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Toxicological Study of Hydroalcohol Leaf Extract of *Acanthospermum hispidum* (Asteraceae)

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Abstract: *Acanthospermum hispidum* is a widely plant used in African traditional medicine. This study aims to assess cardiotoxicity and sub-chronic (28-days) oral toxicity of hydroalcohol leaf extract of *A. hispidum*. The sub-chronic toxicity was evaluated after administering daily oral doses of 500 and 1000 mg/kg body wt., for 28 days to the rats, biochemical and haematological assessments as well as body and relative organ weights of the rats were carried out. The results of the study have shown that blood sodium was significantly ($p < 0.05$) lower in the group treated with 500 and 1000 mg/kg of *A. hispidum* extract and the blood platelet was significantly high. The weekly body and organ weight of the rats showed no significant differences between the control and the rats treated with the extract except for the testis where there was a significant decrease ($p < 0.05$) in rats that received 1000 mg/kg, i.e., 1.16±0.2 g against 0.78±0.22 g for the control. A discreet seminiferous tubular atrophy and fibrosis of the tube wall were observed at this dose. The hydroalcoholic extract of *A. hispidum* at 0.1; 1; 10 and 100 mg/ml on toad heart and isolated atria of guinea pigs have caused a significant increase in cardiac amplitude (positive inotropic) and a significant decrease ($p < 0.0001$) in frequency (negative chronotropism). Our results suggest that the hydroalcohol extract of *A. hispidum* is relatively toxic to the testis and caution must be taken at high doses especially for the heart.

Keywords: *Acanthospermum hispidum*, Phytochemical Screening, Sub-Chronic Toxicity, Cardiotoxicity

Introduction

Acanthospermum hispidum is an annual plant belonging to Asteraceae family. It is widespread in the tropics and among the weeds. It is a plant used in traditional medicine in treating of a lot of diseases. The plant is used in the treatment of yellow fever, malaria and stomach disorders (Mann *et al*., 2003; Chakraborty *et al*., 2012; Agbodeka *et al*., 2016). In some parts of South America, it is used as diuretic. In Ivory Coast, it is use for its many therapeutic properties such us anti-malarial, anti-hypertensive, antispasmodic, anthelmintic and abortion (Yapi, 2013). In Togo, *A. hispidum* is used in the treatment of headache, malaria, typhoid fever and hemorrhoids.

Several studies have shown that *A. hispidum* has antibacterial and antiviral properties (Anani *et al*., 2000; Atindehou *et al*., 2004; Fleischer *et al*., 2003; Hoffman *et al*., 2004), abortifacient and teratogenic (Lemonica and Alvarenga, 1994), anti-malaria (Sanon *et al*., 2003), immunostimulatory (Summerfield and Sallmuller, 1998) antitrypanosomal, antileishmanial, antitrichomonal (Ganfon *et al*., 2012; Adepiti *et al*., 2014), Antihypertensive effects (Palozi *et al*., 2017), antioxidant and hepatoprotective (N'Do *et al*., 2018). It was reported that *A. hispidum* contains some chemicals such as sesquiterpenes and lactones (Jakupovic *et al*., 1986; Arena *et al*., 2011). Secondary metabolites such us acanthospermol galactoside, flavones, cafeic acid, cis-cis-germacranolides, melampolides and β-caryophyllene have been reported to be present in the aerial part of the plant (Edewor and Olajire, 2011). Despite these proven pharmacological properties, caution must be put in its use by the people because little study has focused on his toxicity (Ali and Adam, 1978).

Thus, the present study has the objective to study the cardiotoxicity and sub-chronic (28-days) oral toxicity of hydroalcohol leaf extract of *A. hispidum*.

