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EFFECTS OF EXTRACTS AND FRACTIONS FROM *BRUCEA SUMATRANA* ROXB. (SIMAROUBACEAE) LEAVES ON CASTOR OIL AND MAGNESIUM SULPHATE-INDUCED DIARHHEA IN ANIMAL MODEL

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ABSTRACT
In the present investigation, from experimental data obtained in castor oil and magnesium sulphate-induced diarrhea in Wistar rats, the administration of these two cataractic agents led to the production of copious diarrhea in untreated animals during 4h characterised by a decrease of onset time to 75.2±0.3 and 84.3±0.02 minutes in castor oil and magnesium sulphate induced diarrhea respectively and increase of their all diarrheic parameter levels like wet and hard faeces, and secreted intestinal liquid volume. On the other hand, the administration of lyophilized aqueous extract and its soluble fractions chloroform, ethylacetate, *n*-butanol and residual aqueous phase as well as 80% methanol and total alkaloids extracts from *Brucea sumatrana* leaves, in these diarrheic animals, carried significant increase of onset times from 129.3±0.2 to 162.3±0.2 minutes and of 141.1±0.3 to 178.2±0.1 minutes in castor oil and magnesium sulphate test respectively, at the highest oral dose of 200 mg/kg bodyweight. This effect was characterised by significant reduction of all diarrheic parameter levels cited above and finally the reduction of the production of defecation and diarrrhea in treated animals compared to untreated group. Samples of *B. sumatrana* leaves caused the inhibition production of diarrhea induced by castor oil with percentage inhibitions from 63.3±0.1 to 92.8±0.1% and defecation with 61.0±0.3 to 90.2±0.3% respectively while in magnesium sulphate test, diarrhea and defecation were inhibited by 61.2±0.1 to 90.3±0.2% and 63.1±04 to 85.4±0.2% respectively. At the same oral dose, extracts and fractions from *B summatrana* leaves caused significant reduction of gastro-intestinal motility of charcoal meals, intestine content, volume and intraluminal fluid accumulation in gastro-intestinal motility test. In castor oil induced enteropooling, they caused significant reduction of the mean weight and mean volume of small intestine content (63.01±0.03 to 84.61±0.02% respectively). In all tests, 80% methanolextract showed high activity compared to lyophilized aqueous, its soluble fractions and total alkaloids extract. Thus, they possess good and interesting antidiarrheic activity in experimental animal model. The use of the plant part to treat diarrhea in traditional medicine seemed to be supported and justified by these reported results in animal model in DR-Congo and other African countries where it is used for the same purpose.

KEYWORDS: *Brucea sumatrana*, leaves, antidiarrheic activity.

INTRODUCTION
Diarrhea in the passage of liquid or water stools three or more times per day. It is a major cause of morbidity and mortality for malnourished children because of poor socio-economic status and sanitary in mainly developing countries and others. The etiology of diarrheic disorders is multifactorial, attributed to factors such as infectious agents and their toxins, increased fluid secretion, malabsorption of biliary salts.[1] food allergies and some medications, like antibiotics.[2] Diarrhea is responsible for up to 5 million deaths each year.[3] especially children of less than 5 years, corresponding to 5000000 deaths annually in developing countries and associated with factors such as poor home environments, undernutrition and lack of access to essential services.[4] Although it is well known that diarrhea can have infectious origin provoked by the effects of some bacteria such as *Escherichia colis, Clostridium difficile, Salmonella thyphimurium, Shigella dysenteria, S. flexneri, Staphylococcus aureus* and *Vibrio cholera*, and the parasite *Entamoeba histolytica*, its cause in traditional medicine practises is often unknown because of the lack

of a specific and precise diagnosis. Some chemical like castor oil and magnesium sulphate can also generate the disease by bad use.[5] In addition, some other complex factors related to the disease including the living of people in areas of poor sanitation and socio-economic status, poor life style environmental conditions and the non-availability to guaranteed conventional medical treatment came more again to complicate the situation of patients. The cost of conventional antidiarrheic drugs in villages and sometimes in towns is highly coupled with their non-availability, reason the need for an alternative treatment of the disease easyl accessible to mainly local people.[6]

For the treatment of the disease, antimotility and antisecretory agents are the mainstay in the treatment of diarrhea. Opioids and their derivatives continue to be widely used in the treatment of the disease. Difenoxin, diphenoxylate, immodium, bismuth subsalicylate, kaopectate, pepto-bismol and loperamide etc. are commonly used as antidiarrheal drugs. There are also many other drugs that have antimotility or/and antisecretory effects on the intestine and can be used for the treatment of diarrhea.[7,8] Antibacterial and antiamoebic agents are also able to reduce the severity and duration of infectious diarrhea.[9] Most of the enteropathogens which cause persistent diarrhea are treatable with antimicrobial therapy.[10] Generally, fluoroquinolones have become the drugs of choice for the empirical treatment of acute diarrhea in adults. On the other hand, third-generation cephalosporins have been considered as the best drugs for the treatment of severe acute infectious diarrhea in children.[11]

Taking account of the frequent use of more medicinal plant species in traditional medicine for the treatment of diarrhea by traditional practitioners, scientific investigations in different pharmacological models are nowadays performed to prove their potentiality to cure and stop diarrhea mainly in animal model, or to evaluate other biological activities such as antibacterial and antiamoebic activities, which can at least, justify their antidiarrheic properties. Results from these investigations had shown that many medicinal plants used to treat empirically diarrhea were scientifically reported to possess antidiarrheic properties mainly in animal model.[12-18] or by exhibiting these biological activities at some extents. In some cases, active principles belonging to different chemical groups were isolated and reported.[19-24]

