Anticoagulant Property of Periwinkle (*Catharanthus roseus*) Leaf Extract Formulated as Solution

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Abstract

Coagulopathy is a growing global health concern, often associated with conditions such as hypertension and diabetes, which increase the risk of thrombotic events like deep vein thrombosis and pulmonary embolism. Current anticoagulant therapies, including warfarin and heparin, are effective but pose risks such as bleeding complications and require close monitoring (Harter et al., 2015). This study explored the anticoagulant potential of Periwinkle (*Catharanthus roseus*), a medicinal plant traditionally used to manage high blood pressure and diabetes. Rich in bioactive compounds like alkaloids and flavonoids, periwinkle has the potential to influence blood coagulation pathways. An in vitro experiment was conducted using human plasma samples from females aged 18 to 21 years with blood type A. Ethanol-extracted periwinkle leaf was tested at three concentrations (0.05 g/100 mL, 0.1 g/100 mL, and 0.2 g/100 mL) to evaluate its effect on Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT). Distilled water served as the negative control, while garlic extract was used as the positive control. Results showed that the 0.2 g/100 mL concentration had the most pronounced anticoagulant effect, with mean PT value of 26.6 seconds and mean aPTT value of 53.3 seconds, respectively. Lower concentrations namely 0.1g/100 mL yielded a mean PT of 19.3 seconds and aPTT of 43.7 seconds, while 0.05g/100 mL yielded a mean PT of 17.5 seconds and aPTT of 43.7 seconds, which showed shorter clotting times, confirming a dose-dependent response. Statistical analysis indicated significant differences between experimental and control groups, particularly at the highest concentration ($p < 0.00005$). These findings suggest that *Catharanthus roseus* extract exhibits promising anticoagulant activity and may serve as a natural alternative to conventional anticoagulants, warranting further investigation in clinical settings.

Keywords: Anticoagulants; *Catharanthus roseus*; in-vitro; prothrombin time (PT); activated partial thromboplastin time (aPTT)

1. Introduction

Catharanthus roseus, also known as periwinkle or Tsitsirika for its local name, is a perennial species of flowering plant in the family Apocynaceae. It was formerly in the genus Vinca as Vinca rosea. It is an erect, smooth or slightly hairy, simple or slightly branched plant, 30 to 50 centimeters high. Stems are somewhat woody. Leaves are oblong, 4 to 7 centimeters long, rounded at the tip, and pointed at the base. Flowers are white, pink, or red, or variegated white and red, 3.5 cm to 5 centimeters across, borne in the axils of the leaves. Calyx lobes are green and very slender. about 4 millimeters long. Corolla-tube is slender, 2.5 to 3 centimeters long, and pale green; the limb is spreading with obliquely obovate lobes 1.7 to 2.5 centimeters wide. Fruit is a hairy and cylindrical follicle, 2 to 3 centimeters long (*Tsitsirika*/Periwinkle/ *Catharanthus Roseus*: Herbal Medicinal Plants/ Philippine Alternative Medicine, 2023).

Coagulopathy contributed to considerable morbidity and mortality worldwide. Millions experience bleeding episodes, and underlying conditions like hypertension and diabetes, which are leading causes of death in the Philippines, exacerbate the risk of thrombosis. Conventional treatments for Thromboembolic Diseases, such as deep-vein thrombosis and pulmonary embolism, rely on anticoagulants like warfarin and heparins. While effective, these drugs come with a significant drawback, the risk of bleeding complications. This necessitates careful monitoring and limits their widespread use. Due to the limitations of existing treatments, this leads us to *Catharanthus roseus*, commonly known as periwinkle. Traditionally, it's been used to manage conditions like high blood pressure and diabetes. Periwinkle is rich in bioactive compounds, including alkaloids and flavonoids, that have the potential to influence the blood coagulation pathways.

Thromboembolic disorders, such as deep vein thrombosis (DVT) and pulmonary embolism (PE), pose serious health risks due to abnormal blood clot formation that can block blood flow to vital organs, leading to complications like stroke, organ damage, or death. To reduce morbidity and mortality, anticoagulant therapies—including warfarin, heparins, and factor Xa inhibitors—are essential for both preventing clot formation in high-risk populations (e.g., patients with atrial fibrillation, artificial heart valves, or undergoing surgery) and treating existing clots to prevent progression and recurrence. Without timely intervention, thromboembolism can cause long-term issues such as post-thrombotic syndrome, chronic pain, and swelling, significantly affecting patients' quality of life. Therefore, effective prevention and treatment strategies are critical to managing these life-threatening conditions and minimizing their burden on patients and healthcare systems.

From a healthcare perspective, effective prevention and treatment of thromboembolic disorders help reduce the economic burden linked to extended hospital stays, emergency care, and rehabilitation. Conventional anticoagulant therapies, such as warfarin, require careful management to balance bleeding risks with therapeutic benefits, often necessitating close monitoring like INR testing. Alongside these traditional treatments, there is growing interest in complementary and alternative medicine (CAM) approaches, driven by the complexities and side effects of conventional drugs. Eastern medical systems like Traditional Chinese Medicine and

Ayurveda offer holistic treatments using natural remedies—such as turmeric, ginger, and garlic—with anticoagulant and circulation-enhancing properties, often accompanied by mind-body practices like meditation, yoga, and acupuncture that support stress reduction and blood flow regulation. These CAM modalities may present fewer side effects, improve patient adherence, and enhance quality of life, especially in cultures where holistic medicine is culturally embraced. While conventional anticoagulants remain essential for preventing serious complications, integrating CAM offers multifaceted strategies to manage thromboembolic disorders more comprehensively.

The study of the anticoagulant effects of periwinkle (Catharanthus roseus) is significant due to its potential to address coagulopathy, a common and serious condition in critically ill patients that can lead to bleeding and thrombotic complications such as deep vein thrombosis (DVT), pulmonary embolism (PE), and disseminated intravascular coagulation (DIC). These thromboembolic diseases complicate patient management and increase mortality risk, highlighting the need for safer anticoagulant therapies. While current synthetic anticoagulants are effective, their use is often limited by severe side effects like bleeding. Periwinkle, rich in bioactive phytochemicals, offers a promising complementary and alternative medicine (CAM) option, potentially influencing blood coagulation pathways with fewer adverse effects. Its long history of medicinal use and emerging research into its anticoagulant properties align with the broader goal of developing safer, plant-based treatments for coagulation disorders, addressing the limitations of conventional drugs and meeting the urgent need for effective therapies in conditions such as COVID-19-associated coagulopathy.

2. Objectives

The primary objective of this study is to evaluate the anticoagulant property of Periwinkle (Catharanthus roseus) leaf extract formulated as a solution. Specifically, the study aims to determine the most effective concentration among 0.05g/100mL, 0.1g/100mL, and 0.2g/100mL in terms of prolonged Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT). It also seeks to assess whether there is a significant difference in the anticoagulant activity among the experimental groups treated with Periwinkle extract, the positive control group using Garlic extract, and the negative control group using distilled water. Furthermore, the study intends to evaluate the physicochemical characteristics of the most effective Periwinkle extract solution by analyzing its specific gravity, pH, color, odor, and clarity.

3. Materials and Methods

This section lays out the materials and method in the determination of the anticoagulant properties of the Periwinkle *(Catharanthus Roseus)* Leaf Extract. This section included the processes that were involved in the study, including the research design, population and locale of the study, data gathering tools, data gathering procedures, and the treatment of data.

Research Design

An experimental research design was utilized to determine the anticoagulant property of Periwinkle *(Catharanthus roseus)* extract formulated as a solution. The experimental method in educational research is the application and adaptation of the classical method of experimentation. It is a scientific method of conducting research in which one or more independent variables will be altered and applied to one or more dependent variables in order to determine their influence on the latter. It is an attempt by the researchers to maintain control over all factors that may affect the result of an experiment. The researcher will attempt to determine or predict what may occur (Zubair, 2023).

Collection, Preparation, and Extraction of the Plant Sample

The plant sample used for this study, Periwinkle (*Catharanthus roseus*), specifically, the leaves were collected in San Cristobal, Bangar, La Union where the sample is widely available and abundant. This location was chosen due to the presence of lush vegetation and suitable climate, as periwinkle plants thrive in moderate temperatures between 60–75°F (15–23°C) and perform well in average humidity levels. To ensure sample reliability and prevent contamination, periwinkle leaves were handpicked using sterile gloves and transported in sterile paper bags. The leaves were air-dried for two days, then oven-dried at 40°C for 6–8 hours to preserve sensitive bioactive compounds like flavonoids and alkaloids while effectively removing moisture to prevent microbial growth. This optimized drying process balanced dehydration with minimal nutrient loss. Finally, the dried leaves were ground into a fine powder using clean equipment and stored in airtight containers to maintain quality and prevent moisture absorption (Drugs.com, 2024; Dubey, Shukla, Hussain, & Tasin, 2023; ElGamal et al., 2023; John Innes Centre, 2021; RxList, 2021; Sorraia, 2018).

The powdered periwinkle leaves were soaked in 70% ethanol, then filtered to obtain the extract. This filtrate was concentrated using a rotary evaporator, where the solution was placed in a pre-weighed flask connected to a cooled condenser. Under partial vacuum and gentle rotation in a water bath, the ethanol solvent evaporated and was collected, with careful measures taken to prevent splashing and bumping. After evaporation, the flask was kept under reduced pressure to remove any remaining solvent. The resulting residue was weighed and used for phytochemical analysis and testing of its anticoagulant properties.

Confirmatory Testing of the Constituents Plant Sample

The researchers performed phytochemical analysis as a confirmatory test on Periwinkle (*Catharanthus roseus*) to determine the constituents present in the plant sample, including alkaloids and flavonoids. The presence of alkaloids was confirmed by conducting Mayer's, Wagner's, and Hager's tests. Flavonoids were detected through the Alkaline reagent test and the Lead Acetate test. *(Appendix H and Appendix I)*

Treatment Groups

The study involved five treatment groups to evaluate the anticoagulant activity of Periwinkle (Catharanthus roseus) leaf extract solution. The experimental group consisted of three different concentrations of periwinkle extract—0.05g/100mL, 0.1g/100mL, and 0.2g/100mL—each prepared by diluting the respective amount of extract with distilled water to a final volume of 100 mL. The positive control group used garlic extract, prepared similarly through rotary evaporation, with 0.1g diluted in 100 mL of distilled water. Distilled water served as the negative control to establish a baseline coagulation time without any anticoagulant effect, ensuring that any changes observed in the experimental and positive control groups were due to the active extracts. This setup allowed for a clear comparison of the anticoagulant effects of periwinkle extract against a known anticoagulant and a neutral baseline.

Human Blood Samples

The blood samples were obtained from 3 female volunteer participants with blood type A, specifically within the age range of 18 to 21 years old, as this demographic is more susceptible to thromboembolic diseases. Prior to sample collection, the participants also underwent blood type testing to confirm their ABO classification and ensure consistency in the study group.

A questionnaire was used to assess their health and adherence to the criteria *(Appendix R)*, and informed consent was obtained from all participants to ensure they understood the study's purpose, procedures, risks, and benefits, with confidentiality maintained in accordance with the Data Privacy Act of 2012 (RA 10173). Blood samples of approximately 15 mL were collected by a supervising medical technologist, yielding about 6 mL of plasma per sample. The blood was centrifuged at 1,000-2,000 x g for 10 minutes to separate plasma, which was then carefully transferred into sodium citrate-containing tubes for use in anticoagulant testing.

In-Vitro Testing

In vitro testing of anticoagulants using human blood samples involved critical steps to ensure accurate and reliable results. After sample collection, the subsequent step was testing for anticoagulant activity, which included two primary assays: Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT).

Anticoagulant Activity Testing

Prothrombin Time (PT)

The researchers conducted Prothrombin Time (PT) testing at the University of Baguio laboratory, utilizing their specialized equipment. The PT reagent was prewarmed, and test samples were prepared for each group by mixing 0.1 mL of the respective treatment solution (periwinkle extract at concentrations of 0.05, 0.1, and 0.2 g/100 mL, garlic extract as positive control, or distilled water as negative control) with 0.2 mL of plasma from three participants, with each sample replicated three times. After adding 0.3 mL of PT reagent to each sample, clotting time was measured by tilting the test tubes

every five seconds until clot formation occurred. The clotting times were recorded and compared across all groups, with normal Prothrombin Time expected to be above 13 seconds and an INR range of 2.0 to 3.0 considered therapeutic for anticoagulants.

Activated Partial Thromboplastin Time (APTT)

For determining the Activated Partial Thromboplastin Time, coagulation analyzer was utilized. First, each containing the appropriate treatment group composition was prepared and labeled. The grouping of the test samples was as follows:

The experimental setup involved preparing test samples by mixing 1 mL of treatment solution—periwinkle extract at concentrations of 0.05, 0.1, and 0.2 g/100 mL for the experimental groups, garlic solution for the positive control, and distilled water for the negative control—with 0.1 mL of plasma collected from three participants, each replicated three times. These samples were loaded into a coagulation analyzer, where 0.1 mL of Activated Partial Thromboplastin Time (APTT) reagent containing phospholipid and activator was added to each test tube. The analyzer automatically measured the clotting time, reflecting the function of intrinsic pathway clotting factors (VIII, IX, XI, and XII). The APTT results, typically expected to range from 36 to 70 seconds for anticoagulant activity, were recorded and analyzed to assess the anticoagulant effects of the treatments.

