

**ASSESSMENT OF ACUTE AND SUBACUTE TOXICITY OF
AQUEOUS EXTRACT AND *IN VITRO* SUSCEPTIBILITY OF
EXTRACTS AND ISOLATED INDOLOQUINOLINE ALKALOIDS
FROM *CRYPTOLEPIS SANGUINOLENTA* (LINDL.) SCHLECHTER
(PERIPLOCACEAE) ROOT BARK TO *ENTAMOEBIA HISTOLYTICA***

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ABSTRACT

Aqueous and 80% methanol extracts from *Cryptolepis sanguinolenta* root bark and their alkaloids were submitted to an evaluation for their potential amoebicidal activity against *Entamoeba histolytica*. Results indicated that aqueous and 80% methanol extracts exhibited pronounced amoebicidal activity with minimal amoebicidal concentrations (MAC) of 6.25 and 4.15 µg/ml respectively and with inhibitory concentrations 50 (IC₅₀) of 4.02 and 2.87 µg/ml respectively. Among isolated indoloquinoline alkaloids, cryptolepine, the major alkaloid of this plant part and its isomer neocryptolepine showed pronounced amoebicidal activity with MAC values of 3.12 and 1.87 µg/ml, and IC₅₀ values of 3.27 and 2.07 µg/ml respectively. Other monomeric alkaloids such as cryptolepine HCl, hydrocryptolepine and quindoline displayed this activity with MAC and IC₅₀ values ranging from 4.25 to 10.68 µg/ml with quindoline exhibiting weak activity (MAC = 10.68 and IC₅₀ = 8.65±0.03 µg/ml. The dimeric alkaloids

biscryptolepine and cryptoquindoline also exhibited pronounced

amoebicidal activity with MAC and IC₅₀ values < 10 µg/ml, but their amoebicidal effect was weak compared to monomer alkaloids except quindoline which showed weak activity. The aqueous extract did not significantly modify the concentrations of haematological, biochemical parameters and weights of some vital organs. It increased the concentrations of some electrolytes in treated animals compared to untreated. The extract was found to be non-toxic, safe and well tolerated. These reported results constitute a irrefutable scientific base supporting and justifying the traditional use of *C. sanguinolenta* root bark for the treatment of amoebiasis in traditional medicine without significant toxic effects.

KEYWORDS: *Cryptolepis sanguinolenta*, root bark, amoebiasis, acute and sub-acute toxicity, indoloquinolinique alkaloids.

1. INTRODUCTION

Cryptolepis sanguinolenta is a medicinal plant belonging to the Periplocaceae family. Its roots or root barks are currently used in traditional medicine in many African countries to treat mainly malaria and fever. Historically, the species collected in Congo-Belgium was named *Cryptolepis triangularis*. The first phytochemical studies conducted on its roots resulted in the isolation of a purple substance with alkaloidal nature named cryptolepine with the proposal structure C₁₄H₁₇O₄N₂ (Clinquant, 1929). Afterwards, Delvaux (1931) isolated the same purple base in the same plant material and proposed the formula C₁₇H₁₆O₂N. These two isolated bases are oxygenated compounds. Thus, it was suggested that the first compound isolated by Clinquant can be named cryptolepine C and the second isolated by Delvaux cryptolepine D. But, from the Nigerian species identified as *C. sanguinolenta*, Gellert et al. (1951) isolated the same purple base for which they proposed two non oxygenated formula as C₁₅H₁₀N₂ or C₁₆H₁₂N₂.

However the major purple alkaloid base isolated from the root or root bark of *C. sanguinolenta* (Lindl.) Schlechter growing in West African countries (Ghana) and named also cryptolepine since 1978 by Dumaa-Badu et al., (1978) responded to the formula C₁₆H₁₂N₂ and is not an oxygenated alkaloid base in contrast to the two first mentioned alkaloids above. The formula of the basic compound proposed by Gellert et al., (1951) and Duma-Badu et al., (1978) was after isolated by many other scientific researchers toward the years 1990 in *C. sanguinolenta* root or root bark collected in different countries (Abloedeppey et al., 1990; Cimanga, 1991; Tackie et al., 1991; Crouch and Martin, 1992; Paulo et al., 1994;) and its structure was confirmed by extensive ¹H, ¹³C-NMR, DEPT and COSY spectroscopic

methods as C₁₆N₁₂N₂ named cryptolepine, an non-oxygenated purple alkaloid base for which the salts have a yellow colour. Other phytochemical studies have reported the isolation and structure elucidation of minor indoloquinoline alkaloids from *C. sanguinolenta* root or root bark such as quindolinone (Crouch et al., 1995), isocryptolepine (Pousset et al., 1995), homocryptolepine (Sharaf et al., 1995a), crptolepicarboline (Sharaf et al., 1995b) cryptosanguinolentine and cryptotackieine (Sharaf et al., 1996a,) cryptomisrine (Sharf et al., 1996b), cryptospirolepine (Tackie et al., 1993), neocryptolepine, hydroxycryptolepine, biscryptolepine, cryptoquindoline, hydroxycryptolepine and quindoline (Paulo, 1994, Cimanga et al., 1996; Cimanga, 1997,). Many of the minor alkaloids were isolated in submicrogram amounts.

Cryptolepine, the major alkaloid for the root or root bark of *C. sanguinolenta* was submitted to the evaluation of its different biological activities such as its effects as an adrenoceptor blocking (Noamesi and Bamgbose, 1980, 1982), anti-inflammatory activity (Bamgbose and Noamesi, 1981), its effects as an antagonist of noradrenaline and modification of this effect by calcium ions and prostaglandin on rat isolated mesenteric artery (Noamesi and Bamgbose, 1983a), antispasmodic activity (Noamesi and Bamgbose, 1983b), inhibitor of the tone of prostaglandin production (Noamesi and Bamgbose, 1984), inhibitor of platelets aggregation (Oyekan et al., 1988), its effects on arterial thrombosis alone and in combination with dipyridamole (Oyekan and Okafor, 1989), antibacterial activity (Paulo et al., 1994a; Cimanga et al., 1995, 1996, 1998), antidiarrhoeal bacteria activity (Paulo et al., 1994b), its effects on the morphology and survival of some bacteria and fungi (Sawer et al., 1995), anticomplementary activity (Cimanga et al., 1996), (Cimanga et al., 1996); antihyperglycemic activity (Luo et al., 1998), antifungal activity (Cimanga et al., 1998), antioxidative activity (Cimanga et al., 2000), antitrypanosomal and antileishmanial activities against *Trypanosoma brucei brucei*, *T. cruzi* and *Leishmania donovani* (Jonckers et al., 2002), inhibitor of β -haematin formation and DNA interaction (Van Miert et al., 20004) and *in vitro* and *in vivo* antiplasmodial activity against different *Plasmodium falciparum* sensible and resistant to chloroquine (Noamesi et al., 1991; Dochez et al., 1994; Grellier et al., 1996; Cimanga et al., 1997b,c; Kirby et al., 1995; Van Miert et al., 2005). Other indoloquinoline alkaloids such such neocryptolepine, bicryptolepine cryptoquindoline and quindoline were also submitted to some biological activities such antiplasmodial (Paulo et al., 1994; Cimanga et al., 1997c,b; Jonckers et al., 2002), antibacterial activity (Paulo et al., 1994; Cimanga et al., 1998), anticomplement (Cimanga et al., 1997a), antitrypanosomal and antileishmanial

