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BIOGIGICAL SCREENING OF ANTIDIARRHOEAL PROPERTIES OF *PSEUDOLACHNOSTYLIS MAPEOUNEIFOLIA* PAX (EUPHORBIACEAE) STEM BAK

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ABSTRACT

The purpose of the present study was undertaken to report the antidiarrhoeal properties of aqueous extract of *Pseudolachnostylis maprouneifolia* (Kudu berry) stem bark and its soluble fractions in evaluating their effects on castor oil and magnesium sulphate-induced diarrhoea in Wistar rats *in vivo* and gastrointestinal motility, as well as antibacterial, antiamoebic and spasmolytic activities. In castor oil and magnesium sulphate-induced diarrhoea in animals, results indicated aqueous extract and its fractions significantly delayed diarrhoea onset, decrease the frequency of weight of wet and hard stool, intestinal volume of secreted fluid, defecation and diarrhoea in dose-dependent manner (oral doses of 100, 200 and 400 mg/kg bodyweight). In addition, significant reduction in the gastrointestinal motility in charcoal meal test and intraluminal fluid accumulation in treated Wistar rats were also observed. Based on the antibacterial activity, aqueous extract and its fractions possessed antibacterial activity by inhibiting the growth of all tested bacteria with minimum inhibitory concentrations ranging from 7.75 to 125 µg/ml and minimal bactericidal concentrations ranging from 15.62 to 250 µg/ml. These samples exhibited spasmolytic activity by inhibiting contractions of isolated guinea-pig ileum induced by acetylcholine and depolarizing solution rich in KCl by producing more than 60% inhibition of both agonist effects with the aqueous extract as the most active (> 80% inhibtion). With regard to the antiamoebic activity, aqueous extract and its fractions inhibited the growth of *Entamoeba histolytica* with minimal ameobicidal concentration (MAC) varying from 5.5 to 31.5 µg/ml and inhibitory concentrations 50 (IC_{50}) ranging from 3.57 to11.24 µg/ml. These reported results showed that aqueous extract of *P. maprouneifolia* stem bark and its fractions are able to significantly reduce diarrhoea induced by castor oil and magnesium sulphate in animals, and exhibited antibacterial, spasmolytic and antiamoebic activities *in vitro* which in part, can support and justify its claimed antidiarroeal activity related to its traditional use to treat diarrhoea in traditional medicine in Democratic Republic of Congo and other African countries.

KEYWORDS: *Pseudolachnostylis maprouneifolia*, stem bark, diarrhoea, antibacterial, spasmolytic and antiamoebic activity.

1. INTRODUCTION

Diarrhoea is defined as an increase in the frequency, fluidity or volume of bowel movements and characterized by increased frequency of bowel sound and movement, wet stools, and abdominal pains. It is used to describe increased liquidity of stools, usually associated with increased stool weight and frequency.[1] It is the frequent passing of loose, water and unformed faeces.[2]

Diarrhoea and other gastrointestinal disorders cause morbidity and mortality mainly of childhood under 5 years in developing countries. It is usually as symptom of diseases in the intestinal tract which can be caused by pathogen microorganisms such as *Staphyloccocus aureus*, *Escherichia colis*, *Shigella dysenteria* and so one, virus such as *Rota virus*, *Cytomegalovirus*, *Novovirus*, etc, protozoa, helminths and parasites such as *Entamoeba histolytica*.[3]

In spite of enormous development of synthetic antidiarrhoeal drugs in the mark, people are still relying on the herbal drugs to treat the disease and find some

alleviations. The World Health Organization (WHO) recommends the conventional practices for the treatment of diarrhoea and has given a special emphasis on the use of traditional medicines in the control and magement of the disease, as medicinal plants constitute an indispensable component of traditional medicine practiced worldwide, traditional remedies, health education as well as preventive approaches.[4] WHO also encourages the use of traditional herbal medicines due partly to their economic viability, easy accessibility, ancentral experiences and perceived efficacy to treat the disease.[5]

According to WHO, estimates for 1998, about 7.1 million deaths were caused by diarrhoea and the incidence of the disease still remains high despite the efforts of many international organizations to cure it.[6]

Nowadays, many medicinal plants are frequently investigated in animal models to prove their claimed antidiarroeal properties and many of them were reported to possess this property with different magnitudes.[7,11]

Pseudolacnostylis maprouneifolia is a member of Euphobiaceae family. Each plant part of it has a therapeutic value in traditional medicine. Aqueous decoction of the root barks are taken a purgative to treat stomach aches and abdominal pains, diarrhoea and diabetes, syphilis, pneumonia, sore eyes and to stop nosebleeds. A paste of crushes leaves is used to treat footroted animals.[12,13] Pharmacological and chemical informations of this medicinal plant are not available in the literature because none of its plant part is not yet scientifically investigated. Thus, the present study was undertaken to evaluate the effect of aqueous extract of *P.maprouneifolia* against castor oil and magnesium sulphate-induced diarrhoea in animal, gastrointestinal motility as well as to assess *in vitro* antibacterial, antispasmodic and antiamoebic activities which more can confirm and support its claimed antidiarrhoeal activity.

2. MATERIALS AND METHODS

2.1. Plant material

Stem barks of *Pseudolechonostylis maprouneifolia* (Euphorbiaceae) were collected in Lubumbashi (Katanga-Democratic Republic of Congo) in May 2015. The plant was authentified at the National Institute of Studies and Researchs in Agronomy (NISRA), Department of Biology, Faculty of Sciences, University of Kinshasa by Mr Nlandu Lukebiabo, B. A voucher specimen of the plant NL052015PMSB was deposited in the herbarium of this institute. The plant materials were dried at room temperature and reduced to powder using an electric mixer-grinder. The powder was kept in brown bottles hermetically closed.

