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In Vitro Antiprotozoal and Cytotoxic Activity of the Aqueous Extract, the 80% Methanol Extract and Its Fractions from the Seeds of *Brucea sumatrana* Roxb. (Simaroubaceae) Growing in Democratic Republic of Congo

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ABSTRACT

The *in vitro* antiprotozoal and cytotoxic activity of the aqueous extract, the 80% methanol extract, and its different soluble fractions and subfractions from *Brucea sumatrana* seeds were assessed against two *Trypanosoma* (*T. cruzi* and *T. brucei brucei*), *Leishmania infantum* and chloroquine and pyrimethanine-resistant K1 strain of *P. falciparum* and against MRC-5 cell-lines respectively. Results indicated that the 80% methanol extract showed a cytotoxic effect against MRC-5 cell lines with CC_{50} value of 0.54 µg/ml. It however exhibited pronounced and non selective activity against *T. cruzi* (IC_{50} = 1.52 µg/ml, SI = 0.03) and *L. infantum* (IC_{50} = 2.41 µg/ml, SI = 0.22). It however displayed pronounced and selective effect against *T. brucei brucei* (IC_{50} < 0.25, SI > 2.16) and chloroquine and pyrimethamine-resistant K1 strain of *P. falciparum* (IC_{50} < 0.25 µg/ml, SI > 2.16). All soluble fractions and subfractions from the partition of the 80% methanol extract were found to exhibit an antiprotozoal activity with IC_{50} values ranging from <0.25 to 30 µg/ml. The most active was the alkaline aqueous soluble fraction exhibiting pronounced antiprotozoal activity against *T. cruzi*, *T. b. brucei*, *L. infantum* and chloroquine and pyrimethamine-resistant K1 strain of *P. falciparum* with IC_{50} values of 0.33, <0.25, 0.25, <0.25 µg/ml respectively resulting in high selective index values of 61.36, > 81, 81 and >81 respectively. The chloroform soluble fraction rich in alkaloid was cytotoxic against MRC-5 cell lines (CC_{50} = 27.09 µg/ml) and showed good activity against *T. b. brucei* (IC_{50} = 8.36 and SI = 3.24) and moderate activity against *T. cruzi*, *L. infantum* and chloroquine-pyrimethane-resistant K1 strain of *P. falciparum* (20 < IC_{50} < 30 µg/ml). Although the aqueous extract (decoction) and the total alkaloids extract showed a cytotoxic effect against MRC-5 cell lines (CC_{50} = 1.55 and 0.43 µg/ml respectively), they however displayed pronounced antiprotozoal activity against *T. cruzi*, *T. b. brucei* and chloroquine and pyrimethamine-resistant K1 strain of *P. falciparum* with IC_{50} values ranging from < 0.25 to 0.6 µg/ml with only a selective action against chloroquine and pyrimethamine-resistant K1 strain of *P. falciparum* (SI = >6.2 and >1.72 respectively). These extracts however showed good and low activity respectively against *L. infantum* (IC_{50} = 24.05 and 6.82 µg/ml respectively).

Keywords: *Brucea sumatrana*; Simaroubaceae; Seeds; Antiprotozoal Activity; Cytotoxicity

1. Introduction

The plant *Brucea sumatrana* Roxb., synonyms: *Brucea javanica* (L.) Merr., *B. amarissima* Desv. Ex Gomes, *Gonus amarissimus* Lour or *Loussa amarissima* O. Ktze, is a shrub belonging to the family Simaroubaceae. It is widely distributed throught Asian Pacific regions including China, Malaysia, Thailand, and Indonesia [1-4]. In these regions, the seeds (fruits) are used for the treatment

of malaria, amoebic dysentery, cancer and as insecticide [5-7]. The plant is also found in some African countries and its seeds are used to treat various diseases among which fever including malaria, parasitosis, colonic diseases, headache, nematode diseases, helminthiasis and protozoal diseases [8,9].

