Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(3): 320-324
Received: 07-03-2017
Accepted: 08-04-2017

Maxime M Sangaré
(A). Department of Animal Physiology, Laboratory of Animal Physiology, University of Abomey-Calavi 06 BP 2584 Cotonou, Benin
(B). Laboratory of Animal Physiology and experimental pharmacology, University of Abomey-Calavi 01 BP 526 Cotonou, Benin

Jean-Marc Atègbo
(A). Department of Animal Physiology, Laboratory of Animal Physiology, University of Abomey-Calavi 06 BP 2584 Cotonou (Benin).
(B). Laboratory of Animal Physiology and experimental pharmacology, University of Abomey-Calavi 01 BP 526 Cotonou, Benin

Jean Robert Klotoé
(A). Department of Animal Physiology, Laboratory of Animal Physiology, University of Abomey-Calavi 06 BP 2584 Cotonou, Benin
(B). Laboratory of Research in Applied Biology, Polytechnic School of Abomey-Calavi, University of Abomey-Calavi (LARBA/EPAC/UAC), Benin

Eugène Attakpa
Department of Animal Physiology, Laboratory of Animal Physiology, University of Abomey-Calavi 06 BP 2584 Cotonou, Benin

Félix FD Guinnin
(A). Department of Animal Physiology, Laboratory of Animal Physiology, University of Abomey-Calavi 06 BP 2584 Cotonou, Benin
(B). Laboratory of Animal Physiology and experimental pharmacology, University of Abomey-Calavi 01 BP 526 Cotonou, Benin

Abdoulaye Z Issotina
Laboratory of Animal Physiology and experimental pharmacology, University of Abomey-Calavi 01 BP 526 Cotonou, Benin

Karim Laye Dramane
(A). Department of Animal Physiology, Laboratory of Animal Physiology, University of Abomey-Calavi 06 BP 2584 Cotonou, Benin
(B). Laboratory of Animal Physiology and experimental pharmacology, University of Abomey-Calavi 01 BP 526 Cotonou, Benin

Correspondence
Maxime M Sangare
(A). Department of Animal Physiology, Laboratory of Animal Physiology, University of Abomey-Calavi 06 BP 2584 Cotonou, Benin
(B). Laboratory of Animal Physiology and experimental pharmacology, University of Abomey-Calavi 01 BP 526 Cotonou. Benin

Phytochemical screening and subacute toxicity evaluation of stem leaves of *Monechma depauperatum* (T. Anderson) on Wistar rats liver and kidney functions

Maxime M Sangaré, Jean-Marc Atègbo, Jean Robert Klotoé, Eugène Attakpa, Félix FD Guinnin, Abdoulaye Z Issotina and Karim Laye Dramane

Abstract
The objective main of this study is to perform phytochemical screening and to evaluate *in vivo* the subacute oral toxicity of the aqueous extract of *Monechma depauperatum* (T. Anderson). The screening is carried according to the method of (Houghton and Raman, 1998) and exploratory tests of subacute oral toxicity are carried *in vivo* on Wistar albinos rats in accordance with the OECD guidelines (423). The phytochemical analysis performed on the aqueous extract of *Monechma depauperatum* (T. Anderson) revealed the presence of tannins, anthocyanins, leucoanthocyans, anthraquinones, flavonoids, mucilages, saponosides and terpernes and sterols. The subacute oral toxicity tests of the aqueous extract of *Monechma depauperatum* (T. Anderson) showed no toxic effect on the biochemical parameters studied up to the 2000 mg/kg dose and became toxic at a dose of 2500 mg/kg. The lethal dose is therefore greater than 2000 mg/kg.

