

## Effects of *Pandanus amaryllifolius* Roxb. ethanolic leaf extract on blood coagulation and platelet count using Wistar albino rat as a model

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**Abstract** - Some plants exhibit anticoagulation properties but have not been fully developed. They are used in traditional medicine for they are important sources of many biologically active compounds. As *Pandanus amaryllifolius* Roxb. (Pandan) had been utilized in traditional medical practice. Phytochemical screening of the ethanolic leaf extract revealed the presence of several biochemical constituents such as sterols, triterpenes, flavonoids, alkaloids, saponins, glycosides and tannins. Thus, this study was therefore designed to scientifically investigate the possible effects of the said plant in blood coagulation and platelet activity through assessing the hematological indices namely platelet count, bleeding time, clotting time, prothrombin time, and activated partial thromboplastin time using Wistar albino rat as a model. The results of the present study indicated that there were significant increases ( $p < 0.05$ ) in the clotting time and APTT level of the rats treated with 400 mg/kg and 800 mg/kg concentrations and 200 mg/kg, 400 mg/kg and 800 mg/kg concentrations, respectively, across each group implicating that there was a significant difference observed across each group at  $p$ -value  $< 0.05$  as also observed on the post hoc analysis conducted using Tukey method. On the other hand, clotting time of 200 mg/kg treated groups were found out to be not significantly affected when multiple comparisons on the effects of extract per group were analyzed. Whereas, the platelet count at all doses 200, 400, and 800 mg/kg did not show significant difference ( $p < 0.05$ ) when compared to normal control after the 10<sup>th</sup> day of administration of the plant extract indicating that the effect is just the same. Bleeding time and prothrombin time that were prolonged in all concentrations were found out to be not statistically significant with the control ( $p > 0.05$ ). In conclusion, *Pandanus amaryllifolius* ethanolic leaf extract exhibits anticoagulant property as evidenced in the significant prolongation of

clotting time and activated partial thromboplastin time at 400 mg/ kg and 800 mg/kg concentrations.

**Keywords:** *Pandanus amaryllifolius*, anticoagulation property, bleeding time, prothrombin time, activated partial thromboplastin time, clotting time

## INTRODUCTION

Blood has been considered sacred since ancient times (Tanaka, Key & Levy, 2009). As described by Sheng (2003), as cited by Nebedum, Udeafor & Okeke (2010), blood is said to be the “mother of energy” for it provides the fundamental building materials and fluid constituents that are required to nourish life. The roles of blood are many and varied. It is responsible for delivering material nourishments to different organs and provides the necessary moisture they require to function properly (Nebedum, Udeafor & Okeke, 2010).

Hemostasis refers to the normal response of the vessel to injury or damage by forming a clot that serves to limit hemorrhage or stoppage of blood loss (Azikiwe, Siminialayi, Brambaifa, Amazu, Enye & Ezeani, 2007; Rasche, 2001). This cessation of blood loss from a damaged vessel is termed as blood coagulation and is considered to be a vital component of the host defense mechanism. Hemostasis is regulated by three basic mechanisms specifically the vascular wall, platelets, and the coagulation cascade (Riddel, Aouizerat, Miaskowski & Lillicrap, 2007). Damage on blood vessels causes vasoconstriction and thrombin activation which are accompanied by adhesion and platelet activation. Secondary hemostasis involves activation of coagulation mechanism (Azikiwe et al., 2007 as cited by Tanko, Eze, Jimoh, Yusuf, Mohammed, Balarabe, et al., 2012).

When hemostasis systems are out of balance, thrombosis and hemorrhage occur (Loscalzo, 2003 as cited by Riddel, Aouizerat, Miaskowski & Lillicrap, 2007). In some abnormalities like thrombosis, atherosclerosis, inflammation and metastasis, activation of enzymes in the coagulation cascade and the platelets may also take place (Gou, Ho, Rowlands, Chung & Chan, 2003). Thrombus formation has an important role in the pathogenesis and progression of ischemic heart and brain diseases (Rajput, Khan, Qaz & Feroz, 2012). Thromboembolic disorders are the main causes of morbidity and mortality in developed countries (Kumar, Joseph, George & Sharma, 2011). It is estimated that approximately 17.1 million people died from cardiovascular and cerebrovascular disease (CCVD) in 2011 and the number of deaths would dramatically increase to almost 23.6 million by 2030. As a result, it is significant to research and develop

antithrombotic medicines (Zhang, Xu, Y., Jia, Xu, L., Yin & Peng, 2013).

Anticoagulants play a pivotal role as agents for the prevention and treatment of thromboembolic disorders. For more than five decades, anticoagulant drugs consisting of heparins, vitamin K antagonists, and their derivatives have been the major players in the clinical setting. Although their efficacy remains undisputed, the deleterious life-threatening side effects of these drugs have also been well documented. Plants may serve as the alternative sources for the development of new anticoagulant agents due to their biological activities (Kumar, Joseph, George & Sharma, 2011).

Herbal remedies for diseases had been considered the oldest form of treatment modality known to mankind. The use of such herbal treatment had been passed on from one generation to the next (Adedapo et al., 2005 as cited by Abubakar, 2009). Even in developing countries of the world, most people depend on herbal medical care (Nwinuka, Monanu & Nwiloh, 2008).

For thousands of years, mankind had been using medicinal plant sources to alleviate or treat various diseases, illnesses and conditions (Gupta, Narniwal & Kothari, 2012; Razali et al., 2008 as cited in Rohman, Riyanto, Yuniarti, Saputra, Utami & Mulatsih, 2010). Plants are sources of novel chemical constituents which are of potential use in medicine and other applications (Gupta, Narniwal & Kothari, 2012). These medicinal plants are vital sources of life-saving drugs for the majority of the world's population (Tripathi and Tripathi, 2003 as cited by Odesanmi, Lawal & Ojokuku, 2009). Therefore, the use of medicinal plants should be investigated further to better understand their properties, safety and efficacy (Rajeh, Zuraini, Sasidharan, Latha & Amutha, 2010). One such herbal plant is *Pandanus amaryllifolius*.