activities against *Trypanosoma brucei brucei*, *T. cruzi* and *Leishmania donovani* (Jonckers et al., 2002) and cytotoxic activity (Jonckers et al., 2002). In all evaluated biological activities, cryptolepine and other indoloquinoline alkaloids isolated from *C. sanguinolenta* root bark had given good positive responses, except in antioxidative activity where it was found inactive together with dimeric alkaloids and quindoline.

The root bark of plant used as aqueous decoction known other medical indications such the treatment of dysentery (amoebiasis), rheumatism, fever, enteralgia, colic intestinal disorders, urogenital infections, asthma, anemia, upper respiratory infections, diabetes, hemorrhoids, anorexy, blenorragia and sexual weakness, used as revigorant, analgesic and hypotensive agent (Kerharo and Adam, 1974; le Grand and Wondergem, 1987; Boye and Ampofo, 1990; Neuwinger, 2000; Kambu, 2009).

On the other hand, it is well known that amoebiasis or dysentery provoked by the protozoan parasite *Entamoeba histolytica* is a disease causing morbidity and mortality in the world. It is a major problem in some countries, mainly in developing countries where people contract it because of sanitation, socio-economic status, poor life-style environmental conditions and non-availability of conventional medical cure (Sharma and Sharma, 2001). Metronidazole is the current drug now widely used and recommended in the treatment of dysentery (Towson et al., 1994), but it has side effects such as metallic taste, nausea, transient neutropenia and metallic taste and is less effective in the tissue than in the gut lumen (Moundipa et al., 2005, Behnia et al., 2008). As human is the only relevant host for this parasite, an effective treatment of luminal intestinal infection is necessary to interrupt transmission of the parasite. Taking account to the difficult of access to other synthetic amoebicidal drugs including Metronidazole (family of nitroimidazole), people drive to traditional medicine using different medicinal plants claimed to treat the disease by traditional healer in their daily practices and find some reliefs as the selection of medicinal plants as new source of different drugs is mainly based on herbal remedies used in traditional medicine. These kind of medicinal plants are nowadays scientifically investigated and reported to have antiamoebic activity mainly *in vitro* test (Wright et al., 1989; Ghoshal et al., 1996; Caldaza et al., 1998; 1999; Tona et al., 2000; Sharma and Sharma, 2001). In some studies, active amoebicidal principles were isolated (Ahmed et al., 1966; Bhutani et al., 1987; Cedeno et al., 1987; Keene et al., 1986, 1987; Wright et al., 1988; Wright et al., 1991; Marshall et al., 1994; Cimanga et al., 2006a,b) and some of them were considered as lead compounds.

As no information was available in literature on the amoebicidal activity of *Cryptolepis sanguinolenta* root bark, WHO has encouraged countries to interact with traditional medicine to identify and exploit aspects that provide safe and effective remedies (WHO, 2000). As such, the present study was conducted to assess the *in vitro* amoebicidal activity of extracts and some isolated indoloquinoline alkaloids from *C. sanguinolenta* root bark *in vitro* as well as acute and sub-acute toxicity of aqueous extract and its effects on haematological and biochemical parameters, electrolytes and weights of vital organs of treated Wistar rats.

2. MATERIALS

2.1. Plant material

Root barks of *Cryptolepis sanguinolenta* were collected in Kinshasa-Democratic Republic of Congo (DR Congo) and the plant was authenticated at the herbarium of National Institute of Studies and Researchs in Agronomy (NISRA), Department of Biology, Faculty of Sciences, University of Kinshasa. A voucher specimen of the plant NL062017RBCS was deposited in the herbarium of this institute. Plant materials were dried at room temperature and reduced to powder using an electronic blender.



Figure. 1: *Cryptolepis sanguinolenta* (Lindl.) Schlechter (Periplocaceae).

2.2. Preparation of extracts and isolation of alkaloids

20 g of powdered root bark were mixed with 300 ml distilled water and heat for 15 minutes. After cooling and filtration on a filter Whatman N°1, the filtrate was evaporated *in vacuo* yielding corresponding dried extract denoted as AE (13.25g). Another batch of 20 g of plant material was macerated in 300 ml methanol 80% and treated as described above yielding dried extract denoted as ME (15.68 g). On the other hand, 1000 mg of powdered root bark were macerated in 80% methanol leading to the isolation of six alkaloids by the classical

method acid/base coupled to chromatography on column filled with silicagel (Merck, Germany) eluted with $\text{CHCl}_3/\text{MeOH}$: 0-100% and preparative TLC (thickness layer 1 mm, Merck Germany) using $\text{CHCl}_3/\text{MeOH}$: 3:1 as mobile phase. All isolated alkaloids were identified by different spectroscopic methods (^1H -RMN, ^{13}C -RMN, DEPT, HSQC and COSY) spectra as cryptolepine (major alkaloid), biscryptolepine, crptoquindoline, hydroxycryptolepine, neocryptolepine, quindoline and cryptolepine HCl (Cimanga *et al.* 1996, 1997b, 2000).

2.3. *In vitro* antiamoebic testing

Entamoeba histolytica used in the present study is a laboratory isolated strain from patients with acute dysentery diagnosed in the Tropical Medicine Institute, Faculty of Medicine, University of Kinshasa. The *in vitro* assessment of activity was performed using the methods previously described by Tona *et al.*, (2000) and Cimanga *et al.* (2010).

Briefly, the parasite was grown and cultured in sterile tubes containing 9 ml of diphasic medium (medium N of Pasteur Institute) called Dobbell and Laidlaw medium. The mixture was stirred and incubated for one week at 37°C. The daily examination and counting of amoebae through a optic microscope with the aid of Neubauer's cells were performed in order to monitor the parasitic growth and to detect possible contamination.

