Ethopharmacological approach, in vivo antidiarrhoeic properties of *Mallotus oppositifolium* (Euphorbiaceae) and isolation of antidiarrheal compound

Abstract

Mallotus oppositifolium. Müll. Arg (Euphorbiaceae) is commonly used in the Center Region of Cameroon against diarrheal syndromes and joint pains. In order to provide a scientific basis for its use in the traditional treatment of diarrhea, we proposed to evaluate the effects of aqueous extracts of the leaves of this plant on the characteristic patterns of diarrhea. The ethnopharmacological approach was carried out in Gouifé village in Bafia (Cameroon) by observing traditional therapeutic practices and by the interview of 5 traditional therapists and 11 of their patients about the traditional treatment of diarrheal syndromes. The leaf extracts of M. *oppositifolium* was extracted by decoction and the product at the doses of 31.25, 62.50 and 125 mg/kg body weight was tested *in vivo* on castor oil-induced secretory and motor diarrhea in rats, using standard antidirrhea techniques. The diarrhea was induced in the rats using standard inducing procedure. The decoction leave extract were found to act against castor oil-induced secretory diarrhoea in rats as it increased significantly the time to onset of diarrhoeal stools in a dose-dependent manner at all the doses investigated ($p < 0.01$) compared to animals who received distilled water. The mass and volume of intestinal contents, compared with Loperamide (36.80%) and distilled water 52.50% ($p < 0.05$), decreased significantly by 9.70% and 32. 60% at 32.25 mg/kg, 38.80% ($p < 0.01$) and 49.80% (0.05) at 62.5 mg/kg and 28.90% ($p < 0.05$) and 37.30% at 125 mg/kg, respectively. The decoction extract was also active against motile diarrhea as it increased significantly the time to onset of charcoal-stained diarrheal stools. Compared to distilled water (70 min), 80 min ($p < 0.05$), 118 min ($p < 0.01$) and 207 min ($p < 0.01$) for the extract doses of 32.25, 62.50 and 125 mg/kg animal weight respectively. The mass and the frequency of the stool output were reduced significantly by all the doses of the extract used in this study. This extract slowed fecal progression by 81.80%, 76% ($p < 0.05$) and 72% ($p < 0.01$) at 32.25, 62.5 and 125 mg/kg respectively. A chemical fractionation by separation and purification using various chromatographic methods (column chromatography, thin layer chromatography, "flash" Chromatography on silica gel G60…) of *M. oppositifolium* leaves extract with dichloro methane/ methanol revealed the presence of metabolites such as sterols, quercetin, diosmetin, quercitrin. Some of these components have known antidiarrheal properties that would justify the traditional use of the plant in the treatment of diarrhea.

Keywords: Ethnopharmacology, *Mallotus oppositifolium*, antidiarrheal, castor oil components

1. Introduction

Traditional medicines are more valued in low income countries where the biodiversity of plants constitutes an important source of traditional remedies and more than 80% of the population, at least use natural products for the treatment of the poverty related diseases [1] or in primary health care [2]. Diarrheal diseases are the second leading cause of death in the world [3], despite a 4% annual decrease in mortality rate [4,5], they still constitute 12% of the 3.6 million deaths in children under 5 years of age [6]. In Cameroon, the prevalence of diarrheal diseases is 19.7% in rural areas of Cameroon [7,8] and they are responsible for 14.4% of deaths in children under 5 years of age [9]. Most of the diarrhoeal cases are due to bacterial enteropathogens, diarrhoeagenic *Escherichia coli* being the most common cause in developing countries [10]. The two important bacterial groups causing diarrhea are diarrhoeagenic *E. coli*, mainly enterotoxigenic and enteroaggregative [11] and invasive bacterial pathogens like *Shigella, Campylobacter and Salmonella* [12]. Rotavirus is the most common amongst the viral agents [13]. Oral rehydration therapy (ORT) and antibiotherapy has been the key strategies for effective case management and has been instrumental in reducing diarrhea-related deaths [14]. The problems of unavailability and affordability of pharmaceutical drugs in urban and rural area, fake dugs and increasing rate of resistance of diarrhoeagenic bacteria to conventional drugs prompted the search of cost-effective alternative approaches for the treatment of diarrhea.

To contribute to the management of these diseases, the World Health Organization (WHO) recommends the evaluation of the quality, safety and efficacy of medicinal plants by modern techniques in search of new avenues for the development of new drugs. This organization has even established a program for the control of diarrheal diseases that includes the study of traditional medicinal plants [15]. The ethnopharmacological approach of the use of *Mallotus oppositifolium* (Euphorbiaceae) partly fulfills these aspirations. *Mallotus oppositifolium* (Geiseler) Müll. Arg. (Euphorbiaceae) is a medicinal plant used locally to treat many diseases in Cameroon and other equatorial and tropical African countries. In these regions, the leaves have several properties against helminthiasis, dysentery and acute diarrhea [16]. Phytochemical studies and antioxidant effects [17,18,19], antimicrobial [20,21] and antispasmodic [22] properties of the plant have been conducted. The present study was undertaken to outline an ethopharmacological approach of the use of the plant, evaluate the

antidiarrheal properties of aqueous extracts, fractionate and isolate potentially active compounds

2. Materials and Methods

2.1 Ethnopharmacological approach

The traditional use of the plant was studied in the village of Gouifé, Kiiki District, 17 km from Bafia, the capital of the Mbam and Inoubou Division in the Centre Region of Cameroon. We observed the traditional practices of the traditional practitioners and the clinical evolution of their patients admitted for diarrheal syndromes. Subsequently, the traditional practitioners, patients or their parents were subjected to voluntary interviews using simple questionnaire to obtain data on their perception of diarrheal syndromes, the classification, the etiologies of diarrhea and their management with medicinal plants. Primary data from the questionnaires and secondary data from ethopharmacological literature allowed us to select *M. oppositifolium* and to develop a simple monograph of plant in the traditional management of diarrhea.