Evaluation Test for Periwinkle Extract Solution

The evaluation of the most effective concentration of Periwinkle Extract Solution involved several physicochemical tests to ensure quality and regulatory compliance. Specific gravity was measured using a pycnometer by comparing the weight of the formulated solution to that of distilled water, calculating the ratio to determine relative density. The pH was assessed by dipping pH paper into the solution and comparing the color change to an indicator scale to determine acidity or alkalinity. Color and odor were evaluated through visual and olfactory assessments conducted by six independent respondents—including 2 pharmacists, 2 pharmacy students, and 2 students from different departments at LORMA Colleges—using a structured questionnaire. Lastly, clarity was tested by passing a beam of light through the solution to confirm it was clear and free from particles, with observations also recorded via the questionnaire. These comprehensive tests ensured the solution's physicochemical properties met quality standards.

4. Results

Figure 1. Results of the Most Effective Concentration Based on the Prothrombin Time (PT)

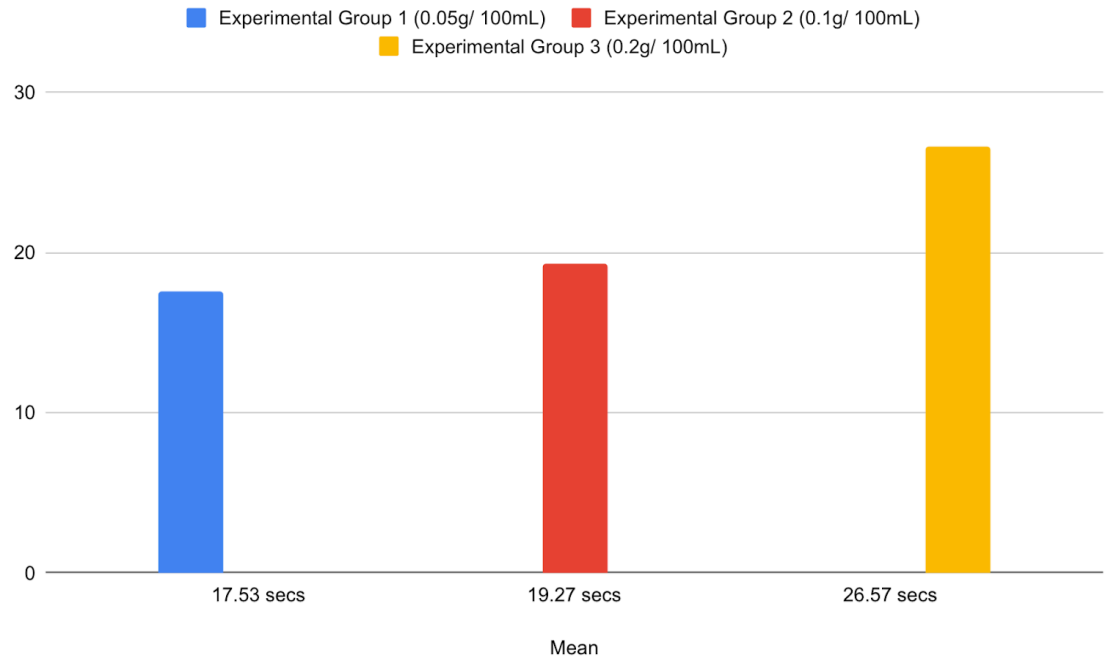

Figure 2. Results of the Most Effective Concentration Based on the Activated Partial Thromboplastin Time (aPPT)

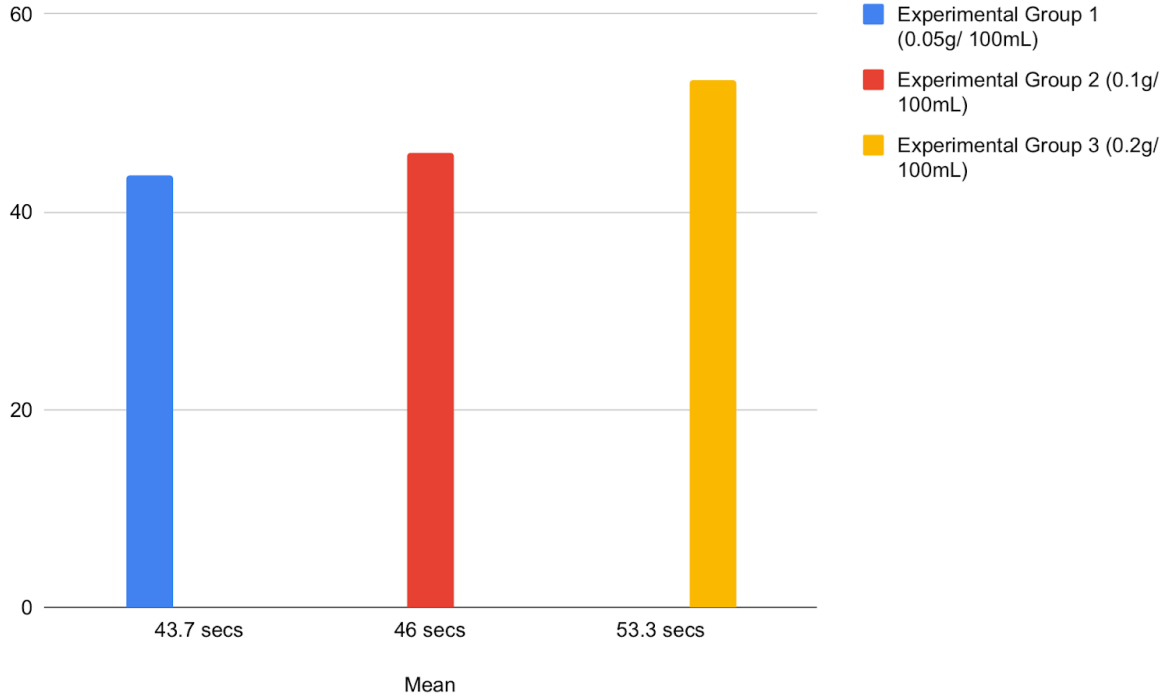

Table 1.a ANOVA Results of the The Difference of the Different Control Group Based on the Prothrombin Time (PT)

One Factor ANOVA					
	Replication 1	**Replication 2**	**Replication 3**	**Mean**	**n**
Experimental Group 1 (0.05 g/100 mL)	18.4 secs	17.7 secs	16.5 secs	17.53	3
Experimental Group 2 (0.1 g/100 mL)	21.7 secs	17.7 secs	18.4 secs	19.27	3
Experimental Group 3 (0.2 g/100 mL)	29.1 secs	28.9 secs	21.7 secs	26.57	3
Positive Group	17.1 secs	16.1 secs	15.6 secs	16.27	3
Negative Group	14 secs	14.2 secs	14.5 secs	14.23	3
				18.77	15
Source	SS	df	MS	F	p-value
Treatment	268.236	4	67.0590	14.03	**0.0004**
Error	47.813	10	4.7813		
Total	316.049	14			

Table 1.b. Results of the Post Hoc Analysis of the Different Control Group Based on the Prothrombin Time (PT)

Post Hoc Analysis **P-values for Pairwise t-test**					
	Experimental Group 1 (0.05g/mL)	**Experimental Group 2 (0.1g/mL)**	**Experimental Group 3 (0.2 g/mL)**	**Positive Group**	**Negative Group**
Experimental Group 1 (0.05g/100 mL)				0.4942	0.0943
Experimental Group 2 (0.1g/100 mL)	0.3545			0.1238	0.0182
Experimental Group 3 (0.2 g/ 100 mL)	**0.0005**	**0.0022**		**0.0002**	**4.15×10^{-5}**
Positive Group					0.2813
Negative Group					

Table 2.a. *ANOVA Results of the Difference of the Different Control Group Based on the Activated Partial Thromboplastin Time (aPPT)*

One Factor ANOVA					
	Replication 1	**Replication 2**	**Replication 3**	**Mean**	**n**
Experimental Group 1 (0.05g/100 mL)	44 secs	43 secs	44 secs	43.7	3
Experimental Group 2 (0.1g/ 100 mL)	48 secs	44 secs	46 secs	46	3
Experimental Group 3 (0.2 g/ 100 mL)	54 secs	53 secs	53 secs	53.3	3
Positive Group	56 secs	53 secs	52 secs	53.7	3
Negative Group	34 secs	33 secs	35 secs	34	3
				46.1	15
Source	SS	df	MS	F	p-value
Treatment	785.73	4	196.433	98.22	**5.54×10^{-08}**
Error	20.00	10	2.000		
Total	805.73	14			

Table 2.b. Results of the Post Hoc Analysis of the Different Control Group Based on the Activated Partial Thromboplastin Time (aPPT)

Post Hoc Analysis **P-values for Pairwise t-test**					
	Experimental Group 1 (0.05g/ 100 mL)	**Experimental Group 2 (0.1g/ 100 mL)**	**Experimental Group 3 (0.2 g/ 100 mL)**	**Positive Group**	**Negative Group**
Experimental Group 1 (0.05g/100 mL)					**7.89×10^{-6}**
Experimental Group 2 (0.1g/ 100 mL)	0.709				**1.21×10^{-8}**
Experimental Group 3 (0.2 g/ 100 mL)	**7.89×10^{-6}**	**0.0001**			**1.21×10^{-8}**
Positive Group	**5.84×10^{-6}**	**0.0001**	0.7787		**1.03×10^{-8}**
Negative Group					

Table 3. The Physicochemical Characteristics of the Most Effective Concentration Solution

Physicochemical Characteristics	Evaluation
Specific Gravity	1.002
pH	pH 6
Color	Light Green- Yellowish Neutral- Moderately Appealing Color
Odor	No Detectable Odor- Mild Herbal Scent Neutral- Acceptable Odor
Clarity	Clear Neutral- Acceptable Clarity

5. Discussion

Figure 1. shows the time (seconds) the blood clotted using the prothrombin time test with the 0.05 g/100 mL, 0.1 g/100 mL, and 0.2 g/100 mL concentration of *Catharanthus roseus* solution. Experimental Groups 1 (0.05g/mL), 2 (0.01g/mL), and 3 (0.02g/mL) yielded means of 17.53 secs, 19.27 secs, and 26.57 secs. An increased time of blood clotting indicates the most effective concentration. Therefore, the 0.2 g/mL concentration, having the highest mean among the three concentrations, is the most effective as to the prothrombin time test.

Although the INR value of Experimental Group 3 does not reach the Therapeutic Anticoagulant Reference Range (INR = 2.0 to 3.0), it can still be effective as an anticoagulant, particularly when the INR values are close to 2.0. Including the INR in the Prothrombin Time (PT) measurement is essential because it standardizes prothrombin time results across different laboratories and reagents, ensuring consistent and reliable monitoring of anticoagulant therapy effectiveness and safety (Favaloro & Lippi, 2020). This claim is supported by a retrospective observational study at Hotel-Dieu Hospital showed that while patients spent on average 68.9% of their time within the classical therapeutic INR range of 2.0 to 3.0, they spent 98.7% of their time within a broader INR range of 1.5 to 3.5, which was considered very safe and effective. The study and supporting literature emphasize that a target INR between 1.5 and 2.0 is regarded as an effective and safe alternative for patients requiring prolonged anticoagulant therapy, especially those at higher bleeding risk. Minor adjustments are typically sufficient when INR values are near the lower limit, such as 1.9, without comparing clinical effectiveness significantly (Nguyen et al., 2013).

The mean PT values demonstrated a dose-dependent increase in clotting time with higher concentrations of periwinkle extract. This finding aligns with studies by Azmi, Anis, & Syaripah (2023) and Alikhani et al. (2017) which both reported that their plant extracts which includes *Momordica charantia*, *Terminalia bellirica*, *Astragalus arbusculinus*, and *Origanum vulgare* significantly prolonged PT values in vitro in a dose-dependent manner, with the strongest effects observed at the highest concentrations, which also indicates potent anticoagulant activity. The

mechanism behind this effect relates to the principle of PT testing: the assay measures the time required for plasma to clot after the addition of tissue factor (thromboplastin) and calcium ions, thereby evaluating the extrinsic and common coagulation pathways. The prolonged clotting time observed is attributed to the inhibition of factors involved in both extrinsic pathway (Factor VII) and common pathway factors (X, V, II, I), further supporting the anticoagulant properties of these plant extracts.

According to Singh et al. (2020), in *Catharanthus roseus*, flavonoids and alkaloids exhibit mechanisms that influence the extrinsic coagulation pathway (Factor VII), as measured by prothrombin time (PT) testing, through their bioactive properties. Alkaloids such as vinblastine, catharanthine, and ajmalicine—key terpenoid indole alkaloids (TIAs) produced by *Catharanthus roseus*, which are known to interfere with enzymatic processes and signaling pathways that could potentially affect coagulation factors. These alkaloids are biosynthesized through pathways involving tryptophan metabolism and monoterpenoid biosynthesis, which are regulated by transcription factors like ORCA3 and MYC2 under jasmonic acid (JA) signaling. The regulation of these pathways may indirectly modulate calcium-binding sites or enzymatic activity required for coagulation, including tissue factor (TF) and factor VIIa in the extrinsic pathway. Additionally, *Catharanthus roseus* flavonoids, derived from phenylpropanoid and flavonoid biosynthesis pathways, have antioxidant and anti-inflammatory properties that inhibit tissue factor expression or activity, reducing activation of the extrinsic pathway (Frontiers in Genetics, 2022; MDPI, 2016). These mechanisms suggest that bioactive compounds in *Catharanthus roseus* could serve as natural anticoagulants by targeting key steps in the extrinsic coagulation cascade.