Uncontaminated tubes containing an average number of 2.5×10^6 amoebae/ml culture medium were selected as test tubes. 10 mg of each test sample was dissolved in 10 ml hydroethanol solvent (eau-ethanol :9:1) to have corresponding stock solutions of 1 mg/ml. These last solutions were diluted in two fold dilutions to give a series of test solutions ranging from 100 to 0.1 $\mu\text{g/ml}$. Next, 1 ml of the test solution with a known test concentration was added to a separated 1 ml of test tubes containing parasites. On the other hand, two tubes were used as controls, one containing parasites in hydroethanol solvent (9:1) without test sample as negative control and another containing test tubes with parasites and Metronidazole or pyrantel (20 to 0.1 $\mu\text{g/ml}$) as positive controls. All tubes were plugged with sterile cotton, vigorously stirred and incubated at 37°C for one week. The daily counting of dead and living amoebae was done described above. The test was considered as positive when the vegetative or kystic forms of amoebae was not microscopically observed. The minimum antiamoebic concentration (MAC) was immediately determined and inhibitory concentrations 50 (IC_{50}) were derived from individual concentration-responses curves by non-linear regression ($n=3$).

2.4. Acute toxicity

The acute toxicity of aqueous extract of *C. sanguinolenta* AE was evaluated in Wistar rats according to the procedure described by the Organization for Economic Cooperation and Development (OECD) guideline for testing chemicals, TG420 (OECD, 2001). Wistar rats (body weight: 145–157 g, aged 8–10 weeks, of either sex) were divided into four groups: Group I (5 rats) orally received 5 ml distilled water and constituted the negative control group. Groups II, III and IV (7 rats each) received orally a single oral dose of 500, 1000 and 5000 mg/kg body weight of the aqueous extract respectively. The animals were observed for toxic symptoms continuously for the first 4 h dosing and were daily weighed. Finally, all animals were then maintained in daily observation and the number of toxic effects and survivor was recorded for further 28 days (Ogbonnia *et al.*, 2008; Kripa *et al.*, 2011; Gandliare *et al.*, 2013).

2.5. Sub-acute toxicity

The sub-acute toxicity of the aqueous extract of *C. sanguinolenta* root bark was evaluated according to the procedure described by Kripa *et al.*, (2011) and Gandhare *et al.*, (2013). Briefly, Wistar rats were used and divided into four groups. Group I (5 rats) orally received daily 5 ml distilled water and constitute the negative control. Groups II, III and IV (7 rats each) orally received daily 200, 400 and 800 mg of aqueous extract for 28 days. Animals were observed for toxic symptoms, change behavior, alteration, digestive troubles, consumption of food and water intake. The body weight was daily recorded. They were observed twice daily for mortality during 28 days period of the investigation.

2.6. Biochemical and hematological parameters analysis

Blood from rats having received 5 g/kg in acute toxicity test was collected from tail vein on Day 28 for analysis. For biochemical parameters, blood was centrifuged at 4000g for 5 min to obtain plasma, which was stored at -20°C : glucose, creatinine, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), serum glutamopyruvate transferase (SGPT), serum glutamooxalate transferase (SGOT), uric acid, total cholesterol, triglycerides, high-density lipoproteins (HDL), low-density lipoproteins (LDL), total and direct bilirubin were quantified using Architect (Abott) automation with Boehringer Ingelheim biochemical kits. Total proteins were estimated using Biuret's method (Saha *et al.*, 2011).

Hematological parameters were analyzed using an automatic hematological analyzer (Coulter STK, Beckam) with appropriated kits. The differential leucocytes count was performed with

an optical microscopy after staining and, in each case, 100 cells were counted (Akpanabiattu *et al.*, 2013).

For mineral elements, 10 ml of blood of animals having received 5000 mg/kg bw of aqueous extract was collected and incinerated at 450°C for 24 h in a muffle and acid digest. The material for analysis was prepared by oxidizing sample with nitric/perchloric acids 2:1. The concentrations of minerals were determined with flame atomic absorption spectrophotometer (Perkin-Elmer 2880 Model) and the inorganic phosphorus was estimated by phosphomolybdovanate method (AOAC, 1990).

2.7. Histopathological analysis

Animals having received 5000 mg/kg bw of AE extract, were sacrificed by cervical displacement followed by exsanguination. Histopathological analysis of vital organs such as heart, kidney, liver, spleen, large intestine and lungs was carried out according to the procedure previously described by Lamb (1981). The organ pieces (5-8 µm) were fixed in 10% formalin for 24 h and washed in running distilled water for 24h. After dehydration in an autotechnicon, the cleared organs were embedded by passing through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the “L” moulds. It was followed by microtome and the slides were stained with haematoxylin-eosin and observed under electronic microscopic. The dried organs were weighted (Akharaiyi *et al.*, 2012; Mafiolet *et al.*, 2013).

2.8. Statistical analysis

The results are reported as mean \pm SD for all values. The significant differences were assessed using one-way analysis of variance (ANOVA) using SPSS software package. P values < 0.05 were considered as significant.

3. RESULTS AND DISCUSSION

3.1. *In vitro* amoebicidal activity

Firstly, hydroethanol 9:1 solvent used as negative control did not inhibit the growth of the parasite *Entamoeba histolytica*. Results presented in Table 1 indicated that aqueous, 80% methanol and total alkaloids extracts, and isolated indoloquinoline alkaloids exhibited pronounced amoebicidal activity with different magnitudes. Aqueous extract AE displayed weak amoebicidal (MAC and IC₅₀ = 6.25 and 4.08 µg/ml respectively) activity compared to 80% methanol extract ME (MAC and IC₅₀ = 4.15 and 2.87 µg/ml respectively) ($p < 0.05$)

suggesting that methanol is a more efficient solvent for extracting active amoebicidal compounds in high amounts in this plant part. The total alkaloids TA extract also exhibited pronounced amoebicidal activity with MAC and IC₅₀ values of 3.75 and 2.05 µg/ml respectively. Its activity was significantly higher ($p < 0.05$) compared to AE and ME extracts. The indoloquinoline monomer alkaloids cryptolepine, neocryptolepine and hydroxycryptolepine exhibited high activity ($p < 0.05$) with MAC values from 3.12 to 7.45 µg/ml and IC₅₀ values from 1.87 to 4.25 µg/ml compared to the dimeric alkaloids biscryptolepine and cryptoquindoline with MAC values of 5.87 and 8.15 µg/ml and IC₅₀ values of 3.51 and 5.63 µg/ml respectively. Cryptolepine exhibited high ($p < 0.05$) amoebicidal activity (MAC and IC₅₀ values of 3.12 ± 0.01 and 1.87 ± 0.02 µg/ml respectively) followed by its isomer neocryptolepine (MAC and IC₅₀ values of 3.87 ± 0.07 and 2.07 ± 0.05 µg/ml respectively ($p < 0.05$) compared to the remaining other indoloquinoline alkaloids (Table 1). The two dimeric alkaloids biscryptolepine (MAC and IC₅₀ = 5.87 ± 0.10 and 3.51 ± 0.01 respectively) and cryptoquindoline (MAC and IC₅₀ = 8.15 ± 0.02 and 5.63 ± 0.13 µg/ml respectively) showed high ($p < 0.05$) activity compared to quindoline with MAC and IC₅₀ values of 10.68 and of 8.65 µg/ml respectively.

