

**IN VITRO HEMOLYSIS INHIBITION POTENTIAL OF ALUGBATI
(*Basella alba*) ETHANOLIC LEAF EXTRACT ON
DIFFERENT BLOOD TYPES**

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**ALLYZA JANE S. DANGGA
ANTHONY M. FULLO II
PRINCESS JANE B. SULITE**

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Misamis University
Ozamiz City
COLLEGE OF MEDICAL TECHNOLOGY



DEPARTMENT OF MEDICAL TECHNOLOGY

CERTIFICATE OF PANEL APPROVAL

The research paper attached hereto, entitled **"IN VITRO HEMOLYSIS INHIBITION POTENTIAL OF ALUGBATI (*Basella alba*) ETHANOLIC LEAF EXTRACT ON DIFFERENT BLOOD TYPES"**, prepared and submitted by **ALLYZA JANE S. DANGGA, ANTHONY M. FULLO II, and PRINCESS JANE B. SULITE**, in partial fulfillment of the requirements for the degree **BACHELOR OF SCIENCE IN MEDICAL TECHNOLOGY**, is hereby recommended for approval.

KARL MAXELO O. LAO, RMT, MSc. MLS

Adviser

Date

ARJADE O. ALCABAZA, RChT

Co-Adviser

Date

Approved by the Defense Panel Committee with a grade of **Passed**.

MARIE ROSELYNN C. ENGUITO, MSc. Bio

Member

Date

MELIZA PARBA, Ph.D.

Member

Date

YUNALYN L. VILLANTES, Ph.D.

Chairman

Date

This research paper is approved in partial fulfillment of the requirements for the degree of **BACHELOR OF SCIENCE IN MEDICAL TECHNOLOGY**.

EVANGELINE M. SENEDO, RMT, MATMRS

Dean, College of Medical Technology

Date

ABSTRACT

Basella alba (Malabar spinach) is a commonly consumed medicinal plant known for its antioxidant, anti-inflammatory, and cytoprotective properties. Despite its recognized therapeutic potential, limited studies have investigated its membrane-stabilizing effects on human erythrocytes of different blood types. This study evaluated the in vitro membrane-stabilizing activity of ethanolic leaf extract of *B. alba* on human erythrocytes of blood types A, B, and AB under hypotonic conditions. A quantitative experimental research design was employed, using a hypotonicity-induced hemolysis assay to determine the extract's protective effects on erythrocyte membranes. The extract was tested at concentrations of 1.0, 1.5, and 2.0 mg/mL, with acetylsalicylic acid serving as the positive control and a hypotonic saline–sodium phosphate buffer solution (pH 7.4) without the extract serving as the negative control. The results demonstrated that the ethanolic extract exhibited measurable membrane-stabilizing activity across all tested blood types and concentrations. A concentration-dependent increase in membrane protection was observed, with the 2.0 mg/mL extract showing the greatest inhibition of hemolysis. Among the blood types tested, blood type B exhibited the highest membrane-stabilizing response. Statistical analysis using two-way ANOVA revealed significant differences in membrane-stabilizing activity among extract concentrations and blood type groups ($p < .001$). These findings suggest that *B. alba* contains bioactive phytochemicals with potential membrane-protective and cytoprotective properties, supporting its possible therapeutic application as a natural source of anti-inflammatory agents.

Keywords: *erythrocyte stability, cytoprotection, hypotonic stress, phytochemicals, therapeutic potential*

DEDICATION

*This paper is dedicated to God Almighty,
whose endless grace, wisdom, and blessings
have guided us throughout this journey.*

And also

*To our beloved parents and siblings,
for their unwavering love, sacrifices, and constant prayers,
which have inspired us to pursue our dreams and never give up*

And

*To our mentors,
for their guidance, patience, and encouragement,
which have strengthened our determination and made this study possible.*

Finally,

*To the College of Medical Technology,
Misamis University, and to all future researchers,
May this work inspire the pursuit of knowledge,
scientific inquiry, and academic excellence.*

**Princess Jane
Allyza Jane
Anthony**

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**Princess Jane
Allyza Jane
Anthony**

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INTRODUCTION

Background of the Study

Medicinal plants have gained increasing attention in recent years as potential sources of therapeutic compounds due to their rich phytochemical content and diverse biological activities. Several studies have associated the consumption of plant-based foods rich in bioactive compounds with reduced risks of chronic diseases, including cardiovascular disorders, cancer, and conditions related to oxidative stress (Liu, 2003). As interest in natural products and plant-derived therapeutics continues to expand, medicinal plants with antioxidant and cytoprotective properties have become important subjects of scientific investigation.

Basella alba L., commonly known as Malabar spinach, Indian spinach, or Ceylon spinach, is a fast-growing perennial vine in the family Basellaceae and is widely cultivated throughout tropical regions of Asia (Haridas et al., 2024). In the Philippines, *B. alba* is locally known as Alugbati and is commonly consumed as a leafy vegetable and used in traditional folk medicine. According to Chaurasiya et al. (2021), *B. alba* possesses significant nutritional and pharmacological value due to the presence of minerals, proteins, carbohydrates, fibers, vitamins, carotenoids, and various bioactive secondary metabolites. Phytochemical analyses of *Basella* species have identified compounds such as betacyanins, carotenoids, bioflavonoids, β -sitosterol, and lupeol, which are reported to exhibit antioxidant, antimicrobial, and anti-inflammatory activities (Moutusi et al., 2019; Chaurasiya et al., 2021).

Recent studies have further demonstrated the potent antioxidant and anti-inflammatory activities of *B. alba*. Rusak et al. (2024) reported that gomphrenin-rich fractions isolated from *B. alba* significantly inhibited pro-inflammatory cytokines and reduced oxidative stress markers and lipid peroxidation in vitro. These findings suggest that the antioxidant compounds present in *B. alba* may help protect cellular membranes against oxidative damage. Oxidative stress is recognized as a major contributor to erythrocyte membrane destabilization, lipid peroxidation, and hemolysis, making antioxidant-rich plant extracts valuable candidates for membrane-stabilizing research. Similar membrane-protective effects have been observed in other polyphenol-rich plant extracts, which were shown to reduce erythrocyte hemolysis and improve membrane stability under oxidative and hypotonic stress conditions (Yesmin et al., 2020).

Traditionally, *B. alba* has been used to manage various ailments, including inflammatory disorders, ulcers, viral infections, skin diseases, and gastrointestinal conditions (Shade et al., 2017; Moutusi et al., 2019). Previous studies have also reported anti-hypoglycemic, wound-healing, antimicrobial, and androgenic activities associated with the plant. In the Philippine setting, Alugbati is widely cultivated in home gardens and incorporated into local diets because of its nutritional and medicinal value. Its consumption has likewise been associated with various nutritional and health benefits due to its rich content of antioxidants and phytochemicals (Felicilda, 2025; Pausanos, 2025). Nutritionally, *B. alba* is rich in vitamins, minerals, antioxidants, and essential amino acids, further supporting its importance as a functional food source (Haridas et al., 2023).

Beyond its nutritional and ethnomedicinal importance, increasing scientific attention has focused on the potential cytoprotective effects of *B. alba*, particularly its

possible role in protecting erythrocyte membranes against oxidative and hypotonic damage. Membrane stabilization is an important biological property because the integrity of the erythrocyte membrane is essential for preventing hemolysis and maintaining normal cellular function. Damage to erythrocyte membranes caused by oxidative stress and osmotic imbalance has been associated with various pathological conditions, including inflammatory disorders and hematologic abnormalities. Consequently, plant extracts with membrane-stabilizing activity are considered promising candidates in natural product and therapeutic research.

Erythrocytes are particularly susceptible to oxidative damage because of their continuous exposure to reactive oxygen species and high polyunsaturated lipid content within their membranes. Oxidative stress may induce lipid peroxidation and structural damage to membranes, leading to increased osmotic fragility and hemolysis (Obeagu et al., 2024). Consequently, compounds that stabilize erythrocyte membranes and reduce oxidative injury are considered important for preventing cellular damage associated with oxidative stress-related conditions. Previous studies have also demonstrated that antioxidant-rich plant extracts exhibit significant antihemolytic and membrane-stabilizing activities, further supporting their potential therapeutic value (Ydyrys et al., 2023).

Despite the increasing body of literature describing the pharmacological activities of *B. alba*, studies specifically evaluating its effects on human erythrocyte membrane stability remain limited. Previous investigations have explored the effects of *B. alba* on erythrocyte osmotic fragility, particularly among individuals with sickle cell disease (Ds et al., 2015). However, there remains a lack of studies examining its effects on normal human erythrocytes, particularly under hypotonic stress. Furthermore, limited information is

available regarding the protective effects of ethanolic extracts of *B. alba* leaves on erythrocyte membranes.

Ethanolic extraction was selected in the present study because ethanol effectively dissolves both polar and moderately nonpolar bioactive compounds, including polyphenols and flavonoids, which are known for their antioxidant and cytoprotective properties. Previous studies involving polyphenol-rich medicinal plants have demonstrated that ethanolic extracts possess substantial antioxidant activity and significant biological potential due to their high phytochemical content (Stanciauskaite et al., 2021). Thus, the use of ethanolic extracts may enhance the recovery of membrane-protective phytochemicals from *B. alba* leaves.

The ethnomedicinal relevance, phytochemical composition, and reported biological activities of *B. alba* provide a strong basis for further scientific investigation. However, studies examining its in vitro protective effects on human erythrocyte membranes remain limited. To date, no study has comprehensively evaluated the effects of ethanolic leaf extracts of *B. alba* across different human blood types under hypotonic-induced hemolytic conditions. Therefore, this study aims to evaluate the membrane-stabilizing activity of ethanolic leaf extracts of *B. alba* on normal human erythrocytes using in vitro assays. The findings of this study may contribute to the growing body of evidence supporting the therapeutic potential of plant-derived bioactive compounds and provide scientific support for the traditional use of Alugbati as a medicinal plant in the Philippines.

Objectives of the Study

This study aimed to evaluate the membrane-stabilizing effects of the ethanolic extract of Malabar spinach (*B. alba*) leaves on human erythrocytes of different blood types (A, B, and AB) using in vitro assays.

