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Antiprotozoal and cytotoxic activities, and the acute toxicity of extracts from of *Brucea sumatrana* Roxb. (Simaroubaceae) leaves collected in Mai-Ndombe, Democratic Republic of Congo

Cimanga R. Kanyanga[1,3]*, Tshodi M. Ehata[1], Nsaka S. Lumpu[1], Kikweta C. Munduku[1], Mbenza A. Phuati[1], Kambu O. Kabangu[1], Cos, P.[2], Maes, L.[2], Apers, S.[3], Vlietinck, A. J. and Pieters, L.[3]

[1]Faculty of Pharmaceutical Sciences, University of Kinshasa, P.O. Box 212, Kinshasa XI, Democratic Republic of Congo.
[2]Faculty of Pharmaceutical Sciences, Biomedical and Veterinary Sciences, Laboratory of Microbiology, Parasitology and Hygiene, University of Antwerp, Universisteitsplein 1, B-2610 Antwerp, Belgium.
[3]Department of Pharmaceutical Sciences, Laboratory of Natural Products and Food-Research and Analysis (NatuRa), University of Antwerp, Universiteitsplein 1, B-2610, Antwerp, Belgium.

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Results from the *in vitro* evaluation of the antiprotozoal activity of the aqueous extract, the 80% methanol extract and its fractions from the leaves of *Brucea sumatrana* against *Trypanosoma brucei brucei*, *Trypanosoma cruzi*, *Lesihmania infantum*, the multidrug-resistant K1 and chloroquine-sensitive NF54 strains of *Plasmodium falciparum* indicated that all samples from the leaves extract presented interesting antiprotozoal activity at different extents. The 80% methanol extract, its chloroform acid, petroleum ether and 80% methanol soluble fractions and the aqueous extract exhibited strong activity against *Trypanosoma b. brucei*, *T. cruzi*, *L. infantum* and the multidrug-resistant K1 strain of *P. falciparum* with IC_{50} values from < 0.25 to 4.35 µg/ml as well as against chloroquine-sensitive NF54 strain of *P. falciparum* with IC_{50} values ranging from < 0.02 to 2.0.4 µg/ml. Most samples were cytotoxic against MRC-5 cell lines (0.2 < cytotoxic concentration 50 (CC_{50}) < 24.5 µg/ml) and showed good selective effect against all tested parasites. In acute toxicity, the aqueous extract was found to be non-toxic and its LD_{50} was estimated to be greater than 5 g/kg. In addition, it did not significantly modify the concentration levels of some evaluated biochemical and hematological parameters in rats. These results constitute a scientific validation supporting and justifying the traditional use of the leaves of *B. sumatrana* for the treatment of malaria, sleeping sickness and at some extent Chaga disease.

Key words: *Brucea sumatrana*, Simaroubaceae, leaves, extracts, antiprotozoal activity, cytotoxic activity, acute toxicity.

INTRODUCTION

Brucea sumatrana Roxb. (synonyms: *Brucea amarissima* Desv. ex Gomes, *Brucea javanica* (L.) Merr., Gonusamarissimus Lour or Loussaamarissma O. Ktze) (Simaroubaceae) is a medicinal plant mainly growing in some Asian countries, such as Cambodia, China, Indonesia and Thailand. It is also found in Panama

(South America). In these countries, the fruit of this medicinal plant is used for the treatment of various ailments such as cancer, malaria and amoebic dysentery (O'Neill et al., 1987; Wright et al., 1988).

Different biological activities of extracts and quassinoids isolated the Asian *B. javanica* fruits including anti-plasmodial (O'Neill et al., 1985, 1987; Pavanand et al., 1986), antileukemic (Lee et al., 1979), antiamoebic (Wright et al., 1988), antiprotozoal (Wright et al., 1993; Bawm et al., 2008), antinematodal (Alen et al., 2000), antidiarrhoeal, (Sawangjaroen and Sawangjaroen, 2005), antibabesial (Subeki et al., 2007) activities were previously reported. This plant species is also found in some African countries and its seeds, leaves and stem bark are used for treatment of various ailments among them protozoal diseases such as malaria, amoebic dysentery and sleeping sickness (Biruniya, 1993; Ngoma and Bikengeli, 1993; Neuwinger, 2000).

In Democratic Republic of Congo (DR Congo), to treat malaria, 4 or 5 seeds are chewed 2 to 3 times per day for the treatment of malaria crisis, while an aqueous decoction of leaves or stem bark is drunk three times/day until the disappearance of fever (Biruniya, 1993). During our ethnopharmacological and ethnobotanical studies conducted in Mai-Ndombe, Bas-Congo province of Democratic republic of Congo (RD Congo) near traditional healers about their knowledge to treat parasitic diseases, the leaves of *B. sumatrana* were frequently cited as starting plant materials used to prepare traditional remedies to treat malaria, amoebiasis and sleeping sickness or human african Trypanosomiasis (HAT) (Musuyu Muganza et al., 2006).

