EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 7
Issue: 8
67-75
Year: 2020

IN VITRO ASSESSMENT OF ANTIBACTERIAL, ANTIAMOEBIC AND ANTISPASMODIC ACTIVITIES OF EXTRACTS FROM *BRUCEA SUMATRANA* ROXB. (SIMAROUBACEAE) LEAVES USED AS ANTIDIARREAL AGENT IN TRADITIONAL MEDICINE IN DEMOCRATIC REPUBLIC OF CONGO

Tshodi E. M.[1], Nsaka L. S.[1], Kambu K. O.[1], Lami N. J.[1], Cimanga K. R.[1]*,[2], Vlietinck A. J.[2] and Pieters L.[2]

[1]Department of Medicinal Chemistry and Pharmacognosy, Laboratory of Pharmacognosy and Phytochemistry, Faculty of Pharmaceutical Sciences, University of Kinshasa, P.O. Box 212, Kinshasa XI, Democratic Republic of Congo.
[2]Department of Pharmaceutical Sciences, Natural Products & Food Research and Analysis (NatuRA), University of Antwerp, Universiteitsplein1, B-2610, Antwerpen, Belgium.

***Corresponding Author: Cimanga K. R.**
Department of Medicinal Chemistry and Pharmacognosy, Laboratory of Pharmacognosy and Phytochemistry, Faculty of Pharmaceutical Sciences, University of Kinshasa, P.O. Box 212, Kinshasa XI, Democratic Republic of Congo.

Article Received on 03/06/2020 **Article Revised on 24/06/2020** **Article Accepted on 14/07/2020**

ABSTRACT
Results from the present study reported the antibacterial, antiaomoebic and antispasmodic activities of samples from *Brucea sumatrana* leaves including lyophilized aqueous extract and its soluble fractions, 80% methanol and total alkaloids extracts. Results from the antibacterial and bactericidal testings, revealed that all extracts and soluble fractions from *B. sumatrana* leaves exhibited antibacterial and bactericidal activities by inhibiting the growth of selected clinically isolated bacteria with minimal and bactericidal concentrations (MIC and MBC) from 7.81 to 250 µg/ml. In antiamoebic testing, all extracts and soluble fractions from the same plant part, were found to possess very good capacity to inhibit the growth of the parasite *Entamoeba histolytica* with minimal amoebicidal concentrations (MAC) from 2.03 to 10.68 µg/ml and inhibitory concentrations 50 (IC_{50}) ranging from 1.14 to 6.35 µg/ml while in antispasmodic testing on isolated guinea-pig ileum, all samples inhibited contractions induced by acetylcholine (ACh) and depolarizing solution rich in KCl (DSR-KCl) by more than 65% when tested at 40 µg/ml. The most active samples were lyophilized aqueous, 80% methanol and total alkaloids extracts, ethylacetate and residual aqueous soluble fractions exhibiting antibacterial activity with MIC and MBC values < 100 µg/ml, displaying antiamoebic activity with MAC and IC_{50} < 10 µg/ml and producing percentage inhibitions of contractions induced by ACh and DSR-KCl more than 80%. These results can partly support and justify the traditional use of *B. sumatrana* leaves in the decoction form for the treatment of diarrhea in traditional medicine in DR-Congo and other African countries according to its origin.

KEYWORDS: *Brucea sumatrana*, Simaroubaceae, leaves, extracts, fractions, antibacterial, antiamoebic and antispasmodic activities, diarrhea

1. INTRODUCTION
Brucea sumatrana Roxb. is a medicinal plant belonging to the Simaroubaceae family. It is found in some African countries under the scientific name *Brucea sumatrana* Roxb. It is widely used in traditional medicine to treat various ailments such as diarrhea, amoebiasis, diabetes, rheumatism, various bacterial infections, fever and malaria, divers pains divers pains using leaves, stem bark or root bark in decoction form, and mainly seeds.[1,2.3]

The same plant is also found in some Asiatic countries under the scientific name *Brucea javanica* (synonyms *Brucea amarissima* Desv. Ex Gomes, *Gonus amarissima* Lour, *Lour amarissima* O. Kze). The Asiatic species was more studied and more biological activities of its seed extracts and isolated principles mainly quassinoids were previously reported. These biological activities included antiplasmodial[4-8], cytotoxicity and antileukemic[9,10], anti-inflammatory, antitrypanosomal[11], antidiabetic and antioxidant.[12,13] Beside quassinoids as major constistuents.[9,10,14] other constituents like alkaloids from suspension cultures of seeds[15, 16] and lignans[17] were reported to be present in the seeds. Ethanol extract from *B. javanica* root bark was reported to be devoid with antibacterial activity against enteropathogenic bacteria like *P. aeruginosa*, *S. aureus* and *Shigella sp.* at the test concentrations from 10 to 30 mg/ml.[18]

The first study on the seeds of *B. sumatrana* growing in some African countries particularly in Democratic

Republic of Congo (RDR-Congo) was conducted by[19] on its phytochemical composition and antiprotozoal activity of lyophilized aqueous extract and its soluble fractions, 80% methanol and total alkaloids extracts. Results from this study revealed that all extracts from the seeds exhibited good antiprotozoal activity against *P. falciparum K1* strain resistant to chloroquine, *Trypanosoma cruzi* and *T. brucei brucei*, and *Leishmania infantum* with IC_{50} < 10 µg/ml. The same results were also shown by soluble fractions from the partition of the lyophilized aqueous extract with solvents of different polarities with IC_{50} values between 5 and 20 µg/ml.[19]

Thus, the present study was initiated to report the phytochemical composition and to assess *in vitro* antibacterial and bactericidal activities against a large game of bacteria involved in diarrhea, antiamoebic activity against the parasite *Entamoeba hystolitica* and antipasmodic activity on isolated guinea-pig ileum of extracts and soluble fractions from *B. sumatrana* leaves collected in Mai-Ndombe in DR-Congo.

