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SHORT COMMUNICATION

Extracts of Selected Plants and Fruits Profoundly Modulate *In Vitro* Coagulation Activity

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Abstract

Background: Plant products treat many diseases and are an important resource for pharmaceutical companies developing new medicines. Hemostasis maintains blood in the fluid state and prevents its loss from damaged blood vessels. Plants have been reported to be sources of anticoagulant and antithrombotic substances and are employed in the management of coagulation disorders. **Aim:** This study investigates the effects of aqueous and ethanolic extracts of bitter leaves, mango leaves, drumstick leaves, pineapples and tomatoes on *in vitro* coagulation activity. **Methods:** Aqueous and ethanolic extracts of leaves of *Mangifera indica*, *Moringa oleifera*, *Vernonia amygdalina*, and fruits of *Ananas comosus* and *Solanum lycopersicum* were prepared using the cold maceration method and lyophilized. The freeze-dried extracts were prepared into four concentrations. Platelet-poor plasma was obtained from ten healthy volunteers. Prothrombin Time (PT) and activated Partial Thromboplastin Time (aPTT) were determined by visual detection of clot formation. The mean and standard deviation were determined and compared with the controls. **Results:** PT values for females were higher for aqueous and ethanolic extracts except for *A. comosus*. Female aPTT values were reduced compared to males except for *V. amygdalina*. There were no changes in PT and aPTT across the concentration gradient. PT control values significantly differ, however, there were no significant differences in aPTT control values between genders. This study revealed that PT and aPTT values differ significantly between genders. **Conclusion:** *Mangifera indica* and *M. oleifera* have procoagulant activity, *A. comosus* has anticoagulant activity, while *V. amygdalina* does not affect coagulation time.

Keywords: Activated Partial Thromboplastin Time, *Ananas comosus*, *Mangifera indica*, *Moringa oleifera*, Prothrombin Time, *Vernonia amygdalina*

1. Introduction

Natural products, mostly plants, have been used extensively in treating many diseases since ancient times; these plant-derived extracts and compounds have played significant roles in disease management. About 35% of global medicine originated directly or indirectly from natural products, including plants. These natural-derived products constitute a very important resource for pharmaceutical companies developing new medicines[1]. Hemostasis is the physiological process by which blood is maintained in the fluid state, and excessive blood loss is prevented from damaged blood vessels through clot formation[2]. It occurs in a cascade of events involving the blood vessels, platelets and clotting factors[3,4]. Any derangement in the hemostatic pathway results in excessive bleeding or clotting, often caused by the deficiency or low activity of one or more clotting factors[2,5].

Impaired clot formation can be genetic, as in haemophilia, or acquired, as in liver disease, with numerous investigations to assess the hemostatic status, including the PT, which assesses the extrinsic pathway and the aPTT, which assesses the intrinsic

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pathway[6,7]. Coagulopathies are one of the leading causes of death globally, there are effective yet expensive drugs such as warfarin and heparin for therapy, but they are relatively toxic and interact with numerous other drugs and food with/producing/resulting in adverse effects ranging from dizziness to severe bleeding, thus, the need to provide less toxic and more affordable treatment options[8-10].

Plants are alternative sources of anticoagulant and antithrombotic substances for the treatment of coagulation disorders because they are rich in many phytochemical compounds with different bioactivities[11,12]. Some of these plants include pineapple, tomato, mango, moringa and bitter leaf. Pineapple (*Ananas comosus*) contains an enzyme, bromelain, therapeutic doses of which are used as a dietary supplement for reducing excessive blood coagulation[13]. Mango (*Mangifera indica*) leaves have been found useful in vitiated conditions of hemorrhage[14], drumstick (*Moringa oleifera*) leaves have been reported to have blood clotting properties[15], and bitter leaves (*Vernonia amygdalina*) have been used in the management of excessive blood loss[16]. Hence, it is imperative to study these plants as potential sources of substances for managing coagulation disorders since plants give hope for future use of organic substances that are more affordable and less toxic[17]. Therefore, this study investigates the effects of aqueous and ethanolic extracts of bitter leaves, mango leaves, drumstick leaves, pineapples and tomatoes on *in vitro* anticoagulant activity.

2. Materials and Methods

2.1 Ethical Considerations

This study was approved by Bowen University, Iwo, Osun State, Nigeria. Ethical approval was obtained from the Institutional Review Board and each participant signed a consent form to willingly participate in the study.

2.2 Source of Plants

Five plants and fruits were used for this research, namely: *M. indica* (mango), *M. oleifera* (drumstick) and *V. amygdalina* (bitter leaf) leaves, as well as *Solanum lycopersicum* (tomato) and *A. comosus* (pineapple) fruits. The fruits were bought from the local market in Iwo, while the leaves were obtained within Bowen University, Iwo premises. For *A. comosus,* the core, peel and pulp parts of the fruit were used. A botanist and pharmacognosist authenticated the identity of the plants and fruits at Bowen University.