Materials and Methods

Extraction of Acanthospermum hispidum

The study was conducted at the Faculty of Health Sciences and the Faculty of Science of the University of Lome, Togo. *A. hispidum* leaves were harvested at Adidogomé (Togo) in October 2017. They were identified by Professor K. Akpagana of the Department of Botany at the University of Lome (Togo) and a specimen was preserved in the herbarium of the Laboratory of Botany and plant Ecology at the Faculty of Science in the reference TOGO15380. The leaves were dried protected from light and reduced to powder. The powder (750 g) was soaked of water-ethanol 95% (50/50: v/v). The mixture was stirred mechanically for 72 hours. The macerate was filtered. The filtrate was evaporated in using a Rotavapor R 210. The yield of this extraction was 16.5%.

Experimental Animals

Male Wistar rats (199.70±6.28 g) were provided by the department of animal physiology of the Faculty of Science of the University of Lome, and used for toxicological tests. Toads (*Bufo marinus*) ranged in body weight from 90-160 g (mean 120 g) were captured in night, in damp places and were used for the study of cardiac activity ''*in vivo*''. The guinea pigs were supplied by the breeding farm of Albatross in Lome (Togo) and used for contractile activity on isolated atria. All these animals were housed in environmental normal conditions and fed standard diets and water.

Chemicals

Digoxin (Sigma) was the reference product used for cardiac activity. The biochemical parameters assay kits and the Mac Ewen physiological solution (NaCl: 15 g; KCl0.24 g; CaCl: 0.48 g; PO4H2Na: 0.29 g; CO3HNa: 2 g; MgCl2: 0.1 g; Glucose 4 g) were used.

Phytochemical Screening

Plant materials were screened for the presence of alkaloids, saponins, tannins, total phenols, anthraquinones, flavonoids, sterols and cardiac glycosides using the methods previously described by Tona *et al*. (1998).

Sub-Chronic Toxicity Study

The repeat dose of oral toxicity study was carried out according to OECD guideline 407 (2008). Three groups (T, D1, D2) of 6 male rats were formed. The control group received distilled water. The groups D1, D2 received the hydroalcohol extract of *A. hispidum* leaves at 500 and 1000 mg/kg body weight respectively. Extract was administered daily for 28 days at the same time. The animals were observed at least twice daily for morbidity and mortality. Body weight of the animals was evaluated daily. At the 29th day, after an overnight fast, rats were anaesthetized with ether and blood sample for haematological and biochemical analysis was collected into tubes with or without EDTA respectively. Haemoglobin (Hb), haematocrit (Ht), Red Blood Cells count (RBC), White Blood Cells count (WBC), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV) and platelet count were determined using automatic counter Sysmex (K21, Tokyo, Japan). Biochemical analysis was performed in serum obtained after centrifugation of total blood without anticoagulant, at 2500 rpm for 15 min. Standardized diagnostic kits (Labkits) and a Biotrons spectrophotometer were used for spectrophotometrical determination of the following biochemical parameters: Alanine aminotransferase (ALT), Aspartate Aminotransferase (AST), creatinine, Alkaline Phosphatise (ALP), Glucose (Glu), total proteins, γGT and urea. Necroscopy of all animals was carried and the organ weights (heart, testis, colon, liver, kidney and spleen) were recorded. Each weighed organ was then standardized for percentage body weight of each rat (relative organ weight). Histological study of organs was done after sacrificing the animals on 29th day. Organs were removed, fixed in 10% formaldehyde, embedded in paraffin and sectioned at 5 mm. Tissue sections were stained with haematoxylin eosin (H and E) for general morphology.

Cardiac Activity Study in Toad

After toads demedullation, a midline ventral incision was made and the pectoral girdle and xiphoid process were removed to expose the heart and major vessels. The apex of the heart with suspended in a fine wire bonded to the transducer coupled to a PowerLab recording system and an application software LabChart all ADI Instruments, listing the frequency and amplitude heart. The software was always calibrated before each experiment. The different doses of the extract and digoxin were administered directly to the heart in vivo. Each test was repeated 5 times.

