

# In-vitro studies of *Bryophyllum pinnatum* Crude Extract on Blood Coagulation Indices: An Investigation on its Traditional Medicine Use

Promise Madu Emeka<sup>1,2,\*</sup>

## ABSTRACT

**Background:** *Bryophyllum pinnatum* extract is used by African natives to stop epistaxis [nose bleeding], postpartum bleeding as well as fresh wounds bleeding. **Aim:** This study was carried out in order to evaluate the coagulant effects and properties of crude *B. pinnatum* extract using *in-vitro* blood coagulation screening tests. **Materials and Methods:** Healthy volunteers were used for the study and the following tests were performed: prothrombin time (PT), Partial Thromboplastin Time with Kaolin (PTTK), Thrombin Time (TT), Euglobulin Lysis Time (ELT), bleeding time (BT) and whole blood coagulation time (WBCT). Blood samples were collected from adult male and female volunteers by trained phlebotomist from the thumb and venous puncture depending on the type of coagulation test. **Results:** *B. pinnatum* extract produced a profound decreased in PT, PTTK, TT and ELT times which were concentration dependent. Extract concentrations of 84 mg/ml produced a highly significant reductions ( $p < 0.05$ ) in PT, PTTK and TT with  $1.68 \pm 0.16$ ,  $7.1 \pm 0.28$ ,  $1.06 \pm 0.19$  sec respectively. Whole blood coagulation time was decreased by 84mg/ml concentration but not significantly. Results also showed that bleeding time was significantly reduced by 84 mg/ml concentration of *B. pinnatum* extract. The effect on plasma showed that the extract had coagulant property without the addition of activating agent to the platelet-poor plasma. **Conclusion:** This investigation confirms the traditional use of this plant extract in the treatment of bleeding episodes, indicating that the extract has blood coagulant properties and therefore could potentially be used in fibrinogen abnormality, deficiency of factors XII, XI, IX, or VIII, heparin in blood.

**Key words:** *Bryophyllum pinnatum*, Coagulation, Blood, Extract, Plasma, Bleeding.

Promise Madu Emeka<sup>1,2,\*</sup>

<sup>1</sup>Department of Pharmaceutical Sciences, College of Clinical Pharmacy, King Faisal University, Hofuf, SAUDI ARABIA.

<sup>2</sup>Department of Pharmacology, College of Medicine, University of Lagos- Lagos, NIGERIA.

## Correspondence

Promise Madu Emeka

Department of Pharmaceutical Sciences, College of Clinical Pharmacy, King Faisal University, Hofuf, SAUDI ARABIA.

Phone no: +966503239033

Email id: pemeka@kfu.edu.sa

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## INTRODUCTION

Herbal remedies are used worldwide for the treatment of variety of diseases, including blood-related disorders. They are often perceived as effective and safe because they are of natural origin,<sup>[1]</sup> and their uses are reported to be on the rise.<sup>[2]</sup> It is reported that nearly one in six adults taking prescription drugs in the United States is concomitantly using at least one herbal remedy.<sup>[3,4]</sup> Medicinal plants are widely used in Nigeria as alternatives to conventional medicines,<sup>[5]</sup> and despite the availability of modern medicines in the treatment of ailments; people still continue to resort to the use of herbal medicines.<sup>[6]</sup> WHO,<sup>[7]</sup> reports that 85% of the sub-Saharan Africa is still dependant on the use of herbal medicinal preparation. The economic situation, high cost of modern drugs and easy accessibility to traditional medicine practitioners drives the trend to traditional medicine.<sup>[8]</sup> Efforts have been made by various workers to discover natural products which have antiplatelet,<sup>[9]</sup> anticoagulant,<sup>[10]</sup> antithrombotic,<sup>[11]</sup> and thrombolytic activity. Coagulation is a complex process by which blood forms clots. It is an important part of hemostasis.<sup>[12]</sup> Disorders of coagulation can

