

**INVESTIGATION OF THE HAEMOSTATIC EFFECTS OF FREEZE DRIED  
EXTRACTS OF SELECTED KENYAN PLANTS**

**BY**

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REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER IN  
SCIENCE IN MEDICAL PHYSIOLOGY OF THE UNIVERSITY OF  
NAIROBI.**

**DECLARATION**

This thesis is my original work and has not been presented for a degree or any other award in any other University.

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**Supervisors Approval:**

We confirm that the work reported in this thesis was carried out by the student under our supervision.

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## **Dedication**

I dedicate this thesis to my parents Stephen Makunyi and Tabitha Mumbi. Their sacrifice to take me to Kajiampau Primary School among other schools was a great step in academic upward movement.

## **Abstract**

**Introduction:** “Hemorrhage is responsible for 30% to 40% of trauma mortality and accounts for almost 50% of the deaths occurring in the initial 24 hours following the traumatic incident”. The approach to “new drugs through natural products has proved to be the single most successful strategy” for the discovery of new drugs. The current study investigated the effect and mechanisms of action of *Tridax procumbens*, *Asphillia africana*, *Euphorbia tirucalli* and *Terminalia brownii* that have been used traditionally to stop bleeding following accidental cuts and after traditional circumcision.

**Setting:** The study was conducted in four institutions. Preparation of freeze dried extracts was carried out at the International Centre for Insect Physiology and Ecology (ICIPE) laboratory. Bleeding and clotting tests were carried out at University of Nairobi, medical physiology laboratory. Prothrombin and activated partial thromboplastin time were carried out at Kenyatta National Hospital, haematology department. Thromboelastography was carried out at Kenyatta University, physiology laboratory.

**Main Objective:** To investigate the effect and mechanism of action of freeze dried extracts of *Tridax procumbens* Linn, *Terminalia brownii*, *Euphorbia tirucalli* and *Asphillia africana* on haemostasis.

**Materials and methods:** Freeze dried extract of the selected plants was prepared and dose determined for the study. Twelve male New Zealand white rabbits were randomly allocated to two groups (control and test). Blood was collected under standard procedures. Duke’s method was used for bleeding time while capillary method was used for clotting time. ACL Elitepro machine was used to do prothrombin time and activated partial thromboplastin time. Thromboelastography

was done for the most potent extracts. Data was analysed using independent t test and results presented as mean  $\pm$  standard error of means. Differences were considered to be significant if  $P < 0.05$ .

**Results:** The percentage yield of the extract was; *Tridax procumbens* (0.8%), *Terminalia brownii* (0.5%), *Euphorbia tirucalli* (0.2%) and *Asphillia Pluriseta* (1.3%).

**Bleeding and Clotting time:** The bleeding time was reduced by freeze dried leaf extract of *Tridax procumbens* ( $p = 0.0068$ ) and by freeze dried bark extract of *Terminalia brownii* ( $p=0.0068$ ). Freeze dried leaf extract of *Asphillia africana* increased the bleeding time ( $p=0.01$ ). The clotting time was reduced by freeze dried leaf extract of *Tridax procumbens* ( $p = 0.038$ ), freeze dried bark extract of *Terminalia brownii* ( $p=0.043$ ) and by freeze dried stem extract of *Euphorbia tirucalli* ( $p=0.01$ ).

**Prothrombin and Activated partial thromboplastin time:** The prothrombin time was reduced by freeze dried leaf extract of *Tridax procumbens* ( $p = 0.004$ ), freeze dried bark extract of *Terminalia brownii* ( $p<0.001$ ) and Freeze dried stem extract of *Euphorbia tirucalli* ( $p=0.001$ ). Activated partial thromboplastin time was reduced by freeze dried leaf extract of *Tridax procumbens* ( $p< 0.001$ ), freeze dried bark extract of *Terminalia brownii* ( $p<0.001$ ) and by Freeze dried stem extract of *Euphorbia tirucalli* ( $p<0.001$ ).

**Thromboelastography:** Four parameters of Thromboelastography were tested. Freeze dried leaf extract of *Tridax procumbens* reduced the r time ( $p =0.04$ ), k time ( $p=0.04$ ) and maximum amplitude ( $p =0.026$ ) but increased the alpha angle ( $p =0.01$ ). Freeze dried bark extract of *Terminalia brownii* did not have statistically significant differences on Thromboelastography variables.

**Conclusion:** *Tridax procumbens* and *Terminalia brownii* have activation effect on the platelets. *Tridax procumbens*, *Euphorbia tirucalli* and *Terminalia brownii* have

activation effect on common coagulation pathway. *Tridax procumbens* has more effect on factor VII which is involved in initiation of clot formation.

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### **List of Abbreviations**

APTT	Activated Partial Thromboplastin Time
BT	Bleeding Time
ICIPE	International Center for Insect Physiology And Ecology
FELASA	Federations for Laboratory Animal Science Associations
PT	Prothrombin Time
TEG	Thrombo-elastography
WHO	World Health Organisation
NTSA	National Transport and Safety Authority

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## CHAPTER ONE: INTRODUCTION

One in seven deaths is associated with traumatic injury and about a quarter of all trauma admissions present with coagulopathy (Mathew and Richard, 2010). It is estimated that more than 1.24 million people die annually as a result of road traffic accidents globally. In addition, fifty million people suffer injuries from these road traffic accidents (NTSA, 2016). In the United States of America, 40% of trauma fatalities are due to bleeding (Mathew and Richard, 2010).

Haemorrhage (bleeding) causes thirty to forty percent of trauma mortality. “It accounts for about fifty percent of mortality in the initial 24 hours following the injuries. On admission, 25% to 35% of trauma patients present with coagulopathy, which is associated with a sevenfold increase in morbidity and mortality”. Coagulopathy is a condition in which the blood's ability to clot is impaired (Charice, 2013).

“According to the World Health Organization (WHO) as many as 80% of the world’s people depend on traditional medicine for their primary health care needs” (Essiett and Akpan, 2013). Plants are an abundant natural source of potential new medicines. “The approach to new drugs through natural products has proved to be the single most successful strategy for the discovery of new drugs”, (Sowmya *et al.*, 2009).

The use of herbal preparations for staunching blood flow and reducing the risk of blood disorders is common worldwide. (Werner *et al.*, 1992). Bleeding in rural set ups can be caused by injuries or traditional circumcision. Many plants have been used to prevent bleeding. The plants are either chewed or crushed and then applied on the

bleeding sites. Many plants are used but this study chose the commonly used plants in Eastern Kenya.

The present study investigated the effect and mechanisms of action of *Tridax procumbens*, *Asphillia africana*, *Euphorbia tirucalli* and *Terminalia brownii* that have been used traditionally to stop bleeding following cuts and after traditional circumcision by eastern Meru and Embu communities. For this study, leaves of *Tridax procumbens* and *Asphillia Africana* were used. The bark of *Terminalia brownii* was used in the study. *Euphorbia tirucalli* the stem was used.

## CHAPTER TWO: LITERATURE REVIEW

The selected plants, the physiology of haemostasis and the coagulation tests are discussed in this chapter.

### **2.1 *Tridax procumbens* Linn**

*Tridax procumbens* Linn grows wildly in tropical areas of three continents; Africa, Asia and Australia (The Wealth of India, 1985). “It is a hardy perennial herb belonging to the family Asteraceae”, (Saxena and Albert, 2005). “It originated in Central America but now occurs throughout the tropics and subtropics. It was reportedly introduced into Nigeria as an ornamental plant in the early 1900s and later spread from there to many other tropical countries”. The plant belongs to the division *Magnoliophyta*, *Astereceae* family, *Tridax* Linn genus and *Tridax procumbens* Linn species (Holm *et al.* (1997). In previous studies, this plant has been found to play a role in wound healing (Udupa *et al.*, 1991), haemorrhage and treatment of diarrhoea, backache (Burkhill, 1985) and bronchial catarrh (Ambasta, 1986). “It exhibits antiseptic, insecticidal and hair growth-promoting properties”, (Chantraine *et al.*, 1998).

