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Phytochemical and toxicity studies of the leaves of *Mangifera Indica*, *Cajanus cajan* and of *Piliostigma thonningii*, acclimated in Benin, used against diarrheal disease

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Abstract
Diarrhoeic diseases caused several million deaths in the world annually, especially in Africa. 52 billion FCFA is annual attributed to this disease in Benin. They are very little scientific workonplants which can curediarrhoeic diseases in Benin. The aim of this study is to perform phytochemical screening, toxicological study of aqueous and ethanolic extracts of *Mangifera Indica, Cajanus cajan and Piliostigma thonningii* leaves and the relationship between the phytochemical compositions.
The leaves of this plants contained notably tannins and flavonoid which show good relationship. Aqueous and ethanolic extracts have $LD_{50} \geq 5000$mg / kg w.b and are not toxic on shrimp larvae. Practically no significant effect was observed on rats (hematological and biochemical parameters: $P>0.05$).
This study demonstrated relationship between the phytochemical compositions of the three plants with no toxic effect. Further antidiarrheal studies of these extracts will provide better insights on this plants and endogenous practice.

Keywords: diarrhea, phytochemical, relationship, sreening, toxicity

Introduction
Diarrhoeic diseases caused several million of deaths in the world annually [1]. In developing countries they are the most common causes of morbidity and mortality [2]. It is well known that diarrhoeic diseases are the leading causes of young children illness and death in developing countries [3]. According to the World Health Organization, there are approximately 2 billion annual cases of diarrhea in the world. Diarrhea is the leading cause of death in children younger than 5 years old and kills 1.5 millions children each year [4]. Umesherported, Rohde and Northrup estimated that annually diarrhoea killed up 5 million children in developing countries [3].
Like developing countries, diarrhoeic diseases are most important in Benin. Each year, Benin loses 52 billion F CFA due to poor sanitation. These loss due of diarrhea are causing by: the premature death of children under five old or during illness; quality of medicinal treatement, etc. [5].
Several treatments are available such as: oral rehydration, antibiotic therapy and others treatments called retarding intestinal transit. Many of these synthetic chemicals like diphenoxylate, loperamide and antibiotics available for diarrhea treatment but some of them have side effects [6]. The antibiotics usually used, such as sulphamides, ampicillin and cyclins, which are not only expensive but are therefore less accessible to populations, are becoming increasingly inactive. In 1981, the strains of *Shigella Dysenteriae* were sensitive to nalidixic acid. In 1992, 100% of these stump were resistant [7].
The search for new treatments accessible and available to the population is necessary and important.
In African traditional environment, the use of plants against various diseases knows an increasing in recent years. It can be explained by cultural reasons, the decline in purchasing power, the high cost of conventional drugs, mistrust of modern synthetic products [8].
Although these plants are commonly used to treat various diseases traditionally, there is a paucity of scientific data to support their effectiveness and user safety. Ethnobotanical studies carried out in several African countries have shown that many of these plants are used by people in the traditional treatment of diarrheal diseases [9, 10]. These data support the hypothesis that the plants reported in this study have antidiarrheal properties.

The purpose of the present study is to reveal the phytochemical families present in the leaves of *Mangifera indica*, *Cajanus cajan* and *Piliostigma thonningii* used against diarrhea in Benin. Specifically, the relationship between these secondary metabolites identified,the antidiarrheal effects and finally the possible risk of toxicity on the patients will be established.

Material and Methods

Material

Plant material

The plant material is contitued of the leaves of *Mangifera indica; Cajanus cajan and Piliostigma thonningii.* Fresh leaves were collected in February 2017 between 11h and 12h30. The leaves of *Mangifera indica* were collected at Abomey-Calavi in the south Atlantic department under a mango tree. The leaves of *Piliostigama thonningii* and *Cajanus cajan* were collected at Abomey in center of Benin. *Piliostigma thonningii* come from plants whose morphology indicates the passage of vegetation fires some time before. *Cajanus cajan* leaves, were collected in a field of plant cultivation after harvesting the seeds during the fallow land period. Plants were identified at National Herbarium of the Faculty of Science and Technology of Abomey-Calavi University. The leaves of each plant were dried separatlyin the shade for two weeks in the Faculty of Pharmacy at the University of Abomey-Calavi and reduced to powder using an electric grinder (Flour Gills NIG: EL. 1827).

