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# IN VITRO ANTICOAGULANT ACTIVITY OF *Zingiber officinale* (GINGER) LEAF EXTRACT ON HUMAN WHOLE BLOOD USING PROTHROMBIN TIME AND ACTIVATED PARTIAL THROMBOPLASTIN TIME

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**Abstract:** This study aims to determine the in vitro anticoagulant activity of *Zingiber officinale* (Ginger) crude leaf extract on human whole blood. Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT) were used to assess the anticoagulant activity of human blood exposed to Heparin, Normal Saline Solution (NSS), and Ginger crude leaf extract. Results showed that blood treated with Heparin had the most prolonged mean PT and aPTT, 67.66±4.68 and 108.59±6.80 seconds, respectively, followed by blood treated with Ginger crude leaf extract, 24.91±4.68 and 58.43±8.50 seconds, respectively. The mean PT and aPTT clotting time of blood treated with NSS were within the normal range, 13.82±0.66 and 33.32±1.42 seconds, respectively. One-way ANOVA showed that the mean PT and aPTT were significantly affected by the type of treatment group, $F$ (1.356, 18.977) = 721.861, $p < 0.5$, $\eta^2$ = 98.1% and $F$(1.391, 19.474) = 510.060, $p < .05$,$\eta^2$ = 97.3%. Bonferroni post-hoc tests showed that the PT and aPTT had significant differences between treatment pairs. With these results, we concluded that the 250 mg/mL of *Zingiber officinale (*Ginger) crude leaf extract has anti-coagulant activity on human blood.

*Keywords*: anticoagulant activity, human whole blood, partial thromboplastin time, prothrombin time, *Zingiber officinale* (Ginger) crude leaf extract

## I. INTRODUCTION

World Health Organization (WHO, 2021) recorded that the two leading global causes of death were ischemic heart disease (IHD) complications such as myocardial infarction. According to Fu et al. (2023), IHD is the most fatal disease worldwide and the foremost cause of death. In the Philippines, IHD had caused approximately 120 deaths out of 100,000 medical conditions in 2019, listed as the top cause of death in Southeast

Asia (Yee, 2020). A study conducted by Shadrack et al. (2019) showed that myocardial infarction and stroke accounted for one in every four deaths globally.

To prevent internal blood clot formation, heparin may be used. Heparin binds to antithrombin enhancing its activity in inhibiting coagulation factors II, V, VIII, and X, thus preventing future clot formation. Chronic heparin administration, however, predisposes to life-threatening adverse effects such as bleeding, thrombocytopenia, and hypersensitivity reaction. Chronic administration of heparin has been shown to be associated with a 30% morbidity (Patriarcheas et al., 2020). With limited medication access in low-income communities and the adverse medical effects of heparin, there is a need to find an affordable alternative drug (Shukar et al., 2021).

Ginger (*Zingiber officinale*) which comes from the family *Zingiberaceae* contains bioactive polyphenol compounds, collectively termed flavonoids, as well as gingerol, shogaol, and terpenoids, which include zingiberene, beta- bisabolene, alpha-farnesene, beta-sesquiphellandrene and α-curcumene. Other bioactive phytochemicals found in ginger include saponins, anthraquinones, tannins, and glycosides, which are said to have multiple pharmacologic effects such as anti-inflammatory, anti-platelet aggregation, antineutrophil, and anticoagulant properties (Akbari et al., 2020; Ilkhanizadeh et al., 2016; Tzeng et al., 2016). Aside from ginger rhizomes being the most studied part of ginger for anticoagulant potential, other parts of ginger also have promising anticoagulant effects; an example would be ginger leaves. Sulfated alkaline plant callus from young leaves of *Zingiber officinale* showed anticoagulation activity at 200 µg/ml (Akbari et al., 2020). The result showed delayed clot formation time. The presence of the sulfate group from the extracts has the ability to enhance the anticoagulant activity of the ginger leaf by inhibition of the coagulation process and clot formation.

