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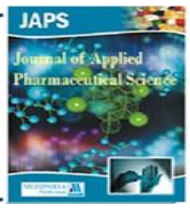

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Hemostatic potential of the sap of *Musa sapientum L*. (Musaceae)

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ABSTRACT

In Benin, *Musa sapientum* is one of the most important medicinal species used in the treatment of bleeding. In this study, hemostatic properties of *M. sapientium* sapand its mechanism of action have been identified through hematologic tests such as Clotting Time, Prothrombin Time, Activated Partial Thromboplastin Time and Milk Precipitation Test; biochemical test (total proteins); macroscopic and microscopic tests performed on different blood products before and after addition of sap. The results obtained showed that *Musa sapientum* reduced significantly clotting Time. However, it has no effect on the individual factors of coagulation. Its mechanism of action results from its ability to form a protein network which is a basis for cellular aggregation stopping bleeding. Moreover, Milk Precipitation Test showed astringent properties of *Musa sapientum* and therefore its vasoconstrictors properties. Phytochemical analyses revealed the presence of alkaloids, tannins, coumarins, reducing compounds, anthocyanins and leucoantocyanes. . *M. sapientum*is great on treatment of bleeding and could help to elaborate new drugs.

Keywords: *Musa sapientum*, coagulation, hemostatic, protein network.

INTRODUCTION

Musa sapientum commonly known as 'banana' is an herbaceous plant of Musaceae family. It has been used for the treatment of gastric ulcer, hypertension, diarrhea, dysentery and diabetes in India (Dikshit *et al.,* 2012). The antidiabetic effect of the leaves, stem, fruit, root and flower has been demonstrated (Pari and Umamaheswari, 2000; Adewoye and al., 2009, 2011). Anti-ulcerative properties have been highlighted in green fruits (Pannangpetch *et al.*, 2001; Prabha *et al.,* 2011). *Musa spientum* is also used in the treatment of excessive menstruation with *Canna indica L. var. speciosa*(Partha, 2007). The fruit of *M. sapientum* showed antimicrobial properties (Fagbemi *et al.,* 2009).

Musa sapientum L. called "Goukokwé" in Benin language (Fon) (de Souza, 1988; Akoègninou *et al.*, 2006) is one of the most widely used medicinal species in the treatment of hemorrhage. The freshly collected sap from the trunk or juice which collects inside the trunk is used for vaginal lavage and / or per os with the addition of kaolin or not. The juice obtained by looting the terminal bud of the regime, is used in oral administration with the addition of kaolin. The fresh sap is also used as local hemostatic for the treatment of external wounds and this plant has been reported by other authors for its hemostatic and wound healing. Thus, in Brazil, its sap is used for treatment of bleeding (Albuquerque *et al.*, 2007). The aqueous extract of the fruit of *Musasapientum* is used in India for the treatment of wounds (Agarwal *et al.*, 2009; Atzingen, 2011). A species of the same genus (*Musa paradisiaca* L.) was reported in Brazil for the same properties (Borges *et al.*, 2005). Despite this wide use as antihemorrhagic, no study has been performed so far on the hemostatic properties of this plant. This is why the objective of this study is to investigate the effect of *Musa sapientum* on hematological and biochemical parameters in order to understand its mechanism of action.

MATERIAL AND METHODS

Vegetal material

M. sapientum's sap was collected directly in sterile bottles after a cut in the trunk of the plant. It was centrifuged at 5000 rpm for 5 minutes and the supernatant was stored at 4°C in Eppendorftubes.

Blood product (whole blood, plasma and serum)

Blood samples were obtained by venipuncture from normal adult human who were volunteers for the study. The blood was collected in a dry tube and a tube containing sodium citrate (0.109 M). Plasma and serum were obtained respectively by centrifugation tubes containing citrate and dry tubes.

Milk

It was about whole milk brand "President" commercially available.

Phytochemical analyses

Phytochemical analyses of *M. sapientum* sap was conducted in the Research Laboratory of Pharmacognosy and Essential Oils from the University of Abomey-Calavi, on the basis of differential staining reactions and precipitation using the method by Houghton & Raman (1998).