*Pandanus amaryllifolius*, also known as pandan, which belongs to the *Pandanaceae* family, is known for its aroma. The plants grow in clumps and have thin wide 4.5 centimeters, length of 40-80



Figure 1. *Pandanus amaryllifolius* (Pandan)

centimeters and also sharply at the edge like sword leaves. In Java, the plants can be found at home yard because of aromatic odor and generally used for fragrant and food colorants (Wardah, 2012). *Pandanus* leaves are used for cooking and to flavor the meat and vegetable products or can be blended with other flavor enhancing sauces (Rayaguru & Routray, 2011). It is

usually cultivated in South East Asia, particularly in India, Thailand, Malaysia and Indonesia (Asmain, 2010).

Besides its aromatic value, *P. amaryllifolius* has a long history of ethnopharmacological uses in alleviating minor ailments such as fever, headache, sore throat and toothache (Chong, Yeap, Rahmat, Akim, Alitheen, & Othman, et al., 2012). Ethnomedical information reveals that the oil of *Pandanus* leaves is used extensively for the treatment of epilepsy and leprosy. This oil is described as purgative, stimulant and brings antispasmodic relief for rheumatism conditions. In Taiwan, *P. amaryllifolius* is used by local communities for its diuretic, cardiostimulant and antioxidant properties (Takayama et al., 2001 as cited by Chong et al., 2012; Yan & Asmah, 2010; Zhang, Guo, Sun, Chen, Yang, Fu, Wu, et al., 2012) and as remedy for thyroid problems and fever (Jong et al., 1998 as cited by Chong et al., 2012). Previously, methanol extracts of *P. amaryllifolius* revealed strong inhibitory rate toward tumor promoter 12-O hexadecanoylphorbol-13-acetate (HPA) induced Epstein- Barr virus (EBV) activation in Raji cells (Murakami et al., 2000 as cited by Chong et al., 2012). Lately, *P. amaryllifolius* extract was found to display selective antiproliferative activity against non-hormone dependent breast cancer cells (Zan et al., 2011 as cited by Chong et al., 2012) and had been proven to have anti-diabetic activity (Sasidharan, Sumathi, Jegathambigai & Latha, 2011). Pandan leaves also contain alkaloids, saponins, flavonoids, tannins, polyphenols, and dye.

Pandamarilactonine-A, pandamari-lactonine-B, norpandamarilactonine-A, norpandamarilactonine-B, pandamarilactone1 and pandanamine are the alkaloids present. In continuing the search of *P. amaryllifolius* alkaloids, new pyrrolidine alkaloids were also found which includes pandamarilactonine-C and pandamarilactonine-D (Asmain, 2010; Lopez & Nonato, 2005).

Phytochemical screening reveals the presence of flavonoids in pandan leaves. Flavonoids, a group of low molecular weight polyphenolic substances including flavones, flavonols, isoflavones, flavonols, flavanones, anthocyanins and proanthocyanins, widely exists in fruits, vegetables and beverages. High content of flavonoids from herbal extracts has been popularly used for the therapy of CCVD (Scalbert & Williamson, 2000 as cited by Zhang et al., 2013).

In light of the above-mentioned statements, current coagulation study had been focusing on the use of biologically active phytochemicals in pandan. The researchers had evaluated the effect of *Pandanus amaryllifolius* ethanolic leaf extract focusing on objective, so as to correlate findings through scientific investigation of the coagulation mechanism of pandan leaves by measuring the following parameters: platelet count, bleeding time, clotting time, activated partial

thromboplastin time, and prothrombin time using normal Wistar albino rats as model.

## **MATERIALS AND METHODS**

### **Plant Materials**

Fresh mature leaves of *P. amaryllifolius*, measuring 40-80 centimeters (Lopez & Nonato, 2005; Wardah, 2012) were collected from Mt. Banoy, Brgy. Talumpok East, Batangas City during the month of July 2013. The taxonomic classification of the plant was performed by Dr. Wilfredo F. Vendivil, Curator II of the Botany Division, National Museum, Manila.

### **Sample Preparation and Extraction**

Fresh leaves of *P. amaryllifolius* were plucked (Rayaguru & Routray, 2011), thoroughly rinsed with tap water (Chong et al., 2012), wiped with a cloth and sliced into small portions (Rayaguru & Routray, 2011). Then, the samples were dried under shade at room temperature for 14 days (Chong et al.,) to achieve extract with good quality (Enechi, Oluka, Ugwu & Zomeh, 2013; Rayaguru & Routray, 2011). The plant samples were later grinded into fine powder using a clean and dry electrical grinder and stored in a clean labeled airtight container until use (Igbinsosa, O., Igbinsosa, E., & Aiyegiro, 2009). One hundred grams (100 g) of dry powder was extracted with 1000 ml ethanol solvent (70%) by maceration at room temperature for 48 hours (Mohammedi, 2011). It was then filtered with Whatman No. 1 filter paper to separate the filtrate from the residue. The filtrate was then concentrated using a rotary vacuum evaporator to obtain the solid mass. The solid extract was then re-dissolved in normal saline and stored in capped bottles in a refrigerator at 4°C until required (Bamidele, Akinnuga, Anyakudo, Ojo, O., Ojo, G., Olorundemi, et al., 2010).

### **Phytochemical Analysis**

The qualitative determination of phytochemical constituents of *P. amaryllifolius* was determined at the Industrial Technology Development Institute of the Department of Science and Technology, Bicutan, Taguig, Metro Manila.