The seeds from the Asian species have been previously investigated for its various biological activities such as antimalarial [10-16], cytotoxic and antileukemic [5,17-23], antiprotozoal against amoeba [24,25], *Toxoplama gondii*, *Giardia intestinal* [25], *Trypanosoma brucei brucei* and *Leishmania donovani* [14], *Blastocystis hominis* [26], *Trypanosoma evansi* [27] and *Babesia gibsoni* [3,28], and anti-inflammatory [29] activities. Different chemical constituents including quassinoids [11,12,17,19,30,31], alkaloids from cell suspension cultures of *B. javanica* seeds [5, 32] and lignans [20] had been reported to be present in the seeds and recognized to be responsible for different biological activities according to the case.

To our knowledge, no plant part of *Brucea sumatrana* growing in African countries was not yet phytochemically and biologically investigated. Thus, the present study deals with the assessment of the *in vitro* antiprotozoal activity of crude extracts, fractions and subfractions from the seeds of *Brucea sumatrana* growing in Democratic Republic of Congo (DR Congo) against *Trypanosoma cruzi*, *T. brucei brucei*, *Leishamania infantum* and chloroquine and pyrimethamine-resistant K1 strain of *P. falciparum*. Their putative cytotoxic effect against MRC-5 cell lines growth was also evaluated.

2. Materials and Methods

2.1. Plant Materiel

Seeds of *Brucea sumatrana* Roxb. (Simaroubaceae) were collected in November 2007 in the district Mayombe's Bas-Congo in DR Congo (**Table 1**). The plant was identified by Mr. Ngoma of the Institut de Recherche en Sciences de la Santé (I.R.S.S.) of Kinshasa, DR Congo. A voucher specimen (NM 2112007) of the plant was deposited in the herbarium of this institute. Seeds were dried at room temperature and reduced to powder.

Table 1. Amount (g) of samples from *Brucea sumatrana* seeds.

Code	Amount
Sd-1	2.98
Sd-1.1	0.932
Sd-1.1.1	0.651
Sd-1.1.2	0.272
Sd-1.2.	1.125
Sd-1.2.1	0.562
Sd-1.2.2	0.426
Sd-2	0.504
Sd-3	0.357

2.2. Preparation of Extracts, Fractions and Subfractions

150 g of powdered seeds were submitted to soxhlet extraction with 80% methanol (500 ml) for 2 h. The extractive solvent was filtererd and evaporated in vacuo yieding a dried extract denoted as Sd-1. An amount of Sd-1 (1.5 g) was fractionated according to the procedure previously described [33] (**Figure 1**). The obtained fractions and subfractions were treated as described above yielding corresponding dried extracts denoted as Sd-1.1 to Sd-1.2.2. On the other hand, 20 g of the plant material were mixed with 100 ml distilled water and boiled for 10 min. After cooling and filtration, the filtrate was evaporated in vacuo yielding dried extract denoted as Sd-2. Another batch of 50 g of plant material was used for the extraction of the total alkaloid extract by the classical acid/base procedure using chloroform as the extractive solvent [34] and denoted as Sd-3.

2.3. Phytochemical Screening

Chemical tests were carried out on the aqueous and 80% methanol extracts from *Brucea sumatrana* seeds, the fractions and subfractions from the partition of the 80% methanol extract using standard procedures [34]. Saponins were identified by using the frothing test. Alkaloids were detected using Draggendorf's and Mayer's reagents resulting in the formation of an orange or yellow-white precipitate respectively as positive test. The presence of flavonoids was determined using aluminium chloride 5% in methanolic solution and Shinoda's reagent (HCl + magnesium turnins) after heating for 10 min giving yellow and purple colour respectively as positive test. The test for anthraquinones was performed by adding ammonia solution (NH_4OH 10% or NaOH 10%) producing red to red-purple colour as positive test. Steroids and terpenes were screened by adding Lieberman-Buchardat's reagent (acetic anhydride/conc. H_2SO_4) followed by heating for 10 min until to the appearance of purplish-blue colour or other colours as positive test. Tannins