The different reactions about active compounds are summarized in Table I.

Morphological evaluations

Action of *M. sapientum*sap on plasma and serum was evaluated in the Research Laboratory of Applied Biology from the University of Abomey-Calavi (UAC) by macroscopic and microscopic observations.

100 µl of sap were added to 1 ml of plasma or serum sample. Each tube was observed macroscopically before and after addition of sap.

Microscopic observations were performed on a microscope mounted on a CETI BELGIUM camera connected to a computer screen. Microscopic preparations were carried out between slide and coverslip by adding 10 µl of sap to 50 µl of plasma.

Evaluation of astringent properties: precipitation of milk test

We used two (02) glass test tubes with one for test and one for control. *M. sapientum* was collected in 1 ml test tube and 1 ml of distilled water was put in the control tube. 100µL milk was then added in each of the two tubes. We homogenized it, allowed to stand for 03 minutes and centrifuged for 01 minute at 3000 rpm. The presence or absence of pellet was noted.

Table. 1: Phytochemical analyses.

Classes of active substances	Specific reagents and reactions
Alkaloids	Mayer → yellowish precipitate
Quinone derivatives	Born-Träger → purplish red color
Cathetic and gallic tannins	-Reagent of Stiasny → pink precipitate - Saturation by acetate of Na+ $FeCl_3$ → blue-dark green or black
Flavonoids	Shinoda → orange color, red or purple
Cyanogenic derived	Guignard (picric acide) → brown coloration
Steroids and triterpenoids	- Libermann-Burchard → violet-blue or green - Kedde → reddish purple or red wine
Saponins	Determination of the Foam Index (MI): positive test if MI >100
Anthocyanins	Red coloration of the filtrate increased in acid medium and blue-violet in alkaline medium
Leuco-anthocyanins	Chloridric alcohol → cherry red color
Mucilages	Absolute alcohol → flocculent precipitate
Reducing compounds	Fehling's hot → brick-red precipitate
Coumarins	Ammonia 25% → intense fluorescence
Anthracenederivatives	Ammonia 50% → intense red color

Table. 2: Procedure for measuring activated partial thromboplastin time

Tubes	T_0	T_1	T_2	T_3	T_4
Plasma	100µl	100µl	100µl	100µl	100µl
Sap		10µl	25µl	50µl	100µl
Cephalin	100µl	100µl	100µl	100µl	100µl
Mix and incubate at 37° C for 2 min					
$CaCl_2$ (0.025M)	100µl	100µl	100µl	100µl	100µl

Table. 3: Procedure for measuring Quick Time.

Tubes	T_0	T_1	T_2	T_3	T_4
Plasma	100µl	100µl	100µl	100µl	100µl
Sap		10µl	25µl	50µl	100µl
		Incubate at 37°C for 01 minute			
Calcium thromboplastin	200µl	200µl	200µl	200µl	200µl

Table. 4: Dilution range of serum pool from the sap of *M. sapientum.*

Dilution	T0	T5%		T10%		T15%		T20%		T25%		T40%		T50%	
		Test	cont	Test	cont	Test	cont	Test	cont	Test	cont	Test	cont	Test	cont
Serum Pool	1000 µl	950 µl	950 µl	900 µl	900 µl	850 µl	850 µl	800 µl	800 µl	750 µl	750 µl	600 µl	600 µl	500 µl	500 µl
Sap	-	50 µl	-	100 µl	-	150 µl	-	200 µl	-	250 µl	-	400 µl	-	500 µl	-
NaCl (0.9%)	-	-	50 µl	-	100 µl	-	150 µl	-	200 µl	-	250 µl	-	400 µl	-	500 µl
Leave the mixture for 15 min and centrifuge at 300 rpm for 5min															

Measurement of Clotting Time (CT)

We used five tubes numbered T0, T1, T2, T3 and T4. T0 is the control tube and T1, T2, T3, T4 received respectively 10, 25, 50 and 100 µl of *Musa sapientum*. After a minute in a water bath at 37°C, 500 µl of blood freshly collected was added to each tube. The timer is immediately triggered and CT of each tube is noted.