On the basis of the aforementioned ethnopharmacological information and the lack of an investigation conducted in this field, it was decided to evaluate *in vitro* the antiprotozoal activity of aqueous extract, 80% methanol extract and its fractions from leaves of *B. sumatrana* against *T. brucei brucei*, *T. cruzi*, *L. infantum*, the multi-resistant K1 and chloroquine-sensitive NF54 strains of *P. falciparum*. The potential cytotoxic effects against MRC-5 cell line (human lung fibroblasts) of all samples as well as the acute toxicity of the aqueous extract which is the used traditional preparation were also assessed.

MATERIALS AND METHODS

Reagents

Methanol from Fischer Scientific (UK) was of high performance liquid chromatography (HPLC) grade. Chloroform and petroleum ether of HPLC grade were obtained from Across Organics (USA). Distilled water was used for the preparation of an aqueous decoction.

Plants

Leaves of *B. Sumatrana* Roxb. (Simaroubaceae) were collected in the district Mai-Ndombe's province Bandundu in DR Congo in December, 2009. The plant was identified by Mr. Bavukinina of the Institut de Recherches en Sciences de la Santé (I.R.S.S.) of Kinshasa, DR Congo. A voucher specimen (B 20122009BSL) was deposited in the herbarium of this institute. Leaves were dried at room temperature and reduced to powder.

Preparation of crude extracts, fractions and subfractions

A 150 g of dried powdered leaves of *B. sumatrana* were submitted to a Soxhlet extraction with 80% methanol (500 ml) for 2 h. The extractive solvent was evaporated *in vacuum* yielding corresponding dried 80% methanol extract denoted as ME-1 (12.53 g). 5 g of this dried 80% methanol extract were dissolved in 100 ml distilled water and fractionated according to the Mitscher's procedure (Figure 1) (Mitscher et al., 1978). The obtained fractions and sub-fractions were treated as described, yielding the corresponding dried extracts denoted as ME-1.1 to ME-1.6. On the other hand, 20 g of the powdered plant material were mixed with 150 ml distilled water and boiled at 100°C for 15 min. After cooling and filtration, the filtrate was treated as described, yielding the dried aqueous extract denoted as AE-1 (5.89 g). The total alkaloids extract (ME-2, 3.06 g) of plant part was obtained using the acid/base procedure described in the literature (Harborne, 1998).

Phytochemical screening

This study was performed by thin layer chromatography (TLC) on precoated silica gel plates F_{254} (thickness later 0.25, mm, Merck, Germany) using different reagents and mobile phases described in the literature to identify major chemical groups such as alkaloids, anthraquinones, coumarins, flavonoids, terpenes and steroids. Hydrochloride acid 0.2 N (aqueous solution of the sample anthocyanins. Froth test and Stiasny's reagent were used to detect saponins and tannins, respectively (Harborne, 1998).

Evaluation of biological activities

The antiprotozoal activity of all the samples obtained from the leaves of *B. sumatrana* was tested *in vitro* against *T. b. brucei*, *T. cruzi* and *L. infantum* from the laboratory of Microbiology, Parasitology and Hygiene of Prof. L. Maes,

*Corresponding author. E-mail: kanyanga.cimanga@ua.ac.be, rkcimanga@gmail.com. Tel. +32 3 265 27 33. Fax: +32 3 265 27 09.

University of Antwerp, Belgium according to the respective procedures previously described by Kuypers et al. (2006) and Tshodi et al. (2012). The antiplasmodial activity against chloroquine and pyrimethamine-resistant K1 strain of *P. falciparum* obtained from the same laboratory, and chloroquine-sensitive NF54 from Tropical Medicine Institute of Antwerp, Belgium, was evaluated according to the lactate deshydrogenase procedure previously described by Makler et al. (1993) with some modifications (Kuypers et al., 2006). The cytotoxic effect against MRC-5 cell lines (human lung fibroblasts) was assessed using the MTT assay previously described by Kuypers et al. (2006). The selective index (SI) as a ratio cytotoxic concentration 50/inhibitory concentration 50 (CC_{50}/IC_{50}) was calculated for each sample to appreciate its effect against the tested parasites and MRC-5 cell lines. SI < 1 indicated a selective effect against the cell line while SI > 1 indicated a selective action against the tested parasite (Camacho et al., 2003; Tshodi et al., 2012). For the purpose of this *in vitro* antiprotozoal screening study, the following criteria were adopted: IC_{50} ≤ 5 µg/ml: strong activity; 5 < IC_{50} ≤ 10 µg/ml: good activity; 10 < IC_{50} ≤ 20 µg/ml: moderate activity; 20 < IC_{50} ≤ 40 µg/ml: weak activity; IC_{50} > 40 µg/ml: inactive.