2.3 Preparation of Plant and Fruit Extracts

The extraction procedure followed the cold maceration method with slight modifications[18,19]. The fruits and leaves were properly washed under running tap water for the aqueous extracts. The leaves were then dried in an oven at 45ºC for 4 hours before blending into fine powder. The fruits were cut into chunks and blended with water in a ratio of 1:16, after which they were properly filtered using a muslin cloth. Fifty grams of the leaves were suspended in 800ml of deionized water for 72 h with frequent agitation. At 72 h, the mixture was sieved with a muslin cloth. The filtrates were centrifuged at 4000rpm for 10 min and refrigerated overnight at 4ºC before lyophilization with Clifton freeze drier.

For ethanolic extracts, 50g of pulverized leaves were soaked in 800ml of 80% ethanol in a ratio of 1:16, while the blended mixture of fruits was soaked in the same ratio and left for 72 hours with frequent agitation. The crude extract was filtered through muslin cloth and centrifuged for 10 min at 4000rpm. Before lyophilization, the residual solvent was evaporated using a rotary evaporator and then refrigerated at 4ºC overnight.

For lyophilization, the extracts were frozen so that the water became ice. Under a vacuum, the ice sublimes directly into water vapour, drawing off the water vapour. Once the ice is sublimated, the extracts are freeze-dried and can be removed from the machine[20]. Each freeze-dried extract was made into a concentration of 2mg/ml in Phosphate-Buffered Saline (PBS) and thereafter diluted to 1mg/ml, 0.5mg/ml, 0.25mg/ml and 0.125mg/ml of aqueous and ethanolic extracts of *V. amygdalina, M. indica, M. oleifera, S. lycopersicum* as well as *A. comosus* core, peel and pulp.

2.4 Recruitment of Subjects and Blood Collection

This study is an *in vitro* assessment of the extracts' capabilities on the intrinsic and extrinsic activity of the coagulation pathway. Ten healthy volunteers were recruited, comprising five males, and five females, each

filled out and signed a consent form to participate in the study willingly. Ten millilitres of blood was collected from each subject and dispensed into a 3.2% trisodium citrate anticoagulant bottle at a ratio of 9:1 for PT and an aPTT assay. Platelet-poor Plasma (PPP) was obtained by centrifuging the blood samples at 1500rpm for 15 min.

2.5 Coagulation Tests

TEClot Diagnostic PT and aPTT test kits were used for the analysis. The tests were carried out manually as described by Cordier *et al.*[21]. For the PT test, 50µL of PPP was added to 50µL of the varying concentrations of extracts (1mg/ml, 0.5mg/ml, 0.25mg/ml and 0.125mg/ml). The mixture was incubated at 37ºC for 5 min. 200µL of PT reagent prewarmed at 37ºC was added to the PPP-extract mixture, and a stopwatch was started. The time taken for clot formation was recorded in sec.

For the aPTT assay, 100µL of aPTT reagent was added to 100µL of PPP-extract mixture. At one minute of incubation at 37ºC, 100µL of calcium chloride prewarmed at 37ºC was added to the mixture, and a stopwatch was started. The time taken for clot formation was recorded in sec. Fifty microliters of PPP and 50µL of PBS were used for the aqueous control, while 50µL of PPP and 50µL of phosphate-buffered ethanol were used for the ethanolic control.

2.6 Data and Statistical Analysis

Mean, and standard deviation at each extract concentration were calculated for the 10 participants using SPSS version 25 at $p<0.05$ significance level. The mean obtained for each extract concentration was compared with the mean obtained from the control using Student's T-test and the p-values were recorded. The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology.

3. Results

Prothrombin time and INR levels for females were higher than males for aqueous and ethanolic extracts of *V. amygdalina, M. indica, M. oleifera and S. lycopersicum* but not for any *A. comosus* extracts (Tables 1 and 2). However, female aPTT values were reduced compared to male values for all extracts except for *V. amygdalina* extracts (Tables 3 and 4). There were also no changes in PT, INR and aPTT values across the concentration gradient. PT and INR control values differ significantly, however, there were no significant differences ($p<0.05$) in aPTT control values between genders.

Furthermore, there were significant differences between gender for PT and INR values for both extracts of *V. amygdalina* and aqueous extract of *S. lycopersicum*. In contrast, there were no significant differences between gender for PT and INR values for all extracts of *A. comosus* and ethanolic extract of *M. oleifera*.

In addition, there were significant differences between genders for aPTT values for extracts of *M. oleifera* and *S. lycopersicum,* as well as aqueous extracts of *V. amygdalina*, *A. comosus* core and ethanolic extract of *M. indica*. However, there is no significant difference between genders for aPTT values of ethanolic extracts of *V. amygdalina* and *A. comosus* as well as aqueous extracts of *A. comosus* pulp.

