

**TOXICOLOGICAL AND PHYTOCHEMICAL SCREENING STUDY OF CRINUM  
SCILIFOLIUM, PLANT OF COTE D'IVOIRE**Koffi Francis Bienvenu<sup>1</sup>, Miezan Bilé Aka Patrice<sup>2</sup>, Okpékon Timothée<sup>3</sup> and Yapi Houphouet Félix\*Pharmacodynamics Biochemical laboratory, UFR Biosciences, Felix Houphouet Boigny University. PO Box 582  
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**ABSTRACT**

The leaves of *Crinum scilifolium*, are used in traditional medicine to treat epilepsy. The present study was carried out to screen aqueous and ethanolic extract of this plant for its phytochemical constituents and acute toxicity profile. Acute toxicity study was evaluated in rodent by OCDE guideline 423 and phytochemical analysis was performed according standard methods. Rats were orally treated by 50, 300, 500, 2000, and 5000 mg/kg body weight with both extracts only once. They were observed for 24H, with special attention given to the first 4H and once daily further for period of 14 day. Phytochemical components were identified in the plants extracts by using standard laboratory grade reagents. The results revealed the presence of sterols, flavonoids, polyphenols and alkaloids. The aqueous one contains the same compounds and saponins. The results of acute toxicity study classified *Crinum scilifolium* above the hazard category 500 mg/Kg < DL50 < 2000 mg/Kg according to global harmonized classification system. The results obtained suggest that the plant extract is considered as slightly toxic. The diversity of phytochemicals found suggest that *Crinum scilifolium* leaves could serve as a source of useful drugs.

**KEYWORDS:** acute toxicity, *Crinum Scilifolium*, phytochemistry, Côte d'Ivoire.**INTRODUCTION**

Traditional medicine was spread in the world and its popularity became extensive, so that 75 to 89% of the population have recourse to the plants to look after it self.<sup>[1]</sup> There is thus an interest growing for the study of the medicinal plants and their use in various areas of world.<sup>[2]</sup> The African continent is equipped with a biodiversity among the rich plants in the world, with a very high number of plants used like grass, natural foods and for therapeutic goals.<sup>[3]</sup> However, traditional medicine not having the capacity to specify the mode of action, the targets biological and the side effects of the molecules. It would expose the populations to serious and sometimes irreversible damages. *Crinum scilifolium* is a plant used much in empirical way to treat epilepsy by traditional medicine; meanwhile, there is no scientific data on the toxicological and phytochemical study of this plant. So the aim of this study is to evaluate the phytochemical profile and the acute toxicity of aqueous and ethanolic extracts of *Crinum Scilifolium* leaves on rats.

**MATERIALS AND METHODS****Plant materials**

The fresh leaves and bulb of *Crinum Scilifolium* were collected in the region of Tomodi, southern of Côte d'Ivoire. The plant was identified at the National

Floristic centre of Felix Houphouet-Boigny, University of Cocody (Abidjan).

**Experimental animals**

Albinos Wistar healthy rats of the same sex, Weighing 150 to 200 g were procured from Animal house. The entire process was approved by OECD Guidelines.<sup>[4]</sup> The animals were kept in clean and dry cages and they were fed with standard pellet diet and water was given as libitum. For experimental purpose, the animals were kept fasting over night but allow for access to water.

**Aqueous Extract Preparation:** The powder of *Crinum Scilifolium* was used to prepare the various extracts. 100g of the powder was extracted in 1L of distilled water. The mixture obtained was then homogenized using a mixer during 24 hours. The homogenate obtained is filtered successively twice on absorbent cotton then once on Wattman filter paper. The filtrate was carried thereafter to evaporation in a drying oven with 50°C during 48 hours. We obtained this way a powder which constituted the aqueous total extract used for the preparation of the various concentrations of the products.<sup>[5]</sup>

**Ethanolic Extract preparation:** 100g of *Crinum Scilifolium* powder were extracted in one liter (1L) of

ethanol-water mixture (70/30). Following unfolds as aqueous extraction.<sup>[6]</sup>

## METHODS

### Study of acute toxicity

The experimentation was carried out sequentially according to OECD guideline 423.<sup>[7]</sup> Healthy Wistar rats (150-200g) were divided into 5 groups of 3 animals each and allow to access water and food throughout the experimentation, except for the fasting period before the oral administration of the single dose of extracts. The five groups of rats received respectively dose of the 50, 300, 500, 2000 and 5000 mg/kg bw of *Crinum scillofolium* extracts. The general behavior and mortality of the rats was continuously monitored for 1 h after dosing periodically during the first 24 h and then daily for 14 days. Changes in the normal activity of rats, sign and symptoms of toxicity study were also recorded.

