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The Effect of *Colocasia esculenta* L. Schott (Taro) Leaf Extract in the Clotting Time and Evaluation of Electrolytes as a Potential Clot Activator

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Abstract. Clot activators are substances added to blood collection tubes to initiate the coagulation cascade, separating blood into serum. Blood chemistry uses serum separator tubes (SST) as they promote rapid clotting and allow serum separation from cellular components through centrifugation. Taro (Colocasia esculenta L.) Schott is a tropical crop widely produced for its edible corms. Researchers used leaves as they remain underutilized, they contain alkaloids, terpenoids, saponins, flavonoids, and phenols, essential to mimic a clot activator and how it influences blood integrity. This study tests different concentrations (12.5, 25, 50, and 100%) of leaf extract to determine its effect on blood. The pre-test also recorded the clotting time and physical observation, whereas (gold top, 12.5%, 25%, 50%, 100%, and red top) had a time of 14:04, 10:45, 11:58, 12:09, 14:29, and 21:33 minutes, respectively. Researchers observed that the clotting time is slower as the concentration increases, and samples that did not hemolyze were used on eight healthy individuals. In each case, they collected 10 mL of blood and placed 2 mL in each respective vacutainer. All 40 specimens were sent to the laboratory for electrolyte testing, specifically for sodium, potassium, and chloride levels. After releasing the results, one-way ANOVA and Tukey's HSD Post-hoc Test were performed, setting the alpha value to 0.05 to determine significant differences between groups. The ANOVA result showed that 50% concentration had the most significant effect compared to 12.5% and 25% in (sodium, potassium, and chloride) with p-values of 6.71E-05, 6.14E-08, and 4.36E-05, respectively. Comparing all setups obtained p-values of 1.95E-13, 1.90E-15, and 1.19E-11, respectively, indicating significant differences among the groups. The findings suggest that taro leaf extract affected clotting time, blood sample integrity, and electrolyte values, with increasing concentrations leading to a more significant effect, showing the potential to act as a clot activator.

Keywords: Clot activator; Taro leaf extract; Clotting time; Sodium; Potassium; Chloride.

1.0 Introduction

Clot activators are substances added to blood collection tubes that initiate the coagulation cascade, causing blood to clot and separate into serum. (Lima-Oliveira et al., 2020). Clinical laboratories widely use serum tubes with clot activators for various diagnostic tests because they promote rapid clotting, which allows for serum separation from cellular components through centrifugation. Serum-separating tubes (SSTs) are test tubes used in clinical chemistry tests requiring blood serum. These test tubes contain micronized silica particles, which aid in the coagulation process of blood before centrifugation, and a gel at the bottom of the tube, which separates the cell components from the scrum (Jo et al., 2021). Clot activators act as catalysts to promote blood clotting, and serum gel separators act as a barrier that enables the diffusion and separation of blood clot contaminants and other cellular components.

Taro (*Colocasia esculenta*) leaves have garnered attention for their potential application as a clot activator in medicine. Mba and Agu (2021) investigated a more practical application of the presence of bioactive compounds in taro leaves that are anti-cancer, anti-inflammatory, and antioxidant. They also contain carbohydrates with properties such as antihyperglycemic and antihyperlipidemic. In comparison, Nwaogwugwu et al. (2020) explored taro leaf's hematological changes and antidiabetic activities in diabetic rats. The study's results proved that taro leaf extract boosts the body weight of the rats, which may help with potential weight loss following complicated diabetes. It also demonstrated that the leaf extract improved diabetic control and management in rats.

Another study by Sjamsudin et al. (2021) shows their therapeutic potential in promoting the healing process of wounds contaminated with *Staphylococcus aureus*, where it has been shown to stimulate hematopoietic cells and enhance healing, which significantly affects wound repair and regeneration. Furthermore, the efficacy of taro leaf extract and its potential as a natural clot activator in clinical applications offer further research to utilizing plant-based alternatives for therapeutic applications. Clot activators in blood collection tubes ensure accurate electrolyte panel values. It has shown that clot activators, such as silica particles, can significantly impact the stability of electrolytes in serum, including (sodium, potassium, and chloride). The delay in specimen processing can occur due to various factors and can lead to changes in these electrolyte values, potentially affecting patient diagnosis and treatment (Fauziah et al., 2021).