### **Test Animals**

Sixteen (16) (Tanko, et al., 2012) healthy male Wistar strain albino rats weighing between 240 and 270 g (Dougnon, Klotoe, Segbo, Ategbbo, Edorh, Gbaguidi, et al., 2012) were obtained from Department of Biology, University of the Philippines, Diliman, Quezon City. They

were placed in different cages for proper identification under standard conditions (Bamidele et al., 2010) at the animal experimentation room at constant temperature of  $22 \pm 1^\circ\text{C}$  with a 12 h light and 12 h in the dark. Prior to experiment proper, all animals were allowed twenty-one (21) days to acclimatize to their environment during which period, they were allowed for free access with standard pellet diet and water ad libitum (Dougnon et al., 2012).

### **Experimental Design**

The rats were randomly divided into four (4) groups consisting of four animals each (Tanko et al., 2012). The baseline hematological parameters were performed first prior to administration of distilled water and/or ethanolic leaf extracts of pandan to the experimental rats.

Group I : The rats in this group served as control. They were administered with 1 ml of distilled water.

Group II : The rats in this group were administered with water, standard feed, and 200 mg/kg of the extract.

Group III : The rats in this group were administered with water, standard feed, and 400 mg/kg of the extract.

Group IV : The rats in this group were administered with water, standard feed, and 800 mg/kg of the extract

Entire administration was carried out orally by gavage feeding for a period of ten (10) days (Tanko et al., 2012).

### **Sample Collection**

Rats were anesthetized for a short period of time using a 300  $\mu\text{l}$  isoflurane placed in cotton inside a jar (Hoff, 2000). Afterwards, venous blood was collected via retro-orbital method. A microhematocrit capillary tube was inserted into the retro-bulbar plexus of the medial canthus of the eye until the bony orbit is contacted, then withdrawn slightly to allow the blood to flow through the tube and into a collection vessel (Ike, Nubila, Ukaejiofo, Nubila, Shu, & Okpalaji 2010; Moore, 2000). Then 0.5 ml of venous blood was collected into a sodium citrate anticoagulant microtainer tube and mixed by gentle inversion for PT and APTT testing (Ike et al., 2010) while 0.2 mL of blood was transferred to EDTA tubes for platelet count determination (Apostol, Gan, Raynes, Sabado, Carigma, Santiago, et al., 2012).

### **Platelet Count Determination**

Pentra 60 Analyzer was used for the test. Thirty (30)  $\mu\text{L}$  of the sampling volume was delivered in to the machine through one button aspiration needle (Horiba ABX).

### **Prothrombin Time Determination (PT)**

DiagnosticaStagoSTart 4 Hemostasis Analyzer was used to perform the prothrombin time test. Blood was immediately transferred into sample vials containing 3.2% sodium citrate in the proportion of 1:9 anticoagulant to blood. The freshly collected samples were gently mixed and centrifuged at 2500 g at 20-22°C for 10 minutes to obtain platelet poor plasma. Cuvettes were placed into A, B, C and D test rows on the coagulometer in accordance to the protocol of the semi-automatic STArt4 coagulometer from DiagnosticaStago. One metal ball was placed into each cuvette and allowed to warm for at least 3 minutes prior to use. Then, 100 µl of the test plasma was added using plastic disposable pipettes and incubated for 120 seconds. The timer was started for each test rows by pressing the timer button. Ten seconds before time is up, the timer starts beeping. Immediately, cuvettes were transferred to PIP row and PIP button position 4 was pressed to activate pipettor. Coagulation time was recorded after the addition of 100 µl of the Neoplastine reagent. Precaution is taken to do the test within 1 h of blood collection given that the labile factor deteriorates rapidly at room temperature (DiagnosticaStago).

### **Activated Partial Thromboplastin Time Determination (APTT)**

DiagnosticaStagoSTart 4 Hemostasis Analyzer was used to perform the activated partial thromboplastin time test. Blood was immediately transferred into sample vials containing 3.2% sodium citrate in the proportion of 1:9 anticoagulant to blood. The freshly collected samples were gently mixed and centrifuged at 2500 g at 20-22°C for 10 minutes to obtain platelet poor plasma. Cuvettes were placed into A, B, C and D test rows on the coagulometer in accordance to the protocol of the semi-automatic STArt4 coagulometer from DiagnosticaStago. One metal ball was placed into each cuvette and allowed to warm for at least 3 minutes prior to use. Then, 50 µl of the test plasma and 50 µl of PTT-A was added using plastic disposable pipettes in cuvettes and incubated for 180 seconds. The timer was started for each test rows by pressing the timer button. Ten seconds before time is up, the timer starts beeping. Immediately, cuvettes were transferred to PIP row and PIP button position 2 was pressed to activate pipettor. Coagulation time was recorded after the addition of CaCl<sub>2</sub> coagulation activation reagent (DiagnosticaStago).

### **Clotting Time Determination**

Slide or Drop method was used to assess the clotting time. The collected drop of blood was placed in a clean glass slide and rubbed with a lancet until fibrin threads were observed. The time from initial

contact with lancet and the development of threads were recorded. The procedure has been repeated during each collection of blood (Apostol et. al., 2012).

### Bleeding Time Determination

Bleeding time was evaluated using Duke's method with some modifications. Three skin punctures were made on the forearm of the animals using a lancet and a stopwatch was started as soon as bleeding started. The puncture was blotted on a filter paper every 15 seconds until the paper no longer stained red with blood. The time from first application until the disappearance of blood was noted. The average was recorded as bleeding time. The procedure was repeated during every blood collection (Weremfo, Adinortey & Pappoe, 2011).