Specifically, it aimed to:

1. assess the membrane-stabilizing activity of the extract on human erythrocytes using 1.0, 1.5, and 2.0 mg/mL concentrations against hypotonic-induced hemolysis, using acetylsalicylic acid as the positive control and the hypotonic saline–sodium phosphate buffer solution without extract as the negative control;
2. compare the membrane-stabilizing effects of the extract among erythrocytes of different blood types (A, B, and AB); and
3. determine the concentration-dependent effect of the extract on erythrocyte membrane stability.

Significance of the Study

This study was significant because it investigated the membrane-stabilizing effects of the ethanolic extract of *B. alba* leaves, a natural plant source that may serve as a safe and accessible therapeutic or dietary supplement. By including erythrocytes of different blood types (A, B, and AB), the study also determined whether blood group variations influenced the extract's effects on red blood cell membrane stability.

Medical and health professionals will benefit from this study, as it will provide insights into the potential use of *B. alba* as a natural therapeutic agent to protect red blood

cells. Understanding its effects across blood types will also support its potential application in personalized or blood-type-specific treatments.

Nutritionists and dietitians will gain useful information on the protective effects of *B. alba* on red blood cells, supporting its inclusion in diets that promote blood health and reduce oxidative stress. Findings based on differences in blood type may also help tailor nutritional recommendations for individuals.

Local communities and the general public will benefit from knowledge of the safety and potential health benefits of consuming *B. alba* in food or traditional medicine. This will help individuals make informed choices regarding its nutritional and medicinal use.

Scope and Delimitations

This study was limited to in vitro testing and did not assess other biological or pharmacological activities of the extract. It focused on determining the membrane-stabilizing effects of *B. alba* leaf ethanolic extract on red blood cells under hypotonic conditions. Normocytic, normochromic human erythrocytes of blood types A, B, and AB were used to evaluate potential variations in membrane-stabilizing responses among different blood groups. Blood type O was excluded from the study because it lacks A and B surface antigens on the erythrocyte membrane, which may limit comparative observations of antigen-associated membrane responses. Additionally, factors such as temperature, pH, sample storage conditions, and pipetting techniques during sample preparation and testing may have influenced the outcome of the experiment. The research was conducted at Misamis University in Ozamiz City, Misamis Occidental, Philippines, during the School Year 2025–2026.

MATERIALS AND METHODS

Research Design

The research employed a quantitative, experimental study design to evaluate the membrane-stabilizing effects of Malabar spinach (*B. alba*) leaf ethanolic extract on normal human erythrocytes of blood types A, B, and AB. Structured quantitative procedures were applied to evaluate the membrane-stabilizing property of the plant extract.

Research Setting

Fresh Malabar spinach (*B. alba*) leaves were cultivated and collected from a privately owned residential property located at Purok 8, Bernad Subdivision, Barangay Aguada, Ozamiz City, Misamis Occidental, to ensure that the plant material was grown without the use of pesticides. Blood extraction and RBC suspension preparation were performed in the Department of Medical Technology at Misamis University under controlled, sterile conditions. The morphological assessment of erythrocytes was performed in a well-equipped clinical laboratory to validate the cellular integrity of the study samples.

Moreover, the preparation of the ethanolic extract from the leaves was conducted at the College of Science and Mathematics, Mindanao State University – Iligan Institute of Technology, which was equipped with the necessary facilities and instruments to support the analysis. Lastly, the membrane-stabilizing activity assay was conducted at the College of Mathematics and Natural Sciences, Northwestern Mindanao State College of Science and Technology.

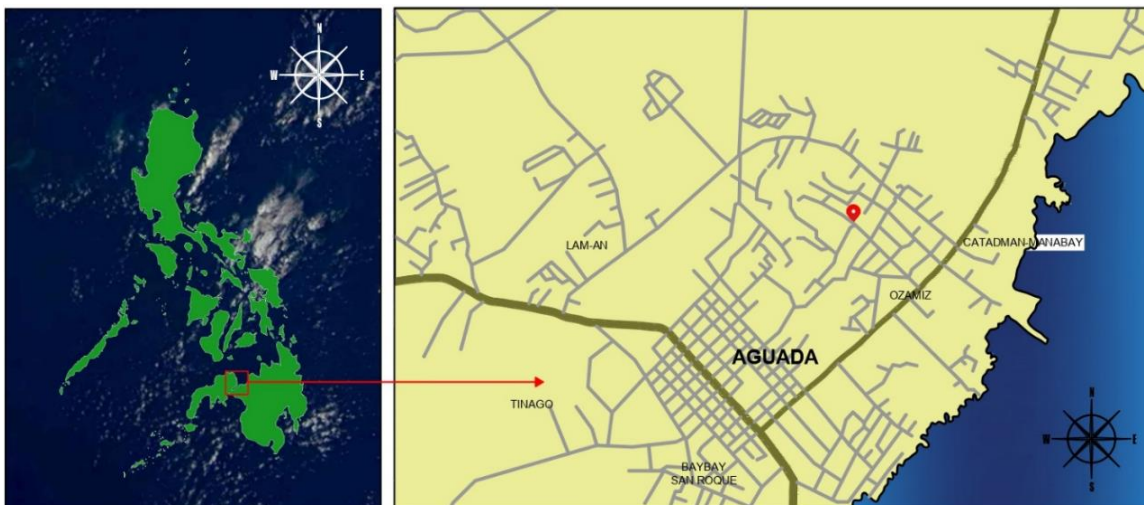


Figure 1. Map of the plant sample collection site in Purok 8, Bernad Subdivision, Barangay Aguada, Ozamiz City, Misamis Occidental

Collection of Plant Materials

Mature leaves of *B. alba* free from visible damage, discoloration, or disease were selected and cut from different parts of the plant using clean scissors to minimize contamination. The collected leaves were carefully placed in autoclavable bags and transported to the laboratory, where they were washed under running water (Jones, 2025). The leaves were then placed on trays and dried in a food dehydrator for 48 h at 43 °C to reduce moisture content (Alonso et al., 2025). Low-temperature drying was employed to preserve heat-sensitive phytochemicals, antioxidants, and bioactive compounds, such as polyphenols and flavonoids, which may contribute to the plant extract's membrane-stabilizing activity (Felicilda, 2025).

Preparation of Ethanolic Extract

The dried leaves were pulverized into a fine powder using a blender (Hanabishi). The resulting powder (100g) was soaked and stirred in 1 L of 70% ethanol and kept for 72

hours in an airtight glass jar with regular manual agitation. After 72 hours, the mixture was decanted through cheesecloth and then filtered through filter paper. The filtrate was concentrated using a rotary evaporator (Elizondo-Luevano et al., 2024). The yields were calculated using Formula (1):

$$\text{Yield (\%)} = \frac{\text{Final Weight}}{\text{Initial Weight}} \times 100 \quad (1)$$

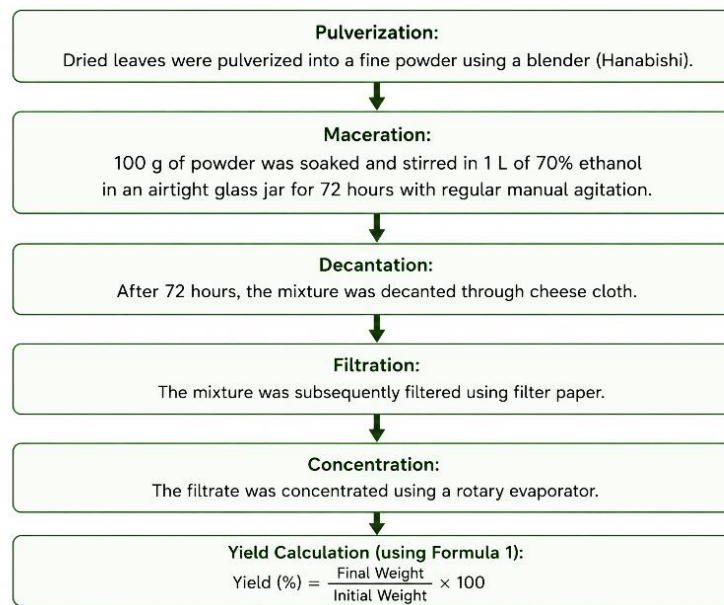


Figure 2. Schematic diagram of the preparation of the plant ethanolic extract

Blood Extraction Procedure

Human blood samples of blood types A, B, and AB were collected from nine (9) healthy volunteers, with three (3) individuals representing each blood type, through venipuncture. A licensed medical technologist performed the procedure in accordance with standard phlebotomy and safety protocols recommended by the Clinical and Laboratory

Standards Institute (CLSI, 2017). Approximately 5 mL of blood was drawn from each donor and transferred into EDTA tubes to prevent clotting (McCall, 2023).

Morphological Assessment of Red Blood Cells

Freshly collected blood samples were first subjected to automated complete blood count (CBC) analysis using the Sysmex KX-1000 Hematology Analyzer to ensure that the erythrocytes used in the assays were morphologically normal. The analyzer operates on the principle of electrical impedance for cell counting and sizing, in which blood cells passing through a small aperture generate changes in electrical resistance proportional to cell size and number (Brown, 2015). Samples with mean corpuscular volume (MCV) values within 80–100 fL, mean corpuscular hemoglobin (MCH) values within 27–31 pg/cell, and mean corpuscular hemoglobin concentration (MCHC) values within 32–36 g/dL were considered morphologically normal, specifically normocytic and normochromic, and were accepted for further use (UF Health, 2024). This ensured that any observed membrane-stabilizing activity was attributable to the plant ethanolic extract rather than to pre-existing abnormalities in red blood cell morphology (Adewoyin et al., 2019).

Preparation of Human Erythrocyte Suspension

Anticoagulated blood samples (2 mL) were transferred into a sterile 15 mL plastic conical centrifuge tube (Sinyomedical) and diluted with 2 mL of 0.9% sodium chloride (normal saline solution, NSS). The contents of the tube were gently mixed to prevent cell damage. The suspension was centrifuged at 3400 rpm for 60 seconds, and the supernatant

was carefully discarded by micropipette aspiration. This washing process was carried out three times using the same volume of NSS until the supernatant became clear (Zan, 2020).

Following the final wash, the red blood cells were resuspended in normal saline and adjusted to a 5% erythrocyte suspension (See Figure 3). The prepared erythrocyte suspension was stored at 4 °C and used within 48 hours to maintain cell viability (Pulliam et al., 2020).