Measurement of activated partial thromboplastin time (aPTT)

We used five glass hemolysis tubes. The procedure used is summarized in Table II. Tube T0 is the control tube and tubes T1, T2, T3 and T4 are the test tubes (which have received different doses of *M. sapientum*). We measure the time of appearance of the clot by tipping every five seconds the tubes at 90°C. The test is repeated five times and averaged.

Time measurement of prothrombin time (PT)

Five glass hemolysis tubes were used. The protocol used is summarized in Table III. T0 is control tube and T1, T2, T3 and T4 are the test tubes. We measure the time of onset of the coagulum by tilting every five seconds tubes at 90°C. The tests were repeated five times and averages were determined.

Determination of Total Proteins

The sap of *M. sapientum* was diluted with a pool of fresh normal human serum in a dilution range from 0% to 50% (Table IV). Total proteins were performed by adding 20µl of supernatant from each tube to 1000 µl of biuret reagent. After 30 min incubation in to dark, reading was done in a spectrophotometer at 550 nm against reagent blank. The tests were repeated five times and the average was determined.

Statistical Analyses

The mean and standard deviations of the different data sets were calculated.The percentage reduction (PR) that evaluates the gain time of the test compared to control was calculated by the following formula:

$$PR = (\text{Time of control} - \text{time test}) / (\text{time of control}) \times 100$$

The time difference between test and control were compared by the Student test. The significance level was set at $P < 0.05$.

These various operations were performed using the software Excel 2007 and XL-Stata version 2011.

RESULTS

Morphological evaluation: Effect of *Musa sapientum* sap on the plasma and serum

Before adding the sap of *M. sapientum*, plasma and serum collected in the tubes were perfectly clear. After the addition of sap, we observed very rapidly (<1s) formation in a network of white substance in the plasma (Figure 1) and serum (Figure 2). This substance is irreversibly formed. Microscopic observations showed that contact between the plasma and sap results in the formation of a substance that is in the form of concentric filaments at a magnification of x100 (Figure 3).

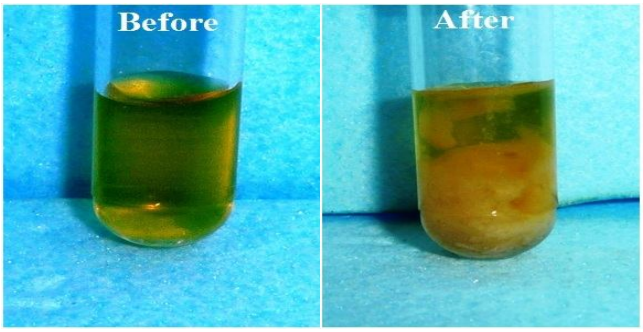

Fig. 1: Appearence of the plasma before and after addition of the sap of *M. sapientum.*

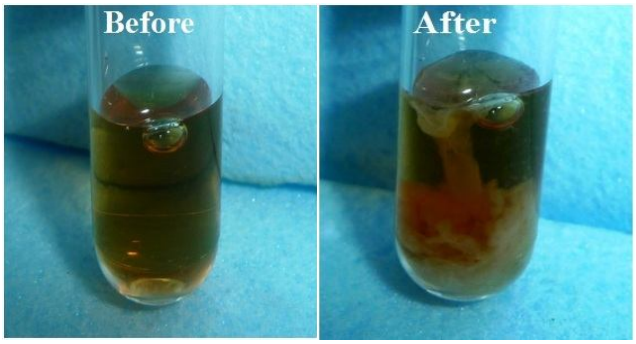

Fig. 2 : Appearence of the serum before and after addition of the sap of *M. sapientum.*