Contractile Activity on Isolated Guinea Pig Atria

The guinea pigs were sacrificed by cervical dislocation after ether anesthesia. The atria were quickly removed and placed in Mac Ewen physiological solution (NaCl: 15 g; KCl0.24 g; CaCl: 0.48 g; PO_4H_2Na: 0.29 g; CO_3HNa: 2 g; $MgCl_2$: 0.1 g; Glucose 4 g) at 37°C. The

atria were then mounted into the vessel body. The right atrium was attached to the hook which is situated at the bottom of the tank and the left atrium is connected to the transducer. The body under a base tension of 1 g was bathed in the physiological Mac Ewen solution maintained at 37°C and aerated with pure oxygen. The atria were washed several times just after installation. After a few minutes of equilibration, cumulative concentrations of 0.1 mg/mL; 1 mg/mL; 10 mg/mL; 100 mg/mL of extract was tested on the amplitude and frequency of contractions. The organ is connected to the transducer coupled to a PowerLab recording system and application software LabChart all ADI Instruments, recording the frequency and amplitude of heart. The effect of each dose on the atria was recorded for 10 minutes. The same operation was repeated three times with each dose.

Statistical Analysis

The software GraphPad Prism 6.02 (Graph Pad Software, Inc. USA) was used to analyze our results. These were expressed as mean value accompanied by the standard error of the mean (m ± SEM). The number (n) of value was specified in each case. The Analysis of Variance (ANOVA) was used to compare different groups. The difference between two groups was determined using the Tukey test. The significance level was set at $p < 0.05$.

Results

Phytochemical Screening

The phytochemical screening was first performed; it showed the presence of traces of alkaloids, flavonoids, saponins, tannins and cardiac glucosides.

Sub-Chronic Toxicity

The hydroalcohol extract of *A hispidum* leaves resulted in no significant change in body weight of rats of different groups (Table 1). But there is a significant decrease ($p < 0.001$) in testis relative weight to the dose of 1000 mg/kg (Table 2).

The extract at 500 and 1000 mg/kg significantly ($p < 0.001$) increased platelet counts after 28 days (Table 3). The Table 4 shows the biochemical analysis. *A. hispidum* significantly ($p < 0.0001$) the decreased blood sodium level. No significant damage was observed in rat liver, kidney, spleen and heart when compared with control. The histological sections of testis have shown a seminiferous tubule atrophy and fibrosis of the tube wall (data was not shown).

Table 1: Effect of hydroalcohol extract of *A. hispidum* leaves on body weight of rats

		Extract dose	
Weeks	Control	500 mg/kg	1000 mg/kg
0	198.5±9.58	197.8±8.05	203.4±17.22
1	209.5±8.66	200.8±9.74	197.6±17.86
2	214.6±8.41	203.1±10.54	199.0±17.88
3	220.8±8.80	204.6±9.88	202.4±15.79
4	215.0±9.99	206.6±11.07	201.4±13.61

Each value represents the mean ± SEM n (the number of animals per group) = 6

Table 2: Effect of hydroalcohol extract of *A. hispidum* leaves on relative organ weight

		Extract dose	
Organs	Control	500 mg/kg	1000 mg/kg
Heart	0.42±0.02	0.35±0.01	0.37±0.02
Rate	0.22±0.02	0.19±0.02	0.19±0.01
Testis	1.16±0.20	0.97±0.19	0.78±0.22*
Liver	3.04±0.10	3.19±0.09	3.21±0.09
Kidney	0.57±0.02	0.61±0.01	0.65±0.03

Each value represents the mean ± SEM. n (the number of animals per group) = 6. *p<0.05 significant difference as compared the control

Table 3: Effect of hydroalcohol extract of *A. hispidum* leaves on hematological parameters