Acute toxicity

In the present study, only the acute toxicity of the aqueous extract was investigated in Wistar rats according to the procedure described by the Organization for Economic Co–operation and Development (OECD) guideline for testing chemicals, TG420 (OECD, 2001). Briefly, groups I (2 rats) orally received 5 ml distilled water and constituted the negative control groups. After, fifteen Wistar rats of either sex, (body weight: 130 to 150 g, aged 8 to 10 weeks) were divided in three groups of 5 rats each noted as groups II, III and IV which orally received a single oral dose of 500, 1000 and 5000 mg/kg body weight (bw), respectively. The animals were observed for toxic symptoms continuously for the first 4 h dosing and were daily weighted. Finally, all animals were then maintained in daily observation and the number of toxic effects and survivor were recorded for 14 days and further 28 days.

Biochemical and hematological parameters analysis

Blood from rats having received 5 g/kg of the aqueous extract (AE-1) was collected from tail vein on Day 28 for analysis. For biochemical parameters, blood was centrifuged at 4000 g for 5 min to obtain plasma, which was stored at –20°C. Glucose, creatinin, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), serum glutamo pyruvate transferase (SGPT), serum glutamo oxalate transferase (SGOT), uric acid, total cholesterol, triglycerides, high-density lipoproteins (HDL), low-density lipoproteins (LDL), total and direct bilirubin were quantified using Architect (Abottâ) automation with Boehringen Ingelheim biochemical kits. Total proteins were estimated using Biuret's method. Hematological parameters analysis was carried out using an automatic hematological analyzer (Coulter STK, Beckam) with appropriated kits. The differential leucocyte count was performed with an optical microscopy after staining and, in each case, 100 cells were counted.

RESULTS AND DISCUSSION

Antiprotozoal and cytotoxic effects

Table 1 represents the antiprotozoal activity and the cytotoxicity of samples from the leaves of *B. sumatrana*. All samples were tested *in vitro* against *T. b. brucei*, *T. cruzi*, *L. infantum*, chloroquine and pyrimethamine-resistant K1 while Table 2 represents the activity of the samples against chloroquine-sensitive NF54 strains of *P. falciparum*. The cytotoxic effects of all samples against MRC-5 cell lines (human lung fibroblasts) (Table 1) and the acute toxicity only of the aqueous extract as the used traditional preparation were also evaluated.

The obtained results indicated that all samples exhibited the evaluated biological activities at different extents. The 80% methanol extract (ME-1) exhibited good activity against *T. cruzi* (6.15 µg/ml) and weak activity against *L. infantum* (24 µg/ml), while it showed strong activity against *T. b. brucei* and the multidrug-resistant K1 strain of *P. falciparum* with IC_{50} values of 4.35 and < 0.25 µg/ml, respectively (Table 1). Interestingly, its chloroform soluble fraction (ME-1.1) rich in waxes, steroids and terpenes and its petroleum ether soluble subfraction (ME-1.2) rich in waxes and lipids had the same antiprotozoal spectra of activity and presented strong activity against all selected protozoa with IC_{50} values ranging from < 0.25 to 1.70 µg/ml (Table 1). The 80% methanol soluble subfraction (ME-1.3) rich in steroids and terpenes exhibited strong activity against *T. b. brucei* and *T. cruzi* with IC_{50} value < 0.25 µg/ml for both parasites, weak activity against chloroquine and pyrimethamine-resistant K1 strain of *P. falciparum,* and was inactive against *L. infantum* (Table1).

The aqueous acid soluble fraction (ME-1.4), its chloroform base soluble subfraction (ME-1.5) rich in alkaloids and aqueous alkaline soluble subfraction (ME-1.6) rich in phenolic compounds had also the same antiprotozoal spectra of activity. All samples displayed good activity