2.2. Preparation of aqueous extract and its fractionation

50 g of powdered plant material were mixed with 500 ml distilled water and boiled on a hotplate for 15 min. After cooling and filtration on a paper filter WatmanN°1, the filtrate was evaporated *in vaccuo* yielding dried extract denoted as Pm-1 (26.38 g). 15g of Pm-1 extract were dissolved in 200 ml distilled water and filtered. The filtrate was exhaustively extracted with solvents of different polarities chloroform, ethylacetate and *n*-butanol. All fractions and the residual aqueous phase were treated as described above yielding corresponding dried extracts denoted as Pm-1 (3.35 g), Pm-2 (3.25 g), Pm-3 (3.89) and Pm-4 (4.15 g) for chloroform, ethylacetate, *n*-butanol and residual aqueous phase respectively.

2.3. Qualitative phytochemical screening

The qualitative phytochemical screening was carried out by TLC method on silica gel plates (thickness layer: 0.25 mm, Merck, Germany) using different mobile phases and chemical reagents described in the literature for the identification major chemical groups such as alkaloids, aminated compounds, anthocyanins, coumarins, flavonoids, tannins, terpenoids steroids reducing sugars and saponins.[14,15]

2.4. Antidiarrhoeal activity against castor oil-induced diarrhoea in Wistar rats

The methods described by[3,10] were followed for this investigation. Wistar rats either sex (130-140 g bodyweight (bw)) were divided into Group I (negative group, 2 rats) and group II (positive group, 2 rats) received orally a vehicle (5 ml water/kg orally) and Loperamide (2 mg/kg) respectively. The test groups III to VIII (6 rats for each oral dose) received 100 and 200 mg/kg bw of aqueous extract Pm-1 and its soluble fractions P1.1 to P1.4 respectively dissolved in distilled water and were placed in Individual cages. One hour after treatment, diarrhoea was induced by oral administration of 0.5 ml castor oil to each rat. The observation of the diarrhoea and defecation production continued up to 4 h on pre-weighted filter paper placed in the individual rat cages (P1) and replaced every hour.

The used filter paper in each cage was reweighed (P2) when it had wet faeces collected after 4hours. The weigt of wet faeces was calculated as (P2-P1) g = P3. Finally, the filter paper was dried at 50°C and was reweighed again (P4). The intestinal fluid excreted was calculated as (P3-P4) g.

The following parameters were observed and recorded: the time elapsed between the administration of the cathartic agent and the excretion of the first diarrheic faeces, the total number of faecal output, the total number of diarrheic faeces and the secreted intestinal fluid by the animals in 4h. The percentage of defecation inhibition and diarrhoea drops were calculated using the following formula respectively:

$$\% \text{ Inhibition of defecation} = \frac{Pc - Ps}{Pc} \times 100$$

Where Pc is the mean number of defecation caused by castor oil and Ps the number of defecation caused by test sample.

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

Where Dc is the mean number of drops caused by castor oil and Ds the mean number caused by the test sample. In addition, a numerical score based on stool consistency washed calculated by taking the sum of the number of "+" rats and twice the number of "++" rats.[16] ++ for copious, + mild and 0 for lack of diarrhoea.

2.5. Antidiarrhoeal activity against magnesium sulphate-induced diarrhoea in rats

A similar protocol as for castor oil-induced diarrhoea was followed. Rats were divided in the same way as in castor-oil test (6 rats for each sample oral dose). But, diarrhoea was induced by oral administration of magnesium sulphate at the dose of 2 g/kg bw to the animals one hour after pre-treatment with vehicle (water, 5 ml/kg) as the control negative group and Loperamide (2 mg/kg) as the positive control group. Aqueous extract and its soluble fractions were orally administered at respective doses of 100 and 200 mg/kg bw to the test groups (6 rats for each sample oral dose) at the same time above. The % inhibition of diarrhoea by tested sample was evaluated in the same way as in castor-oil experiment.[3,10] All experiments were performed according to the current guidelines concerning the care of laboratory animals.

The detannified extract was obtained by column chromatography on polyamide of aqueous extract Pm-1 eluted with methanol. In this way, tannins are retained on the column and no tannic compounds are eluted with methanol.[16]

2.6. Castor oil-induced enteropooling

The methods described by[3,17] were followed for this study. Wistar rats of either sex were fasted for 18 h with free access to food and water grouped as in castor oil-induced diarrhoea test. One hour after administration castor oil 0.5 ml/rat, the animals were sacrificed by cervical dislocation. Their abdomen was open and the whole length of the intestine from the pylorus to the caecum, was ligated, intestines dissected and carefully removed. The small intestines were weighted and the intestinal contents were collected by milking into a graduated tube to measure the volume. The empty intestines were reweighted and the difference between the two weights was calculated. The percentage of reduction of intestinal secretion and weight of intestinal content were determined using the following formula:

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

Where MVISC 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. On the other had, the reduction of intestinal weight was calculated thefollowing formula:

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

Where MWICC 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.

2.7. Gastrointestinal motility

The effect of aqueous extract Pm-1 of *P. maprouneifolia* and its soluble fractions on normal gastrointestinal transit and castor oil-induced intestinal motility was assessed in this test using the methods previously described by[3,18,19] Wistar rats were grouped as described above. They were fasted for 18 h with access to food and water. 30 min after treatment of each sample at the oral dose of 200 mg/kg bw mentioned, each animal was given 1 ml of charcoal suspension for the normal gastrointestinal motility test, and 0.5 ml of castor oil for castor oil-induced intestinal motility assay. After another 30 min, animals were sacrificed under halothane euthanasia, dissected and the total length travelled by the marker charcoal from pylorus to the caecum was measured and recorded. The percentage inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Distance travelled by charoal}}{\text{Total length of intestine}} \times 100$$

2.8. Assessment of spasmolytic activity

Male guinea-pigs were anesthetized and sacrificed by cervical displacement followed by exsanguination. The ileum was dissected out (2-3 cm long), plentifully was with distilled water and suspended in an organ bath (50 ml) containing 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.[20]

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 or depolarizing solution rich in KCl to have tree equivalent contractions and the tissue was plentiful washed with Tyrode's solution to eliminate the presence of agonists in organ bath. 2 mg of each samples were dissolved in distilled water to have respective stock solution of 1 mg/ml. After 2 ml of agonsist were

removed in organ batch replaced by 2 ml of tested samples (40 µg/ml in organ bath) and left in contact with isolated guinea-pig ileum for 15 minutes.

