World Journal of Pharmaceutical ReseaRch
SJIF Impact Factor 5.045
Volume 3, Issue 8, 12-23. Research Article ISSN 2277 – 7105

TOXICOLOGICAL AND PHYTOCHEMICAL SCREENING STUDY OF *TREMA GUINEENSIS* (ULMACEA), PLANT OF CÔTE D'IVOIRE (WEST AFRICA)

Kouakou Yéboué Koffi F[1], Gnahoue Goueh[2], Yapi Houphouet F[1]*, Ayebe Edwige Aka[1], N'guessan Jean D[1], Djaman Allico J [1, 3]

[1]Pharmacodynamics BiochemicalLaboratory, UFR Biosciences, Felix Houphouet Boigny University. PO Box, 582, Abidjan 22- Côte d'Ivoire.

[2]Laboratory of SVT, Higher Teacher training school of Côte d'Ivoire. POBox, 10 Abidjan 08- Côte d'Ivoire.

[3]Laboratory of Basic and Clinical Biochemistry, Pasteur Institute of Côte d'Ivoire. PO. Box, 01 BP 490 Abidjan 01 Biochemical Laboratory, UFR medical Sciences, Félix Houphouet Boigny University. PO Box, 582, Abidjan 22- Côte d'Ivoire.

Article Received on
28 July 2014,

Revised on 21 August 2014,
Accepted on 16 Sept 2014

***Correspondence for Author**
Dr. Yapi Houphouet
Pharmacodynamics BiochemicalLaboratory, UFR Biosciences, Felix Houphouet Boigny University. PO Box, 582, Abidjan 22- Côte d'Ivoire

ABSTRACT

The leaves of *Trema guineensis*are used in traditional medicine to treat several pathologies like the pains, the yellow fever, cough and hypertension. In order to indicate its Tolerance limits, we determined acute toxicity threshold by the Overall Harmonized System of classification (SGH) using predetermined dose method. We have also highlighted principal chemical groups responsible for pharmacological activity. The results allowed us to conclude that any dose of *Trema guineensis*leaves aqueous and ethanol extracts (300 and 2000 mg/kg) was lethal for the inoculated animals. DL50 was thus higher than 2000 mg/kg. That proved clearly that the aqueous and ethanolic leaves extracts of *Trema guineensis*could be used with some degree of safety by intraperitoneal route. The phytochimical analysis indicated the presence of sterol, terpen, polyphenol, flavonoid, tannin, saponosid and alkaloid in the aqueous extract whereas only the polyphenol, The Alkaloid, sterol, terpen were revealed in the ethanolic extract. The presence of these compounds confred several pharmacological activities on the leaves of the plant.

KEYS WORDS: acute toxicity, *Trema guineensis*, phytochemistry, Côte d'Ivoire

INTRODUCTION

Traditional medicine was spread in the world and its popularity became extensive, so that 75 to 89% of the populations 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, 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. It is the case of *Trema guineensis*, which is a plant used much in an empirical way to treat the pains, the fevers, cough and hypertension by traditional medicine [4, 5, 6]. Unfortunately still, up to now, there is no scientific data on the toxicological and phytochimical study of this plant. Its virtues are also little known scientific world. It is thus convenient to develop a scientific approach in order to locate the tolerant limits of its use. The aim of this research, in addition to what precedes, is to know the chemical composition of the leaves of this plant for the optimization of its use by modern medicine.

MATERIALS AND METHODS

Plant Material: The vegetable material is constituted by leaves of *Trema guineensis (Ulmacea)* which were identified by Pr AKE-Assi of the botanical garden at Félix Houphouet Boigny University. This plant is a heliophilous shrub answering the "russet-red" model [7]. It is sarmentous, ramified at the beginning of its growth in savanna, its bark is fibrous and it becomes in forest a tree of about fifteen meter height. These leaves are simple, distiches and its dentils measure 6 to 12 cm length on 2.5 to 5 cm of width [5]. The fresh leaves were collected, dried with the shelter of the sun during two weeks and pulverized with the crushing assistance of type IKAMAG. The powder of leaves obtained, constituted our sample to be analyzed. The aqueous and ethanolic extracts obtained starting from this powder of leaves were used to make the studies of toxicity and phytochemistry.

Experimental Animals: Healthy Wistar Albino rats of female sex, weighing about 150-200g were procured from Animal House. These animals were kept in animalery of the Training and Research Unity of Pharmaceutical and Biological Sciences at FELIX Houphouet Boigny University. The rats were fed with FACI (Fabrication d' Aliments de Côte d'Ivoire) pellets, groundnuts and dried fish. Their drink was tap water. A total of 36 females rats were used in

this study. The care and the conditions of animals' treatment are in conformity with the Hot lines of the Organization for Economic Cooperation and Development (OECD).