Animal material

Animal material is constituted shrimp larvae Artemia salina and Wistar rats. Brine shrimp eggs obtained from JBL society (JBL Gmbh&Co.KG, Germany), were hatched in sea water. After 24 hours incubation at room temperature (22 °C - 29 °C), the larvae were collected.

Wistar rats of either sex (145 - 172 g), provided by the Department of Animal Physiology of University of Lomé were used. They were put in a standard environmental condition with fed rodent in diets standard.

Methods

Extraction

The aqueous extract which is the use form of the different plants by the population against the diarrhea is privileged. To this aqueous extract we added the ethanolic extract in order to extract the maximum of secondary metabolites. Thus, 50 g of the each leaves powder were added to a Erlenmeyer flask with 50 ml distilled water and then heated to boiling for approximately 30 min. Then, the raw extract was filtered in hot condition with Whatman filter paper to remove any fibrous impurities. As the same way, 50g of each leaves powder were macerated in 500ml of ethanol for 48 hours. After filtration, the pure aqueous or ethanolic extracts were then concentrated in a rotavapor under vacuum and stored at -4 °C in a freezer until use.

Determination of phytochemical constituents

Phytochemical screening were carried out directly on the differents leaves powders. The powders were subjected to standard phytochemical analyses for different constituents such as tannins, alkaloids, flavonoids, anthraquinones, glycosides, saponins, phenols... as described by Houghton and Raman [11] adapted of conditions of Pharmacognosy and the Essentials Oils Laboratory of Abomey-Calavi University where the tests were taking place. It's matter of qualitative analyze based on coloration and precipitation reactions.

Brine Shrimp Lethality Assay

The cytotoxic activity of the leaves extracts of the three plants was evaluated using Brine shrimp lethality bioassay. The test is based on the survival of shrimp larvae in sea water in the presence of the test solution. Its interest lies in understanding the possible side effects that would result from traditional use of leaves of *Mangifera indica; Cajanus cajan* or *Piliostigma thonningii* against diarrhoea diseases. 50 mg/ml solution was prepared with aqueous or ethanolic extract of *Mangifera indica*, or *Cajanus cajan* or *Piliostigma thonningii* in distilled water.

From each extract solution (50 mg/ml) a range of ten successive dilutions (49 µg/ml, 98 µg/ml, 195 µg/ml, 391 µg/ml, 781 µg/ml, 1582 µg/ml, 3125 µg/ml, 6250 µg/ml, 12500 µg/ml, 25000 µg/ml) were obtained with sea water. Eggs of *A. salina* were grown in an erlenmeyer containing sea water taken from the Atlantic Ocean and filtered before use. The mixture (eggs and sea water) was left under stirring for 48 hours. Meanwhile, the eggs were hatched to give birth to young larvae (nauplii). Using a pipette, a colony of 16 live larvae was placed in contact with the series of solutions of graded concentrations of aqueous or ethanolic leaves extract of *Mangifera indica; Cajanus cajan* and *Piliostigma thonningii*. These solutions and the controls containing no extract of plant were left stirring and read after 24 hours of incubation. The total death and percentage of mortality (death) at each dose level and control were determined. To assess the degree of toxicity of the different species of plants, the LC_{50} and toxicity corresponding table according to Mousseux [12].

Table 1: Correspondence between LC_{50} and toxicity

CL_{50}	Toxicité
$LC_{50} \geq 100\mu g/ml$ ou 0,1mg/ml	- - (non-toxic)
$100\mu g/ml > LC_{50} \geq 50\mu g/ml$ ou 0,05mg/ml	+ (lowtoxicity)
$50\mu g/ml > LC_{50} \geq 10\mu g/ml$ ou 0,01mg/ml	++ (Moderatetoxicity)
$LC_{50} < 10$ µg/ml	+++ (High toxicity)