### Statistical Analysis

All the data were presented at a 0.05 level of significance employing One-Way Analysis of Variance (ANOVA) with Tukey's HSD method as a post-hoc procedure to determine if there exist significant difference among the control group and *P. amaryllifolius* treated groups (Apostol et al., 2012).

## RESULTS AND DISCUSSION

### Phytochemical Analysis

**Table 1**  
**Phytochemical composition of the ethanolic leaf extract of**  
***Pandanus amaryllifolius* Roxb.**

Constituents	Relative Abundance
Sterols	+
Triterpenes	+
Flavonoids	+
Alkaloids	+
Saponins	+
Glycosides	+
Tannins	+

**Note:** (+) Presence of constituent

(-) Absence of constituents

Table 1 presents the qualitative determination of the phytochemical constituents of *P. amaryllifolius*. Phytochemical analysis of ethanolic leaf extract of *P. amaryllifolius* showed the presence of sterols, triterpenes, flavonoids, alkaloids, saponins, glycosides and tannins. These findings were further supported by the phytochemical analysis made by Dumaoal, Alaras, Dahilan, Depadua, and Pulmones (2010) revealing that similar bioactive compounds were also present.



**Hematological Examination**

Table 2 demonstrates the baseline values of the hematological indices being tested prior to the administration of the *P. amaryllifolius* ethanolic leaf extract together with the hematological changes to varying concentration of pandan ethanolic extract in Wistar albino rat after 10 days of induction of the treatment.

**Table 2**  
**Pre-test and Posttest results on platelet count, bleeding time, clotting time prothrombin time, and activated partial thromboplastin time in rats**

Groups	Platelet Count (x 10 <sup>9</sup> /L)		Bleeding Time (mins)		Clotting Time (mins)		Prothrombin Time (sec)		Activated Partial Thromboplastin Time (sec)	
	Pretest	Posttest	Pretest	Posttest	Pretest	Posttest	Pretest	Posttest	Pretest	Posttest
<b>Control Group</b> (Group I / Distilled Water)	617.705	620.750	2.075	2.085	1.150	0.952	16.725	16.675	13.900	13.900
<b>Treated Group</b> (Group II / 200 mg/kg)	670.000	557.500	2.405	2.907	1.205	1.352	16.800	16.800	13.925	14.025
<b>Treated Group</b> (Group III / 400 mg/kg)	683.250	511.500	2.408	3.080	1.225	2.060	16.525	16.875	13.600	14.250
<b>Treated Group</b> (Group IV / 800 mg/kg)	696.500	495.250	1.658	3.230	1.245	2.315	15.900	17.000	15.000	15.850

The table presents the baseline value for the platelet level of the control group which was 617. 705 x 10<sup>9</sup>/L. It then increased to 620.750 x 10<sup>9</sup>/L after the treatment. Groups II, III, and IV obtained pre-test results of 670.000 x 10<sup>9</sup>/L, 683.2500 x 10<sup>9</sup>/L, and 696.500 x 10<sup>9</sup>/L, respectively. On the other hand, the platelet levels for groups II, III, and IV had reduced to 557.500 x 10<sup>9</sup>/L, 511.500 x 10<sup>9</sup>/L, and 495.250 x 10<sup>9</sup>/L, respectively after the 10<sup>th</sup> day of extract administration. Based on the values presented, it was observed that as the concentration increases, greater reduction in the platelet count was seen. It is noticeable that the values of platelets at all groups decreased. There is the probability that the decrease maybe dose dependent as demonstrated on the values obtained in the increasing concentration of the extract. *P. amaryllifolius* reduced platelet count most probably because of its phytochemical component, saponins which was proven to have antiplatelet effect as demonstrated with the previous study conducted by Klafke, da Silva, Rossato, Trevisan, Walker, Leal, et al. (2012). However, when comparing the statistically evaluated platelet count values of extract treated groups at all doses to those group that

received distilled water, it was found out to be statistically not significant. This observation of antithrombotic property may be due to some effects of biological variables in test animals. The degree of stress to animals might probably affect the blood constituents. Sampling of blood might produce effects on the results obtained as the animals are being exposed to discomfort associated technique (Asika, Idonije, Okhai, & Iribhogbe, 2011).

To further assess the possible effect of *P. amaryllifolius* on platelet activity, the bleeding time on rats was measured. The mean bleeding time in the control group before the experiment proper was 2.075 minutes while those of groups II, III, and IV were 2.405 minutes, 2.408 minutes, and 1.658 minutes, respectively. It was prolonged in the experimental group after the treatment which increased to 2.085 minutes, 2.907 minutes, and 3.080 minutes in groups II, III, and IV, respectively. It was observed that the plant extract increased the time it took for the rats to stop bleeding when compared with the values obtained prior the experiment proper. The rate of increment was dose dependent because the higher the concentration the longer the bleeding time it took. Since bleeding time is an index used to indicate the level of circulating platelet in the blood, it can be implied that the reduction in platelets decrease the tendency for blood to coagulate. It may also be attributed to the vasoconstrictive effect of the blood vessel, formation of hemostatic plug, and platelet activity; thus, resulted to prolongation in bleeding time (Asika, Idonije, Okhai, & Iribhogbe (2011); Hadi, Elderbi, & Mohamed (2010); Kamal, Tefferi, & Pruthi (2007)). However, the extended bleeding time after statistical evaluation was found to be not significant indicating that the effects are the same across the control and experimental groups.