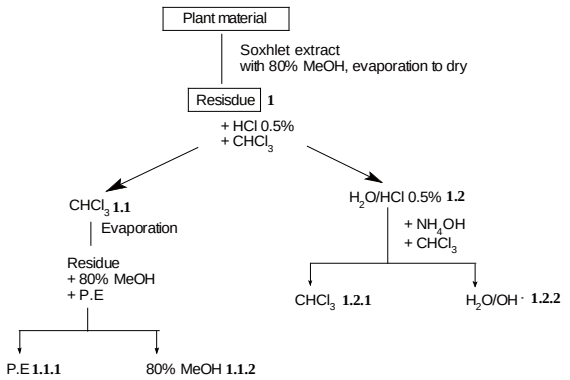

Figure 1. Fractionation of the crude extract [33].

were identified with gelatin and $FeCl_3$ 5%. Moreover, the presence of alkaloids, flavonoids, steroids, terpenoids and anthraquinones was confirmed by TLC (thin layer chromatography) analysis performed on silicagel $60F_{254}$ plates Merck (thickness layer 0.25 mm) using $CHCl_3$/Methanol/NH_3 25%: 8:2:0.5 and Ethyl acetate/*iso*-Propanol/NH_3 25%: 16:3:1 as mobile phases, and Draggendorf's reagent for alkaloids; *n*-Butanol/Acetic acid/Water: 4:1:5 (top layer) as mobile phase and Neu's reagent (1% diphenylboric ethanolamine acid in methanol) for flavonoids; Ethylacetate/Methanol/Water: 8:1:1 as a mobile phase and magnesium acetate 5% in methanol, and NaOH 10% or NH_4OH 10% as reagents for anthraquinones; Chloroforme/Methanol: 9:1 and *n*-Hexane/Dichloromethane: 1:9 as mobile phases and Liebermann-Buchardat's reagent and Vanilline 1%/H_2SO_4 5% in methanol for steroids and terpenoids. Coumarins were detected under UV (366 nm) thanks to their blue fluorescence which becomes intense after spraying KOH 10%. Anthocyanins were detected after heating 0.01 g of each extract dissolved in 10 ml distilled water with 2 M HCl for 5 min at 100˚C producing a red colour which can be extracted into amyl alcohol or by adding 2 M NaOH dropwise giving a blue colour which change to green and becomes slowly fade.

2.4. Assay for *in Vitro* Antitrypanosomal and Antilesmanial Activity

All samples were tested against *Trypanosoma brucei brucei*, *Trypanosoma cruzi* and *Leishmania infantum* bloodstream forms from axenic cultures in HMI-18 medium obtained from Prof. L. Maes of the Laboratory of Microbiology, Parasitology and Hygiene, Faculty of Pharmaceutical Sciences, Biomedical and Veterinary Sciences of the University of Antwerp, Belgium. Assays were performed in 96 well tissue plates, each containing 10 µl aqueous extract dilutions ranging from 100 to 0.01 µg/ml together with 190 µl of the parasite suspension (5×10^4 parasites/ml) in Hirumi (HMI) medium supplemented with 10% foetal calf serum and a solution of 5000 units penicillin/ml and 5000 µg streptomycin/ml, final concentration 2% in medium (2% P/S solution). All plates were incubated for 4 days in humidified atmosphere at 37˚C in 5% CO_2. Two hours before the end of the incubation, 10 µl of Alamar Blue® solution were added. Fluorescence was measured after 4 hours of incubation with the Alamar Blue® in a fluorescence plate reader at 530 nm excitation and 590 nm emission wavelengths. The IC_{50} values were calculated by linear interpolation selecting values above and below the 50% mark. Melarsoprol, Benznidazol and Miltefosine were used as reference products against *T. b. brucei*, *T. cruzi* and *L. infantum* respectively [35].