Figure 3. Schematic diagram of the preparation of human erythrocyte suspension

Membrane-Stabilizing Activity Assay

The membrane-stabilizing activity of *B. alba* extracts was evaluated by analyzing the hemolysis of erythrocyte membranes in a hypotonic solution, as described by Hamiduzzaman et al. (2017). The experiment was conducted by preparing test samples containing 0.50 mL of stock erythrocyte (RBC) suspension and 5 mL of a 40% (v/v) hypotonic sodium phosphate buffer solution (pH 7.4). The *B. alba* extract was tested at concentrations of 1.0, 1.5, and 2.0 mg/mL. Standard acetylsalicylic acid (0.10 mg/mL)

served as the positive control, while the hypotonic saline–sodium phosphate buffer solution without the extract served as the negative control.

The mixtures were incubated at 37 °C for 10 minutes and subsequently centrifuged at 3000 rpm for 10 minutes to obtain the supernatant. The absorbance of the supernatant was measured at 540 nm using a double-beam UV-visible spectrophotometer (Infitek). Triplicate measurements were performed for all treatments and controls to ensure reliability and reproducibility of the results.

The percentage inhibition of RBC membrane hemolysis was calculated using the following Formula (2):

$$\% \text{ inhibition of hemolysis} = 100 \times \frac{(OD_1 - OD_2)}{OD_1} \quad (2)$$

Where OD₁ indicates the absorbance of the hypotonic-buffered solution, and OD₂ indicates the absorbance of the test sample in the hypotonic solution.

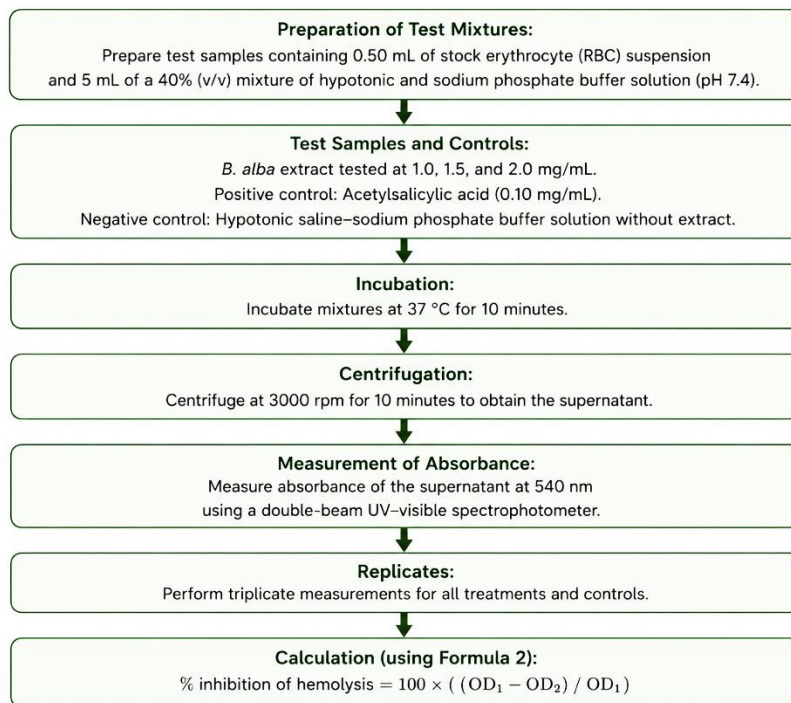


Figure 4. Schematic diagram of the membrane-stabilizing activity assay

The percentage inhibition of hypotonic-induced hemolysis was interpreted relative to the negative and positive controls, with higher values indicating greater membrane-protective activity. Following the protocol of Chaity et al. (2016), inhibition values were classified as high (>70%), moderate (50–70%), or low (<50%) to categorize the membrane-stabilizing activity of the *B. alba* extracts.

Statistical Analysis

The experimental data were presented as mean $\pm$ standard deviation. Data normality was assessed using the Shapiro–Wilk test to determine whether the data were normally distributed. The results indicated that the data were normally distributed, satisfying the normality assumption required for parametric analysis and justifying the use of a two-way analysis of variance. Accordingly, two-way ANOVA was employed to analyze the effects of extract concentration and blood type on the membrane-stabilizing activity of *B. alba* extracts.

Since significant differences were observed, a post hoc Tukey's Honestly Significant Difference (HSD) test was performed to determine pairwise differences among the treatment groups.

Ethical Consideration

This study involved experimental samples consisting of normal human erythrocytes collected for laboratory testing. The study underwent ethical review and was approved by the Misamis University Ethics Committee prior to the conduct of the research (Approval Code: MUREC-UNDERGRAD 2026-002). Informed consent was obtained from each

participant before blood collection. Participants were fully informed about the purpose, procedures, potential benefits, possible risks of the study, and the measures implemented to ensure the confidentiality and privacy of their information.

Participant information and research data, including blood type and laboratory results, were treated with strict confidentiality. Codes were used instead of participants' names to protect their identity. The collected data were securely kept by the researchers solely for research documentation purposes. Access to the data was limited only to the researchers, the research adviser, and the respective participant to ensure privacy and data security. All research data and records will be securely stored for 1 year after the study's completion and thereafter properly discarded and permanently deleted.

In appreciation of their participation, a modest cash incentive was provided, and proper coordination was maintained after blood extraction to monitor their condition and ensure their well-being.

All experimental materials and biohazardous waste generated during the study, including used syringes, needles, gloves, blood-contaminated materials, and specimen containers, were handled and disposed of in accordance with institutional biosafety protocols and standard laboratory waste management guidelines. Sharps were discarded in designated sharps containers, while infectious wastes were disposed of in properly labeled biohazard waste containers provided by the institution.

RESULTS AND DISCUSSION

Membrane Stabilizing Assay

Table 1 presents the estimated marginal means and standard errors of the percentage inhibition of erythrocyte membrane lysis for each blood type group across the three extract concentrations (1.0, 1.5, and 2.0 mg/mL). As shown, the positive control consistently yielded the highest inhibition values across all three concentrations ($M = 0.744 \pm 0.028$, 0.752 ± 0.028 , and 0.745 ± 0.028 at 1.0, 1.5, and 2.0 mg/mL, respectively), confirming the expected membrane-protective activity of acetylsalicylic acid. The negative control showed inhibition values of approximately zero across all concentrations, indicating complete erythrocyte lysis in the absence of any protective agent and serving as the baseline reference for comparison. Across the experimental blood type groups, measurable inhibition of erythrocyte membrane lysis was observed at all extract concentrations tested, indicating that the *B. alba* ethanolic extract protected erythrocyte membranes against hypotonicity-induced hemolysis.

Table 1. Estimated marginal means ($M \pm SE$) of percentage inhibition of erythrocyte membrane lysis by blood type group and extract concentration

Blood Type / Group	1.0 mg/mL $M \pm SE$	1.5 mg/mL $M \pm SE$	2.0 mg/mL $M \pm SE$
Positive Control (PC)	0.744 ± 0.028	0.752 ± 0.028	0.745 ± 0.028
Negative Control (NC)	0.000 ± 0.028	0.000 ± 0.028	0.000 ± 0.028
Blood Type A	0.459 ± 0.016	0.548 ± 0.016	0.638 ± 0.016
Blood Type B	0.582 ± 0.016	0.677 ± 0.016	0.709 ± 0.016
Blood Type AB	0.523 ± 0.016	0.594 ± 0.016	0.666 ± 0.016

Note: M = estimated marginal mean percentage inhibition of erythrocyte membrane hemolysis derived from the two-way ANOVA model. SE = standard error. PC = positive control (acetylsalicylic acid, 0.10 mg/mL); NC = negative control (hypotonic-buffered solution without extract). Complete Tukey HSD post hoc comparisons are provided in Appendix H.

Among the experimental blood type groups, blood type B demonstrated the highest mean inhibition values across all three concentrations ($M = 0.582 \pm 0.016$ at 1.0 mg/mL; $M = 0.677 \pm 0.016$ at 1.5 mg/mL; $M = 0.709 \pm 0.016$ at 2.0 mg/mL), followed by blood type AB ($M = 0.523 \pm 0.016$, 0.594 ± 0.016 , and 0.666 ± 0.016 , respectively) and blood type A ($M = 0.459 \pm 0.016$, 0.548 ± 0.016 , and 0.638 ± 0.016 , respectively). A consistent upward trend in inhibition was observed as extract concentration increased across all blood type groups, suggesting a dose-dependent membrane-protective effect of the *B. alba* ethanolic extract.

Applying the activity classification proposed by Chaity et al. (2016), in which inhibition values above 70% indicate high activity, 50–70% indicate moderate activity, and below 50% indicate low activity, blood type B at 2.0 mg/mL approached the threshold for high membrane-stabilizing activity, while blood type A at 1.0 mg/mL fell within the low-to-moderate range. The majority of experimental groups across all concentrations were classified within the moderate range (0.50–0.70), indicating meaningful but moderate membrane-protective activity relative to the pharmaceutical positive control.

The observed membrane-stabilizing activity of *B. alba* is consistent with the plant's known phytochemical profile. *B. alba* contains betacyanins, bioflavonoids, carotenoids, β -sitosterol, and lupeol, all of which have been reported to exert antioxidant, anti-inflammatory, and cytoprotective properties (Moutusi et al., 2019; Chaurasiya et al., 2021). Among these, the gomphrenin-rich fractions of *B. alba* have been reported to inhibit pro-inflammatory cytokines including interleukin-1 β (IL-1 β) and interleukin-6 (IL-6), reduce oxidative stress markers such as ROS and nitric oxide (NO), and attenuate lipid peroxidation in vitro. Since oxidative stress is a primary contributor to erythrocyte

membrane destabilization and lysis, the antioxidant compounds present in the ethanolic extract may have conferred protection against hypotonic-induced lysis by scavenging free radicals and stabilizing membrane lipid bilayers. Similar membrane-protective effects were reported by Olchowik et al. (2009), who demonstrated that polyphenol-rich plant extracts significantly reduced erythrocyte hemolysis and enhanced membrane stability under oxidative and hypotonic stress conditions. Their findings further suggested that antioxidant phytochemicals may strengthen erythrocyte membrane resistance through interactions with membrane surface components and lipid structures.