Figure 2. shows the time (seconds) that the blood clotted as a partial prothrombin time test with the 0.05 g/100 mL, 0.1 g/100 mL, and 0.2 g/100 mL concentration of *Catharanthus roseus* solution, for the 0.05 g/100 mL concentration, it yielded a mean of 43.7 while for concentration of 0.1 g/100 mL, it yielded a mean of 46 secs, and 0.2 g/100 mL concentration yielded a mean of 53.3 secs. Three of the concentrations belong to the therapeutic reference range for anticoagulants thus, the three concentrations are effective as an anticoagulant. The longest clotting time of blood clotting while still in the therapeutic anticoagulant reference range indicates that it is the most effective concentration, as longer aPTT values within the safe window reflect stronger anticoagulant activity without exceeding the limit that increases bleeding risk. Therefore, the 0.2 g/100 mL concentration, having the highest mean with 53.3 secs among the three concentrations, is the most effective for activated partial thromboplastin time test.

The mean aPTT values showed a dose-dependent increase in clotting time with higher concentrations of Periwinkle extract. The study by Abdallah et al. (2022), describes the aPTT as a measure of the time required for fibrin clot formation, starting from the initiation of the intrinsic coagulation pathway, which is a key component of the blood clotting process. The intrinsic pathway is activated when blood comes into contact with negatively charged surfaces and involves a sequence of clotting factors (XII, XI, IX, and VIII) that activate one another,

ultimately leading to Factor X activation and fibrin clot formation. This finding is consistent with a study conducted by Pouyfung & Sukati (2021), which revealed that increased concentrations of flavonoids are associated with a more significant prolongation of the activated partial thromboplastin time (aPTT). This extension in clotting time is particularly linked to their impact on specific coagulation factors, namely VIII, IX, XI, and XII, which are crucial components of the intrinsic pathway of coagulation. The interaction of flavonoids with these factors appears to disrupt their normal functioning, leading to a delay in the clotting process. Moreover, this effect exhibits a dose-dependent relationship, indicating that higher levels of flavonoids result in more pronounced alterations in aPTT, suggesting a potential therapeutic role for flavonoids in managing conditions related to coagulation disorders.

Evaluation of the Different Control Groups

The three control groups involving the garlic extract (positive control group), distilled water (negative control group), and the periwinkle extract (experimental group) with different concentrations (0.05 g/100 mL, 0.1 g/100 mL, 0.2 g/100 mL) were compared together. This comparison was conducted by analyzing the variables using a One-way ANOVA, followed by a Pairwise t-test for Post-Hoc Analysis to determine any significant differences. The significance level, or the alpha value, is set at 0.05. This threshold is commonly used in statistical tests to determine whether the observed results are statistically significant, allowing researchers to conclude their hypotheses (Johnson, Stone, Bunn, & Navalta, 2020). The results provided valuable insights into the efficacy of periwinkle *(Catharanthus roseus)* leaf extract as an anticoagulant.

Table 1.a provides the average prothrombin times observed for each treatment group. Experimental Group 1 recorded a mean prothrombin time of 17.53 seconds, while Experimental Group 2 had a slightly longer average at 19.27 seconds. Meanwhile, Experimental Group 3 showed a significantly higher mean prothrombin time of 26.57 seconds. In comparison, the positive control group demonstrated an average prothrombin time of 16.27 seconds, whereas the negative control group had a lower average of 14.23 seconds.

The results of the ANOVA analysis yield a p-value of 0.0004, which is substantially lower than the predetermined alpha value of 0.05. This statistically significant result ($p < 0.05$) means that the differences observed in prothrombin time among the control groups are unlikely due to chance. Therefore, the null hypothesis—stating there is no difference among the control groups—is rejected, confirming that at least one group differs significantly in its anticoagulant effect.

Table 1.b presents the comparison of paired treatment groups using the Pairwise t-test, highlighting significant differences in prothrombin time (PT) when using various concentrations of periwinkle extract, garlic extract, and distilled water as a control. The findings suggest that these extracts notably influence PT, indicating potential effects on blood coagulation. Specifically, the results illustrate how different concentrations of periwinkle and garlic extracts can alter PT outcomes. Further details on the results are provided below.

Negative Control Group vs Positive Control Group

The Negative Control Group and the Positive Control Group show no significant difference in coagulation time, with p = 0.2813. The average time for the Negative Control Group is 14.23 seconds, while the Positive Control Group averages 16.27 seconds, exceeding the normal reference range of 11-15 seconds. However, the 2.04 second difference is not statistically significant, indicating a mild elevation in coagulation time without a robust anticoagulant effect. The INR of the Positive Control Group is 1.21, which is below the therapeutic range of 2.0 - 3.0, suggesting it does not demonstrate anticoagulant activity at a clinically effective level.

Negative Control Group vs Experimental Control Group 1

There is no significant difference between the Negative Control and Experimental Group 1 (0.05 g/100 mL), as indicated by a p-value of 0.0943. This suggests that the low concentration lacks sufficient active compounds to alter Prothrombin Time (PT). Studies by Wang et al. (2017) and Zhang et al. (2016) support that effective anticoagulant activity typically requires higher doses or an INR of 2.0–3.0, which was not observed here. Thus, this concentration is likely ineffective for therapeutic use.

Negative Control Group vs Experimental Control Group 2

There is no significant difference between the Negative Control Group and Experimental Group 2 (0.1 g/100 mL concentration), as supported by a p-value above 0.05 and near-normal PT and INR values. Tanaka et al. (2018) note that minor PT changes are clinically negligible if the INR remains below 1.2, which aligns with findings here. Though INR slightly increased, it stayed well below the therapeutic anticoagulation range (2.0–3.0), suggesting the substance's blood-thinning effect is dose-dependent and still too weak at this concentration to be effective.

Negative Control Group vs Experimental Control Group 3

Experimental Group 3 (0.2 g/100 mL) showed a statistically significant anticoagulant effect compared to the Negative Control Group (p = 0.0000415), indicating that this concentration of periwinkle (*Catharanthus roseus*) extract impacts blood clotting. This effect is likely due to its phytochemicals—especially flavonoids and alkaloids—which can disrupt coagulation pathways. According to Pouyfung and Sukati (2021), flavonoids such as quercetin, kaempferol, and luteolin prolong prothrombin time in a dose-dependent manner, influenced by their structural features. Sadowski et al. (2014) also found that flavonoids including procyanidin B2, cyanidin, quercetin, and silybin, inhibit key enzymes like factor Xa and thrombin by binding to their active sites. Together, these findings support the extract's potential as a natural anticoagulant through inhibition of the coagulation cascade.

Positive Control Group vs Experimental Control Group 1

There is no statistically significant difference between the Positive Control Group and Experimental Group 1 (0.05 g/100 mL), as indicated by a p-value above the threshold for significance. Although both groups show slightly prolonged prothrombin times (PT), Experimental Group 1's mean PT of 17.53 seconds is not meaningfully different from the Positive Control's 16.27 seconds, which is already near the lower bound of the therapeutic anticoagulation range (>15 seconds). McRae, Militello, & Refaai (2021) support that low-dose

anticoagulants may have limited additional impact when baseline PT is already elevated. These findings suggest that the 0.05 g/100 mL concentration does not enhance anticoagulation beyond the effect seen in the positive control and that higher doses may be needed for stronger efficacy.

Positive Control Group vs Experimental Control Group 2

The Positive Control and Experimental Group 2 (0.1 g/100 mL) showed no significant difference in anticoagulant effect based on PT values, as indicated by their p-value. This suggests that both treatments have similar, mild anticoagulant activity. Benbott et al. (2023) attributed such effects to alkaloids in *Catharanthus roseus*, while Nuswantoro and Berlianti (2022) found that garlic extract also prolongs PT through its bioactive compounds. Although both groups had PT values exceeding the normal range of 11–15 seconds (Ross, 2019), they remained below the therapeutic INR threshold of 2.0–3.0 (Shikdar & Bhattacharya, 2023), reinforcing the conclusion of modest but comparable anticoagulant action.

Positive Control Group vs Experimental Control Group 3

Experimental Group 3 (0.2 g/100 mL periwinkle extract) significantly differed from the Positive Control Group ($p = 0.0002$), indicating that higher concentrations of periwinkle may modulate coagulation pathways. Pouyfung & Sukati (2021) support this, noting that flavonoid structure—particularly hydroxyl group count—affects anticoagulant activity, primarily via inhibition of intrinsic pathway components.

In contrast, garlic (the positive control) affects clotting differently. Studies by Kim et al. (2018) and Montoya et al. (2024) found that garlic extract more strongly prolongs activated partial thromboplastin time (aPTT) than prothrombin time (PT), targeting intrinsic factors (XII, XI, IX, VIII) rather than the extrinsic pathway. Vaijayanthimala (2017) found that garlic extracts prepared with aqueous or methanol solvents were more effective at prolonging PT—unlike ethanol extracts used in the present study. Omar et al. (2018) confirmed that extraction solvents significantly influence anticoagulant potency, with aqueous and methanolic extracts typically yielding stronger effects than ethanol-based ones, due to differing phytochemical solubility profiles.

Additionally, Abualhasan, Chaurasiya, and Jaradat (2018) highlighted garlic's unique mechanisms, such as enhancing fibrinolysis via t-PA activation and influencing thromboxane A2 production through diallyl trisulfide. These differences underscore the distinct anticoagulant profiles between garlic and periwinkle and the importance of solvent selection in phytochemical extraction.

Experimental Control Group 1 vs Experimental Control Group 2

Experimental Control Groups 1 (0.05 g/100 mL) and Experimental Group 2 (0.1 g/100 mL) showed no significant difference in anticoagulant activity based on aPTT measurements, indicating that both concentrations of *Catharanthus roseus* extract are likely below the effective threshold. This aligns with Alzahrani et al. (2022), who noted that low flavonoid concentrations typically have minimal impact on coagulation. Similarly, Doudi and Setorki (2014) and Kim et al. (2020) emphasized that the anticoagulant effects of flavonoids and alkaloids are dose-dependent, with little to no physiological response at sub-therapeutic levels. These findings

suggest that higher concentrations are needed to observe meaningful effects on intrinsic coagulation pathways.

Experimental Control Group 1 vs Experimental Control Group 3

Furthermore, Experimental control group 1 (0.05 g/100 mL) exhibits a significant difference from experimental group 3 (0.2 g/100 mL) with a p-value of 0.0005, indicating that the higher concentration of the solution is the most effective. This findings aligns with a review reporting that ethanolic extracts of plants such as *Averrhoa bilimbi* significantly prolonged PT and aPTT in vitro in a dose-dependent manner, with the greatest coagulation time prolongation observed at the highest concentrations tested. This anticoagulant effect is attributed to phytochemicals such as flavonoids and saponins, highlighting the therapeutic potentials of these plants as natural anticoagulants (Sari & Suprapti, 2022).

Experimental Control Group 2 vs Experimental Control Group 3

Lastly, Experimental Control Group 2 (0.1 g/100 mL) and Experimental Control Group 3 (0.2 g/100 mL) shows a significant difference with a p-value of 0.0022, which are below 0.05. The trend observed suggests a dose-dependent anticoagulant effect, where increasing concentrations of the extract lead to a more pronounced impact on PT. Notably, the average PT for the 0.2 g/100 mL group was 26.57 seconds, significantly exceeding the normal reference range of 11–15 seconds (Ross, 2019), and approaching the therapeutic anticoagulant range. This finding aligns with previous literature indicating that bioactive compounds in *Catharanthus roseus*, such as flavonoids and alkaloids, can interfere with clotting factors in the extrinsic pathway, thereby extending PT (Singh, 2021; Frontiers in Genetics, 2022).

Table 2.a provides a detailed overview of the average Activated Partial Thromboplastin Time (aPTT) for each treatment group derived from three separate blood samples. The statistical analysis conducted through ANOVA yields a p-value of 5.54×10^{-08} or 0.0000000554, considerably way lower than the alpha value of 0.05. This result suggests rejecting the null hypothesis, emphasizing that the treatment affects clotting times across the different groups.

Table 2.b shows the results of comparing treatment groups using the Pairwise t-test. It highlights important differences in Activated Partial Thromboplastin Time (aPTT) when using different amounts of periwinkle extract, garlic extract, and distilled water as a control. These results suggest that periwinkle and garlic extracts can affect aPTT, which relates to the prolonging of blood clotting. Specifically, the findings show how different amounts of these extracts can change aPTT results. More details about the results are provided below.

Negative Control Group vs Positive Control Group

The Negative Control Group shows a significant difference from the Positive Control Group based on a p-value lower than the alpha value. Garlic extract exhibits significant anticoagulant effects by prolonging activated partial thromboplastin time (aPTT), suggesting that its bioactive compounds, particularly organosulfur molecules, inhibit the intrinsic coagulation pathway, as indicated in a study by Alhamamia et al. (2013).

