

Study of Antiparasitic and Cytotoxicity of the Aqueous, the 80% Methanol Extract and Its Fractions, and the Acute Toxicity of the Aqueous Extract of *Brucea sumatrana* (Simaroubaceae) Leaves Collected in Mai-Ndombe, Democratic Republic of Congo

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Abstract

Results from the *in vitro* evaluation of the antiparasitaire activity of the aqueous extract, the 80% methanol extract and its fractions from the leaves of *Brucea sumatrana* against *Trypanosoma brucei brucei*, *T. cruzi*, *Leishmania infantum*, the multidrug-resistant K1 and chloroquine-sensitive NF54 strains of *Plasmodium falciparum* indicated that all samples from the leaves extract presented interesting antiparasitaire 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 IC₅₀ values from <0.25 to 4.35 µg/ml as well as against chloroquine-sensitive NF54 strain of *P. falciparum* with IC₅₀ values ranging from <0.02 to 2.04 µg/ml. Most samples were cytotoxic against MRC-5 cell lines (0.2 < cytotoxic concentration 50(CC₅₀) < 34.24 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 LD₅₀ was estimated to be greater than 5 g/kg. In addition, it did not significantly modify the concentration levels of some evaluated biochemical

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and hematological parameters in treated 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 Chagas disease.

Keywords

Brucea sumatrana, Simaroubaceae, Leaves, Extracts, Antiprotozoal Activity, Cytotoxic Activity, Acute Toxicity

1. Introduction

Brucea sumatrana Roxb. (synonyms: *B. amarissima* Desv. ex Gomes, *B. javanica* (L.) Merr., *Gonus amarissimus* Lour or *Loussa amarissima* 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 [1] [2].

Different biological activities of extracts and quassinoids isolated the Asian *Brucea javanica* fruits including anti-amoebic [2], antiplasmodial [1] [3] [4], antileukemic [5] antiprotozoal [6] [7], antinematodal [8], anti-diarrhoeal [9], antibabesial [10] activities were previously reported.

This plant species is also found in some African countries where it is named *Brucea sumatrana* Roxb. Its seeds, leaves and stem bark are used for treatment of various ailments among them parasitic diseases such as malaria, amoebic dysentery and sleeping sickness [11]-[13]. 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 [11]. During our ethnopharmacological and ethnobotanically studies conducted in Mai-Ndombe, Bandundu, province of DR Congo near traditional healers about their knowledge to treat parasitic diseases, the leaves of *Brucea sumatrana* were frequently cited as raw plant materials used to prepare traditional remedies to treat malaria, amoebiasis and sleeping sickness or Human African Trypanosomiasis (HAT) [14].

On the basis of the above ethnopharmacological information and the lacking of an investigation conducted in this field, it was decided to evaluate the *in vitro* antiprotozoal activity of aqueous extract, 80% methanol extract and its fractions from leaves of *Brucea sumatrana* against *Trypanosoma brucei brucei*, *T. cruzi*, *Leishmania infantum* and the multi-resistant K1 strain of *Plasmodium falciparum*. The potential cytotoxic effects against MRC-5 cell lines (human lung fibroblasts) of all samples as well as the acute toxicity of the aqueous extract which was the used traditional preparation were also assessed.

2. Materials and Methods

2.1. Reagents

Methanol from Fischer Scientific (UK) was of 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.

2.2. Plant Material



Brucea sumatarana Roxb.

Leaves of *Brucea sumatrana* Roxb. (Simaroubaceae) were collected in the district Mai-Ndombe's province Bandundu in Bas 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, Democratic Republic of Congo (DR Congo). A voucher specimen (20122009BSL) was deposited in the herbarium of this institute. Leaves were dried at room temperature and reduced to powder.

2.3. Preparation of Crude Extracts and Fractions

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**) [15]. The obtained fractions were treated as described above 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 minutes. After cooling and filtration, the filtrate was treated as described above 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 [16].