Table 2 also shows the baseline value of the clotting time during the pre-test. It was found out that the level of clotting time for the control group was 1.150 minutes and decreased to 0.952 minutes after the treatment. Group II with baseline value of 1.205 minutes had increased to 1.352 minutes. On the other hand, group III had a pre-test value of 1.225 minutes had an increased to 2.060 minutes after the treatment. Group IV has a baseline value of 1.245 minutes and was prolonged after the conduction of experiment to 2.315 minutes. Clotting time was prolonged with 200 mg/kg, 400 mg/kg and 800 mg/kg of the extract. According to Asika, Idonije, Okhai, & Iribhogbe (2011) and Dapper, Achinike & Gwotmut (2007), clotting time is an index used to evaluate the prothrombin activator system or thromboplastin level in the blood. It is also used as a qualitative measurement of factors involved in the intrinsic pathway. The prolonged clotting time in this study could be possibly attributed to the

decreased level of circulating thromboplastin, rate of thrombin formation or deficiency in one or more of the clotting factors; thus, producing an increase effect. The result was proven to be significant at p value <0.05 at 400 mg/kg and 800mg/kg concentration (Table 3). This result correlates with the findings of Dapper, Achinike & Gwotmut (2007), who reported an increase in clotting time by gel-like liquid of aloe vera in albino Wistar rats.

The mean prothrombin time in the pretest results in rats of Groups I, II, III, and IV were 16.725 seconds, 16.800 seconds, 16.525 seconds, and 15.900 seconds, respectively. It was noted that the prothrombin time in the control group slightly decreased to 16.675 seconds. On the other hand, groups II, III, and IV showed prolonged prothrombin time which resulted to 16.800 seconds, 16.875 seconds, and 17.000 seconds, respectively. Hadi, Elderbi, & Mohamed (2010), stated that prothrombin time is an index used to measure the overall efficiency of the extrinsic system. When prothrombin time was determined, the values increased as the concentration of the extract increases. This may be caused by the possible alteration in the blood coagulation factors which could be indicated by a decreased level of one or more of the factors involved in the extrinsic pathway. However, the increase in prothrombin time level was found out to be statistically not significant. The reported insignificant increase in prothrombin time of this study contrasted with the observed effect of Gum Arabic on coagulation system of albino of Hadi, Elderbi, & Mohamed, 2010 which showed a significant increase in prothrombin time.

The mean activated partial thromboplastin time (APTT) results from the pre-test of group I, II, III, and IV were 13.900 seconds, 13.925 seconds, 13.600 seconds, and 15.000 seconds, respectively. As shown in Table 2, the APTT level of the control group (I) had remained to 13.900 seconds. In the *P. amaryllifolius* treated groups, APTT levels increased to 14.025 seconds, 14.250 seconds, and 15.850 seconds at doses of 200 mg/kg, 400 mg/kg and 800mg/kg, respectively. Presently, an increase in APTT as result of treatment with high dose of pandan extract was dose dependent and appears parallel to the effect of clotting time. Thus, it led to possible inhibition in the factors involved in the intrinsic coagulation system. In this context, the dissociation of the coagulation system is possible. APTT of the animals treated with 800 mg/kg ethanolic extract was found out to be significantly prolonged than other treatment groups were no significant difference was observed. The reported prolongation of APTT agrees with the observed findings of Ike, et al. (2010) on the effect of crude neem leaf-acetone-water extract on APTT level of Wistar rats.

**Table 3**  
**Comparison on the effect of *P.amaryllifolius***  
**ethanolic leaf extract on hematological parameters**

Hematological Parameter	<i>p</i> -value	Interpretation
Platelet Count	0.964	Not significant
Bleeding Time	0.167	Not significant
Clotting Time	0.011	Significant
Prothrombin Time	0.773	Not significant
Activated Partial Thromboplastin Time	0.001	Significant

\*Significant at  $p$ -value  $< 0.05$ ; Tukey method

Table 3 shows the statistical analysis of the hematological indices when treated according to group. Statistical evaluation revealed that only clotting time and APTT results were significant, with  $p$ -values of 0.011 and 0.001, correspondingly. This means that there is a significant difference observed across each group at  $p$ -value  $< 0.05$ . This was also observed on the post hoc analysis conducted using Tukey method. Significant prolongation in the clotting time and APTT proves that *P. amaryllifolius* ethanolic leaf extract has anticoagulant effect. This is may be due to the alteration in the intrinsic pathway of the coagulation cascade. Similarly, Asika, Idonije, Okhai & Iribhogbe (2011) also observed increased clotting time in one of their studies. They reported that the seeds extract of *Garcinia combogia* has significant ability to decrease the level of circulating thromboplastin in the blood. Consequently, *G. combogia* seed extract shows the same biologically active phytochemical components present in *P. amaryllifolius* ethanolic leaf extract. However, parameters such as platelet count, bleeding time and prothrombin time have  $p$ -values greater than 0.05, indicating that they do not show significant difference and signify that the effects are the same.

Table 4 shows the comparison of the concentration of the *P. amaryllifolius* ethanolic leaf extract between each treatment. For platelet count, it is observed that extract of 200 mg/kg when compared to 400 mg/kg and 800 mg/kg concentrations have  $p$ -values of 0.858 and 0.715, respectively.

On the other hand, when 400 mg/kg of extract is compared with 200 mg/kg and 800 mg/kg gives the  $p$ -values of 0.858 and 0.992, accordingly. Furthermore, when 800 mg/kg concentration is compared against 200 mg/kg and 400mg/kg extract results to the  $p$ -values of 0.715 and 0.992, correspondingly. Thus, the analysis shows that there is no significant difference ( $p$ -value  $>0.05$ ) among the increasing

concentrations of the ethanolic leaf extract and infers that they produce same effect in the treatment.