lead to increased risk of bleeding (hemorrhage) or clotting (thrombosis). In patients with advanced liver disease, bleeding and thrombosis are dangerous complications particularly in those challenged by infection or require surgery.<sup>[12]</sup> Reports have shown that the products of traditionally used plants such as *B. pinnatum* promote healing in experimental animals.<sup>[13]</sup> One of the most popular and commonly used herbal preparations in Africa is *Bryophyllum pinnatum* (Crassulaceae). *B. pinnatum* was first introduced in 1970 at a German complementary/alternative medicine (CAM) centre as treatment for premature labour.<sup>[14]</sup> Clinical outcome in terms of inhibitory activity of the plant product was reported to be similar to those of Fenoterol with its administration reportedly to being a bonus during delivery if contractions are too strong, frequent or painful.<sup>[14]</sup> It is a succulent perennial herb 0.60 – 1.20m high, of Madagascan origin, but spread as an anthropogenic plant tropically. It is naturalized in the territories of the region from Sierra Leone to Southern Nigeria,<sup>[15]</sup> but has not

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penetrated into the drier states. The plant is used in folkloric medicine in tropical Africa, tropical America, India, China and Australia.<sup>[16]</sup> The character of this plant has naturally attracted a lot of different descriptive English names, like, “Never – die, resurrection plant, air-plant, life plant due to the viviparous and tenacious properties of the plant. These generic names appear also to allude to the leaf vivipary: bruo, from Greek meaning, to be full of, or to burst forth, and ‘phullon’, a leaf.<sup>[17]</sup>

The leaves and bark of *B. pinnatum* serve as bitter tonic, an astringent to the bowels, which is useful in the treatment of diarrhea and vomiting.<sup>[18,19]</sup> In traditional medicine, the leave extracts of the plant have been used as antimicrobials,<sup>[20]</sup> antihypertensive.<sup>[21,22]</sup> The leaf extracts have also been shown to have neurosedative and muscle relaxant properties by Yemitan and Salahdeen.<sup>[23]</sup> In East Africa, the slightly heated leaves (heated over fire) are rubbed over the body to treat stiff joint and rheumatism.<sup>[15]</sup> Alkaloids and saponins are present in the aqueous and alcoholic extracts of leaves and lectins in the juice from the fresh leaves.<sup>[24]</sup> The green callus of the plant contains malic acid, quinones and tocopherol.<sup>[25]</sup> The plant is reported to be rich in micro and macro elements, vitamins, calcium, phosphorus, ascorbic acid, insulin.<sup>[26]</sup> The wide range use traditionally, of the plant justifies its being called “life plant”, “resurrection plant”, or “good luck”.<sup>[27]</sup> Most of the users of *B. pinnatum* herbal preparations take it as decoction and in treatment of certain diseases such as wound, bruises and nose bleeding. Its effects on blood coagulation have not been investigated since the plant is also used in stopping bleeding and in wounds. Therefore, the present work was undertaken to evaluate the coagulant effects/properties of *B. pinnatum* crude extract using various *in-vitro* tests.

## MATERIALS AND METHODS

### Collection of Plant

*Bryophyllum pinnatum* plant was collected from Ilorin, Kwara State of Nigeria. A group of research Scientists in the Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, identified the plant.

### Preparation of Extract

18.90g of fresh *B. pinnatum* leaves were weighed out and squeezed to extract the juice thoroughly from the plant. The following procedure was used for the calculation of the concentration of the extract.

$$\text{Concentration} = \frac{\text{Initial weight of leaves} - \text{Final weight of leaves}}{\text{Final volume of extract}}$$

Where, Initial weight of leaves = 18.90g  
Final weight of leaves extract = 12.70g  
Final volume of extract = 14.92ml

$$\text{Concentration} = \frac{18.90 - 12.70}{14.92} = \frac{6.11}{14.92} = 0.41 \text{ g/ml} = 415.5 \text{ mg/ml}$$

Volumes and concentrations of extract used where as follows 0.05 ml (21mg/ml), 0.1ml (42mg/ml) and 0.2 ml (84mg/ml).

### Ethical Issues

Students’ volunteers (18-30 years) male and female were used for the study who indicated their willingness to participate freely to advance Science. Their informed consent was obtained according to the Declaration of Helsinki. Authorization to conduct the study was obtained from the Deanship of Basic Medical Sciences, Department of Pharmacology, College of Medicine Ethical Committee, The University of Lagos, Idi-araba, Lagos. (980702012/2002). Volunteers were accepted based

on their good general health, which included normal blood count and physiological parameters. Blood samples which appeared haemolysed and turbid were discarded and those who were icteric according to the examining physician were not allowed to participate in the study.