2.4. Phytochemical Screening

This study was performed by thin layer chromatography (TLC) on precoated silica gel plates F₂₅₄ (thickness later 0.25, mm, Merck, Germany) using different reagents and mobile phases described in the literature to identify major chemical groups such as

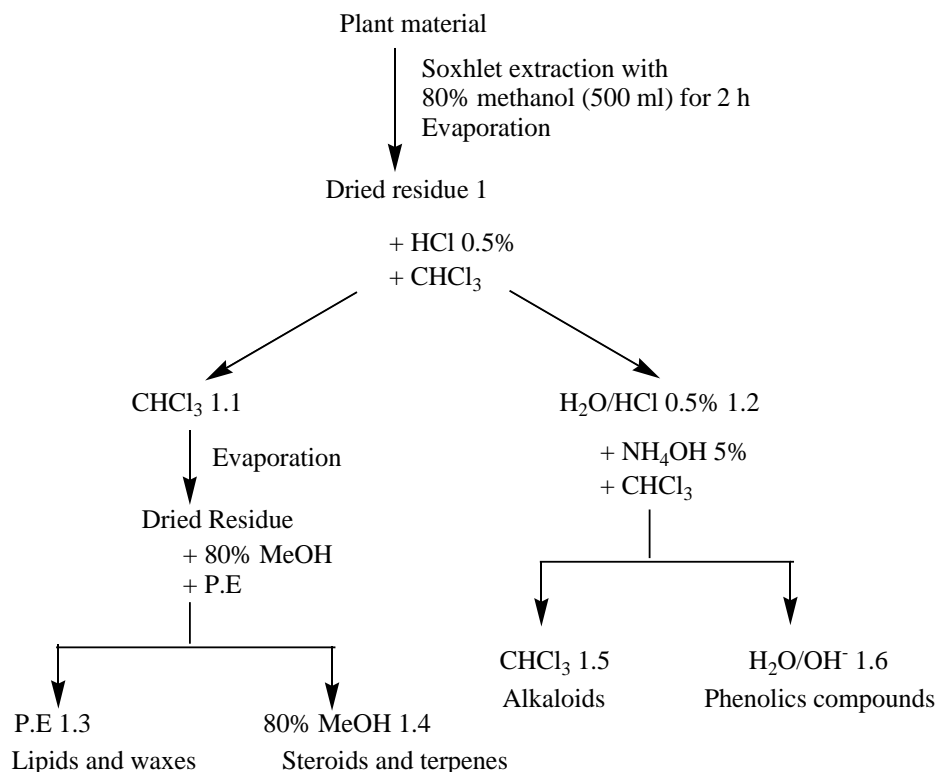


Figure 1. Fractionation of the 80% methanol extract according Mitscher *et al.* (1978).

alkaloids, anthraquinones, coumarins, flavonoids, terpenes and steroids, anthocyanins, saponins and tannins respectively [16].

2.5. Evaluation of Biological Activities

The antiparasitaire activity of all the samples obtained from the leaves of *B. sumatrana* were tested *in vitro* against *T. b. brucei*, *T. cruzi* and *L. infantum* from the laboratory of Microbiology, Parasitology and Hygiene of Prof. L. Maes, University of Antwerp, Belgium according to the respective procedures previously described by [17]. Assays with commercial drugs Metasorprol, Benznidazol and Milteforine (0.1 to 10 µg/ml) were performed to have reference values [17].

The antiplasmodial activity against chloroquine and pyrimethamine-resistant K1 strain of *Plasmodium falciparum* obtained from the same laboratory, and chloroquine-sensitive NF54 from Tropical medicine Institute of Antwerp, Belgium, was evaluated according to the lactate dehydrogenase procedure previously described by [18] with some modifications [17]. All samples dissolved in DMSO 0.1% and Chloroquine (anti-malarial reference product) were tested in sterile microplates at the concentrations from 0.1 to 10 µg/ml.

The cytotoxic effect against MRC-5 cell lines (human lung fibroblasts) was assessed using the MTT assay previously described by [17] and samples were tested at the concentration from 0.1 to 100 µg/ml in sterile microplates. 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 parasites [19] [20].

2.6. 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 [21]. Briefly, group I (2 rats) orally received 5 ml distilled water and constituted the negative control group. After, thirty Wistar rats of either sex, (body weight: 130 - 150 g, aged 8 - 10 weeks) were divided in three groups of 10 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.