Brucea sumtrana have the same medical values compared to the Asiatic species *Bucea javanica* concerning the use of its seeds: treatment of fever, malaria, amoebiasis, abdominal pains, cough, haemorrhoids, corns, warts, diabetes and trypanosomiasis,[25,26] According to informations from traditional practitioners in DR-Congo, leaves of *B. sumstrana* are used as aqueous decoction to treat the same illness including diarrhea, rheumatism, trypanosomiasis, malaria, fever, and amoebiasis, etc. Thus the present investigation was undertaken to evaluate the antidiarrheic activity of lyophilized aqueous extract and its soluble fractions, 80% methanol and total alkaloids extracts from *B. sumatrana* leaves collected in Mai-Ndombe in Democratic Republic of Congo in experimental animal. Recently, its antimicrobial, spasmolytic and antiamoebic activities were reported,[27]

2. MATERIALS AND METHODS

2.1. Plant material

Leaves of *B. sumatrana* Roxb. (Simaroubaceae) were collected in Mai-Ndombe in Democratic Republic of Congo (DR-Congo). It 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^{o} 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 kepted in brown bottles hermetically closed.

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

2.2. Preparation of extracts and fractionation of lyophilized aqueous extract

50 g of powdered leaves were mixed 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). The filtrate was evaporated in vacuum to reduce volume solution which was further lyophilized to give lyophilized aqueous extract denoted as BSLAE-1 (32.54 g). 20 g of BSLAE-1 were dissolved in 200 ml distiller water, filtered as described above and successively and exhaustively extracted with solvents of different polarities chloroform, ethylacetate, *n*-butanol together with the resulting residual aqueous phase.

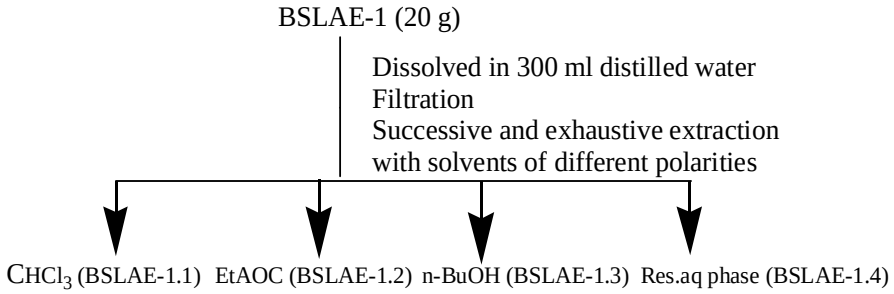

Figure 1: Fractionation of lyophilized aqueous extract BSLAE-1.

All fractions were treated as described above yielding corresponding dried extracts denoted as BSLAE-1.1 (4.51 g, BSLAE-1.2 (5.65 g), BSLAE- 1.3 (3.15 g) and BSLAE-1.4 (6.05 g) [28, 29] corresponding to chloroform, ethylacetate, *n*-butanol and residual aqueous soluble fractions respectively.

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

Detannification of the lyophilized aqueous extract: 10 g of lyophilized aqueous extract BSLAE-1 was dissolved in 10 ml methanol and submitted to column chromatography on Polyamid SC-6 (0.0-0.16 mm, Macherey-Nagel, Germany , 60 x 2 cm) eluted with the same solvent until to obtain unclear eluate. In this way tannins remained on column and eluate no containing tannins was collected and evaporated in vacuum as described above to give a dried detannified extract (3.58 g).[30]

2.3. Qualitative phytochemical screening

The major phytochemical groups in lyophilized aqueous extract BSLAE-1 of *B. sumatrana* leaves were detected in tubes and by TLC (thin layer chromatography on silicagel plates, thikness layer 0.25 mm, Merck, Germany) using different chemical reagents and mobile phases described in literature.[28,29]

2.5. Castor oil-induced diarrhea in Wistar rats

The methods used were previously described by.[16,31,32] Wistar rats of 145-157 g bodyweight (bw) of either sex were divided into 5 groups (6 rats for each oral dose of tested extracts and fractions). Wistar rats having orally received 0.5 ml castor oil produced copious diarrhea. They were fasted for 12 hours and randomly allocated and divided in 5 groups for the treatment as followed:

- Group I orally received distilled water 5 ml/kg bw, (2 rats) as negative control,
- Group II received atropine 5 mg/kg bw, (2 rats) as positive control,
- Groups IIIa and IIIb received lyophilized aqueous extract BSLAE-1 from *B. sumatrana* leaves and group IIIc received the detannified extract BSLAE-1' form BSLAE-1 (5 rats for each oral dose) in the same way 100 and 200 mg/kg bw of respective extract,
- Groups IVa and IVb, Va and VB, VIa and VIb, VIIa and VIIb BSLAE-1.1 to BSLAE-1.4 as chloroform, ethylacetate, *n*-butanol, residual aqueous soluble fractions respectively (5 rats for each oral dose). received orally the same oral doses of respective extract,
- Group VIIIa and b, IXa and b received in the same way oral doses of 100 and 200 mg/kg bw of 80% MeOH et total alkaloids extracts respectively (5 rats for each oral dose).
- Sixty minutes after pretreatment with each selected sample with respective oral doses, each animal was orally administered 0.5 ml castor-oil and the appearance of the first diarrhea drops was noted (the onset time). The treatment of diarrhea severity was assessed for 4 hours with samples from *B. sumatrana* leaves and reference product atropine. The defecation and diarrhea were observed continued up to 4 h. After drying paper filters containing wet faeces at 50°C in steamroom for 1h until constant weight, the mean water content was calculated and the weight of hard faeces was recorded. Other parameters such as the onset time, the number of wet faeces were recorded after 4 h of observation. The percentage inhibitions of diarrhea and defecation drops were calculated using the following formula:

$$\% \text{ Inhibition of diarrhea } \frac{Dc - Ds \times 100}{Dc}$$

Where Dc is the mean number of drops (dry and wet diarrheic droppings) caused by castor oil or magnesium sulphate (negative control group) and Ds the mean number of drops caused by the test samples (treated groups).

$$\% \text{ Inhibition of defecation } = \frac{Dfc - Dfs \times 100}{Dfc}$$

Where Dfc is the mean number of defecation caused by negative control group and Dfs the number of defecation caused by test samples (treated groups).