This research focuses on the potential clot activator properties of Taro (*Colocasia esculenta* L.) Schott leaf extract. Its effects on blood clotting time, physical observations, and electrolyte panel values (sodium, potassium, and chloride) were observed. This study assessed the phytochemical analysis of taro leaves, whereas alkaloids, terpenoids, flavonoids, phenols, and saponins were suspected to influence blood clotting. The researchers examined only the extract's four concentrations (12.5, 25, 50, and 100%). A pre-test was conducted *in vitro to determine which concentrations would be viable to maintain* blood integrity. The blood samples mixed with the extract were compared to those from a commercially available clot activator, serum separator tube (SST), and negative control, red top (no additive). This study only focused on creating an alternative natural clot activator compared to existing ones. Furthermore, the study's findings can help understand the mechanisms behind blood clotting activity, which can contribute more to the knowledge of the clinical application of plant-derived compounds. Further research can help strengthen the effects of plant-derived clot activators and their processes of influencing blood, linking conventional treatments to evidence-based therapy.

2.0 Methodology

2.1 Research Design

The quantitative research design involved a systematic approach to investigating and addressing a specific problem. The method used numerical data and statistical analysis to test theories and generate findings. Furthermore, it enabled the researchers to establish cause-and-effect relationships and make informed conclusions regarding the impact of the manipulated variables. Statistical analyses, like One-way ANOVA and Tukey's HSD hoc test, were used to evaluate the significance of observed differences. The researchers used experimental quantitative data to gather information about the present condition. The scientific method involves modifying one or more variables to observe their effects on another variable, such as experimental research. It is quantitative and provides verifiable, numerical facts. In line with the quantitative and experimental research design, the researchers implemented a two-group design, with the experimental group, taro leaf extract, compared with the positive (serum separator tube) and negative control (no additive). At the end of the study, the objective was to provide an in-depth understanding of the clot-activating properties of taro leaf extract when mixed with the blood samples.

2.2 Research Locale

Researchers conducted the study at the National University-Manila, a private university at 551 F. Jhocson St, Sampaloc, Manila, 1008 Metro Manila.

2.3 Research Participants

Four male and female first-year Medical Technologist Students were willing to get their blood extracted. The researchers collected their samples on the 6th floor of the main building inside the laboratory under the supervision of a registered medical technologist (RMT).

2.4 Research Instrument

This study used laboratory equipment, including a centrifuge, to isolate the serum of the vacutainers obtained from the eight blood collections of the volunteers. The Cornley AFT-800 Electrolyte Analyzer processed the serum samples of the subjects.

2.5 Data Gathering Procedure

Materials

Two kilograms of taro leaves were obtained from Aurora Subdivision, Angono Rizal. All medical supplies were purchased, including vacutainers: gold top (serum separator tube) and red top (no additive), ten cc/mL syringe, tourniquet, alcohol swabs, cotton, micropore, surgical gloves, and 95% ethanol from Bambang, Manila.

Ethanolic Extract of Taro Leaves

Before extraction, a sample was sent on-site for authentication to the Institute of Biology, University of the Philippines, Diliman. Subsequently, taro leaves were washed with distilled water, air-dried for two days, weighed, and ground the resulting weight of the powder. Fifty grams of the powdered sample was taken in a thimble and submerged with 250 mL of 95% ethanol in the Soxhlet apparatus. It took 6 hours to complete. The extract was filtered by Whatman filter paper no. 1 and refrigerated (Deutsch, 2021).

Phytochemical Analysis of Taro Leaf Extract

Standard procedures qualitatively determined the phytochemicals presented in a study by Al-Kaf et al. (2019). Alkaloids: Dragendorff's test. For the Salkowski test for terpenoids. In the Foam test for saponins. For detecting flavonoids using the Shinoda test. In the $FeCl_3$ test for phenols.