### Blood Sample collection

A phlebotomist, assisted in blood collection, collected Blood samples. Different methods were used in the collection of blood samples depending on the type of test, but most were collected by finger pricking and tests were all *in-vitro*.

### Prothrombin Time (PT)

The method of Quick,<sup>[28]</sup> was used in determining PT. To a 0.05ml (21mg/ml) of fresh *B. pinnatum* extract was added 0.1ml of normal plasma and 0.2ml of calcium thromboplastin reagent. The time of appearance of fibrin clot was recorded as PT. Another experiment was set up with the same parameters as above but with different concentrations of fresh *B. pinnatum* extract. For the control experiment, 0.05ml of normal saline was used in place of *B. pinnatum* extract.

### Partial Thromboplastin time with kaolin (PTTK)

Into a glass tube, containing 0.1ml of normal plasma in a water bath was added 0.2ml of a well –shaken kaolin phospholipids mixture. The plasma kaolin phospholipids mixture was left in the water bath at 37°C with occasional shaking. The procedure was timed with the aid of a stopwatch. 0.1ml of calcium chloride solution and 0.05ml (21mg/ml) of the extract of *B. pinnatum* were added to the mixture 10 min later. The time of appearance of a fibrin clot was recorded (PTTK) with the aid of a stopwatch. In the control experiment, 0.05ml of normal saline was used in place of *B. pinnatum* extract.<sup>[29,30]</sup>

### Thrombin Time (TT)

Determination of Thrombin time was according to the method of Hardisty and Ingram.<sup>[31]</sup> To a mixture of 0.05ml (21mg/ml) fresh *B. pinnatum* extract and 0.1ml normal plasma in a clotting tube was added 0.2ml of thrombin solution and the time of appearance of a fibrin clot was recorded as TT. 0.05ml of normal saline was used in place of the *B. pinnatum* extract in the control experiment.

### Euglobulin Lysis Test (ELT)

A slightly modified method of Blix,<sup>[32]</sup> was used in the determination of ELT. Three centrifuge tubes were set up. The first had 0.5ml fresh normal plasma, the second is a mixture of 0.2ml (84mg/ml) of *B. pinnatum* extract and 0.5ml of normal plasma while the third, 0.1ml of normal saline mixed with 0.5ml normal plasma. Into each of these was added 9.01ml distilled water and 0.1ml of 1% of acetic acid and the set up was kept at 4°C for 30 min. Each tube was centrifuged, solution carefully decanted and 0.5ml of borate saline buffer added to the deposit. The mixture was kept at 37°C adding 0.5ml of calcium chloride solution after noting the time of formation of a clot, time of complete lysis of the clot was recorded (ELT).

### Whole Blood Coagulation Time

The modified method of Lee and White as described by de Brito Sousa *et al.*<sup>[33]</sup> Was use in this. evaluation. 1ml each of second stream blood was placed in four cleaned dry standard test tube in a 37°C water bath. Each tube was tilted ½ minute (30 sec) Until they can be inverted without spilling any blood and the time from collecting the blood to tilting for each tube, was noted using a stopwatch. The coagulation time for each tube was recorded separately and the average of the 3 tests was taken as the coagulation time. In another experiment, 0.05ml, 0.1ml and 0.2ml of

the test extract (*B. pinnatum*), representing 21, 42 and 84 mg/ml respectively were added into 3 different standard test tubes each containing 1ml of blood and using the same method of blood sample collection and the time for clotting was noted.

### Bleeding Time (BT)

Bleeding time was observed at 30 sec intervals from a thumb prick, blood was absorbed gently by touching the blood with filter paper, until bleeding ceased. The blots were collected in series along the edge of the filter paper; the numbers of blots divided by 2 gave the bleeding time (BT). Bleeding time was carried out to check the effect of *B. pinnatum* extract on the pricked thumb with a lancet. The fresh crude *B. pinnatum* extract was immediately applied on the pricked thumb and the above procedure was followed and BT was recorded.