### Phytochemical screening

It consists in identifying for a plant, chemical compounds groups showing pharmacological interest. The different extracts obtained with the powder were used to identify and characterize some chemical groups.

### Test for Alkaloids

This description takes place with the reagent of Bouchardat and Dragendorff. 6 mL of plant extract were evaporated. The residue is taken up in 6 mL of 60° alcohol and the alcoholic solution thus obtained is divided in two test tube. In the first tube were added 2 drops of Dragendorff reagent (aqueous solution of potassium iodo-bismuth). The appearance of a precipitate or orange color indicates the presence of alkaloids. In the second tubes were added 2 drops of reagent of Bouchardat (iodo-iodide aqueous solution). The appearance of a reddish brown color indicates the presence of alkaloids.<sup>[8,9]</sup>

### Test for Polyphenols

2 mL plant extract was added a drop of aqueous solution of 2 % ferric chloride. The appearance of a dark green or more less dark blue color indicates the presence of phenolic compounds.<sup>[10,11]</sup>

### Test for Tannins

#### Catechin Tannins

5 mL of extract are evaporated. 15 mL of reagent Stiasny (10 mL 40 % formalin added 5 mL of concentrated HCL) was added to the dry residue. The mixture was kept in the water bath at 80°C for 30 min. The observation of large precipitates flakes characterized catechin tannin.<sup>[10,11]</sup>

#### Gallic Tannins

The solution containing the flakes is filtered and the collected filtrate was then saturated with sodium acetate. 3 drops of ferric chloride 2 % is added to the mixture. The appearance of a deep blue-black color indicated the presence of gallic tannins.

### Test for Flavonoids

2 mL of plant extract is evaporated. After cooling, the residue is taken up in 5 mL of Hydrochloric alcohol (obtained by mixing 10 mL of ethanol at 96 °, 10 mL of distilled water and 10 mL of concentrated hydrochloric acid) diluted 2 times in in test tube. It adds two to three magnesium turning (heat). The addition of 3 drops of iso amyl alcohol intensifies a pink orange or violet, indicating the presence of flavonoids.<sup>[9,11,12]</sup>

### Test for saponosids

10 mL of plant extract are put into a test tube. After stirring for a few minutes, the foam height is measured. The height greater than 1 cm foam indicates the presence of saponin. The saponin may also be highlighted by the persistence of the foam.

### Test for Polyterpens and sterols

Liebermann's reagent is used for this demonstration. 5 mL of plant extract were dried under rotary evaporator. The residue was dissolved in 1 mL of hot acetic anhydride and collected in a test tube. Along the tube, is caused to flow with 0,5 mL of concentrated sulfuric acid. The appearance at the interphase of a purple or purple ring, turning blue to green, indicating the presence of polyterpens and sterol.<sup>[11,12]</sup>

### Test for Quinoid Compounds

Compose quinoid free or compounds are highlighted thanks to the reaction of Borntraeger (ammonium diluted twice).

2 mL of each solution are evaporated dry in a capsule, and the residue is taken again with 5 mL of acid chloridric to the 1 /5. Colouring observe is reversed in a test tube then maintained during 30 min with the Marie bath boiling. After total cooling, 20 mL chloroform is added to the contents of the test tube. Then the recovered chloroformic phase is added to ammonia 0,5 mL diluted twice. The appearance of the colouring going to the red to purple indicates the presence of quinoid compounds.<sup>[9,11,13]</sup>

## RESULTS

### Acute Toxicity

Observing animals during the experiment, no mortality was noticed until amount 2000 mg/kg body weight. During 14 days periods of acute toxicity evaluation, some signs of toxicity have been observed, but they were all quickly reversible. The clinics signs are presented in table 1.

### Phytochemical Screening

Principal chemical groups identified are consigned in the table 2. The result showed that polyphenols, alkaloids, saponosids, sterols and terpens are present in the two extracts (aqueous and ethanol). We find saponosids only in the aqueous extract. Other classes are compounds absent in the two plants: the cardiotonics glycosides, taninns, quinones and leucoanthocyanes (table 2).