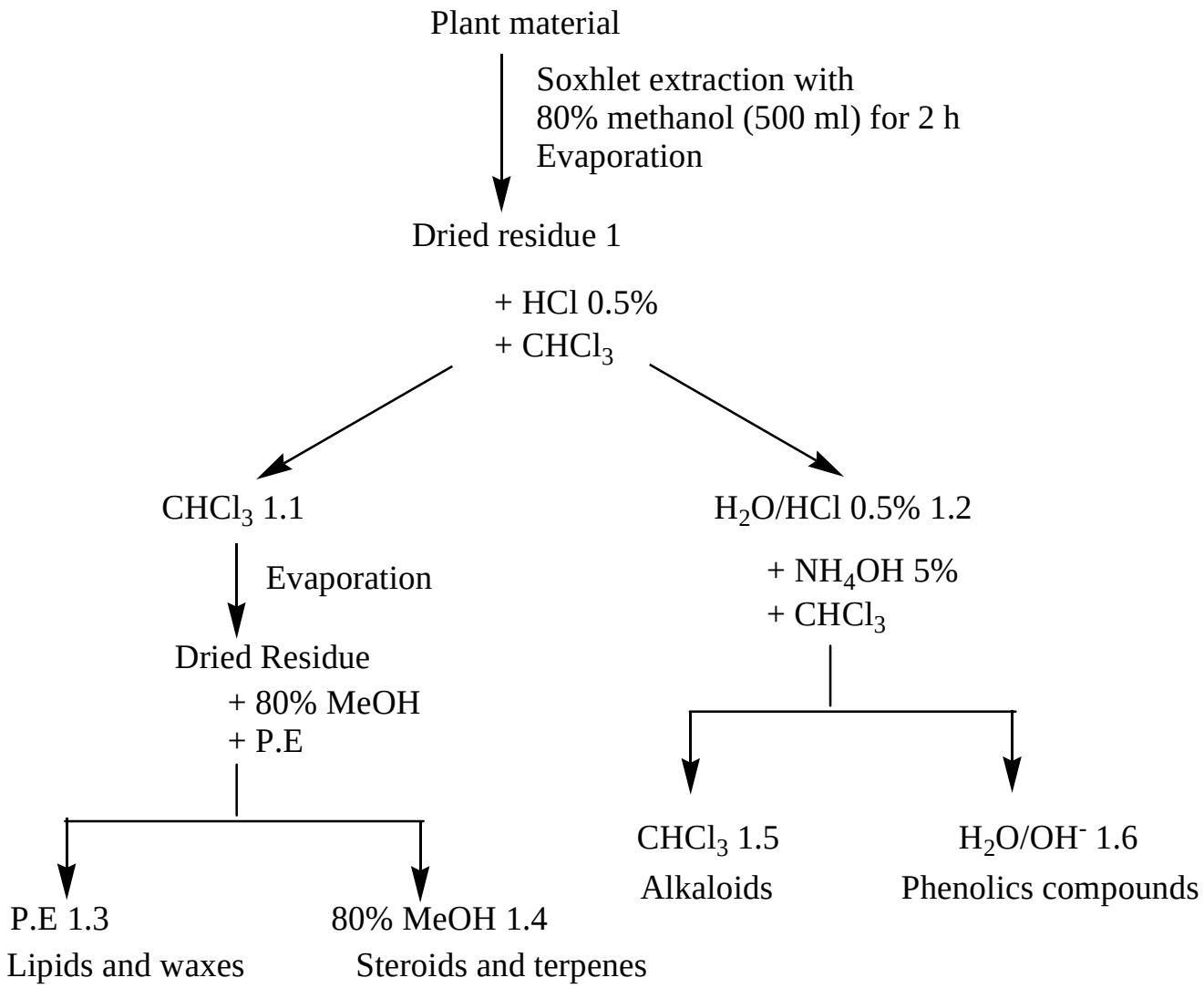

Figure 1. Fractionation of the 80% methanol extract.
Source: Mitcher et al. (1978).

against *T. b. brucei* and the multidrug-resistant K1 strain of *P. falciparum*, weak activity against *T. cruzi* and were inactive against *L. infantum* (Table 1). The aqueous extract (AE-1) which is the used traditional remedy exhibited strong activity against *T. b. brucei*, *T.cruzi* and the multidrug-resistant K1 strain of *P. falciparum* with IC_{50} values ranging from < 0.25 to 0.70 µg/ml (Table 1) and good activity against *L. infantum* (IC_{50} = 5.06 µg/ml). The total alkaloids extract (ME-2) showed good, moderate and weak activity against *T. b. brucei*, *T. cruzi*and *L.* infantum, respectively (Table 1). In addition, it however exhibited strong activity against the multidrug-resistant k1 strain of *P. falciparum* with IC_{50} value of 1.80 µg/ml.

All samples from *B. sumatrana* leaves exhibited strong activity against chloroquine-sensitive NF54 strain of *P. falciparum* with IC_{50} values from < 0.02 to 2.04 µg/ml. The most active samples were ME-1, ME-1.1, ME-1.4, ME-1.5, ME-2 and AE-1 inhibiting its growth with IC_{50} value < 0.25 µg/ml. In addition, ME-1.3 and ME-1.4 also showed strong activity against this *P. falciparum* strain with IC_{50} values of 1.04 and 1.55 µg/ml, respectively. In general, all samples showed strong antiplasmodial activity against this strain of *P. falciparum* (IC_{50} < 5 µg/ml) (Table 2).

With regards to the activity showed by the reference products, it is important to point out that ME-1.1, ME-1.2 and ME-1.5 exhibited higher activity ($0.25 \leq IC_{50} < 0.70$ µg/ml) than benznidazol against *T. cruzi* (IC_{50} = 2.65 µg/ml) whereas the antiplasmodial activity of ME-1, ME-1.1, ME-1.1 and AE-.1 (IC_{50} < 0.25 µg/ml) against the multidrug-resistant K1 strain of *P. falciparum* was

Table 1. Antiprotozoal and cytotoxic activities of samples from *Brucea sumatrana* leaves.

