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Full Length Research Paper

Evaluation of the phytochemical and hemostatic potential of *Jatropha multifida* sap

Tamègnon Victorien Dougnon[1,2]*, Jean Robert Klotoé[1,3], Julien Sègbo[1], Jean-Marc Atègbo[3], Aléodjrodo Patrick Edorh[2,4], Fernand Gbaguidi[5], Armelle Sabine Hounkpatin[2], Carlos Dandjesso[1], Lauris Fah[1], Brice Fanou[1], Karim Dramane[3] and Frédéric Loko[1]

[1]Polytechnic School of Abomey-Calavi, Research Laboratory in Applied Biology, University of Abomey-Calavi, 01 BP 2009 Cotonou, Benin.
[2]Interfaculty Center of Formation and Research in Environment for the Sustainable Development, Laboratory of Toxicology and Environmental Health, University of Abomey-Calavi (UAC), 01 BP 1463 Cotonou, Benin.
[3]Department of Animal Physiology, Faculty of Science and Technology, University of Abomey-Calavi (UAC), 01 BP 526 Cotonou, Benin.
[4]Department of Biochemistry and Cellular Biology, Faculty of Science and Technology, University of Abomey-Calavi (UAC), 01 BP 526 Cotonou, Benin.
[5]Laboratory of Pharmacognosy and Essential Oils (Porto-Novo), Benin.

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Jatropha multifida **is a plant traditionally recognized for its medicinal properties. This study was undertaken not only to assess the hemostatic potential of the sap of *J. multifida*, but also to elucidate its chemical profile. Wistar rats were injured at various locations and time of bleeding was evaluated based on the addition of *J. multifida*'s sap or distilled water. Phytochemical study was performed on sap. *J. multifida'*s sap significantly reduced bleeding time. Indeed, more blood flow was noticed on the side that received physiological water than the one which received the sap. This is even truer when the cut is deep with times of bleeding reduction of 39.06, 47.89, and 46.68%, respectively for superficial wounds, saphenous vein, and the femoral vein clippings. The phytochemical screening of *J. multifida* showed the presence of tannins, flavonoids, saponins, leucoanthocyanes, mucilage and reducing compounds. The power of the hemostatic sap is explained by the strong presence of tannins and flavonoids. All this proves that using the sap of *J. multifida* in traditional medicine is justified.**

Key words: Hemostatic, Wistar rats, tannins, flavonoids, bleeding, *Jatropha multifida.*

INTRODUCTION

The World Health Organization has encouraged research on medicinal plants since 1978 in order to improve, secure and reduce the cost of medical products (OMS, 2002).

No less than 170.000 bioactive molecules have been, to date, identified from plants (OMS, 2002). Despite these results, it should be noted that few plants have been studied for their therapeutic properties, and some pharmacological activities such as hemostatic effects remain unexplored. In Benin, for example, only about 500 plants were studied among the 3000 species of plants (Adjanohoun et al., 1989; Akoègninou et al., 2006). No studies have looked specifically at the inventory of hemostatic plants. The few existing data are from ethnobotanical surveys on the flora as a whole (de Souza, 1988; Adjanohoun et al., 1989; Akoègninou et al., 2006). However, there are some hemostatic plants including *Jatropha multifida* which is used in Vodou ritual practices to stop external bleeding (Adjanohoun et al., 1989).

Interest for hemostatic plants is justified by the fact that hemorrhage is the first cause of early death in surgery bleedings (Sauaia et al., 1995). It is now a leading cause

*Corresponding author. E-mail: victorien88@hotmail.com or dougnonv@yahoo.fr. Tel: 00 (229) 97 73 64 46/64 67 51 19.

of maternal mortality worldwide (Goodburn and Campbell, 2001; Brücker et al., 2006; Fourn et al., 2007). Hemorrhages are unpredictable in 84% of cases (Cinat et al., 1999). Although, it often stops on its own (minor injuries), in many situations, the use of mechanical barriers, thermal and hemostatic drugs is essential (Kozen et al., 2008; Wedmore et al., 2006). Thus, the variability in the severity of bleeding today justifies the existence of a variety of hemostatic. Some hemostatics are human, animal, vegetable or synthetic. These substances are administered topically, orally or by injection (Abaut et al., 2008). It is important for African researchers to investigate African plants known for their therapeutic properties by the legends and traditions. The identification of efficient hemostatic could, for this purpose, improving the management of bleeding in all medical disciplines.