### Statistical analysis

Data is presented as mean  $\pm$  SEM and the number of repeats is denoted as n. Statistical analysis was performed using GraphPad Prism software version 8.2 (San Diego, CA, USA). Comparisons were done between control and *B. pinnatum* crude extract treated groups using the one-way analysis of variance. The differences between the individual test groups were determined using the Tukey's multiple comparison test, hence,  $p < 0.05$  was considered statistically significant.

## RESULTS

### Prothrombin Time (PT)

In this investigation, the results as presented in Table 1 show that with different extract concentrations of *B. pinnatum* of 21mg/ml and 42mg/ml gave a highly significant reduced PT ( $p < 0.05$ )  $4.9 \pm 0.37$  and  $1.68 \pm 0.16$  sec respectively, normal range for PT being 10-14secs. Table 1). The prothrombin time test is a non-specific indicator of the extrinsic blood coagulation mechanism. Deficiencies of Prothrombin and factors, V, VII and X give rise to a prolonged time, as well as the presence of heparin in the blood and hypofibrinogenaemia

### Partial Thromboplastin Time with Kaolin (PTTK)

Partial Thromboplastin Time with Kaolin (PTTK) results show that with varying concentrations of extract *B. pinnatum*; 21mg/ml and 42mg/ml gave a highly significant reduced PTTK ( $p < 0.05$ ) respectively (Table 1).

### Thrombin Time (TT)

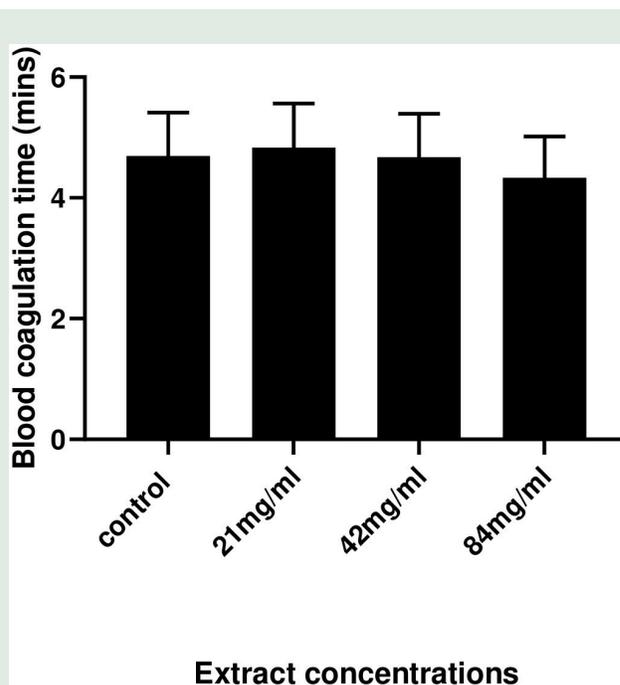
The results obtained for Thrombin Time (TT) in this study, showed that 21 and 42 mg/ml of the extract of *B. pinnatum* also produced a highly significant decreased ( $p < 0.05$ ) TT compared with the control with all concentrations used (Table 1).

### Euglobulin Lysis Time (ELT)

The result obtained from this investigation shows that concentrations of 84mg/ml concentration of *B. pinnatum* extract produced a significant reduction ( $p < 0.05$ ) in ELT (Table 1).

### Whole Blood Coagulation Time

The results for whole blood coagulation time as shown in Figure 1 indicated that the effect of extract of *B. pinnatum* on whole blood coagulation compared to the normal time was not significantly reduced. The extract in concentrations of 84mg/ml was however reduced by 0.36 mins. In this study, when varying volumes 0.05ml, 0.1ml and 0.2ml of *Bryophyllum pinnatum* extract representing 21, 42 and 84 mg/ml concentrations were added to plasma only gave concentration dependent reductions in minutes respectively. Inferring that the extract has coagulant properties independent of the activating agents (Figure 1).