Pre-testing of Different Concentrations

A pre-test was conducted to determine which concentration (12.5, 25, 50, and 100%) affects blood clotting. One random healthy subject willing to have their blood extracted was selected. A syringe method was performed: each vacutainer contained 12.5%, 25%, 50%, and 100% of the extract, respectively. A gold top was used as a positive control, and a red top was used as a negative control. The clotting time of all tubes collected was observed, recorded, and centrifuged. After evaluation, the concentrations (12.5, 25, and 50%) are used.

Blood Extraction on 4 Males and Females

Healthy individuals were identified to get their blood extracted. Unitek College (2022) standard procedures were used during blood extraction, where a Registered Medical Technologist (RMT) was supervised while performing venipuncture using the syringe method. 10 mL of blood was extracted, placing 2 mL into each of the five vacutainers: a gold top (positive control), red tops with the experimental setup (taro leaves extract) at concentrations of 12.5, 25, and 50%, and a red top (negative control), respectively.

In-vitro Blood Chemistry of Taro Leaf Extract, Gold, and Red Top

Forty specimens were obtained and transported to St. Ignatius De Loyola Health Care Services at #253 Shoe Avenue, Sto. Nino, Marikina City. A Registered Medical Technologist (RMT) processed all specimens using a Cornley AFT-800 Electrolyte Analyzer. The vacutainers are placed inside the machine and fed to aspirate the serum and analyze the content of the electrolytes (sodium, potassium, and chloride) of each sample.

Recording Data of the Electrolyte Panel (Sodium, Potassium, and Chloride)

Before carrying out these results, it was crucial to double-check their accuracy. All data were collected inside the clinic laboratory, and statistics were used to find significant differences between the experimental, positive, and negative control groups.

Statistical Analysis and Interpretation of Results
A statistician analyzed and interpreted the collected data, calculating whether there were significant differences between the experimental (taro leaves extract), positive control (serum separator tube), and negative control (no additive) groups. A one-way analysis of variance (ANOVA) and Tukey's HSD Post-hoc Test were used.

2.6 Ethical Considerations
Consent of the participants
The consent form was handed out to 1st Year Medical Technology students at National University-Manila and those who volunteered to have their blood extracted, and their information was kept confidential with no risks of harming the student.

Risks
The study involved sampling four male and female volunteers for blood extraction, which was performed by the researchers, with a registered medical technologist throughout the procedure.

Proper waste disposal
The materials used in the study, such as needles, vacutainers containing blood, and ethanol from taro leaves extraction, were considered hazardous wastes. Therefore, the researchers adequately disposed of the said materials in an orderly, preventing contamination, exposure, and contact with people.

3.0 Results and Discussion

The phytochemical analysis result of the taro leaf extract is found in Table 1. The Alkaloids test showed an orange precipitate. Zubair et al. (2023) reported that alkaloids extracted from taro leaves can enhance platelet aggregation, a crucial step in clotting. The Terpenoids test resulted in a reddish-brown coloration at the interface. A study by Masyita et al. (2022) demonstrated that terpenoids isolated from taro leaves can accelerate the coagulation cascade. This acceleration is achieved by enhancing the activity of coagulation factors, leading to quicker fibrin formation and clot stabilization. The Saponins test produced persistent foam for over ten minutes.

Table 1. Phytochemical analysis of taro leaf extract

Bioactive Compound	Method Used	Result
Alkaloids	Dragendorff's test	+
Terpenoids	Salkowski test	+
Saponins	Foam test	+
Flavonoids	Shinoda test	+
Phenols	$FeCl_3$ test	+

Research by Juang and Liang (2020) highlighted the ability of saponins from taro leaves to promote clot formation. These saponins facilitate the aggregation of red blood cells and platelets, contributing to the rapid formation of a clot. The Flavonoids test showed an orange coloration. Ferdaus et al. (2023) identified that flavonoids such as quercetin and kaempferol enhance platelet function and promote the release of clotting factors. The phenols test showed a blue-green coloration. Christou et al. (2023) reported that phenolic acids in taro leaves can reduce oxidative stress at the injury site, thus preserving the function of clotting factors and platelets.