Table 5 shows no significant differences ($p<0.05$) in PT and INR values between the control and various aqueous and ethanolic extracts except for *A. comosus*. For aPTT, there were significant differences between control values and extracts of *M. indica* and aqueous extracts of *A. comosus* core, as well as ethanolic extract of *S. lycopersicum.* However, there is no significant difference between control and extracts of *V. amygdalina, M. oleifera,* and *A. comosus* peel and pulp (Table 6).

There was a significant increase in PT as concentration reduced for ethanolic extract of *A. comosus* peel (Figure 1) while the aqueous extract of *M. indica* (Figure 2) showed a significant decrease in aPTT compared to the control.

4. Discussion

The rate of clot formation is often estimated using coagulation assays which assess the extrinsic and intrinsic pathways of the coagulation cascade. Several factors, including tissue factors and contact with certain artificial surfaces such as glass, trigger these pathways[22]. One of the challenges faced in using anticoagulants and diagnostic procedures involving people with coagulation disorders is that most medications used in treating and managing these patients are expensive and

Table 1. Effect of aqueous extracts on PT and INR values between genders

Plant	Concentration in mg/ml	Female		Male		p-value
		PT (sec)	INR	PT (sec)	INR	
Control		16 ± 1.3	1.3 ± 0.1	12 ± 1.8	1.0 ± 0.1	< 0.05
V. amygdalina	1	16 ± 0.8	1.3 ± 0.1	12 ± 2.2	1.0 ± 0.1	< 0.05
	0.5	15 ± 1.8	1.2 ± 0.1	12 ± 1.7	1.0 ± 0.1	< 0.05
	0.25	ī17 ± 0.6	1.4 ± 0.1	12 ± 3.3	1.0 ± 0.2	< 0.05
	0.125	16 ± 1.2	1.3 ± 0.1	11 ± 1.5	1.0 ± 0.1	< 0.05
M. indica	1	14 ± 1.1	1.2 ± 0.1	11 ± 1.5	1.0 ± 0.1	< 0.05
	0.5	15 ± 0.5	1.2 ± 0.0	12 ± 2.3	1.0 ± 0.2	< 0.05
	0.25	14 ± 0.6	1.2 ± 0.0	11 ± 0.8	1.0 ± 0.0	< 0.05
	0.125	14 ± 0.0	1.2 ± 0.0	13 ± 1.0	1.1 ± 0.1	>0.05
M. oleifera	1	16 ± 2.1	1.3 ± 0.2	12 ± 1.7	1.0 ± 0.1	< 0.05
	0.5	17 ± 0.6	1.4 ± 0.1	12 ± 1.9	1.0 ± 0.1	<0.05
	0.25	16 ± 0.8	1.3 ± 0.1	12 ± 3.2	1.0 ± 0.2	>0.05
	0.125	16 ± 1.2	1.3 ± 0.1	14 ± 3.9	1.2 ± 0.1	>0.05
S. lycopersicum	1	16 ± 2.1	1.3 ± 0.1	11 ± 1.1	1.0 ± 0.1	< 0.05
	0.5	16 ± 0.6	1.3 ± 0.1	13 ± 1.0	1.1 ± 0.1	<0.05
	0.25	16 ± 1.5	1.3 ± 0.1	12 ± 1.5	1.0 ± 0.1	< 0.05
	0.125	19 ± 1.9	1.5 ± 0.2	11 ± 3.4	1.0 ± 0.2	< 0.05
A. comosus core	1	16 ± 0.5	1.3 ± 0.1	19 ± 2.9	1.5 ± 0.2	>0.05
	0.5	18 ± 1.2	1.5 ± 0.1	19 ± 3.0	1.5 ± 0.2	>0.05
	0.25	18 ± 1.5	1.5 ± 0.1	20 ± 2.9	1.6 ± 0.2	>0.05
	0.125	16 ± 1.7	1.3 ± 0.1	21 ± 2.1	1.7 ± 0.1	>0.05
A. comosus peel	1	19 ± 0.6	1.5 ± 0.0	13 ± 3.0	1.1 ± 0.2	>0.05
	0.5	18 ± 1.2	1.5 ± 0.1	20 ± 4.3	1.6 ± 0.2	>0.05
	0.25	18 ± 1.9	1.5 ± 0.1	20 ± 1.7	1.6 ± 0.1	>0.05
	0.125	17 ± 0.8	1.4 ± 0.1	21 ± 1.8	1.7 ± 0.1	< 0.05
A. comosus pulp	1	16 ± 0.8	1.3 ± 0.1	17 ± 3.8	1.4 ± 0.2	>0.05
	0.5	17 ± 1.6	1.4 ± 0.1	18 ± 5.4	1.5 ± 0.2	>0.05
	0.25	17 ± 2.4	1.4 ± 0.2	17 ± 5.3	1.4 ± 0.2	>0.05
	0.125	19 ± 1.7	1.5 ± 0.1	20 ± 6.2	1.6 ± 0.2	>0.05

relatively toxic to health. It is startling that 80 out of 100,000 people worldwide are dying from conditions caused by thrombosis[23], hence, the need for continuous improvement of intervention. Some plant extracts such as the bitter, mango and drumstick leaves have been discovered to reduce, while pineapple and tomato have been found to increase the rate of thrombus formation. Using these medicinal plants will provide