2.5. Assay for *in Vitro* Antiplasmodial Activity

Extracts, fractions and subfractions were tested against the chloroquine and pyrimethanine-resistant K1strain of *Plasmodium falciparum* obtained from Prof L. Maes of the laboratory of Microbiology, Parasitology and Hygiene, Faculty of Pharmaceutical Sciences, Biomedical and Veterinary Sciences of the University of Antwerp, Belgium. Samples were tested against chloroquine and pyrimethamine-resistant K1 strain of *Plasmodium falciparum* maintained in continuous log phase growth in RPMI-1640 medium supplemented with 2% P/S solution, 0.37 mM hypoxanthine, 25 mM HEPES, 25 mM $NaHCO_3$ and 10% O^+ human serum together with 4% human O^+ erythrocytes according to the method previously described [36]. All cultures and assays were conducted at 37˚C under micro-aerophilic atmosphere (4% CO_2, 3% O_2 and 93% N_2). The *in vitro* antimalarial activity was assessed using an adaptation of the procedure previously described as the lactate dehydrogenase assay [37]. Twenty milligrams of each extract were dissolved in 1 ml DMSO and serially diluted tenfold with culture medium before being added to asynchronous parasite cultures. Assays were performed in 96-well tissue culture plates, each well containing 10 µl of the test sample dilutions together with 190 µl of the parasite inoculum (1% parasitaemia, 2% haematocrit). After 72 hours of incubation at 37˚C, plates were stored at –20˚C until further processing. After thawing, 20 µl of haemolysed parasite suspension from each well was transferred into another plate together with 100 µl Malstat™ reagent and 10 µl of a 1/1 mixture of PES (phenazine ethosulfate, 2 mg/ml) and NBT (nitro blue tetrazolium grade III, 0.1 mg/ml). The plates were kept in the dark for 2 hours and change in colour was measured spectrophotometrically at 655 nm. The results were expressed as percentage reduction in parasitaemia compared to control wells. The concentration causing 50% inhibition of parasite growth (IC_{50}) was calculated from the drug concentration—response curves. Chloroquine diphosphate was used as an antiplasmodial reference drug. Each assay was done in triplicate [35].

2.6. Cytotoxicity Assay

2.6.1. Cell Cultures

Cell lines MRC-5 (human lung fibroblast) were cultured in MEM medium, supplemented with 20 mM L-glutamine, 16.5 mM $NaHCO_3$, 5% foetal calf serum and 2% P/S solution. All cultures were kept at 37˚C and 5% CO_2.

2.6.2. Testing

Assays were performed in sterile 96-well tissue culture plates, each well containing 10 µl of each sample dilutions together with 190 µl of cell suspension (2.5×10^4

cells/ml). After 7 days incubation, cell proliferation/viability was assessed after addition of MTT (Sigma) (50 µl of a 1/ 2.5 solution per well). After 4 hours of incubation at 37˚C, the % absorbance reduction at 540 nm for the treated cultures and untreated control cultures were obtained and compared, and CC_{50} values (50% cytotoxic concentration) were determined [35].

3. Results and Discussion

The aqueous extract (decoction), the 80% methanol extract and its different soluble fractions and subfractions were screened for their potential antiprotozoal activity against *Trypanosoma cruzi*, *T. brucei brucei*, *Leishmania infantum* and chloroquine and pyrimethamine-resistant K1 strain of *P. falciparum* as well as their cytotoxic effect against MRC-5 cell lines. The following criteria were used to appreciate more the level of their activity: $IC_{50} \leq 5$ µg/ml: pronounced activity, $5 < IC_{50} \leq 20$: good activity, $20 < IC_{50} \leq 30$: moderate activity, $30 < IC_{50} \leq 60$: low activity, $IC_{50} > 64$ µg/ml: inactive. A sample is considered as cytotoxic when $CC_{50} < 32$ µg/ml [34]. For the samples tested, the cytotoxicity to MRC-5 cell lines and activity have been compared using the selectivity index (SI) ration (SI = CC_{50} MRC-5 cells/IC_{50} protozoa). A value >1 is considered to be selective against the test protozoa and a value <1 is considered as selective against MRC-5 cell lines [14,38].