Table. 1: Amoebicidal activity of extracts AE, ME and TA, and alkaloids (MAC and IC₅₀, µg/ml) from *C. sanguinolenta* root bark.

Extracts and compounds	MAC	IC ₅₀
AE extract	6.25±0.02	4.08±0.11
ME extract	4.15±0.04	2.87±0.09
TA extract	3.75±0.01	2.05±0.02
Biscryptolepine	5.87±0.10	3.51±0.01
Cryptolepine HCl	4.06±0.06	2.42±0.04
Cryptoquindoline	8.15±0.02	5.63±0.13
Cryptolepine	3.12±0.01	1.87±0.02
Hydroxycryptolepine	7.45±0.12	4.25±0.14
Neocryptolepine	3.87±0.07	2.07±0.05
Quindoline	10.68±0.04	8.65±0.03
Metronidazole	0.50±0.01	0.20±0.01
Pyrantel	0.70±0.02	0.40±0.01
Hydroethanol 9:1	0.00±0.00	0.00±0.00

AE: extract: aqueous extract, ME extract: 80% methanol extract TA extract: total alkaloids extract, MAC: minimal amoebicidal concentrations, IC₅₀: inhibitory concentrations 50.

A structure amoebicidal activity-relationship revealed that the presence of CH₃ group in position C-5 and H in position C-11, and the isomerization of cryptolepine are essential for a

pronounced activity (cryptolepine and neocryptolepine compared to other indoloquinoline monomeric alkaloids). The presence of OH group and CH₃ in C-11 and C-5 position

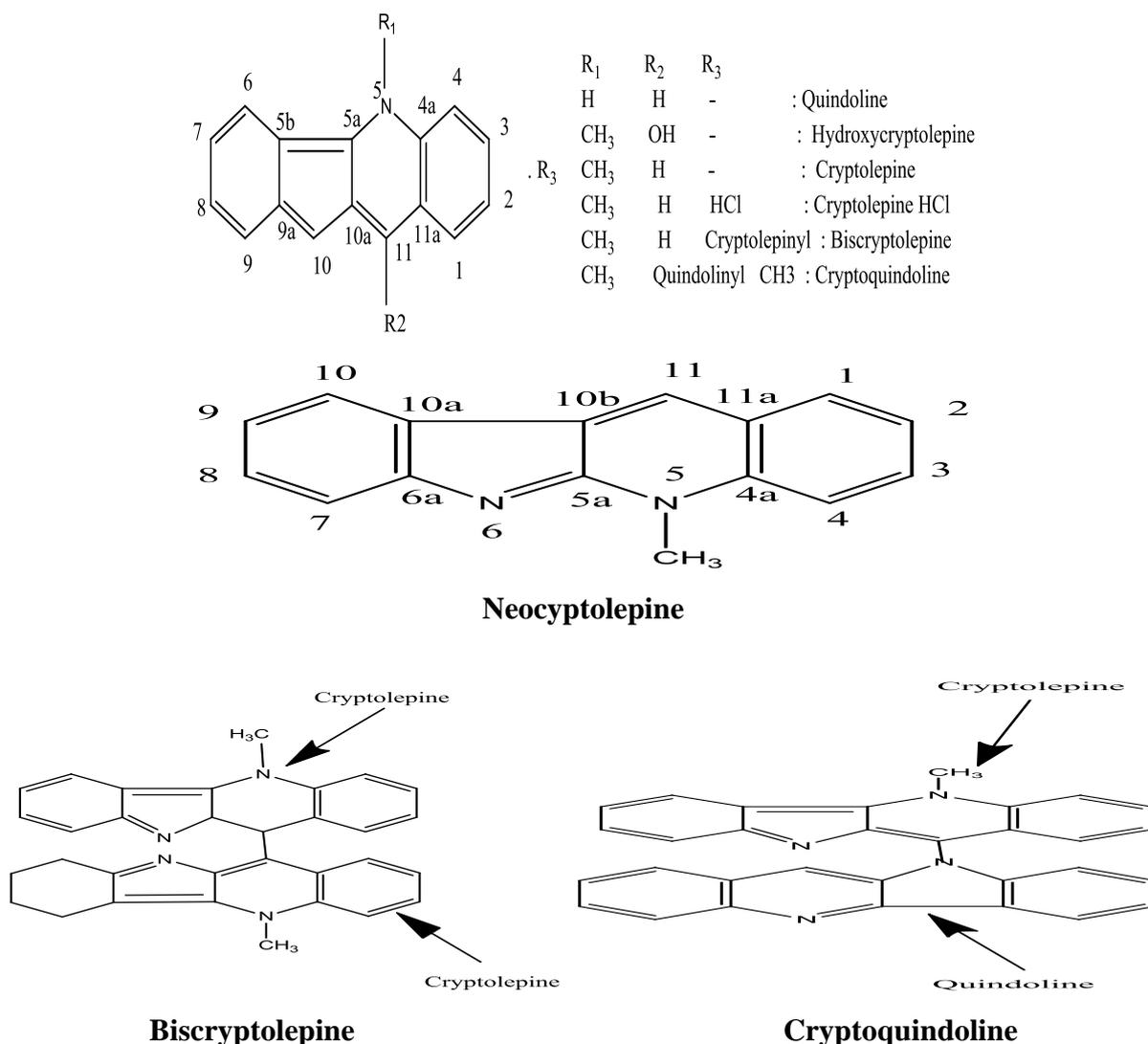


Figure. 2: Structure amoebicidal activity-relationship of indoloquinoline alkaloids from *C. sanguiolenta* root bark.