The effects of extract and fractions on the responses elicited by both agonists were recorded. 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 inhibition of both agonists contractions in the presence of aqueous extract and its fractions was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Cag - Cts}}{\text{Cag}} \text{ x } 100$$

Where Ca is the concentration level of agonist in cm and Cts is the concentration level of tested sample.[20,21,23]

2.9. Antibacterial activity

Clinical microbial isolates included *Escherichia coli*, *Salmonella tiphymurium*, *Salmonella enteritidis*, *Shigella dysenteria*, *Shigella flexenri and Staphylococcus aureus* in patients with microbial infections from Clinic Universitaries of Mont-Amba, University of Kinshasa, Democratic Republic of Congo.

The antibacterial activity was assessed using dilution methods previously described by.[20,24,25] Colonies were suspended into small volume 0.9% saline. 1 ml of respective bacterial suspension was added separately to 1 ml Muller-Hinton. 2 mg of tested samples were dissolved in 2 ml DMSO 0.1% to obtain respective stock solutions. They were further diluted in two fold in two dilutions with the same solvent to have a series of test concentrations ranging from 500 to 0.1 µg/ml. In sterile tubes, 1 ml of bacterial suspension and 1 ml of tested sample with known concentration was added.

On the other hand, a sterile tube containing only bacterial suspension was used as a negative control, and other tubes containing bacterial Ampicillin and Tetracycline (0.1 to 10 µg/ml) were used as positive controls. All test tubes were carefully mixed, plugged with sterile cotton and incubated at 30°C for 24 h.

The lowest concentration of tested samples that inhibited the bacterial visible growth after incubation was taken as the minimum inhibitory concentration (MIC). The minimum bactericidal concentrations (MBC) defined as the lowest concentration yielding negative subcultures or only one colony were determined by sub-culturing 10 µl of the MIC test solutions on the same medium in sterile tubes incubated in the same conditions. (n=3).

2.10. Antiamoebic activity

Entamoeba histolytic used in the present study is a laboratory isolated strain from patients with acute dysentery diagnosed in the Tropical Medicine Institute, Faculty of Medicine, University of Kinshasa. The evaluation of activity was performed using the methods previously described by.[25,26]

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 culture medium were selected as test tubes. 5 mg of each test sample was dissolved in 5 ml hydroethanol solution (eau-ethanol:9:1) to have corresponding stock solutions of 1 mg/ml. These last solutions were diluted two fold dilutions with the same solvent to give a series of test solutions ranging from 500 to 0.1 µg/ml. Next, 1 ml of the test solution with a known concentration was added to a separated 1 ml of test tubes containing parasites. On the other hand, two tubes were used as controls, one containing parasites in hydroethanol (9:1) without test sample as negative control and another containing test tubes with parasites and Metronidazole or Dehydroemetine (10 to 0.1 µg/ml) 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 was done described above. The test was considered as positive when the vegetative or kystic forms of amoebae was not microscopically observed. The minimum amoebicidal concentration (MAC) was determined by using linear-courbes doses-responses (n=3).

2.11. Statistical analysis

The results are reported as mean ± SD for all values. The significant differences were assessed using one-way analysis of variance (ANOVA) using SPSS software package. P values < 0.05 were considered as significant.

4. RESULTS AND DISCUSSION

4.1. Qualitative phytochemical screening

Results from the qualitative phytochemical screening revealed the presence of alkaloids, anthraquinones, flavonoids, aminated compounds, tannins (gallics, cathechics and proantocyanidins), saponins, steroids and terpenoids. Other phytochemical groups such as anthocyanins, cardiotonic glycosides and coumarins were not detected in our experimental conditionsin the aqueous extract. These phytochemical groups were localized in different soluble fractions according to their solubility (Table 1).

Tableau 1: Results of qualitative phytochemical screening.

Chemical groups	Results	Chemical groups	Results
Alcaloids	+++	Reducing sugars	++
Flavonoids	++	Anthocyanins	-
Tannins	+++	Coumarins	-
Gallic tannins	+++	Cardiotonic glycosides	-
Cathechic tannins	+++	Anthraquinones	+
Proanthocyanidins	++	Terpenoids and steroids	++
Aminated compounds	++	Saponins	++

4.2. Effects of aqueous extract Pm-1 of *P. maprouneifolia* and its fractions on castor oil-induced diarrhoea in rat

Castor oil produces diarrhoea due its active principle ricinoleic acid. This metabolite is liberated by the action of lipases in upper part of the small intestine.[27] This acid can stimulate fluid secretion, inhibits water and electrolyte absorption, reduces active Na^+ and K^+ absorption and decreases the level of Na^+, K^+-ATPase in the small intestine and colon, epithelial cells to produce nitric oxide and adenyl cyclase which lead to the production of prostaglandins (E series) inducing diarrhoea.[28,29] Moreover, ricinoleic acid can also cause irritation and inflammation of the intestinal mocosa leading to lead to the release of endogenous prostaglandins which stimulate motility and secretion, enhances motility stimulation and secretion as well as prevention of NaCl and water re-absorption.[12,30] It Stimulate the peristaltic activity in the small intestine, leading to change in the electrolyte permeability of the intestinal mucosa.[10] In some diarrhoea, the secretory components predominate while in other, it is characterized by hyper motility. In general, diarrhoea is a result from an imbalance between the absorptive and secretory mechanisms in the alimentary tract, accompanied by an excess loss of liquid in the faces[31] and alteration of motility of intestinal smooth muscles. It is characterized by faecal urgency and incontinence.[12]

Tableau 2: Effects of aqueous extract Pm-1 of *P. maprouneifolia* and its fractions against castor oil-induced diarrhoea in Wistar rats after 4h of observation.