A study by Clark-Montoya et al. (2024) demonstrated that Snow Mountain garlic extract significantly extended activated partial thromboplastin time (aPTT), with the highest

concentration achieving 1500 seconds, while distilled water showed no effect. Additionally, a narrative review by Hareera et al. (2022) outlined the mechanisms of garlic's anticoagulant effects, including the inhibition of platelet aggregation and interference with thrombin activity, both crucial for fibrin clot formation.

The evidence from in-vivo and in-vitro studies consistently shows a significant difference in their effects on aPTT when comparing garlic extract to distilled water. Garlic extract prolongs clotting time by targeting the intrinsic coagulation cascade, while distilled water remains neutral, having no impact on coagulation factors.

Negative Control Group vs Experimental Control Group 1

Likewise, there is a significant difference between the Negative Control Group and Experimental Group 1 with a concentration of 0.05 g/100 mL, as shown in their p-values lower than 0.05 and their average time. The Negative Control Group has an average of 34 secs (normal range), while Experimental Group 1 has an average of 43.7 secs (therapeutic anticoagulant reference range).

The observed reaction in clotting times is linked to bioactive compounds in periwinkle leaf extract, which inhibit intrinsic pathway factors VIII, IX, and XI, significantly prolonging activated partial thromboplastin time (aPTT) compared to distilled water. Research on medicinal plants similar to the periwinkle profile involving *Aizoon hispanicum* and *Heliotropium maris-mortui* supports these findings, showing that aqueous extracts at 50 mg/mL affect aPTT due to their flavonoids and alkaloids. Flavonoids inhibit factor XII activation, while alkaloids reduce thrombin generation. In contrast, distilled water, serving as a neutral control, maintains normal aPTT values (22–32 seconds) and has no bioactive components, confirming its inert nature.

Negative Control Group vs Experimental Control Group 2

A notable difference was observed between the Negative Control Group and Experimental Group 2, which received 0.1 g/100 mL of *Catharanthus roseus* extract. Although specific activated partial thromboplastin time (aPTT) values were not disclosed, trends suggest an increase in aPTT relative to the control, indicating anticoagulant activity. Yao et al. (2021) reported that flavonoids such as quercetin and kaempferol significantly prolong aPTT by modulating the intrinsic coagulation pathway. Additionally, Gaspar et al. (2019) demonstrated that rutin and hesperidin complexes extend aPTT without affecting prothrombin time (PT) or thrombin time (TT), supporting their selective action on the intrinsic pathway. These findings reinforce the hypothesis that bioactive flavonoids in *Catharanthus roseus* contribute to the observed anticoagulant effect at this dosage.

Negative Control Group vs Experimental Control Group 3

The Negative Control Group and Experimental Group 3 (0.2 g/100 mL) showed a significant difference in anticoagulant activity, as Experimental Group 3 exhibited a mean aPTT of 53.3 seconds-well above the normal reference range of 25–35 seconds (Hammami, 2021) and within the therapeutic anticoagulant range of 36–70 seconds. This result is consistent with findings by Pouyfung & Sukati (2021), who showed that flavonoids significantly prolong aPTT

by inhibiting intrinsic pathway factors, and Benbott et al. (2023), who reported that alkaloids possess strong anticoagulant properties through intrinsic pathway modulation. These results support the presence of bioactive compounds in *Catharanthus roseus*, particularly alkaloids and flavonoids, which are known to exert potent effects on coagulation pathways.

Experimental Control Group 1 vs Experimental Control Group 2

Experimental Group 1 with a concentration of 0.05 g/100 mL shows no significant difference with Experimental Group 2, with a p-value of 0.1896, which exceeds the 0.05 significant threshold. Despite the slightly higher mean aPTT value of Group 2 (46 seconds) compared to Group 1 (43.7 seconds), both values fall within the therapeutic anticoagulant range of 36–70 seconds, indicating effective prolongation of clotting time (Hammami, 2022). This result suggests that while both concentrations enhance anticoagulant activity, the increment from 0.05 g to 0.1 g may not yield a substantial physiological difference. Studies by Pham et al. (2020) and Pouyfung & Sukati (2021) indicating that flavonoids and alkaloids in *Catharanthus roseus* can prolong aPTT through inhibition of intrinsic pathway factors such as VIII, IX, XI, and XII, yet their effect may plateau at lower dosages. These observations imply that the bioactive compounds may reach a threshold of effectiveness at 0.05 g/100 mL, beyond which additional concentration does not significantly enhance aPTT.

Experimental Control Group 1 vs Experimental Control Group 3

Experimental Group 1 (0.05 g/100 mL) showed a statistically significant difference in average activated partial thromboplastin time (aPTT) compared to Experimental Group 3 (0.2 g/100 mL), with means of 43.7 seconds and 53.3 seconds, respectively, both within the therapeutic range. The effect is due to the dose-dependent nature of the treatment.

Studies indicate that plant extracts' flavonoid and alkaloid contents increase with higher extraction doses. For example, research on *Achillea millefolium* revealed that higher doses resulted in greater flavonoid concentrations, with a 1 mg/mL extract showing 39.45 ± 1.84 µg quercetin equivalents (QE)/mg (Hayat et al., 2025). Similarly, a study on *Matricaria pubescens* demonstrated that alkaloid concentrations rose with increased plant material, as higher doses enhance the solubilization of alkaloids in solvents like ethanol or ethyl acetate (Hassiba Metrouh-Amir & Amir, 2023).

Research on *Ephedra nebrodensis* shows that higher doses (e.g., 5 g/kg) result in a greater abundance of alkaloids and increased bioactivity than lower doses (Hamoudi et al., 2022). The dose of plant material during extraction significantly influences the concentration of alkaloids and flavonoids, leading to enhanced activity in the extracts.

Experimental Control Group 1 vs Positive Control Group

Experimental Group 1 (0.05 g/100 mL) demonstrated significant anticoagulant properties, with aPTT values significantly different from the Positive Control Group ($p = 7.89 \times 10^{-06}$). While it exhibits anticoagulant effects, its potency is less than that of the standard anticoagulant. Recent research supports these findings, indicating that plant-derived alkaloids can prolong aPTT by modulating coagulation factors. The aPTT value of 43.7 seconds for Experimental Group 1 is within the therapeutic range (36–70 seconds), indicating potential safe

anticoagulant activity. This aligns with the importance of maintaining moderate clotting times to minimize hemorrhagic risks, as noted in related studies (Kubatka et al., 2022; Mehic, Colling, Pabinger, & Gebhart, 2021; Lamponi, S., 2021)

Furthermore, the statistically significant variance between treatment groups, as shown by the ANOVA results ($F = 98.22$, $p = 5.54 \times 10^{-08}$), further validates the biological activity of the periwinkle extract on coagulation parameters. According to Martial et al. (2022), statistically significant ANOVA outcomes in similar phytochemical studies often correlate strongly with dose-responsive anticoagulant properties, particularly in extracts rich in vincristine and vinblastine which are alkaloids known to affect vascular and hematologic parameters.

Experimental Control Group 2 vs Experimental Control Group 3

Experimental Group 2 (0.1 g/100 mL) had an average clotting time of 46 seconds, significantly shorter than Experimental Group 3 (0.2 g/100 mL), which had an average time of 53.3 seconds. Both concentrations are within the reference range of therapeutic anticoagulants. Higher concentrations result in longer clotting times.

Phytochemical analyses of Vinca species, particularly periwinkle, demonstrate variations in flavonoid and alkaloid content depending on extract concentration and plant variety. A study found that Vinca herbacea exhibited the highest total flavonoid content (TFC) at 151 mg, while higher extract concentrations correlated with increased total alkaloid content (TAC) (Ciorîță et al., 2021). Additionally, research by Doshi, Matthews, & Chaskar (2018) on ethanolic extracts from Catharanthus roseus leaves showed a significant presence of alkaloids, flavonoids, and other secondary metabolites. Their gas chromatography-mass spectrometry (GC-MS) analysis identified 15 key components, revealing that using ethanol as a solvent and higher extract concentrations improved both diversity and quantity of bioactive compounds, underscoring the connection between extraction efficiency and phytochemical yield.

Experimental Control Group 2 vs Positive Control Group

Experimental Group 2 (0.1 g/100 mL) and garlic extract significantly prolonged activated partial thromboplastin time (aPTT), with average times of 46 seconds and 53.7 seconds, respectively.

Research highlights that garlic extract exhibits a potent anticoagulant effect by inhibiting the intrinsic coagulation pathway, with a comparative rodent study showing significant results (Kim et al., 2018). An in vitro study further confirmed a dose-dependent increase in activated partial thromboplastin time (aPTT) with Snow Mountain garlic, where higher concentrations led to greater clotting time prolongation (Clark-Montoya et al., 2024). Similarly, periwinkle extract has been shown to significantly prolong aPTT, attributed to its rich alkaloid and flavonoid content that inhibits intrinsic coagulation factors (Abdallah et al., 2022). While both extracts extend aPTT, garlic's effects stem from organosulfur compounds and polyphenols, while periwinkle's is driven by alkaloids and flavonoids, leading to differences in their anticoagulant potency.

Experimental Control Group 3 vs Positive Control Group

Interestingly, Experimental Control Group 3 (0.2 g/100 mL) demonstrated no significant difference when compared to the Positive Control Group with a p-value of 0.77. This statistical outcome implies that both groups effectively produced similar outcomes in terms of their influence on blood coagulation parameters. This similarity aligns with the observed prolongation of aPTT, suggesting the potential anticoagulant properties of *Catharanthus roseus*. These effects may be attributed to its bioactive compounds, including flavonoids and alkaloids, which have been reported to exert anticoagulant effects by interfering with thrombin generation or activity (Pham, et al., 2020; Sahagun, et al., 2021).

Flavonoids, recognized for their anticoagulant activity, affect clotting factors VIII, IX, XI, and XII, thereby prolonging activated partial thromboplastin time (aPTT) (Benbott et al., 2023). Alkaloids from *Catharanthus roseus,* such as vincristine and vinblastine, also modulate endothelial function, significantly influencing the coagulation cascade. Research suggests that plant-derived anticoagulants may be equally effective as synthetic ones. Garlic *(Allium sativum)*, known for its anticoagulant properties, acts as a positive control, particularly affecting aPTT. Its effects are primarily due to bioactive compounds like allicin and ajoene, which inhibit thrombin formation and promote fibrinolysis, while organosulfur compounds in garlic further prevent platelet aggregation (Senadheera & Perera, 2023).

Table 3 showcases the physicochemical characteristics of the most effective concentration solution. The solution described aligns with the United States Pharmacopeia (USP) standards for botanical extracts, as outlined in USP General Chapter <565>. The solution's specific gravity of 1.002 is within the typical range for liquid formulations, which generally falls between 1.0 and 1.4, indicating proper dissolution of bioactive compounds, supporting its homogeneity and compliance with USP requirements for uniformity in liquid formulations. The pH of the solution is 6.0, which remains slightly acidic and within the generally acceptable range of 5.0 to 7.0 for plant extracts, preventing degradation and enhancing compatibility, making it suitable for potential pharmaceutical formulation. The solution's light green-yellowish color reflects the presence of flavonoids and alkaloids (Fumi, et al), which are known bioactive components of Periwinkle, aligning with the specific color profile for extracts. Its mild herbal odor, consistent with Periwinkle leaf extracts, aligns with consumer expectations for natural products while reinforcing the authenticity of its composition. The clarity of the solution, observed as clear and neutral, meets filtration standards by being free of visible particles, which validates the effectiveness of its preparation process.

6. Conclusion

The results of this study demonstrate that *Catharanthus roseus* (Periwinkle) exhibit significant anticoagulant activity. The findings were rigorously derived from comprehensive analysis of the experimental data and relevant literature. Among the tested concentrations of Periwinkle extract (0.05 g/100 mL, 0.1 g/100 mL, and 0.2 g/100 mL), the 0.2 g/100 mL concentration elicited the most pronounced prolongation of both prothrombin time (PT) and

activated partial thromboplastin time (aPTT), indicating a dose-dependent anticoagulant effect. This concentration also yielded the lowest p-value relative to control groups, suggesting a statistically significant alteration in coagulation parameters. Furthermore, comparative analysis of aPTT results revealed no significant difference between the anticoagulant effects of the 0.2 g/100 mL Periwinkle extract and the positive control (garlic extract), indicating comparable efficacy. Physicochemical characterization of the formulated Periwinkle solution-including specific gravity, pH, odor, color, and clarity-confirmed its stability and suitability for potential therapeutic use, with pH values maintained within physiologically acceptable limits. Collectively, these findings provide compelling evidence for the anticoagulant potential of *Catharanthus roseus* extract, supporting its candidacy as a natural alternative to conventional anticoagulant agents and warranting further investigation in preclinical and clinical contexts.