respectively reached a significant decrease of activity ($p < 0.05$) (hydroxycryptolepine compared to cryptolepine). In addition, the presence of H in position C-5 and C-11 respectively caused significant reduction of activity ($p < 0.05$) (quindoline compared to cryptolepine and hydroxycryptolepine). The addition of cryptolepinyl or quindolinyl group respectively to cryptolepine in C-11 position resulting in the formation of dimeric alkaloids also reached significant decrease of activity ($p < 0.05$) (biscryptolepine and cryptoquindoline compared to cryptolepine). The salt form of cryptolepine is in favour of reduction of activity (cryptolepine HCl compared to cryptolepine) (Table 1, Fig. 1). The calculated IC₅₀ value of

the standard drug metronidazole 0.20 $\mu\text{g/ml}$ is close to the values of 0.22, 0.33 and 0.202 $\mu\text{g/ml}$ obtained in previously antiamoebic experiments by Wright *et al.*, (1998), Mac Graw *et al.*, (2000) and Bharti *et al.*, (2006) respectively.

From these results, it is clear that cryptolepine, the major alkaloid can be considered as responsible for the amoebicidal activity of extracts of *C. sanguinolenta* root bark. Anyway, the remaining minor indoloquinoline alkaloids have also largely contributed to the manifestation of this biological activity taking account of the very good level of their activity which is in general appreciable. The amoebicidal activity of all indoloquinoline alkaloids isolated from *C. sanguinolenta* root bark was weak compared to standard amoebicidal drugs metronidazole and pyrantel (Table 1).

The figure 2 below showed the percentage inhibition of *E. histolytica* growth by aqueous (AE), methanol (ME) 80% and total alkaloids extracts (TA), cryptolepine (Cry) and cryptolepine HCl (CryHCl). It was observed that at the highest concentration of 10 $\mu\text{g/ml}$, all samples inhibited completely the growth of the parasite. The same effect was also obtained with the total alkaloids extract, cryptolepine and its salt when tested at a concentration of 5 $\mu\text{g/ml}$ (100% inhibition). At this concentration AE and ME extraction caused 61 and 87% inhibition of *E. histolytica* growth showing a survival of the parasite at 39 and 13% respectively. At low tested concentration of 2.5 $\mu\text{g/ml}$, all tested samples produced less than 50% of inhibition and the survival of the parasite is between 39 and 70% (Figure 2).

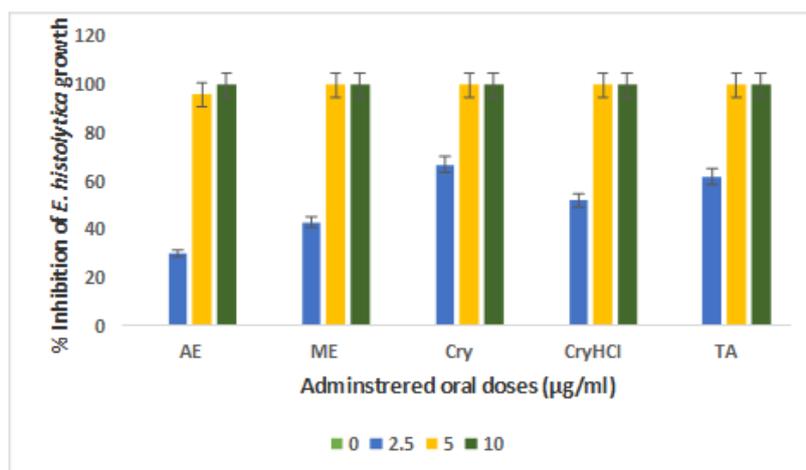


Figure. 2: % Inhibition of *E. histolytica* growth by some samples from *C. sanguinolenta* root bark: AE: aqueous extract, ME: methanol 80% extract, Cry: cryptolepine, CryHCl: cryptolepine HCl, TA: total alkaloids extract.

2.2. Acute toxicity of aqueous extract AE of *C. sanguinolenta* root bark

Acute toxicity is defined by OECD (Organization for Economic and Development) as the adverse effects occurring within a short time of oral administration of a single dose of a substance or multiple doses given within a span of 24 hours. Its purpose is to determine lethal dose 50 (LD₅₀) which help in determining the safe dose range at which the drug can be used such that there is no harmful or lethal effect on treated animals.

Results obtained in acute toxicity test of aqueous extract AE of *C. sanguinolenta* root bark revealed no sign of toxic effects such as alteration of the locomotion activity, change in behaviour and physiological activity, gastrointestinal disturbances appearance, sensory nervous system responses or other abnormalities in treated animals with the highest oral dose of 5000 mg/kg bw after 28 days of observation. The effect of aqueous extract AE on the body weight variation of the treated rats was significantly remarkable ($p < 0.05$) since Wistar rats having received aqueous extract AE gained body weight compared to negative control group (Fig.1). The progressive increase in body weight during the period of treatment may indicate the improvement of the nutritional state of animals and in some case, it might be attributed to the appetite stimulation of the extract on the treated animals (Pieme *et al.*, 2006; Ogonnia *et al.*, 2009).

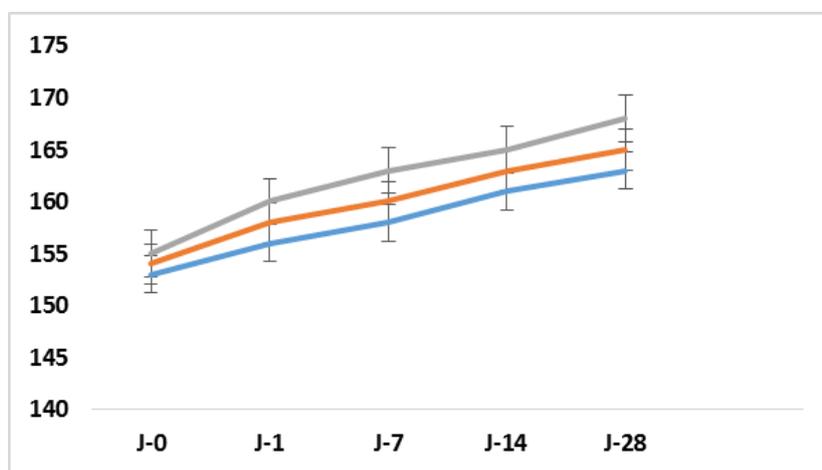


Figure. 1: Variation of body weight of treated animals in acute toxicity test with the oral doses of 1000 (red line) and 5000 (brown line) mg/kg bw, Blue line: negative control.