Keywords: *Monechma depauperatum* (T. Anderson), toxicity, subacute, oral, lethal

1. Introduction
The richness of plant biodiversity and the knowledge of our traditionals therapists and likely to help to improve the management of diseases by opening up new scientific channels for their treatment [1]. *Monechma depauperatum* (T. Anderson) is a plant species of the branch of Magnoliophyta and the large family of Acanthaceae [2]. This plant is traditionally used in northern Benin to cure various pathologies. The aqueous decoction of stem leaves used in drinking and bathing in the treatment of jaundice [3] therefore of the liver affections. It is a herbaceous plant with woody stem, 30-60 cm tall; White or pale yellow leaves. We find the savannahs and fallows derived [3]. Considering the multiple uses of *M. depauperatum* (T. Anderson) and the fact that it has been the object of very few scientific objectiveations, then it is very important to study it's subacute oral toxicity on *in vivo* model of Wistar rats.

2. Material and methods
Plant material
The stem leaves of *M. depauperatum* (T. Anderson) were collected in June 2016 in Djougou (North Benin) and identified in the national herbarium of Benin.

Preparation of aqueous extract
The powder of stem leaves of *M. depauperatum* (T. Anderson) is soaked in water overnight. The aqueous extract is recovered initially after filtration of the mixture with a paper filter allowing obtaining a relevant extract as the crude extract

Animal testing equipment
Subacute oral toxicity tests were performed on Wistar albinos rats (174g-200g), aged 12 to 15 weeks randomly selected. The rats come from the Institute of Applied Biomedical Sciences (ISBA) and are acclimatized in the Animal Physiology laboratory of the Faculty of Science of the University of Abomey-Calavi at least two weeks before the beginning of the experiment at a constant temperature of 22 ± 1 °C with a cycle of 12 hours of light and 12 hours of darkness. They are fed with granulated feed and ad libitum water without discontinuity in feeding bottles.

Phytochemical screening

The phytochemical screening is based on differential characterization (coloring and precipitation) reactions of the main groups of chemical compounds contained in the plant according to the method of [4]. The differents physico-chemical characterization reactions are summarized in the table following.

Table I: Summary of Specific Reactions of Active Ingredients

Table 1: Summary of specific reactions of each compound class

Compound class	Specific reagents and reactions
Alkaloids	-Dragendroff (potassium iodobismuthate) → Red precipitate - Mayer (potassium iodomercurate) → yellow precipitate
Tannins catechiques **Gallic tannins**	- stiasny reagent → precipitate pink - Saturation of acetate of Na + a few $FeCl_3$ drops to 1% → dark blue, green or black
Flavonoids	Shinoda (cyaniding reaction) → Coloration: orange (flavones); red (flavonols) or purple (flavanones)
Anthocyanins	red colouring of filtrate increased in acid medium and blue-violet in alkaline medium
Leucoanthocyanin	Shinoda (hydrochloric alcohol) → Cherry Red
Quinone derivatives	Born-Träger (reaction between Quinone cycles in HN_3 medium) → pink to purplish red colouring
Saponosides	Determination of foam index (positive if IM> 100)
triterpenoids **steroids** **Cardenolides**	-Liberman-Buchard (sulphuric acid-acetic anhydride) violet colour with blue or green - Kedde (Dinitrobenzoic acid in ethanol + 2% NaOH (1N) → purple red wine stain or wheel) -Dinitrobenzene 1% in ethanol + 20% NaOH → blue colour
Cyanogenic derivatives	Gugnard (paper soaked in picric acid) orange to brown colouring)
Mucilages	study of the viscosity of the infused or decocted
Reducing compounds	Hot Liqueur of Fehling → brick-red precipitate
coumarin	Ammoniac 25% → intense fluorescence
Anthracenic derivatives	Chloroform + ammoniac → intense red coloration
o-heterosides	Hydrolyzed + $FeCl_3$ + Chloroform + ammoniac → red colour
C-heterosides	Aqueous phase + $FeCl_3$ + Chloroform + ammoniac → red colour

Source: Houghton and Raman, 1998

Exploratory tests of *in vivo* toxicity of extracts

The tests were performed in accordance with the Guideline of the Organization for Economic Cooperation and Development (OECD) for the testing of chemicals through Method 423 [5]. This trial required tree animals per stage. The aqueous extract of this plant is dissolved in physiological water and administered to the rats at a rate of 1 ml/100 g of body weight. The rats are labeled for individual identification. The rats were divided into three batches of three rats after blood tests to ensure homogeneity of the batches and to serve as a control. Lots (I), lot (II), lot (III) respectively received 300mg/kg, 2000 mg/kg and 2500 mg/kg of *M. depauperatum* (T. Anderson) extract.