Acute toxicity test with haematological and biochemical parameters dosage

Acute oral toxicity assay was performed using the limit test dose according to Woo-SikJeong. *et al*., [13]. Twenty-one female wistar rats randomly divided into seven groups (n = 3) were constitued. The rats of each group were individually administered a single oral dose of 5000 mg/kg of extract (aqueous or ethanolic) of *Mangifera indica, Cajanus cajan* and *Piliostigma thonningii* (one after the other at a grace observation period of 24 h). The control group received water vehicle (10 ml/kg). Animals were observed individually for a period of 4 h for immediate signs of toxicity and mortality and at least once daily for 14 days for delayed mortality and toxic symptoms, such as changes in skin and fur, eyes, mucous membranes, convulsion, salivation, diarrhea, lethargy, sleep and coma. Body weights were measured on the day of dosing (Day 0) prior to treatment, 1 to 14 days after dosing. On the 15th day after administration, the survivor animals were weighed and blood samples were collected from overnight fasted rats under anesthesia by retro-orbital bleeding into tubes with and without EDTA for hematological and biochemical analyses and the rats were sacrificed. Then

the vital organs including heart, lungs, livers, kidneys, spleen, and sex organs were grossly examined. The blood tubes without EDTA were centrifuged at 3000 rpm for 10 min and serum was separated and stored at -20 °C for biochemical analyses.

Blood analysis

White blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) and platelet count (PLT) were determined as hematological parameters using an automatic hematological analyzer (BC-2800, Mindray- China).

Biochemical analysis

The following marker enzymes were measured in the serum as biochemical indicators for liver injury/dysfunction: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (TB), total protein (TP), cholesterol and triglycerides. Kidney dysfunction was indicated by creatinine and blood urea levels. Glucose was assessed to evaluate pancreatic function. Standardized diagnostic kits purchased from Human GmbH. D-65205, Wiesbaden, Germany were used for spectrophotometric determination of the biochemical parameters.

Statistical Analysis

Cytotoxicity test: Statistical analysis for each extract or sample the lethal concentration that causes 50% death (LC_{50}) was calculated at 95% confidence interval by linear regression analysis and also by using the probit analysis method following Ullah *et al*., [14]. A regression line equation was derived for each extract with the mortality data obtained and, it was then used to calculate the LC_{50} value.

Acute toxicity test: The results are expressed as mean ± standard error of mean (SEM). Data were analysed by one-way analysis of variance followed by Tukey post-hoc test. Results were considered to be significant at $P<0.05$. All statistical analyses were carried out using GraphPad Prism 5.00 (GraphPad Software Inc., CA, USA).

Results

a. Pants extracts

Two extracts (aqueous and ethanolic) were obtained for each plant. A total 6 plants extracts color and different aspects were obtained and used. Extraction yields for aqueous extracts were 14.10% for *Mangifera indica*, 13.033% for *Cajanus cajan* and 17.11% for *Piliostigma thonningii*. As for the ethanolic extracts, the yields obtained are respectively 14.94%, 12.67% and 19.29% for *Mangifera indica*, *Cajanus cajan* and *Piliostigma thonningii*.

b. Phytochemical analysis of powder leaves *M. ind; C. cajan and P. thonningii*

Phytochemcalcomponds identified in differents plants powders is summer in Table 2.

Table 2: Phytochemical screening results of powder leaves of *M.indica, C. cajan and of P. thonningii*

Chemical Compounds	Species of plant leaves studied		
	C. cajan	***M. indica***	***P. thonningii***
Alkaloids	-	+	+
Cathetic tannins	++	++	+++
Gallic tannins	++	++	+++
Flavonoids	++	++	++
Anthocyanins	-	+	++
Leucoanthocyanes	++	+	++
Quinone derivatives	-	++	++
Saponins	-	+	-
Triterpenoids	-	-	-
Steroids	-	-	+
Cyanogenicderivatives	-	-	-
Mucilage	+	+	++
Coumarins	-	+	-
Reducingcompounds	++	++	+
Free anthracenederivatives	+	+	++
Combined anthracene derivatives O-heterosides	-	+	-
Combined anthracene derivatives C-heterosides	-	-	-

Legends: - = absent ; + = less present ; ++ = abundant ; +++ = very abundant

These results showed that the powdery samples of the three species (*M. indica, C. cajan and P. thonningii*) contain the tannins (catechics and gallics) more abundant in the *P. thonningii* leaves than in others, flavinoids, leucoanthocyanins, mucilages, reducing compounds and free antracenic derivatives. Triterpenoids, cardenosides, cyanogenic derivatives and C-heterosides were absents in all samples. For other secondary metabolites (alkaloid, Anthocyanins, quinone derivatives, saponosides, steroids, coumarins and O-heterosides), the results vary from powder to another (Table 2).

c. Brine Shrimp Lethality Assay

The aqueous and ethanolic extracts of the three plants (*M. indica, C. cajan* and *P. thonningii*) showed positive results (lethality) on the Brine Shrimp larvae indicating that the test samples are biologically active.The lethal concentration (LC_{50}) graphically determined (figures 1a, 1b, 1c and 1d) are in table3.