There has been an increase in anticoagulant studies focusing on ginger rhizomes but only a few anticoagulation studies. The anticoagulant activity of *Zingiber officinale* leaves was the focus for this study, specifically, through (1) determining prothrombin time (PT) and activated partial thromboplastin time (aPTT) of human blood exposed to three treatment groups: Heparin, Normal Saline Solution, and Ginger Crude Leaf Extract, and (2) determining the significant difference between treatment groups.

## II. METHODOLOGY

This study utilized a repeated-measures experimental study design to determine the effect of in vitro anticoagulation by *Zingiber officinale* crude leaf extract on human blood. The experimental group was treated with 250 mg/mL of *Zingiber officinale* crude leaf extract, the positive control group was treated with 200 IU/mL Heparin, and the negative control group was treated with normal saline solution (NSS).

The extraction and assay for this study was conducted at the Experimental Research Laboratory of Cebu Doctors' University. The approval for implementation was obtained from the Cebu Doctors' University Institutional Ethics Review Committee (IERC). The subjects were oriented on the procedures and the possible risks of blood extraction (including but not limited to dizziness, allergic reaction to latex, hematoma formation and/or inflammation at the site of venipuncture). A licensed medical technologist was hired to conduct the blood extraction procedure.

A total of 15 whole blood samples from healthy screened volunteers (8 mL from each volunteer), who were between 22 and 29 years old, male or female medical students enrolled in Cebu Doctors'

University, with no history or diagnosis of bleeding or cardiovascular disease, or recent viral exposure or infection prior to the blood extraction, verified using a checklist based on the World Health Organization's Blood Donor Selection: Guidelines on Assessing Suitability for Blood Donation, were obtained. The blood samples of each volunteer was divided into four equal parts of 2 mL each and placed in buffered sodium citrate tubes. The tubes containing 2 mL of blood were then centrifuged at 1500 rpm for 15 minutes to obtain platelet-poor plasma. This was done to make sure that the recorded Prothrombin Time and Partial Thromboplastin Time reflected the clotting time due to coagulation factor activities that were present in the plasma with minimal platelet interference. One buffered sodium citrate tube from each volunteer was used in conducting the PT and aPTT baseline tests; only those that had results within the normal range (N.V. 12-15 seconds and 25-35 seconds, respectively) were included in the study. The first of the three remaining samples of those that fulfilled the inclusion criteria were the samples to be exposed to the ginger crude leaf extract, the second remaining sample to the positive control, and the third remaining sample to the negative control. Master data sheets were used to record all the results that were acquired during the experiment.

Fresh *Zingiber officinale* (ginger) leaves were obtained from local vegetable farms within the vicinity of Calamba, Misamis Occidental, and its taxonomy was identified and verified by the Municipal Agriculturist of the Municipality of Calamba, Misamis Occidental. The ginger leaves were air dried and subsequently pulverized. The pulverized leaves were then macerated with 3 liters of 70% ethanol for 48 hours at room temperature (27±2°C) and were sieved through Whatman no. 1 filter paper. Ethanol solvent reduction was done using a rotary evaporator at 40°C for 3 hours. A regulated water bath at 60°C for 30-45 minutes was then used for final elimination and drying until the extract became slightly viscous, had a non-alcoholic scent, and had no cooling sensation upon touch. To confirm the complete elimination of ethanol, a flame test was done on the extract. Phytochemical screening was done to evaluate for qualitative determination of saponins, alkaloids, glycosides, tannins, flavonoids, steroids, and triterpenoids.

In vitro anticoagulant activity of *Zingiber officinale* crude leaf extract was assessed through clotting time using PT and aPTT based on the study of Taj et al. (2021) and HORIBA Medical Instructions for Use: Yumizen G PT Liq 4 Prothrombin Time reagent (HORIBA Medical, 2021) and HORIBA Medical Instructions for Use: Yumizen G APTT Liq 2 Activated Partial Thromboplastin Time reagent (HORIBA Medical, 2021).