The animals were observed individually at least once during the first 30 minutes and at least twice during the first 24 hours after treatment. Particular attention was paid to them daily for 30 days after the administration of the extract. All observations were systematically recorded. Particular attention has been paid to observing the various manifestations of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. The following parameters will be searched:

Body Weight

The individual weight of each rat is determined one hour before administration of the test substance and then at least once a week.

Biochemical examinations

Blood samples are taken from all rats by retro-orbital puncture for biochemical examinations at the Animal Physiology laboratory of the Faculty of Science of the University of Abomey-Calavi. The biochemical tests are carried out by the kinetic method according to the methodology of [6] using the Semi-Automate brand Rayto. This is the dosage of transaminases (ASAT, ALAT), glucose, urea, creatinine.

Statistical Analyzes

All data is processed using Microsoft Excel 2010 and Minitab version 16.FR. The latter was used for the analysis of the variance (ANOVA to a de-stacked factor) for the comparison of the averages. The threshold of significance is 5%.

2. Results and discussion

2.1 Phytochemical Screening

The results of the phytochemical screening are summarized in the following table.

Table 1: Results of the characterization reactions of the aqueous extract of *M. depauperatum* (T. Anderson)

		***M. depauperatum* (T. Anderson)**
Tannins	Catechic	+
	Gallic	+
Anthocyanins		+
Leucoanthocyanins		+
Anthraquinones		+
Alkaloids		-
Flavonoids		+
Mucilages		+
Saponosides		+
Terpenes and sterols		+
Coumarines		-
Reducing compounds		-

The results are interpreted as follows: +: presence; -: absence

The phytochemical analysis carried from the aqueous extract of *M. depauperatum* (T. Anderson) (Table 1) revealed the presence of: Tannins, Anthocyanins, Leucoanthocyanins, Anthraquinones, Flavonoids, Mucilages, Saponosides and Terpernes and Sterols. Indeed, the steroids and terpenes used for their antipyretic and analgesic properties [7], the flavonoids known for their hepatoprotective activities [8], are capable of reducing high blood pressure and protecting The liver [9], saponosides whose spermicidal, analgesic, imuno-modulatory

and cytoprotective activities are often evoked [10]. The biological properties of *M. depauperatum* (T. Anderson) are therefore inferred by its richness of active chemical compound.

Effect of the aqueous extract of *M. depauperatum* (T. Anderson) on the average weight of Wistar rat

T0: Before the first gavage, T + 5: The day after the last gavage, T + 30: 30 days after the last gavage

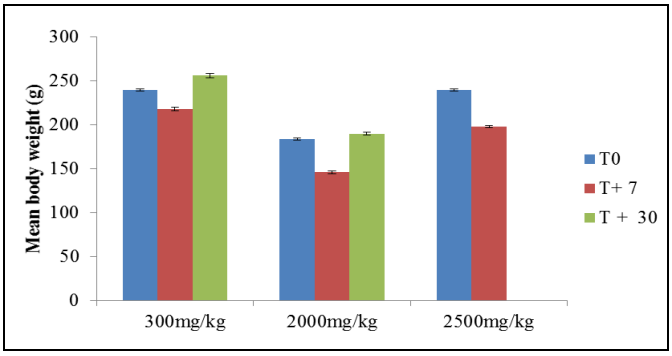

Fig 1: Effect of the aqueous extract of *M. depauperatum* (T. Anderson) on weight

The animals lost weight after 7 days of treatment. This weight loss is not significant (p>0.05). Among the rats given the dose of 2500mg/kg one died after 96h and the other two respectively 24 and 72 after the last treatment. The aqueous extract of *M. depauperatum* (T. Anderson) therefore has no influence on the variation of the weight of the animals. Previous work has shown that the presence of polyphenols such as tannins can be responsible for poor assimilation of food and may lead to a reduction in weight. These results are similar to [11] in the study of the sub-chronic toxicity of Argemone mexicana.