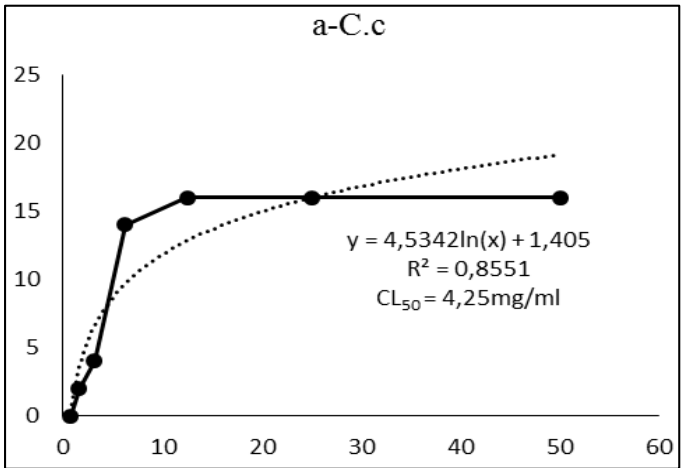

Fig a: Aqueous extract of *Cajanus cajan*

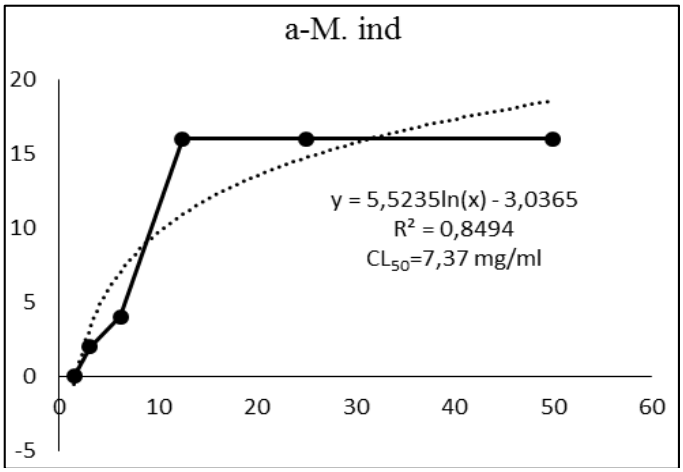

Fig b: Aqueous extract of *Mangifera*

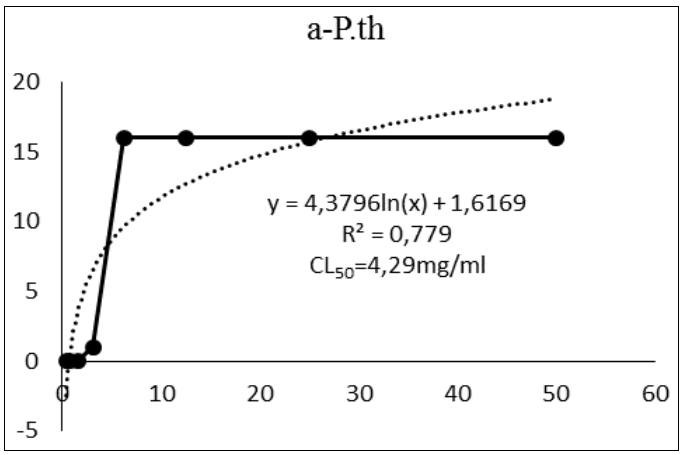

Fig c: Aqueous extract of *Piliostigma thonningii*

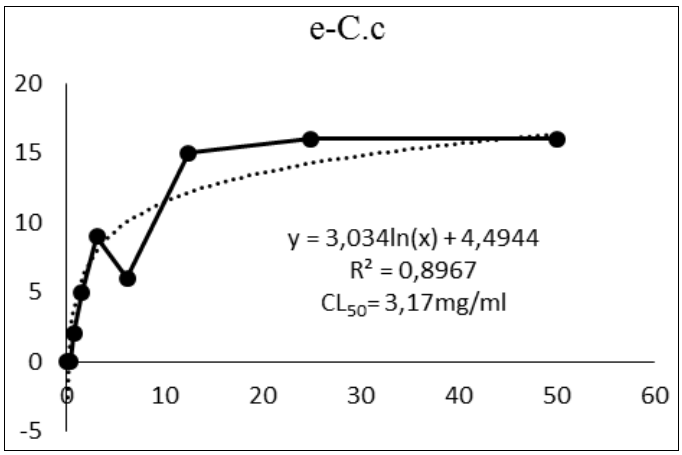

Fig d: Ethanolic extract of *Cajanus cajan*

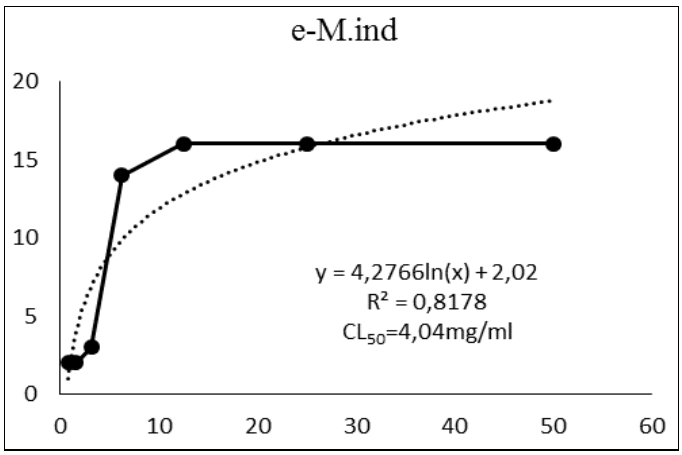

Fig e: Ethanolic extract of *Mangifera indica*

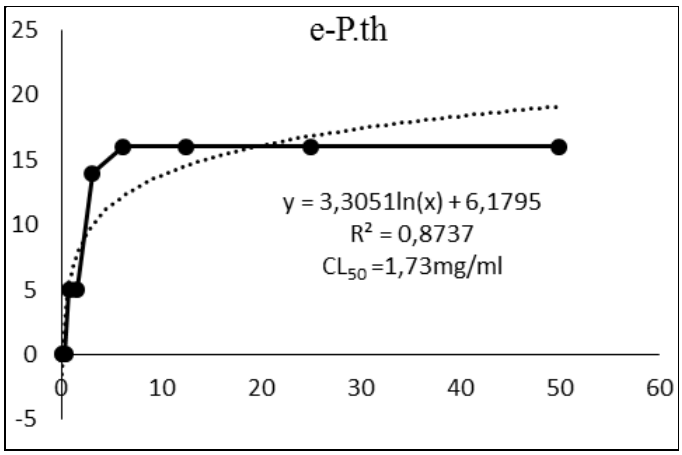

Fig f: Ethanolic extract of *Piliostigma thonningii*

Fig 1: Sensivity curves of the differents leaves extracts of the three plants studied on shrimp larvae

Table 3: CL50 values of the extracts on *M. indica*, *Cajanus cajan* and *P. thonningii*

Extraits des feuilles	LC_{50} (mg/ml)
Aqueous extract of *Cajanus cajan*	4,28
Aqueous extract of *Mangifera indica*	7,37
Aqueous extract of *Piliostigma thonningii*	4,29
Ethanolic extract of *Cajanus cajan*	3,17
Ethanolic extract of *Mangifera indica*	4,04
Ethanolic extract of *Piliostigma thonningii*	1,73

d. Acute toxicity test with haematological and biochemical parameters dosage

After the rats were orally given a single dose of the extracts at 5000 mg/kg of *Magifera indica*, *Cajanus cajan* and *Piliostigma thonningii* no death of rats was recorded for a period of 4 h. Also any behavioral changes and lethargy was not observed in treated groups for 14 days post-treatment. Body weight treated rats wasn't significantly changed relative to that of the control group table 4. No macroscopic abnormalities of the internal organs were observed at the end of the observations.