Code sample	MRC-5	*T. b. brucei*	*T. cruzi*	*L. infantum*	*P. falc*. K1
ME-1	2.34	4.35	6.15	24.00	< 0.25
ME-1.1	< 0.25	< 0.25	< 0.25	0.32	< 0.25
ME-1.4	< 0.25	< 0.25	0.31	1.70	< 0.25
ME-1.3	> 64	< 0.25	< 0.25	> 64	35.33
ME-1.4	34.24	12.35	27.57	> 64	10.00
ME-1.5	10.32	8.11	21.22	> 64	7.05
ME-1.6	24.05	10.04	25.06	> 64	8.15
ME-2	7.00	8.30	12.70	24.00	1.80
AE-1	0.46	0.57	0.70	5.06	< 0.25
Tamoxifen	10.5				
Metarsoprol		0.02			
Benznidazol			2.65		
Multeforine				3.56	
Chloroquine	> 64				0.18

ME-1 : 80% methanol extract, ME-1.1 : chloroform acid soluble fraction, ME-1.2 : petroleum ether soluble subfraction, ME-1.3 : methanol 80% soluble subfraction, ME-1.4 : aqueous acid soluble fraction, ME-1.5 : chloroform base soluble subfraction, ME-1.6 : aqueous alkaline soluble subfraction, ME-2 : totum alkaloids extract,AE-1 : aqueous (decoction) extract, *T.b. brucei: Trypanosome bruceibruce, T. cruzi: Trypanosome cruzi, l. infantum: leishmania infantum, P. falc. K1: Plasmodium falciparum K1.*

comparable to that of chloroquine used as an antimalarial reference product (IC_{50} = 0.18 µg/ml). In addition, samples ME-1, ME-1.1, ME-1.2 and AE-1 were the most cytotoxic samples and were 4.49, < 42, < 42 and 23 times more cytotoxic, respectively (CC_{50} = 2.34, < 0.25, < 0.25 and 0.46 µg/ml, respectively) than tamoxifen (CC_{50} = 10.5 µg/ml) (Table 1).

Cytotoxic effects of samples of *B. sumatrana* leaves against MRC-5 cell line

In the cytotoxic studies, it was observed that except ME-1.3 and ME-1.4 samples devoid of cytotoxic effect against MRC-5 cell line (CC_{50} > 64 and 34.24 µg/ml, respectively) since their CC_{50} were greater than 32 µg/ml, the remaining samples were cytotoxic against this cell line with CC_{50} values ranging from < 0.25 to 24.05 µg/ml, since they were lower than 32 µg/ml (Kuypers et al., 2006). By assessing their respective selectivity index (SI), it was observed that the activities of ME-1, ME-1.1, ME-1.2, MS-2 and AE-1 against *T. b. brucei*, *T. cruzi* and *L. infantum* were correlated to their cytotoxic effect against MRC-5 cell line, since their SI were lower than 1 (Camacho et al., 2003; Tshodi et al., 2012). They however presented good selective action against the multidrug-resistant K1 strain of *P. falciparum,* since their respective SI were greater than 1 (Camacho et al., 2003; Tshodi et al., 2012) (Table 3). ME-1.3 had the highest selectivity index against *T. b. brucei* and *T. cruzi* since its SI value was > 256. Its selective action against *L. infantum* and the strain K1 of *P. falciparum* was also appreciable (Table 3).

Against chloroquine-sensitive NF54 of *P. falciparum*, most samples showed good selective action. The best selective action was observed with ME-1.4 (SI = 1721), followed by ME-1.5 (SI = 516), ME-2 (SI = 350) and ME-1 (SI ≥ 117). The inhibitory effect of ME-1.2 on the growth of this parasite was correlated to its cytotoxic effect since it SI was lower than 1 (SI < 0.24) (Table 2) (Camacho et al., 2003; Tshodi et al., 2012).

Effects of the aqueous extract of *B. sumatrana* leaves on the concentration levels of haematological parameters

The hematological parameter profile is presented in Table 4. The reported results indicated that the oral administration of the aqueous extract of *B. sumatrana* leaves (AE-1) at the oral dose of 5 g/kg bw in acute toxicity, did not affect concentration levels of evaluated hematological parameters and no significant difference between the treated animals compared to untreated was deduced ($p > 0.005$). They remained all in normal limits and presented no sign of a particular pathologic state.

Table 2. Antiplasmodial activity of samples from *B. sumatrana* leaves against chloroquine-sensitive NF54 strain of *P. falciparum.*

Code sample	MRC-5	*P. falciparum. NF54*	*Selectivity index*
ME-1	2.34	< 0.02	> 117
ME-1.1	< 0.25	< 0.02	< 12.5
ME-1.4	< 0.25	1.04	< 0.24
ME-1.3	> 64	1.55	> 41.29
ME-1.4	34.24	< 0.02	> 1712
ME-1.5	10.32	< 0.02	> 516
ME-1.6	24.05	2.04	11.79
ME-2	7.00	< 0.02	> 350
AE-1	0.46	< 0.02	> 23
Chloroquine	> 64	0.15	> 426.67