2.6. Magnesium sulphate-induced diarrhea

Methods used, were the same as for castor oil-induced diarrhea.[16, 31, 32] But in this case, diarrhea was induced

by oral administration of magnesium sulphate (2 g/kg bw) to animals grouped in the same way as described above. 30 minutes after pretreatment with each selected sample at the oral doses of 100 or 200 mg/kg respectively, pretreated received orally 5 ml of magnesium sulphate and produced copious diarrhea in 4 h of observation. They were afterwards treated with extracts and fractions from *B. sumatrana* samples as well as the reference product atropine. The mean onset time, total number of wet and hard faeces and secreted intestinal fluid in 4 h were recorded. The percentage inhibitions of diarrhea and defecation by tested samples were calculated using the same above formula described in castor-oil experiment.

2.7. Gastro-intestinal motility test

The gastro-intestinal motility was evaluated according to the methods previously described by [33-35]. Wistar rats were fasted for 18 h in individual cages and divided in the same groups as described above. Animals were grouped as followed and orally administered each sample:

1. Groups I received 5% gum acacia as negative control,
2. Group II received atropine (5 mg/kg bw) as positive control,
3. Groups IIIa and IIIb received 100 and 200 mg/kg of lyophilized aqueous extract BSLAE-1 form *B. sumatrana* leaves and group IIIc received the detannified extract from BSLAE-1 at oral dose of 200 mg/kg bw,
4. Groups IVa and IVb, Va and Vb, VIa and VB, VIIa and VIIb received each extract of soluble fraction at the same oral doses as mentioned above.
5. Groups VIIIa and VIIb, IXa and IXb received 100 and 200 mg/kg bw of total alkaloids BSTA and 80% methanol BSME extracts respectively (5 rats for each oral dose).

Thirty minutes after administration with test samples, each animal in each group was administered with 1 ml charcoal meal (5% activated charcoal in 5% gum acacia) as peristaltic marker. After 30 min, all animals were under anesthesia and killed. The intestine was removed. The total length of the small intestine (TLSI=A), and the distance covered by charcoal meal in the small intestine (DCCM=B) were recorded. The intestinal length moved by the charcoal meal from the pylorus towards the caecum was measured. The percentage inhibition transit was calculated using the following formula:

% Inhibition transit = A- B/ x 100.

Where A is the distance travelled by charcoal in negative group and B the distance travelled by charcoal in treated groups.

2.8. Castor oil induced-enteropooling

Intraluminal fluid accumulation was determined by methods described by.[16,35,36] Animals were fasted for 24 h, but allowed free access to water. Rats were divided in groups of 5 animals each and were administered orally 200 mg/kg bw of extracts and fractions from *B. sumatrana* leaves:

- Group I received normal saline (2 ml/kg, p.o) as a negative control,
- Group II received atropine (5.0 mg/kg p.o.) as positive control,
- Groups III received lyophilized aqueous extract BSLAE-1,
- Groups IV, V, VI an VII received the same oral doses of respective extracts of soluble fractions,
- Groups VIII and IX received orally the same oral dose of 80% MeOH et total alkaloids extracts respectively.

One hour after the administration of castor oil 0.5 ml/rat, animals were sacrificed by cervical dislocation. Their abdomen was open and the whole length of the intestine from the pylorus to the caecum, was binded, intestine dissected, carefully removed and washed with distilled water. The small intestine was weighted and the intestinal content was collected by milking into a graduated tube to measure the volume. The empty intestine was reweighted and the difference between the two weights was calculated. The percentage of reduction of intestinal secretion and weight of intestinal content were determined by using the following formula:

$$\% \text{ Inhibition of secretion by using MVSIC} = \frac{\text{MVICC - MVICT}}{\text{MVICC}} \times 100$$

Where MVSIC is the mean volume of the small intestinal content, MVICC is the mean volume of the intestinal content of the negative control group and MVICT is the mean volume of the intestinal content of the treated animals.

$$\% \text{ Inhibition of weight by using MWSIC} = \frac{\text{MWICC - MWICT}}{\text{MWICC}} \times 100$$

Where MWSIC is the mean weight of the small intestinal content, MWICC is the mean weight of the intestinal content of the negative control group and MWICT is the mean weight of the intestinal content of treated animals.

2.11. Statistical analysis

Data were analyzed using SPSS statistical software, version16. Results were expressed as means ± SEM. Comparisons between groups were made using ANOVA followed by post hoc Tukey's multiple comparison test. At 95% confidence interval of $p < 0.05$, the difference between the compared groups was considered as statistically significant.

3. RESULTS AND DISCUSSION

3.1 Phytochemical screening

Results from the phytochemical screening of lyophilized aqueous extract BSLAE-1 of *B. sumatrana* leaves indicated the presence of alkaloids, flavonoids, steroids and terpenoids, catechic and gallic tannins, proanthocyanidins, amined compounds, saponins,

reductor sugars, polysaccharides. Anthocyanidins, coumarins, amthraquinones and cardiotonic heterosides were not detected in this extract in our experimental conditions. Our results are in good agreement with Tshodi et al.[27]

Table 1: Results of qualitative chemical screening.