Results of the different set-ups (Gold Top, 12.5%, 25%, 50%, 100%, and Red Top) are presented in Table 2, where the pre-testing of blood in their clotting time and physical observation were taken. It is shown that among the four concentrations, as it goes lower, so does the time needed for blood to clot. However, the 100% concentration was not viable in further testing for the electrolyte panel because the sample was hemolyzed, per standard laboratory procedure.

Table 2. Physical observation and clotting time of set-ups

Set-up	Observation	Clotting Time (minutes)
Gold Top (SST)	Clear and Not Hemolyzed	14:04
Red Top (12.5%)	Clear and Not Hemolyzed	10:45
Red Top (25%)	Clear and Not Hemolyzed	11:58
Red Top (50%)	Clear and Not Hemolyzed	12:09
Red Top (100%)	Reddish and Hemolyzed	14:29
Red Top (No additive)	Clear and Not Hemolyzed	21:33

Azman et al. (2019) explained that when red blood cells undergo hemolysis, they release intracellular contents into the serum or plasma. This can lead to falsely elevated levels of certain electrolytes, particularly potassium, while diluting others like sodium.

The effect of the taro leaf extract on the blood clot with different set-ups in the electrolyte sodium is presented in Table 3. The values are expressed in millimole per liter (mmol/L). The extract has increased sodium levels, as the concentration, potentially impacting sodium regulation within the samples. Yuen et al. (2021) used measuring sodium concentrations. Their findings showed a dose-dependent relationship, with sodium levels showing distinct patterns across different concentrations.

Table 3. Comparison of electrolyte: Sodium levels on experimental, positive, and negative control

Positive Control	12.5%	25%	50%	Negative Control
138.7	124.8	130.0	132.6	137.4
140.6	127.0	129.2	133.0	139.4
139.2	130.6	135.2	135.8	137.8
135.9	125.3	129.0	131.0	136.6
136.2	126.2	123.5	133.7	139.5
137.7	127.0	130.0	133.2	136.2
135.1	126.4	128.0	132.4	135.3
137.2	125.2	128.2	131.8	138.3

Table 4 shows that when subjected to one-way ANOVA, the analysis between the F value's experimental, positive, and negative values was 45.78. By using an alpha of 0.05, the F0.05 was 4,35 = 2.64. Since the test statistic is much larger than the critical value and the *p-value* for 2.64 is 1.95×10^{-13}, the test statistic is significant at that level. Therefore, there is a significant difference in the sodium electrolyte activity.

Table 4. ANOVA analysis of sodium levels between set-ups

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	781.04	4	195.26	45.78	1.95E-13	2.64
Within Groups	149.27	35	4.2648			
Total	930.31	39				

Furthermore, Table 5 presents a post-hoc test, or Tukey's Honestly Significant Difference (HSD) test, was performed. A significant difference between the sodium levels of positive control compared to 12.5%, 25%, and 50% concentration resulted in a p-value = 0.001. There is a significant difference between 12.5%, 25%, and 50% compared to the negative control resulting in a p-value = 0.001. When compared between concentrations, there is no significant difference between 12.5% and 25%, with a p-value = 0.086. There is a significant difference between 12.5% and 50% concentrations with a p-value = 0.001. Additionally, there is a significant difference between 25% and 50% concentrations with a p-value = 0.009. Abubakar and Sule (2010) reported that higher doses of the extract led to significant increases in serum potassium levels and notable decreases in serum sodium levels, indicating a complex interaction where the concentration of plant extract influences sodium balance in the body.