2. MATERIALS AND METHODS

2.1. Vegetal material

Leaves of *B. sumatrana* Roxb. (Simaroubaceae) were collected in Mai-Ndombe in Democratic Republic of Congo (DR-Congo). The plant was identified at the National Institute of Studies and Researchs in Agronomy (NISRA), Departement of Biology, Faculty of Sciences, University of Kinshasa. A voucher specimen of the plant N° BSL2209014NL had been deposited in the herbarium of this institute. Leaves were dried at room temperature and reduced to powder using an electronic blender and were kept in brown bottles hermetically closed.

Figure 1: *Brucea sumatrana* leaves, immature and mature fruits.

2.2. Preparation of extracts and fractionation of lyophilized aqueous extract

50 g of powdered leaves were soaked with 300 ml distilled water and boiled on hotplate for 15 minutes. The mixture was cooled and filtered on a filter paper F001 grade (CHLAB GROUP, 08205, Barcelona, Spain) and the filtrate was evaporated to reduce volume to 10 ml which, was further lyophilized to give dried lyophilized aqueous extract denoted as BSLAE-1 (32.54 g). 20 g of BSLAE-1 extract were dissolved in 200 ml distilled water, filtered as described above, successively and exhaustively extracted with solvents of different polarities including chloroform, ethylacetate, *n*-butanol together with the resulting residual aqueous soluble fractions. They were treated as described above to yield corresponding dried extracts named as BSLAE-1.1 (4.51 g), BSLAE-1.2 (4.65 g), BSLAE- 1.3 (4.15 g) and BSLAE-1.4 (6.05 g) for chloroform, ethylacetate, *n*-butanol and residual aqueous soluble fractions respectively.[20,21]

On other hand the same amount of plant material was macerated with 80% methanol for 24 h and after filtration, the marc was exhaustively percolated with the same solvent. The macerate and percolate were combined and evaporated in vacuum to give dried extract named as BSME (38.02 g).

2.3. Qualitative phytochemical screening

The detection of major phytochemical groups such as alkaloids, flavonoids, tannins (catechic and gallic), proanthocyanidins, anthraquinones, anthocyanins, coumarins, saponins, steroids, terpenoids, reducing sugars and polysaccharides in lyophilized aqueous extract BSLAE-1of *B. sumatrana* leaves was carried out in tubes and by TLC (thin layer chromatography on silica gel 60 GF254, thickness layer 0.25 mm, Merck, Germany) using different chemical reagents and mobile phases described in literature.[19, 20]

2.4. Evaluation of biological activities

2.4.1. In vitro antibacterial activity testing

Dilution methods proposed by[22,23] were used to evaluate antibacterial and bactericidal activities of extracts and fractions of *B. sumatrana*. Clinically isolated microorganisms from Cliniques Universitaires du Mont-Amba, Kinshasa, Democratic Republic of Congo including *Escherichia coli*, *Staphylococcus aureus*, *Shigella flexneri*, *Shigella dysenteria*, *Shigella enteritidis*, *Shigella thyphimurium* were used.

Extracts and fractions from *B. sumatrana* leaves were tested at concentrations from 500 to 0.1 µg/ml. Ampicillin and Tetracycline were used as positive controls in tubes containing bacterial suspension. All tubes were incubated at 37°C for 24 h. The inhibition of

bacteria growth was evaluated by comparison with normal bacteria growth in the control holes (ethanol/water 9:1) prepared without test samples. The minimum inhibitory concentrations (MIC) were determined as the lowest concentrations of the test samples that completely inhibited macroscopic growth of bacteria. To determine the minimum bactericidal concentrations (MBC), the two lowest concentrations which inhibited bacteria growth were plated out on a nutrient agar and incubated at 37°C for 24 h. The minimum bactericidal concentrations *(MBC)* were the lowest concentrations of antibacterial agents required to kill a particular bacteria.

The test for fungi was similar to that for bacteria, except for the used medium. A suspension of fungi was prepared from a tube-culture of 3 weeks old in Sabouraud agar. Minimum inhibitory antifungal (MIA) and fungicidal (MFC) concentrations were determined.

2.4.2. *In vitro* antiamoebic testing

Entamoeba histolytica used in the present study is a laboratory isolated strain from patients with acute dysentery diagnosed in the Tropical Medicine Institute, Antwerpen, Belgium. The evaluation of the activity was performed using methods previously described by.[24, 25]

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

Uncontaminated tubes containing an average number of $2.5.x10^6$ amoebae/ml in culture medium were selected as test tubes. Samples from *B. sumatrana* leaves dissolved in ethanol/water 9:1 were tested at concentrations from 1 to 50 µg/ml. Metronidazole or Dihydroemetine respectively (20 to 0.1 µg/ml) were used as positive controls.

All tubes were plugged with sterile cotton, vigorously stirred and incubated at 37°C for one week. The daily counting of dead and living amoebae were recorded The test was considered as positive when the vegetative or kystic forms of the parasite (amoeba) was not microscopically observed. The minimum antiamoebic concentrations (MAC) were immediately determined and inhibitory concentrations 50 (IC_{50}) were derived from concentrations-responses curves by non-linear regression.