**Table 1: Clinical signs observed during the first 24 hours after administering of aqueous and ethanolic extracts of *Crinum scilifolium*.**

	36 FEMALS RATS EXPLOITED						
	Number of treated animals	Number of dead animal	CLINICAL SIGNS				
			Abdominal constrictions	Immobility	Food	Fast breathing	Difficult displacement
<b>AQUEOUS EXTRACT</b>							
Control (NaCl)	3	0	-	-	+	-	-
Group 1 (50 mg/kg)	3	0	-	-	+	-	-
Group 2 (300mg/kg)	3	0	-	-	+	-	-
Group 3 (500 mg/kg)	3	0	-	-	+	-	-
Group 4 (2000mg/kg)	3	3	+	+	-	-	+
Group 5 (5000mg/kg)	3	3	+	+	-	-	+
<b>ETHANOLIC EXTRACT</b>							
Control (NaCl)	3	0	-	-	+	-	-
Group 1 (50 mg/kg)	3	0	-	-	+	-	-
Group 2 (300mg/kg)	3	0	-	-	+	-	-
Group 3 (500mg/kg)	3	0	-	-	+	-	-
Group 4 (2000mg/kg)	3	2	+	+	-	-	+
Group 5 (5000mg/kg)	3	3	+	+	-	-	+

+ : presence of sign - : Absence of signs

**Table 2: Preliminary phytochemical screening of *Crinum scilifolium*.**

IDENTIFIED CHEMICALS GROUPS	EXTRAITS DE FEUILLES	
	Aqueous extract	Ethanolic extract
Polyphenols	+	+
Flavonoïds	++	+
Saponosids	++	-
Taninns	Gallic	-
	Catéchin	-
Leucoanthocyane	-	-
Alcaloïds	Dragendorf	++
	Boucharddat	++
Cardiotonics Glycosides	-	-
Stérols et Terpens	+	++
Quinones	-	-

+ : Present - : Absent

## DISCUSSION

### Acute toxicity

The method of predetermined dose does not aim precise value of DL50 calculation, but determines product SGH category.<sup>[14,15]</sup> The presence of death observed with dose 2000 mg/Kg enable us to classify our extracts in category 4 of the SGH. That means what follow:

- 500 mg/Kg < DL50 Aqueous extract < 2000 mg/Kg body weight.
- 500 mg/Kg < DL50 Ethanolic extract < 2000 mg/Kg body weight

We can thus say according to the scale of toxicity of Hodge and Sterner in the rat that our products are slightly toxic.<sup>[16]</sup>

### Phytochemical Screening

Within sight of the results obtained, it could be possible that the two extracts express biological and pharmacological activities. Meanwhile, the presence of certain compounds such us saponosids only in the

aqueous extract would make it possible to use this extract like most active.

The saponosids and polyphenols are more abundant in the aqueous extract. The saponosids are heterosides whose core is either steroidien, or triterpenic or pentacyclic. They express haemolytic, antimicrobial, insecticidal, molluscicidales properties,<sup>[17]</sup> anti-inflammatory and analgesics properties.<sup>[18]</sup>

The flavonoids are metabolites which express a good antioxydant activity according to N'guessan and Zhi.<sup>[19,20]</sup> The flavonoids are largely known by their antiviral, antiplasmodic, anti-inflammatory, anti-hypertensives and anti-microbial activities.<sup>[21,22,23,24]</sup> The tannins are used as vasodilator and haemostatic.<sup>[13,25,26]</sup>

The alkaloids are natural organic compounds (generally of vegetable origin), heterocyclic with nitrogen like heteroatom, of complex molecular structures more or less basic and endowed with marked physiological

properties even with low dose.<sup>[27]</sup> They have several pharmacological applications at the human: they are indeed antitumor, antalgic and antipaludic.<sup>[28]</sup>

Sterol have several functions which allow the manufacture of many drugs such as the contraceptive and anti-inflammatory drugs.<sup>[29]</sup>

## CONCLUSION

The study of toxicity was carried out to locate the tolerant limits of the leaves of *Crinum scilifolium*. According to this study, it could be concluded that the administering of ethanolic and aqueous extract of *Crinum scilifolium* in rats is safe at any dose less than or equal to 500 mg/kg body weight. For the phytochemical screening, we can say that the leaves of *Crinum scilifolium* is rich in following active compounds: sterols, flavonoids, polyphenols, alkaloids and saponosids. This abundance in active elements confers to this plant remarkable properties. After these phytochemical screening and toxicological studies on *Crinum scilifolium* it is desirable to evaluate its pharmacological effects on immunity.

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