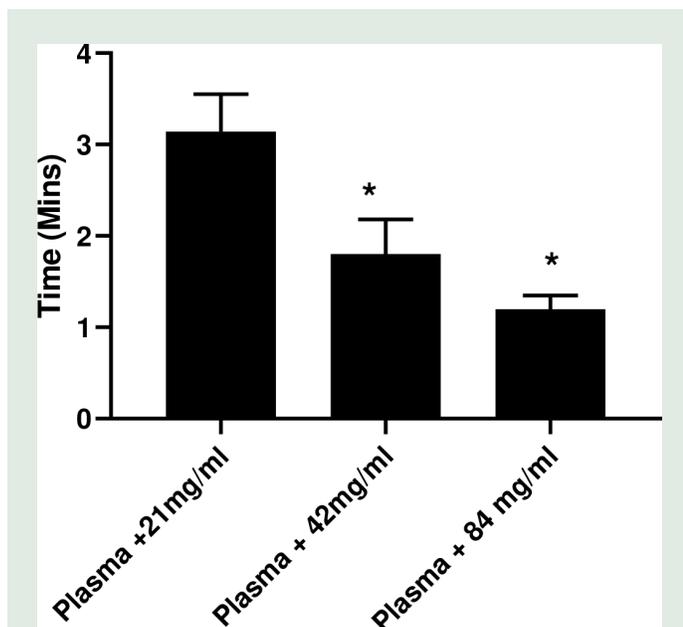
**Figure 1:** Effect of different concentrations of crude extract of *B. pinnatum* on whole blood coagulation time. Showing the effect of crude extract of *B. pinnatum* concentrations, 21, 42 and 84 mg/ml, on whole blood coagulation time, with 84 mg/ml concentration showing a reduction in human whole blood coagulation time. Values represent Mean  $\pm$  SEM, n=5.

**Table 1:** Effect of *B. pinnatum* concentrations on basic coagulation screening tests.

TEST	Normal Saline + Plasma + Reagent (control)	Plasma + Reagent	21mg/ml Extract + Plasma + Reagent	42mg/ml Extract + Plasma + Reagent	84mg/ml Extract + Plasma + Reagent
PT (sec)	13.4 $\pm$ 0.13	13.4 $\pm$ 0.049	4.9 $\pm$ 0.37*	1.68 $\pm$ 0.16*	-
PTTK (sec)	42.5 $\pm$ 0.045	43.0 $\pm$ 0.102*	30.5 $\pm$ 0.60*	7.1 $\pm$ 0.28*	-
TT (sec)	11.2 $\pm$ 0.068	10.7 $\pm$ 0.106*	3.2 $\pm$ 0.2*	1.06 $\pm$ 0.19*	-
ELT (min)	96.5 $\pm$ 1.05	93.1 $\pm$ 1.15*	-	-	81.0 $\pm$ 1.3*

Values represent Mean  $\pm$  SEM, n=8 \*  $P < 0.05$  compared to control

Effects of different concentrations of *B. pinnatum* crude extract on basic coagulation *in-vitro* screening tests: PT (Prothrombin time) in sec, PTTK (Partial Thromboplastin Time with Kaolin) in sec, TT (Thrombin Time) in sec, and ELT (Euglobulin lysis time) in mins. Values represent Mean  $\pm$  SEM, n=8. \* represents  $p < 0.05$  compared to control as significant.



**Figure 2:** Effect of crude extract of *B. pinnatum* concentrations on plasma only coagulation

Showing the effect of crude extract of *B. pinnatum* concentrations, 21, 42 and 84 mg/ml, on plasma only coagulation, with 42 and 84 mg/ml concentrations showing significant reductions in plasma clotting time. Values represent Mean  $\pm$  SEM, n = 8. \* represents  $p < 0.05$  compared to control.

### Plasma clotting time

In this study, plasma clotting time was examined with different concentrations of *B. pinnatum* extracts (21, 42 and 84 mg/ml) were added to plasma only. The clotting time was recorded in minutes. Concentrations of 42 and 84 mg/ml crude extract of *B. pinnatum* significantly reduced plasma-clotting time, as shown in Figure 2. Inferring that the extract has coagulant properties independent of the activating agents.

