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TOXICOLOGICAL AND PHYTOCHIMICAL SCREENING STUDY OF CRINUM SCILIFOLIUM, PLANT OF COTE D'IVOIRE

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ABSTRACT

The leaves of *Crinum scilifolium*, are used in traditional medicin 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 evalauted 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 ony 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 standart laboratory grade reagents. The results revealed the presence of sterols, flavonoids, polyphenols and alkaloids. The aqueous one countains 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

Traditionnal 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.[1] There is thus an interest growing for the study of the medicinal plants and their use in various areas of world.[2] 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.[3] However, traditionnal 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 ampirical way to treat epilepsy by traditionnal medicine; meanwhile, there is no scientific data on the toxicological and phytochimical study of this plant. So the aim of this study is to evaluate the phytochimical 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, Weigthin 150 to 200 g were procured from Animal house. The entire process was approved by OECD Guidelines.[4] 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 Preparetion: 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 mixtor 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 varions concentrations of the products.[5]

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.[6]

METHODS

Study of acute toxicity

The experimentation was carried out sequentially according to OECD guideline 423.[7] 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 scilifolium 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 cconsists in identitifying 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 an 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.[8,9]

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 presesnce of phenolic compounds.[10,11]

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.[10,11]

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.[9,11,12]

Test for saponosids

10 mL of plant extract are put into a test tube. After stirrig for a few minutes, the foam height is mesured. The height greater than 1 cm foam indicates the presence of sapononins. The saponins 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.[11,12]

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 toltal cooling, 20 mL chloroform is added to the contents of the test tube. Then the recorvered chloroformic phase is added to ammonia 0,5 mL diluted twice. The appearance of the colourng going to the red to purple indicates the presence of quinoid compounds.[9,11,13]

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 thee first 24 hours after administering of aqueous and ethanolic extracts of *Crinum scilifolium.*

	36 FEMALS RATS EXPLOITED						
	Number of treated animals	Nomber of dead animal	CLINICAL SIGNS				
			Abdominal constrictions	Immobility	Food	Fast breathing	Difficult displacement
	AQUEOUS EXTRACT						
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	+	+	-	-	+
	ETHANOLIC EXTRACT						
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 cathegory.[14,15] 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.[16]

Phytochemical Screening

Within sight of the results obtained, it could be possible that the two extracts express biological and pharmacogical 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, antimicrobic, insecticidal, molluscicidales properties,[17] anti-inflammatory and analgesics properties.[18]

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

The alkaloids are natural organiccompounds (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.[27] They have several pharmacological applications at the human: they are indee antitumor, antalgic and antipaludic.[28]

Sterol have several functions which allow the manifacture of many drugs such as the contraceptic and anti-inflammatory drugs.[29]

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 conferts to this plant remarquable properties. After these phytochemical screening and toxicological studies on *Crinum scilifolium* it is desirable to evaluate its pharmacological effects on immunity.