2.7. Histopathological Study

Histopathological study of vital organs of treated rats 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 against 24 h. 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 microtone and the slides were stained with hematoxylin-eosin and observed under electronic microscopic. The state of each vital organ of treated animals was observed and compared to negative control groups [22].

2.8. 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, 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 (Abbott) automation with Boehringer 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 [23].

3. Statistical Analysis

Results were expressed as the mean of parameters \pm standards error of the mean (SE). Differences between means were evaluated using the Student-t test. ANNOVA tests to determine multiple comparisons were also used. Differences are significant at $p < 0.05$ [24].

4. Results and Discussion

4.1. Antiprotozoal and Cytotoxic Effects

The antiparasitaire activities and the cytotoxicity of samples from the leaves of *B. sumatrana* and respective references products are listed in **Table 1**. All samples were tested *in vitro* against *T. b. brucei*, *T. cruzi*, *L. infantum*, chloroquine and pyrimethamine resistant K1 and chloroquine-sensitive NF54 strains of *P. falciparum*. The cytotoxic effects of all samples against MRC-5 cell lines (human lung fibroblasts) and the acute toxicity only of the aqueous extract as the used traditional preparation were also evaluated. For the purpose of this *in vitro* antiparasitaire screening study, the following criteria were adopted to more appreciate the level of activity of each tested sample: $IC_{50} \leq 5 \mu\text{g/ml}$: strong activity; $5 < IC_{50} \leq 10 \mu\text{g/ml}$: good activity; $10 < IC_{50} \leq 20 \mu\text{g/ml}$: moderate activity; $20 < IC_{50} \leq 40 \mu\text{g/ml}$: weak activity; $IC_{50} > 40 \mu\text{g/ml}$: inactive.

Table 1. Antiprotozoal and antileishmanial (IC_{50} , $\mu\text{g/ml}$) and cytotoxic (CC_{50} , $\mu\text{g/ml}$) activities of samples from *Brucea sumatrana* leaves.

Code samples	MRC-5	<i>T. b. brucei</i>	<i>T. cruzi</i>	<i>L. infantum</i>
ME-1	2.34	4.35	6.15	24.00
ME-1.1	<0.25	<0.25	<0.25	0.32
ME-1.2	<0.25	<0.25	0.31	1.70
ME-1.3	>64	<0.25	<0.25	>64
ME-1.4	34.24	12.35	27.57	>64
ME-1.5	10.32	8.11	21.22	>64
ME-1.6	24.05	9.15	25.06	>64
ME-2	7.00	8.30	12.70	15.07
AE-1	0.46	0.57	0.70	4.85
Tamoxifen	10.5			
Metarsoprol		0.02		
Benznidazol			2.65	
Milteforine				3.56

Legend: ME-1: 80% methanol extract, ME-1.1 to ME-1.6: chloroform acid, petroleum ether, methanol 80% and aqueous acid soluble fractions, chloroform base, aqueous alkaline soluble subfractions respectively from the partition of ME-1 extract, ME-2: total alkaloids extract, AE-1: aqueous (decoction) extract, *T. b. brucei*: *Trypanosoma brucei brucei*, *T. cruzi*: *Trypanosoma cruzi*, *L. infantum*: *Leishmania infantum*, *P. falc.* K1: *Plasmodium falciparum* K1.

Results indicated that all samples exhibited the evaluated biological activities at different extents. The 80% methanol extract (ME-1) exhibited good **antitrypanosomal activity** against *T. cruzi* (6.15 µg/ml) and showed strong activity against *T. b. brucei* (**Table 1**) indicating that the growth inhibition was higher against *T. b. brucei* than *T. cruzi*. Interestingly, its chloroform soluble fraction (ME-1.1) rich in waxes, steroids and terpenes and its aqueous acid soluble subfraction (ME-1.2) rich in alkaloids and phenolic compounds had the same antitrypanosomal spectra of activity and presented strong activity against both *Trypanosoma* species with IC₅₀ values < 0.25 µg/ml (**Table 1**). The petroleum ether soluble fraction (ME-1.3) was rich in waxes and lipids showed pronounced activity against *T. b. brucei* and *T. cruzi* (IC₅₀ < 0.25 µg/ml). The activity of the fraction ME-1.4 was significantly weaker (p < 0.05) compared to other fractions, 80% methanol, aqueous and total alkaloid extracts. The activity of this last fraction ME-1.4 was rich in steroids and terpenes and showed pronounced activity against *T. b. brucei* and *T. cruzi* (IC₅₀ < 0.25 µg/ml) (**Table 1**). The activity of this last fraction was significantly weaker (p < 0.05) compared to other fractions and parent extract.