**Table 4**  
**Comparison of the concentration of the *P. amaryllifolius* ethanolic leaf extract between each treatment**

	Concentration		p-value	Interpretation
Platelet Count	200 mg/kg	400 mg/ kg	0.858	Not significant
		800 mg/ kg	0.715	Not significant
	400 mg/kg	200 mg/kg	0.858	Not significant
		800 mg/ kg	0.992	Not significant
Bleeding Time	800 mg/kg	200 mg/kg	0.715	Not significant
		400 mg/ kg	0.992	Not significant
	200 mg/kg	400 mg/ kg	0.072	Not significant
		800 mg/ kg	0.131	Not significant
Clotting Time	400 mg/kg	200 mg/kg	0.864	Not significant
		800 mg/ kg	0.983	Not significant
	800 mg/kg	200 mg/kg	0.975	Not significant
		400 mg/ kg	0.983	Not significant
Prothrombin Time	200 mg/kg	400 mg/ kg	0.380	Not significant
		800 mg/ kg	0.136	Not significant
	400 mg/kg	200 mg/kg	0.380	Not significant
		800 mg/ kg	0.015	Significant
Activated Partial Thromboplastin Time	800 mg/kg	200 mg/kg	0.136	Not significant
		400 mg/ kg	0.015	Significant
	200 mg/kg	400 mg/ kg	0.994	Not significant
		800 mg/ kg	0.750	Not significant
Activated Partial Thromboplastin Time	400 mg/kg	200 mg/kg	0.994	Not significant
		800 mg/ kg	0.879	Not significant
	800 mg/kg	200 mg/kg	0.750	Not significant
		400 mg/ kg	0.879	Not significant
Activated Partial Thromboplastin Time	200 mg/kg	400 mg/ kg	0.999	Not significant
		800 mg/ kg	0.007	Significant
	400 mg/kg	200 mg/kg	0.999	Not significant
		800 mg/ kg	0.005	Significant
800 mg/kg	200 mg/kg	0.007	Significant	
	400 mg/ kg	0.005	Significant	

Comparing the 200 mg/kg extract with 400 mg/kg and 800 mg/kg on the bleeding time test, it reveals to have p-values of 0.072 and 0.131, respectively. On the same scenario, it is observed that when 400 mg/kg is compared against 200 mg/kg and 800 mg/ kg of the extract gives the p-values of 0.864 and 0.983, correspondingly. Moreover, 800 mg/kg when compared to 200 mg/kg and 400 mg/kg have p-values of 0.975 and 0.983, respectively. Hence, it demonstrates that there was no significant difference (p-value <0.05) among the three

concentrations and shows that they give same effect on the bleeding time test.

On the clotting time test, when 200 mg/kg is compared with 400 mg/kg and 800 mg/kg results to the p-values of 0.380 and 0.136, correspondingly. When 400 mg/kg is compared against 200 mg/kg and 800 mg/kg, it gives the p-values of 0.015 and 0.136. Furthermore, 800 mg/kg compared to 200 mg/kg and 400 mg/kg have p-values of 0.136 and 0.015, respectively. 400 mg/kg and 800 mg/kg concentrations shows to have significant difference (p-value <0.05) on the effect when computed statistically. Therefore, it can be noted that *P. amaryllifolius* ethanolic leaf extract exhibited an anticoagulant effect (as evidenced by prolonged clotting time discussed in Table 2) at doses of 200 mg/kg and 800 mg/kg. This result agrees with the reported findings of increase in clotting time by gel-like liquid of aloe vera in albino Wistar rats conducted in the study of Dapper, Achinike & Gwotmut (2007).

For the prothrombin time determination, it shows that when 200 mg/kg is compared to 400 mg/kg and 800 mg/kg have p-values of 0.994 and 0.750, respectively, which infers no significant difference at p-value <0.05 level. The same effect is observed when comparing 400 mg/kg against 200 mg/kg and 800 mg/kg that are found to have p-values of 0.994 and 0.879, correspondingly. This is also similar when 800 mg/kg was compared with 200 mg/kg and 400 mg/kg.

Comparing 200 mg/kg against 400 mg/kg and 800 mg/kg on the activated partial thromboplastin time determination, gives the p-values of 0.999 and 0.007, respectively. On the other hand, 400 mg/kg when compared to 200 mg/kg and 800 mg/kg have p-values of 0.999 and 0.005, accordingly. Moreover, when 800 mg/kg concentration is compared against 200 mg/kg and 400mg/kg extract results to the p-values of 0.007 and 0.005, respectively. Statistically, it was found out that there is significant difference (p-value <0.05) at concentrations of 200 mg/kg, 400mg/kg and 800 mg/kg when compared to each other. Thus, it can be inferred that at minimum dosage of 200 mg/kg of the extract already produces beneficial effect. All the concentrations generate the same effect in the treatment. Eventually, this indicates that the low and high doses of the *P. amaryllifolius* ethanolic leaf extract have the similar effects in prolonging the APTT (signifies its anticoagulant activity as discussed in Table 2). This findings is correlated to the reports of Thomas, Ernest, Nkoyo, Elvis, Chukwubuzor, Ayodele et al. (2013) in their investigation on the effects of *Telfaira occidentalis* on blood coagulation in albino rats. In their study, APTT showed a statistically significant increase in groups treated with the extract. This could be attributed to the inhibitory effect of the coagulation proteins by the extract, which invariably is most

likely to prolong coagulation, more especially the intrinsic coagulation pathway. In addition, the most probable reason could be due to the phytochemical composition of *T. occidentalis*, flavonoid, that was proven to be an effective inhibitor of platelet aggregation and also possesses antithrombotic activities.

### **CONCLUSION**

Based on the study, it may then be concluded that *Pandanus amaryllifolius* ethanolic leaf extract possesses anticoagulant property as evidenced by significant prolongation in the clotting time and APTT at 400 mg/kg and 800 mg/kg concentrations and 200 mg/kg, 400 mg/kg and 800 mg/kg concentrations, respectively, which may be of value in thrombotic states and cardiovascular diseases.