Groups	CS	OD	ETD	TNWF	TNHF	IFV	% IDia	% IDef
I	C. N:	5	87.2±0.3	11.2±0.6	10.7±0.5	8.5±0.3	0	0
II	Loperamide	2.5	154.3±0.2	1.1 ± 0.1	1.2 ± 0.2	0.3 ± 0.1	90.3±0.1	89.0±0.3
III	Pm-1	100	110.3±1.5	2.2 ± 0.3	2.4 ± 1.1	3.5 ± 1.3	80.3 ±0.1	77.6 ± 0.5
		200	144.6 ±0.4	1.9 ± 0.6	2.2 ± 0.4	3.2 ±0.6	83.0 ±0.6	79.4 ± 0.3
IV	Pm-1.1	100	119.4.3±.3	3.6 ±0.5	3.9 ± 0.2	4.3 ± 0.8	67.8 ±0.7	63.6 ± 0.4
		200	134.7 ±0.5	3.2 ±0.8	3.7 ± 0.8	4.1 ± 0.5	71.4 ±1.1	65.4 ± 1.2
V	Pm-1.2	100	114.6 ±0.3	3.2 ±0.2	3.4 ± 0.8	4.0 ± 0.7	71.4 ±0.2	68.2 ± 0.4
		200	141.7±0.2	2.7 ±0.4	3.2 ± 0.8	3.8 ± 0.2	75.8 ±1.1	70.1 ± 0.9
VI	Pm-1.3	100	129.2 ±0.2	3.3 ±0.6	3.7 ± 0.2	4.1 ± 0.7	70.5 ±1.4	65.4 ± 0.8
		200	134.2 ±0.5	3.1 ±1.0	3.5 ± 0.4	3.6 ± 0.7	72.3 ±0.3	67.3 ± 0.5
VII	Pm-1.4	100	138.4±0.4	2.4 ±0.8	2.8 ± 0.5	3.3 ± 0.3	78.6 ±0.5	73.8 ± 0.2
		200	143.5 ±0.6	2.2 ±0.1	2.6 ± 0.7	3.0 ± 0.6	80.3 ±0.5	75.7 ± 0.6
VIII	Pm-1’	200	115.4±0.5	6.6 ± 0.3	6.8 ± 1.5	5.5 ± 0.5	41.1 ±0.3	36.4 ± 0.5

Pm-1 :aqueous extract,: Pm-1’ : detannified extract from Pm-1, Pm-1.1 to Pm-1.4 : chloroform, ethylacetate, *n*-butanol et aqueous residual phase respectively from the partition of aqueous Pm-1 extract, CS: code samples, OD: oral dose (mg/kg bw), ETD: explusion time of diarrhoea (min), TNWF: total number of wet faces, TNHF: total number of hard faces, IFV: intestinal fluid volume, % IDia: % Inhibition of diarrhoea, % IDef: % Inhibition of defecation.

In the present study, the oral administration of castor in Wistar rats receiving only vehicle as negative control group (5 ml water/kg body weight), produced copious diarrhoea, with an onset time of 87.2 ± 0.3 min, and characterized by the total number of hard faeces and the total number of wet faeces of 11.2 ± 0.6 and 10.7 ± 0.5 respectively, and an intestinal fluid volume of 8.5 ± 0.3 ml during 4 h. It showed a maximum score of 10 indicating that it does not possess antidiarrhoeal activity.

On the other hand, the oral administration of aqueous extract of *P. maprouneifolia* and its soluble fractions at all oral test doses of 100 and 200 mg/kg body weight, significantly showed a dose-dependent delay on the onset of diarrhoea in treated animals (110±0.5 to 144.6±0.4 min) compared to untreated group (87.2±0.3 min) in 4 h. (Tables 1) with the aqueous extract exhibiting the better effect. Their effects were also characterized by significant decrease of total number of hard faeces, total number of wet faeces and intestinal fluid volume compared to untreated groups ($p < 0.01$) (Table 1). It

possessed a score of 2 when administered at 100 mg/kg and 1 when administered a 200 mg/kg indicating its good and strong antidiarrhoeal activity respectively.

At the highest oral test dose of 200 mg/kg bodyweight, aqueous extract (Pm-1) and its fractions Pm-1.1 to Pm-1.4 produced more than 70% inhibition of diarrhoea induced by castor oil in rat with aqueous extract (Pm-1 = 83.0 ± 0.5%) as the most active sample, followed by the residual aqueous phase Pm-1.4 rich in polyphenolic compounds (80.3 ± 0.5%), ethylacetate soluble fraction Pm-1.2 rich in flavonoids (75.8 ± 1.1%), *n*-butanol soluble fraction Pm-1.3 rich in saponins (72.3 ± 0.3%) and the chloroform soluble fraction Pm-1.1 rich in steroids and terpenoids (71.4 ± 1.1). In addition, these samples produced good inhibition of the defecation from 67 to 80% at this highest tested oral dose of 200 mg/kg body weight. The most active samples was aqueous extract Pm-1 (79.4 ± 0.5%) followed by Pm-1.4 soluble fraction (75.7 ± 0.6%) and Pm-1.2 (70.5 ± 0.9. Pm-1.1 and Pm-1.3 also produced good inhibition of defecation of 67.3 ± 0.5 and 65. 4 ± 0.2% respectively.