For prothrombin time determination, 100 µL each of the crude leaf extract from *Zingiber officinale,* Heparin (positive control), and Normal Saline Solution (negative control) were added to 50 µL of platelet-poor plasma. The test tubes were incubated in a water bath at 37°C for five minutes. The PT reagent (rabbit brain thromboplastin, calcium chloride, and sodium azide preservative) was prewarmed for 10 minutes at 37°C using a water bath before being added to each treatment tube. One hundred microliters of PT reagent were added to the mixture (50 uL of plasma + 100 uL of experimental solution or positive control or negative control) and the stopwatch timer was started. Continuous gentle tilting with clot formation inspection was done. Clot formation was described as the presence of clumping at the bottom of the tube or loss of fluidity when shook at which point, the stopwatch timer was stopped, and clotting time was recorded by the assigned researcher.

For activated partial thromboplastin time determination, 50 µL of crude leaf extract from *Zingiber officinale,* Heparin (positive control), and Normal Saline Solution (negative control) were added to 50 µL of platelet-poor plasma. The test tubes

were incubated in a water bath at 37°C for five minutes. The aPTT reagent (phospholipid and ellagic acid contact activator) and calcium chloride reagent were prewarmed for two minutes at 37°C before being added to each treatment tube. Fifty microliters of aPTT reagent were then added to the mixture (50 uL of plasma + 50 uL of experimental solution or positive control or negative control) and incubated for three minutes. Upon addition of 50 uL of calcium chloride reagent to the mixture, the stopwatch timer was started. The mixture was mixed and placed in the water bath. The test tubes were then gently tilted and observed for clot formation every five seconds until clot formation was seen. Clot formation was described as the presence of clumping at the bottom of the tube or loss of fluidity of plasma when shook, at which point, the stopwatch timer was stopped, and clotting time was recorded by the assigned researcher.

A Repeated-Measures Analysis of Variance (ANOVA) was used to determine the significant difference in the mean clotting time of the human blood specimens, found on the PT and aPTT, among the treatment groups at .05 level of significance. If the *F* test result was significant, a Bonferroni post-hoc test for pairwise comparison was performed to determine the significant difference in the mean clotting time of the human blood specimen, based on the PT and aPTT, between each pair of treatment groups.

## III. RESULTS AND DISCUSSION

Phytochemical screening showed positive results for the presence of flavonoids, saponins, and tannins in *Zingiber officinale* (Ginger) leaves.

The in vitro anticoagulant activity of *Zingiber officinale* (Ginger) crude leaf extract on human blood is presented in the tables below.

**Table 1. Differences in Mean Clotting Time (sec.) of Human Whole Blood using PT among the Treatment Groups (*n* = 15)**

| Treatment Group | Mean and SD | Type III Sum of Squares | *df* | Mean Square | *F* | Conclusion |
|---|---|---|---|---|---|---|
| 250 mg/mL *Zingiber officinale* (Ginger) Crude Leaf Extract | 24.91 ± 4.68 | 24249.679 | 1.356 | 17889.548 | 721.861 | Significant |
| 200 IU/mL Heparin (Positive Control) | 67.66 ± 4.68 | | | | | |
| NSS (Negative Control) | 13.82 ± 0.66 | | | | | |

Table 1 shows the mean and standard deviation of PT among treatment groups. The human blood treated with 200 IU/mL of Heparin (Positive Control) had the longest time of 67.66 (SD=4.68) followed by the treatment group of ginger crude leaf extract, which was 24.91 seconds (SD=4.68). The mean PT of 13.82 seconds (SD= 0.66) of NSS means that the clotting time is within the normal clotting time range of 12-15 seconds and 25-35 seconds. The differences in PT clotting time were significant, $F(1.356, 18.977) = 721.861$, $p < .05$, $\eta^2 = 98.1\%$. This means that the PT reagent had different responses depending on the type of treatment group exposed to the blood. Heparin vs. Normal Saline Solution showed the highest significant

mean difference of 53.84 seconds (SE=1.25, 95% CI [50.46, 57.23]). Heparin, as a commercial anticoagulant drug, inhibits thrombin through antithrombin-heparin complex formation, causing the inactivation of the extrinsic coagulation pathway (Oduah et al., 2016). Based on the result, Heparin showed a longer mean PT clotting time as compared to ginger crude leaf extract. The ginger crude Leaf extract vs. NSS solution was significant for 11.09 seconds (SE = 1.18, 95% CI [7.89, 14.29]).