The extract did not induce any death of treated animals after 28 days of observation at the highest oral dose of 5000 mg/kg bw. Therefore, the LD₅₀ of the extract was estimated to be greater than 5000 mg/kg bw. Substances that present LD₅₀ higher than 2000 or 5000 mg/kg bw via oral route, may be considered as practically non-toxic and regarded as being safe and

well tolerated (Kennedy *et al.*, 1986; OECD, 2001). Other authors suggest that chemical substance with LD₅₀ within the range 5000-15.000 mg/kg bw is considered as practically non-toxic (Loomis and Hayes, 1996). This finding suggested that aqueous extract AE of *C. sanguinolenta* root bark is practically non-toxic by oral route.

3.6. Sub-acute toxicity of aqueous extract AE of *C. sanguinolenta* root bark

In this test, it was observed that the animals fed the aqueous extract from *C. sanguinolenta* root bark AE at all administered daily oral doses were healthy. No unusual changes in behaviour pattern, salivation, diarrhoea, lethargy, sleep, coma, locomotion activity as well as no ataxis convulsions, tremors, aggressiveness, respiratory and circulatory problems and no other signs of intoxication was observed during the 28-Day period of observation. All parameters used for evaluation toxicity were found to be normal in treated animals compared to untreated. Moreover, no change in fur coat, eyes and mucous membrane was observed. Animals which have received extracts at all tested oral doses gained body weight compared to the negative control group for the same reasons evoked above. No death of animal was recorded at all daily administered oral doses. These parameters of toxicity were not also observed in acute toxicity (Narayan and Mittal, 2015; Zahi *et al.*, 2015).

2.3. Effects of aqueous extract AE of *C. sanguinolenta* root bark on some haematological parameters

Results presented in Table 2 revealed that the haemoglobin and red blood cells (RBC) concentrations of treated rat groups were significantly increased compared to untreated group.

This may be due to the increased absorption of iron and copper or to the immunopotentiating effect of the extract as also previously reported for some medicinal plant extracts (Ameyaw and Owusu-Ansah, 1998) (Table 2).

The concentration of haematocrit did not show significant change compared to negative control group ($p > 0.05$) while it was observed significant increase of WBC animals compared to untreated group ($p < 0.001$), but its concentration remained in the normal physiological ranges (Table 2).

A significant decrease of platelets level was observed in treated animals compared to untreated suggesting that *C. sanguinolenta* aqueous extract AE could precipitate

thrombocytopenia which is the presence of low platelets level in the circulation system as also reported for other medicinal plant extracts (Adedapo et al., 2008). Moreover, with a decrease of this parameter count, there is an increase risk of bleeding (Slichter, 2004). Taken together, this finding suggested that aqueous extract of *C. sanguinolenta* root bark exerts an anti-haematopoiesis activity as also previously reported by Mukinda and Eagles (2010) for aqueous extract of *Polygala fruticosa* leaves.

Table. 2. Effects of aqueous extract AE of *C. sanguinolenta* root bark on the concentrations of hematological parameters

Parameters	Negative control	AE: 5000 mg/kg bw	Reference values
RBC ($\times 10^6 \mu\text{L}^{-1}$)	8.3 ± 0.4	9.4 ± 0.5	7.6-10.29
Hemoglobin (g/dL)	16.1 ± 0.3	17.6 ± 0.4	15-18.2
Haematocrit (%)	48.5 ± 0.3	49.1 ± 0.2	40.7-50
Platelets ($\times 10^3 \mu\text{L}^{-1}$)	1281.0 ± 0.6	1268.5 ± 0.2	995-1713
WBC ($\times 10^3 \mu\text{L}^{-1}$)	16.7 ± 0.3	17.9 ± 0.5	6.6-20.5
Neutrophils (%)	20.2 ± 0.3	22.8 ± 0.3	3-24.7
Basophils (%)	0.0	0.0	0.0
Eosinophils (%)	1.3 ± 0.1	1.5 ± 0.3	0-2
Lymphocytes (%)	89.2 ± 1.6	90.3 ± 0.1	58.8-94
Monocytes (%)	2.8 ± 1.1	3.1 ± 1.2	0-4
Segmented leucocytes s (%)	17.6 ± 0.6	22.2 ± 2.2	-

RBC ; red blood cells, WBC : white blood cells, AE: aqueous extract of *C. sanguinolenta* root bark (Nsaka et al., 2012).

The decrease of platelets level in circulatory system of treated animals caused by aqueous extract AE of *C. sanguinolenta* root bark supported its anticoagulant property also reported for other medicinal plant extracts (Adedapo et al., 2008; Li et al., 2010; Mukinda and Eagles, 2010; Gandhare et al., 2013).

The remaining evaluated haematological parameters such as neutrophils, eosinophils, lymphocytes and segmented leucocytes showed significant increase in treated animals according to the case, with no significant difference compared to control group ($p > 0.05$). In general the reported concentrations of these selected haematological parameters remained in physiological ranges throughout the treatment period (28 days) (Table 2) (Nsaka et al., 2012; Cimanga et al., 2015).

2.4. Effects of aqueous extract AE of *C. sanguinolenta* root bark AE on biochemical parameters

Table 3 shows the effects of the oral administration of the aqueous extract AE (decoction) of *C. sanguinolenta* root bark on the concentration levels of biochemical parameters of treated Wistar rats. Results indicated that the oral administration of aqueous extract AE of *C. sanguinolenta* root bark at the highest oral dose of 5000 mg/kg bw in acute toxicity induced remarkably significant decrease of plasma glucose level in treated animals compared to that seen in untreated animals ($p < 0.05$) especially at the highest dose of 5000 mg/kg bw. This decrease may be due probably to its hypoglycemic properties as also previously reported for other medicinal plant extracts (Ogbonnia et al, 2008; Kripa et al., 2011. Nsaka et al., 2013; Cimanga et al., 2015) and gave credence to the use of this extract AE as a hypoglycemic agent.

Alanine transferase (ALAT) and aspartate transferase (ASAT) are two liver enzymes known as good indicators of hepatic and renal functions and as biomarkers predicting possible toxicity of liver and kidney (Mukinda and Eagles, 2010; Kripa et al, 2011). AST is present in a wide variety of tissues including heart, kidney, skeletal muscle and liver whereas ALT is primarily localized in the liver (Kripa et al., 2001). The analysis of these parameters is important since there are several reports of liver and kidneys toxicity related to the use of phytotherapeutic products (Ozolua et al., 2009; Kripa et al., 2011). Also, the elevation of concentrations of the both transaminases in the blood indicates the damage to parenchymal liver cells (Mukinda et al., 2010).