Table 4: Effect of extracts on the animals mean weight 14 days after administration of the extracts

Control	Groups treated orally with the different extracts at the single dose of 5000mg / kg b.w.					
Distilled water	a-M.ind	a-C.c	a-P.th	e-M.ind	e-C.c	e-P.th
176 ± 0,98	177 ± 2,27	172 ± 2,41	175 ± 0,02	170 ± 0,58	169 ± 2,31	178 ± 1,50

Each value represents the mean ± ESM (n=3) of the animals body weight at the end of the observation; b.w. = body mass of animals. No significant difference between the control and treated groups (P>0.05, ANOVA). a-M. ind = aqueous leaves extract of *Mangifera indica*; a-C.c = aqueous leaves extract of *Cajanus cajan*; a-P.th = aqueous leaves extract of *Piliostigma thonningii*; e-M.ind = ethanolic leaves extract of *Mangifera indica*; e-C.c = ethanolic leaves extract of *Cajanus cajan*; e-P.th = ethanolic leaves extract of *Piliostigma thonningii*.

Analysis of the hematological parameters showed no significant difference between treated groups and control group (P>0, 05) except for the white blood cell count of the rats treated with *Mangifera indica* aqueous extract which experienced significant increaseand the red blood cells rate of the animals treated with *Cajanus cajan* ethanolic extract which experienced significant decrease compared with the control group (P<0.05). (Table5).

Table 5: Effect of the extracts of a-M.ind; a-C.c; a-P.th; e-M.ind; e-C.c and of e-P.th on of the hematological parameters rate

Paramters	Control	Groups treated orally with the different extracts at the single dose of 5000mg / kg b.w.					
	Eau distillée	a-M.ind	a-C.c	a-P.th	e-M.ind	e-C.c	e-P.th
GB (10^3/µL)	10,83 ±1,33	16,67 ±1,09*	11,87 ± 1,42	10,47 ± 0,65	13,30±0,47	16,87±0,43*	13,10 ±1,17
GR (10^6/µl)	08,8 3± 0,08	07,64± 0,20*	08,14±0,41	08,68 ± 0,23	08,27±0,18	08,16 ± 0,14	08,46 ± 0,28
HGB (g/dl)	15,5 ± 0,15	14,63 ± 0,13	14,67±0,50	15,17 ± 0,41	15,63±0,38	14,53 ± 0,35	15,4 ± 0,55
(HCT)%	47,3 ± 0,49	41,77 ± 0,79	43,53±2,10	46,37 ± 0,90	46,9±0,00	46,9 ± 0,00	46,9 ± 0,00
MCV(fL)	53,53 ± 0,23	54,73 ± 0,62	53,53±1,24	53,47 ± 0,94	55,53±0,43	52,43 ± 1,0	54,6 ± 0,70
MCH (pg)	17,57 ± 0,03	19,2 ± 0,67	18,07±0,29	17,5±0,35	18,9±0,36	17,83 ± 0,52	18,2 ± 0,29
MCHC (g/dl)	32,77 ± 0,09	35,1 ± 1,00	33,77±0,75	32,7±0,26	34,03±0,44	34 ± 0,36	33,33 ± 0,35
PLT (10^3/µl)	963,3±64,18	884 ± 168,5	864,7 ±52,52	964,3 ±140,1	779 ±72,19	930,7±18,78	814,3 ±118,4

Values are expressed as mean ± standard error of mean (SEM, n = 3); **P<0.01, *P<0.05 vs. control (one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test).

Oral single-dose administration of 5000 mg / kg body weight of the extracts a-M.ind; a-C.c; a-P.th; e-M.ind; e-C.c and e-P.th caused no significant changes in the blood levels of AST, ALT, PAL, creatinine, total protein, total bilirubin, bilirubin C, and Glucose levels in the treated groups compared to the control group (P>0.05) (Table6).