On the other hand, variation in body weight is used as an indicator of adverse effects of chemical compounds [12]. This weight loss can be explained by a reduction in the consumption of food, but also by the possibility of dose/absorption interactions and by the reduction in the amount of food absorbed.

T0: Before the first gavage, T + 5: The day after the last gavage, T + 30: 30 days after the last gavage

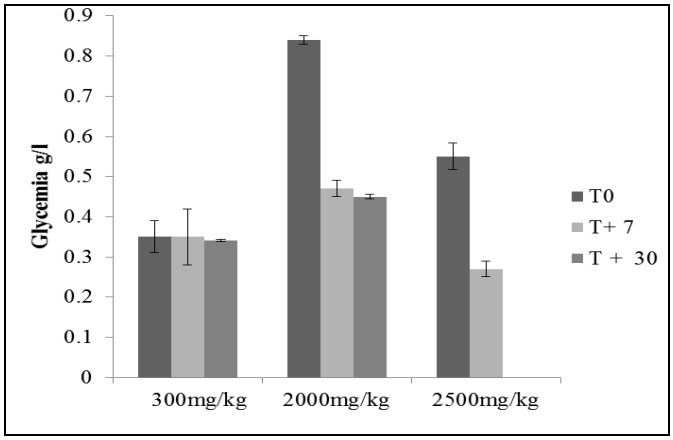

Fig 2: Effect of the aqueous extract of *M. depauperatum* (T. Anderson) on blood glucose in rats

The Figure 2 shows the evolution of blood glucose in rats during the experiment. Animals receiving the 2000 mg/kg dose and those receiving the 2500 mg/kg dose experienced a blood glucose drop. This decrease is not significant. (P> 0.05).

T0: Before the first gavage, T + 5: The day after the last gavage, T + 30: 30 days after the last gavage

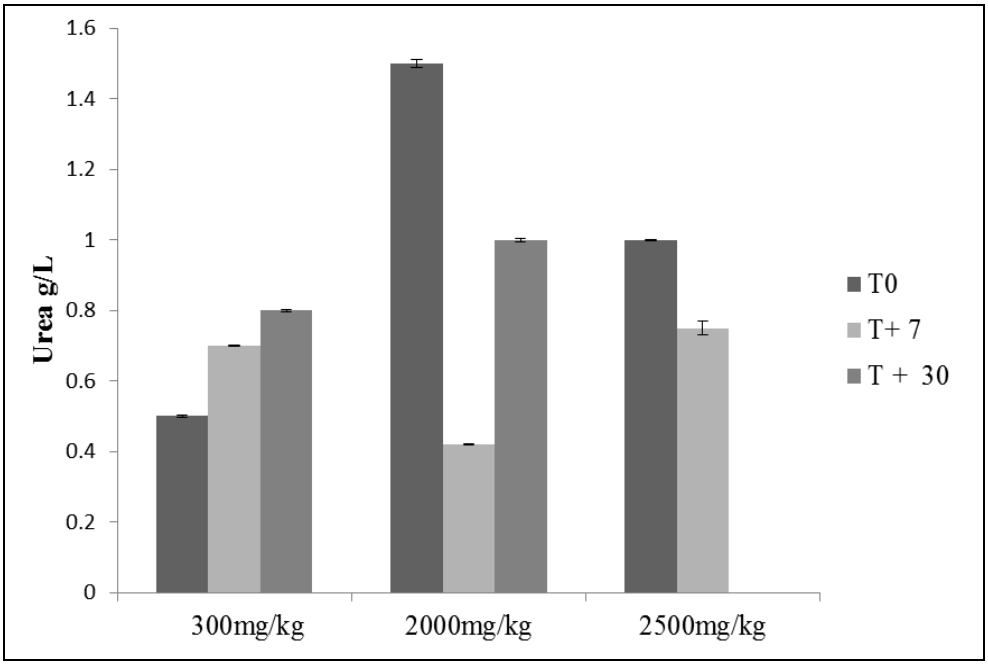

Fig 3: Effect of the aqueous extract of *M. depauperatum* (T. Anderson) on the urea of rats

The rats who received a dose of 2000mg/kg had a significant decrease in urea (p <0.05), while those receiving 300 mg/kg and 2500 mg/kg showed a non-significant variation (p > 0.05).