J. multifida, in traditional medicine, is used as a hemostatic, usually to treat wounds. It is also used as herbal tea to treat microbial infections (Adjanohoun et al., 1989). In Benin, no scientific study has, to date, proved hemostatic potential of this plant. However, preliminary studies and ongoing work has shown good results of this sap on *in vitro* tests (Dougnon, 2012); hence, the need to assess its potential phytochemical and its effects *in vivo*.

MATERIALS AND METHODS

Phytochemical tests were conducted at the Laboratory of Pharmacognosy and Essential Oils of Benin Center for Scientific and Technical Research. Hemostatic and biochemical tests were conducted at the Laboratory for Research in Applied Biology at the University of Abomey-Calavi (UAC).

Plant

The plant material is made from the sap of *J. multifida*. It was collected directly into Eppendorf tubes after leaf cutting. Samples were kept refrigerated at 4°C.

Animals

Seven male Wistar rats of 16 to 20 weeks and weighing between 240 and 270 g were kept in the Research Laboratory in Applied Biology at constant temperature of 22 ± 1°C with a 12 h light and 12 h in the dark. They were fed with pellets and water *ad libitum*.

Phytochemical tests

Phytochemical analysis is a qualitative test based on staining reactions and/or differential precipitation of the major groups of chemical compounds of plants. This analysis was performed on the sap using the methodology described by Houghton and Rama (1998). The different reactions of active compounds are summarized in Table 1.

Hemostatic potential of *J. multifida's* sap

Minor cuts, deep cuts (type 1), and deep cuts (type 2) were performed in both legs simultaneously on seven Wistar rats. Wistar rats were intramuscularly anesthetized with ketamine (80 mg/kg). The anesthesia was supplemented by a local administration of 2% Xylocaine (10 mg/kg) on the chosen site for a total stunning of the animal.

Superficial cuts included carrying out the incisions of a millimeter in length and 3 mm deep at the plantar surface of rats localized at 1 cm below the knee. A sterile blade was used. The cuts were made simultaneously at the two legs. Deep cuts of type 1 consisted of sectioning saphenous vein (Figure 1) to two inches above the knee. The two veins of the animal were sectioned simultaneously after partial dressing of the rat. Deep cuts of type 2 consisted of severing femoral vein (Figure 1) which is an extension of the saphenous vein at the groin. The two veins of the animal were sectioned simultaneously after partial dressing of the rat.

After each cut, sap was applied topically to the wound located on one of the legs and the physiological water (0.9% NaCl) on the other leg. The volumes applied were 50, 150, and 200 µl for superficial cuts, deep cuts of type 1, and type 2, respectively. Bleeding time was determined in all three cases with a stopwatch. It was defined as bleeding time, elapsed time between the onset of bleeding (from the cut) and stopping blood flow.

Statistical analyses

Comparisons of comparing two by two the average using the Student t test, p (T>t) = 0.05 were made. The softwares used are Microsoft Excel 2010 and 2011 XL Stat.

RESULTS

Chemical composition of the sap of *J. multifida*

The phytochemical tests carried out on the sap of *J. multifida* have revealed the presence of tannins (gallic and cathechic), flavonoids, saponins, leucoanthocyanes, mucilage, and reducing compounds (Table 2).

Assessment of bleeding time

Averages are recorded as shown in Figure 2. The effect of applying the sap of *J. multifida* on bleeding time is reflected by Figure 3. The cavity marked by blue arrow received physiological water, while the one marked by the green arrow has received sap after section of the femoral vein. More blood flow toward the blue arrow has been noticed than the other. The blood clotted faster on the side receiving the sap.