Table 6: Mean blood clinical chemistry value of control and treated rats with different extracts in oral acute toxicity

Parameters	Controle	Groups traited with each extract of 5000mg/kg of PC					
	Distilled water	a-M.ind	a-C.c	a-P.th	e-M.ind	e-C.c	e-P.th
PT	47,83±7,25	46,87±4,86	53±7,95	54,03±6,36	56,83±3,66	52,83±2,48	45,07±1,14
BIL T	0,167±0,032	0,12±0,04	0,39±0,07	0,38±0,10	0,22±0,052	0,29±0,094	0,32±0,072
BIL C	0,01±0,00	0,03±0,00	0,03±0,01	0,03±0,01	0,03±0,01	0,04±0,01	0,04±0,01
ALAT	44,43±5,91	46,63±3,32	54,3±3,90	52,87±3,28	43,03±5,95	32,80±7,67	28,17±5,48
ASAT	63,17±3,36	74,77±10,20	75,23±12,92	69,87±7,39	86,60±3,92	59,47±1,10	57,50±4,51
GLUC	1,472±0,07	1,52±0,13	1,41±0,14	1,33±0,16	1,39±0,22	1,23±0,07	1,20±0,13
CREJ2	0,57±0,05	0,51±0,02	0,48±0,06	0,48±0,02	0,65±0,04	0,57±0,01	0,50±0,07
PAL	111±11,85	126,3±24,91	140,7±10,11	166±24,11	78,67±8,19	108,30±38,97	65,33±16,19

Discussion

In african traditional field, the use of plants against various diseases has grown more and more in recent years. Although these plants were traditionally used against a variety of diseases, there are paucity scientific data in support of their efficacy and safety.

The phytochemical analysis of the powder samples from *Mangifera indica, Cajanus cajan* and *Piliostigma thonningii* leaves revealed the presence tannins (catechics and gallics) more abundant in the *Piliostigma thonningii* leaves than in others, flavonoids, leucoanthocyanins, mucilages, reducing compounds and free antracenic derivatives in all samples (Table 1). These results confirm those obtained by several researchers on the leaves of *Mangifera indica:* [15-17]; on *Cajanus cajan*: [18-21] and on the leaves of *Piliostigma thonningii*: [22-24].

The leaves powders of the three plants showed a phytochemical profile somewhat similar to those reported on other antidiarrheal plants such as *Suwertiachirata* [25] and *Buchholzia* [26].

In contrast, other authors for the same plants did not report the presence of tannins or flavonoids in their samples [27]. The absence of favonoids in *Cajanus cajan* and *Piliostigma thonningii* leaf extracts harvested in Nigeria wasalso noticed [19; 28]. This difference in composition compared to our results could be explained by the chemotype phenomenon (different geographical origins: Benin and Nigeria of the studied plants) or by the method of extraction or detection used.

The leaf powders of *Mangifera indica*, *Cajanus cajan* and *Piliostigma thonningii* acclimated in Benin all remarkably contain tannins and flavonoids. Many previous studies on the antidiarrhoeal properties of various plants indicate tannins and flavonoids as responsible for the antidiarrheal effects noticed [29-34]. Tannins have the ability to denature proteins in the intestinal mucosa by forming tannates that make the intestinal mucosa more resisxtant to chemical alterations thus reducing

secretion [35]. As for flavonoids, their antidiarrheal effects are reflected in the inhibition of intestinal motility and hydroelectrolitic secretion [36]. Theses secondary metabolite are mainly present in our three powders samples (*Mangifera indica, Cajanus cajan* and *Piliostigma thonningii*), we can speculate that there is a relationship between the phytochemical composition of these plants acclimated in Benin and diarrhea.

Many previous researches reported that the use of medicinal plants have toxicity risk, [37, 38]. We find it necessary to evaluate the toxicity of our plants extracts in order to protect people healthy. The larval toxicity test of leave aqueous and ethanolic extracts of leaves of *Mangifera indica, Cajanus cajan* and *Piliostigma thonningii* revealed, based on the LC_{50} and the table of toxicity correspondence (Table 1) established by Mousseux [12], show that ours extracts are no toxic on shrimp larvae because theirs LC_{50} are less than 0.1 mg / ml. These observations corroborate those reported by Anand *et al.* [39], on the *Mangifera indica* leaf extract; Njeruand *et al.* [40], on *the Piliostigma thonningii* leaf extract by Bussmann and *et al.* [41], on the *Cajanus cajan* leaf extract. However, the ethanolic extracts of our three plants appeared more toxic on the shrimp larvae (*Artemiasalina*) than the aqueous extracts. The same observations were made by Bussmann and *et al*. [41] who reported that the alcohol extracts of Peru plants were found to be more toxic than their aqueous extracts.