### **2.2 *Terminalia brownii***

“*Terminalia brownii* is a leafy deciduous tree with an attractive somewhat layered appearance, usually 4-15 (25) m high with a rounded, flat topped, spreading crown, and a straight bole; branches reaching close to the ground”, (Orwa *et al.*, 2009). It is a medicinal plant commonly used by herbalists in Mbeere, and Embu districts of Eastern province, Kenya. It is the most used medicinal plant in Embu and Mbeere. It is used for the management of eye problems, allergies, kidney problems and bleeding. “It is widely used in traditional medicine to treat fungal and viral infections. The traditional use of *Terminalia brownii* extracts to treat diarrhoea, cut wounds and



gonorrhoea”, (Kareru *et al.*, 2007). It is used to treat nematodes in animals and human in Mbeere community, Embu county. The stems and branches gaseous substances are used to treat rheumatic and back pains (Khalid *et al.*, 1996). It has antibacterial activity. Chemical analysis of the different parts of the plant revealed abundant amounts of tannins and flavonoids (Thoria *et al.*, 2011).

### **2.3 *Euphorbia Tirucalli L.***

*Euphorbia tirucalli* L. “belongs to the genus *Euphorbia*. It is can grow up to 7 – 12 M high. It is found in tropical areas. *Euphorbia tirucalli* is evergreen. The tree is rarely fed on by herbivores. It produces white poisonous latex, which accounts for its low herbivore pressure and medicinal features”, (Julius *et al.*, 2013).

The bark/latex of *E. tirucalli* has pharmacological activities as antibacterial, molluscicide and antimutagenic. In Brazil, the latex of *E. tirucalli* is used as a folk medicine against syphilis. *Euphorbia tirucalli* contains a large quantity of terpenes and sterols among its constituents and the following substances; alcohol eufol, alfaeuforbol, and taraxa sterol etirucallol have been isolated (Gupta *et al.*, 2013).

### **2.4 *Aspilia africana Adams***

The genus *Aspilia* is in the family *Compositae* in the major group *Angiosperms* (Flowering plants). *Aspilia africana* is a very rapid growing, semi-woody herb producing usually annual stems about 2 meters tall from a perennial woody root-stock. It has a somewhat aromatic carrot smell. It is widely gathered from the wild and used locally in traditional medicine (Burkill, 1985).

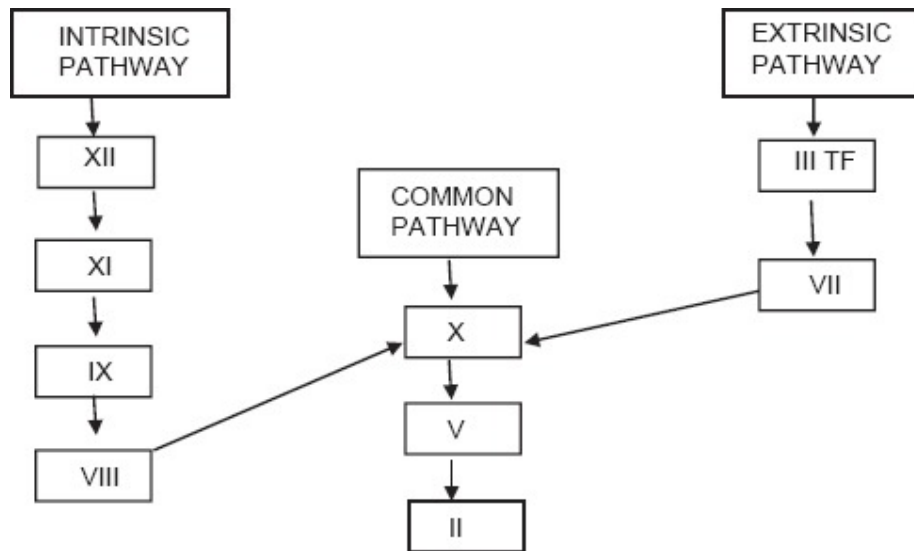
In African ethnomedicine, *Asphillia africana* is widely used to stop bleeding. It is also used in wound healing (Dimo *et al.*, 2002). Anticoagulant effects have also been elucidated (Hanna and Niemetz, 1987). “Infusion of a liquid made from the leaves is taken by children and can also be mixed with clay as a medicine for stomach trouble”.

Researchers have also found anti-malarial effects (Okokon *et al.*, 2006). *Asphillia africana* has palliative properties. “Their leaves possess constituents capable of decreasing wound bleeding, inhibiting the growth of microbial wound contaminants and accelerating wound healing which suggest good potential for use in wound care”, (Okoli *et al.*, 2007). In Uganda, it is used to treat gonorrhoea. The plant has a wide reputation and use as a haemostatic, and in Liberia it was found to arrest bleeding of severed artery. The leaf-sap is also an eye – medicine in Tanganyika. “The crude extract has inhibitory activity on *Candida albicans*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Aspergillus flavus*”, (Kazeem *et al.*, 2013).

## **2.5 Physiology of Haemostasis**

Coagulation was conventionally explained as a function of the intrinsic and extrinsic pathway. The two link into common pathway. Currently it thought via the cell based model to involve platelets and other cellular components. In the intrinsic pathway, the initial reaction is the conversion of inactive factor XII to active factor XIIa. Factor XII is activated in vitro by exposing blood to foreign surface (glass test tube). Activation in vivo occurs when blood is exposed to collagen fibers underlying the endothelium in the blood vessels.

The extrinsic pathway requires contact with tissue factors external to blood. This occurs when there is trauma to the vascular wall and surrounding tissues. The extrinsic system is triggered by the release of tissue factor (thromboplastin from damaged tissue), that activates factor VII. The tissue thromboplastin and factor VII activate factor X (Hoffman and Monroe, 2005). Figure 2.1 shows the coagulation cascade.



**Figure 2. 1: Coagulation Cascade**

Source: Hoffman and Monroe (2005)

“Normal haemostasis consists of a delicate balance between the blood vessels, platelets, and coagulation proteins, a relationship known as the “triad” of haemostasis”, (Hoffman and Monroe, 2007). “While each component of the triad plays a very specific role in the hemostatic process, they all work together to preserve the integrity of the fluid state of the blood”, (Johns, 2004).

In the cell based model of coagulation, the first step is initiation of coagulation on tissue factor bearing cells. “This process is initiated when Tissue Factor (TF) bearing cells are exposed to blood at a site of injury. Tissue factor is a transmembrane protein that acts as a receptor and cofactor for Factor VII. Once bound to TF, zymogen FVII is rapidly converted to FVIIa by involving FXa or noncoagulation proteases. The resulting FVIIa/TF complex catalyzes activation of FX and activation of FIX. The factors Xa and IXa formed on the TF-bearing cells have distinct and separate functions in initiating blood coagulation. The FXa formed on the TF-bearing cell interacts with its cofactor Va to form prothrombinase complexes and generates a small amount of thrombin on the TF cells. By contrast, the FIXa activated by

FVIIa/TF does not act on the TF-bearing cell and does not play a significant role in the initiation phase of coagulation (Hoffman and Monroe, 2005).

If an injury has occurred and platelets have adhered near the site of the TF-bearing cells, the FIXa can diffuse to the surface of nearby activated platelets. It can then bind to a specific platelet surface receptor, interact with its cofactor, FVIIIa, and activate FX directly on the platelet surface”, (Hoffman and Monroe, 2005).

The second step is amplification of procoagulant signal by thrombin. “The small amounts of thrombin generated on the TF-bearing cells are responsible for: (1) activating platelets, (2) activating FV, (3) activating FVIII and dissociating FVIII from VWF, and (4) activating FXI. The activity of the FXa formed by the FVIIa/TF complex is restricted to the TF-bearing cell, because FXa that dissociates from the cell surface is rapidly inhibited by Tissue Factor Plasminogen Inhibitor (TFPI) in the fluid phase. In contrast to FXa, FIXa can diffuse to adjacent platelet surfaces because it is not inhibited by TFPI and is inhibited much more slowly by AT than is FXa”, (Hoffman and Monroe, 2005).

The third step is “propagation of thrombin generation on the platelet surface. Platelets play a major role in localizing clotting reactions to the site of injury because they adhere and aggregate at the sites of injury where TF is also exposed. They provide the primary surface for generation of the burst of thrombin needed for effective hemostasis during the propagation phase of coagulation. Platelet localization and activation are mediated by vWF, thrombin, platelet receptors, and vessel wall components, such as collagen. Once platelets are activated, the cofactors Va and VIIIa are rapidly localized on the platelet surface. The FIXa formed by the FVIIa/TF complex can diffuse through the fluid phase and also bind to the surface of activated

platelets. The platelet-produced thrombin also stabilizes the clot”, (Hoffman and Monroe, 2005).