Chemical groups	Results	Chemical groups	Results
Alkaloids	++	Tannins	++
Anthocyanidins	-	Catechic tannins	++
Anthraquinones	_	Gallic tannins	++
Coumarins	-	Proantocyanidins	++
Cardiotonic heterosides	_	Reductor sugars	++
Polysaccharides	++	Steroides and terpenoides	++
Amined compounds	++	Saponins	+

3.2. Effects of *B. sumatrana* samples against castor oil-induced diarrhea in Wistar rats

In the present study, it was observed that the oral administration of castor-oil induced copious diarrhea in untreated Wistar rats during 4h of observation characterized by the decrease of onset time 75.2±0.3 minutes and increase of their all diarrheic parameter levels (Table 2). The active constituent of this oil from *Ricinus comminus* L. (Euphorbiaceae) inducing diarrhea is ricinoleic acid liberated from the action of lipases on the oil. It is well known that this acid produced irritation and inflammatory effects on the intestinal mucosa leading to the release of prostaglandins. This condition induced an ion increasing in permeability of the mucosal cells and change in electrolytes transport which resulted in a decrease of Na^+ and K^+ absorption, stimulating thus, the peristaltic activity and caused diarrhea.[37]

Considering the severity of diarrhea provoked in untreated groups by the administration of castor oil, the administration of *B. sumatrana* lyophilized aqueous extract BSLAE-1 and its soluble fractions BSLAE-1.1 to BSLAE-1.4 as well as the detannified aqueous, 80% methanol and total alkaloids extracts in these diarrheic Wistar rats, led to significant increase of onset times from 120.3±0.3 to 162.3±0.2 minutes and caused dose-dependent reduction of their all diarrheic parameter levels like wet and hard (dry) faeces, and secreted intestinal liquid volume. These effects resulted finally in significant reduction of diarrhea and defecation intensity or production induced by castor oil in treated Wistar rats at different extents compared to untreated group (Table 2).

At the highest oral dose of 200 mg/kg bw, the administration of lyophilized aqueous extract BSLAE-1 significantly ($p < .05$), increased the onset time at 151.6±0.4 min of treated diarrheic animals compared to negative control with 75.2±0.03 min, and significantly ($p < 0.05$) lowered their all diarrheic parameter levels compared to untreated group (Table 2) resulting in remarkable reduction of diarrhea by 91.36±0.02% and of defecation by 85.84±0.04% after 4h of observation.

Its soluble fractions caused also to the same effects as the increase of onset times from 129.3±0.2 to 158.2±0.2 min compared to untreated group with an onset time of 75.2±0.3 min and caused significant decrease of diarrheic parameter levels at different extents of treated diarrheic animals compared to untreated group (Table 2). They produced percentage inhibitions of diarrhea from 64.7±0.3 to 84.9±0.1% with the ethylacetate BSLAE-1.2 soluble fraction rich in flavonoids as the most active (84.9±0.01%) followed by the residual aqueous BSLAE-1.4 soluble fraction rich in phenolic compounds other than flavonoids (83.4.±0.1%) and *n*-butanol BSLAE-1.3 soluble fraction rich in saponins (71.6±0.01%). Chloroform.

Table 2: Effects of *B. sumatrana* leaves samples on castor oil-induced diarrhea in Wistars rats.

Sample codes	D	OT	WF	HF	IFV	% IDE	% IDIA
Negative control I	5 ml water	75.2±0.3	13.9±0.3	11.3±0.1	4.3±0.1	-	-
Atropine II	5	192.3±0.1	0.6.±0.1	0.4±0.2	0.2±0.0	94.7.±0.2	97.1±0.1
BSLAE-1 IIIa	100	134.3±0.1	1.9±0.1	1.7±0.3	0.2±0.0	83.8±0.2	87.7±0.3
IIIb	200	151.6±0.4	1.6±0.1	1.4±0.2	0.4±0.0	85.8±0.4	91.7±0.2
BSLAE-1' IIIc	200	89.02±0.1	9.9±0.3	7.8±0.3	1.2±0.3	30.9±0.4	28.7±0.1
BSLAE-1.1 IVa	100	120.3±0.3	5.1±0.2	4.8±0.3	0.3±0.1	57.5±0.3	63.3±0.1
IVb	200	129.3±0.2	4.9±0.3	4.4±0.2	0.5±0.0	61.0±0.1	64.7±0.2
BSLAE-1.2 Va	100	142.4±0.2	3.2±0.1	2.5±0.2	0.7±0.0	71.6±0.2	82.0±0.3
Vb	200	158.2±0.2	2.6±0.0	2.1±0.2	0.5±0.1	73.4±0.3	84.9±0.1
BSLAE-1.3 VIa	100	137.2±0.2	4.7±0.2	4.1±0.3	0.6±0.1	63.7±0.01	66.1±0.1
VIb	200	148.2±0.1	4.5±0.4	3.2±0.1	1.3±0.3	71.6±0.1	67.6±0.2
BSLAE-1.4 VIIa	100	139.8±0.1	3.4±0.4	2.4±0.2	1.0±0.1	70.0±0.3	83.4±0.4

VIIb	200	152.5±0.2	2.7±0.1	2.1±0.3	0.6±0.0	76.1±0.3	83.4±0.1
BSTA VIIIa	100	161.2±0.1	1.6±0.1	1.5±0.2	0.1±0.0	86.7±0.2	88.4±0.1
VIIIb	200	173.3±0.3	1.4±0.0	1.3±0.1	0.1±0.0	88.5±0.1	90.0±0.0
BSME IXa	100	151.8±0.1	1.4±0.2	1.2±0.3	0.2±0.0	87.6±0.3	91.3±02
IXb	200	162.3±0.2	1.1±0.0	1.0±0.1	0.1±0.0	90.2±0.3	92.8±0.1

BSLAE-1: lyophilized aqueous extract (decoction 20%), BSLAE-1': detannified extract from BSLAE-1, BSLAE-1.1 to BSLAE-1.4 chloroform, ethylacetate, *n*-butanol and residual aqueous soluble fractions respectively from the partition of extract BSLAE-1, D: doses (mg/kg bw) OT: onset time (minutes), TNWF: total number of wet faeces and TNHF: total number of hard faeces in 4 h, IFV: intestinal fluid volume in 4h, IDE: inhibition of defecation, IDIA; inhibition of diarrhea.