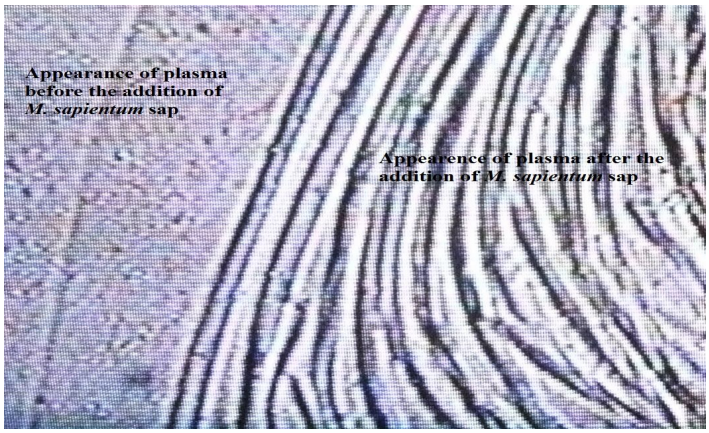

Fig. 3: Microscopic view of the plasma before and after contact with the sap of *M. sapientum*(magnification x100).

Studies astringent properties: precipitation test milk

We observed the formation of a pellet in the tube containing the sap while the control tube remained clear (Figure 4). Sap of *M. sapientum* has precipitated milk proteins, so it has an astringent property.

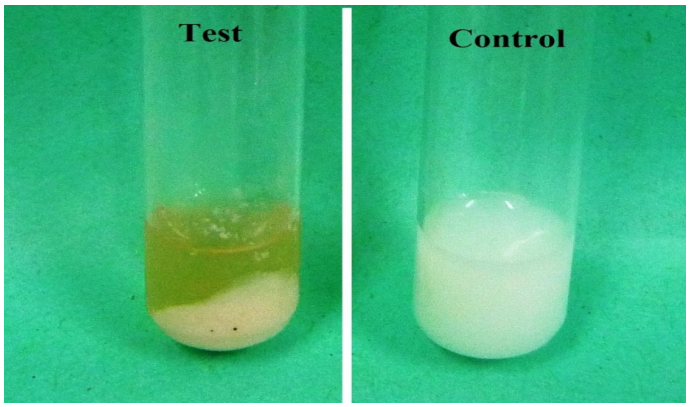

Fig. 4 : Precipitation of milk proteins under the action of the sap of *M. sapientum*

Measurement of Clotting Time (CT)

Averages of CT obtained are presented in Figure 5. Sap significantly reducedCT ($p < 0.05$) with percentages ranging from 18.33% to 27.22%.

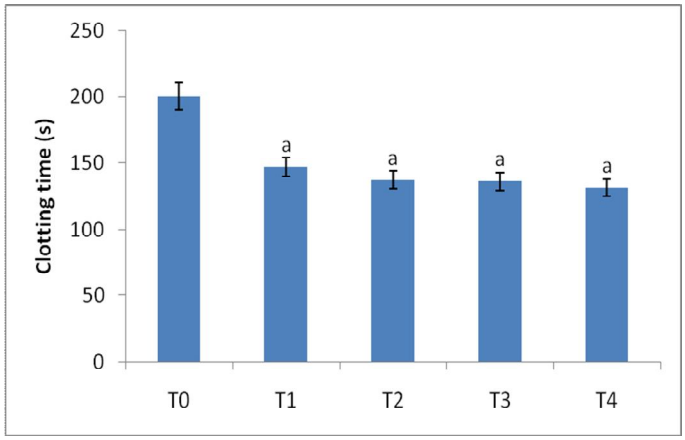

Fig. 5 : Effect of the sap of *M. sapientum* on Clotting Time.

Measurement of activated partial thromboplastin time (aPTT)

aPTT's average obtained are 45 ± 1seconds in all tubes. No different signification was observed between tubes which received sap and control tubes ($p > 0.05$).

Time measurement of prothrombin time (PT)

Averages obtained are 15 ± 1s in all tubes. No difference significance was observed between tubes which received sap and control tubes ($p > 0.05$).