T0: Before the first gavage, T + 5: The day after the last gavage, T + 30: 30 days after the last gavage

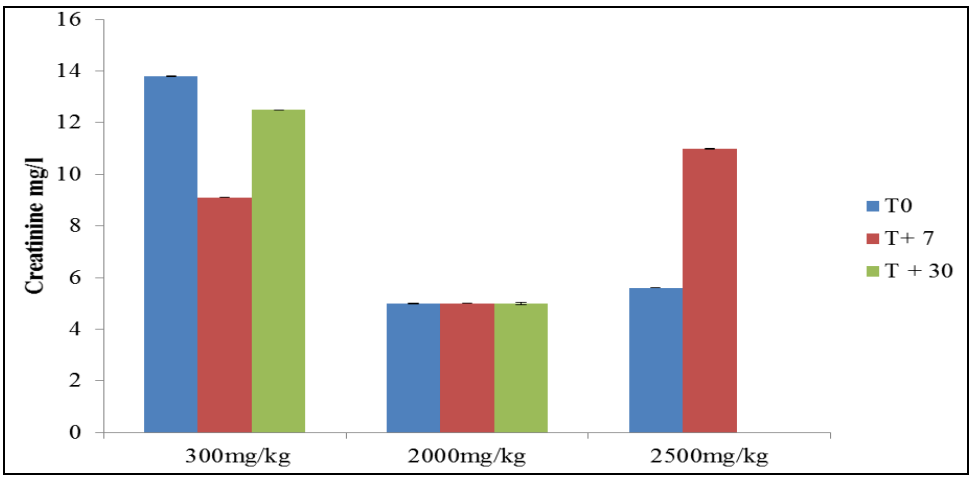

Fig 4: Effect of the aqueous extract of *M. depauperatum* (T. Anderson) on creatinine in rats

An insignificant decrease (p>0.05) was observed with rats taking 300 mg/kg. Creatinine of rats taking the 2500mg / kg dose increased significantly (p <0.05).

T0: Before the first gavage, T + 5: The day after the last gavage, T + 30: 30 days after the last gavage