BSLAE-1.1 soluble fraction rich in steroids and terpenoids also showed good diarrhea inhibition between 58 and 62% (Table 1). They also exhibited good inhibition of defecation with percentage inhibitions from 57.5±0.3 to 96.1±0.3% with the same order of activity after 4 h of observation.

Total alkaloids BSTA and 80% methanol BSME extracts showed high antidiarrheic activity by inhibiting diarrhea and defecation production with percentage inhibitions of 90.0±0.0 and 88.5±0.1%, and 90.2±02 and 92.8±0.1% respectively after 4h of observation, and lowered highly all diarrheic parameters of treated diarrheic animals compared to other samples. Taking account of their effects, it was suggested that these two last extracts contained high amounts of active antidiarrheic principles (not the same) compared to lyophilized aqueous extract.

On the other hand, several mechanisms of castor-oil to produce diarrhea were well known and some of them can be mentioned as the inhibition of the motility and secretion of diarrhea by association of dual effects on gastro-intestinal motility, absorption reduction of water and electrolytes transport characterized by their across of the intestinal mucosal.[36,38] In addition, ricinoleic acid initiated the production of diarrhea via irritation of gastro-intestinal mucosa, leading to the release of prostaglandins which stimulated gastro-intestinal motility and electrolytes secretion, reduction absorption of electrolytes from the intestine and colon.[31,33] and stimulation of prostaglandin formation and caused diarrhea [30]. Sometimes, prostaglandins also caused contractions in intestines, which can cause a range of GI (gastro-intestinal) symptoms, including diarrhea. They also reduced the intestine's rate of food absorption, which makes food pass through colon faster. Moreover, active constituent ricinoleic led to the production of diarrhea by stimulating secretory processes and intestinal motility secondary to irritation and inflammatory [37]. These conditions suggested that *B. sumatrana* samples acted as antidiarrheic agent either by antimotility and antisecretory mechanisms, by increasing reabsorption of electrolytes and water, or by inhibiting induced intestinal accumulation of fluid and inhibition of prostaglandin synthesis as also reported for other antidiarrheic medicinal plant extracts and fractions.[12-20]

Four pathophysiologic processes including increased luminal osmolarity, electrolytes secretion, decreased electrolytes absorption and abnormal intestinal motility causing reduction in intestinal transit time were associated in the production of diarrhea. In the intervention of diarrhea, antimotility and antisecretory agents remained as the main agents used to decrease such pathophysiologic changes.[39] as also it can be suggested in the case of *B. sumatrana* samples.

Castor oil had been widely used for induction diarrhea in animals model and to study antidiarreheic activity of natural and synthetic products. Therefore, the antidiarrheic activity of the studied plant extract in the present study, might be due also to the activities that opposed the actions of castor oil for induction of diarrhea or pathophysiologic processes leading to diarrhea. The extract had been shown to decrease the intestinal fluid accumulation suggesting that it may decrease water and electrolytes secretion to the intestinal lumen. It promoted their absorption causing in turn, a decrease intestinal overload and distension, which led to reduction of intestinal motility, electrolytes and water contents of the faecal drops and hence overall reduction in the total number of defecation instances and diarrheic drops in treated groups as also reported by [32] for 80% methanolic extract leaf extract of *Justicia schimperiana*. In addition, the studied extract may have an anticholinergic activity and cause reduction in intestinal motility and secretion, which is in agreement with the action of papaverine and atropine on isolated intestine.[40] Fortunately, this anticholinergic or spasmolytic activity of *B. sumatrana* leaves extracts and fractions was recently reported by Tshodi et al.,[27]

Interestingly, the second administration of the same oral doses of lyophilized aqueous extract of *B. sumtrana* of 100 and 200 mg/kg bw in treated diarrheic rats after 4 h of observation, led to stop completely diarrhea and defecation after 1h15' and 1h 32 respectively.

3.3. Effects of *B. sumatrana* leaves against magnesium-sulphate induced diarrhea in Wistar rats

Magnesium sulphate acted by the osmotic properties preventing reabsorption of water ions, leading to increment of the intestinal content volume. This salt also promoted the liberation of cholecystokinin from the duodenal mucosa. In magnesium sulphate test induced diarrhea in the present study, animals in control groups receiving this cataractic agent at oral dose of 2 g/kg bw produced copious diarrhea characterized by an onset time

of 84.3±0.2 and increase of all diarrheic parameter levels cited above of untreated rats.

In the present investigation, the oral administration of lyophilized aqueous extract from *B. sumatrana* leaves and its soluble fractions in Wistar rats with provoked copious diarrhea by magnesium sulphate, caused significant increase of the onset times from 145.6±0.3 to 162.3±0.3 min compared to untreated group showing an onset time of 84.3±0.2 min ($p < 0.05$). At the same time, they caused remarkable decrease of their all diarrheic parameters, and finally resulting in significant reduction of diarrhea and defecation instances in treated animals compared to negative control (Table 3).