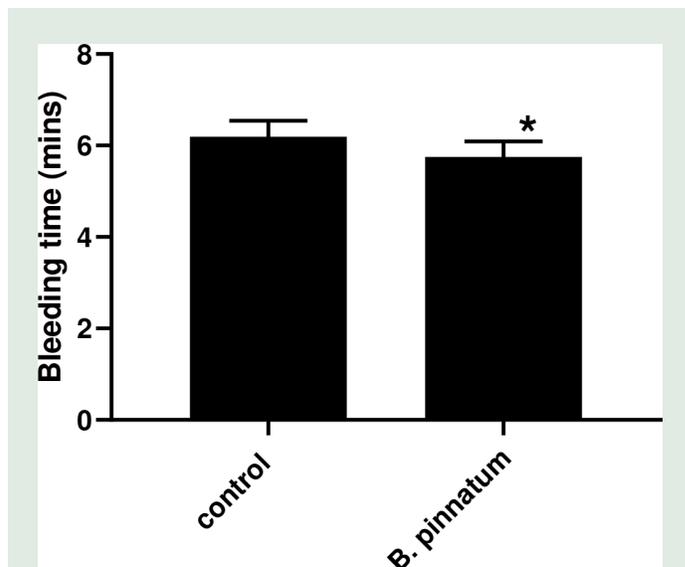
### Bleeding Time (BT)

The results of extract *B. pinnatum* on bleeding time was performed on both whole blood as presented in Figure 3, produced a significant reduction with a  $p$  value of 0.0243. This means that there is a significant difference between *B. pinnatum* treated and control with a reduction in bleeding time by 0.44mins (Figure 3).

## DISCUSSION

The measurement of bleeding time, prothrombin time, thrombin, time, partial thromboplastin time with kaolin, Euglobulin lysis time are all screening tests of haemostasis. These are non-specific tests designed to assess overall haemostatic function and which are useful for the screening of patients who may have a bleeding disorder. In this present study, *B. pinnatum* crude extract was investigated for its coagulating properties.

The prothrombin time test is a non-specific indicator of the extrinsic blood coagulation mechanism. Deficiencies of prothrombin and factors V, VII and X give rise to a prolonged bleeding time, as well as the presence of heparin in the blood and hypofibrinogenaemia. The present investigation using *B. pinnatum* crude extract, produced a reduced PT, indicating that the extract, has the potential to produce coagulation in a shorter time. Normal range of PT is 10-14secs.<sup>[34]</sup> Partial Thromboplastin Time with Kaolin (PTTK), also known as the Activated Partial Thromboplastin



**Figure 3:** Effect of *B. pinnatum* concentration (84mg/ml) on human bleeding time.

Showing the effect of crude extract of *B. pinnatum* concentration, 84 mg/ml on human bleeding time, showing significant reduction in human bleeding time. Values represent Mean  $\pm$  SD, n = 8 with a \* $P$  value of 0.0243 compared to control.

Time (APTT) and the Kaolin – Cephalin Clotting Time (KCCT), is another blood screening test. It is a simple screening test for first stage plasma clotting factor deficiencies. When the results fall within normal range, it indicates normal clotting function. Reports has shown that PTTK is prolonged in deficiencies of factors XII, XI, X, IX, VIII, V or II.<sup>[35]</sup> However, a normal PTTK result can be obtained with factor VII deficient plasma. Shorter than normal times have been reported in hyper-coagulable states as well as in cancer patients who are reported to develop shortened PTTK time. A condition of shortened PTTK, could make surgery difficult. The test is more useful in the detection of hemophilia A and B (Christmas disease).<sup>[36]</sup> Severe hemophilia is usually described as frequent bleeding into the joints, muscles and soft tissues without any known cause. Also, patients with hemophilia can suffer from bleeding episodes that is life-threatening, like intracranial hemorrhages.<sup>[37]</sup>

In this study, we found that crude extract of *B. pinnatum* reduced PTTK time, indicating an activation of coagulation. Likewise, Ahmed *et al.*<sup>[34]</sup> Reported that APTT normal time to be 34 sec, *B. pinnatum* crude extract gave a  $30.5 \pm 0.60$  and  $7.1 \pm 0.28$  sec respectively for 21 and 42 mg/ml concentrations.