The chloroform base soluble subfraction (ME-1.5) and aqueous alkaline soluble subfraction (ME-1.6) had also the some antitrypanosomal spectra of activity. All samples displayed good activity against *T. b. brucei* and weak activity against *T. cruzi* (**Table 1**). ME-1.5 showed higher activity (p < 0.05) than ME-1.6.

The aqueous extract (AE-1) which is the used traditional remedy exhibited strong activity against *T. b. brucei* and *T. cruzi* with IC₅₀ values 0.57 and 0.70 µg/ml respectively suggesting that the growth inhibition of *T.b. brucei* was higher (p < 0.05) than that of *T. cruzi*. The total alkaloids extract (ME-2) showed good and moderate activity against *T. b. brucei*, *T. cruzi* respectively (**Table 1**) and its activity was significantly weaker (p < 0.05) compared to 80% methanol and aqueous extracts. It is important to point out that the antitrypanosomal activity of compounds such as quassinoids isolated from *Brucea javanica* fruit, a plant species different to those used in the present study, were found to produce growth inhibition of trypomastigotes of *Trypanosoma evansi* [6] (**Table 2**).

Concerning the **antileishmanial activity**, some samples displayed leishmanial effects. It was observed that the 80% methanol (ME-1) and the total alkaloid extracts

Table 2. Selectivity index (SI = CC₅₀/IC₅₀) of samples from *Brucea sumatrana* leaves.

Code samples	MRC-5/ <i>T.b.brucei</i>	MRC-5/ <i>T. cruzi</i>	MRC-5/ <i>L. infantum</i>
ME-1	0.53	0.38	0.10
ME-1.1	<1	<1	<0.80
ME-1.2	<1	<0.80	<0.14
ME-1.3	>256	>256	ND
ME-1.4	2.77	1.24	ND
ME-1.5	1.27	0.48	ND
ME-1.6	2.40	0.96	ND
ME-2	0.84	0.55	0.29
AE-1	0.80	0.66	0.09

Legend: See **Table 1**, ND: not determined because the tested sample was inactive.

(ME-2) exhibited low and moderate activity respectively against *L. infantum* (Table 1). In contrary, the chloroform (ME-1.1) and aqueous acid (ME-1.2) soluble fractions from the partition of ME-1 displayed strong antileishmanial activity against this parasite with IC₅₀ values of 0.32 and 1.70 µg/ml, their activity was remarkably higher compared to that of the parent extract (ME-1). For the remaining soluble fractions, no relevant anti-leishmanial effects were found at the highest tested concentration of 64 µg/ml against this parasite (Table 1). However, the aqueous extract (AE-1) exhibited strong activity against this parasite with IC₅₀ value of 4.85 µg/ml, and its effect was significantly higher ($p < 0.05$) than that of ME-1 and ME-2 extracts, but weaker ($p < 0.05$) compared to the activity of the two soluble fractions cited above.

Results from the **antiplasmodial activity** assays indicated that the highest activity was that of samples ME-1, ME-1.1, ME-1.2, ME-2 and AE-1 showing strong growth inhibition of *P. falciparum* K-1 strain with IC₅₀ values ranging from < 0.25 to 1.80 µg/ml (Table 1). The soluble fractions ME-1.3 and ME-1.4 exhibited low and good activity respectively and their activity was weaker ($p < 0.05$) compared to other samples cited above (Table 1). In addition, all samples from *B. sumatrana* leaves exhibited strong activity against chloroquine-sensitive NF54 strain of *P. falciparum* with IC₅₀ 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 IC₅₀ values <0.25 µg/ml (Table 3). In general, in the present study, it was observed that the pre-purification of the crude extract can lead to the obtaining of some fractions with high antiprotozoal activity compared to that of the parent extract.