		Extract dose	
Parameters (unit)	Control	500 mg/kg	1000 mg/kg
WBC (10^3/UL)	6.53±0.97	5.73±0.54	4.94±0.34
Haemoglobin (g/dL)	14.13±0.41	14.05±0.30	14.28±0.20
RBC (10^6/UL)	8.27±0.15	8.30±0.18	8.40±0.05
Haematocrit (%)	42.95±1.19	42.16±1.12	43.10±0.56
MCV (fL)	51.91±0.67	50.88±0.63	51.34±0.42
MCH (pg)	17.01±0.26	16.86±0.13	16.94±0.16
MCCH (g/dL)	32.85±0.11	33.30±0.38	33.08±0.15
Platelet (10^3/UL)	696.83±60.13	717.16±72.75*	798.80±66.02**

Each value represents the mean ± SEM. n (the number of animals per group) = 6. ** p <0.001 significant difference in the dose 1000 mg/kg compared with the control. * p < 0.05 significant difference as compared to control

Table 4: Effect of hydroalcohol extract of *A. hispidum* leaves on biochemical parameters

		Extract dose	
Parameters (Unit)	Control	500 mg/kg	1000 mg/kg
Urea (g/L)	0.21±0.09	0.20±0.01	0.19±0.01
Creatinine (mg/L)	7.66±0.49	7.16±0.16	7.20±0.20
ASAT (UI/L)	109.00±23.96	83.66±7.89	91.80±6.86
ALAT (UI/L)	58.00±13.54	48.16±3.89	54.00±3.64
ɣGT (UI/L)	3.50±0.22	3.50±0.56	2.60±0.60
Alkaline phosphatase (UI/L)	174.50±13.05	141.50±11.18	164.40±13.70
Glucose (mg/dL)	88.66±5.76	100.83±4.67	106.00±11.52
Na^+ (mmol/L)	427.08±61.72	416.66±57.44***	307.29±51.52***
Cl^- (mmol/L)	9.96±2.16	15.75±2.66	14.42±1.45
K^+ (mmol/L)	15.40±2.10	18.44±1.37	13.22±1.99
Ca^{2+} (mmol/L)	6.92±2.37	3.93±0.94	2.89±0.42

Each value represents the mean ± SEM. n (the number of animals per group) = 6. *** p <0.0001 significant difference as compared with the control

Table 5: Effect of hydroalcohol extract of *A. hispidum* leaves on the frequency and amplitude of the heart

		Extract dose			
Parameters	Control	0.1 mg/ml	1 mg/ml	10 mg/ml	100 mg/ml
Frequency	71.20±3.51	68.80±3.95***	65.40±4.38**	58.80±5.33	47.60±5.00***
Amplitude	0.76±0.12	0.79±0.12	0.81±0.12	0.84±0.14	0.95±0.17

Each value represents the mean ± SEM. n (the number of reply) = 5. *** p <0.0001 significant difference as compared to the control. ** p <0.01 significant difference as compared to the control.

Table 6: Effect of hydroalcohol extract of *A. hispidum* leaves on the frequency and amplitude of isolated guinea pig atria

		Extract dose			
Parameters	Control	0.1 mg/ml	1 mg/ml	10 mg/ml	100 mg/ml
Frequency	143±2.64	95±2.64***	74±5.29***	5±5***	0±0***
Amplitude	0.29±0.01	0.36±0.02	0.40±0.02	0.1±0.1	0±0

Each value represents the mean ± SEM. n (the number of reply) = 5. *** p <0.0001 significant difference as compared to the control.

The Effect of A. hispidum on the Toad Heart

The hydroalcohol extract of *A. hispidum* at 0.1; 1; 10 and 100 mg/mL caused an increase in toad cardiac amplitude (positive inotropic) and a significantly decrease ($p < 0.0001$) the heart rate (negative chronotropism) (Table 5).

The Effect of A. hispidum on Guinea Pig Isolated Atria

The extract at concentrations of 0.1 mg/ml and 1 mg/ml on isolated guinea pig atria, resulted in an increase of the amplitude (positive inotropic) and a significant decrease ($p < 0.0001$) of the frequency (negative chronotropic) (Table 6). At concentrations of 10 mg/mL and 100 mg/mL the extract caused a cardiac arrest.