At the highest oral dose of 200 mg/kg bw, lyophilized aqueous extract BSLAE-1 significantly ($p < 0.05$) increased the onset time to 156.6±0.03 min of treated animals compared to untreated group showing an onset time of 84.3±0.02 min. It also markedly ($p < 0.05$) lowered their all diarrheic parameters compared to untreated group (Table 3) resulting in the inhibition of diarrhea and defecation by 77.6±0.2 and 78.6±04% respectively after 4h of observation.

Table 3: Effects of *B. sumatrana* leaf samples on magnesium sulphate-induced diarrhea in Wistar rats.

Sample codes	D	OT	TNWF	TNHF	IFV	% IDE	%IDIA
NC water I	10 ml	84.3±0.2	13.4±1.3	10.3±0.7	3.1±0.1	-	-
Atropine II	2.5	195.3±0.2	0.8±0.1	0.9±0.2	0.2±0.0	93.0.±0.2	95.6±0.1
BSLAE IIIa	100	148.4±0.1	4.2 ± 0.3	2.2 ±0.1	2.0±0.2	74.75±0.0	73.8±0.5
IIIb	200	156.6±0.3	4.0 ± 0.2	1.8 ±0.2	2.2±0.1	78.64±0.4	77.6±0.2
BSLAE-1' IIIc	200	98.3.6±0.2	9.2 ± 0.3	6.5 ± 0.1	2.5±0.2	38.8±0.1	35.8±0.1
BSLAE1.1 IVa	100	146.3±0.4	6.2 ± 0.2	4.4 ± 0.2	1.8±0.0	63.1±0.4	61.9±0.1
IVb	200	151.4±0.5	5.1 ± 0.3	3.7± 0.0	1.4±0.0	66.2±0.1	64.9±0.3
BSLAE-1.2 Va	100	156.6±1.7	4.6 ± 0.1	3.3 ± 0.0	1.3±0.2	68.9±0.2	67.6±0.3
Vb	200	162.3±0.3	3.3 ± 0.1	2.8 ± 0.2	0.5±0.0	73.8±0.4	72.8±0.2
BSLAE1.3 VIa	100	132.3±0.4	4.0 ± 0.2	3.4 ± 0.4	0.6±0.1	67.9±0.1	64.8±0.1
VIb	200	145.6±0.3	3.5 ± 02	3.1 ± 0.3	0.4±0.2	67.9±0.2	66.4±0.1
BSLAE1.4VIIa	100	141.1±0.3	3.5 ± 0.3	2.8 ± 0.2	0.7±0.0	71.8 ±0.1	70.8±0.3
VIIb	200	158.3±0.2	3.0 ± 0.2	2.6 ± 0.3	0.4±0.2	72.8±0.3	72.3±0.4
BSTA VIIIa	100	167.8±0.1	2.3±0.1	2.1±0.2	0.2±0.1	77.7±0.2	84.3±0.2
VIIIb	200	175.3±0.3	2.0±0.2	1.8±0.1	0.2±0.1	80.5±0.3	86.5±0.1
BSME IXa	100	170.1±0.2	1.8±0.1	1.6±0.2	0.2±0.2	82.5±0.2	88.0±0.3
IXb	200	178.2±0.1	1.5±0.1	1.3±0.1	0.2±0.1	85.4±0.4	90.3±0.2

See Table 1,

All soluble fractions had the same effects by causing more marked increase of onset time from 145.6±0. to 162.3±0.3 min compared to untreated group with onset time of 84.3±0.3 min. They also significantly led reduction ($p < 0.05$) all diarrheic parameters of treated animals compared to untreated group (Table 3) leading to the reduction of production of diarrhea and defecation with percentage inhibitions between 66.4±0.1 and 72.8±0.2%, and 67.9±0.2 to 73.8±0.4% respectively. The ethylacetate BSLAE-1.2 soluble fraction was the most active, followed by the residual aqueous and *n*-butanol soluble fractions (Table 3). Chloroform BSLAE-1.1 soluble fraction also showed appreciable inhibition of diarrhea and defecation (Table 3).

Also interestingly, the second administration of the same oral doses of the lyophilized aqueous extract of *B. sumatrana* led to stop completely diarrhea and defecation at 1h45'and 2h05 with 200 and 100 mg/kg bw of the lyophilized aqueous extract respectively.

In both antidiarrheic tests, at the highest oral dose of 200 mg/kg body weight, the detannified aqueous extract BSLAE-1' produced low antidiarrheic activity than 50% inhibition of defecation and diarrhea induced by castor oil and magnesium sulphate respectively compared to the parent lyophilized aqueous extract BSLAE-1 (Tables 2 and 3). Its activity was thus weak compared to the parent extract (Tables 2 and 3). It completely stoped diarrhea and defecation after 3h08' and 3h 45' after the second administration of 200 and 100 mg/kg bw of the extract respectively. Its effect is due to the production of low onset time and increase of others diarrheic parameters compared to the parent extract. This finding clearly showed the important role played by tannins in the manifestation of the evaluated antidiarrheic activity because the absence of tannins in the extract markedly reduced the activity and suggested that tannins can partly be considered as active principle.

The total alkaloids BSTA and 80% methanol BSME extract prominently increased the onset time and led to reduction of all diarrheic parameters of treated animals compared to untreated group (Table 3). At the high oral dose of 200 mg/kg bw, they provoked an inhibition of diarrhea and defecation more than 80%. The 80% methanol BSME showed high activity compared to lyophilized aqueous extract and total alkaloids extract

and seemed to be the better solvent for the extraction of active principles in both tests.