The intestinal fluid volume excreted by animals after the treatment of 4 h is also a good parameter used to more appreciate the level of antidiarrhoeal activity of tested sample. In the present case, it was observed that all samples from *P. maprouneifolia* significantly decrease the volume of this parameter in a dose-dependent manner compared to untreated group (Table 1).

Table 3: Scores of aqueous extract Pm-1 of *P. maprouneifolia* and its fractions in castor oil- induced diarrhoea in Wistar rats.

Treatment	Oral dose (mg/kg bw)	Diarrhoea	score		Total score
		++	+	0	
Négative control	5 ml water	6	0	0	10
Pm-1	100	0	2	4	2
	200	0	1	5	1
Pm-1.1	100	3	2	1	8
	200	3	1	2	7
Pm-1.2	100	2	1	3	5
	200	1	2	3	4
Pm-1.3	100	2	1	3	5
	200	1	2	3	4
Pm-1.4	100	1	1	4	3
	200	0	2	4	2
Pm-1’	100	3	2	1	8
Atropine	5	0	1	0	1

See Table 1. Score: 0-1: pronounced activity, 2-4: good activity, 5-7: moderate activity, 8-9: weak activity, 10: inactive

4.3. Effect of aqueous extract Pm-1 of *P. maprouneifolia* stem bark and its fractions on magnesium sulphate induced diarrhoea in Wistar rats

Magnesium sulphate is known to induce diarrhoea by increasing the volume of intestinal content through the prevention of reabsorption of water. It also promotes the liberation of cholecystokinin from the duodenal mucosa, which increases the secretion and the motility of the small intestine resulting in the prevention of the reabsorption of sodium chloride and water.[32,33] It also causes an increase in electrolyte secretion by creating an osmotic imbalance.[34]

In this pharmacological model used in the present study, all test samples from *P. maprouneifolia* stem bark administered at oral doses of 100 and 200 mg/kg body weight, were found to alleviate the diarrhoea conditions in a dose-dependent manner since significant delay of onset time of diarrhoea and defecation appearance and significant decrease of all other diarrhoeal parameters were observed in treated Wistar rat groups compared to untreated groups ($p < 0.01$) (Table 2).

At the test oral doses of 100 and 200 mg/kg bw, the inhibition of diarrhoea and defecation by aqueous extract Pm-1 was characterized by significant increase of the latency time of onset of diarrhoea (136.7 ± 0.7 and 145.7 ± 0.4 min respectively, significant ($p < 0.001$) decrease of the total number of faeces, total number of wet faeces and intestinal fluid volume.

Tableau 4: Effects of aqueous extract Pm-1 of *P. maprouneifolia* and its fractions against sulphate de magnesium-induced diarrhoea in Wistar rats.

CS	OD	ETD	TNWF	TNHW	IFV	% IDia	% IDef
N.C. eau	5 ml water	93.3 ± 0.4	9.4±0.4	8.4±0.5	7.8±0.2	0.0	0.0
Pm-1	100	136.2±0.7	2.2±0.3	2.4±0.4	3.1±0.6	76.6±0.7	71.4±0.2
	200	145.7±0.4	1.9±0.7	2.1±0.1	2.9±0.8	79.8±0.5	75.0±0.3
Pm-1.1	100	119.5±0.2	3.8±0.4	4.0±0.3	4.5±0.5	59.6±0.3	52.4±0.2
	200	123.4±1.1	3.5±0.3	3.8±0.3	3.6±0.8	62.8±0.5	54.8±0.5
Pm-1.2	100	138.7±1.3	3.4±0.6	3.6±0.5	4.2±0.2	63. ± 1.2	57.1±1.3
	200	151.6±1.1	3.0±0.2	3.2±0.4	3.4±0.2	68.1±0.2	62.0±0.4
Pm-1.3	100	132.4±0.5	3.3±0.4	3.5±0.5	4.3±0.8	60.7±0.2	58.3±0.1
	200	137.4±1.9	3.1±0.2	3.3±0.2	4.0±0.3	67.0±0.6	60.7±0.2
Pm-1.4	100	141.3±0.6	2.4±0.3	2.8±0.9	3.5±0.4	74.5±0.8	66.7±0.3
	200	147.3±0.9	2.1±0.6	2.4±0.8	3.1±1.5	77.6±1.2	71.4±1.4
Pm-1’	200	123.4±0.5	6.9±0.2	7.1±0.8	5.1±0.5	26.6±0.3	15.5±0.7
Loperamide	2.5	196.7±0.2	1.3±0.1	1.5±0.2	0.5±0.1	87.5±0.3	84.0±0.6

See Table 2.

Compared to untreated groups compared to negative control group (Table 2). It had shown scores of 3 and 2 indicating its good antidiarrhoeal activity at all tested oral doses.

At the same oral doses, all soluble fractions Pm-1.1 to Pm-1.4 also showed significant ($p < 0.05$) increase of the latency time of onset diarrhoea (119.5±0.5 to 147.3±0.9) min compared to untreated groups (93.3±0.3 min). Briefly, the administration of the highest test oral dose of 200 mg/kg bodyweight showed that all fractions showed more than 60% inhibition of diarrhoea (Table 2). Pm-1.4 soluble fraction showed higher activity ($p < 0.005$) than other fractions (Table 2) while the remaining soluble fractions produced more than 60 and 52% inhibition of diarrhoea and defecation respectively. Based on the scores, score of 3 and 4 where seen with the fractions Pm-1.4 and Pm-1.2 respectively indicating their good antidiarrhoeal activity, followed by Pm-1.3 (score 5) with moderate activity while Pm-1.1 had a score of 8 showing its low activity comparable to that of the detannified extract Pm-1’.

