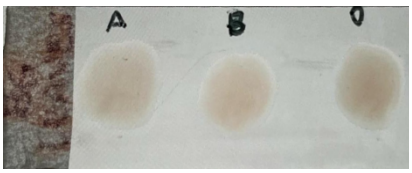
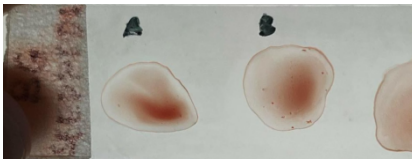
Controls	 method			slide	 tube method			
	Results	**Agglutination Reaction**			**Results**	**Grading**		
		A	**B**	**O**		**A**	**B**	**O**
a. Seeds		+	+	+		4+	4+	4+
b. Stem		-	+	-		0	4+	0
c. Leaves		-	-	-		0	0	0
d. Seed pods		+	+	+		4+	4+	4+

Table 3 shows agglutination reaction (in slide method) and grading (in tube method) of Bitter gourd plant shoot extracts with the ABO blood group system. The extracts from bitter gourd seeds, seed pods, and stem consistently produced strong reactions with the ABO blood group system in both slide and tube methods. Seeds and seed pods are marked positive (+) with blood type A, B, and O, and the stem reacts positive (+) with blood type B using a slide method. In the tube method, seeds and seed pods reacted with blood type A (4+), blood type B (4+), and blood type O (4+), while those from the bitter gourd stem reacted with blood type B (4+). In contrast, no agglutination was observed from leaves: negative (-) in the slide method, and 0 grading in the tube method.

Mitra (2018) reported that bitter gourd contains lectins capable of agglutinating red blood cells, making it potentially useful for blood typing and transfusion studies. Other parts of the plant—such as stems, seed pods, and leaves—also have bioactive compounds like saponins,

glycosides, and alkaloids that can cause agglutination, though to a lesser degree. Bitter gourd lectins are especially known for binding to specific carbohydrates on cell or pathogen surfaces.

The findings confirm Mitra (2018) that bitter gourd contains lectins with hemagglutination properties. Seed and seed pod extracts showed strong agglutination across all ABO blood groups, while stems showed weaker reactivity. The absence of agglutination in leaves indicates that lectin concentration may be too low or inactive to induce visible red blood cell clumping.

Table 4

Hemagglutination Reaction and Grading of Black beans

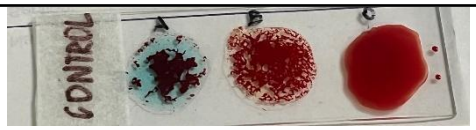
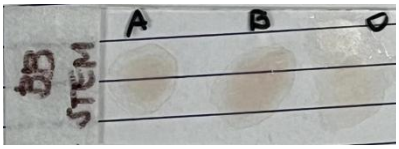
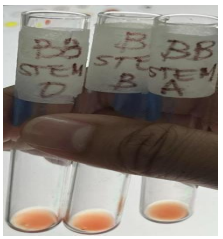
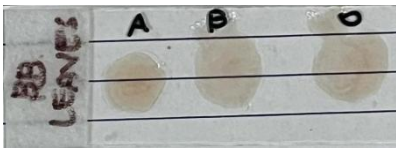
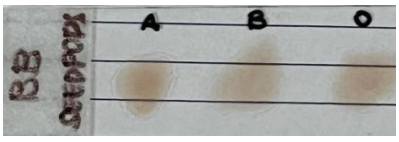
Controls	 slide method			 tube method				
	Results	**Agglutination Reaction**			**Results**	**Grading**		
		A	**B**	**O**		**A**	**B**	**O**
a. Stem		-	-	-		**0**	**0**	**0**
b. Leaves		-	-	-		**0**	**0**	**0**
c. Seed pods		-	-	-		**0**	**0**	**0**

Table 5 presents the findings of the agglutination reaction between P. vulgaris and the ABO blood group using the slide and tube methods. While black bean seeds have been reported by Awuchi (2020) to exhibit hemagglutination with the ABO blood group system, the seed pods, stems, and leaves in the current study do not produce any reaction with the ABO blood group in both slide and tube methods. It is considered as negative (-), and 0 grading.

Black beans are a rich source of plant-based protein, dietary fiber, and phenolic compounds. One of their key components is lectin, a plant protein that binds to carbohydrate

receptors on cell surfaces, such as red blood cells, and causes hemagglutination. According to Ebere (2016), crude lectin extracts from legumes like red kidney beans, black-eyed peas, soybeans, green beans, and peanuts show varying levels of hemagglutination activity, with some being blood type–specific. Awuchi (2020) also reported positive hemagglutination results from different legume lectin extracts. Since black beans belong to the Fabaceae family, they are likely to share similar hemagglutination properties with other legumes.

Although Awuchi (2020) reported hemagglutination activity in black bean seeds, this study found no such activity in the seed pods, stems, or leaves when tested with the ABO blood group system. This suggests that hemagglutinating extracts are likely concentrated in the seeds rather than evenly distributed throughout the plant. Further studies are needed to isolate these active components and explore their potential use in hemagglutination-based diagnostics.

CONCLUSIONS AND RECOMMENDATIONS

Conclusion

This study determined the hemagglutination reactions of the shoot systems of malunggay, bitter gourd, and black bean extracts using the ABO blood group system. The results revealed that the plants: bitter gourd seed pods yielded the highest percent extract, followed by malunggay leaves, indicating a greater concentration of active soluble compounds. The findings revealed that the hemagglutination reaction and grading of plants using malunggay leaf extract demonstrated specific hemagglutination activity with blood type A, whereas the seeds, seed pods, and stem did not produce any visible reaction. Bitter gourd extracts from seeds, seed pods, and stem showed strong agglutination across all blood types tested (A, B, and O), whereas its leaves did not exhibit any reaction. On the other hand, black bean shoot parts showed no hemagglutination activity with any of the blood types tested. These results indicate that malunggay and bitter gourd shoot systems, particularly the leaves and seed-containing parts, possess potential active compounds that can induce blood agglutination. Therefore, the use of crude plant extracts, especially from malunggay and bitter gourd, may serve as promising and accessible alternatives in the formulation of plant-based blood typing reagents.

Recommendations

1. Establishing a consistent, reproducible method for preparing plant-based antisera is crucial. Researchers should optimize parameters like extraction solvents, pH, temperature, and centrifugation settings. This standardization would allow scalability of production and facilitate comparisons across various studies.
2. Future researchers are encouraged to use protein purification techniques such as affinity chromatography, Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS-PAGE), and gel filtration chromatography to isolate the specific lectins responsible for hemagglutination. Once isolated, mass spectrometry (e.g., MALDI-TOF) and amino acid sequencing can be employed to identify and characterize these proteins. This will provide a molecular-level understanding of their structure, their specificity for ABO antigens, and their potential for diagnostic use.
3. It is recommended that future research investigate the thermal stability, shelf-life, and appropriate storage conditions of the crude extracts. Techniques such as freeze-drying (lyophilization) should be tested to enhance the portability and durability of plant-based reagents, especially for use in field settings or rural clinics.

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