Table 2. Effect of ethanolic extracts on PT and INR values between genders

Plant	Concentration in mg/ml	Female		Male		p-value
		PT (sec)	INR	PT (sec)	INR	
Control		15 ± 0.8	1.2 ± 0.1	12 ± 1.3	1.0 ± 0.1	<0.05
V. amygdalina	1	17 ± 2.1	1.4 ± 0.2	10 ± 1.1	1.0 ± 0.1	<0.05
	0.5	19 ± 1.7	1.5 ± 0.1	12 ± 0.9	1.0 ± 0.1	<0.05
	0.25	18 ± 1.5	1.5 ± 0.1	12 ± 2.4	1.0 ± 0.1	<0.05
	0.125	17 ± 1.1	1.4 ± 0.1	12 ± 2.9	1.0 ± 0.2	<0.05
M. indica	1	18 ± 1.8	1.5 ± 0.1	12 ± 1.1	1.0 ± 0.2	<0.05
	0.5	17 ± 1.1	1.4 ± 0.1	12 ± 1.5	1.0 ± 0.1	<0.05
	0.25	19 ± 0.8	1.5 ± 0.1	14 ± 2.1	1.2 ± 0.1	<0.05
	0.125	16 ± 0.8	1.3 ± 0.1	15 ± 2.3	1.2 ± 0.2	>0.05
M. oleifera	1	13 ± 1.5	1.1 ± 0.1	12 ± 3.8	1.0 ± 0.1	>0.05
	0.5	13 ± 0.8	1.1 ± 0.1	12 ± 1.6	1.0 ± 0.1	>0.05
	0.25	12 ± 1.3	1.0 ± 0.1	12 ± 2.8	1.0 ± 0.2	>0.05
	0.125	12 ± 1.0	1.0 ± 0.1	12 ± 2.2	1.0 ± 0.1	>0.05
S. lycopersicum	1	17 ± 3.2	1.4 ± 0.2	12 ± 1.5	1.0 ± 0.1	>0.05
	0.5	17 ± 2.3	1.4 ± 0.2	14 ± 3.0	1.2 ± 0.2	>0.05
	0.25	17 ± 1.1	1.4 ± 0.1	13 ± 1.9	1.1 ± 0.1	<0.05
	0.125	17 ± 1.3	1.4 ± 0.1	16 ± 3.1	1.3 ± 0.2	>0.05
A. comosus core	1	19 ± 4.0	1.5 ± 0.1	16 ± 4.5	1.3 ± 0.2	>0.05
	0.5	20 ± 2.1	1.6 ± 0.1	17 ± 4.2	1.4 ± 0.2	>0.05
	0.25	19 ± 2.5	1.5 ± 0.2	17 ± 6.0	1.4 ± 0.1	>0.05
	0.125	20 ± 2.2	1.6 ± 0.2	16 ± 5.6	1.3 ± 0.2	>0.05
A. comosus peel	1	17 ± 2.8	1.4 ± 0.2	20 ± 4.1	1.6 ± 0.2	>0.05
	0.5	17 ± 1.9	1.4 ± 0.2	20 ± 4.2	1.6 ± 0.2	>0.05
	0.25	20 ± 2.4	1.6 ± 0.2	23 ± 3.5	1.8 ± 0.1	>0.05
	0.125	19 ± 3.5	1.5 ± 0.2	23 ± 4.4	1.8 ± 0.2	>0.05
A. comosus pulp	1	16 ± 3.6	1.3 ± 0.1	21 ± 6.8	1.7 ± 0.2	>0.05
	0.5	19 ± 1.7	1.5 ± 0.1	18 ± 4.1	1.5 ± 0.2	>0.05
	0.25	17 ± 2.1	1.4 ± 0.1	20 ± 5.6	1.6 ± 0.2	>0.05
	0.125	17 ± 1.3	1.4 ± 0.1	20 ± 5.6	1.6 ± 0.2	>0.05

an intervention that is less toxic and free from side effects[17].