There is final, fibrinolysis. “The final effector of the fibrinolytic system is plasmin, which cleaves fibrin into soluble degradation products. Plasmin is produced from the inactive precursor plasminogen by the action of two plasminogen activators: urokinase-type plasminogen activator (uPA) and tissue- type plasminogen activator (tPA)”, (Hoffman and Monroe, 2005).

“Fibrinolysis is essential for removal of clots during the process of wound healing and for removing intravascular clots that might otherwise be manifest as thrombosis. An effective fibrinolytic system therefore tends to protect against the chronic process of atherosclerotic vascular disease and the acute process of thrombosis”, (Hoffman and Monroe, 2005).

## **2.6 Coagulation Tests**

### **Bleeding time**

This test measures the capillary integrity and platelet function. Haemostatic mechanism is activated by a slit on the skin. “Without the aid of external pressure, bleeding usually stops within 7 to 9 minutes” (Walker *et al.*, 1990). “This test measures the time taken for blood vessel constriction and platelet plug formation to occur. No clot is allowed to form, so that the arrest of bleeding depends exclusively on blood vessel constriction and platelet action”, (McKenzi *et al.*, 2010).

### **Clotting time**

It measures the time taken to generate thrombin. “In order for blood to clot, the enzyme thrombin must be generated from the plasma precursor prothrombin. Thrombin converts soluble fibrinogen into insoluble fibrin. Generation of thrombin involves the sequential activation of a number of other plasma clotting factor, this

process is also being assisted by calcium ions and by factors released by platelets and damaged tissues . If the plasma concentration of prothrombin or of some of the other factors is low, clotting time will be prolonged. The expected range for clotting time is 4-10 mins”, (Walker *et al.*, 1990).

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

“The PT measures the time necessary to generate fibrin after activation of factor VII. It measures the integrity of the "extrinsic" and "common" pathways (factors VII, V, X, prothrombin, and fibrinogen)” (Walker *et al.*, 1990).

### **Activated Partial Thromboplastin Time (aPTT)**

“The aPTT measures the time necessary to generate fibrin from initiation of the intrinsic pathway. Activation of factor XII is accomplished with an external agent (like kaolin) capable of activating factor XII without activating factor VII. Since platelet factors are necessary for the cascade to function normally, the test is performed in the presence of a phospholipid emulsion that takes the place of these factors. The classic partial thromboplastin time depends on contact with a glass tube for activation”, (Walker *et al.*, 1990).

### **Thromboelastography**

This measures the clot formation/dissolution kinetics and tensile strength of the clot. Thromboelastography (TEG) is a method of testing the efficiency of blood coagulation. The test sample is placed in the oscillating cup at 37° C. A pin is suspended from torsion wire into blood sample and the development of fibrin strands couple the motion of the cup to the pin. This coupling is directly proportional to the clot strength. Increased tension in the wire is detected by the electromagnetic transducer and electrical signal amplified to create a trace which is displayed on

computer screen. Deflection of the trace is proportional to clot strength (Thakur and Ahmed, 2012).

### **Parameters of measurement in thromboelastography**

#### **I. r-time**

Represents period of time of latency from start of test to initial fibrin formation. Normal range in native blood is 15 to 23 minutes while in kaolin activated blood it is 5 to 7 minutes (Thakur and Ahmed, 2012).

#### **II. k-time**

Represents time taken to achieve a certain level of clot strength of 20 mm. Normal range when using native blood is 5 to 10 minutes while with kaolin activated blood it is 1 to 3 minutes (Thakur and Ahmed, 2012).

#### **III. $\alpha$ -angle**

It measures the speed at which fibrin build-up and cross-linking takes place. It measures clot strengthening; rate of clot formation. The normal is 22 to 38 degrees when using native blood while it is 53 to 67 degrees when using kaolin-activated (Thakur and Ahmed, 2012).

#### **IV. Maximum amplitude**

“Maximum amplitude is a direct function of the maximum dynamic properties of fibrin and platelet bonding via GPIIb/IIIa and represents the ultimate strength of the fibrin clot and which correlates to platelet function: 80% platelets; 20% fibrinogen. Normal range: 47 to 58 mm (native blood); 59 to 68 mm (kaolin-activated)”, (Thakur and Ahmed, 2012).

## CHAPTER THREE: MATERIALS AND METHODS

### 3.1 Extract Preparation

The plants were collected and their identity verified at the University of Nairobi Herbarium, Department of Botany, School of Biological sciences and voucher specimens of the plants deposited therein. The plants were air dried after which they were milled and then macerated with distilled water in a weight volume ratio of 1:4. The resulting suspension filtered with cotton wool after which Whatmans filter paper was used. The resulting filtrate was frozen, using the *Hot Point* deep freezer. The frozen filtrate was freeze dried at the International Centre for Insect Physiology and Ecology (ICIPE). The resulting freeze dried extract was weighed and then stored in the deep freezer

### 3.2 Animal Preparation and Welfare

Male adult Zealand white rabbits were locally obtained and used for study. The male rabbits were used because they have almost constant hormonal levels contrary to females. All the animals weighed (2.0-2.5 Kg). They were housed in spacious cages in the animal house, Department of Medical Physiology, University of Nairobi. The room temperature was maintained between 15-25°C and relative humidity of 45-65%, a normal 12 hours dark/12hours light cycle. The animals were handled humanely. Selection of rabbits as the experimental animal model was to ensure adequate blood volume is achieved. Adult male New Zealand white rabbits, 8-12 weeks old, weighing 2.0-2.5Kg and healthy were included in the study while sick male rabbits were excluded. They were kept under standard laboratory conditions as recommended by The Federation of European Laboratory Animal Science Associations (FELASA) guidelines (Weiss et al., 2010).



### 3.3 Experimental Protocol

Blood was collected using the standard method of bleeding the rabbit from the ear (Duke, 1981). For prothrombin time, activated partial thromboplastin time and thromboelastography, blood was collected in clean citrated bottles and tested within two hours.

The tests were performed using blood from six rabbits for each group (control and test groups). The dose of the freeze dried extracts was determined using titration method. The dose of freeze dried extracts used in most of the tests was 10mg/ml. The dose for freeze dried extract of *Terminalia brownii* for thromboelastography was reduced to 2.5 mg/ml because doses of 10 mg, 7.5 mg and 5 mg/ml were too potent that they only indicated a straight line on thromboelastography. Duke's method of bleeding time was used (Janzarik *et al*, 1988). Capillary method of clotting time was used (Kumar *et al*, 2013). Prothrombin time and APTT were done at Kenyatta National Hospital Hematology laboratory. The freeze dried extracts of *Tridax procumbens* and *Terminalia brownii* which were the most potent were evaluated in the thromboelastography stage.

For thromboelastography, 200  $\mu$ l of sodium citrate was mixed with 1800  $\mu$ l of rabbit's blood. The extract and rabbit blood were mixed at a ratio of 1:4 respectively after which 360  $\mu$ l of the mixture is loaded into a thromboelastography cup. The ratio is similar to the ratio of the reagents used in Thromboelastography. Calcium chloride, 0.2 M was added and the test is run for one hour.

This was an *in vitro* experiment. Blood was drawn from the rabbit ear after which the freeze dried extract was added as a reagent in the various tests.

### **3.4 Main Objective**

To investigate the effects of freeze dried extracts of *Tridax procumbens*, *Terminalia brownii*, *Euphorbia tirucalli* and *Asphillia africana* on haemostasis.

### **3.5 Specific Objectives**

1. To prepare freeze dried extracts of *Tridax procumbens*, *Terminalia brownii*, *Euphorbia tirucalli* and *Asphillia africana*.
2. To determine the effect of *Tridax procumbens*, *Terminalia brownii* and *Asphillia africana* on bleeding time.
3. To determine the effect of *Tridax procumbens*, *Terminalia brownii* and *Euphorbia tirucalli* on clotting time.
4. To determine the effect of *Tridax procumbens*, *Terminalia brownii* and *Euphorbia tirucalli* on prothrombin time.
5. To determine the effect of *Tridax procumbens*, *Terminalia brownii* and *Euphorbia tirucalli* on activated partial thromboplastin time.
6. To determine the effect of *Tridax procumbens* and *Terminalia brownii* on thromboelastography.