In both tests, the standard drug atropine exhibited more marked reduction in the number of defecation and diarrhea instances, and all diarrheic parameters of treated animals compared to the vehicle and *B. sumatrana* samples-treated groups (Table 2 and 3). In general, the antidiarrheic activity of samples from *B. sumatrana* leaves was weak compared to atropine used as an antidiarrheic reference product producing 94.7± 0.2 and 97.1±0.1%, and 93.0±0.2 and 95.6±0.1% inhibition of defecation and diarrhea in castor oil and magnesium sulphate induced diarrhea respectively, at an oral dose of 5 mg/kg bw in both tests (Tables 2 and 3).

3.4. On gastro-intestinal motility

Lyophilized aqueous extract BSAE-1 and its soluble fractions as well as the total alkaloid BSTA and 80% methanol BSME extracts from *B. sumatrana* leaves showed prominent decrease of the motility against gastro-intestinal motility of charcoal meal movement as presented in Table 5.

Interestingly, compared to negative control, all plant extracts and fractions showed prominent inhibition of gastro-intestinal transit of charcoal meal at the highest oral dose of 200 mg/kg bw ($p < 0.01$) from 76.18±0.02 to 83.00±0.03%. The 80% methanol BSME extract was considered as the most active compared to lyophilized aqueous extract BSAE-1 and its soluble fractions as well as the total alkaloids BSTA extract (Table 5).

At the highest oral dose of 200 mg/kg bw, lyophilized aqueous extract BSLAE-1 suppressed by 76.18±0.02% intestinal transit. The soluble fractions from the partition of lyophilized aqueous extract BSLAE-1 acted in the same manner in producing percentage inhibitions of gastro-intestinal transit of charcoal movement from 35.83±0.02 to 73.71±0.04% with the ethylacetate soluble fraction BSLAE-1.2 as the most active compared to other remaining soluble fractions (Table 5). The total alkaloids BSTA and 80% methanol BSME extracts showed more than 80% suppression of charcoal motility. Results from the charcoal meal test revealed that all *B. sumatrana* samples produced a remarkable anti-motility effect (inhibition of intestinal transit of charcoal) ($p < 0.001$) compared to negative control.

Table 5: Effects of extracts and fractions from *B. sumatrana* on gastro-intestinal motility.

Sample codes	TTT (dose: mg/kg bw)	TLSI (mm)	DCCM (mm)	% IIT
Negative control	2 ml 10% GA	90.20 ±0.03	88.30 ± 0.02	0
Atropine	5	89.10±0.01	12.56±0.03	85.90±0.01
BSLAE-1	100	80.74±0.04	25.87±0.03	70.70±0.03
	200	72.54±0.01	21.03±0.02	76.18±0.02
BSLAE-1’	200	85.00±0.02	60.21±0.01	32.0± 0.04
BSLAE-1.1	100	77.54±0.03	48.66±0.03	35.83±0.02
	200	73.05±0.02	44.11±0.02	41.00±0.01
BSLAE-1.2	100	82.00± 0.04	27.0±0.01	69.42±0.02
	200	76.25±0.02	23.21±0.02	73.71± 0.04
BSLAE-1.3	100	84.02±0.04	28.2±0.02	68.06±0.02
	200	78.24±0.02	26.0±0.01	70.55±0.03
BSLAE-1.4	100	80.14±0.01	26.1±0.01	70.44±0.02
	200	74.00±0.02	23.0±0.03	73.40±0.01
BSTA	100	71.23±0.01	20.01±0.02	77.33±0.03
	200	67.21±0.03	17.35±0.02	80.35±0.01
BSME	100	62.34±0.01	15.06±0.01	83.00±0.03
	200	60.01±0.03	13.02±0.03	85.25±0.01

See Table 1, TTT; treatment, GA; gum acacia, TLSI: total length small intestine, DCCM: distance covered by charcoal meal, IIT: inhibition of intestinal transit.

Lyophilized aqueous extract BSLAE-1 and its fractions BSLAE-1.1 to BSLAE-1.4 as well as 80% MeOH BSME and total alkaloids extracts showed that activated charcoal avidly absorbed drugs and chemicals on the surface of charcoal meal particles preventing absorption. By this test, it was demonstrated that samples from *B. sumatrana* leaves inhibited in dose-dependent manner peristaltic movements of charcoal since there suppressed its propulsion and increased the absorption of water and electrolytes as also previously described for other medicinal plant extracts.[16,41]

Total alkaloids BSTA and 80% methanol BSME extracts exhibited remarkable antidiarrheic activity by decreasing the gastro-intestinal motility. They statistically induced marked dose-dependent decrease of the propulsion of the charcoal meal passing through the gastro-intestinal tract at all administered oral doses (100 and 200 mg/kg bw respectively) compared to untreated group ($p < 0.01$). The percentage inhibitions of charcoal movement produced by all samples from *B. sumatrana* leaves is higher than 50% at all administered oral doses, excepted

the detannified aqueous extract BSLAE-1' which demonstrated low percentage inhibition of 38.55±0.01% at the highest oral dose of 200 mg/kg bw compared to the parent extract. Atropine used as a reference produced high reduction of the motility of charcoal (85.90±0.01% inhibition of intestinal transit) compared to tested *B. sumatrana* leaf samples ($P < 0.01$) (Table 5).

3.5. Castor oil induced-enteropooling

In the castor oil induced-enteropooling, at the highest tested oral dose of 200 mg/kg bw, lyophilized aqueous extract BSLAE-1 and its soluble fractions chloroform BSLAE-1.1 to residual aqueous phase BSLAE-1.4 were found to be able to decline the mean weight of small intestine content (MWSIC), the mean volume of small intestine content (MVSIC) and the intraluminal fluid accumulation (IFA) compared to negative control group (Table 6).