2.4.3. Assessment of antispasmodic activity on isolated guinea-pig ileum

The antispasmodic activity of samples from *B. sumatrana* leaves was evaluated on isolated guinea-pig ileum after sacrification male guinea-pigs (220-250 g body weight) according to methods described by.[26, 27] The ileum was dissected out, plentifully washed with distilled water, cuted (3 cm of ileum) and suspended in an organ bath containing 50 ml of Tyrode's solution (mM: KCl: 2.2, $MgCl_2$: 0.11, $NaH_2PO_4.2H_2O$: 0.42, $CaCl_2$:1.8, NaCl:137, $NaHCO_3$:11, glucose:5.6) or depolarizing solution rich in KCl (mM: NaCl: 2.7, KCl:100, $NaHCO_3$:15, $CaCl_2$:1.25, $MgCl_2$:12.5, glucose:11) gassed with 95% O_2 and 5% CO_2 according the case.

The isolated tissue was allowed to equilibrate for 30 minutes under a resting tension of 0.5 g before exposure to drugs and tested samples. To evaluate antispasmodic activity, the tissue was first exposed to 5.10^{-7} M acetylcholine (ACh) or depolarizing solution rich in KCl (DSR KCl) to have tree equivalent amplitudes of contractions and was after plentifully washed with Tyrode's solution to eliminate the presence of agonists in the organ bath. 2 mg of tested samples were dissolved in 2 ml distilled water to have stocks solution of 1 mg/ml. 3 ml of Tyrode solution were removed and 2 ml of each tested sample corresponding to 40 µg/ml of tested sample were added in organ bath and left in contact with isolated guinea-pig ileum for 15 minutes. The effects of tested samples on the responses elicited by both agonists were recorded after restimulation separately ileum with 1 ml of respective agonists in the presence of tested samples. The responses were recorded via a frontal writing lever on kymograph paper (Scientific and Research Instruments Ltd. England). The experiment was repeated tree times and mean percentage inhibitions of both agonist contractions in the presence of test samples were calculated using the following formula.

$$\% \text{ Inhibition} = \frac{ACag - ACts}{ACag} \times 100$$

Where ACag is the amplitude level of the contractions (cm) induced by agonists and ACts is the amplitude level of the contractions (cm) induced by each tested sample. Effective doses 50 (ED_{50}) were derived from linear doses-responses curves. Atropine sulphate and papaverine hydrochloride were used as reference antispasmodic products and tested at a concentration of 20 µg/ml in organ batch.

3. RESULTS AND DISCUSSION

3.1. Qualitative phytochemical screening

Results from qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, gallic and catechic tannins, proanthocyanidins, saponins, steroids, terpenoids, reducing sugars and polysaccharides. Anthocyanins, anthraquinones, coumarins and cardiotonic heterosides were not detected in the examined extract in our experimental conditions. Our results are in good agreement with.[28]

Table 1: Results from qualitative phytochemical screening of the lyophilized aqueous extract BSLAE-1 of *B. sumatrana* leaves.

Chemical groups	Results	Chemical groups	Results
Alkaloids	++	Gallic tannins	++
Anthocyanins	-	Proanthocyanidins	++
Anthraquinones	-	Saponins	+
Cardiotonic glycosides	-	Steroids	+++
Coumarines	-	Terpenoids	+++
Flavonoids	+++	Reducing sugars	+++
Catechic tannins	++	Polysaccharides	++

3.2. *In vitro* antibacterial activity of lyophilized aqueous extract, its soluble fractions, 80% methanol and total alkaloid extracts

In some cases, diarrhea was caused particularly by pathogen microorganisms. In these conditions, extracts and soluble fractions from *B. sumatrana* leaves were tested *in vitro* against a large game of bacteria implicated in diarrhea. For the present study, following criteria were adopted for good understanding and interpretation of results: MIC (minimal inhibitory concentrations) and MBC (minimal bactericidal concentrations) ≤ 10 µg/ml: pronounced activity, 10 < MIC, 10 < MBC ≤ 100 µg/ml: good activity, 100 < MIC, MBC ≤ 250 µ/ml; moderate activity, MIC, 250 < MBC ≤ 500 µg/ml: weak activity, MIC, MBC > 500 µg/ml inactive.

Results indicated that the lyophilized aqueous extract BSLAE-1 exhibited good antibacterial and bactericidal activities since it inhibited the growth all selected bacteria with MIC and MBC < 100 µg/ml.[22, 28] It only showed moderate activity against *S. aureus* (MIC and MBC = 125 and 250 µg/ml respectively). Chloroform soluble fraction BSLAE-1.1 rich in steroids and terpenoids showed good activity against 5 bacteria with MIC and MBC values < 100 µg/ml)[22, 28] (Table 2). This fraction exhibited moderate activity against *E.coli*, *S. thyphi* et *S. aureus* with MIC and MBC values of 125 or 250 µg/ml respectively according to the case. Ethylacetate soluble fraction BLAE-1.2 rich in flavonoids displayed good antibacterial and bactericidal activities against all selected clinically isolated microorganisms with MIC and MBC values < 100 µg/ml.[22, 28] *n*-butanol soluble fraction BSLAE-1.3 rich in saponins exhibited good antibacterial and bactericidal activity against *E. coli*, *S. dysenteria* and *S. flexneri* (MIC and MBC < 100 µg/ml[22, 28], it also displayed good antibacterial and moderate bactericidal activities against *S. enteritidis* and moderate antibacterial and bactericidal effects against *S. typhi* and *S. aureus* (Table 2) while the residual aqueous phase BSLAE-1.4 rich in phenolic compounds than flavonoids showed good antibacterial and bactericidal activity against *S. flexneri*, *S. dysenteria* and *S. enteritis* with MIC and MBC values < 100 µg/ml[22,28], good antibacterial activity against *S. dysenteria* with moderate bactericidal effect, moderate antibacterial and bactericidal effects against *E. coli* and *S. aureus* (Table 2).