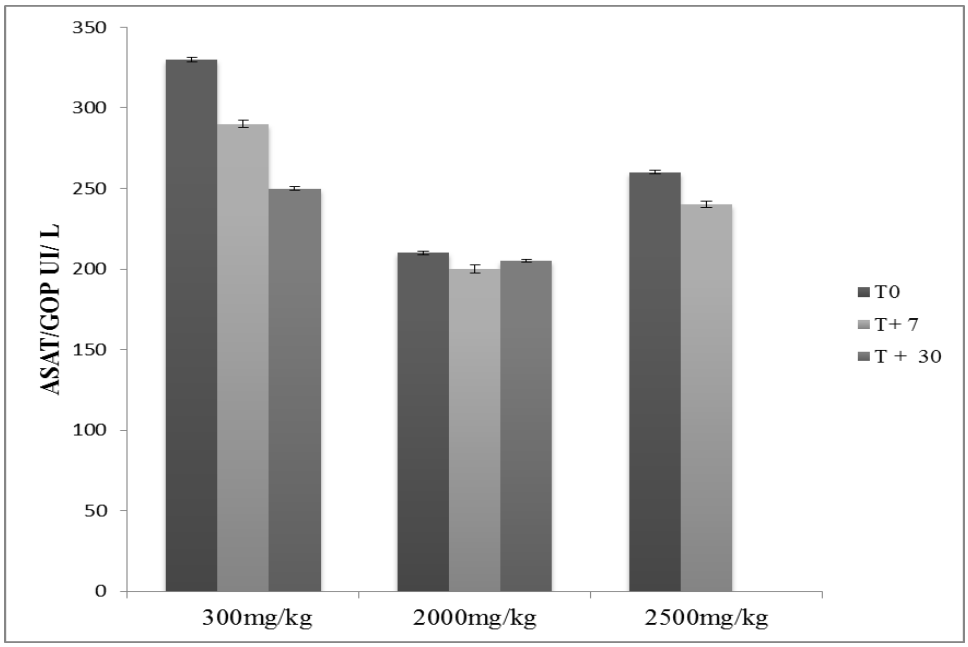

Fig 5: Effect of the aqueous extract of *M. depauperatum* (T. Anderson) on the ASAT / GOP of the rats. There was no significant difference in AST in animals during the experiment (p> 0.05).

T0: Before the first gavage, T + 5: The day after the last gavage, T + 30: 30 days after the last gavage

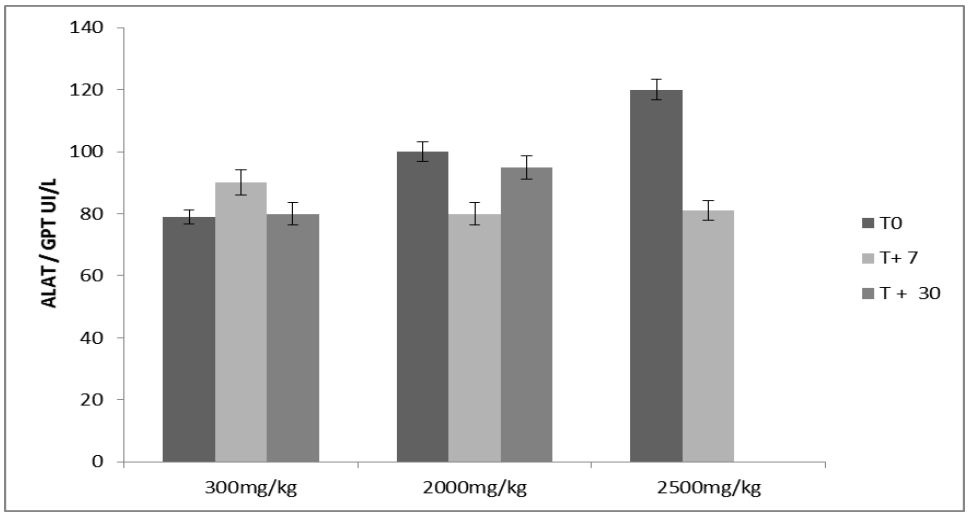

Fig 6: Effect of the aqueous extract of *M. depauperatum* (T. Anderson) on the ALAT / GPT of rats

The figure above shows the evolution of the ALAT/GPT during the experiment. No significant variation was observed at any dose.

Except urea and creatinine doses of 2000 mg/kg and 2500 mg/kg, which show a statistically significant difference ((p <0.05) at the end of 7 days, Other biochemical parameters such as ALAT/GTP transaminases (Fig.6) And ASAT/GOP (Fig. 5), Glucose (Fig.2), Show a statistically insignificant difference (p> 0.05) The variations of urea and creatinine in these test batches can be linked to the result between inputs (food, synthesis, mobilization of reserves) and outputs (storage, catabolism, elimination). The aqueous extract of *M. depauperatum* (T. Anderson) had no effect on plasma biochemical parameters up to the 2000 mg/kg dose and became toxic at a dose of 2500 mg/kg.