Maximal inhibition of MWSIC and MVSIC was observed with 80% methanol BSME extract (83.56±0.00 and 84.61±0.02% inhibition respectively) and total alkaloids BSTA extract (79.45±0.02 and 81.53±0.03% inhibition respectively) followed by lyophilized aqueous extract BSLAE-1 (75.35±0.02 and 76.92±0.03% inhibition respectively), ethylacetate soluble fraction BSLAE-1.2 and residual aqueous phase BSLAE-1.4 producing percentage inhibitions of 73.84 ±0.01 and 72.60±0.05%, and 70.76±0.04 and 68.48±0.03% respectively. Chloroform BSLAE-1.1 and *n*-butanol BSLAE-1.3 soluble fractions also showed good inhibition of both intestine parameters more than 63% with the last fraction as the most active sample compared to the first one. The detannified aqueous extract showed weak activity by producing only 38.00±0.03 inhibition of enterpooling induced by castor oil.

Similarly, in the enteropooling test, the lyophilized aqueous, 80% MeoH and total alkaloids extracts (at all tested doses) ($p < 0.01$) produced a significant decline in the weight and volume of intestinal contents as already mentioned above. The high effect was shown by atropine used a reference product compared to samples from *B. sumatrana* leaves (Table 6).

From these results, all samples from *B. sumatrana* leaves produced remarkable antidiarrheic activity and can show their efficacy in treatment of diarrheic conditions in human. They can be considered as alternative natural remedies for the treatment of diarrhea.

Table 6: **Effects of extracts and fractions from *B. sumatrana* leaves on castor oil induced-enteropooling of Wistar rats.**

Groups	TTT(200 mg/kg bw)	MWSIC (g)	% Inhibition	MVSIC (ml)	% Inhibition
I	Castor oil	0.73±0.02	-	0.65±0.07	-
II	Atropine	0.15±0.02	80.00±0.04	0.11±0.04	83.07±.002
III	BSLAE-1	0.18±0.04	75.34±0.02	0.15±0.02	76.92±0.03
IV	BSLAE-1.1	0.27±0.02	63.01±0.04	0.22±0.04	66.15±0.02
V	BSLAE-1.2	0.16±0.01	78.08±0.02	0.13±0.03	80.00±0.01
VI	BSLAE-1.3	0.25±0.01	65.75±0.03	0.20±0.01	69.23±0.02
VII	BSLAE-1.4	0.23±0.02	68.49±0.03	0.19±0.04	70.76±0.04
VIII	BSTA	0.14±0.02	80.82±0.01	0.12±0.02	81.53±0.01
IX	BSME	0.11±0.03	85.00±0.00	0.10±0.01	84.61±0.02

The study demonstrated that extracts and solvent fractions from *B. sumatra* leaves contained bioactive constituents that exhibited antidiarrheic activity in animal model. Therefore, this study provided a scientific support for the acclaimed traditional use of *B. sumatrana* leaves for the treatment of diarrheic diseases.

Moreover, lyophilized aqueous extract BSLAE-1 and its soluble fractions (BSALAE-1.1 to 1.40) as well as the total alkaloids BSTA and 80% methanol BSME extracts, like the standard antidiarrheic agent atropine statistically produced prominent inhibition of the frequency of defecation and diarrhea, and reduction of all diarrheic parameters at different magnitudes, declined gastro-intestinal motility and enteropooling caused by castor oil in treated animals compared to untreated groups in both models ($p < 0.01$). Thus, these *B. sumatrana* samples possessed good and interesting antidiarrheic activity and were considered to have atropine-like effects.

In general, it is well reported that the antidiarrheic activity of some medicinal plants is due to the presence of tannins, saponins, coumarins, flavonoids, alkaloids, steroids and terpenoids.[42,43] Tannins and tannic acid acted as antidiarrheic agents by the denaturation of proteins in the intestinal mucosa forming protein tannates which make the intestinal mucosa more resistant to chemical alterations. They reduced the peristaltic movements and intestinal secretion. Flavonoids acted as antidiarrheic agents by their ability to inhibit intestinal motility and hydroelectrolytic secretions, and have antispasmodic effects.[36,44] Steroids and triterpenes are useful for the treatment of diarrhea and may increase intestinal absorption of Na^+ and water.[45]

Other reports in the literature indicated that tannins, steroids, terpenoids and flavonoids have antispasmodic activity and muscle relaxant effects. Flavonoids inhibited prostaglandin E2-induced intestinal secretion, saponins and terpenoids inhibited histamine release and the release of prostaglandins respectively. Phenolic compounds

declined intestinal secretion and transit, and had astringent action. All these actions led to the inhibition of diarrhea by the decrease intestinal secretion and motility [42-44]. Therefore, the antiadiarrheic activity of aqueous extract of *B. sumatrana* leaves and its soluble fractions, as well as for 80% methanol and total alkaloids extracts reported in the present study, may be due to the presence of these phytochemical groups identified in this plant part as evidenced by phytochemical screening results, which in part, can react in synergistic manner for the manifestation of these evaluated biological activities.

4. CONCLUSION

Results from this study clearly demonstrated for the first time, that extracts and soluble fractions) from *B. sumatrana* leaves possessed remarkable capacity to reduce diarrhea induced by castor-oil and magnesium sulphate in Wistar rats, as well gastro-intestinal motility of charcoal meal and castor oil- induced-enteropooling showing thus their antidiarrheic activity. Tannins as shown in the present study, contribute in part to the observed activities and can be considered partly as active antidiarrheic principles. The use of this medicinal plant part in traditional medicine for the treatment diarrhea in African countries seemed to be supported and justified by these reported pharmacological properties.

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