Aqueous Extract Preparation: The powder of *Trema guineensis* 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 mixor during 24 hours. The homogenate obtained is filtered successively twice on absorbent cotton then once on Wattman N°1 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 [8].

Ethanolic Extract Preparation: 100g of *Trema guineensis* powderwere extractedin oneliter (1L) of ethanol-water mixture (70/30, v / v). Followingunfolds asaqueous extraction [9].

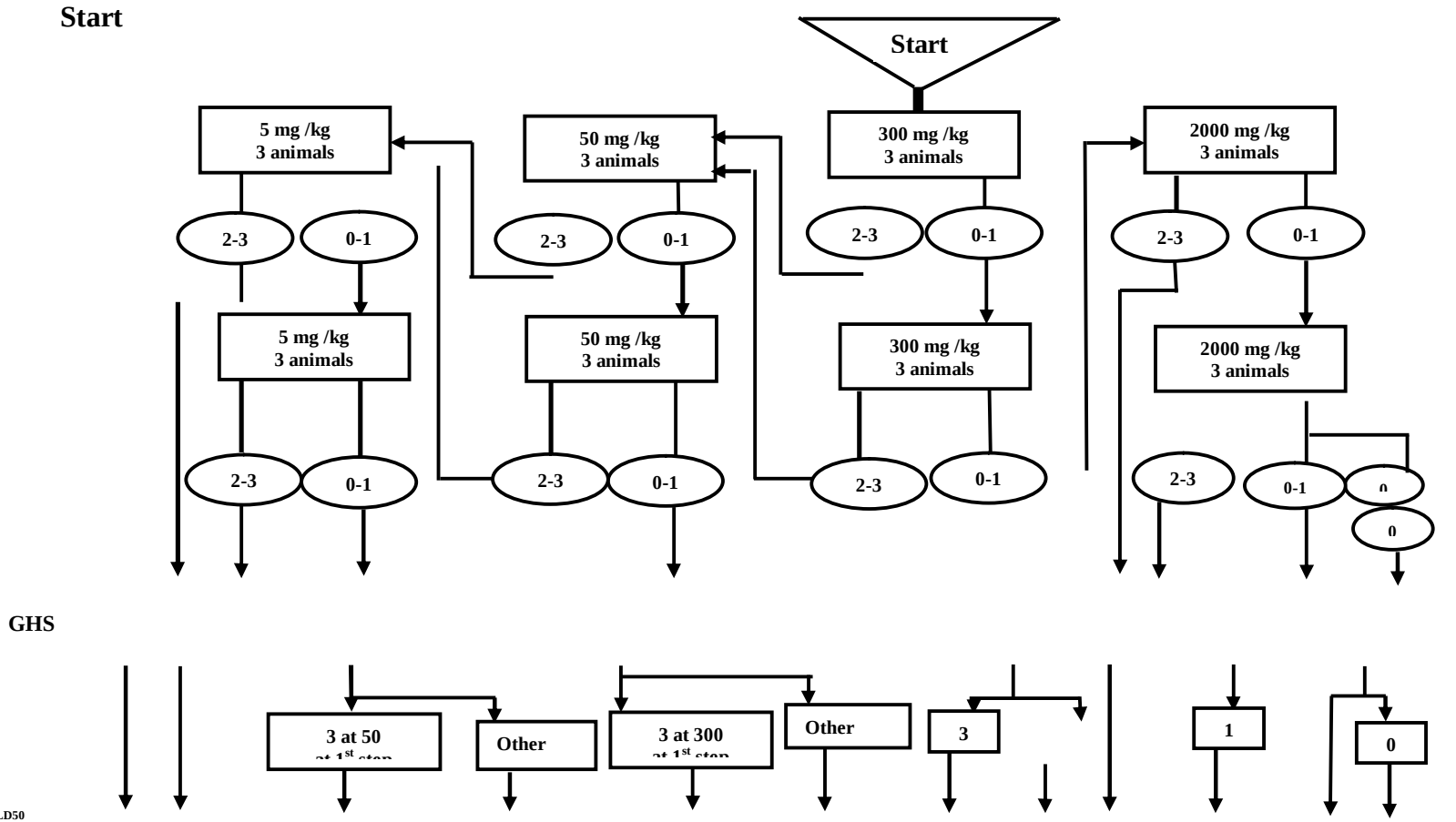
METHODS

Study of Acute Toxicity

Preparation of concentrations to be administered to the rats: The concentrations of aqueous and ethanol Extract of *Trema guineensis* were prepared on the basis of principle that the concentrations To be administered, must be associated to the body weight of the rats, considering that the administered doses are expressed in mg/kg body weight. Thus, we injected 0.5 mL of aqueous or Ethanolic extract to all the female rats.