### **3.6 Data Analysis and Presentation**

Data were entered into STATA Version 11 and were analyzed using independent t-test. Results were expressed as means  $\pm$  standard error of means (SEM). Differences were considered to be significant if  $P < 0.05$ .

## CHAPTER 4: RESULTS

### 4.1 Extract yield

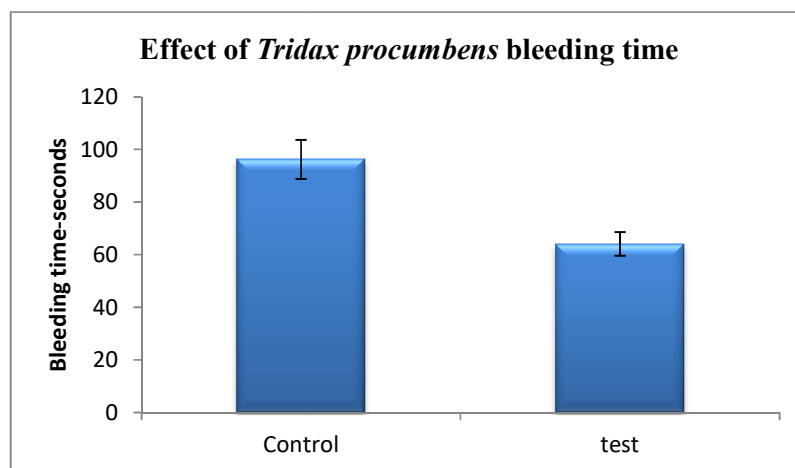
The weight of the grounded product was taken. After the freeze drying, the weight of the extract was also taken. The percentages were then calculated. Table 4.1 shows the percentage yield of the extract that is; *Tridax procumbens* leaf (0.8%), *Terminalia brownii* bark (0.5%), *Euphorbia tirucalli* stem (0.2%) and *Asphillia africana* leaf (1.3%).

**Table 4:1: Extract yield**

No.	Plant	Weight of grounded product	Weight of extract obtained	Percentage yield
1	<i>Tridax procumbens</i> leaves	500 gm	4 gm	0.8
2	<i>Terminalia brownii</i> bark	2 kg	10 gm	0.5
3	<i>Euphorbia tirucalli</i> stem	1 kg	2 gm	0.2
4	<i>Asphillia Africana</i> leaves	300 gm	4 gm	1.3

#### 4.2.1 Effect of *Tridax procumbens* on bleeding time

The results show that the bleeding time is reduced by freeze dried leaf extract of *Tridax procumbens* with statistically significant differences in the means ( $93.6 \pm 7.4$ (c) vs.  $64.2 \pm 4.5$  (t) seconds,  $P = 0.0068$ ,  $t = 3.39$ ).



**Figure 4.2: Effect of *Tridax procumbens* on bleeding time**

#### 4.2.2: Effect of *Terminalia brownii* on bleeding time

Freeze dried bark extract of *Terminalia brownii* reduced the bleeding time with statistically significant difference in the means ( $100.3 \pm 7$  (c) vs.  $82.6 \pm 4.3$  (t) seconds,  $p=0.0068$ ,  $t=3.39$ ).

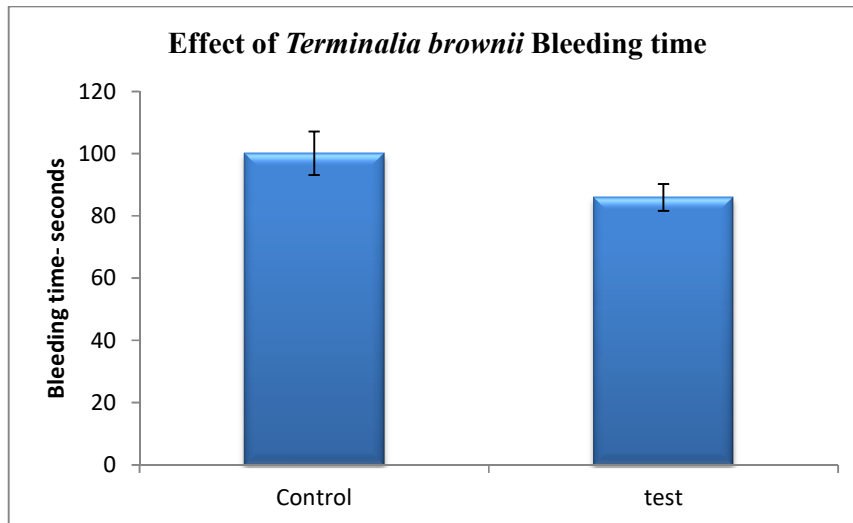
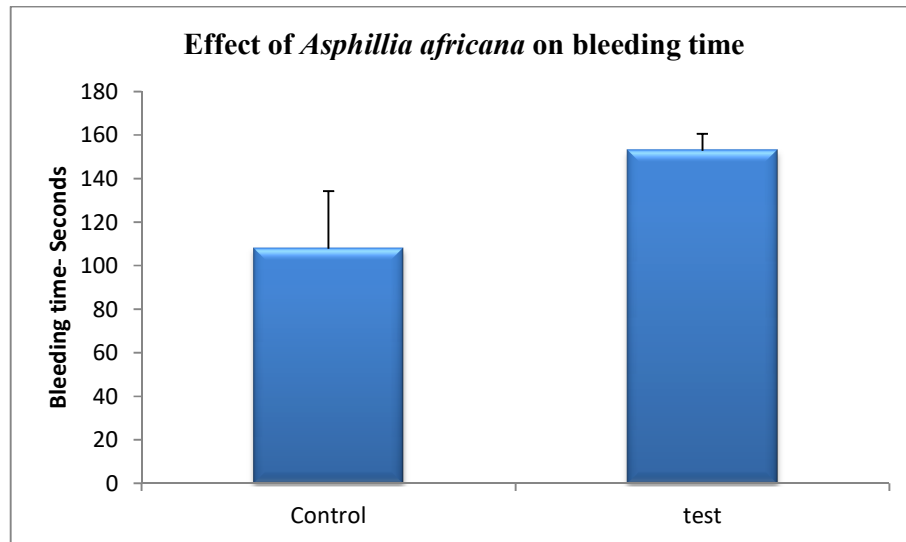


Figure 4.3: Effect of *Terminalia brownii* on bleeding time

#### 4.2.3 Effect of *Asphillia africana* on bleeding time

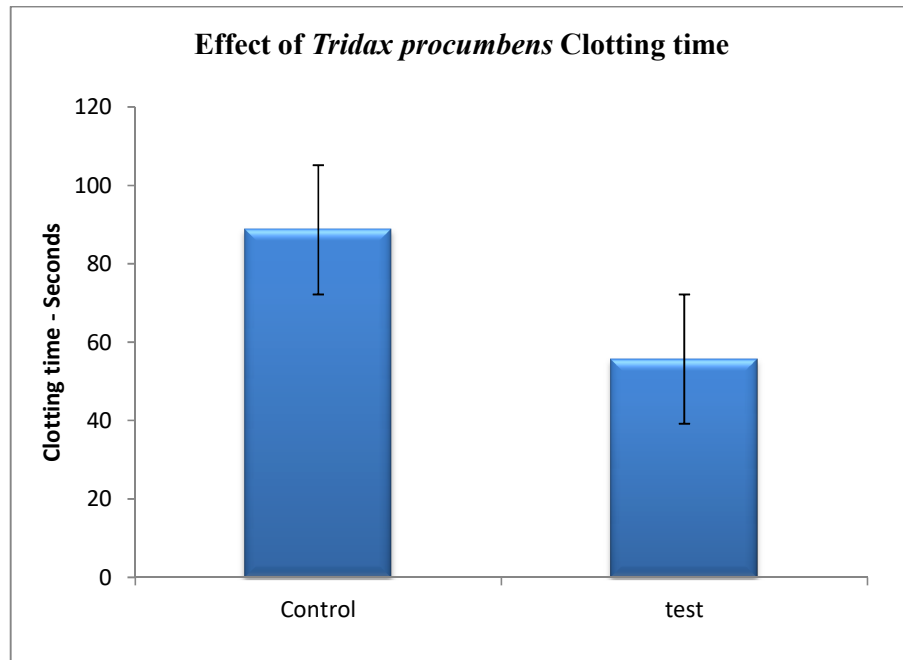
Freeze dried leaf extract of *Asphillia africana* increased the bleeding time with statistically significant difference in the means ( $107.8 \pm 10.5$  (c) Vs.  $152.8 \pm 9.2$  (t) seconds,  $t=3.1$   $p=0.01$ ).