Since the aqueous and 80% methanol extracts of *B. sumatrana* leaves are crude extracts and showed interesting antiprotozoal activity in the present study and have a very

Table 3. Antiplasmodial (IC₅₀, µg/ml) and cytotoxic (CC₅₀, µg/ml) activities, and selective index of samples from *B. sumatrana* leaves against chloroquine-sensitive NF54 and chloroquine-resistant K1 strains of *P. falciparum* (IC₅₀, µg/ml).

Code samples	MRC-5	<i>P. falc. NF54</i>	<i>SI/P.falc.NF54</i>	<i>P.falc.K1</i>	<i>SI/P.falc.K1</i>
ME-1	2.34	<0.02	>117	<0.25	>9.36
ME-1.1	<0.25	<0.02	<12.5	<0.25	<1
ME-1.2	<0.25	1.04	<0.24	<0.25	<1
ME-1.3	>64	1.55	>41.29	35.33	>1.81
ME-1.4	34.24	<0.02	>1712	10.00	4.86
ME-1.5	10.32	<0.02	>516	7.05	1.46
ME-1.6	24.05	2.04	11.79	8.15	2.95
ME-2	7.00	<0.02	> 350	1.80	3.89
AE-1	0.46	<0.02	>23	<0.25	<1.84
Chloroquine	>64	0.15	>426.67	0.18	>355.55

Legend: See Table 1. *SI/P.falc. NF54* and *SI/P. falc. K1*: selective index towards *Plasmodium falciparum* NF54 and K1 strains respectively.

complex composition, purification might lead to the isolation pure compounds with highly increased activity. This observation is also applied on fractions having exhibited high antiprotozoal activity in the present study.

Moreover, our results suggested that the most promising extracts and fractions in the antitrypanosomal, antileishmanial and antiplasmodial assays were the aqueous, methanol 80% and the total alkaloid extracts, the chloroform acid, the petroleum ether and aqueous acid soluble fractions from the partition of the 80% methanol extract exhibiting these activities with $IC_{50} < 2 \mu\text{g/ml}$ since they have higher activity than the respective reference products as summarized below and in **Table 1**.

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 ($0.25 \leq IC_{50} < 0.70 \mu\text{g/ml}$) ($p < 0.05$) than benznidazol against *T. cruzi* ($IC_{50} = 2.65 \mu\text{g/ml}$) whereas the antiplasmodial activity of ME-1, ME-1.1 and AE-1 ($IC_{50} < 0.25 \mu\text{g/ml}$) against the multidrug-resistant K1 strain of *P. falciparum* was comparable ($p > 0.05$) to that of chloroquine as an antimalarial reference product ($IC_{50} = 0.18 \mu\text{g/ml}$). 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 ($p < 0.05$) respectively ($CC_{50} = 2.34, <0.25, <0.25$ and $0.46 \mu\text{g/ml}$ respectively) than tamoxifen ($CC_{50} = 10.5 \mu\text{g/ml}$) (**Table 1**).

4.2. Cytotoxic and Selective 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 with cytotoxic effect against MRC-5 cell line ($CC_{50} > 64$ and $34.24 \mu\text{g/ml}$ respectively) since their CC_{50} are greater than $32 \mu\text{g/ml}$, the remaining samples were cytotoxic with CC_{50} values ranged from <0.25 to $24.05 \mu\text{g/ml}$ since their respective CC_{50} were weaker than $32 \mu\text{g/ml}$ [17].

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*, and that of ME-1.5 and ME-1.6 against *T. b. brucei* and *T. cruzi* were correlated to their cytotoxic effect against MRC-5 cell line, since their SI were weaker than 1 [19] [20]. 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**).