This study revealed that PT and INR values differ significantly between genders; the values were higher in females than males. On the other hand, males had greater aPTT values than females, though this was not statistically significant. However, it has been reported that many factors influence hemostatic changes, and gender plays an important role due to the influence of steroid sex hormones. Experimental studies also indicate that 17β-estradiol acts as a regulator for blood coagulation factor genes, inducing transcription of

Table 3. Effect of aqueous extracts on aPTT values between genders

Plant	Concentration in mg/ml	Female	Male	p-value
Control		36 ± 4.1	40 ± 7.9	>0.05
V. amygdalina	1	35 ± 1.0	27 ± 2.6	<0.05
	0.5	35 ± 5.4	28 ± 3.8	<0.05
	0.25	33 ± 2.4	27 ± 1.9	<0.05
	0.125	36 ± 1.4	32 ± 4.5	<0.05
M. indica	1	23 ± 4.0	33 ± 8.1	<0.05
	0.5	25 ± 2.0	33 ± 6.0	<0.05
	0.25	26 ± 4.6	33 ± 3.8	<0.05
	0.125	26 ± 4.2	30 ± 4.0	>0.05
M. oleifera	1	27 ± 7.5	36 ± 4.7	<0.05
	0.5	23 ± 7.2	41 ± 6.1	<0.05
	0.25	25 ± 7.1	37 ± 5.4	<0.05
	0.125	23 ± 6.2	37 ± 8.6	<0.05
S. lycopersicum	1	22 ± 3.3	40 ± 6.6	<0.05
	0.5	23 ± 3.3	40 ± 3.3	<0.05
	0.25	21 ± 0.6	41 ± 6.4	<0.05
	0.125	18 ± 0.0	38 ± 6.1	<0.05
A. comosus core	1	27 ± 3.7	35 ± 4.0	<0.05
	0.5	20 ± 1.2	39 ± 1.5	<0.05
	0.25	29 ± 3.1	37 ± 3.2	<0.05
	0.125	26 ± 1.5	38 ± 4.3	<0.05
A. comosus peel	1	27 ± 3.2	42 ± 1.2	<0.05
	0.5	29 ± 4.5	37 ± 6.6	<0.05
	0.25	36 ± 6.4	39 ± 6.4	>0.05
	0.125	31 ± 7.4	42 ± 2.5	>0.05
A. comosus pulp	1	28 ± 6.8	36 ± 4.0	>0.05
	0.5	30 ± 4.6	35 ± 5.4	>0.05
	0.25	28 ± 5.3	35 ± 4.9	>0.05
	0.125	25 ± 2.1	32 ± 2.1	>0.05

factors, such as FXII (Hageman factor), which plays an essential role in the activation of proteolytic pathways of the coagulation cascade[24]. The findings of this study are like a study which reported that PT values differ significantly between genders, but aPTT values do not[25]. Moreover, in contrast to a study which showed that aPTT values differ significantly between genders but no significant difference in PT values[26]. The findings of this study are also at variance with a study conducted in Turkey with significantly higher male PT and INR values than females; however, the study also reported no significant difference between genders for aPTT[27].

Table 4. Effect of ethanolic extracts on aPTT values between genders

Plant	Concentration in mg/ml	Female	Male	p-value
Control		39 ± 4.0	33.0 ± 6.3	>0.05
V. amygdalina	1	45 ± 7.2	35 ± 7.5	>0.05
	0.5	39 ± 2.7	33 ± 4.0	>0.05
	0.25	43 ± 3.1	29 ± 5.0	>0.05
	0.125	41 ± 2.0	37 ± 2.7	>0.05
M. indica	1	21 ± 4.2	30 ± 5.7	<0.05
	0.5	22 ± 4.0	34 ± 3.4	<0.05
	0.25	22 ± 4.9	38 ± 9.0	<0.05
	0.125	21 ± 3.4	34 ± 7.8	<0.05
M. oleifera	1	17 ± 3.1	42 ± 9.1	<0.05
	0.5	19 ± 5.1	36 ± 5.4	<0.05
	0.25	18 ± 3.2	39 ± 5.2	<0.05
	0.125	16 ± 2.4	43 ± 1.5	<0.05
S. lycopersicum	1	22 ± 4.4	35 ± 9.8	<0.05
	0.5	22 ± 4.4	33 ± 5.5	<0.05
	0.25	22 ± 4.9	36 ± 5.4	<0.05
	0.125	22 ± 3.6	33 ± 7.0	<0.05
A. comosus core	1	27 ± 4.0	32 ± 8.0	>0.05
	0.5	28 ± 2.7	33 ± 7.5	>0.05
	0.25	29 ± 5.0	37 ± 7.8	>0.05
	0.125	24 ± 2.1	34 ± 6.4	>0.05
A. comosus peel	1	36 ± 5.5	36 ± 9.7	>0.05
	0.5	40 ± 8.4	34 ± 9.4	>0.05
	0.25	37 ± 5.2	36 ± 4.7	>0.05
	0.125	36 ± 9.5	36 ± 6.4	>0.05
A. comosus pulp	1	32 ± 6.9	40 ± 9.4	>0.05
	0.5	28 ± 5.9	34 ± 5.3	>0.05
	0.25	28 ± 7.5	36 ± 8.1	>0.05
	0.125	25 ± 6.0	38 ± 6.9	>0.05