The use of ethanolic extraction in the present study may have enhanced the recovery of these bioactive compounds. Ethanolic extraction specifically enhances the yield of polyphenols and flavonoids compared to aqueous extraction, a pattern similarly reported in polyphenol-rich plants such as *Populus balsamifera* L. (Stanciauskaite et al., 2021), further supporting the suitability of the 70% ethanol solvent system employed in this study.

To determine whether the observed differences in membrane-stabilizing activity among blood types and extract concentrations were statistically significant, a two-way ANOVA was conducted to evaluate the main effects of blood type and extract concentration, as well as their interaction, on percentage inhibition of hemolysis.

Two-Way ANOVA: Effects of Blood Type and Extract Concentration on Membrane-Stabilizing Activity

Prior to conducting the two-way ANOVA, the assumptions of normality and homogeneity of variances were evaluated. The Shapiro–Wilk test indicated a statistically significant deviation from normality ($W = 0.971$, $p = .029$), while Levene’s test for homogeneity of variances was likewise statistically significant, $F(14, 84) = 4.21$, $p < .001$,

indicating heterogeneity of variances across groups. Despite these violations, the two-way ANOVA was retained as the primary analysis because the experimental design was fully balanced, with equal group sizes across all treatment conditions, and ANOVA is generally considered robust to moderate violations of assumptions under such conditions (Field, 2018).

The observed heterogeneity of variances may partly reflect inherent biological variability among donors. Hadjesfandiari et al. (2021) reported that differences in erythrocyte membrane composition, oxidative stress response, and antioxidant defense capacity can influence red blood cell susceptibility to hemolysis and membrane damage, potentially contributing to variability in membrane-stabilizing responses among donor groups.

The results of the two-way ANOVA are presented in Table 2. The overall model was statistically significant ($F(14, 84) = 114.10, p < .001$), indicating that the combination of blood type and extract concentration accounted for substantial systematic variance in membrane-stabilizing activity.

Table 2. Two-way analysis of variance for percentage inhibition of erythrocyte membrane lysis

Source	SS	df	MS	F	p	η^2	η^2p
Blood Type	3.421	4	0.855	365.72	< .001	.904	.946
Concentration	0.102	2	0.051	21.86	< .001	.027	.342
<i>Blood Type</i> × <i>Concentration</i>	0.065	8	0.008	3.49	.002	.017	.249
Residuals	0.196	84	0.002	—	—	—	—

Note: SS = sum of squares; df = degrees of freedom; MS = mean square; F = F-ratio; p = p-value; η^2 = eta-squared (proportion of total variance); η^2p = partial eta-squared. Dependent variable: percentage inhibition of erythrocyte membrane lysis.

The main effect of blood type was statistically significant, $F(4, 84) = 365.72, p < .001, \eta^2 = .904, \eta^2p = .946$. The large eta-squared value suggests that blood type explained a substantial proportion of the variance in membrane-stabilizing activity, including the

positive and negative control conditions. The large partial eta-squared ($\eta^2p = .946$) further indicates that blood type contributed substantially to the variation in membrane-stabilizing activity within the overall model. These findings confirm that the membrane-stabilizing effects of the *B. alba* ethanolic extract varied significantly across blood type groups. It should be noted that each blood type group consisted of three biological donors, and the reported means represent values averaged across these biological replicates.

The main effect of extract concentration was also statistically significant, $F(2, 84) = 21.86$, $p < .001$, $\eta^2 = .027$, $\eta^2p = .342$. The eta-squared value ($\eta^2 = .027$) indicates that concentration accounted for a relatively small proportion of total variance in the overall model. However, the partial eta-squared ($\eta^2p = .342$) reflects a moderate effect of concentration when considered in isolation from other sources of variance, indicating that concentration-related differences in membrane-stabilizing activity were statistically reliable and directionally consistent, though secondary in overall magnitude to between-blood-type variation.

The Blood Type $\times$ Concentration interaction was statistically significant, $F(8, 84) = 3.49$, $p = .002$, $\eta^2p = .249$, indicating that the rate of concentration-dependent increase in membrane-stabilizing activity was not uniform across blood type groups. The biological implications of this interaction are discussed in the following section.

Post Hoc Analysis

Given the statistical significance of both main effects, Tukey's HSD post hoc analysis was conducted to identify specific pairwise differences. The complete post hoc comparison tables for blood type and extract concentration are provided in Appendix F.

Post Hoc Comparisons Among Blood Type Groups

For blood type comparisons, all pairwise differences between the positive control and the experimental blood type groups were statistically significant ($p < .001$), confirming that acetylsalicylic acid produced uniformly greater inhibition than all extract-treated groups. All comparisons between the negative control and the experimental blood-type groups were likewise statistically significant ($p < .001$), confirming that the *B. alba* extract conferred measurable membrane protection above the untreated hemolysis baseline across all blood types and concentrations.

Among the experimental blood type groups, blood type B exhibited the highest inhibition and differed significantly from blood type A (Mean Difference = -0.108 , SE = 0.013 , $p < .001$, Cohen's $d = -2.234$) and blood type AB (Mean Difference = 0.062 , SE = 0.013 , $p < .001$, Cohen's $d = 1.277$). Blood type A and blood type AB also differed significantly from each other (Mean Difference = -0.046 , SE = 0.013 , $p = .006$, Cohen's $d = -0.957$). Complete pairwise comparisons are available in Appendix F.

In the present study, blood type B consistently demonstrated the highest inhibition values, while blood type A exhibited the lowest across all concentrations. This pattern is consistent with the previously discussed variability in erythrocyte membrane characteristics among ABO blood groups. Human erythrocytes differ across ABO blood types in the composition of their surface glycoproteins, glycolipid-associated antigens, and membrane properties, which may influence their susceptibility to hypotonic stress (Hoffbrand et al., 2019). Consistent with this, Suleiman et al. (2021) reported significant differences in osmotic fragility among ABO blood groups. Such differences in baseline membrane characteristics could influence the extent to which plant-derived bioactive

compounds interact with the erythrocyte membranes and may partly account for the differences in membrane-stabilizing responses observed in the present study. However, the precise mechanisms underlying these differences remain unclear, and caution is warranted in attributing the observed patterns solely to blood type-specific membrane characteristics. Nevertheless, the statistically significant between-group differences ($\eta^2p = .946$ for the blood type main effect) suggest that ABO blood group status may represent a biologically relevant variable in future studies evaluating the erythroprotective activity of plant extracts.

Post Hoc Comparisons Among Extract Concentrations

For concentration comparisons, all pairwise differences among the three extract concentrations were statistically significant. The 1.0 mg/mL versus 2.0 mg/mL comparison yielded the largest mean difference (Mean Difference = -0.090 , SE = 0.014 , $p < .001$, Cohen's $d = -1.861$), followed by 1.0 mg/mL versus 1.5 mg/mL (Mean Difference = -0.053 , SE = 0.014 , $p < .001$, Cohen's $d = -1.093$) and 1.5 mg/mL versus 2.0 mg/mL (Mean Difference = -0.037 , SE = 0.014 , $p = .022$, Cohen's $d = -0.767$). These findings confirm a stepwise increase in membrane-stabilizing activity with each successive increase in extract concentration.

These findings suggest that increasing extract concentration enhanced membrane protection, likely due to greater availability of bioactive compounds that protect erythrocyte membranes from hypotonicity-induced damage. Within the tested concentration range (1.0–2.0 mg/mL), higher concentrations of the *B. alba* ethanolic extract produced progressively greater inhibition of hemolysis, indicating a dose-dependent membrane-protective effect. Similar concentration-dependent responses have

been reported in other studies of membrane-stabilizing plant extracts. For example, Yesmin et al. (2020) observed comparable membrane stabilization in the ethanolic root extract of *Piper chaba*, attributing the effect to increased interactions between polyphenolic compounds and the erythrocyte membrane. Collectively, these findings support the concentration-dependent protective activity of polyphenol-rich ethanolic plant extracts, suggesting that this response may not be unique to *B. alba* alone.

Interaction Effect: Blood Type $\times$ Extract Concentration

The significant Blood Type $\times$ Concentration interaction, $F(8, 84) = 3.49$, $p = .002$, $\eta^2p = .249$, indicates that the pattern of concentration-dependent change in membrane-stabilizing activity was not uniform across the three blood type groups, and its interpretation is aided by the interaction plot presented in Figure 5.

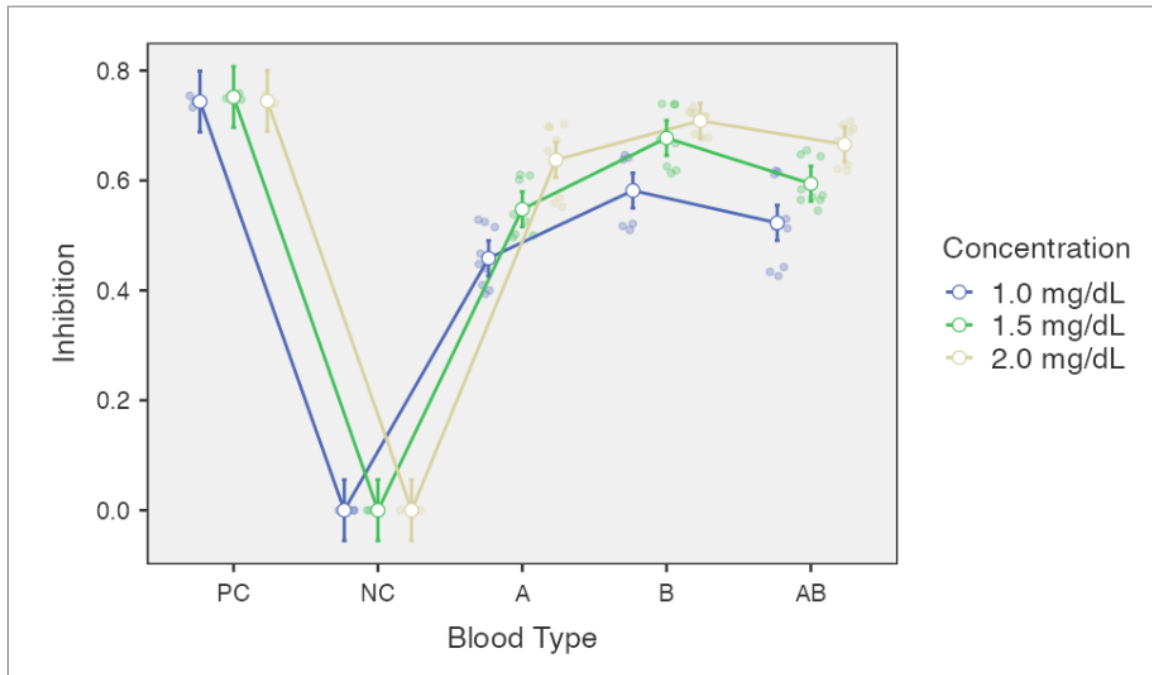


Figure 5. Interaction plot of blood type and concentration

As depicted, the positive control (PC) and negative control (NC) remained relatively stable across all three concentration levels, consistent with their respective roles as reference conditions. Among the experimental blood type groups, a general upward trend in mean inhibition was observed from 1.0 to 2.0 mg/mL, indicating that higher concentrations of the *B. alba* extract tended to produce greater membrane protection across all blood types. However, the slope and magnitude of this increase varied among the groups, which accounts for the statistically significant interaction effect.