In both antidarrhoeal models, it can be suggested that *P. maprouneifolia* stem bark and its soluble fractions may have increased the reabsorption of electrolytes and water from the gastrointestinal tract, since they delay the gastrointestinal transit in treated animals as compared to negative control group.[33,35] The delay in gastrointestinal transit prompted by these samples might have contributed to some extents to their antidiarrhoeal activity by allowing a greater time of absorption. This finding is in good agreement with Paredes et al., (2016). The antidiarrhoeal activity of aqueous extract of *P. maprouneifolia* stem bark and its fractions may probably due to their ability to inhibit intestinal motility and to decrease reabsorption of water and electrolytes as also reported for other medicinal plants extracts.[8,19,29,33]

Interestingly, in both castor oil and magnesium sulphate-induced diarrhoea, at oral doses of 100 and 200 mg/kg, and 200 mg/kg body weight respectively, the detannified aqueous extract (Pm-1’) produced low antidiarrhoeal activity compared to that seen with the parent extract ($p < 0.001$). At the highest tested oral dose of 200 mg/kg, it produced 40.7 ± 0.4 and 36.7 ± 0.8%, and 32.9 ± 0.2 and 21.3 ± 0.5% inhibition of diarrhoea and defecation in castor oil-induced and magnesium sulphate diarrhoea in animals respectively. The intestinal fluid volume excreted by animals after treatment of 4 h is also a good parameter used to more appreciate the level of antidiarrhoeal activity of a tested sample. In the present case, it was observed that all samples from *P. maprouneifolia* significantly decrease the volume of this parameter in a dose-dependent manner compared to untreated group (Tables 2 and 4).

Other diarrhoeal parameters such onset time, total number of wet faeces, total number of faeces and volume of intestinal fluid excreted are low compared to that seen with the parent extract. It had a score of 8 in castor-oil and 9 in magnesium sulphate induced diarrhoea as a sign of its low antidiarrhoeal activity.

This finding clearly showed the important role played by tannins in the level of the antidiarrhoeal activity of the parent sample and could be considered as the active principle as also previously reported in other studies.[6,19,33,36,37]

Table 5: Scores of *P. maprouneifolia* stem bark samples against magnesium sulphate-induced diarrhoea in Wistar rats.

Treatment	Dose (mg/kg)	Diarrhoea	score		Total score
		++	+	0	
Negative control	5 ml water	6	0	0	12
Pm-1	100	1	1	4	3
	200	0	2	4	2
Pm-1.1	100	4	1	1	9
	200	3	2	1	8
Pm-1.2	100	2	1	2	5
	200	1	2	1	4
Pm-1.3	100	3	1	1	7
	200	2	1	3	5
Pm-1.4	100	2	1	1	5
	200	1	1	4	3
Pm-1’	100	3	3	0	9
	200	4	1	1	9
Loperamide	5	0	0	5	0

In the small intestinal transit test, during the experiment, the charcoal meal procedure was choiced to follow the displacement of the gastrointestinal content as the decrease of gastrointestinal motility is known as a one mechanism action of antidiarrhoeal agents.[38] Results in Table 6 showed that all tested samples have a high capacity to inhibit intestinal motility compared to negative control group. They significantly ($p < 0.05$) reduced intestinal transit as observed by the reduction of gastrointestinal motility of the marker charcoal suggesting their action on all parts of the intestine. Thus, the decrease of the intestinal propulsive movement of the marker may be due to the anti-motility effect[12,39] of tested aqueous extract and its fractions and demonstrate that they may be able to reduce the frequency of stool in diarrhoeal conditions as also reported by[40] for the effect of *Maranta arundinacea* leaves methanolic extract. Moreover, the delay of gastrointestinal transit prompted by tested samples might have contributed, at least to some extents their antidiarrhoeal activity by allowing a greater time for absorption and this observation is in good agreement with.[39,40]

Table 6: Effect of aqueous extract Pm-1 of *P. maprouneifolia* stem bark and its fractions on castor oil-induced small intestinal transit in Wistar rats at oral dose of 200 mg/kg bw.

Groups	Treatment	MLSI	MDTC	%IW
I	Castor oil (NC)	110.60±0.05	90.87±0.12	-
II	Loperamide (2 mg)	104.30±0.03	79.65±0.05	76.36±0.04
III	Pm-1	108.23±0.02	77.35±0.03	71.46±0.05
IV	Pm-1.1	106.78±0.05	60.25±0.07	56.42±0.02
V	Pm-1.2	107.54±0.10	75.23±0.11	69.95±0.13
VI	Pm-1.3	104.89±0.07	58.23±0.09	55.51±0.14
VII	Pm-1.4	107.98±0.06	74.25±0.10	68.76±0.06

NC: negative control, TLI: Mean length of small intestine (cm), DTC: mean distance travelled by charcoal (cm), % IW: % inhibition of intestinal weight.

The observed inhibition of the intestinal transit by these samples from *P. maprouneifolia* stem bark can be used to establish that they possess the ability to relax intestinal smooth muscles as also reports for other antidiarrhoeal medicinal plant extracts.[41,42,43] These reported results are similar to those previously described by.[43] The delay in gastrointestinal transit prompted by the samples might have contributed to some extents to their antidiarrhoeal activity by allowing a greater time of absorption of water and electrolytes. This finding is in good agreement with.[33] Compared to Loperamide treated Wistar rats, higher percentage inhibition of intestinal motility transit was observed in aqueous extract Pm-1 treated animals with inhibitory potentials of 71.46% followed by Pm-1.2 and Pm-1.4 soluble fractions with 69.95 and 68.78% respectively. Pm-1.1 and Pm-1.3 soluble fractions showed more than 50% inhibition (Table 6).