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9. Appendices

APPENDIX A
Timetable

TASKS	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr
Chapter 1: Introduction								
Background of the Study								
Conceptual Framework								
Operational Paradigm								
Statement of the Problem								
Statement of the Hypothesis								
Scope and Limitations								
Chapter 2: Methodology								
Research Design and Method								
Population and Locale of the Study								
Data Gathering Tools								
Data Gathering Procedures								
Ethical Considerations								
Treatment of Data								
Chapter 3: Results & Discussion								
Chapter 4: Conclusions & Recommendations								
Appendices								
Literatures Cited								

APPENDIX B
List of Materials, Equipment, and Reagent

Plant Specimen	Periwinkle (*Catharanthus roseus)* Leaves
Laboratory Apparatus and Equipment	Rotary Evaporator Round-Bottom Flask Weighing Balance Distilled water Mortar and Pestle or Blender Filter Paper or Gauze Test Tubes Fibrometer Testing Machine Incubator Centrifuge ph paper Pycnometer Purple-Top Tubes Test Tube Rack Syringes Erlenmeyer Flasks Beaker Oven pH paper/ pH strips
Reagent	Ethanol Hager's Reagent (Saturated Picric Acid) Mayer's Reagent (Mercuric-Potassium Iodide) Wagner's Reagent (Iodide in Potassium Iodide) 2% Sodium Hydroxide Solution Dilute HydrochloricAcid Lead Acetate Solution apTT Reagent PT Reagent

APPENDIX C
Identification Certificate Of Plant Material

DON MARIANO MARCOS MEMORIAL STATE UNIVERSITY
North La Union Campus
Sapilang, Bacnotan 2515, La Union
COLLEGE OF AGROFORESTRY AND FORESTRY

IDENTIFICATION CERTIFICATE OF PLANT MATERIAL

RELEASED NLUC-RECORDS FO4-1624 DATE 11/28/2024

This is to certify that **Irish Glean N. Almojeula, Jessie Anne M. Cahimari, Trisha Keith B. Macario, Marielle Jan M. Balagot, Luvly Arnie M. Casuga, Wala O. Omar** of the College of Pharmacy, Lorma Colleges, City of San Fernando, La Union have brought plant species for proper authentic identification. After a thorough and closer examination on the morphological and botanical characteristics of the specimen, it was identified and described as follows.

Common Name - Periwinkle
Scientific Name - *Catharanthus roseus* (L.) G.Don
Family Name - Apocynaceae

This certification is issued **Irish Glean N. Almojeula, Jessie Anne M. Cahimari, Trisha Keith B. Macario, Marielle Jan M. Balagot, Luvly Arnie M. Casuga, Wala O. Omar** for all legal intentions and purposes.

Issued this 26th day of November 2024, College of Agroforestry and Forestry, Don Mariano Mariano Marcos Memorial State University, North La Union Campus, Bacnotan, La Union.

Prepared and examined by:

FOR. RUBY ANNE G. OLBINADO
Dendrologist/Faculty, CAFF

Noted:

FOR. JAY MARK G. CORTADO
Dean, CAFF

DR. JUNIFER REY E. TABAFUNDA
Chancellor

APPENDIX D
LC-REC Approval Letter

LORMA COLLEGES

LC-REC Form #024
APPROVAL LETTER

REC Reference #: 2025-088

March 11, 2025

To: Irish Glean Almojuela, Marielle Jan Balagot, Jessie Anne Cahimari, Luvly Arnie Casuga, Trisha Keith Macario, Wala Omar
LORMA Colleges, College of Pharmacy

Subject: Approval of the Research Study "ANTICOAGULANT PROPERTY OF PERIWINKLE *(CATHARANTHUS ROSEUS)* LEAF EXTRACT FORMULATED AS SOLUTION" by the Research Ethics Committee (REC).

Dear Researchers,

The Research Ethics Committee (REC) has reviewed your application to conduct the above-mentioned research study in the LOCALE OF STUDY with you as the Principal Investigators within the duration of March 11, 2025 to March 11, 2026.

The Following documents have been reviewed and approved:

1. Letter of Intent to Conduct the Study
2. Endorsement of the Research Technical Panel
3. Title and Statement of the Problem/ Objective
4. Literature Review
5. Methods and Procedures
6. Population and Locale
7. Exclusion/Inclusion Criteria
8. Data Analysis
9. Ethical Considerations

We approve the study to be conducted in the presented form provided the following are integrated in the final research protocol:

1. In the questionnaire, specify the medications in question number 2 which affect blood coagulation as aspirin, warfarin or coumadin and the questionnaire needs validation.
2. In the manuscript, provide statements on who can access and the deletion plan for the data collected, the funding of the research and the steps that the researchers take when participants experience an after-effect from the blood extraction such as dizziness, headache and others.
3. Provide justification for why the plasma extraction will be conducted in Baguio.
4. State the informed consent form as if researchers directly communicate with the respondents.

None of the Investigators participating in this study took part in the decision making and voting procedure for this study.

The Institutional REC expects to be informed about the progress of the study, any revision in the protocol before implementation and participants'/respondents' information/informed consent. Likewise, you are required to provide the Board a copy of the final report.

Yours Sincerely,

RYAN JAY G. MUSTOLES, MASE, RMT
Interim Chairman, Lorma Colleges-Research Ethics Committee

APPENDIX E
Preparation Of Plant Sample

Collection and Air Drying of Periwinkle (*Catharanthus roseus)* Leaves		
Oven Drying and Pulverization of Periwinkle (*Catharanthus roseus)* Leaves		

APPENDIX F
Extraction Of Plant Sample

Soaking of Pulverized Periwinkle (*Catharanthus roseus)* Leaves	
Filtration of Periwinkle (*Catharanthus roseus)* Extract	 

APPENDIX G
Rotary Evaporation

Removal of Solvent through Rotary Evaporation	

APPENDIX H
Phytochemical Screening Test Procedures

Test for Alkaloids			
Test	**Reagent**	**Procedures**	**Positive (+) Results**
Hager's Test	Hager's Reagent (Saturated Picric Acid)	1. Measure 2-3 ml of the sample extract. 2. Add 1-2 drops of the Hager's reagent	Formation of a creamy white precipitate
Mayer's Test	Mayer's Reagent (Mercuric-Potassium Iodide)	1. Measure 2-3 ml of the sample extract. 2. Add 1-2 drops of the Mayer's reagent	Formation of a creamy white/yellow precipitate
Wagner's Test	Wagner's Reagent (Iodide in Potassium Iodide)	1. Measure 2-3 ml of the sample extract. 2. Add a few drops of the Wagner's reagent	Formation of brown/reddish precipitate

Test for Flavonoids			
Test	**Reagent**	**Procedures**	**Positive (+) Results**
Alkaline Reagent Test	2% Sodium Hydroxide Solution and Dilute HydrochloricAcid	1. Measure 1 ml of the sample extract. 2. Add 2ml of the 2% Sodium Hydroxide Solution and a few drops of Dilute Hydrochloric Acid.	Formation of an intense yellow colour, becomes colourless in addition of dilute acid.
Lead Acetate Test	Lead Acetate Solution	1. Measure 1 ml of the sample extract. 2. Add a few drops of Lead Acetate Solution.	Formation of yellow precipitate.

APPENDIX I
Phytochemical Screening Documentation

Test for Alkaloids

TYPE OF TEST	RESULTS
Hager's Test	(+)
Mayer's Test	(+)
Wagner's Test	(-)

LEGEND: (+) Presence; (-) Absence

∴ Alkaloids are detected in the extract.

Test for Flavonoids

TYPE OF TEST	RESULTS
Alkaline Reagent Test	(-)
Lead Acetate Test	(+)

LEGEND: (+) Presence; (-) Absence

∴ Flavonoids are detected in the extract.

APPENDIX J
Formulation Of Different Solution Concentrations Procedure

Periwinkle Solution: 0.05 g/100 mL Concentration

Periwinkle *(Catharanthus roseus)* …….…… 0.05g (extract)

Distilled Water ……..…….…… qs ad100mL

Periwinkle Solution: 0.1 g/100 mL Concentration

Periwinkle *(Catharanthus roseus)* ……...…… 0.1g (extract)

Distilled Water ……...……. qs ad100mL

Periwinkle Solution: 0.2 g/100 mL Concentration

Periwinkle *(Catharanthus roseus)* ……...…… 0.2g (extract)

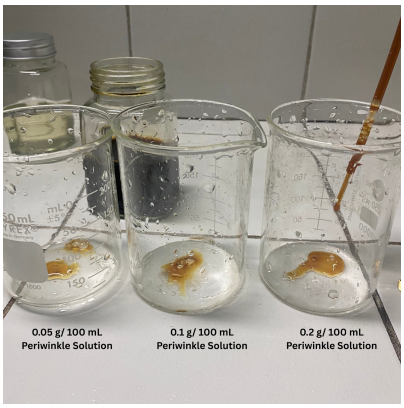
Distilled Water …….…..……. qs ad100mL

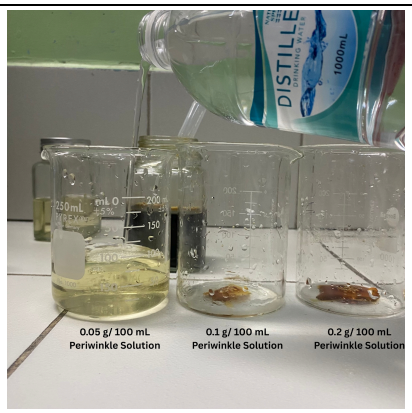
Garlic Solution: 0.01 g/100 mL Concentration

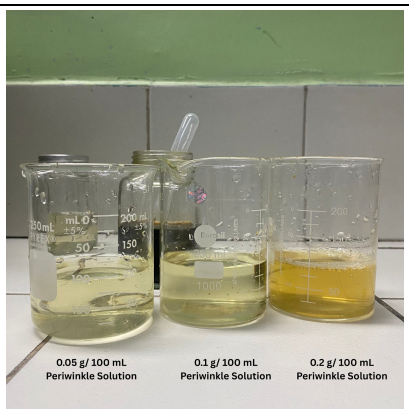
Garlic *(Allium sativum)* ……. 0.1g (extract)

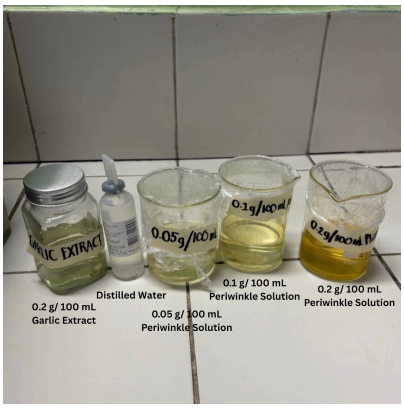
Distilled Water ……..…..…… qs ad100mL

APPENDIX K
Formulation Of Different Solution Concentration Documentation









APPENDIX L
Participants' Questionnaires And Certificate Of Consent

PARTICIPANT A

PARTICIPANT'S QUESTIONNAIRE

Direction: Fill up the following blank with the appropriate response.

Vital Signs

Body Temperature: 37 °C

Pulse Rate: 69 bpm

Blood Pressure: 120 | 80 mmHg

Health Status

Questions	Yes	No
1. Have you been diagnosed with any medical conditions (e.g., diabetes, hypertension, etc.)? **If yes, please specify.**		/
2. Are you currently taking any medications? **If yes, please list them**		/
3. Have you consumed alcohol within 12 hours?		/
4. Have you experienced any recent illnesses or infections?		/
5. Are you pregnant? **If yes, how long?**		/
6. As of now, are you having your menstruation?		/

CERTIFICATE OF CONSENT:

I have read the information stated herein or it was properly explained to me. I was provided with a chance to ask questions relative to it. All questions I asked were answered properly; therefore, I consent and voluntarily participate in this study.

Name of Participant: Participant A

Signature of Participant: [signature]

Date: March 22 2025

Statement from the Researcher/Person Obtaining the Consent

All information pertaining to this study was explained to the possible participant and made sure that I fully understood what I have to do in the research.

Similarly, I affirm that the potential participant was given a chance to ask questions which I have answered accurately to the best of my ability.

Likewise, I affirm that the participant was not coerced or forced in giving consent. That I have voluntarily provided the consent.

Accomplished by: [signature] Date Submitted: 03-22-2025

(Signature over Printed Name)

PARTICIPANT B

PARTICIPANT'S QUESTIONNAIRE

Direction: Fill up the following blank with the appropriate response.

Vital Signs

Body Temperature: 36.5 °C

Pulse Rate: 65 bpm

Blood Pressure: 120 | 80 mmHg

Health Status

Questions	Yes	No
1. Have you been diagnosed with any medical conditions (e.g., diabetes, hypertension, etc.)? **If yes, please specify.**		/
2. Are you currently taking any medications? **If yes, please list them**		/
3. Have you consumed alcohol within 12 hours?		/
4. Have you experienced any recent illnesses or infections?		/
5. Are you pregnant? **If yes, how long?**		/
6. As of now, are you having your menstruation?		/

CERTIFICATE OF CONSENT:

I have read the information stated herein or it was properly explained to me. I was provided with a chance to ask questions relative to it. All questions I asked were answered properly; therefore, I consent and voluntarily participate in this study.

Name of Participant: Participant B

Signature of Participant: [signature]

Date: March 22, 2025

Statement from the Researcher/Person Obtaining the Consent

All information pertaining to this study was explained to the possible participant and made sure that I fully understood what I have to do in the research.

Similarly, I affirm that the potential participant was given a chance to ask questions which I have answered accurately to the best of my ability.

Likewise, I affirm that the participant was not coerced or forced in giving consent. That I have voluntarily provided the consent.