Among the experimental groups, all blood types showed increased membrane stabilization as extract concentration increased; however, the magnitude of this increase varied across groups. Blood type B consistently exhibited the strongest membrane-protective response, whereas blood type A showed comparatively lower inhibition values across concentrations. Blood type AB displayed intermediate responses. These non-parallel concentration-response patterns account for the statistically significant interaction effect and suggest that erythrocyte responsiveness to the *B. alba* extract may vary according to blood type-associated membrane characteristics. Nevertheless, given the limited number of biological replicates per group ($n = 3$), these findings should be interpreted cautiously and validated in larger studies.

Orbach et al. (2017) reported that red blood cells with lower membrane deformability are more susceptible to osmotic stress and membrane damage. These differences in erythrocyte membrane properties may explain why some donor groups in the present study showed a sharp increase in membrane stabilization as extract concentration increased. In contrast, others exhibited only minimal changes or plateau-like responses at higher concentrations.

Taken together, the findings demonstrate that both blood type and extract concentration significantly influenced the membrane-stabilizing activity of the *B. alba* ethanolic extract. The extract produced measurable inhibition of hypotonic-induced hemolysis across all blood types and concentrations tested, supporting its erythroprotective potential in vitro. These findings are consistent with previous reports describing the antioxidant and membrane-protective properties of *B. alba* phytochemicals (Moutusi et al., 2019; Chaurasiya et al., 2021).

The present findings extend earlier work by Ds et al. (2015), who examined the effect of aqueous *B. alba* leaf extract on the osmotic fragility of erythrocytes in subjects with sickle cell anemia and reported a reduction in red blood cell fragility. The current study extends these observations to normal erythrocytes of three ABO blood types using an ethanolic extract, a preparation with demonstrably higher yields of polyphenols and flavonoids. It establishes that membrane-protective activity is present in morphologically normal erythrocytes under hypotonic stress conditions. The inhibition values produced by the *B. alba* extract, while consistently lower than those of the acetylsalicylic acid positive control at all concentrations tested, were substantially and significantly elevated above the negative control baseline across all blood type groups and concentration levels.

Several limitations of the present study should be acknowledged. The in vitro design precludes direct extrapolation to in vivo physiological contexts, where bioavailability, hepatic metabolism, and systemic distribution of *B. alba* phytochemicals may substantially alter the effective concentrations that reach erythrocyte membranes. The sample size of three donors per blood type, while acceptable for a preliminary experimental investigation, limits statistical power for within-group analyses. Future studies should

increase donor representation per blood type, include blood type O for a complete ABO comparison, and conduct phytochemical profiling and fractionation to identify the specific constituents responsible for the observed membrane-protective activity. Additional assay models, such as heat-induced hemolysis, would further characterize the nature and breadth of membrane-stabilizing effects.

Overall, the present findings are consistent with the ethnomedicinal uses of *B. alba* in conditions associated with oxidative stress and blood disorders, including anemia and inflammatory conditions (Felicilda, 2025; Pausanos, 2025), and support the potential of this plant as a natural source of bioactive phytochemicals with erythroprotective and membrane-stabilizing properties.

CONCLUSIONS AND RECOMMENDATIONS

The study demonstrated that the ethanolic leaf extract of *B. alba* exhibited membrane-stabilizing activity against hypotonic-induced erythrocyte membrane lysis across blood types A, B, and AB. The extract showed a dose-dependent effect, with higher concentrations yielding greater inhibition, although its activity remained lower than that of the positive control. Variations in responses across blood types were observed, suggesting that erythrocyte membrane characteristics may influence the extract's effectiveness. Overall, the findings support the potential of *B. alba* as a natural source of bioactive compounds with membrane-protective properties.

It is recommended that future researchers increase the number of donors per blood type and include blood type O to allow a more comprehensive comparison among ABO groups. Additional studies may employ heat-induced hemolysis assays alongside hypotonic models to further evaluate membrane-stabilizing activity under different conditions. Future investigations may also explore the anti-inflammatory potential of *B. alba* through in vivo studies and analysis of inflammatory markers to further establish its potential therapeutic applications. Advanced phytochemical profiling and fractionation studies may also be conducted to identify the specific bioactive compounds in *B. alba* responsible for its potential to inhibit hemolysis. Furthermore, strict adherence to proper pipetting techniques is recommended to minimize experimental variability and improve the accuracy and reliability of results.

DECLARATION OF THE USE OF AI TOOLS

The researchers acknowledge the use of ChatGPT, developed by OpenAI, in assisting with the refinement of grammar, sentence structure, clarity, coherence, and academic phrasing during the preparation of this research paper/thesis.

Prompt/s Used:

The following prompts were entered during the writing and revision process:

- “Revise this paragraph for academic tone and clarity.”
- “Help me understand what this statistical result means.”
- “Help improve the transition between paragraphs.”
- “Check the flow and redundancy of this discussion section.”
- “Improve the grammar and coherence of this statement.”

Use of the Output:

The generated outputs were used as writing assistance for language refinement, clarification of statistical concepts, improvement of paragraph flow, and enhancement of academic readability. All content was reviewed, modified, and validated by the researchers prior to inclusion in the final manuscript.

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APPENDICES

APPENDIX A. Approved letters and consent

 **Misamis University**
Ozamiz City

MISAMIS UNIVERSITY RESEARCH CENTER
CERTIFIED: ISO 21001: 2018 Educational Organizations Management System by DNV
ACCREDITED: Philippine Association of Colleges and Universities Commission on Accreditation (PACUCOA)

MU-RC-042/13 November 2024

Thesis/Research Data Gathering Approval Form

Date: January 20, 2026

Name of Authors: Princess Jane B. Sulite, Anthony M. Fullo II, Allyza Jane S. Dangga

College/Department: College of Medical Technology

Research Title: In Vitro Hemolytic and Membrane-Stabilizing Effects of Malabar Spinach (Basella alba) Leaves Ethanolic Extract on Human Erythrocytes of Different Blood Types (A, B, and AB)

Data Gathering Procedure:

a. Type of Research: (Kindly check)

☒ Quantitative	☐ Qualitative	☒ Experimental
☐ Approved Research Title & Problem/Objectives	☐ Approved Data Gathering Procedure	☐ Approved Data Gathering Instrument/Data Sheets

b. Respondents/Participants/Subject/Samples to be Collected: Basella alba leaves and human erythrocytes from healthy donors (blood types A, B, and AB).

c. Procedure:

☐ Survey	☐ Interview	☒ Experimentation	☒ Laboratory Testing
---------------------------------	------------------------------------	---	--

d. Place of Implementation/Study Area: Misamis University and MSU-IIT, Iligan City.

If to be conducted outside the school campus, indicate the nature of transportation to be used:

☒ Own private vehicle	☐ public utility vehicle	☐ rental	☐ Others, please indicate _____
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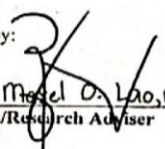
e. Date/s of Implementation/Sampling: January - February 2026

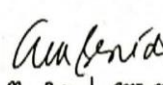
Attachments:

☒	Approved Letter of Consent from the designated authority in the area/place where the study is to be conducted
☒	Signed Parents' Consent

The thesis proposal mentioned above is hereby recommended for the commencement of data gathering implementation.

Recommended by:

 <u>Karl Maged O. Lao, RMT, MSc. MLS</u> Thesis/Research Adviser	 <u>Nelfa D. Canini, PhD</u> Thesis/Research Instructor
---	--

Approved by: 
Evangelina M. Sencido, RMT, MATMRS
College Dean

CONTROLLED DOCUMENT

APPENDIX A Cont'd



MISAMIS UNIVERSITY
Ozamiz City
Department of Student Affairs & Services

MU-DSAS-006/02 June 2023

PARENT'S CONSENT

Misamis University
Sponsoring Group/Organization

I am allowing my son/daughter/ward, Allyza Jane S. Dangga, to join the conduct of undergraduate research laboratory work (activity) to be held on January - February, 2026 at MSU-IIT, Iligan City, under the supervision of Mr. Karl Maxel O. Lao (Adviser/Faculty).

I understand that Misamis University exercises the necessary safety precautions in this activity.

In consideration of the benefits to be derived from the above activity, I expressly waive any and all claims against the administration or any member of the faculty and staff of the Misamis University on account of any unforeseen accident or injury that my son/daughter/ward might incur in connection with the aforementioned activity.

Lorna S. Dangga
Signature over printed name of
Parent/Guardian

January 18, 2026
Date

Allyza Jane S. Dangga
Signature over printed name of
Student

January 18, 2026
Date

Important: This form shall be submitted to the DSAS Office at least two (2) days before the conduct of the activity.

SUBSCRIBED AND SWORN to before me, this JAN 21 2026 day of JANUARY at MISAMIS CITY, Philippines, that the herein affiant personally came and appeared with his/her _____, as evidence of his/her personal identity.