Results in **Table 2** show that the 80% methanol extract (Sd-1) possessed a cytotoxic effect against MRC-5 cell lines growth with a CC_{50} value of 0.54 µg/ml. It however exhibited pronounced antiprotozoal activity against *Trypanosoma cruzi*, *T. b. brucei*, *Leishmania infantum* and chloroquine and pyrimethamine-resistant K1strain of *Plasmodium falciparum* with IC_{50} values of 1.52, <0.25, 2.41 and <0.25 µg/ml respectively. In evaluating the selectivity index (SI) of this extract (ratio CC_{50}/IC_{50}), it was observed that the resulting values are <1 indicating its selective action against MRC-5 cell lines than against *T. cruzi* and *L. infantum*, and confirmed that the observed activity against these two protozoa is due to the cytotoxic effect of the extract since the extract had high selective action against MRC-5 cell lines than the test parasites [14,38]. Its activity against *T. b. brucei* and chloroquine and pyrimethamine-resistant K1 strain of *P. falciparum* was selective since the SI value was >1 (**Table 3**) [14,38]. In a previous study, a methanol extract from the fruits of *B. javanica* collected in Thailand showed a cytotoxic effect against KB cells ($CC_{50} = 9.20 \pm 0.23$ µg/ml) and exhibited an activity against *Leishmania donovani* and *Trypanosoma brucei brucei* trypamastigotes with IC_{50} values of 246.68 ± 1.20 and 500.00 ± 2.60 µg/ml respectively. This activity was related to its cytotoxic effect since the selectivity index values were 0.037 and 0.018 respectively [14].

In the present investigation, all soluble fractions and subfractions from the partition of the 80% methanol extract seeds (Sd-1) exhibited antiprotozoal activity at different extents. Briefly, the chloroform soluble fraction (Sd-1.1) from Sd-1 was devoid of cytotoxic effect against MRC-5 cell lines, and showed low and selective activity against *T. cruzi* and *T. b. brucei* with IC_{50} values $30 < IC_{50} < 60$ µg/ml and was inactive against *L. infantum* ($IC_{50} > 64$ µg/ml) (**Table 2**). It however displayed pronounced activity against chloroquine and pyrimethamine-resistant K1 strain of *P. falciparum* with IC_{50} value of 2.25 µg/ml. In assessing its selectivity index, the resulting value was high (>28.24) indicating its high selective effect against this parasite. It had also good selective effect against *T. cruzi* and *T. b. brucei* since the selectivity index value were appreciable (**Table 3**). The petroleum ether (Sd-1.1.1) rich in lipids and waxes and

Table 2. Antiprotozoal activity of samples from *Brucea sumatrana* seeds (IC_{50}, µg/ml).

Samples (codes)	MRC-5: CC_{50}, µg/ml	*T. cruzi*	*T. b. brucei*	*L. infantum*	*P. falciparum K*1
Sd-1	0.54	1.52	<0.25	2.41	<025
Sd-1.1.	>64	57.8	34.86	>64	2.25
Sd-1.1.1	7.90	12.70	8.30	21.05	1.86
Sd-1.1.2	15.24	30.24	9.6	>64	9.12
Sd-1.2	13.10	9.04	5.64	13.05	8.64
Sd-1.2.1	27.09	23.86	8.36	27.27	21.50
Sd-1.2.2	20.25	0.33	< 0.25	0.25	< 0.25
Sd-2	1.55	2.60	2.11	24.05	< 0.25
Sd-3	0.43	1.30	0.54	6.82	< 0.25
Tamoxifen	10.5	-	-	-	-
Benznidazol	-	2.65	-	-	-
Miltesfosine	-	-	-	4.76	-
Melarsoprol	-	-	0.05	-	-
Chloroquine	-	-	-	-	0.18

Table 3. Selective index (SI) values of samples from *Brucea sumatrana* seeds.