**Figure 4.4: Effect of *Asphillia africana* on bleeding time**

#### **4.3.1 Effect of *Tridax procumbens* on clotting time**

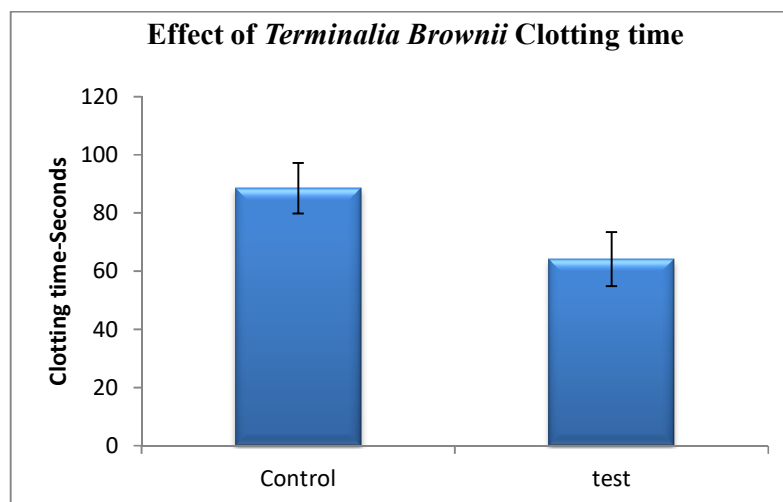
The result show that the clotting time is reduced by freeze dried leaf extract of *Tridax procumbens* with statistically significant difference in means ( $88.7 \pm 7.8$  (c) vs.  $55.7 \pm 26.5$  (t) seconds,  $t = 2.45$ ,  $p = 0.0338$ ).



**Figure 5.4: Effect of *Tridax procumbens* on clotting time**

#### 4.3.2 Effect of *Terminalia brownii* on clotting time

Freeze dried bark extract *Terminalia brownii* reduced the clotting time with a statistically significant difference in the means (88.5±8.7 (c) vs. 64.2±9.3 (t) seconds,  $t=1.9$   $p=0.043$ ).



**Figure 4.6: Effect of *Terminalia brownii* on clotting time**

### 4.3.3 Effect of *Euphorbia tirucalli* on clotting time

Freeze dried stem extract of *Euphorbia tirucalli* reduced the clotting time with a statistically significant difference in means ( $88.5 \pm 8.7$  (c) vs.  $57 \pm 9.7$  (t) seconds,  $t=2.4$   $p=0.01$ ).

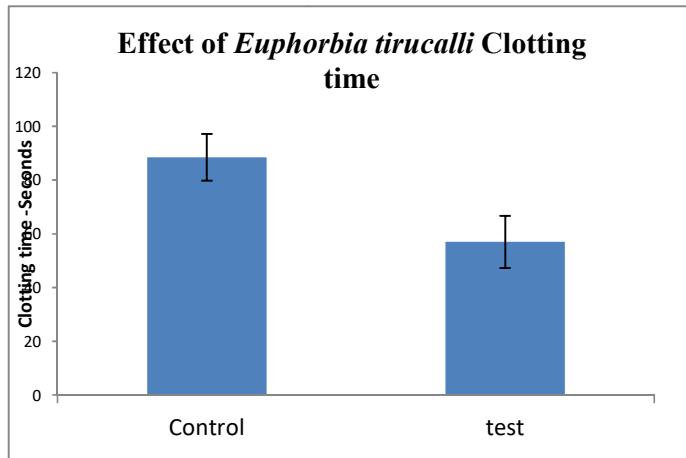


Figure 4.7: Effect of *Euphorbia tirucalli* on clotting time

### 4.4.1 Effect of *Tridax procumbens* on prothrombin time

The results show that the prothrombin time is reduced by freeze dried leaf extract of *Tridax procumbens* with statistically significant difference in the means ( $9.4 \pm 0.17$  (c) vs.  $5.3 \pm 0.17$  (t) seconds,  $t=5.12$ ,  $p = 0.004$ ).

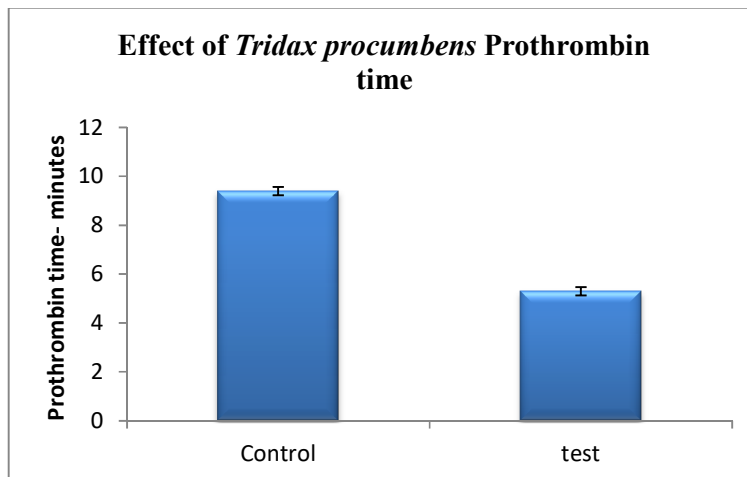


Figure 4.8: Effect of *Tridax procumbens* on prothrombin time

#### 4.4.2 Effect of *Terminalia brownii* on prothrombin time

Freeze dried bark extract of *Terminalia brownii* reduced the prothrombin time with a statistically significant difference in the means ( $9.4 \pm 0.78$  (c) vs.  $3.8 \pm 0.38$  (t) seconds,  $t=6.4$ ,  $p<0.001$ ).

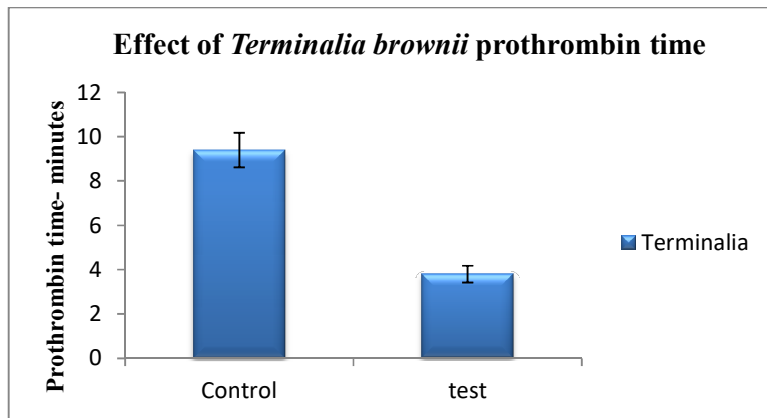


Figure 4. 9: Effect of *Terminalia brownii* on prothrombin time

#### 4.4.3 Effect of *Euphorbia tirucalli* on prothrombin time

Freeze dried stem extract of *Euphorbia tirucalli* reduced the prothrombin time with statistically significant difference in the means ( $5.8 \pm 0.17$  (c) vs.  $9.4 \pm 0.78$  (t) seconds,  $t=4.6$ ,  $p=0.001$ ).