Doc. No. 731
Page No. 74
Book No. 14
Series of 2024

ATTY. REY B. BINAG, RMT
Notarial Commission No. 2025-18
Notary Public for Ozamiz City and Clann
City Hall Drive, Barnad Subdivision, Aguada, Ozamiz City
PTR No. 6007751; G1/05/2026; Ozamiz City
IRP O.R. No. 576165; 12/29/2025; Pasig City
Attorney's Roll No. 70648
MCPS Compliance No. V.II-C014127; Valid until 04/2027
Notary Public

(For DSAS Personnel only)

Attested by:

RODEL L. BALDADO, MHist, MAEd
OIC, Department of Student Affairs & Services

Date: 01/22/26

APPENDIX A Cont'd

	MISAMIS UNIVERSITY Ozamiz City Department of Student Affairs & Services
---	--

MU-DSAS-006/02 June 2023

PARENT'S CONSENT

Misamis University
Sponsoring Group/Organization

I am allowing my son/daughter/ward, Anthony M. Fullo II, to join the conduct of undergraduate research laboratory work (activity) to be held on January - February, 2026 at MSU - IIT, Iligan City, under the supervision of Mr. Karl Mayel O. Lao (Adviser/Faculty).

I understand that Misamis University exercises the necessary safety precautions in this activity.

In consideration of the benefits to be derived from the above activity, I expressly waive any and all claims against the administration or any member of the faculty and staff of the Misamis University on account of any unforeseen accident or injury that my son/daughter/ward might incur in connection with the aforementioned activity.

F. M. Fullo
Signature over printed name of
Parent/Guardian

January 18, 2026
Date

Anthony M. Fullo II
Signature over printed name of
Student

January 18, 2026
Date

Important: This form shall be submitted to the DSAS Office at least two (2) days before the conduct of the activity.

SUBSCRIBED AND SWORN to before me, this JAN 21 2026 day of JANUARY at MISAMIS CITY, Philippines, that the herein affiant personally came and appeared with his/her _____, as evidence of his/her personal identity.

Doc. No. FD-70
Page No. 70
Book No. 18
Series of 20 20

ATTY. KEY B. BINAG, RMT
Notarial Commission No. 2025-18
Notary Public for Ozamiz City and Clarin
City Hall Drive, Bernad Subdivision, Aguada, Ozamiz City
FTR No. 6007751; 01/03/2026; Ozamiz CityTR No. _____
IUP O.R. No. 576165; 12/29/2025; Pasig CityTR No. _____
Attorney's Roll No. 70640 TIN No. _____
M.C. Compliance No. VIII-0014127; Valid until 04/18/2028 ROP No. _____

(For DSAS Personnel only)

Attested by:

RODEL T. BALDADO, MHist, MAEd
OIC, Department of Student Affairs & Services
Date: 01/22/26

APPENDIX A Cont'd



MISAMIS UNIVERSITY
Ozamiz City
Department of Student Affairs & Services

MU-DSAS-006/02June2023

PARENT'S CONSENT

Misamis University
Sponsoring Group/Organization

I am allowing my son/daughter/ward, Princess Jane B. Sulite, to join the conduct of undergraduate research laboratory work (activity) to be held on January - February 2026 at MSU-IIT, Iligan City, under the supervision of Mr. Karl Maxel O. Lao (Adviser/Faculty).

I understand that Misamis University exercises the necessary safety precautions in this activity.

In consideration of the benefits to be derived from the above activity, I expressly waive any and all claims against the administration or any member of the faculty and staff of the Misamis University on account of any unforeseen accident or injury that my son/daughter/ward might incur in connection with the aforementioned activity.

Rozel B. Sulite
Signature over printed name of
Parent/Guardian

January 18, 2026
Date

Princess Jane B. Sulite
Signature over printed name of
Student

January 18, 2026
Date

Important: This form shall be submitted to the DSAS Office at least two (2) days before the conduct of the activity.

SUBSCRIBED AND SWORN to before me, this JAN 21, 2026 at OZAMIZ CITY, Philippines, that the herein affiant personally came and appeared with his/her _____, as evidence of his/her personal identity.

Doc. No. 171
Page No. 711
Book No. 71
Series of 2024

ATTY. RUEL D. BINAG/RMT
Notarial Commission No. 2025-18 Notary Public
Notary Public for Ozamiz City and Iligan
City Hall Drive, Bernad Subdivision, Agup, Ozamiz City
PTR No. 6007751; 01/05/2026; OZAMIZ CITY
IBP O.R. No. 576165; 12/29/2025; OZAMIZ CITY
Attorney's Roll No. 7064 OZAMIZ CITY
MCLE Compliance No. VIII-0014127; Valid until 04/14/2028

(For DSAS Personnel only)

Attested by:

RODEL L. BALDADO, MHist, MAEd
OIC, Department of Student Affairs & Services
Date: 01/22/26

APPENDIX A Cont'd



Misamis University
H. T. Feliciano St., Ozamiz City, 7200 Philippines
MISAMIS UNIVERSITY RESEARCH ETHICS COMMITTEE
Phone: +6388 521 0367 | Fax: +6388 521 2917
Email: muresearchethics@mu.edu.ph

MU-REB-001/03 March 2022

February 4, 2026

MS. PRINCESS JANE B. SULITE
MR. ANTHONY M. FULLO II
MS. ALLYZA JANE S. DANGGA
Principal Investigators

MUREC-UNDERGRAD Code 2026-002

**IN VITRO HEMOLYTIC AND MEMBRANE-STABILIZING EFFECTS OF MALABAR
SPINACH (*Basella alba*) LEAVES ETHANOLIC EXTRACT ON HUMAN
ERYTHROCYTES OF DIFFERENT BLOOD TYPES (A, B, AND AB)**

Dear Investigators:

After careful review of your research protocol, the MU Research Ethics Committee (MUREC) has determined that the study poses **minimal risk** to the research participants. The Committee is therefore pleased to **grant ethical approval**, subject to compliance with the recommendations outlined below. You may proceed with data collection once these recommendations have been appropriately addressed.

The following are the recommendations based on the Committee's review:

Ethical Considerations

- Specify who will be responsible for performing the blood collection (e.g., licensed medical personnel or trained staff).
- Clearly describe the procedures for data storage, including security measures and the duration of data retention.
- Describe how experimental materials, particularly biohazardous waste, will be handled and disposed of in accordance with institutional and regulatory guidelines.

Sincerely yours,


YUNALYN VILLANTES, PhD
Chair, MUREC

APPENDIX B. Validity of test results



+63 916 900 5332
aguilar.abstat@gmail.com
fb.com/lyssbncglr
San Juan I, Noveleta, Cavite

CERTIFICATION OF STATISTICAL TREATMENT

Research Title: IN VITRO MEMBRANE-STABILIZING EFFECTS OF MALABAR SPINACH (BASELLA ALBA) LEAVES ETHANOLIC EXTRACT ON HUMAN ERYTHROCYTES OF DIFFERENT BLOOD TYPES (A, B, AND AB)

Research Authors: PRINCESS JANE B. SULITE
ANTHONY M. FULLO II
ALLYZA JANE S. DANGGA

This is to certify that the data presented in the above-mentioned study conducted at Misamis University H.T. Feliciano St., Ozamiz City 7200 Philippines, were subjected to appropriate statistical treatment. All responses gathered were processed, analyzed, and interpreted using established statistical procedures deemed suitable for the nature and objectives of the research.

The application of statistical methods was conducted with diligence and objectivity, ensuring the accuracy and reliability of the results presented. The treatment of data adhered to recognized standards of academic and research integrity.

Issued this 1st day of May, 2026.


ALYSSA BIANCA B. AGUILAR, LPT
Statistics Practitioner

APPENDIX C. Complete blood count (CBC) results of participants



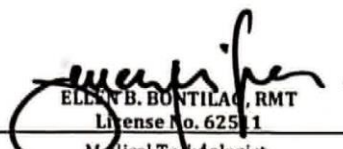
TOBIAS FELICIANO FAITH GENERAL HOSPITAL INC.

(formerly FAITH HOSPITAL)
Feliciano St., Aguada, Ozamis City
Tel. No. (088) 521-0018

SECTION OF HEMATOLOGY

PID : 0025562	Lab No. : 2606984
Patient Name : [REDACTED]	OR No. : 71782
Age : [REDACTED] DOB : Mar-01-2005	Room No. : OPD
Sex : [REDACTED]	Exam Date : Apr. 29, 2026 12:08PM
Requesting Physician :	
Remarks :	

TEST	RESULT	UNIT	REFERENCE RANGE
Complete Blood Count			
Hematocrit	L 34.9	%	35.0 - 50.0
Hemoglobin	12.1	g/dL	12.0 - 16.5
RBC	4.07	X10 ¹² /L	3.8 - 5.8
WBC	7.4	X10 ⁹ /L	4.0 - 10.0
Platelet Count	288	X10 ³ /uL	140 - 440
Red Cell Indices			
MCV	85.7	fL	80.0 - 99.0
MCH	29.7	pg	26.5 - 33.5
MCHC	34.7	%	32.0 - 36.0
RDW-CV	12.3	%	10.0 - 15.0
RDW-SD	38.6	fL	35.0 - 56.0
Differential Count			
Neutrophil	58.6	%	55.0 - 65.0
Lymphocytes	31.7	%	25.0 - 35.0
Monocytes	7.1	%	4.0 - 9.0
Eosinophil	1.9	%	0.0 - 7.0
Basophil	0.7	%	0.0 - 2.0


ELEAN B. BONTILAO, RMT
 License No. 62511
 Medical Technologist


HAROLD L. TEE, MD., FPSO., FPSP
 License No. 0077234
 Pathologist

APPENDIX C Cont'd



TOBIAS FELICIANO FAITH GENERAL HOSPITAL II

(formerly FAITH HOSPITAL)
Feliciano St., Aguada, Ozamis City
Tel. No. (088)521-0018

SECTION OF HEMATOLOGY

PID : 0025531 Lab No. : 2606933
Patient Name : [REDACTED] OR No. : 71685
Age : [REDACTED] DOB : Dec-08-2004 Room No. : OPD
Sex : [REDACTED] Exam Date : Apr. 28, 2026 04:56PM
Requesting Physician :
Remarks :

TEST	RESULT	UNIT	REFERENCE RANGE
Complete Blood Count			
Hematocrit	38.0	%	35.0 - 50.0
Hemoglobin	13.4	g/dL	12.0 - 16.5
RBC	4.40	X10 ¹² /L	3.8 - 5.8
WBC	7.2	X10 ⁹ /L	4.0 - 10.0
Platelet Count	295	X10 ³ /uL	140 - 440
Red Cell Indices			
MCV	86.4	fL	80.0 - 99.0
MCH	30.5	pg	26.5 - 33.5
MCHC	35.3	%	32.0 - 36.0
RDW-CV	12.9	%	10.0 - 15.0
RDW-SD	40.7	fL	35.0 - 56.0
Differential Count			
Neutrophil	57.1	%	55.0 - 65.0
Lymphocytes	33.3	%	25.0 - 35.0
Monocytes	6.6	%	4.0 - 9.0
Eosinophil	2.4	%	0.0 - 7.0
Basophil	0.6	%	0.0 - 2.0