Results reported here indicated that there was slight increase of the concentrations of these both enzymes, but this did not show significant difference compared to the negative control ($p > 0.05$). Based on this finding, it was suggested that hepatocytes of the treated rats were not damaged, the hepatic and renal functions of the treated animals were maintained safe as this effect can be also considered for other organs since the administered extract doses not.

Table. 3. Effects of the aqueous extract AE of *C. sanguinolenta* root bark AE on the concentrations of some biochemical parameters at oral dose 5000 mg/kg body weight.

Parameters	Negative Control	AE: 5000 mg/kg bw
Glucose (mg/dL)	85.5 ± 0.4	74.9 ± 1.4
Creatinine (mg/dL)	0.85 ± 0.03	0.86 ± 0.08
AST (UI/L)	176.6 ± 0.3	178.9 ± 0.2
ALT (UI/L)	51.2 ± 1.2	49.9 ± 0.2
Total cholesterol (mg/dL)	102.5 ± 1.3	101.9 ± 1.2
Triglycerides (mg/dL)	44.7 ± 0.8	43.9 ± 0.5
Total bilirubin (mg/dL)	0.6 ± 0.1	0.5 ± 0.3
Direct bilirubin (mg/dL)	0.2 ± 0.0	0.2 ± 0.0
Total Proteins (g/dL)	7.9 ± 0.3	8.2 ± 1.1
Albumin (g/dL)	3.2 ± 0.5	3.4 ± 0.3
ALP (IU/L)	143.4 ± 1.0	144.3 ± 0.4
HDL-cholesterol (mg/dL)	60.3 ± 0.3	62.6 ± 0.8
LDL-cholesterol (mg/dL)	34.5 ± 1.1	31.2 ± 0.3
Uric acid (mg/dL)	1.81 ± 0.8	1.96 ± 0.5
SGOT (UI/L)	117.3 ± 0.6	119.4 ± 0.2
SGPT (UI/L)	28.7 ± 0.3	27.3 ± 1.2
Urea (mmol/L)	6.1 ± 0.8	6.9 ± 0.6

AST : aspartate transferase, ALT : alanine transferase, ALP : alkaline phosphate, HDL : high-density lipoproteins, LDL : low-density lipoproteins, SGOT : serum glutamooxalate transferase, SGPT : serum glutamopyruvate transferase, AE: aqueous extract of *C. sanguinolenta* root bark.

possess significant deleterious effects in treated animals as also previously reported for other medicinal plant extracts in treated animals (Pieme et al., 2006; Ogbonnia et al., 2010; Akharainyi et al., 2012; Nsaka et al., 2013; Cimanga et al., 2015). In addition, it implied that the extract might not have caused any toxic effect on the liver and heart tissues of treated animals.

Creatinine, SGPT and SGOT are also considered as hepatic biomarker enzymes. It was observed an insignificant difference between the treated group and negative control group compared ($p > 0.05$), and suggested that aqueous extract of *C. sanguinolenta* root bark did possess not significant deleterious effect on hepato-renal functions or against the function of liver and kidney of treated animals (Kripa et al., 2011; Rajasekaran and Kannabiran, 2012). The aqueous extract did not interfere with the renal capacity to excrete the metabolite as also reported by Kripa et al., (2011). Results suggested that aqueous extract AE of *C. sanguinolenta* root bark did possess significant deleterious effect on hepato-renal functions or against the function of liver and kidney of treated animals (Kripa et al., 2011; Rajasekaran

and Kannabiran, 2012). As any damage was observed on these vital organs, it may be concluded that the administered aqueous extract did not induce significant observable toxicity on these two vital organs.

Cholesterol, LDL and triglycerides ($p < 0.05$) in treated rat groups showed slight decrease compared to untreated rat groups which might be attributed to the presence of hypolipidemic substances in aqueous extract AE of *C. sanguinolenta* root bark conferring its hypolipidemic properties (Ogbonnia et al., 2010), and in some times to the increase of the secretion of thyroid hormones T3 and T4 (Arijo et al., 2005) and slight increase of HDL (anti-atherogenic agent). Thus, the decrease of LDL and increase of HDL concentrations are of great significance in cardiovascular diseases management. From this, aqueous extract AE of *C. sanguinolenta* root bark has beneficial effects in the prevention and reducing of these diseases and correlated risk factors which contribute to the death of mainly diabetic patients (Ogbonnia et al., 2008; Akpanabiattu et al., 2013).

Albumin is a protein produced in the liver and have high concentration in plasma. Its decrease in serum may arise from liver and kidney diseases (Lima et al., 2009). Fortunately, its level in treated animals compared to untreated rat groups did not show statistically significant difference ($p > 0.05$). In addition, there was not significant change observed in the concentration of the total and direct bilirubin in treated animals compared to control groups ($p > 0.05$) suggesting that jaundice could not be resulted in the intake of this aqueous extract AE of *C. sanguinolenta* root bark as also reported by Coolborn et al., (2012) for ethanol extract of *Spathodea campanulata* leaf. The total proteins level significantly increased in treated rats compared to untreated group ($p < 0.05$) suggesting a supplement apport of an exterior supply of this element.

A significantly increase of urea concentration at in treated groups compared to untreated groups ($p < 0.01$) was observed, but this was not found as a sign of insufficiency renal because its concentration level remained within the normal physiological ranges (2.5– 7.5 mmol/L). As urea production in mammals occurs specially in liver, its concentration level could also be used as an indicator of hepatic function (Arijo et al., 2005). Thus, our results more confirmed good maintenance of hepatic and renal functions of treated animals as already demonstrated above with the concentration of other biomarkers for these vital organs. Together, the normal values of liver and kidney biomarkers including creatinine, urea, uric acid, SGPT and SGOPT suggested that aqueous extract AE of *C. sanguinolenta* root bark did

not produce any sort of disturbance in hepatic and renal functions, and hence was safe on its chronic use in treatment of various ailments, as also previously reported for various medicinal plant extracts (Saha et al., 2011; Rajasekaran and Kannabiran, 2012; Akpanabiattu et al, 2013; Gandhare et al., 2013).

Serum ALP is a sensitive detector for intrahepatic and extrahepatic bile obstruction. From the obtained results, no significant difference in the concentration of ALP in treated rat groups compared to untreated group was recorded ($p > 0.05$) (Table 3). As the presence of infiltrative diseases of the liver and all bones diseases is associated with osteoplastic activity (Vasudevan and Sreekumari (2005), it is likely that the highest oral dose used in this study for the aqueous extract *C. sanguinolenta* root bark leaves did not abnormally interfere with the calcification or metabolic activities involving the liver. This finding is in good agreement with Pieme et al. (2006) and Eden and Usoh (2009) concerning the effect of other plant extracts on ALP concentration in treated animals.