**Table 2**. **Differences in Mean Clotting Time (sec.) of Human Whole Blood using aPTT among the Treatment Groups (*n*=15)**

| Treatment Group | Mean and SD | Type III Sum of Squares | *df* | Mean Square | *F* | Conclusion |
|---|---|---|---|---|---|---|
| 250 mg/mL *Zingiber officinale* (Ginger) Crude Leaf Extract | 58.43 ± 8.50 | 44057.877 | 1.391 | 31674.254 | 510.060 | Significant |
| 200 IU/mL Heparin (Positive Control) | 108.59 ± 6.80 | | | | | |
| NSS (Negative Control) | 33.32 ± 1.42 | | | | | |

In Table 2, the 200 IU/mL of Heparin group has the longest mean clotting time of 108.59 seconds (SD=6.80). This was followed by ginger crude leaf extract mean aPTT of 58.43 (SD=8.50). With more than 13 seconds of aPTT human blood clotting time, this demonstrated above normal values and inhibits intrinsic coagulation pathway and clotting time. The difference between the aPTT clotting time is considered significant, $F(1.391, 19.474) = 510.060$, $\eta^2 = 97.3\%$. With differing times for the treatment groups, the aPTT reagent had responded differently when exposed to blood. The Bonferroni Post-Hoc Test showed that Heparin vs. NSS had the highest significant mean aPTT difference of 75.27 seconds (SE = 1.77, 95% CI [70.46, 80.08]). The prolonged aPTT clotting time for Heparin showed that the commercial anticoagulant drug has the capacity to inhibit intrinsic coagulation pathway while normal saline solution did not the capacity to inhibit the intrinsic coagulation pathway. The ginger crude leaf extract vs. NSS showed a significant mean difference of 25.11 seconds (SE = 2.16, 95% CI [19.23, 30.99]). The ginger crude leaf extract had a longer aPTT clotting time than the normal saline solution. This phenomenon is due to the anticoagulant phytochemical properties of ginger leaf extract. It has the capability to inhibit the intrinsic coagulation pathway as compared to the NSS which does not have the capacity to inhibit the intrinsic coagulation pathway (El-Hameid et al., 2020).

## IV. CONCLUSION

Based on the results, the mean PT and aPTT clotting times showed ginger leaf extract (250 mg/mL) was able to prolong PT and aPTT above normal upper limit values indicating the presence of anticoagulant activity. Ginger leaf extract (250 mg/mL) has in vitro anticoagulant activity on human whole blood.

For future studies, recommendations would include the use of the soxhlation method and a higher concentration of ethanol for a higher yield of desired phytochemicals in ginger plant extraction. Conducting *in vivo* assays is also recommended for further assessment of its anticoagulant potential.

## References


Akbari, A., Nasiri, K., & Heydari, M. (2020). Ginger (*Zingiber officinale* Roscoe) extract can improve the levels of some trace elements and total homocysteine and prevent oxidative damage induced by ethanol in rat eye. *Avicenna Journal of Phytomedicine*, *10*(4), 365. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7430965/pdf/AJP-10-365.pdf

El-Hameid, A., Asmaa, R., Abo El-kheir, Z. A., Abdel-Hady, M. S., & Helmy, W. A. (2020). Identification of DNA variation in callus derived from Zingiber Officinale and anticoagulation activities of Ginger Rhizome and callus. *Bulletin of the National Research Centre*, *44*(1), 1-8. https://doi.org/10.1186/s42269-020-0281-9