In this study, it was also observed that ethanolic extracts had a more significant effect on *in vitro* coagulation activity than aqueous extracts. This could be due to the solubility of different phytochemicals in these solvents[28], as organic solvent is often more effective for extraction[29]. Aqueous extraction products mainly contain metals, ions, highly hydrophilic compounds, water-soluble enzymes, glycoproteins and peptides, while alcohol extraction products are mainly composed of very polar, neutral, basic and acidic compounds as well as natural oils. Using hot water in the aqueous extraction method may improve the extraction of compounds

Table 5. Effect of aqueous and ethanolic extracts on PT and INR values

Plant	Concentration in mg/ml	Aqueous			Ethanolic		
		PT (sec)	INR	p-value	PT (sec)	INR	p-value
Control		14 ± 2.5	1.2 ± 0.2		14 ± 1.7	1.2 ± 0.1	
V. amygdalina	1	14 ± 2.5	1.2 ± 0.2	>0.05	14 ± 3.8	1.2 ± 0.3	>0.05
	0.5	14 ± 2.5	1.2 ± 0.2	>0.05	15 ± 3.9	1.2 ± 0.3	>0.05
	0.25	14 ± 3.3	1.2 ± 0.2	>0.05	15 ± 3.7	1.2 ± 0.3	>0.05
	0.125	14 ± 2.8	1.2 ± 0.2	>0.05	14 ± 3.1	1.2 ± 0.2	>0.05
M. indica	1	13 ± 1.9	1.1 ± 0.1	>0.05	15 ± 3.6	1.2 ± 0.1	>0.05
	0.5	13 ± 2.2	1.1 ± 0.1	>0.05	15 ± 2.9	1.2 ± 0.2	<0.05
	0.25	13 ± 1.8	1.1 ± 0.1	>0.05	16 ± 3.3	1.3 ± 0.2	<0.05
	0.125	15 ± 1.6	1.2 ± 0.1	>0.05	15 ± 2.0	1.2 ± 0.2	<0.05
M. oleifera	1	14 ± 2.9	1.2 ± 0.2	>0.05	12 ± 2.7	1.0 ± 0.2	>0.05
	0.5	15 ± 3.1	1.2 ± 0.2	>0.05	13 ± 0.8	1.1 ± 0.1	>0.05
	0.25	14 ± 3.0	1.2 ± 0.2	>0.05	12 ± 2.1	1.0 ± 0.1	>0.05
	0.125	15 ± 2.9	1.2 ± 0.2	>0.05	12 ± 1.6	1.0 ± 0.1	>0.05
S. lycopersicum	1	14 ± 2.9	1.2 ± 0.2	>0.05	14 ± 3.5	1.2 ± 0.2	>0.05
	0.5	14 ± 3.4	1.2 ± 0.1	>0.05	15 ± 3.0	1.2 ± 0.2	>0.05
	0.25	14 ± 2.3	1.2 ± 0.2	>0.05	15 ± 2.4	1.2 ± 0.2	<0.05
	0.125	15 ± 4.8	1.2 ± 0.3	>0.05	17 ± 2.5	1.4 ± 0.2	<0.05
A. comosus core	1	19 ± 3.2	1.5 ± 0.2	<0.05	18 ± 4.2	1.5 ± 0.3	<0.05
	0.5	20 ± 3.5	1.6 ± 0.2	<0.05	18 ± 3.6	1.5 ± 0.3	<0.05
	0.25	19 ± 2.3	1.5 ± 0.2	<0.05	18 ± 4.5	1.5 ± 0.3	<0.05
	0.125	18 ± 3.4	1.5 ± 0.2	<0.05	18 ± 4.5	1.5 ± 0.3	<0.05
A. comosus peel	1	17 ± 4.2	1.4 ± 0.3	<0.05	19 ± 3.7	1.5 ± 0.3	<0.05
	0.5	21 ± 6.3	1.7 ± 0.3	<0.05	19 ± 3.5	1.5 ± 0.3	<0.05
	0.25	20 ± 4.6	1.6 ± 0.3	<0.05	21 ± 3.3	1.7 ± 0.3	<0.05
	0.125	20 ± 3.8	1.6 ± 0.2	<0.05	21 ± 4.2	1.7 ± 0.3	<0.05
A. comosus pulp	1	18 ± 5.2	1.5 ± 0.2	>0.05	18 ± 5.8	1.5 ± 0.3	<0.05
	0.5	16 ± 4.4	1.3 ± 0.3	>0.05	18 ± 3.0	1.5 ± 0.2	<0.05
	0.25	17 ± 3.9	1.4 ± 0.3	<0.05	19 ± 4.2	1.5 ± 0.2	<0.05
	0.125	20 ± 4.4	1.6 ± 0.3	<0.05	17 ± 2.2	1.4 ± 0.2	<0.05

responsible for the coagulation activity, provided the extracted active compounds are thermally stable[30].