Acute Toxic Class Method: Acute toxicity study was performed according to OECD guidelines 423 modified (Organization of Economic Cooperation and Development); the substances of test were given to rats via intraperitoneal route instead of the oral. It is a stepwise procedure with three animals of single sex per step and thus, three female rats whose average weight is 180 were used in each one of these stages. Depending on the mortality and morbidity status of animals, 4 steps were necessary to allow judgment on our test substance. The procedure is to fix a minimal number of animals, which allows acceptable database scientific conclusion. The method uses different defined doses (5, 50, 300, 2000mg/kg body weight) and the results allow a substance to be ranked and classified according to the "Globally Harmonized System "(GHS) for the classificationof extracts which cause acute toxicity.

DIAGRAM1: Annex 2c of OECD guidelines 423, showing test procedure with a starting dose of 300 GHS and LD50 mg/kg body weight.



-per step three animals of single sex (normally females) are used
-0, 2, 3: Number of moribund or dead animals at each step
-GHS: Globally Harmonized Classification System (mg/kg b.w)

-: unclassified
-Testing at 5000 mg/kg b.w.:see

Procedure

The rats were fasted prior to dosing by removing food during the night but not water. No estimate of the substance's lethality is available; so dosing was initiated at 300 mg/kg of body weight because of the wellbeing of the animals. Like it of did not die there after 48 hours, we repeated the same dose for the confirmation. We made of it in the same way for the dose 2000 mg/kg of body weight. The animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours), and daily thereafter, for a total of 14 days. All these rats were regularly observed during 14 days according to recommendations of OECD[10] and we noted the mortality or the symptoms of intoxication (clinical signs). The following flow chart depicts the procedure adopted for this method.

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 (alcohol content) and the alcoholic solution thus obtained is divided in two test tubes. 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 tube were added 2 drops of reagent Bouchardat (iodo - iodide aqueous solution). The appearance of a reddish brown color indicates the presence of alkaloids[11, 12].

Test for Polyphenols

2 mL of plant extract was added a drop of aqueous solution of 2 % ferric chloride. The appearance of a dark green or more or less dark blue color indicates the presence of phenolic compounds.[12, 13]

Test for Tannins

Catechin Tannins

5 mL of extract 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 a water bath at 80°

C for 30 min. Cool under running water. The observation of large precipitates flakes characterized catechin tannins. [12, 13]

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 indicates 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 a test tube. It adds two to three magnesium turnings (heat). The addition of 3 drops of iso amyl alcohol intensifies a pink - orange or violet, indicating the presence of flavonoids.[11, 13, 14]

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 saponins. 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 sterols.[13, 14]

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 5mL of acid chloridric to the 1/5. Colouring obtained 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 a colouring going of the red to purple indicates the presence of quinoid compounds. [11, 13, 15]

RESULTS

Acute Toxicity Studies

During the acute toxicity study, aqueous and ethanolic extracts were administered and animals were observed for mortality and behavioral responses. During the 14 days of observation, there was no mortality observed even at 2000mg/Kg for the both extracts. All the animals were found to be normal and there were no significant behavioral changes till the end of the observation period. This observation revealed that the aqueous and ethanolic extract of the leaves were found to be very safe up to 2000mg/kg of body by acute toxicity model study as per OECD guidelines 423 (table 1).

Table 1: Clinical signs observed during the first 24 hours after administering of the aqueous and ethanolic extracts of *trema guineensis*

	36 FEMALS RATS EXPLOITED						
	Number of treated animals	number of dead animals	CLINICAL SIGNS				
			Abdominal constrictions	Immobility	food	fast breathing	Difficult displacement
	AQUEOUS EXTRACT						
Control (NaCl)	6	0	-	-	-	-	-
Group 1 (300mg/kg)	6	0	+	+	+	+	+
Group 2 2000mg/kg)	6	0	+	+	+	+	+
ETHANOLICEXTRACT							
Control (NaCl)	6	0	-	-	-	-	-
Group 1 (300mg/kg)	6	0	+	+	+	+	+
Group 2 (2000mg/kg)	6	0	+	+	+	+	+

+:Presence of signs light at the beginning and fast disappearance of these signs at theend of the time of observation