### **RECOMMENDATIONS**

Additional studies and researches must be conducted in order to investigate other plants with the same phytochemical constituents. Further studies should also be designed to describe the exact anticoagulant mechanism of the extract. Extraction of particular constituent of plant is also suggested. It is also suggested to rule out the possible presence of the circulating anticoagulant. Lastly, liver function screening test should be carried out to confirm the probable cause of prolonged PT and APTT in the study.

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### **REFERENCES**

Abubakar, E. M. M. (2009). Antibacterial activity of crude extracts of *Euphorbia hirta* against some bacteria associated with enteric infections. *Journal of Medicinal Plants Research* 3(7), 498-505

- Apostol, J.G., Gan, J. V. A., Raynes, R. J. B., Sabado, A. A. S., Carigma, A. Q., Santiago, L. A., et al. (2012). Platelet-Increasing Effects of *Euphorbia hirta* Linn. (Euphorbiaceae) in Ethanol-Induced Thrombocytopenic Rat Models. *International Journal of Pharmaceutical Frontier Research* 2(2), 1-11
- Asika, E.C., Idonije, B.O., Okhai, O., and Iribhogbe, I.O. (2011). Preliminary investigation of antithrombotic activities of methanolic seed extracts of *Garcinia Combogia* in rats. *Scholars Research Library, Annals of Biological Research* 2 (3), 333-346.
- Asmain A. B., (2010). Extracts of the subterranean root from *Pandanus amaryllifolius* (Doctoral Dissertation, *Universiti Teknologi Mara*).
- Azikiwe, C. C. A., Siminialayi, I. M., Brambaifa, N., Amazu, L. U., Enye, J. C., and Ezeani, M. C. (2007). Anticonvulsant activity of the fractionated extract of *Crinum jagus* bulbs in experimental animals. *Asian Pacific Journal of Tropical Disease* S446-S452
- Bamidele, O., Akinnuga, A. M., Anyakudo, M. M. C., Ojo, O. A., Ojo, G. B., Olorundemi, J. O., et al. (2010). Haemostatic effect of methanolic leaf extract of *Ageratum conyzoides* in albino rats. *Journal of Medicinal Plants Research* 4(20), 2075-2079
- Chong, H. Z., Yeap, S. K., Rahmat, A., Akim, A. M., Alitheen, N. B., Othman, F., et al. (2012). In vitro evaluation of *Pandanus amaryllifolius* ethanol extract for induction of cell death on hormone dependent human breast adenocarcinoma MDA-MB-231 cell via apoptosis. *BMC Complementary and Alternative Medicine* 12(134)
- Dapper, D.V., Achinike, P. N., and Gwotmut, M. D. (2007). The effects of Aloe vera [gel] on clotting time, prothrombin time and plasma fibrinogen concentration in albino Wistar rats. *Port Harcourt Medical Journal* 2, 56-60
- Dougnon, T. V., Klotoe, J. R., Segbo, J., Ategbo, J. M., Etorh, A. P., Gbaguidi, F., et al. (2012). Evaluation of the phytochemical and hemostatic potential of *Jatropha multifida* sap. *African Journal of Pharmacy and Pharmacology* 6(26), 1943-1948  
doi:10.5897/AJPP12.351
- Dumaol, O. S., Alaras, L. D., Dahilan, K. G., Depadua, A. A., and Pulmones, C. J., J. (2010). In vitro Activity of Pandan (*Pandanus amaryllifolius*) Leaves Crude Extract Against Selected Bacterial Isolates. *JPAIR Multidisciplinary Journal* 4, 285-307
- Enechi, O.C., OlukaIkenna H., Ugwu Okechukwu P.C. and Zomeh Yusuf S. (2013). Effect of Ethanol Leaf Extract of *Alstonia Boonei* on the Lipid Profile of Alloxan Induced Diabetic Rats. *World Journal of Pharmacy and Pharmaceutical Sciences*, 2(3), 782-795.



- Gou, Y.L., Ho, A.L.S, Rowlands, D.K., Chung, Y.W., and Chan, H.C. (2003). Effects of BakFoong Pill on Blood Coagulation and Platelet Aggregation. *Biological and Pharmaceutical Bulletin* 26(2) 241—246
- Gupta, A., Narniwal, N., and Kothari, V. (2012). Modern Extraction Methods for Preparation of Bioactive Plant Extracts. *International Journal of Applied and Natural Sciences* 1(1), 8-26
- Hadi, A.A., Elderbi, M. A., and Mohamed, A. H. (2010). Effect of Gum Arabic on Coagulation System of Albino Rats. *International Journal of PharmTech Research*, 2(3), 1762-1766
- Hoff, J. (2000). Methods of Blood Collection in the Mouse. *Lab Animal*, 29(10)
- Igbinsosa, O. O., Igbinsosa, E. O., and Aiyegiro, O. A. (2009). Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn). *African Journal of Pharmacy and Pharmacology* 3(2), 058-062
- Ike, S., Nubila, T., Ukaejiofo, E., Nubila, I., Shu, E., and Okpalaji, B. (2010). The Effects of Crude Neem Leaf Acetone-Water Extract on Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) in Albino Wistar Rats. *The Nigerian Health Journal*, 10(1-2).
- Kamal, A. H., Tefferi, A., and Pruthi, R. K. (2007). How to Interpret and Pursue an Abnormal Prothrombin Time, Activated Partial Thromboplastin Time, and Bleeding Time in Adults. *Concise Review For Clinicians* 82(7), 864-873
- Klafke, J. Z., da Silva, M.A., Rossato, M. F., Trevisan, G., Walker, C. I. B., Leal, C. A. M., et al. (2012). Antiplatelet, Antithrombotic, and Fibrinolytic Activities of *Campomanesia xanthocarpa* Evidence-Based Complementary and Alternative Medicine doi:10.1155/2012/954748
- Kumar, S., Joseph, L., George M., and Sharma A. (2011). A review on anticoagulant/antithrombotic activity of natural plants used in traditional medicine. *International Journal of Pharmaceutical Sciences Review and Research* 8(1), 70-74
- Lopez, D. C., and Nonato, M. G. (2005). Alkaloids from *Pandanus amaryllifolius* Collected from Marikina, Philippines. *Philippine Journal of Science*, 134(1), 39-44
- Mohammedi, Z. (2011). Impact of Solvent Extraction Type on Total Polyphenols Content and Biological Activity from *Tamarixaphylla* (L.) Karst. *International Journal of Pharma and Bio Sciences* 2(1)
- Moore, D. M. (2000). Rats and Mice: Biology. *Laboratory Animal Medicine and Science Series II*

