



In-Vitro study of the anticoagulant Property of *Terminalia catappa* (Talisay) leaf extract using *Gallus gallus* (Chicken) Blood

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Abstract

Prothrombin time is a test used to help detect and diagnose a bleeding disorder or excessive clotting disorder. It is important to know the prothrombin time because it checks to see if five different blood clotting factors are present. Lack of Vitamin K and having Liver problems are one of the factors that can decrease the prothrombin time of the blood. This research aims to investigate the potential ability of the Terminalia catappa (Talisay) leaf extract to increase prothrombin time of chicken's blood. The research methods used by the researchers is experimental. A research design where the results are being observed and determined when the independent variable took effect on the dependent variable. . The materials used are ethanol, calcium chloride, leaves and chicken blood. The method that was used in the study is in-vitro, a process that is performed in a test tube, or elsewhere outside a living organism. The 0.5 ml plasma sample was separated into four tubes and the first group of plasma was tested first to determine the initial prothrombin time while the other three groups with the different volumes of plant extract (0.2, 0.4, 0.6 mg/ml) was added separately. The tubes were tilted at about 45° until the permanent clots was obtained. These are recorded as the prothrombin time. The research showed that the Terminalia Catappa leaf extract is effective on increasing the normal prothrombin time of the blood.

Introduction

Every five minutes someone dies from a blood clot or deep vein thrombosis. One of four people worldwide dies from conditions caused by thrombosis, making it a leading global cause of death and disability. In a study conducted by Benjamin et al (2017) the total number of deaths increases and it is mainly caused by heart attack and stroke. In an article by inquirer, shows that here in the Philippines 276 dies from heart attack everyday while 1 person dies every minute from stroke. The number of heart attacks and strokes are expected to rise in the near future. Herbal medicine is one of the frequently used alternatives for anticoagulation medicine. *Terminalia catappa* was used due to its phytochemicals that helps on delaying the blood coagulation (Muhammad, et al., 2011).

Blood coagulation is a major problem that needs a treatment immediately. The fibrins which are proteins in the blood work with platelets that forms clot. A semi-solid mass of blood, that is called blood clot can be stationary (thrombosis) and block the flow of the blood hemostasis chemical and biological properties, clinical applications (Ayodele, et al., 2019). It may result to cardiovascular disease and can be life threatening depending on their location and severity. The total number of death increased and it is mainly caused by heart attack and stroke.

Currently, the cardiovascular disease is a leading cause of death all over the world. Anticoagulant has the effect of delaying or inhibiting the clotting of the blood and it is called blood “thinners”. It is a defense system to preserve an ideal circulatory system in mammals after vascular injury (Gou, et. al, 2003; Asha 2017), in a study examined the In-vitro anticoagulant activity and demonstrated leaf extract of *Nelumbo nucifera* (lotus) is able to restrain blood clotting disorders through its active anticoagulant components. There is a potential use of *Nelumbo nucifera* (lotus) leaf as supplementary source of natural anticoagulant in the future.

Over the years, herbal medicine has attracted not few scholars. It is becoming popularized due to its lesser side effects (Padh & Patel, 2001). *Terminalia catappa* is an example of a herb medicine. This plant species can be seen all over the Philippines and it is considered to be the one of the most common inland trees. The plant leaves contain vitamin A, B, and C and also carbohydrates, proteins, fat, fiber, ash and moisture. It contain a variety of phytochemicals like flavonoids, tannins, saponins, phytosterols, cyanogenic, glycocides, alkaloids, phenols, and steroids (Offor et al., 2015). It can cure many diseases such as diabetes, diarrhea, and tumor. In the study of Mgbemene & Ohiri (1999) there is an increase in the prothrombin time with extract concentration of *Terminalia catappa*. This shows that the extract has an anticoagulant property that made the clotting time of the human blood longer.

Prothrombin time (PT) is a blood test that measures how long the coagulation of the blood takes. It is an in-vitro laboratory assay for common pathway of coagulation and extrinsic (Sox et. al,1990). This can be used to check for bleeding problems and monitoring of patients on oral anticoagulation therapy (Davidson & Henry, 1969). Based on the study conducted by Philippine Journal of Otolaryngology-Head and Neck Surgery, the subjects used for their study showed the effectiveness of *Moringa olefera* (Malunggay) leaf extract in the bleeding time of the rabbit’s wound where the former bleeding time was significantly shorter than the latter.

Methodology

Research Design

The research methods used by the researchers is experimental. A research design where the results are being observed and determined when the ethanolic extract of leaf of *terminalia catappa* took effect on coagulation process of chicken’s blood. The experimental design refers to how the units are distributed to different conditions.