Table 5. Tukey's HSD Post-hoc test analysis of sodium levels between set-ups

Pairwise comparison	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD interference
Positive control vs 12.5%	15.06	0.001005	p<0.05
Positive control vs 25%	11.54	0.001005	p<0.05
Positive control vs 50%	6.344	0.001005	p<0.05
Positive control vs Negative control	0.017	0.899995	insignificant
12.5% vs Negative control	15.04	0.001005	p<0.05
25% vs Negative control	11.52	0.001005	p<0.05
50% vs Negative control	6.327	0.001005	p<0.05

The effect of the taro leaf extract on the blood clot with different set-ups in the electrolyte potassium is presented in Table 6. The values are expressed in millimole per liter (mmol/L). The results align with the study of Zubair et al. (2023), which shows its significant role in regulating potassium levels due to its high mineral content. The study highlights taro leaves with high potassium content as crucial for various physiological functions. This supports the observed dose-dependent increase in potassium levels when different concentrations were applied. It suggests that taro's substantial potassium content can effectively modulate potassium levels, reinforcing its potential applications in dietary and medicinal contexts for maintaining electrolyte balance and proper cellular function.

Table 6. Comparison of electrolyte: Potassium levels on experimental, positive, and negative control

Positive Control	12.5%	25%	50%	Negative Control
3.92	4.41	5.42	6.75	3.70
3.94	4.98	5.51	6.82	3.82
4.01	4.50	5.29	6.35	4.01
4.19	4.54	5.45	6.45	4.33
3.49	4.37	6.28	6.04	3.61
3.69	4.25	5.01	6.06	3.70
4.06	4.48	5.42	6.23	4.19
4.47	5.59	5.97	7.10	4.74

Table 7 shows that when subjected to one-way ANOVA, the analysis between the experimental, positive, and negative values of the F value was 62.47. By using an alpha of 0.05, the F0.05 was 4,35 = 2.64. Since the test statistic is much larger than the critical value and the p-value for 2.64 is 1.90×10^{-15}, the test statistic is significant at that level. Therefore, there is a significant difference in the potassium electrolyte activity.

Table 7. ANOVA analysis of Potassium levels between set-ups

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	36.87	4	9.217	62.47	1.90E-15	2.641
Within Groups	5.164	35	0.147			
Total	42.03	39				

Furthermore, Table 8 presents a post-hoc test, or Tukey's Honestly Significant Difference (HSD) test, which was performed. A significant difference between the sodium levels of positive control when compared to 12.5%, 25%, and 50% concentration resulted in a p-value = 0.001. There is a significant difference between 12.5%, 25%, and 50% when compared to the negative control resulting in a p-value = 0.001. When compared between concentrations 12.5%, 25%, and 50%, there is a significant difference between the potassium levels with a p-value = 0.001. Koukoui et al. (2022) examined various plant extracts, noting that higher concentrations of certain extracts, mainly from *Heliotropium indicum* and *Ipomoea fistulosa*, significantly increased serum potassium levels in rats. Their research emphasizes these plants' potassium-rich nature and potential therapeutic effects, which correlates with the study by Ferdaus et al. (2023), where taro leaves are a highly nutritious crop rich in carbohydrates, dietary fiber, and essential minerals such as potassium, magnesium, and calcium.

Table 8. Tukey's HSD Post-hoc test analysis of Potassium levels between set-ups

Pairwise comparison	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD interference
Positive control vs 12.5%	4.924	0.011106	p<0.05
Positive control vs 25%	11.57	0.001005	p<0.05
Positive control vs 50%	18.43	0.001005	p<0.05
Positive control vs Negative control	0.303	0.899995	insignificant
12.5% vs Negative control	4.620	0.019315	p<0.05
25% vs Negative control	11.27	0.001005	p<0.05
50% vs Negative control	18.13	0.001005	p<0.05

The effect of the taro leaf extract on the blood clot with different set-ups in the electrolyte chloride is presented in Table 9. The values are expressed in millimole per liter (mmol/L). In support of the recorded results of the research study, Christou et al. (2023) conducted an ultrasound-assisted extraction of the chemicals and antioxidants found in taro leaf extracts. Taro, in general, is widely known as a practical resource of proteins, potassium, calcium, phosphorus, iron, and polyphenolic compounds. In addition, chloride is one of the most abundant components in taro.