Discussion

The phytochemical screening showed the presence of alkaloids, flavonoids, saponins, tannins and cardiac glucosides. These chemicals would be involved in the different biological activities of this extract. Our result confirms those of Roy *et al*. (2010), which have already noted the presence of these chemical groups in the leaves of *A. hispidum*. Edewor and Olajire (2011), have noted the lack of alkaloids and cardiac glycosides in the aerial parts of *A. hispidum*. Our result has noted the lack of anthracene in our extract. This result confirms those of Edewor and Olajire (2011), which also noted the lack of anthracene.

The hydroalcohol extract of *A hispidum* leaves induced no significant change in body weight of rats of different groups. The administration of hydroalcohol extract of *A hispidum* leaves to rats resulted in no significant change in body weight of rats of different groups, but there is a significant decrease ($p < 0.001$) in testis relative weight at the dose of 1000 mg/kg. The assessment of the weight of organs such as the liver, kidney, spleen, testes, heart, pancreas, brain and language is very important in toxicological studies. The weight of a body or more, the relative weight is an important index used in physiology and toxicology (Dybing *et al*., 2002). The decrease in testis relative weight translates a testicular atrophy. The same result

was reported by Ali and Adam (1978). This reduction in the relative weight of the testis was confirmed by the histological sections of testis showing a seminiferous tubule atrophy and fibrosis of the tube wall.

Achieving Blood Counts (CBC) is also very important in toxicological studies. The hematopoietic system is one of main targets of toxic substances and consequently, an important parameter of the physiology of the human or animal (Olson *et al*., 2000; Diallo *et al*., 2008). The extract at 500 and 1000 mg/kg significantly ($p < 0.0001$) increased platelet counts after 28 days. The thrombocytosis can be attributed to increased production and secretion of thrombopoietin, the major regulator of platelet production (Kaushansky, 1995).

The measurement of some biochemical parameters such as enzyme activities in tissues and body fluids plays a major role in the study of disease, diagnosis and assessment of toxicity. The liver, kidneys and lungs are the main organ affected by the metabolic reaction caused by toxic substances. The liver is the organ most affected because it is the most important organ of detoxification. Our result has shown that *A. hispidum* significantly ($p <0.0001$) decreased blood sodium. This hyponatremia may be due to the diuretic effect of this extract hence its use as an antihypertensive (Tirloni *et al*., 2017).

On toad heart, *A. hispidum* has increased the cardiac amplitude (positive inotropic) and a significantly decrease ($p < 0.001$) the cardiac frequency (negative chronotropism). These effects may be due to cardiac glycosides contained in *A. hispidum* leaves.

The contractile activity on isolated guinea pig atria with *A. hispidum* leaves extract resulted in an increase of the amplitude (positive inotropic) and a significant decrease ($p < 0.0001$) of the frequency (negative chronotropic). These effects are similar to the effect of cardiac glycosides which are also present in our extract. Cardiac glycosides increase the contractile force (positive inotropic effect) by increasing the intracellular calcium during systole. They bind to a specific site of the Na-K-ATPase. Depolarization occurs at the opening of fast sodium channels. In addition, calcium flux induces the release of calcium from the sarcoplasmic reticulum, producing contraction (Sellers *et al*., 2007).

Conclusion

After this work, *A. hispidum* essentially contains alkaloids, flavonoids, saponins, tannins and cardiac glycosides. He induced a significant decrease in relative testes weight, blood sodium and increased platelets. Cardiac disorders were observed, characterized by an increase in the force of contraction, a significant decrease in the frequency and increased heart muscle tone, due to the presence of cardiac glycosides.

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Author's Contributions

All authors equally contributed in this work.