Accomplished by: [signature] Date Submitted: 03/22/2025

(Signature over Printed Name)

PARTICIPANT C

PARTICIPANT'S QUESTIONNAIRE

Direction: Fill up the following blank with the appropriate response.

Vital Signs

Body Temperature: 36.5°C

Pulse Rate: 69 bpm

Blood Pressure: 120|80 mmHg

Health Status

Questions	Yes	No
1. Have you been diagnosed with any medical conditions (e.g., diabetes, hypertension, etc.)? **If yes, please specify.**		✓
2. Are you currently taking any medications? **If yes, please list them**		✓
3. Have you consumed alcohol within 12 hours?		✓
4. Have you experienced any recent illnesses or infections?		✓
5. Are you pregnant? **If yes, how long?**		✓
6. As of now, are you having your menstruation?		✓

CERTIFICATE OF CONSENT:

I have read the information stated herein or it was properly explained to me. I was provided with a chance to ask questions relative to it. All questions I asked were answered properly; therefore, I consent and voluntarily participate in this study.

Name of Participant: Participant C

Signature of Participant: [signature]

Date: March 22, 2025

Statement from the Researcher/Person Obtaining the Consent

All information pertaining to this study was explained to the possible participant and made sure that I fully understood what I have to do in the research.

Similarly, I affirm that the potential participant was given a chance to ask questions which I have answered accurately to the best of my ability.

Likewise, I affirm that the participant was not coerced or forced in giving consent. That I have voluntarily provided the consent.

Accomplished by: [signature] (Signature over Printed Name) Date Submitted: 03-22-2025

APPENDIX M
Blood Collection

PARTICIPANT A	
PARTICIPANT B	
PARTICIPANT C	

APPENDIX N
Prothrombin Time Test Raw Data

	DOCUMENT: **MASTERLIST OF FORMS**	SUBJECT: **RESULT FORM**	
	DOCUMENT NO:	SUPERSEDES:	CC: **INTRANET DCO**
	EFFECTIVITY DATE: **JUNE 1, 2024**	DATED:	

This is a controlled document. Photocopying or printing of this document, without the controlled mark, shall make this document an uncontrolled copy.

Telefax No.: (074) 442-3071 E-mail Address: medtech@ubaguio.com E-mail Address: medtech@ubaguio.com

RESULT FORM - HEMATOLOGY

Lab No. 25-3389 **Date** 3/22/25 4:36 PM

Name A, B, C **Age** N/A **Sex** N/A

Course/Dept. OPD

COAGULATION TESTS

ID	Tests	Result 1 (seconds)	INR	Result 2 (seconds)	INR	Result 3 (seconds)	INR	Reference Range (seconds)
Negative control	Prothrombin Time	14.0	0.97	14.2	0.99	14.5	1.01	11-13
Positive control	Prothrombin Time	17.1	1.28	16.1	1.20	15.6	1.16	11-13
Experimental Control A	Prothrombin Time	18.4	1.29	17.7	1.24	16.5	1.15	11-13
Experimental Control B	Prothrombin Time	21.7	1.54	17.7	1.24	18.4	1.29	11-13
Experimental Control C	Prothrombin Time	29.1	2.09	28.9	2.08	21.7	1.54	11-13

Remarks: _____ ****Test principle: Viscosity-based clot detection*

Legend:

1 Participant A
2 Participant B
3 Participant C
Experimental Control A 0.05g/100mL
Experimental Control B 0.1g/100mL
Experimental Control C 0.2g/100mL

APPENDIX O
Activated Partial Thromboplastin Time Test Raw Data

	DOCUMENT: **MASTERLIST OF FORMS**	SUBJECT: **RESULT FORM**	
	DOCUMENT NO:	SUPERSEDES:	CC: **INTRANET DCO**
	EFFECTIVITY DATE: **JUNE 1, 2024**	DATED:	

This is a controlled document. Photocopying or printing of this document, without the controlled mark, shall make this document an uncontrolled copy.

Telefax No.: (074) 442-3071 Website: www.ubaguio.edu E-mail Address: medtech@ubaguio.com

RESULT FORM - HEMATOLOGY

Lab No. 25-3389 **Date** 3/22/25 4:41 PM

Name A, B, C **Age** N/A **Sex** N/A

Course/Dept. OPD

COAGULATION TESTS

ID	Tests	Result 1 (seconds)	Result 2 (seconds)	Result 3 (seconds)	Reference Range (seconds)
Negative control	Activated Partial Thromboplastin Time	34	33	35	25-35
Positive control	Activated Partial Thromboplastin Time	56	53	52	25-35
Experimental Control A	Activated Partial Thromboplastin Time	44	43	44	25-35
Experimental Control B	Activated Partial Thromboplastin Time	48	44	46	25-35
Experimental Control C	Activated Partial Thromboplastin Time	54	53	53	25-35

Remarks: *>200 = no detectable clot formation*

****Test principle: Viscosity-based clot detection*

Legend:

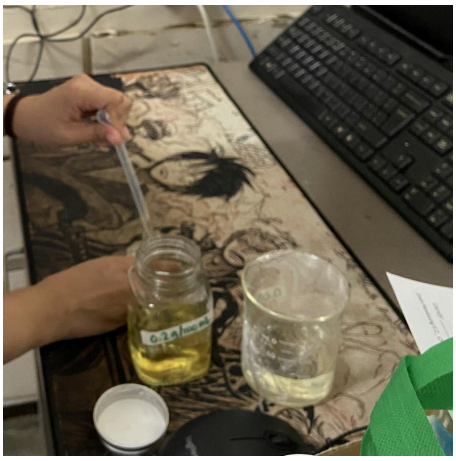
1 Participant A
2 Participant B
3 Participant C
Experimental Control A 0.05g/100mL
Experimental Control B 0.1g/100mL
Experimental Control C 0.2g/100mL

Digitally signed by Farah Jane Jamili Musni
Date: 2025.03.22 16:42:29 +08'00'

Farah Jane J. Musni, RMT
License No: 0100685
Medical Technologist

Rhesa Michelle M. Wong, MD, DPSP
License Number: 0111589
Clinical Pathologist

APPENDIX P
Physicochemical Properties

SPECIFIC GRAVITY- PYCNOMETER	
	

$$Specific\ Gravity = \frac{W2}{W1}$$

Where:

W1= Weight of Water

W2= Weight of the Formulated Solution

$$Specific\ Gravity = \frac{42.78}{42}$$

$$= 1.01$$

pH TESTING	
	
The range goes from 0 - 14, pH of 6 indicates slightly acidic. pH of less than 7 indicate acidity pH of greater than 7 indicates a basicity	

ORGANOLEPTIC TEST EVALUATION

Pharmacists

Questionnaire for Organoleptic Testing (Odor, Color, and Clarity) of Periwinkle *(Catharanthus roseus)* Leaf Extract Formulated as Solution

Please evaluate the following characteristics of the Periwinkle leaf extract formulation. Your honest feedback is valuable for assessing its quality. Select the most appropriate response based on your perception.

Sensory Evaluation	
Odor Assessment	How would you describe the odor of the formulation? (Check all that apply) ☐ No detectable odor ☐ Mild herbal scent ☐ Strong herbal scent ☐ Unpleasant odor ☐ Other (please specify): _____
	How acceptable is the odor of the formulation? ☐ Highly acceptable ☑ Acceptable ☐ Neutral ☐ Unacceptable
Color Evaluation	What is the perceived color of the formulation? ☐ Light green ☐ Dark green ☐ Brownish-green ☑ Yellowish ☐ Other (please specify): _____
	How appealing is the color of the formulation? ☐ Very appealing ☐ Moderately appealing ☑ Neutral ☐ Unappealing
Clarity Evaluation	How would you describe the clarity of the formulation? ☑ Clear (no particles or cloudiness) ☐ Slightly cloudy ☐ Moderately cloudy ☐ Very cloudy (visible particles)

	How acceptable is the clarity of the formulation? ☑ Highly acceptable ☐ Acceptable ☐ Neutral ☐ Unacceptable
General Comments	What aspects of the formulation's odor, color, or clarity would you improve? _____ _____ _____
	Would you recommend this formulation for further testing based on its organoleptic properties? ☑ Yes ☐ No ☐ Unsure (please explain) _____ _____ _____

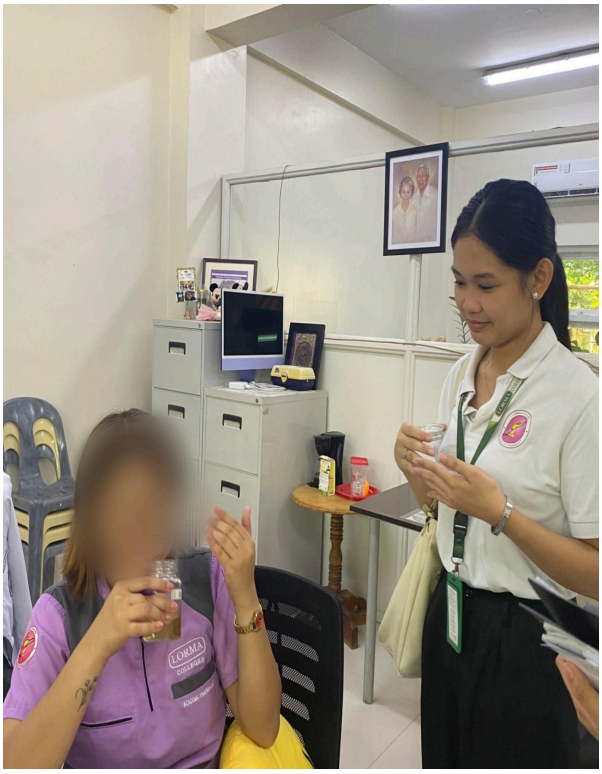
_____
Signature of the Evaluator

Questionnaire for Organoleptic Testing (Odor, Color, and Clarity) of Periwinkle *(Catharanthus roseus)* Leaf Extract Formulated as Solution

Please evaluate the following characteristics of the Periwinkle leaf extract formulation. Your honest feedback is valuable for assessing its quality. Select the most appropriate response based on your perception.

Sensory Evaluation	
Odor Assessment	How would you describe the odor of the formulation? (Check all that apply) ☑ No detectable odor ☐ Mild herbal scent ☐ Strong herbal scent ☐ Unpleasant odor ☐ Other (please specify): _____
	How acceptable is the odor of the formulation? ☐ Highly acceptable ☐ Acceptable ☐ Neutral ☐ Unacceptable
Color Evaluation	What is the perceived color of the formulation? ☑ Light green ☐ Dark green ☐ Brownish-green ☐ Yellowish ☐ Other (please specify): _____
	How appealing is the color of the formulation? ☐ Very appealing ☐ Moderately appealing ☑ Neutral ☐ Unappealing
Clarity Evaluation	How would you describe the clarity of the formulation? ☑ Clear (no particles or cloudiness) ☐ Slightly cloudy ☐ Moderately cloudy ☐ Very cloudy (visible particles)
	How acceptable is the clarity of the formulation? ☐ Highly acceptable ☑ Acceptable ☐ Neutral ☐ Unacceptable
General Comments	What aspects of the formulation's odor, color, or clarity would you improve? _____ _____ _____
	Would you recommend this formulation for further testing based on its organoleptic properties? ☑ Yes ☐ No ☐ Unsure (please explain) _____ _____ _____

_____
Signature of the Evaluator



BS Pharmacy Students

Questionnaire for Organoleptic Testing (Odor, Color, and Clarity) of Periwinkle *(Catharanthus roseus)* Leaf Extract Formulated as Solution

Please evaluate the following characteristics of the Periwinkle leaf extract formulation. Your honest feedback is valuable for assessing its quality. Select the most appropriate response based on your perception.

Sensory Evaluation	
Odor Assessment	How would you describe the odor of the formulation? (Check all that apply) ☑ No detectable odor ☐ Mild herbal scent ☐ Strong herbal scent ☐ Unpleasant odor ☐ Other (please specify): _____
	How acceptable is the odor of the formulation? ☐ Highly acceptable ☑ Acceptable ☐ Neutral ☐ Unacceptable
Color Evaluation	What is the perceived color of the formulation? ☑ Light green ☐ Dark green ☐ Brownish-green ☐ Yellowish ☐ Other (please specify): _____
	How appealing is the color of the formulation? ☐ Very appealing ☐ Moderately appealing ☑ Neutral ☐ Unappealing
Clarity Evaluation	How would you describe the clarity of the formulation? ☑ Clear (no particles or cloudiness) ☐ Slightly cloudy ☐ Moderately cloudy ☐ Very cloudy (visible particles)
	How acceptable is the clarity of the formulation? ☐ Highly acceptable ☑ Acceptable ☐ Neutral ☐ Unacceptable
General Comments	What aspects of the formulation's odor, color, or clarity would you improve? _____
	Would you recommend this formulation for further testing based on its organoleptic properties? ☐ Yes ☐ No ☐ Unsure (please explain) _____

Signature of the Evaluator

Questionnaire for Organoleptic Testing (Odor, Color, and Clarity) of Periwinkle *(Catharanthus roseus)* Leaf Extract Formulated as Solution

Please evaluate the following characteristics of the Periwinkle leaf extract formulation. Your honest feedback is valuable for assessing its quality. Select the most appropriate response based on your perception.