To better explain the relationship between the depth of the wound and the bleeding time, the percentage reductions are summarized in Table 3.

DISCUSSION

The power of hemostatic sap is really explained by the strong presence of tannins and flavonoids. Indeed, the tannins can stop the bleeding and fight against infections. The tannins-rich plants are used to make

Table 1. Summary of specific reactions of the activeplant.

Classes of active substance	Specific reagent and reaction
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
Anthracene derivatives	Ammonia 50% → intense red color

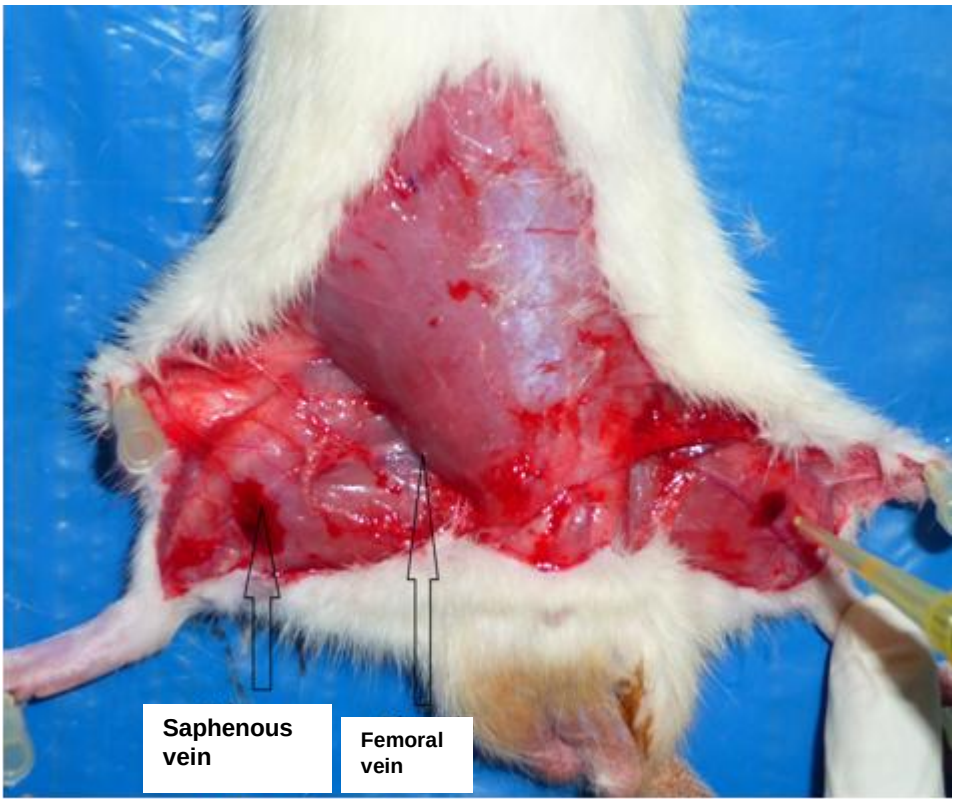

Figure 1. Sections of saphenous and femoral veins.

tissues soft, as in the case of varicose veins, to drain the excess secretions and to repair tissue damaged by burns or eczema (Djabou, 2006). Rira (2006) has also shown that the biological properties of tannins are mainly related

Table 2. Result of reactions to characterize the sap of *Jatropha multifida*.