Samples (codes)	*T. cruzi*	*T. b. brucei*	*L. infantum*	*P. falciparum K*1
Sd-1	0.03	>2.16	0.22	>2.16
Sd-1.1.	>1.11	>1.84	>1	>28.44
Sd-1.1.1	0.62	0.95	0.37	4.25
Sd-1.1.2	0.50	1.6	<0.24	1.67
Sd-1.2	1.45	2.32	1.00	1.51
Sd-1.2.1	1.14	3.24	1.00	1.26
Sd-1.2.2	61.36	> 81	81	>81
Sd-2	0.60	0.73	0.06	>6.2
Sd-3	0.33	0.80	0.06	>1.72

Legend: Sd-1: 80% methanol extract, Sd-1.1 and Sd-1.2: $CHCl_3$ and aqueous acid solube fractions respectively, Sd-1.1 and Sd-1.1.1 and Sd-1.1.2 soluble subfractions from Sd-1, Sd-1.2.1 and Sd-1.2.2: soluble subfractions from Sd-1.2.

the 80% methanol (Sd-1.1.2) soluble subfractions rich in steroids and terpenes from Sd-1.1 had the same antiprotozoal spectrum. They possessed a cytotoxic effect against MRC-5 cell lines ($7 < CC_{50} < 16$ µg/ml). Sd-1.1.1 showed good activity against *T. b. brucei* and *T.cruzi* (IC_{50} = 8.30 and 7.90 µg/ml respectively) and moderate activity against *L. infantum* (IC_{50} = 21.05 µg/ml). Regarding its selective index values against these protozoa, it was concluded that the observed activity was due to its cytotoxity since the SI were <1. In contrast, this subfraction, exhibited pronounced antiplasmodial activity against the multidrug-resistant K1 strain of *P. falciparum* with IC_{50} value of 1.86 µg/ml and with appreciable selectivity index (**Table 3**). Sd-1.1.2 displayed good activity against *T. b. brucei* and chloroquine and pyrimethamine-resistant K1 strain of *P. falciparum* with IC_{50} values of 9.6 and 9.12 µg/ml with appreciable selectivity index (**Table 3**). It showed low activity against *T. cruzi* and was inactive against *L. infantum* (**Table 2**). The acid aqueous soluble fraction (Sd-1.2), its chloroform (Sd-1.2.1) and alkaline-aqueous phase (Sd-1.2.2) soluble fraction rich in alkaloids and phenolic compounds respectively, had also the same antiprotozoal spectrum. These samples were however cytotoxic against MRC-5 cell lines since their CC_{50} values are less than 32 µg/ml (**Table 2**) [34]. Sd-1.2 showed good activity against *T. cruzi*, *T. b. brucei* and chloroquine and pyrimethamine-resistant K1 strain of *P. falciparum* with IC_{50} value of 9.04, 5.64 and 8.64 µg/ml respectively with selective effect (SI > 1), and showed good activity against *L. infantum* (**Table 2**). Sd-1.2.1 exhibited good and selective activity against *T. b. brucei* with IC_{50} value of 8.36 µg/ml and SI value of 3.24, moderate and selective activity against *T. cruzi*, *L.infantum* and chloroquine and pyrimethamine-resistant K1 strain of *P. falciparum* ($20 < IC_{50} < 28$ µg/ml, SI > 1). Interestingly, Sd-1.2.2 displayed pronounced activity against *T. cruzi*, *T. b. brucei*, *L. infantum* and multidrug-resistant K1 strain of *P. falciparum* with $IC_{50} < 1$ µg/ml (**Table 2**). In evaluating its selectivity index values, the resulting values were high values of 61.36 against *T. cruzi*, 81 against *L. infantum* and >81 against *T. b. brucei* and multidrug-resistant K1 strain of *P. falciparum* indicating its high selecive action against these protozoa. This finding suggested that the active constituents may be phenolic compounds.