Table 9. Comparison of electrolyte: Chloride levels on experimental, positive, and negative control

Positive Control	12.5%	25%	50%	Negative Control
100.1	92.6	90.6	95.7	100.0
104.0	87.7	90.0	95.9	96.9
102.1	91.6	96.7	96.5	101.0
101.6	89.8	93.4	96.5	98.5
108.5	90.7	90.7	99.0	104.1
104.2	92.8	95.5	98.1	106.2
102.3	94.9	93.4	97.0	99.2
101.0	89.9	92.6	96.3	98.4

Table 10 shows, when subjected to One-way ANOVA, the analysis between the experimental, positive, and negative of the F value of 34.23 and by using an alpha of 0.05, the $F_{0.05;4,35}$ = 2.64. Since the test statistic is much larger than the critical value and the *p-value* for 2.64 is 1.19 x 10^{-11}, the test statistic is significant at that level. Therefore, there is a significant difference in the activity of electrolytes in chloride.

Table 10. ANOVA Analysis of Chloride Levels Between Set-ups

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	786.8925	4	196.7231	34.23015	1.19E-11	2.641465
Within Groups	201.1475	35	5.747071			
Total	988.04	39				

Furthermore, Table 11 presents a post-hoc test, or Tukey's Honestly Significant Difference (HSD), performed. A significant difference between the sodium levels of positive control compared to 12.5%, 25%, and 50% concentration resulted in a p-value = 0.001. There is a significant difference between 12.5% and 25% compared to the negative control, resulting in a p-value = 0.001. In comparison, there is a significant difference between 50% and negative control with a p-value = 0.033. When compared between concentrations, there is no significant difference between 12.5% and 25% with a p-value = 0.26. There is a significant difference between 12.5% and 50% with a p-value = 0.001. Additionally, there is a significant difference between 25% and 50% with a p-value = 0.002. In correlation to sodium levels presented in Table 3 and chloride levels in Table 9, Osonuga et al. (2023) study examined the effects of *Phyllanthus amarus* leaf extract on serum electrolyte levels, including chloride. Their results indicate that while there was a non-significant difference in serum sodium and potassium levels, there were slight increases in chloride levels with higher doses of the extract. It suggests that plant extracts can influence the bloodstream's electrolyte balance, including chloride levels. The results of sodium levels using the different concentrations of taro leaf extracts are lower than the positive and negative control.

Table 11. Tukey's HSD post-hoc test analysis of Chloride levels between set-ups

Pairwise comparison	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD interference
Positive control vs 12.5%	13.83	0.001005	p<0.05
Positive control vs 25%	11.93	0.001005	p<0.05
Positive control vs 50%	7.197	0.001005	p<0.05
Positive control vs Negative control	2.875	0.271804	insignificant
12.5% vs Negative control	10.95	0.001005	p<0.05
25% vs Negative control	9.055	0.001005	p<0.05
50% vs Negative control	4.321	0.032629	p<0.05

4.0 Conclusion

The following conclusions were drawn based on the researchers' findings concerning the effect of taro leaf extract on the blood samples. Researchers observed that using taro leaf extract affected blood and clotting time. Compared to the serum separator tube (SST) and red top (no additive), the three concentrations (12.5, 25, and 50%) displayed a faster time of clotting. It also separated the blood components without causing hemolysis of the red blood cells and obtained serum. They established that the 100% was not part of the electrolyte panel testing procedure because it underwent hemolysis after centrifugation during the pre-testing phase. The three concentrations (12.5, 25, and 50%) affected the blood chemistry, mainly the electrolyte panel (sodium, potassium, and chloride). The researcher noted that as the concentration increased, so did the values of the electrolyte panel. The data analysis shows that the different concentrations of taro leaf extract had a significant effect in evaluating the blood chemistry of the electrolyte panel (sodium, potassium, and chloride). The researchers conclude that there are significant differences in comparing the experimental set-up (taro leaf extract), positive control (serum separator tube), and negative control (no additive) in the activity of the electrolyte panel (sodium, potassium, and chloride).

5.0 Contributions of Authors

All authors gave equal contributions, reviewed, and approved for the final work.

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7.0 Conflict of Interests

The authors declare no conflict of interest in publishing the paper.

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