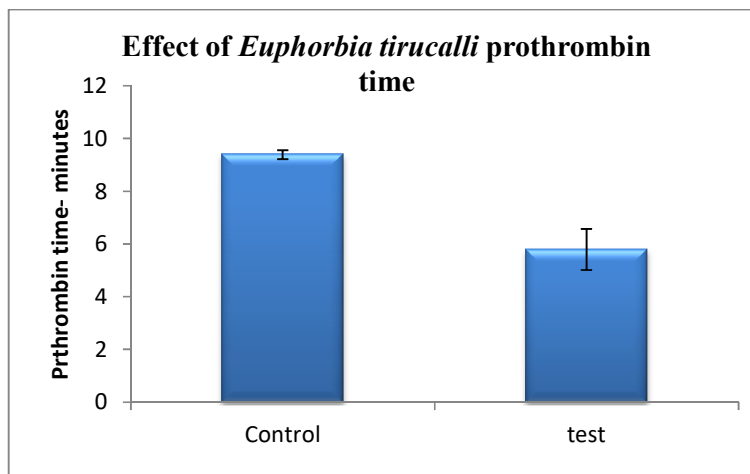
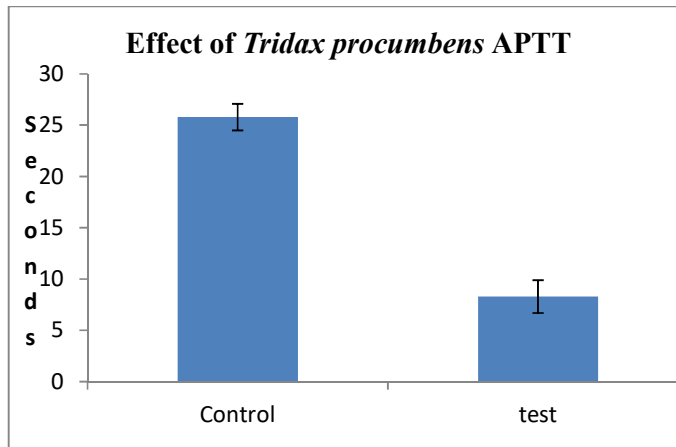


Figure 4.10: Effect of *Euphorbia tirucalli* on prothrombin time



#### 4.5.1 Effect of *Tridax procumbens* on Activated partial thromboplastin time

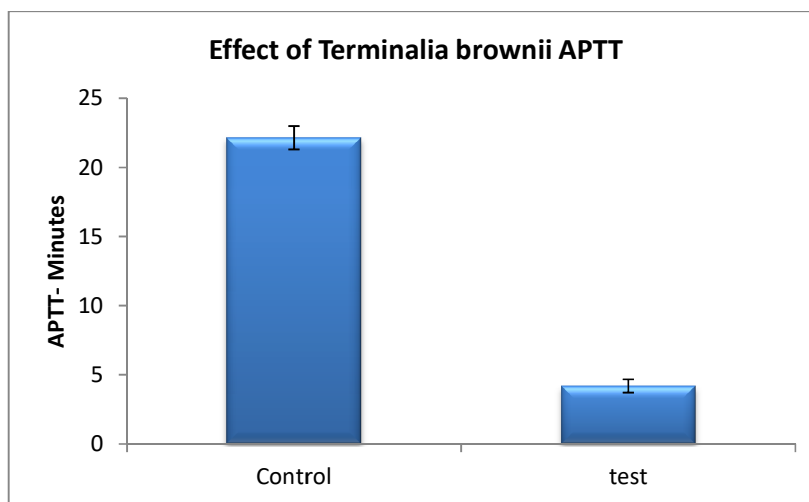
The result showed that the APTT was reduced by freeze dried leaf extract of *Tridax procumbens* with statistically significant difference in the means ( $25.8 \pm 1.3$  (c) vs.  $8.3 \pm 1.6$  (t) seconds,  $t = 8.53$ ,  $p < 0.001$ ).



**Figure 4.11: Effect of *Tridax procumbens* on Activated Partial Thromboplastin Time**

#### 4.5.2 Effect of *Terminalia brownii* on Activated partial thromboplastin time

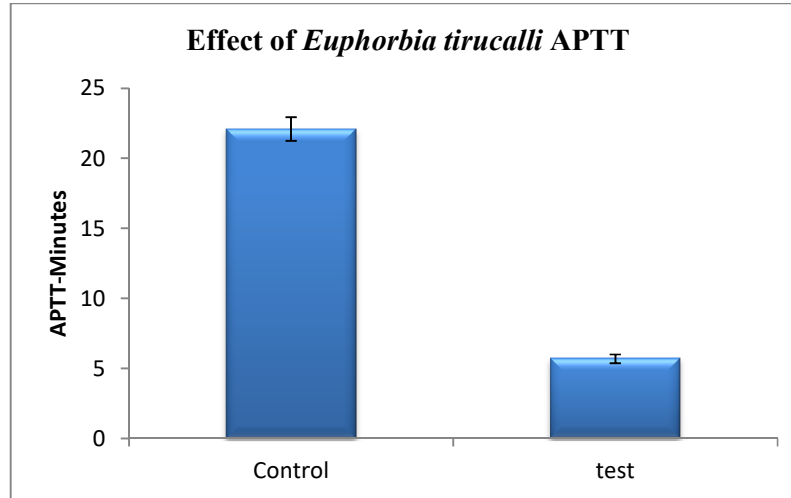
Freeze dried bark extract of *Terminalia brownii* reduced the APTT with statistically significant difference in means of the treatment group ( $22.14 \pm 0.84$  (c) vs.  $4.2 \pm 0.48$  (t) seconds,  $t = 18$ ,  $p < 0.001$ ).



**Figure 4.12: Effect of *Terminalia brownii* on Activated Partial Thromboplastin Time**

#### 4.5.3 Effect of *Euphorbia tirucalli* Activated partial thromboplastin time

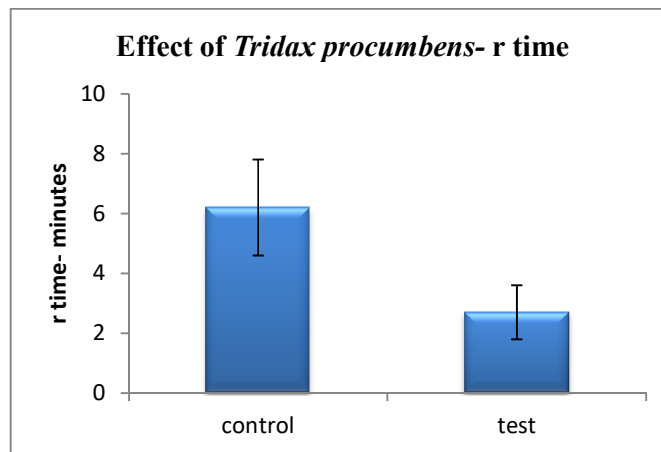
Freeze dried stem extract of *Euphorbia tirucalli* decreased the APTT with statistically significant difference in means ( $22.1 \pm 0.84$  (c) vs.  $5.7 \pm 0.31$  (t),  $t=18$ ,  $p < 0.001$ ).



**Figure 4.13: Effect of *Euphorbia tirucalli* Activated Partial Thromboplastin Time**

#### 4.6.1 Effect of *Tridax procumbens* on r- time

The results indicate that freeze dried leaf extract of *Tridax procumbens* decreased the time with the statistically significant difference in the means ( $6.2 \pm 1.6$  (c) vs.  $2.7 \pm 0.49$  (t) minutes,  $t=2.08$ ,  $p = 0.04$ ).



**Figure 4. 14: Effect of *Tridax procumbens* on r- time**

#### 4.6.2 Effect of *Terminalia brownii* on r-time

Freeze dried bark extract of *Terminalia brownii* did not elicit a statistically significant difference in the means of *r* time ( $4.9 \pm 1.6$  (c) vs.  $5.3 \pm 0.9$  (t) minutes,  $t=0.17$   $p=0.86$ ).

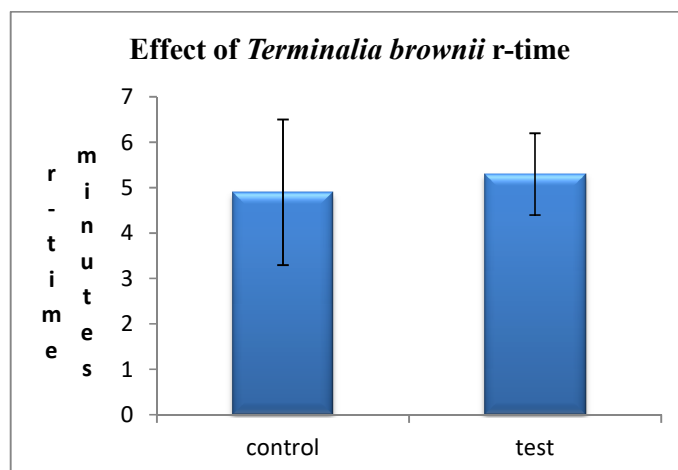


Figure 4.15: Effect of *Terminalia brownii* on r-time

#### 4.6.3 Effect of *Tridax procumbens* on K- time

The results indicate freeze dried leaf extract of *Tridax procumbens* decrease the *k* time with the statistically significant difference in the means ( $3.7 \pm 1.1$  (c) vs.  $1.4 \pm 0.18$  (t) minutes,  $p=0.04$   $t=2.03$ ).