REFERENCES

1. Sofowora, A. Medicinal plants and Traditionnal Medicines in Africa. Spectrum Books LTD, Sunshine House 1, Emmanuel Alayade Street, P.M.B 5612, Ibadan, Nigeria, 1996.
2. Muthu C. Ayyanar M, Raja N, Ignacimuthu S. Medicinal plants used by traditionnal healars in Kancheepuran District of Tamil Nadu, India. Journal of Ethnobiology and Ethnomedicine, 2006; 2: 43.
3. Aké Assi L, Guinko S. Plantes utilisées dans la médecine traditionnelle en Afrique de l'ouest. Edition Roche, 1991; 151.
4. OECD, Acute orale toxic class method guideline 423 adopted 23.03.1996. In: Eleventh Addendum to the, OECD, guidelines for the testing of chemicals. *Organisation for Economical Co-Operation and developpement*, Paris, June, 2000.
5. Guédé-Guina F, Vangah-Manda M, Harouna D. Potencies of MISCA, a plant source concentrate against fungi. Submitted to *J. of Ethnopaharmacol*, 1993; 14(2): 45-53.
6. Zihiri GN, Grellier P, Guédé-Guina F, Bodo B, Lengo M. Isolation, caractérisation and antiplasmodial activity of steroidal alkaloids from *Funtumia elastic* (Preuss) Staph. *Bioganic and Medicinal Chemistry Letters*, 2005; 15: 2637-2640.
7. OECD, Acute oral toxicity. Acute oral toxicity class method guideline 423 adopted 23.03.1996. in: Eleventh Addendum to the OECD guidelines for the testing of chemical. *Organisation for Economical Co-Operation and developpement,* Paris, June, 2000.
8. Nemlin J. Brunel JF. Facicule de travaux pratiques de matière médicale (3ème année). Université nationale de Côte d'Ivoire. Faculté de pharmacie. Département de Pharmaconosie, Laboratoire de Phytologie, 1995; 47.
9. Rafael F. Elena C., Marcedes DRC. Pharmaconosie, Phytochimie, Plante méddicinales. Phytochemistry, 2005; 66: 175-185.
10. Rafael F. Elena C., Marcedes DRC. Pharmaconosie, Phytochimie, Plante méddicinales. Phytochemistry, 2005; 66: 175-185.
11. N'guessan K. Plantes médicinales et pratiques médicales traditionnelles chez les Abbey et Krobou du département d'agboville (Côte d'Ivoire). PhD dissertation, University of Cocody-Abidjan, 2008.
12. Brock A, Herzfeld T, Paschke R, Koch M, Drager B. Pharmacognosie, Phytochimie, Plantes médicales. Phytochemistry, 2006; 67(18): 2050-2057.
13. Gbeassor M., Kossou Y, Amegbo K, Souza C, Koumaglo K, Denke A. Antimalarial effects of eight african medicinal plants. Ethnopharm, 1989; 25: 115-118.
14. OCDE (Organisation de Cooperation et de developpement Economique). Harmonised Integrated Hazard Classification for human Health and Environment Effects of Chemical Substances as endorsed by the 28th Joint Meeting of the Chemicals Committee and the working Party on Chemicals, 1998; 2: 11.
15. OCDE (Organisation de Cooperation et de developpement Economique). Guideline for testing of chemicals. Guideline 420. Acute oral toxicity-Fixed Dose Procedure, 2001; 15: 254-365.
16. Hodge A. Sterner B. toxicity Classes In :Canadian Centre for Occupational Health and Safety. Copyright @ 1997-2010. Retrieved from (http:// www.ccohs.ca/osshanswers/chemical/LD50.htm.on 3/5/2010.
17. vincken JP, Heng LA, De Groot H. Gruppen, Review Saponins, classification and occurrence in the plant kingdom. Phytochemistry, 2007; 68: 275-297.
18. Donatien K. Enquête ethnobotanique de six plantes médicinales Malienne-Extraction, Identification d'alcaloids-Caractérisation, Quantification de polyphénols: Etude de leur activité antioxydante. Thèse de l'université de bamako, Faculté des Sciences et Techniques, Spécialité Chimie Organique, 2009: 23-31.
19. N'Guessan JD, Zihiri GN, Kra AKM, Kouakou K, Djaman AJ, Guédé-Guina F. Free radical scavenging activity, flavonoids and phenolic contents of selected Ivorian plants. IJONAS, 2007; 4: 425-429.
20. Zhi PR, Liang LZ, Yi ML. Evaluation of the antioxydant activity of Syzygium cumini leaves molecules, 2008; 13: 2545-2556.
21. Das HC, Wang JH, Lien E. Carcinogene city and cancer preventing activities of flavonoids: a structure system-activity relationship analysis. *Journal of Food Engineering*, 1994; 69: 133-136.
22. Formica JV, Regelson W. Review of the biology of quercetine and related bioflavonoids. Food ChemToxicol, 1995; 33: 1061-1080.
23. Yochum L, KusliL, Meyer K, Folson A. Dietary flavonoid intake and risk of cardiovascular desease

in postmenopausal women. *Am J Epiderma*, 1999; 149: 943-949.
24. Kim HP, Son KH, Chang HW, Kong SS. Anti-inflammatory plant flavonoids and cellular action mecanism. *J Pharmaco. Sci*, 2004; 96: 229-254.
25. Ouédraogo Y, Nacoulma O, Guissou IP, Guédé GF. Evaluation in vivo et in vitro de la toxicité des extraits aqueux d'écorses de tige et de racines de *Mitragyna inermis* (Wild.) O. Pharma. Med. Trad. Afr, 2001; 11: 13-29.
26. Traoré H, Balansard G, Pauli AM, Scotta AM. Pharmacological in Alkaloids from leaves of *Mitragyna inermis* (Rubiaceae). J. Ethnopharm, 2002; 14: 35-65.
27. Bruneton J. Pharmacognosie, phytochimie et plantes medicales. Edition Tec et Doc, Paris, 1999: 1120.
28. Donatien K. E nquête ethnobotanique de six plante médicinales Malienne-Extraction, Identification d'alcaloids-Caractérisation, Quantification de polyphénols: Etude de leurs activités antioxydante. These de l'université de Bamako, Faculté des Sciences et Techniques, Spécialité Chimie Organique, 2009: 23-24.
29. Bruneton J. Pharmacognosie, phytochimie et plantes medicales 2ème edition; 1993: 203-642.