In general, all reported concentrations of haematological and biochemical parameters evaluated in the present study were within the physiological ranges (Barry et al., 1995; Feldamn et al., 1997; Nsaka et al., 2012; Cimanga et al., 2015).

Table 4 shows the effect of the aqueous extract of *C. sanguinolenta* root bark on some electrolytes. Results revealed that the administration of the extract at all administered oral doses of 1000 and 5000 mg/kg bw induced significant increase of calcium, chloride, iron, potassium and sodium in treated animals compared to untreated animals ($p < 0.05$). This increase had shown significant difference between both groups ($p < 0.01$) mainly remarkable at both administered oral doses (Table 4).

Table. 4. Effects of the aqueous extract AE of *C. sanguinolenta* root bark on the concentration levels on some electrolytes (mg/dL) in Wistar rats in acute toxicity.

Electrolytes	N. control	AE: 1000 mg/kg bw	AE: 5000 mg/kg bw
Calcium	98 ± 0.2	101.6 ± 0.2	104.8 ± 0.1
Chloride	72.8 ± 0.4	74.7 ± 0.2	78.9 ± 0.4
Inorganic phosphorus	4.0 ± 0.7	3.8 ± 0.2	4.3 ± 0.3
Iron	7.5 ± 0.1	8.9 ± 0.4	9.4 ± 0.5
Potassium	70.8 ± 0.1	74.2 ± 0.5	77.9 ± 0.4
Sodium	74.5 ± 0.4	77.3 ± 1.2	82.2 ± 1.4

N. control: negative control, AE: aqueous extract of *C. sanguinolenta* root bark

For the effect of aqueous extract AE on organ weight, a significant difference was only observed in the testicles weight in treated animals compared to untreated animals ($p < 0.05$) at all administered oral doses. For other organs, no significant difference in treated animals compared to untreated was observed ($p > 0.05$). But in general, it was observed that aqueous extract AE of *C. sanguinolenta* root bark has no significant effect on these animal organs and it did not detrimentally affect the weight, organ-to-body weight ratio and there was not change in the colour of various organs of treated animals compared to negative control in acute toxicity (Table 5). However, slight changes in the various organs specially heart, kidney, lung pancreas spleen an ovaries occurred only in animals that received the highest oral dose of 5000 mg/kg bw, but did not show significant difference compared to negative control group ($p > 0.05$).

Table. 5. Effects of aqueous extract AE of *C. sanguinolenta* root bark AE on the organ weights (g/kg) of Wistar rats.

Organs	Negative control	AE:1000 mg/kg bw	AE: 5000 mg/kg bw
Brain	3.71 ± 0.11	3.72 ± 0.36	3.75 ± 0.48
Heart	0.94 ± 0.40	0.97 ± 0.25	0.99 ± 0.42
Kidneys	2.34 ± 0.12	2.36 ± 0.44	2.38 ± 0.21
Lungs	3.01 ± 0.71	3.07 ± 0.42	3.09 ± 0.40
Pancreas	1.57 ± 0.43	1.58 ± 0.20	1.61 ± 0.73
Spleen	0.71 ± 0.52	0.73 ± 0.22	0.75 ± 0.72
Testicles	9.12 ± 0.42	9.24 ± 0.73	9.26 ± 0.37
Ovaries	0.24 ± 0.37	0.26 ± 0.11	0.29 ± 0.04

See Table 4.

They showed normal architecture. Our results are in good agreement with other studies (Kripa et al., 2010; Pillai et al., 2011).

2.5. Histopathological analysis

Histological examination revealed no changes in any vital organ of treated animals compared to untreated animals. The macroscopic and histological analysis of the organs of treated animals with the highest oral dose of 5000 mg/kg bw of the aqueous extract of *C. sanguinolenta* root bark did not show any changes in colour compared to control group rat's organs. It revealed no anatomical and/or structural changes in major organs as also reported for other medicinal plant extracts (Saha et al., 2011; Coolborn et al., 2012; Mafioleti et al., 2013). No detectable abnormalities such as hypertrophy of organs were also observed by pathological examination of the tissues. No alterations in cell culture or unfavourable effects were seen in the microscopic examination of the internal organs using multiple magnification

powers. No pathologies were recorded in the histological sections of the vital organs such as heart, spleen, kidney, liver and lung. Histopathological analysis of these vital organs in the negative control group and treated Wistar rats showed normal architecture and absence of any gross pathological lesions. Heart, lung, brain and spleen of the treated rats also did not demonstrate significant changes in morphology and were comparable to negative control group, indicating the protective effect of AE extract of *C. sanguinolenta* root bark on these tissues. In addition, stomach parts of treated rats did not show the development of ulcerative spots.

The present study provided evidence for the total safety and tolerability profile of the aqueous extract AE of *C. sanguinolenta* root bark suggesting its safe use in single oral dose treatment as well as for long term use for the treatment of various ailments, without producing any significant toxic effects.

4. CONCLUSION

This is the first report of the amoebicidal activity of aqueous, methanol 80% and total alkaloids extracts, and some isolated indoquinoline alkaloids of *C. sanguinolenta* root bark, and acute and subacute toxicity of aqueous extract as well as its effects on haematological and biochemical parameters of Wistar rats. This study provides valuable scientific data on amoebicidal activity of *C. sanguinolenta* root bark attributed to its alkaloids content. Acute and sub-acute oral toxicity profile of aqueous extract of that should be very useful for any future *in vivo* and clinical trials of this medicinal plant part. The extract was considered as safe and well tolerated in animals without visible toxic effects and did not induced mortality in animals. It was found to have no significant influence on the concentrations of biochemical and haematological parameters and can be considered as a non-source of any pathologic disease. It caused significant increase or decrease of some electrolytes in treated animals and did not significantly modify the weight of some vital organs of treated animals. It did not cause or produce severe toxicological effects on selected body organs in acute and sub-acute toxicity. Thus, aqueous extract AE from *C. sanguinolenta* root bark can be considered practically non-toxic *per os*. These reported results constitute a irrefutable scientific base supporting and justifying the traditional use of the studied plant part of *C. sanguinolenta* for the treatment of dysentery (amoebiasis) and at some extents, diarrhoea in traditional medicine in Democratic Republic of Congo and other African countries without significant side effects in human.

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