Table 2: Antibacterial and bactericidal activities of extracts and soluble fractions from *B. sumatrana* leaves against clinically isolated microorganisms implicated in diarrhea.

A/B	*E.coli*		*S. dysenteria*		*S. enteritidis*		*S. flexneri*		*S.thyphi*		*S aureus*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
BSLAE-1	31.25	62.50	31.25	62.50	62.50	62.50	15.63	31.25	62.50	62.50	125	250
BSLAE-1.1	62.50	155	62.50	62.50	31.25	62.50	31.25	62.50	125	125	250	250
BSLAE-1.2	31.25	62.50	62.50	62.50	62.50	125	62.50	125	62.50	125	31.25	62.50
BSLAE-1.3	31.25	62.5	31.25	62.50	62.50	125	31.25	62.50	125	250	125	250
BSLAE-1.4	125	250	62.50	125	31.25	31.25	31.25	62.50	62.50	62.50	125	250
BSTA	7.81	15.6	31.2	62.50	31.25	31.25	15.6	31.25	31.25	62.50	31.25	62.50
BSME	15.63	31.25	15.63	31.25	31.25	62.50	7.81	15.63	31.25	62.50	31.25	62.50
Ampicilline	1.95	1.97	0.48	0.97	0.97	0.97	0.97	1..97	1.95	3.90	0.97	1.95
Tetracycline	1.56	3.25	0.97	1.95	1.95	1.95	0.97	1.95	1.90	3.90	0.97	1.95

A; microorganism, B; sample codes; BSLAE-1 : lyophilized aqueous extract, BSLAE-.1 to BSLAE-1.4: chloroform, ethylacetate, *n*-butanol and residual aqueous soluble fractions respectively form the partition of BSLAE-1 extract. E. *coli: Escherichia coli, S. dysenteria: Shigella dyesenteria, S. enteritidis: Shigella enteritidis, S. flexneri: Shihella flexneri, S. typhi: Salmonella thyphimururim* and *S. aureus: Stahylococcus aureus*, MIC: minimal inhibitory, concentration, MBC: minimal bactericidal concentration.

Moreover, it was observed that 80% methanol BSME and total alkaloids BSTA extracts exhibited good antibacterial and bactericidal against all selected clinically isolated with MIC and MBC < 100 µg/ml.[22,28] BSTA and BSME extracts seemed to have the same level of antibacterial and bactericidal activities which were

high compared to lyophilized aqueous extract BSLAE-1 (Table 2). In comparison the activities of tested samples from *B. sumatrana* leaves to antibiotic reference products, it were observed the activities of the first one were weak than the second one.

3.3. Antiamoebic activity of extracts and fractions from B. *sumatrana* against the parasite *Entamoeba hystolitica*

For good interpretation of results, following criteria were adopted: MAC, $IC_{50} \leq 10$ µ/ml: pronounced activity, 10 < MA, $IC_{50} \leq 20$ µg/ml: good activity, 20 < MAC, $IC_{50} \leq$ 30 µg/ml: moderate activity, 30 < MAC, $IC_{50} \leq 50$ µg/ml: weak activity, MAC, $IC_{50} > 50$; inactive.

Results indicated that all extracts and fractions were found to inhibit the 100% growth of the parasite *E. hystolytica* with minimal amoebicidal concentrations (MAC) < 10 µg/ml). Their effects on 50% population of the parasite were also marked with inhibitory concentrations 50 (IC_{50}) ranging from 1.85±0.02 to 6.35±0.07 µg/ml. Their activity was considered thus as pronounced. The most active samples were the total alkaloids extract BSTA showing these effects with MAC and IC_{50} values of 2.03±0.05 and 1.14±0.06 µg/ml respectively, followed by 80% methanol BSME extract (MAC and IC_{50} values of 2.75±0.02 and 1.52±0.04 µg/ml respectively) and lyophilized aqueous extract BSLAE-1 (MAC and IC_{50} values of 3.52±0.01 and 1.85±0.02 µ/ml respectively). All soluble fractions BSAE-1.1 to BSLAE-1.4 exhibited also pronounced antiamoebic activity since they inhibited the 100 and 50% of the growth of *E. hystolitica* with MAC and IC_{50} values < 10 µg/ml (Table3). All samples from *B. sumatrana* leaves were devoid with cytotoxic effects against Vero cells and produced good selective index suggesting their selective action against *E. hystolitica* with selective index values from 15.74 to 54.05. In general, the aniamoebic activity displayed by *B. sumatrana* leaves sample was weak compared to metronidazole and dihydroemetine used antiamoebic reference drugs (Table 3).

Table 3: Antiamoebic activity of extracts and fractions from *B. sumatrana* leaves against the parasite *E. hystolitica* (MAC and IC_{50}, µg/ml).