In the castor oil induced enteropooling test, all tested samples were found able to significantly ($p < 0.05$) reduce both the mean weight of small intestine content (MWSIC) and mean volume of small intestine content (MVSIC) at the highest tested oral dose of 200 mg/kg bw and the intraluminal fluid accumulation compared to negative control group (Table 7).

Maximal inhibition of MWSIC and MVSIC was observed with aqueous extract Pm-1(74.64 and 72.72% respectively) followed by soluble fractions Pm-1.2 (69.01 and 70.90% respectively) and Pm-1.4 (64.78 and

69.09% respectively). Pm-1.1 and Pm-1.3 soluble fractions also showed good inhibition more than 60% on MVISC and more than 50% on MWSIC. The remarkable antidiarrhoeal effect of aqueous extract of *P. maprouneifolia* and its soluble fractions proved to their efficacy in extensive range of diarrhoeal conditions can be considered as an alternative natural drug for the treatment of diarrhoea.

Table 7: Effects of aqueous extract Pm-1 of *P. maprouneifolia* stem bark and its fractions on castor-oil-induced enteropooling of Wistar rats at oral dose of 200 mg/kg bw.

Groups	Treatment	MWSIC(g)	% Inhibition	MVSIC	%Inhibition
I	Castor oil	0.71±0.05	-	0.55±0.11	-
II	Loperamide	0.18±0.02	74.64	0.13±0.04	76.36
III	Pm-1	0.22±0.04	69.01	0.15±0.12	72.72
IV	Pm-1.1	0.27±0.02	61.97	0.19±0.04	65.45
VI	Pm-1.2	0.24±0.16	66.62	0.16±0.17	70.90
VI	Pm-1.3	0.30±0.01	57.74	0.22±0.10	60.00
VII	Pm-1.4	0.25±0.12	64.78	0.17±0.14	69.09

Moreover, tannins and tannic acid act as antidiarrhoeal agents by the denaturation of proteins in the intestinal mucosa forming protein tannates which make the intestinal mucosa more resistant to chemical alterations and thus reduce the secretions[43,45] and more resistance to chemical alteration and hence reduce peristaltic movements and intestinal secretion.[46] Flavonoids have been ascribed to their ability to inhibit intestinal motility and hydroelectrolytic secretions which are known to be altered in diarrhoea conditions.[47,48] Steroids and triterpenes are useful for the treatment of diarrhoea and may increase intestinal absorption of Na^+ and water.[3]

4.4. Spasmolytic activity of aqueous extract of *P. maprouneifolia* and its fractions

Results in Table 3 showed the percentage inhibition tested samples on the contractions of isolated guinea-pig ileum induced by acetylcholine (Ach) and depolarizing solution rich in KCl (DSR KCl). They revealed that all tested samples were able to significantly inhibit these contractions induced by both agonist on isolated guinea-pig ileum at the tested concentration of 40 µg/ml in organ bath. Aqueous extract Pm-1 displayed high spasmolytic activity by producing 85.6 ± 0.5 and 82.4 ± 0.8% inhibition of contractions induced by Ach and DSR KCl respectively. Pm-1.2 and Pm-1.4 soluble, Pm-1.1 and Pm-1.3 soluble fraction fractions produced more than 75 and 60% inhibition of contractions induced by both agonist respectively (Table 6).

Tableau 8: Percentage inhibition of contractions induced by Ach and DSR KCl by aqueous extract Pm-1 of *P. maprouneifolia and its* fractions.

Code samples	% Inhibition of Ach	% Inhibition of DSR KCl
Pm-1	85.64 ± 0.51	82.41 ± 0.83
Pm-1.1	66.57 ± 0.24	61.77 ± 0.71
Pm-1.2	79.65 ± 0.11	76.54 ± 1.24
Pm-1.3	69.32 ± 1.43	66.91 ± 1.10
Pm-1.4	82.64 ± 1.21	78.68 ± 0.44
Atropine	100.00 ± 0.0	0.00 ± 0.00
Papaverine HCl	98.48 ±0.45	97.74 ± 012

See Tableau 2

The most active soluble fraction was Pm-1.2 rich in flavonoids followed by Pm-1.4 rich other phenolic compounds than flavonoids. Thus, the resultant soluble fractions were obtained in the following order of potency for their spasmolytic effect: ethylacetate Pm-1.2 > aqueous residual phase Pm-1.4 > *n*-butanol Pm-1.3 and chloroform Pm-1.1.

All samples significantly reduced the maximal effect of Ach suggesting a non-competitive antagonism over the cholinergic contraction as also reported by[49] for extracts of *Aloysia polystachya* and *Alosia gartissima* leaves. The spasmolytic activity of these samples was completely reversible after washing isolated guinea-pig ileum with plentiful Tyrode's solution and restimulation separately with both agonists suggesting that their spasmolytic activity was not possibly accompanied with binding to Ca^{2+} channels or entering to the smooth muscle cells.

Compared to the antispasmodic activity of atropine and papaverine used as reference products, it was concluded that these samples from *P. maprounefolia* stem bark have a papaverinique-like effect and their efficiencies were lower compared to reference products.

4.5. Antibacterial activity of aqueous extract of *P. maprouneifolia* and its fractions

Results from the antibacterial testing are presented in Table 7. For a good interpretation, following criteria were adopted: MIC,MBC ≤ 100 µg/ml :good activity, 125≤ MIC, MBC ≤ 250 µg/ml :moderate activity, 250 < MIC, MBC ≤ 500 µg/ml : week activity, MIC, MBC > 500 µg/ml : inactive.