Fu, X., Wang, J., Jiang, S., Wu, J., Mu, Z., Tang, Y., Wang, S., Fu, H., Ma, H., & Zhao, Y. (2023). Mortality trend analysis of ischemic heart disease in China between 2010-2019: A Joinpoint analysis. *BMC Public Health,* (23), 644. https://doi.org/10.1186/s12889-023-15549-3

HORIBA Medical. (2021, January 26). *Instructions for Use: Yumizen G APTT Liq 2 Activated Partial Thromboplastin Time Reagent*. https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&cad=rja&uact=8&ved=2ahUKEwicxKbc2JODAxXarVYBHZCbCHQQFnoECAkQAQ&url=https%3A%2F%2Ftoolkits.horiba-abx.com%2Fdocumentation%2Fdownload.php%3Fid%3D349540&usg=AOvVaw0ivAylcbfyG1iqLkyZgldW&opi=89978449

HORIBA Medical. (2021, January 27). *Instructions for Use: Yumizen G PT Liq 4 Prothrombin Time Reagent*. https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&ved=2ahUKEwicxKbc2JODAxXarVYBHZCbCHQQFnoECAoQAQ&url=https%3A%2F%2Ftoolkits.horiba-abx.com%2Fdocumentation%2Fdownload.php%3Fid%3D341460&usg=AOvVaw0ASE9o-phH8-EW4HlBRpH2&opi=89978449

Ilkhanizadeh, B., Shirpoor, A., Ansari, M.H.K., Nemati, S., & Rasmi, Y. (2016). Protective effects of ginger (Zingiber officinale) extract against diabetes-induced heart abnormality in rats. *Diabetes & Metabolism Journal,* *40*(1), 46-53. https://doi.org/10.4093/dmj.2016.40.1.46

Patriarcheas, V., Pikoulas, A., Kostis, M., Charpidou, A., & Evangelos, D. (2020). Heparin-induced thrombocytopenia: Pathophysiology, diagnosis and management. *Cureus, 12*(3). https://doi.org/10.7759/cureus.7385

Shadrack, K., Faraj, A., Alex, M. K., & Kenneth, N. (2019). Anti-thrombotic effect of *Zingiber officinale* (Ginger) in Sprague Dawley rats. *International Journal of Research in Medical Sciences*, *7*(9), 3239–3245. https://doi.org/10.18203/2320-6012.ijrms20193905

Shukar, S., Zahoor, F., Hayat, K., Saeed, A., Gillani, A.H., Omer, S., Hu, S., Babar, Z.U.D., Fang, Y., & Tang, C. (2021). Drug shortage: Causes, impact and mitigation strategies. *Frontiers in Pharmacology,* *21*. https://doi.org/10.3389/fphar.2021.693426

Taj, S., Kashif, A., Fatima, S. A., Imran, S., Lone, A. & Ahmed, Q. (2021). Role of hematological parameters in the stratification of COVID-19 disease severity. *Annals of Medicine & Surgery,* 62, 68-72. Elsevier. https://doi.org/10.1016/j.amsu.2020.12.035

Tzeng, T.-F., Liou, S.-S., Tzeng, Y.-C., & Liu, I-M. (2016). Zerumbone, a phytochemical of subtropical ginger, protects against hyperglycemia-induced retinal damage in experimental diabetic rats. *Nutrients*, *8*(8), 449. https://doi.org/10.3390/nu8080449

World Health Organization. (2012, January 1). *Blood donor selection: Guidelines on assessing donor suitability for blood donation.* https://www.who.int/publications/i/item/9789241548519

World Health Organization. (2021, June 11). *Cardiovascular diseases (CVDs)* https://www.who.int/en/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)

Yee, J. (2020, December 13). Heart Disease in PH worst in Southeast Asia-WHO. *Inquirer.net*. https://newsinfo.inquirer.net/1371108/heart-disease-in-ph-worst-in-sea