ME-1 : 80% methanolextract, ME-1.1 : chloroform acid soluble fraction, ME-1.2 : petroleum ether soluble subfraction, ME-1.3 : methanol 80% soluble subfraction, ME-1.4 : aqueous acid soluble fraction, ME-1.5 : chloroform base soluble subfraction, ME-1.6 : aqueous alkaline soluble subfraction, ME-2 : totumalkaloidsextract,AE-1 : aqueous (decoction) extract, *T.b. brucei: Trypanosome bruceibruce, T. cruzi: Trypanosome cruzi, l. infantum: leishmania infantum, P. falc. K1: Plasmodium falciparum K1.*

Table 3. Selectivity index (SI= CC_{50}/IC_{50}) of samples from*Brucea sumatrana* leaves.

Code sample	MRC-5/*T.b.brucei*	MRC-5/*T. cruzi*	MRC-5/*L. infantum*	MRC-5/*P. falc.*K1
ME-1	0.53	0.38	0.10	> 9.36
ME-1.1	< 1	<1	< 0.80	< 1
ME-1.2	< 1	< 080	> 0.14	< 1
ME-1.3	> 256	> 256	ND	> 1.81
ME-1.4	2.77	1.24	ND	4.86
ME-1.5	1.27	0.48	ND	1.46
ME-1.6	2.40	0.96	ND	2.95
ME-2	0.84	0.55	0.29	3.89
AE-1	0.80	0.66	0.09	< 1.84
Chloroquine	> 64	-	-	> 355.55

ME-1 : 80% methanol extract, ME-1.1 : chloroform acid soluble fraction, ME-1.2 : petroleum ether soluble subfraction, ME-1.3 : methanol 80% soluble subfraction, ME-1.4 : aqueous acid soluble fraction, ME-1.5 : chloroform base soluble subfraction, ME-1.6 : aqueous alkaline soluble subfraction, ME-2 : totumalkaloidsextract,AE-1 : aqueous (decoction) extract, *T.b. brucei: Trypanosome bruceibruce, T. cruzi: Trypanosome cruzi, L. infantum: Leishmania infantum, P. falc. K1: Plasmodium falciparum K1.* ND: not determined because the tested sample was inactive.

Effects the aqueous extract of *B. sumatrana* leaves on the concentration levels of evaluated biochemical parameters

Table 5 shows the effects of the oral administration of the aqueous extract (decoction, AE-1) of *B. sumatrana* leaves on the concentration levels of some biochemical parameters of Wistar rats. The obtained results indicated that the oral administration of the extract at the highest oral dose of 5 g/kg bw in acute toxicity test produced significant decrease of the concentration level of glucose in treated groups compared to untreated groups ($p < 0.05$). This decrease may be due to the hypoglycaemic and antidiabetic properties of the extract as also previously reported for other plant extracts (Okoli et al., 2010; Luka et al., 2012).

Alanine amino transferase (ALAT) also called alanine transaminase (ALT) and aspartate transaminase (AST) also known as aspartate amino transferase (ASAT/AspAT/AAT) are two liver enzymes associated in the hepatocellular damages and thus considered as indicators of liver damages. ALAT is only specific for liver functions and ASAT is mostly found in the myocardium, skeletal muscle, kidneys and brain (Wasan et al., 2001; Crook et al., 2006). Although a slight decrease was observed, the results reported here indicated that

Table 4. Effects the aqueous extract of *B. sumatrana* leaves (AE-1) at oral dose of 5 g/kgbw on the concentration levels of haematological parameters.

Parameter	Negative control	*B. sumatrana*: 5g/kgbw	*Reference values*
RBC (×10^6 µME-1)	8.1 ± 0.8	8.4 ± 1.2	7.6-10.29
Hemoglobin (g/dl)	14.2 ± 0.2	16.4 ± 1.2	15-18.2
Hematocrit (%)	43.2 ± 0.1	47.2 ± 2.0	40.7-50
Platelets (×10^3 µME-1)	1421.0 ± 0.2	1404.2 ± 0.2	995-1713
WBC (x10^3 µME-1)	13.3 ± 0.3	16.0 ± 0.5	6.6-20.5
Neutrophils (%)	18.8 ± 0.7	23.2 ± 1.2	3-24.7
Basophils (%)	0.0	0.0	0.0
Eosinophils (%)	1.5 ± 0.1	1.5 ± 0.4	0-2
Lympocytes (%)	89.2 ± 1.1	88.3 ± 0.1	58.8-94
Monocytes (%)	3.4 ± 1.1	3.6 ± 1.2	0-4
Segmented leucocytes (%)	15.1 ± 0.6	19.3 ± 2.1	-

RBC; red blood cells, WBC : white blood cells

Table 5. Effects the aqueous extract of *B. sumatrana* leaves (AE-1) at oral dose of 5 g/kgbw on the concentration levels of biochemical parameters.