Ethics

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

Adepiti, A.O., C.O. Adewumi and J.M. Agbedahunsi, 2014. Antitrichomonal activity of *Acanthospermum hispidum* DC (Asteraceae). Afr. J. Biotechnol., 13: 1303-1307. DOI: 10.5897/AJB2013.13064

Agbodeka, K., H.E. Gbekley, S.D. Karou, K. Anani and A. Agbonon *et al*., 2016. Ethnobotanical study of medicinal plants used for the treatment of malaria in the plateau region, Togo. Pharmacognosy Res., 8: S12-S18. DOI: 10.4103/0974-8490.178646

Ali, B. and S.E.I. Adam, 1978. Effects of *Acanthospermum hispidum* on goats. J. Comp. Pathol., 88: 533-544. DOI: 10.1016/0021-9975(78)90007-5

Anani, K., J.B. Hudson, C. de Souza, K. Akpagana and G.H.N. Tower *et al*., 2000. Investigation of medicinal plants of Togo for antiviral and antimicrobial activities. Pharm. Biol., 38: 40-45. DOI: 10.1076/1388-0209(200001)38:1;1-B;FT040

Arena, M.E., E. Cartagena, N. Gobbato, M. Baigori and J.C. Valdez *et al*., 2011. *In vivo* and *in vitro* antibacterial activity of acanthospermal B, a sesquiterpene lactone isolated from *Acanthospermum hispidum*. Phytother. Res., 25: 597-602. DOI: 10.1002/ptr.3300

Atindehou, K.K., C. Schmid, R. Brown and M.W.D Kone, 2004. Traore Antitrypanosomal and Antiplasmodial activity of medicinal plants from Ivory Coast. Ethnopharmacol. J., 90: 221-227. DOI: 10.1016/j.jep.2003.09.032

Chakraborty, A.K., A.V. Gaikwad and K.B. Singh, 2012. Phytopharmacological review is *Acanthospermum hispidum*. J. Applied Pharm. Sci., 2: 144-148.

Diallo, A., M. Gbeassor, A. Vovor, K. Eklu-Gadegbeku and K. Aklikokou *et al*., 2008. Effect of Tectona grandis is phenylhydrazine-induced anemia in rats. Phytotherapy, 79: 332-336. DOI: 10.1016/j.fitote.2008.02.005

Dybing, E., J. Doe, J. Groten, J. Kleiner and J. O'Brien *et al*., 2002. Hazard characterization of chemicals in food and diet: dose response, mechanisms and from extrapolation. Food Chem. Toxicol., 40: 237-282. DOI: 10.1016/S0278-6915(01)00115-6

Edewor, I.T. and A.A. Olajire, 2011. Two flavones from *Acanthospermum hispidum* DC and their antibacterial activity. Int. J. Org. Chem., 01: 132-141. DOI: 10.4236/ijoc.2011.13020

Fleischer, T.C., E.P.K. Ameade and I.K. Sawer, 2003. Antimicrobial activity of the leaves and flowering tops of Acanthospermum hispidum. Fitoterapia, 74: 130-132. DOI: 10.1016/S0367-326X(02)00290-3

Ganfon, H., J. Bero, A.T. Tchinda, F. Gbaguidi and J. Gbenou *et al*., 2012. Antiparasitic activities of two sesquiterpenic lactones isolated from *Acanthospermum hispidum* DC. Ethnopharmacol. J., 141: 411-417. DOI: 10.1016/j.jep.2012.03.002

Hoffman, B.R., H. DelasAlas K. Blanco N. Wiederhold and R.E. Lewis *et al*., 2004. Screening of antibacterial and antifungal activities of ten medicinal plants from Ghana. Biol. Pharm., 42: 13-17. DOI: 10.1080/13880200490504925

Jakupovic, J., R.N. Baruah, F. Bohlmann and J.D. Msonthi, 1986. Further acanthospermolides from *Acanthospermum hispidum*. Planta Med., 52: 154-155. DOI: 10.1055/s-2007-969104

Kaushansky, K., 1995. Thrombopoietin: The primary regulator of megakaryocyte and platelet production. Thromb. Haemost., 74: 521-525. DOI: 10.1055/s-0038-1642732

Lemonica, I.P. and C.M.D. Alvarenga, 1994. Abortifacient and teratogenic effect of *Acanthospermum hispidum DC*. and pigeonpea (L.) Millps. in pregnant rats. Ethnopharmacol. J., 43: 39-44. DOI: 10.1016/0378-8741(94)90114-7

Mann, A., M. Glate and N.A. Umar, 2003. Medicinal and Economical plants of Nupeland. 1st Edn., Jube Evans Published Books and Bida, pp: 276.