-: Absence of signs

Phytochemical Screening

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

Table 2: Preliminary phytochemical screening of *Trema guineensis*

IDENTIFIED CHEMICALS GROUPS		LEAVES POWDER EXTRACTS	
		Aqueous extract	Ethanolic Extract
Polyphenols		++	+
Flavonoids		+	-
Saponosids		++	-
Taninns	**Taninn catechin**	-	-
	Taninn gallic	-	-
Leucoanthocyane		-	-
Alcaloids	**Dragendorf**	+	+
	Bouchardat	+	+
Cardiotonics Glycosides		-	-
Sterols and Terpens		+	+
Quinones		-	-

+: Present, -: Absent

DISCUSSION

Acute Toxicity

The method of predetermined dose does not aim precise value of LD50 calculation, but determines product SGH category[16, 17]. The absence of death observed with doses 300 and 2000mg/kg body weight enables us to classify our extracts in category 5 of the SGH; that means what follows:

2000mg/kg body weight < DL50 Aqueous extract< 5000mg/kg body weight

2000mg/kg body weight < DL50 Ethanolic extract< 5000mg/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 [18].

Phytochemical Screening

Within sight of the results obtained, the two extracts would express biological and pharmacogical activities. However, the presence of flavonoids, saponosids, taninns only in the aqueous extract would make it possible to use this extract like most active. 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 [19].

They have several pharmacological applications at the human; they are indeed antitumor, antalgic and antipaludic[20]. The polyphenols and saponosids are more abundant in the aqueous extract. The saponosids are heterosides whose core is either stéroidien, or triterpenic or pentacyclic. They express haemolytic, antimicrobic, insecticidal, molluscicidales properties [21], anti-inflammatory and analgesics properties [20].

This work also showed that *Trema guineensis*leaves contain sterols, polyterpens, polyphenols, flavonoids, taninns and alkaloids in variable proportion like that was noted with an another specie of Ulmacea suck as *Trema orientalis*[22].

The flavonoids are metabolites which express a good antioxydant activity according to N'Guessanand Zhi [23, 24]. The flavonoids are largely known by their antiviral, antispasmodic,anti-inflammatory, anti-hypertensives and antimicrobic activities[25, 26, 27, 28]. As for the taninns, they are also used as vasodilator and haemostatic[15, 29, 30]. Sterols have several functions which allow the manufacture of many drugs such as the contraceptic and anti-inflammatory drugs [31].

CONCLUSION

The study of toxicity was carried out to locate the tolerant limits of the leaves of *Trema guineensis*. According to this study, the aqueous and ethanolic extracts did not record any mortality by predetermined dose; from the observation made, it could be concluded that the administering of *Trema guineensis* in rats is safe at any dose less than or equal to 2000 mg/kg body weight. However, extremely high doses by intraperitoneal route may not be advisable. For the phytochimical screening we can say the leaves of *Trema guineensis*are rich in following active compounds: sterols, flavonoids, taninns, alkaloids and saponosids. This abundance in active elements confers to the plant, remarkable properties. That could justify the many therapeutic indications for which it is used in traditional medicine. After these phytochemical screening and toxicological studies on *Trema guineensis*leaves, it is desirable to evaluate its pharmacological effects on the anti-inflammatory activity; this in order to include the allotted virtues to this vegetable species.

REFERENCES

1. Sofowora, A. Medicinal plants and Traditional 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 traditional healers in Kancheepuram 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. Editions Roche 1991; 151.
4. Aké Assi L. Quelques plantes utilisées dans le traitement des maladies cardiaques en Côte d'Ivoire. Bull. Méd. Trad. Pharm. ACCT. Paris 2, 1988; (1): 96-100.
5. N'Guessan, K. Contribution à l'étude ethnobotanique en pays Krobou (République de Côte D'ivoire). Thèse 3eme cycle, Faculté sciences et Techniques de l'Université Nationale. 1995; 557.
6. Kerharo, J, Bouquet A. Plantes médicinaleset et toxiques, de la Côte d'Ivoire-Haute Volta. Mission d'Etude de la Pharmacopée Indigène en A.O.F.Ed. Vigot Frère:1950; 1011.
7. Halle, F,Odeman RAA. Essai sur l'architecture et la dynamique de croissance des arbres tropicaux. Masson et ed. 1970; 179.
8. Guédé-Guina F, Vangah-Manda M, Harouna D, Bahi. Potencies of MISCA, a plant source concentrate against fungi. Submitted to *J. of Ethno pharmacol*, 1993; 14(2): 45-53.
9. Zirihi GN, Grellier P, Guédé-Guina F, BodoB, Lengo M. Isolation, characterisation and antiplasmodial activity of steroidal alkaloids from *Funtumia elastic* (Preuss) Stapf. *Biorganic and Medicinal Chemistry Letters*, 2005; 15: 2637-2640.
10. OCDE. (Organisation de Coopération et de Développement Economiques). Guideline for testing of chemicals. Guideline 420: Acute Oral Toxicity-Fixed Dose Procedure, 2001; 15: 254-365.
11. Nemlin J, Brunel JF. Fascicule de Travaux Pratiques de Matière Médicale (3ème année). Université Nationale de Côte d'Ivoire: Faculté de Pharmacie. Département de Pharmacognosie, Laboratoire de Phytologie, 1995; 47.
12. Rafael F, Elena C,Marcedes DRC. Pharmacognosie, Phytochimie, Plantes médicinales. Phytochemistry, 2005; 66: 175-185.
13. N'Guessan K. Plantes médicinales et pratiques médicales traditionnelles chez les peuples Abbey et Krobou du Département d'Agboville (Côte d'Ivoire). PhD dissertation, University of Cocody-Abidjan Côte d'Ivoire, 2008.