Sensory Evaluation	
Odor Assessment	How would you describe the odor of the formulation? (Check all that apply) ☐ No detectable odor ☑ Mild herbal scent ☐ Strong herbal scent ☐ Unpleasant odor ☐ Other (please specify): _____
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	How acceptable is the clarity of the formulation? ☐ Highly acceptable ☑ Acceptable ☐ Neutral ☐ Unacceptable
General Comments	What aspects of the formulation's odor, color, or clarity would you improve? _____ _____ _____
	Would you recommend this formulation for further testing based on its organoleptic properties? ☐ Yes ☐ No ☐ Unsure (please explain) _____ _____ _____

_____
Signature of the Evaluator

Non BS Pharmacy Students

Questionnaire for Organoleptic Testing (Odor, Color, and Clarity) of Periwinkle *(Catharanthus roseus)* Leaf Extract Formulated as Solution

Please evaluate the following characteristics of the Periwinkle leaf extract formulation. Your honest feedback is valuable for assessing its quality. Select the most appropriate response based on your perception.

Sensory Evaluation	
Odor Assessment	How would you describe the odor of the formulation? (Check all that apply) ☐ No detectable odor ☑ Mild herbal scent ☐ Strong herbal scent ☐ Unpleasant odor ☐ Other (please specify): _____
	How acceptable is the odor of the formulation? ☐ Highly acceptable ☑ Acceptable ☐ Neutral ☐ Unacceptable
Color Evaluation	What is the perceived color of the formulation? ☑ Light green ☐ Dark green ☐ Brownish-green ☐ Yellowish ☐ Other (please specify): _____
	How appealing is the color of the formulation? ☐ Very appealing ☐ Moderately appealing ☑ Neutral ☐ Unappealing
Clarity Evaluation	How would you describe the clarity of the formulation? ☑ Clear (no particles or cloudiness) ☐ Slightly cloudy ☐ Moderately cloudy ☐ Very cloudy (visible particles)
	How acceptable is the clarity of the formulation? ☐ Highly acceptable ☑ Acceptable ☐ Neutral ☐ Unacceptable
General Comments	What aspects of the formulation's odor, color, or clarity would you improve? _____ _____ _____
	Would you recommend this formulation for further testing based on its organoleptic properties? ☐ Yes ☐ No ☐ Unsure (please explain) _____ _____ _____

Signature of the Evaluator

Questionnaire for Organoleptic Testing (Odor, Color, and Clarity) of Periwinkle *(Catharanthus roseus)* Leaf Extract Formulated as Solution

Please evaluate the following characteristics of the Periwinkle leaf extract formulation. Your honest feedback is valuable for assessing its quality. Select the most appropriate response based on your perception.

Sensory Evaluation	
Odor Assessment	How would you describe the odor of the formulation? (Check all that apply) ☐ No detectable odor ☑ Mild herbal scent ☐ Strong herbal scent ☐ Unpleasant odor ☐ Other (please specify): _____
	How acceptable is the odor of the formulation? ☐ Highly acceptable ☑ Acceptable ☐ Neutral ☐ Unacceptable
Color Evaluation	What is the perceived color of the formulation? ☑ Light green ☐ Dark green ☐ Brownish-green ☐ Yellowish ☐ Other (please specify): _____
	How appealing is the color of the formulation? ☐ Very appealing ☐ Moderately appealing ☐ Neutral ☐ Unappealing
Clarity Evaluation	How would you describe the clarity of the formulation? ☑ Clear (no particles or cloudiness) ☐ Slightly cloudy ☐ Moderately cloudy ☐ Very cloudy (visible particles)
	How acceptable is the clarity of the formulation? ☐ Highly acceptable ☑ Acceptable ☐ Neutral ☐ Unacceptable
General Comments	What aspects of the formulation's odor, color, or clarity would you improve? _____ _____ _____
	Would you recommend this formulation for further testing based on its organoleptic properties? ☐ Yes ☐ No ☐ Unsure (please explain) _____ _____ _____

Signature of the Evaluator

APPENDIX Q
Rec-Informed Consent Form And Certificate Of Consent

LORMA COLLEGES

INFORMED CONSENT FORM

INSTRUCTION: Please accomplish the form and ensure that all necessary documents are included in your submission.

GENERAL INFORMATION:

Title of the Study: Anticoagulant Property of Periwinkle (*Catharanthus roseus*) Leaf Extract Formulated as Solution

REC Code : _____ No. of Study Participants: 3

Study Site : Campus for Health Sciences, Lorma Colleges, Carlatan, San Fernando La Union and University of Baguio

Name of Researcher/s: Almojuela, Irish Glean N., Balagot, Marielle Jan M., Cahimari, Jessie Anne M., Casuga, Luvly Arnie M., Macario, Trisha Keith B., Omar, Wala O.

Contact Information : Telephone Number: _____ Mobile Number: 0945 772 1196

Fax Number: _____ Email : jessieanne.cahimari@lorma.edu

Name of Institution: Lorma Colleges

Institution's Address : Campus for Health Sciences, Lorma Colleges, Carlatan, San Fernando La Union

Type of Study:
- ☐ Sponsored Clinical Trial
- ☐ Biomedical Research
- ☐ Researcher-Initiated Clinical Trials
- ☐ Stem Cell Research
- ☐ Health Operations Research
- ☐ Genetic Research
- ☐ Social or Behavioral Research
- ☑ Others: Experimental Research
- ☐ Public Health or Epidemiologic

Source of Funding :
- ☑ Self-Funded
- ☐ Scholarship/Research Grant
- ☐ Government-Funded
- ☐ Institution-Funded
- ☐ Sponsored by Pharmaceutical Company
- ☐ Others: _____

Duration of the Study: Start Date: September 2024 End Date: May 2025

INTRODUCTION (Use Extra Sheet if Necessary)

The study aims to explore the anticoagulant properties of the periwinkle plant *(Catharanthus roseus)*. Anticoagulants are critical in preventing and managing medical conditions caused by blood clots, such as heart attacks, strokes, and deep vein thrombosis (DVT). These conditions are particularly prevalent

among those who are often at a higher risk of developing blood clot-related complications due to age, gender, and specific blood characteristics.

Anticoagulants work by reducing the risk of clot formation, helping to maintain healthy blood flow and preventing potentially life-threatening complications. For individuals in this age range, anticoagulants can be life-saving, offering significant benefits in managing cardiovascular and circulatory health. This study aims to identify and better understand the anticoagulant potential of the periwinkle plant as a natural alternative, which could lead to safer and more effective treatments for these conditions.

PURPOSE OF RESEARCH (Use Extra Sheet if Necessary)

The research aims to determine the anticoagulant properties of periwinkle *(Catharanthus roseus)* extract as an anticoagulant , specifically their flavonoid and alkaloid compounds. By investigating bioactive compounds, the study seeks to advance the understanding of their effects on blood coagulation. The research focuses on evaluating these extracts using standard coagulation tests, specifically aPTT and prothrombin time, to assess their impact on coagulation pathways. The study includes two experimental groups, with a negative control group receiving distilled water, and a positive control group receiving garlic extract, which is known for its anticoagulant properties. By comparing the effects of periwinkle extracts with those of garlic extract, the study aims to establish a baseline for the potential anticoagulant activity of periwinkle. The extracts are obtained through rotary evaporation to preserve the bioactive flavonoids and alkaloids while concentrating the compounds for evaluation. This research is expected to contribute to the growing body of knowledge surrounding natural anticoagulants and provide insights into their safety and efficacy. Ultimately, the goal is to explore the possibility of periwinkle extracts as a viable, natural alternative to conventional synthetic anticoagulants. This could pave the way for the development of innovative therapies for coagulation disorders. If successful, this research could lead to safer, plant-based treatments that reduce the risks associated with current anticoagulant medications.

TYPE OF RESEARCH INTERVENTION (Use Extra Sheet if Necessary)

1. Participant Selection

The participants in this study will include volunteers who will agree to take part in the study and fit to the set criteria for selecting the participants.

The following will be the inclusion criteria: (1) Eligible participants must be female; (2) the age must range from 18-2118-21 years old;(3) the participants must have blood type A; and (4) willing to participate in this study. These criteria are crucial to the research design, as variations outside these parameters could introduce inconsistencies that may impact the validity of the findings. Ensuring uniformity in the participant pool helps maintain the integrity of the research outcomes.

2. Voluntary Participation

Voluntary participation means that you as our respondent are fully aware of the procedures and purpose of the study. You are free to decide whether or not to take part in the study. You have the freedom and will to choose whether or not to participate, and you are free to stop at any moment without facing any consequences.

3. Procedures

Before commencing data collection activities, the research proposal will be validated and approved by three faculty validators from the College of Pharmacy, the Research Technical Panel, and the Research Ethics Committee (REC). Once approved, the researchers will obtain authorization from the Dean of the College of Pharmacy at Lorma Colleges. Following this, the researchers will coordinate with designated faculty representatives to secure the list of potential participants and obtain consent for conducting the study.

Blood collection will be conducted by qualified personnels of the University of Baguio under sterile conditions, ensuring participant safety and adherence to ethical guidelines. Participants will be fully informed about the study objectives, procedures, and their rights through an informed consent form provided before the study begins. All data collected will be carefully recorded, compiled, and analyzed in line with the research objectives. The researchers will ensure that the privacy and confidentiality of the participants are maintained throughout the study, and any inquiries from participants will be addressed directly by the research team.

4. Risks

Needle pain is a recognized risk associated with participation in this study, as it may occur during certain procedures. However, every effort will be made to minimize discomfort, and trained professionals will be administering the injections or taking blood samples. You will be provided with clear instructions and support throughout the process to ensure their comfort and safety. Despite this potential risk, all procedures will be conducted with the utmost care to prioritize your well-being.

5. Benefits

The study aims to determine the efficacy of periwinkle extract in exhibiting anticoagulant properties. By participating, you contribute to advancing medical knowledge and potentially paving the way for safer, more effective treatments for coagulation disorders. While there may not be immediate personal health benefits for participants, your involvement supports the development of innovative therapies that could benefit individuals with clotting issues in the future. Your contribution will play a crucial role in improving healthcare outcomes and enhancing the quality of life for countless individuals. All potential benefits will be clearly communicated before you consent to participate, ensuring you make an informed

decision. Ethical guidelines will be followed to ensure that the anticipated benefits outweigh any associated risks.

6. Reimbursements

The study is purely self-funded and you as our respondent in this study will not incur any financial cost or receive any compensation for participating in the study.

7. Confidentiality

The researchers will guarantee your autonomy by furnishing them with comprehensive details, including the study's objectives, benefits, significance, and the individuals who could obtain the study's findings. Additionally, to minimize the risk of your identification through personal details, only information necessary for the study will be included. Furthermore, the researchers will ensure that all the details provided by you as our participant will be treated with utmost confidentiality, and will be used for this study only. Hence, their identities will be anonymous and kept unknown during the research study. Rest assured that your responses will remain anonymous and be accessible only to the researchers, the research adviser, and the research instructor, in order to protect the confidentiality of the data. The data that will be collected will be safely disposed of and will destroy the data responses after the study is finished.

8. Sharing of Results

The findings of this study will be shared with respondents and published in academic journals or conferences to inform nursing education and practice. You may request a summary of the study results. However, you will not be identified in any shared materials.

9. Right to Refuse or Withdrawal

You have the right to refuse or withdraw from a study at any time without penalty. We as researchers understand your autonomy. If you decide to withdraw, data collection from you will stop, though previously collected data may be used if prior consent allows. Your declining to continue with the study would not have any impact on our relationship with you, the researchers or the institution.

10. Who to Contact For any questions, concerns or additional information about the study, please contact:

Jessie Anne M. Cahimari

Email address: Jessieanne.cahimari@lorma.edu

Phone number: 09457721196

We are available to address any questions you may have promptly and ensure your comfort throughout the research process.

CERTIFICATE OF CONSENT:

I have read the information stated herein or it was properly explained to me. I was provided with a chance to ask questions relative to it. All questions I asked were answered properly; therefore, I consent and voluntarily participate in this study.

Name of Participant: _____

Signature of Participant: _____

Date: _____

Statement from the Researcher/Person Obtaining the Consent

All information pertaining to this study was explained to the possible participant and made sure that I fully understood what I have to do in the research.

Similarly, I affirm that the potential participant was given a chance to ask questions which I have answered accurately to the best of my ability.

Likewise, I affirm that the participant was not coerced or forced in giving consent. That I have voluntarily provided the consent.

Accomplished by: _____ Date Submitted: _____

(Signature over Printed Name)

APPENDIX R
Letter Of Intent To Conduct Research At Ub Laboratory

March 01, 2025

DR. JANICE KAYLYN K. LONOGAN
Office of the Vice President for Academic Affairs
University of Baguio

okay
03/14/25

THRU: MS. JACQUELINE HERNANDEZ, CSSP, CSSM, MPH
Head, UB Central Supply Room

Dear Ma'am,

Greetings!

We, the third-year student researchers of Lorma Colleges - College of Pharmacy are currently conducting a research entitled **"Anticoagulant Property of Periwinkle *(Catharanthus roseus)* Leaf Extract Formulated as Solution"**. Our study aims to explore the anticoagulant properties of periwinkle leaf extract in a solution and assess its potential therapeutic applications in managing blood coagulation disorders.