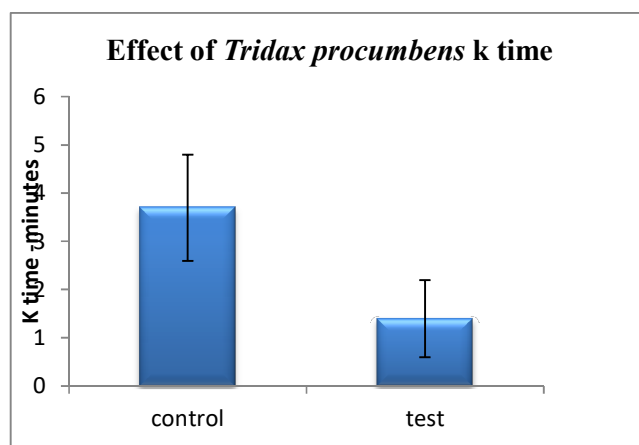


Figure 4.16 : Effect of *Tridax procumbens* on K- time

#### 4.6.4 Effect of *Terminalia brownii* on k time

Freeze dried bark extract of *Terminalia brownii* did not elicit a statistically significant difference in the means of *k* time ( $1.3 \pm 0.26$  (c) vs.  $2.2 \pm 0.6$  (t) minutes,  $t=1.47$ ,  $p=0.21$ ).

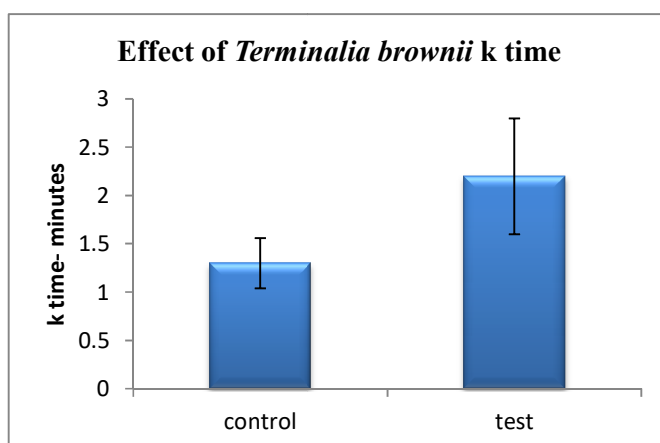


Figure 4.17: Effect of *Terminalia brownii* on k time

#### 4.6.5 Effect of *Tridax procumbens* on alpha angle

The results indicate freeze dried leaf extract of *Tridax procumbens* increase the *alpha* angle with the statistically significant difference in the means ( $43.3 \pm 6.9$  (c) vs.  $69.9 \pm 2.51$  (t) degrees,  $t=3.65$ ,  $p=0.01$ ).

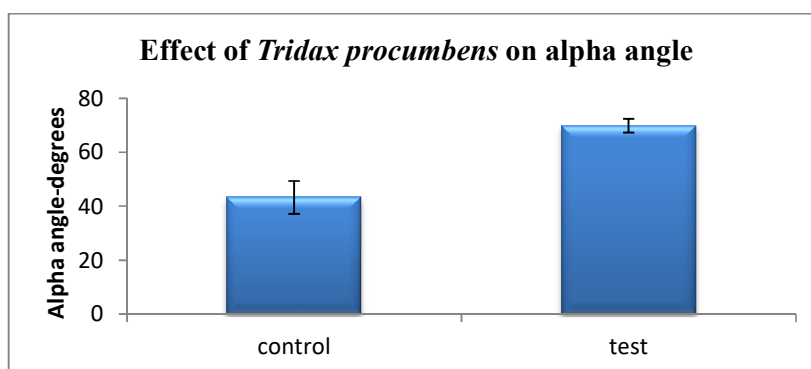


Figure 4. 18: Effect of *Tridax procumbens* on alpha angle

#### 4.6.6 Effect of *Terminalia brownii* on alpha angle

Freeze dried bark extract of *Terminalia brownii* did not elicit a statistically significant difference in the means of *alpha angle* ( $69.1 \pm 3.4$  (c) vs.  $51.3 \pm 10.8$  (t) degrees,  $t=1.6$   $p=0.08$ ).

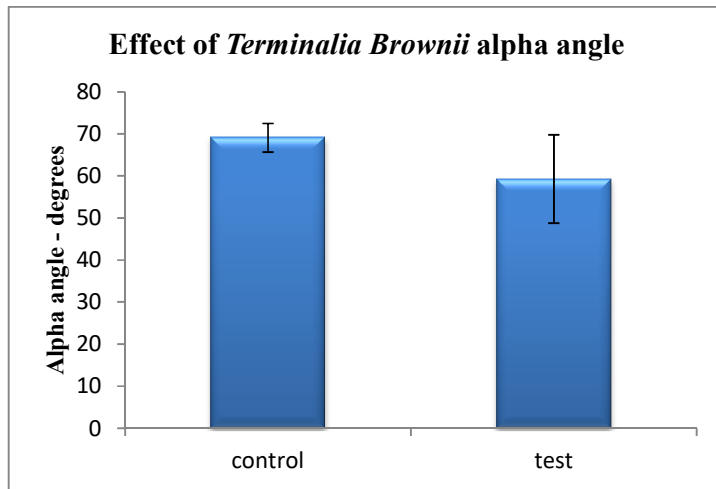


Figure 4.19: Effect of *Terminalia brownii* on alpha angle

#### 4.6.7 Effect of *Tridax procumbens* on Maximum amplitude

The results indicated freeze dried leaf extract of *Tridax procumbens* increased the *maximum amplitude* with the statistically significant difference in the means ( $62.4 \pm 5.6$  (c) vs.  $34.8 \pm 7.6$  (t) mm,  $t=3.65$ ,  $p=0.026$ ).

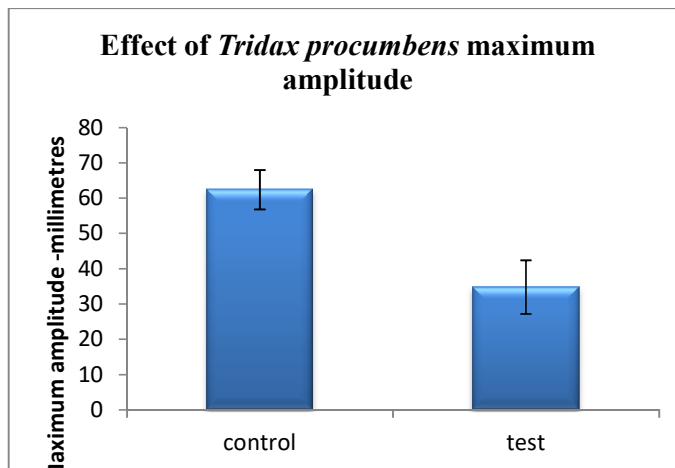


Figure 4:20 : Effect of *Tridax procumbens* on Maximum amplitude

#### 4.6.8 Effect of *Terminalia brownii* on maximum amplitude

Freeze dried bark extract of *Terminalia brownii* did not elicit a statistically significant difference in the means of *maximum amplitude* ( $61.5 \pm 2.8$  (c) vs.  $46.9 \pm 9.7$  (t) mm,  $t=1.44$ ,  $p=0.19$ ).

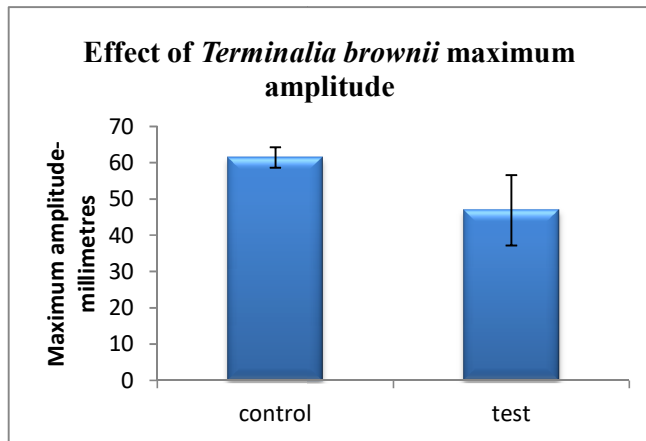


Figure 4: 21 : Effect of *Tridax procumbens* on Maximum amplitude

## CHAPTER 5: DISCUSSION

“Coagulation requires complex interactions of cellular and molecular components that mainly involve platelets, plasma and red blood cells”, (Hoffman and Monroe, 2007). Initially clotting was seen as involving intrinsic and extrinsic pathways with a common pathway at the end but lately it has been noted to be due to a balance between the pro-coagulants and anti-coagulants (Hoffman and Monroe, 2007). It involves the interaction of coagulation factors and platelets. Coagulation status can be measured by means of laboratory tests. The haemostatic effects of four Kenyan plant extracts (*Tridax procumbens*, *Terminalia brownii*, *Euphorbia Tirucalli* and *Asphillia africana*) were elucidated using five laboratory tests; bleeding time, clotting time, prothrombin time, activated partial thromboplastin time (APTT) and thromboelastography. Bleeding time assesses the capillary integrity and platelet function. Clotting time measures the time taken to generate thrombin. Prothrombin time mainly measures the effect on the extrinsic pathway and is more sensitive to factor VII. Activated partial thromboplastin time evaluates the effect of the intrinsic pathway factors.