- Nebedum, J. O., Udeafor, P. C., and Okeke, C. U. (2010). Comparative effects of ethanolic extracts of *Ficus carica* and *Mucuna pruriens* leaves on haematological parameters in albino rats. *Biochemistri*, 22(2)
- Nwinuka, N. M., Monanu, M. O., and Nwiloh, B. I. (2008). Effects of Aqueous Extract of *Mangifera indica* L. (Mango) Stem Bark on Haematological Parameters of Normal Albino Rats. *Pakistan Journal of Nutrition* 7(5), 663-666
- Odensami, S. O., Lawal, R. A., and Ojokuku, S. A. (2009). Effects of Ethanolic Extract of *Tetrapleura tetraptera* in Rabbits. *International Journal of Tropical Medicine* 4(4), 136-139
- Rajeh, M. A. B., Zuraini, Z., Sasidharan, S., Latha, L. Y., and Amutha S. (2010). Assessment of *Euphorbia hirta* L. Leaf, Flower, Stem and Root Extracts for Their Antibacterial and Antifungal Activity and Brine Shrimp Lethality. *Molecules* 15, 6008-6018, doi: 10.3390/molecules 15096008
- Rajput, M.A., Khan, R.A., Qaz, N., and Feroz, Z. (2012). Effect of Methanol Extract of Ajwain (*Trachyspermum Ammi* L) on Blood Coagulation in Rats. *Journal of Liaquat University of Medical and Health Sciences* 2(2), 105-108
- Rasche, H. (2001). Haemostasis and thrombosis: an overview. *European Heart Journal Supplements* 3, Q3-Q7
- Rayaguru, K. and Routray, W. (2011). Microwave drying kinetics and quality characteristics of aromatic *Pandanus amaryllifolius* leaves. *International Food Research Journal* 18(3), 1035-1042
- Riddel, J. P., Aouizerat, B. E., Miaskowski, C., and Lillicrap D. (2007). Theories of Blood Coagulation. *Journal of Pediatric Oncology Nursing* 24(3), 123-131
- Rohman, A., Riyanto, S., Yuniarti, N., Saputra, W. R., Utami, R. and Mulatsih, W. (2010). Antioxidant activity, total phenolic, and total flavonoid of extracts and fractions of red fruit (*Pandanus conoideus Lam*). *International Food Research Journal* 17, 97-106
- Sasidharan, S., Sumathi, V., Jegathambigai, N. R., and Latha, L. H. (2011). Antihyperglycemic effects of ethanol extracts of *Carica papaya* and *Pandanus amaryfollius* leaf in streptozotocin-induced diabetic mice. *Natural Product Research: Formerly Natural Product Letters* 25(20), 1982-1987
- Tanaka, K. A., Key, N. S., and Levy, J. H. (2009). Blood Coagulation: Hemostasis and Thrombin Regulation. *Anesthesia and Analgesia* 108:1433-46
- Tanko, Y., Eze, E. D., Jimoh, A., Yusuf, K., Mohammed, K. A., Balarabe, F., et al. (2012). Haemostatic effect of aqueous extract of

- mushroom (*Ganoderma lucidum*). *European Journal of Experimental Biology* 2(6), 2015-2018
- Thomas, N., Ernest, U. O., Nkoyo N. I., Elvis, S. N., Chukwubuzor, O. U., Ayodele U. C., et al. (2013). Effects of methanolic seed extract of *Telfairia occidentalis* on blood coagulation in Albino rats. *Nigerian Journal of Experimental and Clinical Bioscience*, 1(1), 10-13
- Wardah, F. M. S. (2012). Ethnobotanical study on the Genus *Pandanus* L. f. in certain areas in Java, Indonesia. *Biodiversitas* 10(3), 146-150
- Weremfo, A., Adinortey, M. B. and Pappoe A. N. M. (2011). Haemostatic Effect of the Stem Juice of *Musa paradisiaca* L. (Musaceae) in Guinea Pigs. *Advances in Biological Research* 5 (4), 190-192
- Yan, S.W., and Asmah, R. (2010). Comparison of total phenolic contents and antioxidant activities of turmeric leaf, pandan leaf and torch ginger flower. *International Food Research Journal* 17, 417-423
- Zhang, X., Guo P., Sun, G., Chen, S., Yang, M., Fu, N., Wu, H., et al (2012). Phenolic compounds and flavonoids from the fruits of *Pandanus tectorius soland*. *Journal of Medicinal Plants Research* 6(13), 2622-2626
- Zhang, S., Xu, Y., Jia, Y., Xu, L., Yin, L., and Peng, J. (2013). Antithrombotic effects of the flavonoids from *Rosa laevigata* Michx fruit assessed by in-vivo models of thrombosis. *Phytopharmacology* 4(3), 481-491