Common causes of prolonged thrombin time (TT) include the presence of heparin, fibrinogen/fibrin degradation products, or depletion of fibrinogen. In chronic liver disease, the thrombin time is often prolonged and the clots are transparent and bulky. The defect is caused by abnormalities of fibrin polymerization. Abnormally polymerization is also the cause of a long thrombin time in some congenital dysfibrinogenaemia and in multiple myeloma. The thrombin time is usually a few seconds longer in plasma from new-born infants than in normal adult plasma.<sup>[38]</sup> In this study, we observed a significantly reduced TT with extracts of *B. pinnatum*. ( $1.06 \pm 0.19$  sec) compared to the normal range of 17.3 sec.<sup>[34]</sup> Euglobulin Lysis Time (ELT) test measures predominantly plasminogen activator activity. The time for this activity was reduced by crude extract of *B. pinnatum*. Bleeding time is often prolonged in the condition of thrombocytopenia. A prolonged bleeding time is more likely with

low platelet count due to defective bone marrow production than due to increased platelet deficiency. Other common cause of acquired platelet dysfunction is aspirin ingestion. Documented evidence show that aspirin could result in prolongation of the bleeding time up to 20 min, and therefore a lower dose can affect the bleeding time.<sup>[39]</sup> In this investigation, results show that different concentrations of the extract of *B. pinnatum*, non-significantly though, decreased the coagulation time compared with the control. However, the bleeding time was significantly reduced by the highest concentration of 84 mg/ml, with a time of  $5.75 \pm 0.342$ . Bleeding and coagulation time tests are done to detect hemostatic disorders and when they are normal, it excludes hemostatic disorders.<sup>[40]</sup> The extracts of *B. pinnatum* could be evaluated for use in hemostatic disorders such as uncontrolled bleeding. This study further supports the use of this plant extract in traditional medicine in stopping bleeding episodes.

## CONCLUSION

The findings of the current study indicate that, the use of crude extract of *B. pinnatum* in bleeding episodes by traditional healers, has some merit and is well founded by the results presented here. Therefore, this plant extract has the potential to decrease bleeding time and affect overall blood coagulation process.

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## CONFLICT OF INTEREST

The author declares no conflict interest with this manuscript.

## ABBREVIATIONS

**APTT:** Activated Partial Thromboplastin Time; **BT:** Bleeding time; **ELT:** Euglobulin Lysis Time; **KCCT:** Kaolin – Cephalin Clotting Time; **PT:** Prothrombin time; **PTTK:** Partial Thromboplastin Time with Kaolin; **TT:** Thrombin Time; **WBCT:** Whole blood coagulation time.

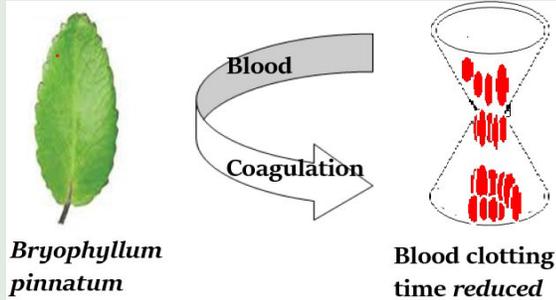
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### GRAPHICAL ABSTRACT



### SUMMARY

The measurement of bleeding time (BT), prothrombin time (BT), thrombin time (TT), partial thromboplastin time with kaolin (PTTK), Euglobulin lysis time (ELT) are all screening tests designed to assess overall haemostatic function and which are useful to screen patients who may have bleeding disorders. *B. pinnatum* (Crassulaceae), is one of the commonly used herbal preparations employed by traditional healers to stop bleeding. The aim of this study was to evaluate the coagulant properties of *B. pinnatum* crude extract using in-vitro screening tests. The results confirmed that *B. pinnatum* extract exhibited coagulant properties; an action that is compatible with its use as a haemostatic agent. This coagulant effect of *B. pinnatum* is not limited to either the intrinsic or common pathways only. The results show that the addition of different concentrations of *B. pinnatum* crude extract to normal plasma gave a highly significant difference ( $p < 0.05$ ) time difference compared to the control in PT, PTT, TT and ELT screening tests.

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