ANGELA CHRISTA MARIE C. CORTES, RMT
License No. 132442
Medical Technologist


HAROLD L. TEE, MD., FPSP, FPSP
License No. 0077234
Pathologist

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(formerly FAITH HOSPITAL)
Feliciano St., Aguada, Ozamis City
Tel. No. (088)521-0018

SECTION OF HEMATOLOGY

PID : 0025533	Lab No. : 2606935
Patient Name : [REDACTED]	OR No. : 71687
Age : [REDACTED] DOB : Apr-26-2005	Room No. : OPD
Sex : [REDACTED]	Exam Date : Apr. 28, 2026 05:04PM
Requesting Physician :	
Remarks :	

TEST		RESULT	UNIT	REFERENCE RANGE
Complete Blood Count				
Hematocrit	L	31.2	%	35.0 - 50.0
Hemoglobin	L	10.5	g/dL	12.0 - 16.5
RBC		3.83	X10 ¹² /L	3.8 - 5.8
WBC		5.0	X10 ⁹ /L	4.0 - 10.0
Platelet Count		243	X10 ³ /uL	140 - 440
Red Cell Indices				
MCV		81.5	fL	80.0 - 99.0
MCH		27.4	pg	26.5 - 33.5
MCHC		33.7	%	32.0 - 36.0
RDW-CV		12.5	%	10.0 - 15.0
RDW-SD		37.1	fL	35.0 - 56.0
Differential Count				
Neutrophil	L	27.5	%	55.0 - 65.0
Lymphocytes	H	62.7	%	25.0 - 35.0
Monocytes		7.8	%	4.0 - 9.0
Eosinophil		1.6	%	0.0 - 7.0
Basophil		0.4	%	0.0 - 2.0


ANGELA CHRISTA MARIE C. CORTES, RMT
 License No. 132442
 Medical Technologist


HAROLD L. TEE, MD., FPSO., FPSP
 License No. 0077234
 Pathologist

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(formerly FAITH HOSPITAL)
Feliciano St., Aguada, Ozamis City
Tel. No. (088) 521-0018

SECTION OF HEMATOLOGY

PID	: 0025536	Lab No.	: 2606938
Patient Name	: [REDACTED]	OR No.	: 71688
Age	: [REDACTED] DOB : Sep-05-2004	Room No.	: OPD
Sex	: [REDACTED]	Exam Date	: Apr. 28, 2026 05:05PM
Requesting Physician	:		
Remarks	:		

TEST	RESULT	UNIT	REFERENCE RANGE
Complete Blood Count			
Hematocrit	39.2	%	35.0 - 50.0
Hemoglobin	13.9	g/dL	12.0 - 16.5
RBC	4.78	X10 ¹² /L	3.8 - 5.8
WBC	9.1	X10 ⁹ /L	4.0 - 10.0
Platelet Count	303	X10 ³ /uL	140 - 440
Red Cell Indices			
MCV	82.0	fL	80.0 - 99.0
MCH	29.1	pg	26.5 - 33.5
MCHC	35.5	%	32.0 - 36.0
RDW-CV	12.4	%	10.0 - 15.0
RDW-SD	37.5	fL	35.0 - 56.0
Differential Count			
Neutrophil	H 73.4	%	55.0 - 65.0
Lymphocytes	L 18.9	%	25.0 - 35.0
Monocytes	6.0	%	4.0 - 9.0
Eosinophil	1.4	%	0.0 - 7.0
Basophil	0.3	%	0.0 - 2.0


ANGELA CHRISTA MARIE C. CORTES, RMT
License No. 132442
Medical Technologist


HAROLD L. TEE, MD., FPSO., FPSP
License No. 0077234
Pathologist

APPENDIX C Cont'd



TOBIAS FELICIANO FAITH GENERAL HOSPITAL INC.

(formerly FAITH HOSPITAL)
Feliciano St., Aguada, Ozamis City
Tel. No. (088) 521-0018

SECTION OF HEMATOLOGY

PID	: 0025537	Lab No.	: 2606939
Patient Name	: [REDACTED]	OR No.	: 71691
Age	: [REDACTED] DOB : Nov-24-2005	Room No.	: OPD
Sex	: [REDACTED]	Exam Date	: Apr. 28, 2026 04:56PM
Requesting Physician	:		
Remarks	:		

TEST	RESULT	UNIT	REFERENCE RANGE
Complete Blood Count			
Hematocrit	39.7	%	35.0 - 50.0
Hemoglobin	13.0	g/dL	12.0 - 16.5
RBC	4.52	X10 ¹² /L	3.8 - 5.8
WBC	H 10.1	X10 ⁹ /L	4.0 - 10.0
Platelet Count	286	X10 ³ /uL	140 - 440
Red Cell Indices			
MCV	87.8	fL	80.0 - 99.0
MCH	28.8	pg	26.5 - 33.5
MCHC	32.7	%	32.0 - 36.0
RDW-CV	13.0	%	10.0 - 15.0
RDW-SD	41.9	fL	35.0 - 56.0
Differential Count			
Neutrophil	56.1	%	55.0 - 65.0
Lymphocytes	31.6	%	25.0 - 35.0
Monocytes	8.0	%	4.0 - 9.0
Eosinophil	3.8	%	0.0 - 7.0
Basophil	0.5	%	0.0 - 2.0

ANGELA CHRISTA MARIE C. CORTES, RMT
License No. 132442
Medical Technologist

HAROLD L. TEE, MD., FPSO., FPSP
License No. 0077234
Pathologist

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(formerly FAITH HOSPITAL)
Feliciano St., Aguada, Ozamis City
Tel. No. (088)521-0018

SECTION OF HEMATOLOGY

PID	: 0025539	Lab No.	: 2606941
Patient Name	: [REDACTED]	OR No.	: 71693
Age	: [REDACTED] DOB : May-25-2005	Room No.	: OPD
Sex	: [REDACTED]	Exam Date	: Apr. 28, 2026 04:57PM
Requesting Physician	:		
Remarks	:		

TEST	RESULT	UNIT	REFERENCE RANGE
Complete Blood Count			
Hematocrit	40.9	%	35.0 - 50.0
Hemoglobin	14.2	g/dL	12.0 - 16.5
RBC	4.79	X10 ¹² /L	3.8 - 5.8
WBC	7.8	X10 ⁹ /L	4.0 - 10.0
Platelet Count	293	X10 ³ /uL	140 - 440
Red Cell Indices			
MCV	85.4	fL	80.0 - 99.0
MCH	29.6	pg	26.5 - 33.5
MCHC	34.7	%	32.0 - 36.0
RDW-CV	11.3	%	10.0 - 15.0
RDW-SD	35.2	fL	35.0 - 56.0
Differential Count			
Neutrophil	56.6	%	55.0 - 65.0
Lymphocytes	30.6	%	25.0 - 35.0
Monocytes	H 9.3	%	4.0 - 9.0
Eosinophil	2.7	%	0.0 - 7.0
Basophil	0.8	%	0.0 - 2.0


ANGELA CHRISTA MARIE C. CORTES, RMT
License No. 132442
Medical Technologist


HAROLD L. TEE, MD., FPSO., FPSP
License No. 0077234
Pathologist

APPENDIX C Cont'd



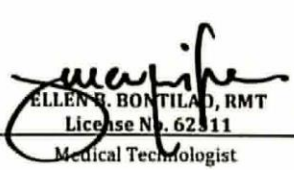
TOBIAS FELICIANO FAITH GENERAL HOSPITAL INC.

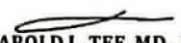
(formerly FAITH HOSPITAL)
Feliciano St., Aguada, Ozamis City
Tel. No. (088)521-0018

SECTION OF HEMATOLOGY

PID : 0025563 Lab No. : 2606985
Patient Name : [REDACTED] OR No. : 71781
Age : [REDACTED] DOB : May-09-2003 Room No. : OPD
Sex : [REDACTED] Exam Date : Apr. 29, 2026 12:08PM
Requesting Physician :
Remarks :

TEST	RESULT	UNIT	REFERENCE RANGE
Complete Blood Count			
Hematocrit	36.8	%	35.0 - 50.0
Hemoglobin	12.8	g/dL	12.0 - 16.5
RBC	4.30	X10 ¹² /L	3.8 - 5.8
WBC	6.4	X10 ⁹ /L	4.0 - 10.0
Platelet Count	269	X10 ³ /uL	140 - 440
Red Cell Indices			
MCV	85.6	fL	80.0 - 99.0
MCH	29.8	pg	26.5 - 33.5
MCHC	34.8	%	32.0 - 36.0
RDW-CV	12.4	%	10.0 - 15.0
RDW-SD	38.8	fL	35.0 - 56.0
Differential Count			
Neutrophil	62.4	%	55.0 - 65.0
Lymphocytes	30.3	%	25.0 - 35.0
Monocytes	4.8	%	4.0 - 9.0
Eosinophil	1.7	%	0.0 - 7.0
Basophil	0.8	%	0.0 - 2.0


ELLEN B. BONTILAD, RMT
License No. 62311
Medical Technologist


HAROLD L. TEE, MD., FPSO., FPSP
License No. 0077234
Pathologist

APPENDIX C Cont'd



TOBIAS FELICIANO FAITH GENERAL HOSPITAL INC.