Our results are contrary to those obtained by Mazharal and *et al.* [42] who found during their studies that the leaf and root extracts of *Cajanuscajan* showed a high degree of toxicity on shrimp larvae. In 2013, Ngutaand *et al.* [43], demonstrated the possibility of cytotoxicity variability between leaf extracts of *Piliostigma thonningii* harvested in Kenya and Malawi.

Taking into account the established correlation between the toxicity of shrimp larvae and that of human cellslines particularly lung carcinoma (A-549) and colon carcinoma (HT-29) [44], the use of leaves of *Mangifera indica, Cajanus cajan* and *Piliostgma thonningii* harvested in Benin may be considered less toxic.

Concerning the acute oral toxicity of aqueous and ethanolic extracts of leaf of *Mangifera indica, cajanus cajan* and *Piliostigma thonningii* reveal an LD_{50}> 5000mg / kg of the body weight of the rats.Thus, referring to the Hodge and Stemer [45], scale the orally administered the aqueous and ethanolic extracts of the leaves of *Mangifera indica, Cajanus cajan* and *Piliostigma thonningii* could be considered practically non-toxic. These results support the findings of Adamu *et al.* [46], and Ukwuani *et al.* [47], who demonstrated that leaf extract of *Piliostigma thonningii* did not cause oral toxicity in rats at dose hight than 5000 mg/kg. The same trend was obtained [48; 49], using respectively aqueous stem bark extract of *Mangifera indica* at the dose of 5000 mg/kg in Wistar rats and ethanolic leaf extract of *Cajanus cajan* at the DL50> 16.000 mg/kg of the body weight of the rats.

The haematological indices obtained in this study suggest that our extracts are not toxic on haematological parameters. However, the significant increase in the white blood cell count of the rats treated with the aqueous extract of *Mangifera indica* and the ethanolic extract of *Cajanus cajan* indicate that these extracts stimulate the immune system. These extracts can therefore be used against neutropenia. On the other hand, leaf aqueous extract of *Mangifera indica* administrated of oral unique dose of 5000mg/kg caused anemia after 14 days in the treated animals, which suggests interference of this extract with the production of red blood cells in the treated rats.

The results obtained in this study indicate that the aqueous and ethanolic extracts of *Mangifer aindica*; *Cajanus cajan* and *Piliostigma thonningii* administered orally in high doses did not induce liver damage. Blood levels of AST, ALT, PAL, creatinine, total protein, total bilirubin, bilirubin C, and glucose in the treated groups did not increase Significant compared to the control group (Table 6). These observations corroborate those recently made by Muthupillai and *et al.* [50] on the aqueous and methanolic extracts of *Mangifera indica* and Dasofunjo K. and *et al.* [37] on the ethanolic extract of *Piliostigma thonningii* which showed that the extracts of these plants play a hepato protective role. The cyanogenic derivatives are real poisons [51]; their absence in our leaf extracts would explain in part the non-toxic effects observed.

The acute toxicity study of aqueous and ethanolic extracts of *Mangifera indica, Cajanus cajan* and *Piliostigama thonningii* extended to the determination of haematological and biochemical parameters similar to the work of Woo-SikJeong *et al.* [13] on oil red ginseng is a first to our knowledge. In this study, larval toxicity on *Artemia salima* and acute toxicity on rats were carried out as the first step of safety evaluation, which provides first safety information on the investigated plants.

Conclusion

At the end, we note that the powders of the leaves of *Mangifera indica; Cajanus cajan* and *Piliostigma thonningii* contain secondary metabolites related to diarrhea. The aqueous and ethanolic extracts of the leaves of the three plants showed practically no toxicity on larvae of Artemia. The safety of the six extracts is demonstrated at an oral dose greater than 5000mg / kg body weight. These results confirm the use of leaves of *Mangifera indica, Cajanus cajan* and *Piliostigma thonningii* against diarrhea without risk of toxicity. Further studies on the active fractions of the extracts and their mode of action on diarrhea are needed and will provide further insights into endogenous practices in relation to leaf use of these plants against diarrhea.

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