Code samples	MAC, µg/ml	IC_{50}, µg/ml	CC_{50}, µg/ml Vero cells	SI :CC_{50}/IC_{50}
BSLAE-1	3.52±0.01	1.85±0.02	> 100	> 54.05
BSLAE-1.1	8..54±0.05	5.26±0.03	> 100	> 19.01
BSLAE-1.2	4.28±0.02	2.85±0.04	> 100	> 35.08
BSLAE-1.3	6.42±0.03	4.68±0.01	> 100	> 21.36
BSLAE-1.4	5.75±0.01	3.15±0.02	> 100	> 31.74
BSTA	2.03±0.01	1.14±0.02	> 100	> 87.72
BSME	2.75±0.02	1.52±0.04	> 100	> 65.79
BSLAE-1’	10.68±0.03	6.35±0.03	> 100	> 15.74
Metronidazole	0.05±0.02	0.02±0.01	> 100	> 5000
Dihydroemetine	0.07±0.01	0.04±0.01	> 100	> 2500

See Table 2, SI: selective index

Figure 2 showed the percentage inhibitions of 100% *E. hystolitica* growth in the presence of some extracts and fractions from *B. sumatrana* tested at concentrations from 1 to 5 µg/ml. The inhibition of the parasite growth was dose-dependent. It was observed that all samples tested at the highest concentration of 5 µg/ml caused 100 % mortality of the parasite with 0% of survival. With 2.5 µg/ml, lyophilized aqueous, 80% methanol and total alkaloids extracts produced 100% mortality and 0% of survivals

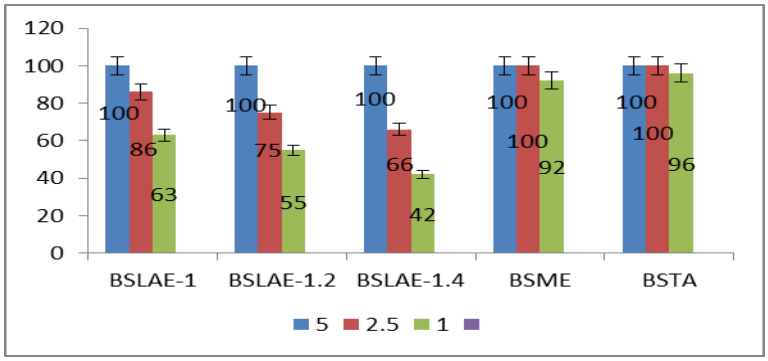

Figure 2: Percentage inhibitions of *E. hystolitica* growth by some extracts and fractions from *B. sumatrana* leaves alkaloids extracts provoked 86, 100 and 100 % of mortality with a survival of 24, 0 and 0% respectively of the parasite.

while the ethylacetate and residual aqueous soluble fractions caused 75 and 66% of mortality with a parasite survival of 25 and 37%. The mortality of the parasite significantly ($p < 0.05$) decreased with the decrease of tested concentrations (Fig. 2). At the lowest tested concentration of 1 µg/ml the survival percentage of the parasite was 37, 8, 4, 45 and 4% when lyophilized aqueous BSLAE-1, 80% methanol BSME and total alkaloids BSTA extracts, ethylacetate and residual aqueous soluble fractions, were tested respectively suggesting that BSME and BSTA extracts remained again more active in the inhibition of the parasite growth since the parasite.

3.4. Antispasmodic activity of extracts and fractions from *B. sumatrana* on isolated guinea-pi ileum

Results from the evaluation of the antispasmodic activity of *B. sumatrana* extracts and fractions were presented in Table 4. Tested at the highest tested concentration of 40 µg/ml in

Table 4: Inhibition percentages of extracts and fractions from *B. sumatrana* leaves on isolated guinea-pig ileum.

Code samples	% inhibition ACh	ED_{50}	% Inhibition of DSR-KCL	ED50
BSLAE-1	86.61±0.01	22.78±0.02	84.40±0.03	24.36±0.01
BSLAE-1.1	68.55±0.03	35.45±0.01	66.81±0.0	37.02±0.04
BSALAE-1.2	83.62±0.03	25.06±0.03	80.52±0.02	27.36±0.01
BSLAE-1.3	77.71±0.04	28.41±0.01	70.90±0.01	30.12±0.03
BSLAE-1.4	79.60±0.02	27.03±0.02	75.22±0.02	28.82±0.01
BSLAE-1’	65.70±0.05	37.65±0.02	62.34±0.04	38.00±0.004
BSAT	90.36±0.03	18.65±0.02	88.74±0.03	20.03±0.03
BSME	88.06±0.04	20.02±0.03	86.24±0.02	22.36±0.01
Atropine	100.00±0.00	10.03±0.01	-	-
Papaverine	99.25±0.03	12.03±0.01	98.65±0.01	14.03±0.03