The aqueous extract (decoction, Sd-2) and the total alkaloids extract (Sd-3) also showed the same wide range of antiprotozoal spectrum. Although they showed a cytotoxic effect against MRC-5 cell lines (CC_{50} = 1.55 and 0.43 µg/ml respectively), they exhibited pronounced activity against *T. cruzi*, *T. b. brucei* and chloroquine and pyrimethamine-resistant K1 strain with IC_{50} values in the range of 1 to 3 µg/ml (**Table 2**). Except for chloroquine and pyrimethamine-resistant K1 strain for which the activity of these fractions was selective ($1 < SI < 7$), it was however seen that their effect against the remaining protozoa was correlated to their cytotoxic effect and was not selective (SI < 1). In contrast, the aqueous extract (macerate) from the seeds *of B. javanica* collected in Thailand was found to be inactive against *L. donovani* and *T. b. brucei* (IC_{50} > 500 µg/ml) [14]. The difference in activity of this aqueous extract specially against *T. brucei brucei* as reported by these authors compared to our results can be only attributed in part to the type of preparation (macerate *versus* decoction).

In comparison of the IC_{50} values recorded for all samples to selected parasites in the present study, it was observed that most samples had smaller IC_{50} values against *T. T. b. brucei* (<0.25 to 8.36 µg/ml), *T. cruzi* (0.43 to 1.55 µg/ml) and chloroquine-resistant and pyrimethamine-resistant K1 strain of *P. falciparum* (<0.25 to 2.25 µg/ml) indicating that these three parasites are more sensitive than *L. infantum* (0.25 to >64 µg/ml). These samples had in addition appreciable selective action against sensitive parasites (**Table 2**).

With respect to the level of activity showed by respecttive reference products against these selected protozoa, it was however seen that the samples Sd-1, Sd-2 and Sd-3 showed higher cytotoxic effect (19.4, 6.8 and 24.4-times more cytotoxic) than tamoxifen, against MRC-5 cell lines growth, Sd-1, Sd-1.2.2 and Sd-3 exhibited higher activity (1.7, 8 and 2-times more cytotoxic) than benznidazol against *T. cruzi* growth, while the activity of Sd-2 was comparable to that of this reference product against this parasite. Sd-1 and Sd-1.2.2 displayed higher activity (2 and 19-times more cytotoxic) than miltesfosine against *L. infantum* growth. The activity of Sd-1, Sd-1.2.2, Sd-2 and Sd-3 was comparable to that of chloroquine against chloroquine and pyrimethamine-resistant K1strain of *P. falciparum*. On the other hand, it is well known that compounds responsible for the antiplasmodial activity of *B. sumatrana* seeds growing in Asian regions are quassinoids. These compounds were also recognized be responsible for the antiprotozoal activity against *Trypanosoma evansi* infecting animals [27], but compounds active against other *Trypanosoma* and *Leishmania* specie from this plant part are still unknown. This finding demonstrates that these samples need extensive phytochemical investigations leading to the isolation and structural elucidation of active principles which would be considered as lead compounds.

Phytochemical screening conducted on the aqueous and 80% methanol extract from *B. sumatrana* seeds in the present study by TLC using different mobile phases and chemical reagents reported in the literature [34], revealed the presence of steroids and/or terpenes, alkaloids and phenolic compounds as major constituents, which can account for the observed biological activity.

4. Conclusion

This is the first report of the *in vitro* antiprotozoal activity of extracts, fractions and subfractions from *Brucea sumatrana* seeds growing in DR Congo, an African country, on a large number of protozoa species. In addition, this study shows that the aqueous extract, the 80% methanol extract and its different soluble fractions and subfractions possessed potential and interesting antiprotozoal activity against the selected protozoa according to the case. Most samples were found to exhibit pronounced or good activeity which might be contributed in reducing infection. Based on this study, fractions and subfractions with high activity will be further phytochemically studied to isolate and identify active constituents.

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