Except ME-1 and ME-2 samples for which the activity was not selective against chloroquine-sensitive and the multidrug-resistant K1 strain of *P. falciparum* ($SI < 1$) suggesting that their activity is due to cytotoxic activity toward MRC-5 cell lines, the remaining samples presented good selective action against these parasites ($SI > 1$) (**Table 3**) [19] [20]. 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 selective index of these samples obtained towards the multidrug-resistant K1 of *P. falciparum* was appreciable (**Table 3**). These results constitute important information

gained from the present study showing good selective antiprotozoal action of tested samples to form *B. sumatrana* leaves and open new doors for the discovery of safe and tolerated antiprotozoal agents since it is well known that current antiprotozoal medicines used have very low therapeutic windows, limiting their frequent use.

4.3. Effects of the Aqueous Extract of *B. sumatrana* Leaves on the Concentration Levels of Haematological Parameters

The haematological 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 the concentration levels of evaluated haematological parameters and no significant difference between the treated animals compared to untreated was deduced ($p > 0.005$). The values of all evaluated parameters reported in the present study remained in the normal limits (**Table 1**) [23].

4.4. 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 group compared to untreated group ($p < 0.05$). This decrease may be due to the hypoglycaemic and antidiabetic properties of the extract as also previously reported for other plant extracts in previous studies [25] [26].

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

Parameters	Negative control	<i>B. sumatrana</i> : 5 g/kg bw	Reference values
RBC ($\times 10^6 \mu\text{l}^{-1}$)	8.1 \pm 0.8	8.4 \pm 1.2	7.6 - 10.29
Hemoglobin (g/dL)	14.2 \pm 0.2	16.4 \pm 1.2	15 - 18.2
Hematocrit (%)	43.2 \pm 0.1	47.2 \pm 2.0	40.7 - 50
Platelets ($\times 10^3 \mu\text{l}^{-1}$)	1421.0 \pm 0.2	1404.2 \pm 0.2	995 - 1713
WBC ($\times 10^3 \mu\text{l}^{-1}$)	13.3 \pm 0.3	16.0 \pm 0.5	6.6 - 20.5
Neutrophils (%)	18.8 \pm 0.7	23.2 \pm 1.2	3 - 24.7
Basophils (%)	0.0	0.0	0.0
Eosinophils (%)	1.5 \pm 0.1	1.5 \pm 0.4	0 - 2
Lymphocytes (%)	89.2 \pm 1.1	88.3 \pm 0.1	58.8 - 94
Monocytes (%)	3.4 \pm 1.1	3.6 \pm 1.2	0 - 4
Segmented leucocytes (%)	15.1 \pm 0.6	19.3 \pm 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/kg bw on the concentration levels of biochemical parameters.

Parameters	Negative control	<i>B. sumatrana</i> : 5g/kg bw
Glucose (mg/dL)	245.5 ± 0.4	206.3 ± 1.4
Creatinine (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
Total cholesterol (mg/dL)	54.2 ± 1.1	53.3 ± 2.2
Triglycerides (mg/dL)	47.7 ± 1.8	46.3 ± 3.5
Total bilirubin (mg/dL)	0.6 ± 0.1	0.6 ± 0.1
Direct bilirubin (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: alkaline phosphate, HDL: high-density lipoproteins, LDL: low-density lipoproteins, SGOT: serum glutamooxalate transferase, SGPT: serum glutamate transferase.

Alanine aminotransferase (ALAT) also called alanine transaminase (ALT) and aspartate transaminase (AST) also known as aspartate aminotransferase (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 [27] [28]. Although a slight decrease was observed, the results reported here indicated that 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 these tested oral doses may not cause liver, heart or kidney damages as also previously reported by [29] [30]. Moreover, the hepatic function of these animals can be considered to be maintained [31]. In addition, the concentration levels of creatinine 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 did not show significant difference compared to control negative group ($p > 0.05$) (Table 5). This effect may be due to the hypolipidimic property of the aqueous extract and to the increase secretion of thyroid hormones T3 and T4 [31]. A

slight decrease in concentrations of HDL and 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 beneficial effects by reducing some risk factors related to cardiovascular diseases [32].