See Table 2

organ bath of 50 ml, high activity was shown by total alkaloids, 80% methanol and lyophilized aqueous extracts producing more than 84% inhibition of contractions induced by acetylcholine (ACh) and depolarizing solution rich in KCl (DSR-KCl). For this, it was observed that against ACh contractions, lyophilized aqueous BSLAE-1, 80% methanol BSME and total alkaloids BSTA produced percentage inhibitions of 86.61±0.01, 88.06±0.04 and 90.36±0.02% respectively, and against DSR-KCl, they caused 84.40±0.03, 86.24±0.02 and 88.74±0.03% percentage inhibitions respectively of contractions induced by this agonist on isolated organ. In both cases, it appeared that BSTA extract exhibited high antispasmodic activity compared to BSLAE-1 and BSME extracts as well as all soluble fractions. Concerning soluble fractions, the high activity was developed by ethylacetate soluble fraction causing a percent inhibition of 83.62±0.03 and 80.52±0.02 of contractions induced by ACh and DSR-KCl on isolated organ respectively. It was followed by the residual aqueous and *n*-butanol soluble fractions showing 79.60±0.02 and 77.71±0.04 % inhibition of contractions induced by ACH and 75.22±0.02 70.90±0.01% inhibition of the same effect against DSR-KCl on isolated organ respectively. Chloroform soluble fraction showed also good inhibition of contractions induced by both agonists from 66 to 69% (Table 4). As papaverine used as an antispasmodic reference drug, all samples from *B. sumatrana* posessed papaverine-like effects.[26,28]

To know whether if the antispasmodic activity shown by samples from *B. sumatrana* leaves was due the blockage of Ca^{2+} channel, the high concentration of K^+: mM as KCl was used for the preparation in DSR-KCl. For this, it was well known that the concentration of K^+ higher than 30 mM as proposed by[29] caused contractions of smooth muscles through the dependent voltage of L-type of Ca^{2+} channels provoking thus an extracellular influx of Ca^{2+} ions causing a contractile effect.[29] Thus, a substance that caused inhibition of high concentration K^+ inducing contractions was considered as a blocker of Ca^{2+} influx.[30] In the present study, high concentration of 100 mM KCl was used. Our results showed that all samples from *B. sumatrana* leaves relaxed this high concentration ions by inhibiting contractions of isolated organ induced by DSR-KCl. They were thus considered as antagonists of Ca^{2+} producing relaxation of smooth muscles via the decrease of free intracellular Ca^{2+} through respective mechanisms of hyperpolarization of membrane and inhibition of Ca^{2+} ion towards channels blockage as suggested by.[31] Antagonisms of calcium formed an important therapeutic group and their common characteristic was their inhibition of slow entrance of Ca^{2+} which was dose-dependent and their capacity of change of this effect by Ca^{2+}.[32] Thus, the spasmolytic activity showed by samples from *B. sumatrana* leaves served as mediators towards probably the effect of Ca^{2+} which explained their therapeutic uses in hyperactive disorders like addominal colics and diarrhea as particularly the lyophilized aqueous extract from *B. sumatrana* leaves was used in these disorders as also previously reported by Gliani et al (2006a)[30] for the hydro-aqueous extract of *Morinda citrifolia* roots.

The antispasmodic activity displayed by samples from *B. sumatrana* leaves seemed to be due to the presence of some secondary metabolites identified in this plant part like alkaloids, flavonoids, steroids and terpenoids saponins and tannins because these metabolites or phytochemical groups which were previously reported to

exhibit antispasmodic activity at different extents in different pharmacological models.[33-46]

4. CONCLUSION

The present study had revealed for the first time the antibacterial, antiamoebic and antispasmodic activities of extracts and fractions from *B. sumatrana* leaves collected in Mai-Ndombe in Democratic Republic of Congo. Results indicated these samples from *B. sumatrana* leaves exerted antibacterial and bactericidal activities with different magnitudes against a large game on microorganisms implicated in diarrhea. In addition to their antispasmodic and antiamoebic activities, these reported biological activities can partly support the use of the plant part in popular medicine (traditional medicine) in DR-Congo and other African countries for the treating of diarrhea according its origin.