Parameters	Negative control	*B. sumatrana*: 5 g/kg bw
Glucose (mg/dl)	245.5 ± 0.4	206.3 ± 1.4
Creatinin (mg/dl)	0.90 ± 0.05	0.87 ± 0.02
AST (UI/L)	180.6 ± 0.3	178.2 ± 0.5
ALT (UI/L)	52.2 ± 2.2	53.5 ± 1.2
Totalcholesterol (mg/dl)	54.2 ± 1.1	53.3 ± 2.2
Triglycerides (mg/dl)	47.7 ± 1.8	46.3 ± 3.5
Total bilurbin (mg/dl)	0.6 ± 0.1	0.6 ± 0.1
Direct bilurbin (mg/dl)	0.2 ± 0.0	0.2 ± 0.0
Total proteins (g/dl)	8.0 ± 0.3	8.4 ± 1.1
Albumin (g/dl)	3.6 ± 0.5	3.5 ± 0.6
ALP (IU/L)	148.4 ± 1.6	146.3 ± 2.4
HDL (mg/dl)	65.3 ± 1.3	65.6 ± 1.3
LDL (mg/dl)	39.5 ± 2.1	38.5 ± 0.4
Uric acid (mg/dl)	1.8 ± 0.1	2.2 ± 0.5
SGOT (UI/L)	128.3 ± 1.6	126.4 ± 0.2
SGPT (UI/L)	32.7 ± 2.3	33.3 ± 1.2
Urea (mmol/L)	6.1 ± 0.8	6.6 ± 1.6

AST: aspartate transferase, ALT : alanine transferase, ALP : alkalinephospahte, HDL : hight-densitylipoproteins, LDL : low-densitylipoproteins, SGOT : serumglutamoxatetransferase, SGPT : serum glutamate transferase

concentration levels of these both enzymes in treated animals did not show a significant difference compared to the negative control ($p > 0.05$). This finding showed that the aqueous leaf extract at this tested oral dose may not cause liver, heart or kidney damages as also previously reported by Pieme et al. (2006) and Lima et al. (2009). Moreover, the hepatic function of these animals can be considered to be maintained (Arüjo et al., 2005). In addition, the concentration levels of creatinin and SGPT which did not show significant difference in treated rat groups compared to untreated rat groups ($p > 0.05$) well support this observation.

The slight decrease of the concentration levels of cholesterol and triglycerides in treated rat groups was not

significant ($p > 0.05$) (Table 5). This effect may be due to the hypolipidimic property of the aqueous leaves extract and to the increased secretion of thyroid hormones T3 and T4 (Arüjo et al., 2005). A slight decrease in concentrations of high density lipoprotein (HDL) and low density lipoprotein (LDL) in treated animals was also observed, but, it did not show significant difference compared to the control groups ($p > 0.05$). It can be considered as a consequence of the decrease of total cholesterol. These results suggest that the extract has some beneficial effects by reducing some risk factors related to cardiovascular diseases (Ameyaw and Owusu-Ansah, 1998).

Albumin is a protein with high concentration in plasma. Since it is produced in the liver, its decrease in serum may arise from liver and kidney diseases (Lima et al., 2009). Fortunately, a slight decrease of the concentration level of albumin was observed, but did not show significant difference compared to untreated groups ($p > 0.05$). In addition, although a slight decrease for the concentration levels of the total and direct bilirubin was observed, there was no significant difference of this biochemical parameter in treated animals compared to control groups ($p > 0.05$). The level of total proteins slightly increased ($p < 0.05$), suggesting an external supply. A slight increase of the concentration level of SGOT and SGPT in treated animals was observed. Nevertheless, it did not show significant difference compared to the control groups ($p > 0.05$) indicating that the heart and liver were not affected. No significant difference in the concentration level of ALP in treated rat groups compared to untreated groups was recorded although a slight decrease or increase in treated groups according to the case was observed ($p > 005$) (Table 5). As the presence of infiltrative diseases of the liver and all bones diseases is associated with osteoplastic activity, it is likely seen that the oral dose used in this study for the aqueous extract of *B. sumatrana* leaves did not abnormally interfere with the calcification or metabolic activities involving the liver. The intrahepatic and extrahepatic bile functions did not know an obstruction (Vasudevan and Sreekumari, 2005). This finding is in good agreement with results reported concerning the effect of other plant extracts on ALP concentration level in animals (Pieme et al., 2006; Eden and Usoh, 2009).

As urea production in mammals occurs specially in liver, its concentration level could also be used as an indicator of hepatic function. In our study, the urea concentration level significantly increased in treated groups compared to untreated groups ($p < 0.02$), but this observation was not found as a sign of insufficiency renal because its concentration level remained within the normal limits (2.5 to 7.5 mmol/L). Therefore, our results showed good hepatic function of treated animals for the aforementioned reasons (Arüjo et al., 2005).

In general, all concentration levels of biochemical and haematological parameters evaluated in the present study were within the normal ranges (Barry et al., 1995; Feldams et al., 1997).