Dosage of Total proteins

After adding sap, there was a significant decrease ($p < 0.05$) of the total proteins concentration in all test tubes compared to controls tubes. The decreasedpercentage in protein concentration is higher with dilutions of 20% and 25% (Fig. 6).

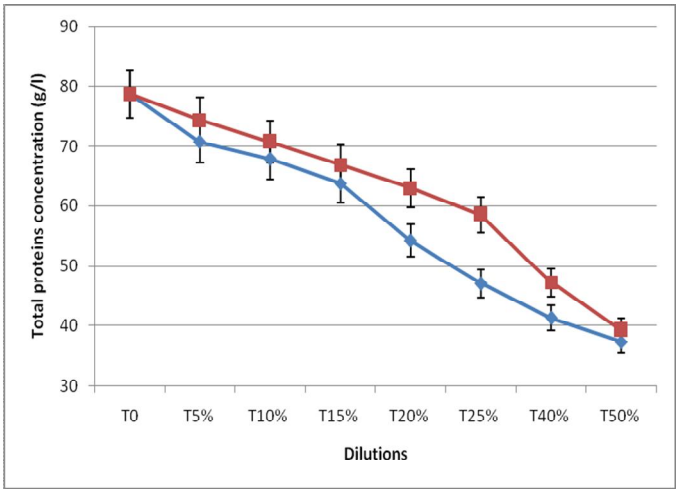

Fig.6: Effects of *Musa sapientum* sap on total proteins

Phytochemical analyses

The phytochemical analysis showed the presence of alkaloids, tannins, coumarins, reducing compounds, anthocyanins and leucoantocyanes.

DISCUSSION

The use of *M. sapientum* in the treatment of bleeding is very common in the South - Benin. Indeed, this plant is used as a hemostatic in Brazil (Albuquerque *et al.*, 2007) and India (Agarwal *et al.*, 2009; Atzingen, 2011). But this study is the first that has focused on assessing the power of hemostatic *M. sapientum*.The addition of sap in whole blood induced a reduction of CT significance with an effect not dose dependent. The action of sap on the CT does not appear related to the typical cascade of coagulation reactions typical of coagulation since the addition of sap in plasma did not alter the prothrombin time nor the activated partial thromboplastin time. The sap therefore has no effect on clotting factors (II, V, VII, VIII, IX, X, XI, XII and XIII) of both intrinsic and extrinsic ways (Hoffman and Monroe, 2007). This particular mechanism of action registered sap in the same category as the hemostatic with nonspecific action on the coagulation

cascade (Abaut and Basle, 2008). The decreasing proteins concentration in the supernatant serum after the addition of sap indicates that proteins have become networks. This interaction between sap-protein seems linked to the presence of tannins in the sap. Studies on the hemostatic properties of *Jatropha multifida* and *Annonasenegalensis* have already underlined the role of tannins on the blood proteins. Indeed, the tannins have the ability to transform certain soluble proteins insoluble because of the chemical bonds that develop between them and the tannins (Wolberg, 2007). They are bristling with phenolic hydroxyl groups capable of reacting with strong hydrogen bonds with the atoms of the peptide binding protein (Crozier *et al.*, 2009) which rendered insoluble proteins increases blood viscosity and inhibit the movement of red blood cells. That's what facilitates their aggregation (Bishop *et al.*, 2000). Like fibrin, we believe that the protein network formed behaves like a net that traps red blood cells but also platelets and leukocytes (Wolberg, 2007). The cell aggregation has a significant effect on hemodynamics *in vivo* (Bishop *et al.*, 2000). Increased aggregation produced locally in each capillary will immediately disrupt blood flow (Mchedlishvili *et al.*, 2002). This could lead to a reduction in time and volume of bleeding. The haemostatic effect of sap can be enhanced by its astringent properties. Indeed astringent activity favors vasoconstriction, which is an important parameter in hemostasis.

CONCLUSION

The traditional use of sap of *M. sapientum* in the treatment of bleeding is warranted. And its mechanism of action results from a part of it causes vasoconstriction and secondly from the formation of a protein network that serves as a focal point to cell aggregation and the bleeding stops.

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