REFERENCES

1. Ngoma B, Kikenge M. Therapeutic application of *Brucea sumatrana* Roxb. (Simaroubaceae). Al-Birunya, 1993; 9 910: 101-108.
2. Briruniya AL. Recueil des plantes utilisées en médecine taditinnelle Zairoise. Rev Med Pharm, 9(1): 101-115.
3. Neuwinger HD. African traditional medicine. A dictionary of plant uses and application. Medipharm Scientific Publishers. Stuttgart.
4. O'Neill MJ, Bray DH, Boardman P, Chan KL, Phillipson JD, Warhurst DC, Peters W. Plants as a sources of antimalarial drugs. Part 4; Activity of *Brucea javanica* fruits against chloroquine-resistant *Plasmodium falciparum* in vitro and *Plasmodium berghei*. J Nat Prods, 1987; 50(1): 41-48.
5. Camacho MDR, Phillipson JD, Croft SL, Solis PN, Marshall SJ, Ghazamfar SA. Screening of plant extracts for antiprotozoal and cytotoxic activities. J Ethnopharmacol, 2003; 89(2-3): 185-191.
6. Nguyen-Poulin J, Tran H, Tran H, Pha TA, Dlecek C, Farrar J, Tran TH, Caron R, Bodo BM Grelleir P. 2007. Antimalarial and cytotoxic activities of ethnopharmacologically selected medicinal plants from South Vietnam. J Ethnopharmacol, 2007; 109(3): 417-427.
7. Sriwilaijaroen N, Kondo S, Nanthasri P, Auparakkitamon S, Suzuki Y, Vilariat P. Antiplasmodial effects of *Brucea javanica* (L.) Merr. and *Eurycoma longifolia* Jack and their combination with chloroquine and quinine on *Plasmodium falciparum* culture. Trop Med Health, 2010; 38(1): 61-68.
8. Chumkaeuw P, Srisawat. Antimalarial and cytotoxic quassinoids from roots of *Brucea javanica*. Int Asian Nat Pprod, 2017; 19(3): 247-253.
9. Lee KH, Imakura Y, sumida Y, Wu RY, Haal IH, Huang HC. 1979. Antitumor agents. 33. Isolation and structure elucidation of bruceoside A et B, novel antileukemic quassinoid glycosides and brucein D and E from *Brucea java*nica. J. Org. Chem., 1979, 44(13): 2180–2185.
10. Sakadi T, Yoshimura S, Tsuyuki T, Takahashi T, Honda T. Yadanzioside P. 1986. Yadanzioside P. A new antileukemic quassinoid glycoside from *Brucea javanica* (L.) Merr with the 3-O-(beta-D-glucopyranosyl)-bruceantin. Structure. Chem Pharm Bull, 34(10): 4447-4450.
11. Bawn BS, Matssura H, Elkhateeb A, Nabeta K, Subeki, Nonaka N, Oku Y, Katakura K, 2008. Antitrypanosomal activities of quassinoids compounds from the fruits of a medicinal plant, *Brucea javanica*. Vet Parasitol, 158(4): 288-294.
12. Ablat A, Mohamad J, Awang K, Slipi JA, Arya A. Evaluation of antidiabetic and antioxidant properties of *Brucea javanica* seed. The Scient World J. 2014; http://dx.doi.org/10.1155/20214:786130**,** 1-8.
13. Ablat A, Halabi MF, Mohamad J, Hasnan MHH, Hazni H, Slipi JA, Mohamed Z, Awang K. 2017. Antidiabetic effects of *Bruces javanica* seeds in type diabetic rats. BMC Complement Alternat Med, 2017; 17(1): 1-31.
14. Pan L, Chin LW, Chai HB, Ninh TN, Soejario DD, Kinghorn AD. 2009. Bioactivity-guided isolation of cytotoxic constituents of *Brucea javanica* collected in Vietnam. Bioorg Med Chem, 17(6): 2219-2224.
15. Karin CS, Liu, Shi-Lin Yang, Margaret FR, J. David Phillipson JD. Canthin-6-one alkaloids from cell suspension cultures of *Brucea javanica*. Phytochemistry, 1990; 29(1): 141-143.
16. Wagih ME, Alam G, Wiryodagdo S, Attia K. 2008. Improved production of the indole alkaloid canthin-one from cell suspension culture of *Brucea javanica* (L.) Merr. Indian Sci Technol, 2008; 1(1): 1-6.
17. Luyengi L, Suh N, Fong HHS, Pezzuto JM, Kinghorn AD. 1996. A lignan and four terpenoids from *Brucea javanica* that induce differentiation with cultured HI-60 promyelocytic leukemia cells. Phytochemistry, 43: 409-412.
18. Helmi H, Agung NA, Susanti I. Antibacterial activity of belilik (*Brucea javanica* (L). Merr) and benta (*Wikstroemia androsaemofolia* Decne) to inhibit the growth of enteropathogenic bacteria, 2016; DOI: 10.23869/bphjbr.21.1.20154.
19. Tshodi EM, Mbenza PA, Nsak LS, Kikweta MC. Bakan PD. Tona LG, Kamu KO, Cimanga KR., Matheussen A, Cos, P, Apes s, Pieters L, Maes L, Vlietinck AJ. 201. *In vitro* antiprotozoal and cytotoxic activity of aqueous extract, the 80% methanol extract and its fractions from the seeds of *Brucea sumatrana* Roxb. (Simaroubaceae) growing in Democratic Republic of Congo. Chinese Med, 2012; 3(1): 65-71.
20. Harborne JB. 1998. Phytochemical methods. A guide to modern techniques of plants analysis. Chappama & Hall. London.
21. Trease GE, Evans MC. Textbook of Pharmacognosy, 14th edition. London. Bailliere, Tindal, 2000.
22. Vanden Berghe D.A., Vlietinck, AJ. Screening of antibacterial and antiviral agents In: Plant