(formerly FAITH HOSPITAL)
Feliciano St., Aguada, Ozamis City
Tel. No. (088) 521-0018

SECTION OF HEMATOLOGY

PID : 0025538	Lab No. : 2606940
Patient Name : [REDACTED]	OR No. : 71692
Age : [REDACTED] DOB : Nov-04-2003	Room No. : OPD
Sex : [REDACTED]	Exam Date : Apr. 28, 2026 05:05PM
Requesting Physician :	
Remarks :	

TEST	RESULT	UNIT	REFERENCE RANGE
Complete Blood Count			
Hematocrit	40.6	%	35.0 - 50.0
Hemoglobin	13.8	g/dL	12.0 - 16.5
RBC	4.78	X10 ¹² /L	3.8 - 5.8
WBC	6.8	X10 ⁹ /L	4.0 - 10.0
Platelet Count	306	X10 ³ /uL	140 - 440
Red Cell Indices			
MCV	84.9	fL	80.0 - 99.0
MCH	28.9	pg	26.5 - 33.5
MCHC	34.0	%	32.0 - 36.0
RDW-CV	11.9	%	10.0 - 15.0
RDW-SD	36.8	fL	35.0 - 56.0
Differential Count			
Neutrophil	L 45.2	%	55.0 - 65.0
Lymphocytes	H 44.8	%	25.0 - 35.0
Monocytes	7.8	%	4.0 - 9.0
Eosinophil	1.6	%	0.0 - 7.0
Basophil	0.6	%	0.0 - 2.0


ANGELA CHRISTA MARIE C. CORTES, RMT
 License No. 132442
 Medical Technologist


HAROLD L. TEE, MD., FPSO., FPSP
 License No. 0077234
 Pathologist

APPENDIX C Cont'd



TOBIAS FELICIANO FAITH GENERAL HOSPITAL INC.

(formerly FAITH HOSPITAL)
Feliciano St., Aguada, Ozamis City
Tel. No. (088)521-0018

SECTION OF HEMATOLOGY

PID : 0025541		Lab No. : 2606944
Patient Name : [REDACTED]		OR No. : 71690
Age : [REDACTED]	DOB : Mar-02-2005	Room No. : OPD
Sex : [REDACTED]		Exam Date : Apr. 28, 2026 05:05PM
Requesting Physician :		
Remarks :		

TEST		RESULT	UNIT	REFERENCE RANGE
Complete Blood Count				
Hematocrit	L	34.2	%	35.0 - 50.0
Hemoglobin	L	11.8	g/dL	12.0 - 16.5
RBC		4.03	$\times 10^{12}/L$	3.8 - 5.8
WBC		6.7	$\times 10^9/L$	4.0 - 10.0
Platelet Count		209	$\times 10^3/uL$	140 - 440
Red Cell Indices				
MCV		84.9	fL	80.0 - 99.0
MCH		29.3	pg	26.5 - 33.5
MCHC		34.5	%	32.0 - 36.0
RDW-CV		12.4	%	10.0 - 15.0
RDW-SD		38.7	fL	35.0 - 56.0
Differential Count				
Neutrophil	L	51.8	%	55.0 - 65.0
Lymphocytes	H	38.7	%	25.0 - 35.0
Monocytes		6.4	%	4.0 - 9.0
Eosinophil		2.7	%	0.0 - 7.0
Basophil		0.4	%	0.0 - 2.0


ANGELA CHRISTA MARIE C. CORTES, RMT
 License No. 132442
 Medical Technologist


HAROLD L. TEE, MD., FPSO., FPSP
 License No. 0077234
 Pathologist

APPENDIX D. Documentation



D1. Collection of Malabar Spinach



D2. Drying Process and Preparation of Ethanolic Extract



D3. Filtration process

APPENDIX D Cont'd



D4. Pre-experimentation Process



D5. Performing the Membrane-Stabilizing Activity Assay at NMSCST

APPENDIX E. Raw results

	1.0 mg/dL			1.5 mg/dL			2.0 mg/dL		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
PC	0.425	0.4663	0.4561	0.4044	0.4162	0.4205	0.4142	0.4358	0.4567
NC	1.7303	1.7463	1.7802	1.678	1.6463	1.6802	1.7025	1.7143	1.7059
A1	0.8226	0.8466	0.8392	0.6689	0.6408	0.6566	0.5575	0.5926	0.6263
A2	1.0218	1.0476	1.0794	0.8451	0.8228	0.8366	0.7328	0.7564	0.7644
A3	0.9681	0.9642	0.9486	0.762	0.781	0.7754	0.5062	0.5174	0.5169
AB1	0.8476	0.8361	0.8597	0.6284	0.637	0.6414	0.4715	0.4556	0.4685
AB2	0.6204	0.6318	0.6297	0.4371	0.4298	0.4393	0.4856	0.4843	0.4721
AB3	0.7127	0.7202	0.7364	0.5574	0.5248	0.5467	0.5488	0.5514	0.5381
B1	0.9926	0.9889	0.9923	0.7218	0.7485	0.7317	0.6316	0.6497	0.6516
B2	0.6612	0.6785	0.6841	0.6984	0.7161	0.7176	0.4975	0.512	0.5208
B3	0.8426	0.846	0.8362	0.5793	0.586	0.5923	0.5296	0.5618	0.5835

APPENDIX F. Complete Tukey HSD post hoc comparison

Table F1. Complete Tukey HSD post hoc comparisons among blood type groups for percentage inhibition of erythrocyte membrane lysis

Comparison	Mean Difference	SE	t	pTukey	Cohen's d	95% CI Lower	95% CI Upper
PC – NC	0.747	0.023	32.76	< .001*	15.445	14.210	16.679
PC – A	0.199	0.019	10.69	< .001*	4.113	3.121	5.105
PC – B	0.091	0.019	4.88	< .001*	1.879	1.061	2.697
PC – AB	0.153	0.019	8.20	< .001*	3.156	2.250	4.062
NC – A	–0.548	0.019	–29.44	< .001*	–11.331	–12.957	–9.706
NC – B	–0.656	0.019	–35.25	< .001*	–13.566	–14.899	–12.233
NC – AB	–0.594	0.019	–31.93	< .001*	–12.289	–13.786	–10.791
A – B	–0.108	0.013	–8.21	< .001*	–2.234	–2.875	–1.594
A – AB	–0.046	0.013	–3.52	.006*	–0.957	–1.518	–0.397
B – AB	0.062	0.013	4.69	< .001*	1.277	0.702	1.853

Note: Mean Difference = difference in estimated marginal means of percentage inhibition. SE = standard error of the mean difference. t = t-statistic for the pairwise comparison. pTukey = p-value adjusted for multiple comparisons using Tukey's HSD procedure. Cohen's d = standardized effect size. 95% CI = 95% confidence interval for Cohen's d (Lower, Upper). PC = positive control (acetylsalicylic acid, 0.10 mg/mL); NC = negative control (hypotonic-buffered solution without extract). A = Blood Type A; B = Blood Type B; AB = Blood Type AB. df = 84.0 for all comparisons. Comparisons are based on estimated marginal means averaged across concentration levels. *p < .05 (Tukey's HSD corrected).

Table F2. Complete Tukey HSD post hoc comparisons among extract concentrations for percentage inhibition of erythrocyte membrane lysis

Comparison	Mean Difference	SE	t	pTukey	Cohen's d	95% CI Lower	95% CI Upper
1.0 mg/mL – 1.5 mg/mL	–0.053	0.014	–3.87	< .001*	–1.093	–1.680	–0.506
1.0 mg/mL – 2.0 mg/mL	–0.090	0.014	–6.58	< .001*	–1.861	–2.490	–1.230
1.5 mg/mL – 2.0 mg/mL	–0.037	0.014	–2.71	.022*	–0.767	–1.340	–0.192

Note: Mean Difference = difference in estimated marginal means of percentage inhibition. SE = standard error of the mean difference. t = t-statistic for the pairwise comparison. pTukey = p-value adjusted for multiple comparisons using Tukey's HSD procedure. Cohen's d = standardized effect size. 95% CI = 95% confidence interval for Cohen's d (Lower, Upper). df = 84.0 for all comparisons. Comparisons are based on estimated marginal means averaged across all blood type groups. Negative mean differences indicate that higher concentrations produced greater inhibition values. *p < .05 (Tukey's HSD corrected).

APPENDIX G. Grammarly report and Turnitin results

Report: JANE SULITE

JANE SULITE

by Misamis University

General metrics

50,535	6,894	450	27 min 34 sec	53 min 1 sec
characters	words	sentences	reading time	speaking time

Writing Issues

 No issues found

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CURRICULUM VITAE

PERSONAL INFORMATION

Name : Allyza Jane S. Dangga
Age : 21
Birthdate : September 05, 2004
Civil Status : Single
Nationality : Filipino
Home Address : Poblacion, Purok 3, Kolambugan, Lanao del Norte
Current Address : Lapu-Lapu Street, Aguada, Ozamiz City
Contact Number : 09639361461
E-mail Address : allyzjanedangga4@gmail.com

EDUCATIONAL BACKGROUND

Tertiary School : Bachelor of Science in Medical Technology
Misamis University
Ozamiz City, Misamis Occidental
2026 – Present

Secondary School : Holy Cross High School Kolambugan, Inc.
Kolambugan, Lanao del Norte
2022 – 2023

Primary School : Holy Cross Elementary School Kolambugan, Inc.
Kolambugan, Lanao del Norte
2016 – 2017

CURRICULUM VITAE

PERSONAL INFORMATION

Name : Anthony M. Fullo II

Age : 21

Birthdate : December 28, 2004

Civil Status : Single

Nationality : Filipino

Home Address : Poblacion II, Clarin, Misamis Occidental, Mindanao, 7201

Current Address : Poblacion II, Clarin, Misamis Occidental, Mindanao, 7201

Contact Number : 09173108307

E-mail Address : anthonyfullo2@gmail.com

EDUCATIONAL BACKGROUND

Tertiary School : Bachelor of Science in Medical Technology
Misamis University
Ozamiz City, Misamis Occidental
2026 – Present

Secondary School : Misamis University
Ozamiz City, Misamis Occidental
2022-2023

Primary School : Fernandez Zacal Learning Center
Ozamiz City
2016 – 2017

CURRICULUM VITAE

PERSONAL INFORMATION

Name : Princess Jane B. Sulite
Age : 21
Birthdate : March 2, 2005
Civil Status : Single
Nationality : Filipino
Home Address : Purok 3, Lala Proper, Lala, Lanao del Norte
Current Address : Laurel Street, Ozamiz City, Misamis Occidental
Contact Number : 09752484144
E-mail Address : jaynesulite@gmail.com

EDUCATIONAL BACKGROUND

Tertiary School : Bachelor of Science in Medical Technology
Misamis University
Ozamiz City, Misamis Occidental
2026 – Present

Secondary School : Lala National High School
Maranding, Lala, Lanao del Norte
2022 – 2023

Primary School : Lala Proper Integrated School
Lala Proper, Lala, Lanao del Norte
2016 – 2017