Classes of active ingredients sought	Result
Alkaloids	-
Quinone derivatives	-
Cathetic and gallic tannins	+++
Flavonoids	+++
Cyanogenic derived	+++
Steroids and triterpenoids	+++
Saponins	-
Anthocyanins	-
Leuco-anthocyanins	+++
Mucilages	-
Reducing compounds	+++
Coumarins	+++
Anthracene derivatives	+++

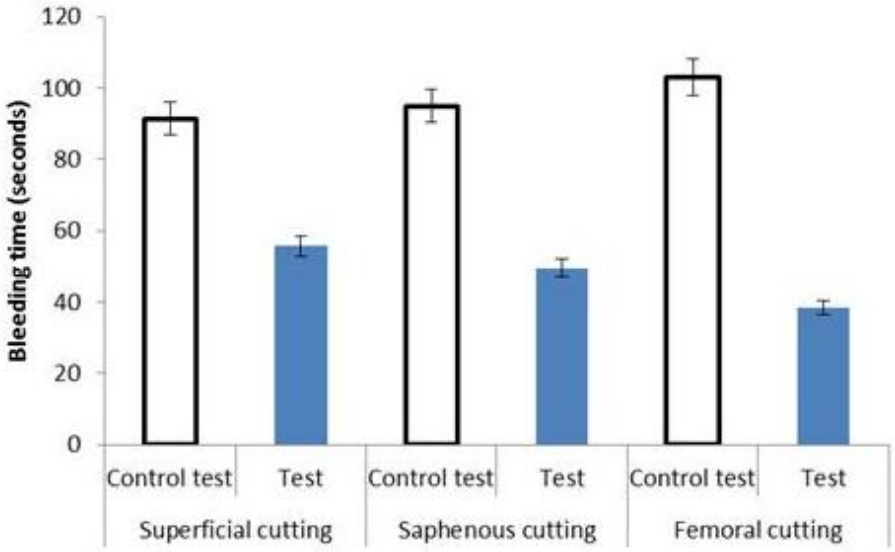

Figure 2. Bleeding time variation by type of cuts.

to their ability to form complexes with macromolecules, especially proteins. Tannins have astringent properties. They are also anti-inflammatory in burns (Paris and Moyse, 1965). Nacoulma (1996) also showed that flavonoids are able to reduce bleeding. They are particularly active antioxidant substances in maintaining blood circulation. They contribute to the increased production of nitric oxide in blood platelets, which limits the formation of clots by preventing platelets from sticking together (Diallo, 2005).

The study of the hemostatic properties of the sap of *J. multifida* reinforces the potential of plants used in traditional hemostasis. According to Kerharo and Adam (1974), the decoction of the bark of *Entada africana*'s trunk is regarded as hemostatic, and tannins and flavonoids were found there. The decoction of its root or stem is also used to clean wounds and the powder of the same parts of the plant is applied on wounds (Sangaré, 2005). Flavonoids were also found in *Calandula officinalis*, a medicinal plant (Hussain et al., 2012).

The results of this study showed that the sap of *J. multifida* significantly reduced the bleeding time. This is even more true when the cut is deep, probably because the blood volume charged at this time is important. The effect of the sap of *J. multifida* on bleeding time is comparable to Ankaferd Blood Stopper, a mixture of traditional plants (*Thymus vulgaris*, *Glycyrrhiza glabra*, *Vitis vinifera*, *Alpinia officinarum,* and *Urtica dioica*) acting on hemostasis reducing bleeding time (Duz et al., 2010).

Traditional knowledge deserves to be appreciated. This enhancement involves the production of improved traditional medicines. After the study, it was proved that

Figure 3. Effects of the sap of *Jatropha multifida* on bleeding time.

Table 3. Percentage reductions in bleeding time according to the type of injury.

Tube	Superficial cutting		Saphenous cutting		Femoral cutting	
	Control	Test	Control	Test	Control	Test
Means ± Standard deviations	91.42 ± 33.07[a]	55.71 ± 23.22[b]	95 ± 37.26[a]	49.5 ± 28.76[b]	103.16 ± 25.82[a]	55 ± 38.35[b]
Percentage of reduction	39.06		47.89		46.68	

Means with the same letters are not significantly different at significance level α = 0.05.

the traditional use of the sap of *J. multifida* is justified as a hemostatic. Its effects on bleeding time were investigated. The different constituents of this substance have been revealed and its hemostatic potential has been well explained. This offers interesting perspectives in therapy, because the sap of *J. multifida* could be used as local hemostatic in both normal subjects as those with coagulation disorders such as hemophilia.

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