Biotechmistry. Vol. 6. Assays for Bioactivity. Ed. K. Hostettmann. Academic Press, London, 1991.
23. Cimanga K., De Bruyne T, Van Poel Bart, Pieters L, Claeys M, Vanden Berghe D, Tona L. Kambu K, Vlietinck AJ. 1998. Antibacterial and antifungal activities of neocryptolepine, biscryptolepine and cryptoquindoline, alkaloids from *Cryptolepis sanguinolenta*. Phytomedicine, 5: 2009-214.
24. Tona L, Kambu O, Mesia K, Cimanga K, Apers S, De Bruyne T, Pieters L, Totté J, Vlietinck AJ. Biological screening of traditional preparations from some medicinal plants used as antidiarrhoeal in Kinshasa, Congo. Phytomedicine, 1998; 6(1): 59-66.
25. Cimanga K, Kambu K, Tona L, Hermmans N, Totté J, Pieters L, Vlietinck AJ. 2006a. Antiamoebic activity of iridoids from *Morinda moridoides* leaves. Planta Med, 72(8): 751-753.
26. Cimanga K., Mukenyi PNK, Kambu K, Tona L, Apers S, Totté J, Pieters L, Vlietinck AJ. 2010. The spasmolytic activity of extracts and some isolated compouds from the leaves of *Morinda morindoides* (Baker) Milne-Redh. (Rubiaceae). J Ethnopharmacol, 2010; 127(2): 215-220.).
27. Bashir S, Memon R, AnwarH.Gilani A. Antispasmodic and antidiarrheal activities of *Valeriana hardwickii* Wall. rhizome are putatively mediated through calcium channel blockade. Evid-Based Complement Alternat Med 2011; Article ID 304960, 6 pages, doi:10.1155/2011/304960
28. Cimanga KR, Tshodi EM, Nsaka LS, Kikweta MC, Mbenza AP., Cos P, Maes L. Kambu K O, Apers S, Vlientick AJ, Pieters L. Antiprotozoal and cytotoxic activities, and acute toxicity of extracts from *Brucea sumatrana* leaves collected in Mai-Nombe, democratic Republic of Congo. J Pharmacgno Phytother, 2015; 7(4): 34-44.
29. Rio JL, Recio MC. 2005. Medicinal plants and antibacterial activity. J Ethnopharmacol, 100; (1-2): 80-84.
30. Gilani AH, Khan AK, Ghyur MN, Ali SF, Herzig JW 2006a. Antispasmodic effects of rooibos tea (*Aspalathus lienearis*) is mediated predominantly through K^+ channel action. Basic Clin Pharmacol Toxicol, 2006a; 99(5): 365-373.
31. Godfraind T, Miller R, Wiino M. 1986. Calcium antagonism and calcium entry blockade. Pharm Rev, 38(4): 321-416.
32. Kaaraki H, Ozaki M, Hori M, Mtsui-Sito K, Amano K, Haradan S, Miyamoto H, Nakazawa KJ, WO KS. 1997. Calcium movements, distribution and functions in smooth muscle. Pharmacol Rev, 49(2): 157-230
33. Fleckensein A. 1997. Specific pharmacology od Ca^{++} In myocardium cardiac pacemakers and vascular smooth muscle. Rev Pharmacol Toxicol**,** 17(1): 149-166**.**
34. Calixto JB, Unes RA, Neto AS, Valle RM, Rae GA. 1984. Antispasmodic effects of an alkaloid extract from *Phyllanthus sellowuianus*; a comparative study with papaverine. Bazilian J Biol, 1984; 17(2): 313-321.
35. Ogomgbamila FO, Samuelson, G. 1990. Smooth muscle relaxation flavonoids form *Alchornea cordifolia*. Acta Pharmaceutical Nordica, 2(6): 421-422.
36. Capasso A Pinto a, Sorrentino R, Capasso F. 1991. Inhibitory effects of quercetin and other Flavonoids electrically-induced contractions on guinea-pig isolated ileum. J Ethnopharmacol, 1991; 34(2-3): 279-281.
37. Capasso A, de Feo V, de Simone F Sorrentrino L. 1997. Activity-directed isolation of spasmolytic (anticholinergic) alkaloids from *Brugmania arbórea* (L.) Lagerheim. Int J Pharmacogn, 1997; 35(1): 43-48.
38. Aoki K, Alma Rosa Cortés AR, Ramírez MDC, MGHFJ. Pharmacological Study of Antispasmodic Activity of *Mirabilis jalapa* Linn flowers. J Ethnopharmacol, 2008; 106(1): 96-101.
39. de Medeiros CLC, Thomas G, Mukkhereje R. 1991. The source of Ca^{+2} for spasmolytic actions of longicaudine, a bisindole alkaloid isolated from *Strychnos trinevis* (Vell.) Mart. (Loganiaceae). Phytother Res, 5(1): 24.-28.
40. Da Silva BA, De Arraújo Filho AP, Mukerjee R, Chiappeta A. 1993. Bisnorhidroxydihydroxytoxiferine and vellosimine from *Strychnos divacans* root: spasmolytic properties of bisnordihydroxitoferine. Phytother Res, 1993; 7(6): 419-424.
41. Bigoniya P**.** Antidiarrhoeal and antispasmodic activity of steroidal alkaloid fraction from *Wrightia tinctoria* bark, 2009; Pharmacologyonline, 2009; 3: 298-310.
42. Corea G, Fattorusso E, Lanzotti, Capasso R, Izzo AA. 2005. Antispasmodic saponins from bulbs of red onion, *Allium cepa* L. var. *Tropea*. J Agricult Food Chem, 53(4): 935-940.
43. Gilani AH, Mankukhail SR, Iqball J, Yasinzai M, Aziz N, Khan A, Najeed-ur Rhman. 2006b. Antispasmodic and vasodilator activities of Morinda citrifolia root extract are mediated through blockade of voltage dependent calcium channels. BMC Complement Alternat Med, 2006b; 10(1): 3-9.
44. Naz SB, Chaudhary B, Reman MS. 2016. Dual receptor blocker mechanism attributes smooth muscle relaxant od *Polypodium vulgare* Linn. Bandaglesh J Pharm, 11(2): 414-420.
45. Zhao M., Xian YF, Ip SP, Fong HHS, Che CT. 2010. A new weakly antispasmodic protoberrine alkaloid from *Rhina cotidis*. Phytother Res, 24(9): 1414-1416.
46. Asifa S, Mueen A, Madiha H, Alangeer Safur RM, 2017. Pharmacological evaluation of antispasmodic and bronchodilator effects of *Spinacia oleraceaes*. Ame J Phytomed Clin Ther, 5(1): 1-5.
47. Silva FL, da Silva JLV, Silva JM, Marcolin LSA, Nouaillhetas VLA, Yoshida M, Vendramini H, Eberlin MN, Barbosa-Filho JM, Moreno PRH. 2017.

Antispasmodic activity of *Sejania carasana* fractions and their safety. Rev Brasiliera de Farmacogn, 27(1): 1-15.