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 [29]. 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 not significant difference in the concentration level of these biochemical parameters in treated animals compared to control negative 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 a significant difference compared to the control group ($p > 0.05$) 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 in treated groups was observed ($p > 0.05$) (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 [33]. This finding is in good agreement with results reported concerning the effect of other medicinal plant extracts in previous studies [30] [34].

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 renal insufficiency because its concentration level remained within the normal limits (2.5 - 7.5 mmol/L). Therefore, our results showed good hepatic function of treated animals for the above reasons as also previously reported by [31].

In general, all concentration levels of biochemical and haematological parameters evaluated in the present study were within the normal ranges [23] [35] [36].

4.5. Acute Toxicity of the Aqueous Extract of *B. sumatrana* Leaves in Wistar Rats

Animals were treated with single oral doses of the aqueous extract (AE-1) (500, 1000 and 5000 mg/kg body weight respectively, 10 animals for each oral dose). In this test, no sign of toxicity such as alteration of the locomotion activity and gastrointestinal disturbances were observed. Rats received all tested oral doses significantly gained body weight compared to negative control group. According to [30], the growth response ef-

fect could be considered as a result of increased food and water intake. On Day 21, one death (10%) was noted in the third group receiving 5000 mg/kg bw of the extract and not death was observed with other administered oral doses until to 28th day of observation. This last percentage of mortality is weaker than 50%. According to [37], substances that present LD₅₀ higher than 5 g/kg body weight via oral route, may be considered as practically non-toxic. Therefore, it may be suggested that acute toxicity of *B. sumatrana* aqueous leaves extract is practically null via oral route. Therefore, the LD₅₀ 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 group indicating that their state was well maintained at the administered oral dose. After histopathological examination of vital organs such as liver, kidney, spleen, large intestine, heart and lungs, no abnormality was observed in treated animals compared to negative control groups. All examined organs were in good state without any visible modification.

On the other hand, the acute toxicity of leaves ethanol extract of *Brucea javanica* Merr. collected in Indonesia on mice was previously reported [38]. In this investigation, it was reported that vital organs and body average weights of treated leaves ethanol extract did not show no difference compared to control group. In addition, gross examination of the vital organs revealed no pathologic abnormality compared to control group on the microscopic observation. These finding were in good agreement with our observations for the aqueous extract of *B. sumatrana* leaves studied in the present investigation.

4.6. The Chemical Composition of *B. sumatrana* Leaves

Some plant parts of *B. javanica* growing in Asian regions were chemically previously investigated. Other triterpenoids and quassinoids were isolated from the combined plant material leaves, twigs and inflorescences. Flavonoids were also detected [39] or isolated from the leaves [40].

To obtain information on the type of compounds which could be responsible for these evaluated these biological activities, we reviewed the literature, but little is known about the African *B. sumatrana* since to our knowledge, this species was never investigated before our present study. 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. Several phytomolecules in the tested samples including flavonoids, alkaloids, steroids, terpenes and phenolic compounds isolated from other medicinal plants have been previously reported as potent antiprotozoal agents [41]-[50]. The presence these secondary metabolites identified in the studied plant extracts and fractions may be contributed to the observed antiprotozoal activity, particularly, different terpenes belonging to the quassinoids groups mainly isolated from the seeds of *B. javanica* growing in Asian regions are known as active principles for various evaluated biological activities already men-

tioned above [1]-[10]. However, the observed antiprotozoal activity of the aqueous and 80% methanol extracts of *B. sumatrana* leaves might be attributed to either the individual class of compounds present in respective extract or to the synergistic action that each class of compounds exert to give the observed biological activity as also previously reported by [51].

5. Conclusions

In conclusion, the present investigation has described for the first time the antiprotozoal and cytotoxic activities, and acute toxicity of extracts, fractions of the African *B. sumatrana* leaves collected in DR Congo on a large game 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 current used traditional preparation was found to be non-toxic and did not affect the concentration levels of evaluated biochemical and hematological parameters 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 remedies for the treatment of parasitic diseases such as malaria, sleeping sickness, leishmaniosis and in some extent American trypanosomiasis named Chagas disease, with no apparent toxic effects in patients. Further studies are in progress on the most active extracts and fractions leading to the isolation active constituents.

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