Acute toxicity of the aqueous extract of *B. sumatrana* leaves in rats

Animals were treated with single oral doses of the aqueous extract (AE-1) (500, 1000 and 5000 mg/kg body weight, respectively). In this test, no sign of toxicity such as alteration of the locomotion activity and gastro-intestinal disturbances were observed. Rats received all tested oral doses significantly gained body weight compared to negative control groups. According to Pieme et al. (2006), the growth response effect could be considered as a result of increased food and water intake. On Day 21, one death (6.66%) was noted in the third group receiving 1000 mg/kg bw of the extract and on Day 22, 3 deaths (20%) were recorded in the fourth group having received 5000 mg/kg body weight of the extract. This last percentage of mortality is weaker than 50%. According to Kennedy et al. (1986), substances that present LD_{50} higher than 5 g/kg body weight via oral route, may be considered as practically non-toxic. Therefore, it may be suggested that the acute toxicity of *B. sumatrana* aqueous leaves extract is practically null via oral route. Therefore, the LD_{50} of the extract was estimated to be greater than 5000 mg/kg body weight. In addition, histopathological examination of vital organs of treated animals did not show any abnormality compared to untreated groups indicating that their state was well maintained at the administered oral dose.

On the other hand, the acute toxicity of leaves ethanol extract of *Brucea javanica* Merr. collected in Indonesia on mice was previously reported (Marissa et al., 2012). Results from this study indicated that the oral administration of the leaves ethanol extract at oral doses of 562.5, 1125, 2250 and 4500 mg/kg body weight, respectively, were unable to induce acute toxic effects in mice. But at the highest oral dose of 4500 mg/kg body weight, 26% mortality occurred after 14 days, and its DL_{50} was determined to be 1003.65 mg/kg body weight. This DL_{50} determined by these authors seems not to be correct because not only 26% of death observed at the highest oral dose of 4500 mg/kg bw was weaker than 50% of death, but also not percentage death was reported after the administration of 1125 and 2250 mg/kg bw, respectively, suggesting that these oral doses did not induce mortality of animals. Taking account of these important observations, the DL_{50} of the administered extract in this previous study must be greater than 4500 mg/kg bw and not 1003.65 mg/kg bw as reported. Interestingly, in this same investigation, it was reported

that vital organs and body average weights of treated animals did not show any difference compared to control groups. In addition, gross examination of the vital organs revealed no pathologic abnormality compared to control groups on the microscopic observation. These finding were in good agreement with our observations for the aqueous leaves extract tested in the present study. From these results of this study, these authors concluded that the extract can be considered to be slightly toxic since the damage caused by leaves ethanol extract is minor and not permanent considering the administered oral dose (Marissa et al., 2012).

The chemical composition of *B. sumatrana* leaves

Some plant parts of *B. javanica* growing in Asian regions were chemically previously investigated. Triterpenoids and quassinoids were isolated from the combined plant materials leaves, twigs and inflorescences. Flavonoids were also detected (Ismail et al., 2012) or isolated from the leaves (Dong et al., 2013). Results from our phytochemical screening conducted on *B. sumatrana* leaves collected in Mai-Ndombe, DR Congo, revealed the presence of alkaloids, steroids, terpenoids, flavonoids and tannins in the leaves. Anthraquinones, anthocyanins and coumarins were not detected in our experimental conditions. The presence mainly of alkaloids, flavonoids, steroids and terpenoids may largely contribute to the observed antiprotozoal activity because more chemicals belonging to these phytochemical groups present in other medicinal plant species had previously been reported to exhibit antiprotozoal activity at different extents (Wright et al., 1994; Camacho et al., 1998; Christensen and Kharazmi, 2001; Hoet et al., 2004: Bero et al., 2009; Bero and Quetin-Leclercq, 2011). Particularly, different terpenes belonging to the quassinoid groups mainly isolated from the seeds of *B. javanica* growing in Asian regions are known as active principles for various evaluated biological activities already mentioned earlier.

Conclusion

The present investigation has described for the first time the antiprotozoal and cytotoxic activities, and acute toxicity of extracts, fractions and sub-fractions from *B. sumatrana* leaves from RD Congo on a large spectrum of protozoa. The reported results showed that all samples possessed interesting *in vitro* antiprotozoal activity at different extents. The aqueous extract (AE-1), which is the used traditional preparation, was found to be non-toxic and did not affect the concentration levels of evaluated biochemical and hematological parameters, and can be considered as a sign of no appearance of pathologic abnormality. The reported results can partly justify and support the use of this plant part of *B. sumatrana* as raw material for the preparation of traditional remedy for the treatment of parasitic diseases such as malaria, sleeping sickness, leishmaniosis and in some extents American trypanosomiasis named Chagas disease, with no apparent toxic effects in patients. Further studies are in progress on the most active fractions, sub-fractions and the total alkaloids extract, leading to the isolation and structural elucidation of active constituents.

Conflict of interest

Authors declare that there are no conflicts of interests

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