The freeze dried leaf extract of *Tridax procumbens* significantly reduced the bleeding time. These findings confirm those of Ikese *et al.*, (2015) who found that a freeze dried extract of *Tridax procumbens* significantly decreased bleeding time. The results are also similar to the ethanolic extracts of the same plant that were shown to reduce the bleeding time (Manjusha Borde *et al.*, 2014) which reflects platelet function (Kumar *et al.*, 2013). This plant has potential activation effects on the platelets.

Freeze dried bark extract of *Terminalia brownii* reduced the bleeding time. These are new findings on this plant that have previously not been reported. Bleeding time

indicates the platelet function (Kumar et al., 2013) and therefore this plant may have activation effects on the platelets.

The freeze dried leaf extract of *Asphillia africana* increased the bleeding time. These findings were unexpected because other studies demonstrated that it reduces the bleeding time. These findings are contrary to those found by Okoli *et al.*, (2007) that showed that methanol and hexane extracts reduced bleeding time. Bleeding time indicates the platelet function (Kumar et al., 2013) and therefore this plant may have inhibitory effect on platelet function.

The freeze dried leaf extract of *Tridax procumbens* significantly reduced the clotting time. This confirms the results of a similar study in which an aqueous extract reduced the clotting time (Ikese *et al.*, 2015). The results are also similar to those of pet ether extract of the same plant that showed a significant reduction the clotting time (Manjusha Borde *et al.*, 2014). The results are also similar to those of Mayuraa *Et al.*, (2008) that showed that ethanolic leaf extracts reduced the clotting time. Sowmya B. J. *Et al.*, (2015) also demonstrated that the plant extract reduced the clotting time. Clotting time reflects time taken to generate clotting factors specifically thrombin to form a clot (Hoffman and Monroe, 2007) and therefore this plant may have activation effects to clotting factors that lead to thrombin formation.

Freeze dried bark extract of *Terminalia brownii* reduced the clotting time. These are new findings on this plant that have previously not been reported. Clotting time reflects time taken to generate clotting factors specifically thrombin to form a clot (Hoffman and Monroe, 2007) and therefore this plant could have activation effects to clotting factors that lead to thrombin formation.



The freeze dried stem extract of *Euphorbobia Tirucalli* reduced the clotting time. These are new findings on this plant that have previously not been reported. Clotting time reflects time taken to generate clotting factors specifically thrombin to form a clot (Hoffman and Monroe, 2007) and therefore this plant may have some activation effects to clotting factors that lead to thrombin formation.

The freeze dried leaf extract of *Tridax procumbens* reduced the prothrombin time. These are new findings that have previously not been reported. Prothrombin time evaluates the extrinsic pathway (Hoffman and Monroe, 2007) and therefore this plant extract may have some activation effects on the extrinsic pathway coagulation factor.

Freeze dried bark extract of *Terminalia brownii* reduced the prothrombin time. These are new findings on this plant that have previously not been reported. Prothrombin time evaluates the extrinsic pathway (Hoffman and Monroe, 2007) and therefore this plant extract may have activation effects on the extrinsic pathway coagulation factor.

The freeze dried stem extract of *Euphorbobia Tirucalli* significantly reduced the prothrombin time. These are new findings on this plant. Prothrombin time evaluates the extrinsic pathway (Hoffman and Monroe, 2007) and therefore this plant extract may have activation effects on the extrinsic pathway coagulation factor.

The freeze dried leaf extract of *Tridax procumbens* reduced the Activated partial Thromboplastin time. These are new findings that have previously not been reported. Activated partial Thromboplastin time evaluates the intrinsic pathway (Hoffman and Monroe, 2007) and therefore this plant extract has activation effect on intrinsic pathway factors.

Freeze dried bark extract of *Terminalia brownii* reduced the Activated partial Thromboplastin time. These are new findings on this plant that have previously not been reported. Activated partial Thromboplastin time evaluates the intrinsic pathway

(Hoffman and Monroe, 2007) and therefore this plant extract has activation effect on intrinsic pathway factors.

The freeze dried stem extract of *Euphorbia Tirucalli* reduced the Activated partial Thromboplastin time. These are new findings on this plant that have previously not been reported. Activated partial Thromboplastin evaluates the intrinsic pathway (Hoffman and Monroe, 2007) and therefore this plant extract has activation effect on intrinsic pathway factors.

Freeze dried extracts of *Terminalia brownii* and *Tridax procumbens* were noted to be most potent among the group and were used during thromboelastography. Thromboelastography generated four variables; *r*, *k* times, *alpha* angle and maximum amplitude. The *r* time evaluated the time of initiation of a clot of 2 mm amplitude. At 'initiation', Tissue Factor binds to circulating FVIIa and acts with FV to generate FIXa and FXa. The *k*- time evaluated the time taken for clot amplitude of 2 mm to reach 20 mm. This involves the interaction of clotting factors and platelets. During 'amplification' of a clot thrombin triggers reactions on the surface of activated platelets, where more FVIIa is produced. Thrombin activates co-factors FV and FVIII. The *alpha* angle measured the speed of clot strengthening. This mainly involves interaction of fibrin and platelets. The maximum amplitude measures the ultimate clot strength. This mainly involves platelet function. In hypercoagulable state, the *r* and *k* times are decreased while *alpha* angle and the maximum amplitude are increased.

The freeze dried leaf extract of *Tridax procumbens* significantly reduced the *r*-time, *k*-time and maximum clot amplitude but increased the *alpha* angle. These are novel findings that have previously not been reported. Thus the plant extract acts on the clotting factors mainly in extrinsic pathway and does not have much effect on the platelets.

The main factor in the initiation of clot formation in the extrinsic pathway is factor VII (Hoffman and Monroe, 2007).

Freeze dried bark extract at a dose of 2.5 mg/ml of *Terminalia brownii* increased the r-time, k-time and the maximum amplitude but it decreased the alpha angle through none of the changes were statistically significant. Doses of 10 mg/ml, 7.5 mg/ml and 5 mg/mg all caused very rapid coagulation that indicated a straight line on thromboelastography. The fact that it the lower dose produced opposite results may be due to disproportional interaction of the extract molecules and the coagulation factors. This is the first time that this study has been done on this plant.

The limitation of the study was that the platelet aggregation test that would have indicated the effect of *Tridax procumbens* on coagulation was not possible because of time and logistical considerations.

### **Conclusion**

Freeze dried leaf extracts of *Tridax procumbens* and freeze dried bark extract of *Terminalia brownii* have some activation effect on the platelets and have stimulatory effect on the capillary muscles. Freeze dried extract of *Asphillia africana* has anticoagulant effect contrary to traditional perceptions of it being pro-coagulant. Freeze dried leaf extract of *Tridax procumbens*, freeze dried stem extract of *Euphorbia tirucalli* and freeze dried bark extract of *Terminalia brownii* have some activation effect on both extrinsic and intrinsic coagulation pathways.

Freeze dried leaf extract of *Tridax procumbens* has more effect on factor VII which is involved in the initiation of clot formation. *Tridax procumbens*, *Terminalia brownii* and *Euphorbia tirucalli* are potential plants for development of drugs that can be used to reduce bleeding.

The present research recommends that further studies on *Tridax procumbens* to assess the active molecule in coagulation which can further be evaluated for drug development. Platelet aggregation test for the freeze dried extracts should also be carried out to verify the low platelet effect seen in thromboelastography.

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