The result of this study revealed that aqueous and ethanolic extracts of *V. amygdalina* did not affect PT; however, the ethanolic extract increased aPTT values compared to controls but was only statistically significant at 0.125mg/ml. This suggests that *V. amygdalina* does not affect the extrinsic pathway of blood coagulation,

Table 6. Effect of aqueous and ethanolic extracts on aPTT values

Plant	Concentration in mg/ml	Aqueous	p- value	Ethanolic	p-value
Control		38 ± 6.4		36 ± 5.8	
V. amygdalina	1	32 ± 5.6	>0.05	39 ± 9.2	>0.05
	0.5	34 ± 7.3	>0.05	38 ± 7.1	>0.05
	0.25	32 ± 7.0	>0.05	40 ± 9.6	>0.05
	0.125	35 ± 4.6	>0.05	42 ± 9.1	<0.05
M. indica	1	28 ± 7.9	<0.05	23 ± 4.5	<0.05
	0.5	29 ± 6.2	<0.05	25 ± 5.4	<0.05
	0.25	30 ± 5.6	<0.05	25 ± 6.8	<0.05
	0.125	29 ± 7.3	<0.05	24 ± 6.8	<0.05
M. oleifera	1	33 ± 6.9	>0.05	29 ± 13.2	>0.05
	0.5	36 ± 9.9	>0.05	27 ± 8.4	>0.05
	0.25	34 ± 6.6	>0.05	28 ± 10.7	>0.05
	0.125	32 ± 9.5	>0.05	29 ± 14.2	>0.05
S. lycopersicum	1	36 ± 9.4	>0.05	28 ± 6.8	>0.05
	0.5	31 ± 9.7	>0.05	25 ± 4.1	<0.05
	0.25	29 ± 10.5	>0.05	26 ± 6.6	<0.05
	0.125	27 ± 10.3	<0.05	26 ± 6.1	<0.05
A. comosus core	1	31 ± 5.4	<0.05	29 ± 6.4	>0.05
	0.5	30 ± 8.7	<0.05	31 ± 5.9	>0.05
	0.25	31 ± 6.4	<0.05	33 ± 7.6	>0.05
	0.125	31 ± 5.8	<0.05	32 ± 8.5	>0.05
A. comosus peel	1	34 ± 7.8	>0.05	36 ± 7.5	>0.05
	0.5	33 ± 7.2	>0.05	37 ± 8.9	>0.05
	0.25	31 ± 8.7	>0.05	36 ± 4.7	>0.05
	0.125	36 ± 8.3	>0.05	36 ± 7.6	>0.05
A. comosus pulp	1	32 ± 6.6	>0.05	36 ± 8.8	>0.05
	0.5	32 ± 6.2	>0.05	31 ± 6.3	>0.05
	0.25	32 ± 6.1	>0.05	32 ± 8.5	>0.05
	0.125	35 ± 8.2	>0.05	31 ± 9.0	>0.05

however, it may possess some anticoagulant activity at a reduced concentration *in vitro*. This is discordant with previous studies reporting *V. amygdalina* to cause a significant reduction in PT but no significant difference in aPTT compared to controls[31,32].

In this study, aqueous extract of *M. indica* at 1, 0.5 and 0.25mg/ml showed reduced PT and INR values; however, this was not statistically significant. Moreover, aqueous and ethanolic extracts of *M. indica* showed significantly reduced values compared to the control

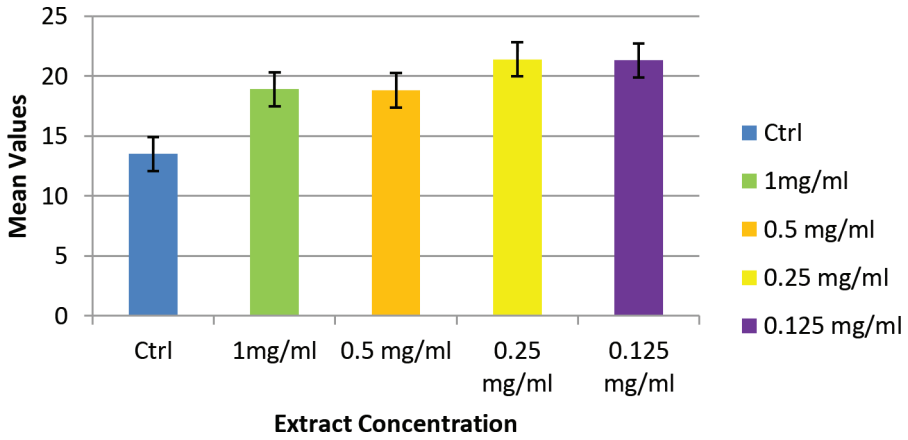


Figure 1. Effect of ethanolic extract of *Ananas comosus peel* on prothrombin time.