Plant Materials

The falling leaves of *Terminalia catappa* (Talisay) were collected and identified by Mr. Edwino S. Fernando, a botanical expert in the Institute of Biology, University of the Philippines Diliman. After collection, the leaves were oven dried. They were chopped and pulverized into 100 grams. The subject has undergone extraction process after the said method.

Preparation of Plant Extract

The powderized leaves were macerated for 48 hours with a mixture of 300 miligrams ethanol. After two days, the mixture was filtered using whatman filter paper no.1. The filtrate was transferred into a separating funnel. The ethanol extract was separated and concentrated using a hot plate and vacuum for 8 hours. The extract solution was heated at a constant temperature

of 40°. After separating the ethanol, the plant extract was diluted with 7 ml of distilled water. The residue was stirred until it dissolved.

Preparation of Calcium chloride Solution

The 100g calcium chloride was bought from a chemical depot and weighed. The 0.25g of calcium chloride and 0.25ml distilled water was mixed and stirred until it dissolved.

Collection of Blood samples

The chicken was bought from a slaughter house. Fresh blood was collected by slaughtering process in a syringe in a volume of 0.5 ml and was transferred into a centrifuge tube containing 0.5 ml calcium chloride solution and placed separately to prevent the clotting process. It was centrifuged at 3000 x g for 10 minutes to separate the blood cells from plasma to obtain pure platelets plasma for calculation of prothrombin time.

Anticoagulation Assay

The plasma sample was separated into four tubes. The first group of plasma, was tested first to determine the normal prothrombin time. The other three groups including three volumes of plant extract (0.2, 0.4, 0.6 mg/ml) was added separately. The tubes were tilted gently at an angle about 45° until permanent clots were obtained when the tubes could be completely inverted without spilling the contents. These were recorded as the prothrombin time.

Ethical Considerations

The research was approved by a concerned authority before doing the experiment. The researchers underwent different recommendations and suggestions from the veterinarian, Dr. Evangeline Esguerra , in doing the experiment and was guided.

Results and Discussion

Table 1: Effect of ethanolic extract of leaf of terminalia catappa on coagulation process of chicken's blood

Blood (ml)	Extract (ml)	0.25 g CaCl ₂ (ml)	Prothrombin Time (min/sec)
0.5	0.0	0.0	15 secs.
0.5	0.2	0.5	1 min. and 36 secs.
0.5	0.4	0.5	3 mins. and 51 secs.
0.5	0.6	0.5	5 mins. and 48 secs.

Source: Primary Data, 2020

Table 1 shows the effectiveness of Terminalia Catappa's leaf extract on increasing the prothrombin time of chicken's blood.



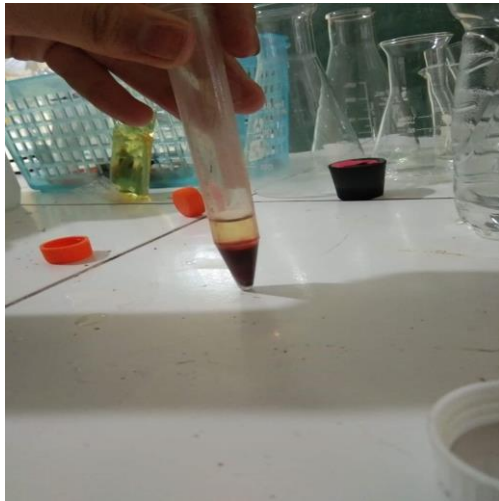
Figure 1. shows the blood sample before centrifugation process



For Normal Prothrombin Time



Tube A



Tube B



Tube C

Figure 2. Blood sample after the centrifugation process.

Figure 2 shows the separated pure platelets plasma from the red blood cells. The test was adapted to the study of Mgbeneme et al., The following procedures including the collection of blood, the proper use of materials and laboratory equipments were also adapted from Chegus et al. (2018). The results of the effect of the *Terminalia catappa* (Talisay) leaf extract are presented in Table 1. It showed that the prothrombin time was increased with the given extract concentration (0.2%, 0.4%, 0.6%). The higher concentration of leaf extract the longer the prothrombin time it takes. This results was also proven in a study conducted by Mgbemene & Ohiri (1999) in which the talisay leaf extract was also used as an anticoagulant and it showed a positive effect that increases the clotting time process.

Conclusion

The study shows that, the plant extract of the *Terminalia catappa* (Talisay) effectively slows the coagulation time compare to its normal clotting time. It exhibits the increase in the

clotting time of the blood thus, it concludes the potential of the *Terminalia catappa* leaf extract as an alternative medicine treatment to slow down the clotting time of the blood. The researchers would recommend the following method for the improvement of the study. Increasing the amount of the leaf extract that will be added in the blood sample to prolong the prothrombin time of the blood.

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