N'do, J.Y., A. Hilou, N. Ouedraogo, E.N. Sombie and T.K. Traore, 2018. Phytochemistry, antioxidant and hepatoprotective potential of *Acanthospermum hispidum* DC extracts against diethylnitrosamine-induced hepatotoxicity in rats. Medicines (Basel), 5: 42-42. DOI: 10.3390/medicines5020042

Olson, H., G. Betton, D. Robinson, K. Thomas and A. Monro *et al*., 2000. Concordance of toxicity of pharmaceuticals in humans and animals. Regul. Toxicol. Pharmacol., 32: 56-67. DOI: 10.1006/rtph.2000.1399

OECD, 2008. Repeated Dose Oral Toxicity Test Method. In: OECD Guidelines for Testing of Chemicals, N°407. Organization for Economic Cooperation and Development, Paris, France.

Palozi, R.A.C., M.I. Schaedler, C.A.S. Tirloni, A.O. Silva and F.A.D.R. Lívero *et al*., 2017. Roles of nitric oxide and prostaglandins in the sustained antihypertensive effects of *Acanthospermum hispidum* DC. on ovariectomized rats with renovascular hypertension. Evid. Based Complement Alternat. Med. DOI: 10.1155/2017/2492483

Roy, H., A. Chakraborty, S. Bhanja, B.S. Nayak and S.R. Mishra *et al*., 2010. Preliminary phytochemical investigation and anthelmintic activity of *Acanthospermum hispidum* DC. J. Pharm. Sci. Technol., 2: 217-221.

Sanon, S., N. Azas, M. Gasquet, E. Ollivier and V. Mahiou *et al*., 2003. Antiplasmodial activity of alkaloid extracts from Pavetta crassipes (K. Schum) and *Acanthospermum hispidum* (DC), two plants used in traditional medicine in Burkina Faso. Parasitol. Res., 90: 314-317. DOI: 10.1007/s00436-003-0859-9

Sellers, R.S., D. Morton and B. Michael, 2007. Society of toxicologic pathology position paper: OrganWeight recommendations for toxicology studies. Toxicol. Pathol., 751-755. DOI: 10.1080/01926230701595300

Summerfield, A. and A. Saalmüller, 1998. Interleukin-2 dependent selective activation of γδ T cells by pig year extract from the leaves of *Acanthospermum hispidum*. Int. J. Immunopharmacol., 20: 85-89. DOI: 10.1016/S0192-0561(98)00016-2

Tirloni, C.A.S., F.A.D.R. Lívero, R.A.C. Palozi, R.C.A. Silveira and P.C.P. Vasconcelos *et al*., 2017. Ethnopharmacological investigations of the cardio-renal properties of a native species from the area of Pantanal, state of Mato Grosso do Sul, Brazil. J. Ethnopharmacol., 206: 125-134. DOI: 10.1016/j.jep.2017.05.027

Tona, L., K. Kambu, N. Ngimbi, K. Cimanga and A.J. Vlietnick, 1998. Antiamoebic and phytochemical screening of some Congolese medical plants. J. Ethnopharmacol., 61: 57-65. DOI: 10.1016/S0378-8741(98)00015-4

Yapi, A.B., 2013. Inventory of medicinal plants in the Asteraceae markets Abobo (Abidjan, Ivory Coast). MSc Thesis, University Houphouët Boigny Abidjan Felix Ivory Coast.