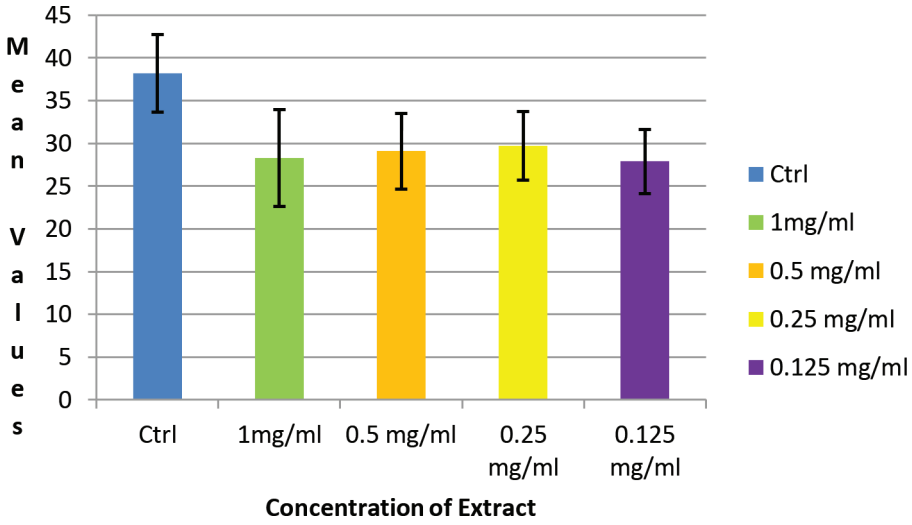


Figure 2. Effect of aqueous extract of *Mangifera indica* on activated partial thromboplastin time.

for aPTT, suggesting procoagulant activity as the time taken for coagulation in the intrinsic pathway was prolonged. This finding does not align with a previous report that *M. indica* shows a strong anticoagulant effect on plasma coagulation time by extending the time required for clot formation[33].

Ethanolic extracts of *M. oleifera* showed reduced values for PT and INR compared to controls, though this was not statistically significant. Thus, *M. oleifera* can be considered to have procoagulant activity. This agrees with a study which observed that *M. oleifera* exhibited procoagulant activity due to decreased time required for clot formation. This effect may result from the high proteolytic activity in the leaf[34]. This finding disagrees with a study that reported *M. oleifera* to increase PT significantly, suggesting that *M. oleifera* leaves have an anticoagulant effect[35]. Another study reported *M. oleifera* to act as an anticoagulant by prolonging the time required for blood coagulation[36]. Furthermore, aqueous and ethanolic extracts of *M. oleifera* reduced the time taken for clot formation compared to the control in the intrinsic coagulation pathway (assessed by aPTT), though this was not statistically significant.

Ethanolic extracts of *S. lycopersicum* at 0.5mg/ml, 0.25mg/ml, and 0.125mg/ml showed significantly reduced values compared to the control for aPTT, however, there was no effect across the concentration gradient. This suggests *in vitro* procoagulant activity as the time required for coagulation via the intrinsic pathway was prolonged. The result of this study contradicts a previous study which stated that *S. lycopersicum* fruit significantly increased clotting time[37]. Several studies have also reported lycopene in tomatoes to have an antithrombotic effect due to its effect on platelets, not coagulation factors[38-40].

Aqueous extracts of *A. comosus* core and peel at all concentrations, as well as the pulp at 0.25 and 0.125mg/ml, showed a significant increase compared to the control for PT and INR values, however, there was no concentration effect. This indicates *in vitro* anticoagulant activity because of the delay in clot formation via the extrinsic pathway. The prolonged PT could be due to clotting inhibitors in the extract, which interfere with the integrity of the coagulation proteins[41,42]. The result of this study differs from the findings of a study that *A. comosus* does not affect PT[26]. However, the findings of this study are in accord with a study that reported prolonged PT with the administration of *A. comosus* extract, thus reducing thrombus formation[43]. Furthermore, ethanolic extracts of *A. comosus* core, peel and pulp at all concentrations showed a significant increase in PT and INR values compared to the control. Hence, *A. comosus* can be considered to have *in vitro* anticoagulant properties as the time required for clot formation was significantly prolonged. Another study in the Philippines reported *A. comosus* as a viable anticoagulant[30]. *A. comosus* contains bromelain, which has been found to prevent blood coagulation via the fibrinolytic system by amplifying the conversion of plasminogen to plasmin. It also limits fibrin formation by reducing factor X and prothrombin intermediates of the clotting cascade resulting in delayed clot formation and prolonged PT and aPTT[44,45].

In conclusion, this study revealed that PT, INR and aPTT values differ significantly between genders. *M. indica* and *M. oleifera* have procoagulant activity, *A. comosus* has anticoagulant activity while *V. amygdalina* does not affect coagulation. Consequently, these leaves and fruit being natural products, could be better agents for managing coagulation disorders as the related side effects would be less and, therefore, safer. Further studies are needed to identify the bioactive compound(s) responsible for the coagulation activity and the coagulation factors affected as well as understand the mechanism by which the bioactive compound(s) affect coagulation activity.

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