The analysis of the results obtained leads us to deduce that the aqueous *M. depauperatum* (T. Anderson) was found to be non-toxic to the tested parameters up to the 2000 mg/kg dose, thus not influencing blood tissue and then on vital organs such as the liver and Kidneys for doses below 2000 mg/kg. Serum enzymes ASAT, ALAT are enzymes synthesized in the cytoplasm of the cell and discharged into the circulation in the case of damaged cells [13]. These are considered good indicators of hepatic cytolysis. Thus, high levels of liver enzymes, including ALAT and ASAT, are frequently attributed to the metabolic and/or toxic effects of different drugs such as psychotropic drugs [14]. The realization of the histological sections will allow us to confirm these observations. These results are comparable to those obtained by [11] with the deciles of *Argemone mexicana L*., where they did not notice changes in biochemical parameters during sub-chronic toxicity. This shows the safety of the aqueous extract of this plant.

Conclusion

The phytochemical analysis performed on the aqueous extract of *M. depauperatum* (T. Anderson) revealed the presence of tannins, anthocyanins, leucoanthocyans, anthraquinones, flavonoids, mucilages, saponosides and terpernes and sterols. The subacute oral toxicity tests of the aqueous extract of *M. depauperatum* (T. Anderson) showed no toxic effect on the biochemical parameters studied up to the 2000 mg/kg dose and became toxic at a dose of 2500 mg/kg. The lethal dose is therefore greater than 2000 mg/kg. Further works on the determination of therapeutic dose and chronic toxicity tests for extracts *M. depauperatum* (T. Anderson) should be carried out in order to confirm the dosage to be adapted for the use of this plant.

References

1. Tounkara B. Etude phytochimique et activités biologiques de cinq plantes utilisées dans le traitement traditionnel du paludisme au Mali. Thèse d'Etat de Pharmacie, FMPOS, Université de Bamako, 2008, 127.
2. APG, An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Botanical Journal of the Linnean Society of London. 2009; 61:105-121.
3. Akoègninou A, van der Burg WJ, van der Maesen LJG. Flore Analytique du Bénin. 2006 Backhuys Publishers: Wageningen, 1034.
4. Houghton PJ, Amala RA. Laboratory Handbook for the Fractionation of Natural Extracts, 1998.
5. OCDE. Sous-comité d'experts du système général harmonisé de classification et d'étiquetage des produits chimiques : dangers pour la santé et l'environnement – toxicité aiguë. UN/SCEGHS/2/INF. 2001; 11:13.
6. Sodipo OA, Abdulrahman FI, Alemika TE, Gulani IA. Chemical composition and biological properties of the petroleum ether extract of *Solanum macrocarpon* L. (Local Name: Gorongo). British Journal of Pharmaceutical Research. 2012; 2(2):108-128.
7. Sakande J, Nacoulma OG, Nikiema JB, Lompo M, Bassene E, Guissou IP. Médécine d'Afrique Noire. 2004; 51(5):280-282.
8. Wegner T, Fintelmamann V. Parmacologie properties and therapeutic profile of artichoke (*Cynara scolymus* L.). Wien Med Wochenschr. 1999; 149(8-10):141-247.
9. Gazola R, Machado D, Ruggiero C, Singi G, Macedo Pharmacol. 2004; 50:477-480.
10. Marston A, Hostettmann K. Plant saponins: chemistry and molluscicidal action. In Ecological, chemistry and biochemistry of plant terpenoids. Harbone, IB. eds. Clarendon Press, Oxford. 1991, 264-286.
11. Guirou C. Etude de la toxicité sub-chronique de *Argemone mexicana* utilisée dans le traitement traditionnelle du paludisme. Thèse de Pharmacie, FMPOS. Université de Bamako. 2008, 82.
12. Hilaly JE, Israili ZH, Lyouss B. Acute and chronic toxicological studies of *Ajuva Iva* in experimental animals. Journal of Ethnopharmacology. 2004; 91:43-50.
13. Ozturk IC, Ozturk F, Gul M, Ates B, Cetin A. Protective effects of ascorbic acid on hepatotoxicity and oxidative stress caused by carbon tetrachloride in the liver of Wistar rats. Cell Biochemistry and Function. 2009; 27:309-315.
14. Himmerich H, Kaufmann C, Schuld A, Pollmacher T. Elevation of liver enzyme levels during psychopharmacological treatment is associated with weight gain. Journal of Psychiatric Research. 2005; (39):35-42.