In this regard, given the University of Baguio's expertise and advanced laboratory facilities, we are writing this letter to formally request your assistance in conducting Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT) testing on our in-vitro human blood samples to be conducted on March 15, 2025. This analysis is critical in determining the anticoagulant efficacy of our formulated extract at varying concentrations (0.05g/100mL, 0.1g/100mL, 0.2g/100mL).

We kindly seek your guidance regarding the necessary requirements, protocols, and procedures for sample submission. Additionally, we would appreciate information on any fees, schedules, and documentation required to facilitate this request.

The table below is a list of the materials and reagents that will be needed to conduct the PT and aPTT.

Quantity	Materials
1	Coagulometer | APTT Analyzer
30 mL	APTT Reagent
30 mL	PT Reagent
6	Blue Top Tube
35	Test Tube
5	100 mL Graduated Cylinder
1	Centrifuge
8	Pipette

Should you require further information or clarification, the researchers would be happy to assist by reaching us in this email jessieanne.cahimari@lorma.edu.

We are looking forward to a favorable response regarding our request. Thank you for your support and God bless!

Respectfully Yours,

ALMOJUELA, ERISH GLEAN N.
Student Researcher

BALAGOT, MARIELLE JAN M.
Student Researcher

CAHIMARI, JESSIE ANNE M.
Student Researcher

CASUGA, LUVLY ARNIE M.
Student Researcher

MACARIO, TRISHA KEITH B.
Student Researcher

OMAR, WALA O.
Student Researcher

Noted by:

02-26-2025
BEVERLY BAGAYAO-BARUT, RPh
Faculty Researcher

02/26/25
IVY ROSE C. OROZCO, RPh, IP
Thesis Instructor

02-26-2025
ELLEN MAE P. ABIQUI, RPh, MSPharm, CPT
Dean, College of Pharmacy
Lorma Colleges

APPENDIX S
Certificate From Statistician

LORMA COLLEGES

GENERAL EDUCATION DEPARTMENT
Campus for Health Sciences
Carlatan, San Fernando City, La Union

April 7, 2024

CERTIFICATION

This is to certify that the research entitled **"Anticoagulant Property of Periwinkle (Catharanthus roseus) Leaf Extract Formulated as Solution "** by Almojuela, Irish Glean N., Balagot, Marielle Jan M., Cahimari, Jessie Anne M., Casuga, Luvly Arnie M., Macario, Trisha Keith B., and Omar, Wala O., third year students taking the degree Bachelor of Science in Pharmacy of LORMA Colleges, had had been checked and statistically analyzed by the undersigned.

This certification is issued to ensure that the institution received quality research work. Signed this 7th day of April, 2025.

JEROME P. VERA
Statistician

APPENDIX T
Certificate Of English Critic

CERTIFICATION

This is to certify that the research entitled **"Anticoagulant Property of Periwinkle *(Catharanthus roseus)* Leaf Extract Formulated as Solution"** by **Almojuela, Irish Glean N., Balagot, Marielle Jan M., Cahimari, Jessie Anne M., Casuga, Luvly Arnie M., Macario, Trisha Keith B., Omar, Wala,** third year students currently taking the degree of Bachelor of Science in Pharmacy of LORMA Colleges, had been checked for its grammatical structure and format by the undersigned.

This certification is issued to ensure that the institution received quality research work. Signed this 28th day of May 2025.

MICHELLE B. MACARIO, LPT, MA English
Master Teacher 1
Division Research Evaluator
Division of Abra

APPENDIX U
Grammarly Report

Chapter I

by Z

General metrics

38,685	5,352	269	21 min 24 sec	41 min 10 sec
characters	words	sentences	reading time	speaking time

Score

99

This text scores better than 99% of all texts checked by Grammarly

Writing Issues

7	6	1
Issues left	Critical	Advanced

Plagiarism

1 %

0 source

1% of your text matches 0 sources on the web or in archives of academic publications

Report was generated on Wednesday, May 28, 2025, 09:55 PM

Page 1 of 25

Chapter II

by Z

General metrics

27,140	3,951	252	15 min 48 sec	30 min 23 sec
characters	words	sentences	reading time	speaking time

Writing Issues

No issues found

Plagiarism

This text seems 100% original. Grammarly found no matching text on the Internet or in ProQuest's databases.

Chapter III

by Z

General metrics

37,681	5,337	425	21 min 20 sec	41 min 3 sec
characters	words	sentences	reading time	speaking time

Writing Issues

No issues found

Plagiarism

This text seems 100% original. Grammarly found no matching text on the Internet or in ProQuest's databases.

Chapter IV

by Z

General metrics

7,750	1,111	50	4 min 26 sec	8 min 32 sec
characters	words	sentences	reading time	speaking time

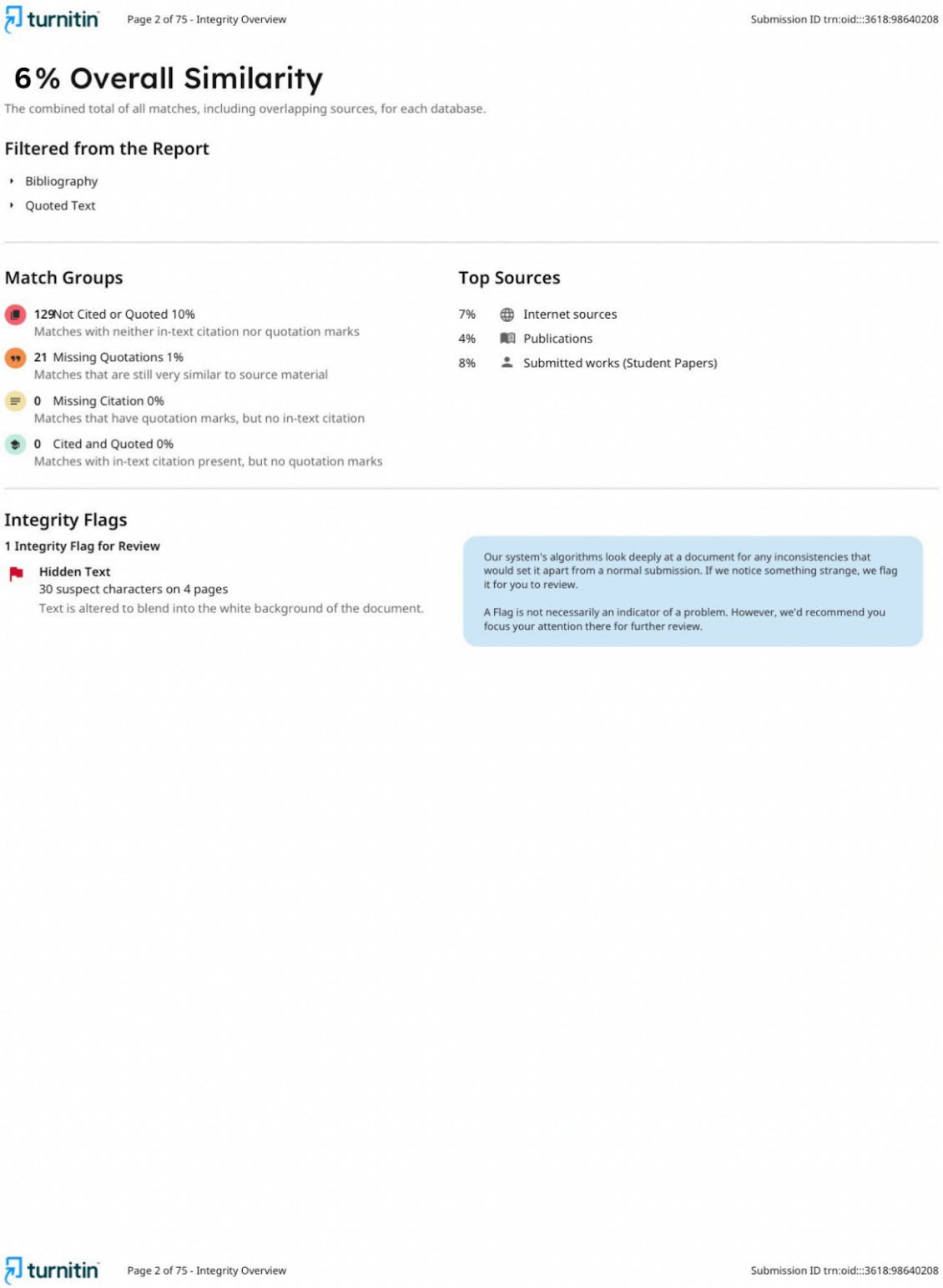
Writing Issues

No issues found

Plagiarism

This text seems 100% original. Grammarly found no matching text on the Internet or in ProQuest's databases.

APPENDIX V
AI And Plagiarism Checker Report



turnitin Page 2 of 75 - Integrity Overview Submission ID trn:oid:::3618:98640208

6% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

Filtered from the Report

- Bibliography
- Quoted Text

Match Groups

- **129** Not Cited or Quoted 10%
  Matches with neither in-text citation nor quotation marks
- **21** Missing Quotations 1%
  Matches that are still very similar to source material
- **0** Missing Citation 0%
  Matches that have quotation marks, but no in-text citation
- **0** Cited and Quoted 0%
  Matches with in-text citation present, but no quotation marks

Top Sources

- 7% Internet sources
- 4% Publications
- 8% Submitted works (Student Papers)

Integrity Flags

1 Integrity Flag for Review

Hidden Text
30 suspect characters on 4 pages
Text is altered to blend into the white background of the document.

Our system's algorithms look deeply at a document for any inconsistencies that would set it apart from a normal submission. If we notice something strange, we flag it for you to review.

A Flag is not necessarily an indicator of a problem. However, we'd recommend you focus your attention there for further review.

turnitin Page 2 of 75 - Integrity Overview Submission ID trn:oid:::3618:98640208

8 % detected as AI

The percentage indicates the combined amount of likely AI-generated text as well as likely AI-generated text that was also likely AI-paraphrased.

Caution: Review required.

It is essential to understand the limitations of AI detection before making decisions about a student's work. We encourage you to learn more about Turnitin's AI detection capabilities before using the tool.

Detection Groups

21 AI-generated only 7%
Likely AI-generated text from a large-language model.

1 AI-generated text that was AI-paraphrased 0%
Likely AI-generated text that was likely revised using an AI-paraphrase tool or word spinner.

Disclaimer
Our AI writing assessment is designed to help educators identify text that might be prepared by a generative AI tool. Our AI writing assessment may not always be accurate (it may misidentify writing that is likely AI generated as AI generated and AI paraphrased or likely AI generated and AI paraphrased writing as only AI generated) so it should not be used as the sole basis for adverse actions against a student. It takes further scrutiny and human judgment in conjunction with an organization's application of its specific academic policies to determine whether any academic misconduct has occurred.

Frequently Asked Questions

How should I interpret Turnitin's AI writing percentage and false positives?
The percentage shown in the AI writing report is the amount of qualifying text within the submission that Turnitin's AI writing detection model determines was either likely AI-generated text from a large-language model or likely AI-generated text that was likely revised using an AI-paraphrase tool or word spinner.

False positives (incorrectly flagging human-written text as AI-generated) are a possibility in AI models.

The AI writing percentage should not be the sole basis to determine whether misconduct has occurred. The reviewer/instructor should use the percentage as a means to start a formative conversation with their student and/or use it to examine the submitted assignment in accordance with their school's policies.

What does 'qualifying text' mean?
Our model only processes qualifying text in the form of long-form writing. Long-form writing means individual sentences contained in paragraphs that make up a longer piece of written work, such as an essay, a dissertation, or an article, etc. Qualifying text that has been determined to be likely AI-generated will be highlighted in cyan in the submission, and likely AI-generated and then likely AI-paraphrased will be highlighted purple.

Non-qualifying text, such as bullet points, annotated bibliographies, etc., will not be processed and can create disparity between the submission highlights and the percentage shown.

10. Author(s)

Beverly Bagayao-Barut, RPh, serves as the Academic Coordinator of the College of Pharmacy at Lorma Colleges. Her outstanding academic achievements and professional credentials reflect her unwavering dedication to excellence. A proud Lormanian Pharmacist, she holds a Master of Science in Pharmacy from Saint Louis University, Baguio City, underscoring her commitment to advancing pharmaceutical education and practice.

Irish Glean N. Almojuela is a Pharmacy student at Lorma Colleges. She is known for her hardworking nature and strong attention to detail, consistently demonstrating dedication to her studies and academic responsibilities.

Marielle Jan M. Balagot is a Pharmacy student at Lorma Colleges. She is recognized for her creativity and curiosity, actively engaging in learning and showing a clear and thoughtful approach to her academic work.

Jessie Anne M. Cahimari is a Pharmacy student at Lorma Colleges. She has shown confidence and leadership skills, often taking initiative in group activities while maintaining strong academic performance.

Luvly Arnie M. Casuga is a Pharmacy student at Lorma Colleges. She is enthusiastic and positive, known for her cooperative spirit and motivation to excel both academically and in teamwork.

Trisha Keith B. Macario is a Pharmacy student at Lorma Colleges. She demonstrates discipline and determination, focusing on mastering details and consistently striving for academic success. She also served as the President of College of Pharmacy - Student Body Organization.

Wala O. Omar is a Pharmacy student at Lorma Colleges. She is thoughtful and open-minded, showing empathy and a willingness to consider different perspectives in her academic journey.