14. Brock A,Herzfeld T, Paschke R, Koch M, Drager B.Pharmacognosie, Phytochimie, Plantes médicinales. Phytochemistry. 2006; 67 (18): 2050-2057.
15. Gbeassor M.,Kossou Y,Amegbo K, Souza C, Koumaglo K, Denke A.Antimalarial effects of eight african medicinal plants. Ethnopharm, 1989; 25: 115-118.
16. OCDE (Organisation de Coopération et de Développement Economiques). 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.
17. OCDE (Organisation de Coopération et de Développement Economiques). Guideline for testing of chemicals. Guideline 420: Acute Oral Toxicity-Fixed Dose Procedure. 2001; 15: 254-365.
18. Hodge A, Sterner B. Toxicity Classes In: Canadian Centre for Occupational Health and safety. Copyright @ 1997-2010. Retrieved from (http: //www.ccohs.ca/oshanswers/chemicals/Ld50.htm. on 3/5/2010.
19. Bruneton, J. Pharmacognosie, Phytochimie et plantes médicales. Editions Tec et Doc, Paris, 1999: 1120.
20. Donatien, K. Enquête ethnobotanique de six plantes médicinales Maliennes - Extraction, Identification d'alcaloïdes -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.
21. Vincken, J.P., L. Heng, A., De Groot,H. Gruppen,Review Saponins, classification and occurrence in the plant kingdom. Phytochemistry, 2007; 68: 275–297
22. N'Guessan K., Kadja, Zirihi, B G N, Traoré D, L. Aké-Assi.Screening phytochimique de quelques plantes médicinales ivoiriennes utilisées en pays Krobou (Agboville, Côte-d'Ivoire). *Sciences &Nature.* 2009; *6*(1): 1 – 15.
23. N' Guessan JD, Zirihi GN, Kra AKM, Kouakou K, Djaman AJ, Guédé-Guina F.Free radical scavenging activity, flavonoid and phenolic contents of selected Ivoirian plants. IJONAS. 2007; 4: 425-429.
24. Zhi PR, Liang LZ, Yi ML.Evaluation of the Antioxydant Activity of *Syzygium cumini* Leaves Molecules. 2008; 13:2545-2556.
25. 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.

26. Formica JV, Regelson W. Review of the biology of quercetine and related bioflavonoids. *Food ChemToxicol*.1995; 33: 1061-1080.
27. Yochum L, Kusli L, Meyer K, Folsom A. Dietary flavonoid intake and risk of cardiovascular disease in postmenopausal women. *Am J Epiderma*. 1999; 149: 943-949.
28. Kim H P, Son KH, Chang HW, Kong SS.Anti-inflammatory plant flavonoids and cellular action macanism. *J Pharmaco. Sci.* 2004; 96: 229-254.
29. Ouédraogo Y, Nacoulma O, Guissou IP, Guédé GF. Evaluation *in vivo* et *in vitro* de la toxicité des extraits aqueux d'écorces de tige et de racines de *Mitragyna inermis* (Willd.) O. Pharma. Med. Trad. Afr. 2001; 11: 13-29.
30. Traoré H,Balansard G, Pauli AM, Scotta AM. Pharmacological in alkaloids from leaves of *Mitragyna inermis* (Rubiaceae). J.Ethnopharm. 2002; 14: 35-65.
31. Bruneton J. Pharmacognosie, Phytochimie, Plantes médicinales 2ème édition; 1993:203-642.