Results indicated that aqueous extract Pm-1 exhibited good antibacterial and bactericidal activities against a wide range of tested bacteria with CMI and CMB values < 100 µg/ml.[23,50] All soluble fractions displayed antibacterial activity at different extents. Some of them such as etylacetate Pm-1.2, *n*-butanol Pm-1.3 and residual aqueous phase Pm-1.4 also exhibited good antibacterial and bactericidal activities against a wide range of selected bacteria[23,50] or showed moderate and weak activities against other bacteria, or they were inactive according to the case (Table 7). Moreover, it was observed that the selected *S. aureus* in the present study presented a resistance against all tested plant samples including Ampicillin and Tetracycline, antibiotic uses as references. This bacteria would be a resistant microorganism to antibiotics.

Tableau 9: Antibacterial activity of aqueous extract Pm-1 of *P. maprouneifolia* stem bark and its fractions.

A/B	*E.c.*		*S.f.*		*S.d*		*S.e.*		*S.t*		*S.a.*	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Pm-1	62.50	125	15.62	31.25	31.25	62.50	62.50	125	31.25	62.5	>500	>500
Pm-1.1	125	250	31.25	62.50	>500	>500	125	250	>500	>500	>500	>500
Pm-1.2	15.62	31.25	7.75	15.62	31.25	62.50	>500	>500	31.25	62.50	>500	>500
Pm-1.3	31.25	62.50	15.62	31.25	625	125	>500	>500	62.50	62.50	>500	>500
Pm-1.4	62.5	125	31.25	62.5	>500	>500	125	250	250	500	>500	>500
Ampicillin	1.95	3.90	0.97	0.48	0.48	0.97	0.97	1.95	1.95	3.90	>500	>500
Tetracycline	15.62	31.25	0.97	1.95	0.97	1.95	1.95	3.90	1.90	3.90	>500	>500

A : microorganisms, B : sample codes (see Table 2), *E.c.*: *Escherichia colis, S. f.: Shigella flexneri, S. d.: Shigella dysenteria, S.t.; Salmonella tiphymurium, S.a.: Staphylococcus aureus.*

In general, the antibacterial and bactericidal activities of *P. maprouneifolia* stem bark samples were weaker compared to antibiotics Ampicillin and Tetracycline used as reference products (Table 7).

4.6. Antiameobic activity of aqueous extract Pm-1 of *P. maprouneifolia* and its fractions

Results from the antiameobic testing are listed in Table 8. For a good interpretation, following criteria were adopted: MAC, IC_{50} < 10 µg/ml: pronounced activity, 10 ≤ MAC, IC_{50} < 20 µg/ml: good activity, 20 ≤ M AC, IC_{50} < 30 µg/ml: moderate activity, 30 ≤ MAC, IC_{50} < 40 µg/ml: weak activity, MAC, IC_{50} > 50 µg/ml inactive.

Results indicated that aqueous extract Pm-1 and its soluble fractions Pm-1.2 and Pm-1.4 exhibited pronounced antiamoebic activity with MAC and < IC_{50} values < 10 µg/ml. The most activity sample was Pm-1 extract with MAC and IC_{50} values of 5.55±0.12 and 3.71±0.04 µ/ml respectively. Pm-1.1 soluble fraction showed good antiamoebic activity on the inhibiting *E. histolytica* growth with MAC value of 17.25±0.07 µg/ml and pronounced activity by inhibiting 50% population of the parasite with IC_{50} value of 9.24±0.05 µg/ml. Pm-1.3 soluble fraction showed moderate activity on the growth of this parasite and good activity by inhibiting 50% population with MAC and IC_{50} values of 21.56±0.11 and 11.57±0.09 µg/ml respectively.

Table 10: Amoebicidal activity of aqueous extract Pm-1 of *P. maprouneifolia* and its fractions.

Sample codes	MAC, µg/ml	IC_{50}, µg/ml
Pm-1	5.55±0.12	3.57±0.14
Pm-1.1	17.25±0.07	9.24±0.05
Pm-1.2	7.15±0.02	5.24±0.04
Pm-1.3	21.56±0.11	11.57±0.09
Pm-1.4	9.45±0.08	6.21±0.05
Metronidazole	0.05±0.01	0.03±0.01
Dehydroemetine	0.07±0.02	0.04±0.01

MAC: minimum ameobicidal concentration, IC_{50}: inhibitory concentration of 50% population.

The antiamoebic of these samples were weaker compared to Metronidazole and Dehydroemetine used as antiamoebic reference products (Table 8).

These biological activities displayed by samples from *P. maprouneifolia* stem bark are due to presence of secondary metabolites such as alkaloids, flavonoids, tannins, saponins, steroids and terpenes detected in this plant part which have largely contributed to their manifestation since they were previously reported to

exhibit these evaluated biological activities at different extents.[33,37,44,45,51,60] As the responsible active principles are not yet determined, these chemical groups may act in a synergetic manner to enchance the level of observed activity. In general, it is well reported that the antidiarrhoeal activity of some medicinal plants is due to the presence of tannins, saponins, coumarins, flavonoids, alkaloids, steroids and terpenoids.[37,43,44]

5. CONCLUSION

The present study have demonstrated that aqueous extract Pm-1 of *P. maprouneifolia* and its soluble fractions exhibited various biological activities such as antibacterial, antiamoebic and antispasmodic *in vitro*with different magnitudes. In addition all samples from this medicinal plant were able to significantly reduce diarrhoea induced by castor oil and magnesium sulphate, gastrointestinal motility and enteropoling in treated animals with various magnitudes. This is the first time to report some biological activities of extracts of *Pseudolacnostylis maprouneifolia* stem bark Thus, these findings constitute scientific bases that can explain and support the medicinal use of *P. mapouneifolia* in traditional medicine in Democratic Republic of Congo and other African countries for the treatment of diarrhoea according to its origin.

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