2.2 Plant material

Fresh leaves of *M. oppositifolium* were collected in Gouifé Center Region of Cameroon in march 2021 between 7h:00 and 9h:00 AM before the rising sun. The species was subsequently identified and authenticated at the National Herbarium of Cameroon where a specimen was deposited under the number HNC 16619. The leaves were washed with tap water before drying in the shade at room temperature (22-26°C) for 7 days. They were then crushed into powder using an electric machine and stored in closed glass bottles (3200 g). In the traditional Gouifé pharmacopoeia, the leaves were boiled (decoction) or macerated in cold water, or placed in boiled water (infusion). The infusion or decoction was administered orally in the treatment of diarrheal syndromes. This preparation guided us in the elaboration of the extraction and administration protocols of the plant. 500 g of leaf powder of this plant were macerated in 5 L of distilled water for two hours. The macerated sample was boiled in water bath for 15 minutes. The solution obtained was filtered with Whatman No. 1 filter paper. The filtrate obtained was concentrated in a rotary evaporator at 40-45 °C to obtain 28.6 g of a brown semi-solid extract of *Mallotus oppositifolium* yielding 5.72%.

2.3 Experimental animals

The experiments were carried out on 10 to 12 weeks old albino Wistar rats of both sexes weighing between 150 to 220 g. Animals were raised at room temperature, with sufficient ventilation, in the animal houses in the Faculty of Medicine and Biomedical

Sciences (FMSB) of the University of Yaoundé 1. These animals were watered *ad libitum* with tap water with the addition of a few drops of vitamin B complex (5 drops per liter).

Their diet consisted of a mixture of corn meal (60%), wheat meal (10%), fish meal (12%), soybean meal (15%) and 3% palm oil [23]. All experiments on animals were conducted according to the recommendations of the OECD guidelines 425, and the European Union guidelines (EEC Directive of 1986; 86/609/EEC).

2.4 Chemicals

All chemicals used in this study were of analytical quality and from standard companies and the water used was double distilled. The evaluation of the effects of the aqueous extract on secretory and motor diarrhea was studied with the following reagents: double distilled water (ED), castor oil (HR-Sigma-Aldrich laboratory), Loperamide 5mg (Imodium®: Janssen Cilaq laboratory), activated charcoal (AC) dissolved in 1% carboxymethylcellulose (CM) (Sigma-Aldrich laboratory).

2.5 Evaluation of the activity of *M. oppositifolium* on castor oil-induced diarrhea

The effects of *M. oppositifolium* decoction on castor oil-induced diarrhea were evaluated on 18 hours fasted animals with access to distilled water *ad libitum* according to the method of Yakubu et al. [24]. Animals were divided and treated as follow: the first group (negative control) received distilled water (10 mL/kg), animals of the second group (positive control) received Loperamide (5mg/kg) and the other groups were treated with *Mallotus oppositifolium* decoction at doses of 31.25, 62.5 and 125 mg/kg animal body weight respectively. 30 minutes after the administration of the different treatments, each animal was orally administered castor oil (2 mL/kg body weight) through an esophageal tube. The animals were then placed individually in metabolic cages on pre-weighed filter paper racks that was changed every hour for 6 hours. The time of onset of diarrhea was observed and the frequency of diarrhea was assessed every hour for 6 hours. The number of normal and diarrheal feces excreted as well as their frequency and change in mass were expressed compared to the negative and positive control groups. The percentage of diarrheal defecation inhibition in each group was also calculated according to the formula:

Inhibition of diarrheal feces = (Mt-Me) /Mt x100 [24]

Mt: Mass of diarrheal feces excreted by rats treated with distilled water (negative control)

Me: Mass of diarrheal feces excreted by rats treated with Loperamide (positive control) or the extract.

2.6 Evaluation of the of effect *M. oppositifolium* on castor oil-induced gastrointestinal fluid accumulation

The effects of *M. oppositifolium* decoction on castor oil-induced intestinal secretions were tested on fasted rats as described previously.

Rats were distributed in 5 cages of 6 animals each (3 males and 3 females) and treated as follows [25]: Animals in the first group (negative control) received distilled water, animals of the second group (positive control) received Loperamide (5mg/kg), the other groups were treated with *Mallotus oppositifolium* decoction at doses of 31.25, 62.5 and 125 mg/kg respectively. One hour after the administration of the different treatments, each animal received orally castor oil (2 mL/kg body weight) through an esophageal tube. Two hours after the *per os* administration of the cathartic agent, rats were sacrificed by cervical dislocation. The located intestine, the pyloric end, and the ileo-caecal junction were ligated with a wire, and the resulting pudding was collected and weighed. The contents of each intestine were emptied into a graduated cylinder and the volumes recorded. The emptied bowel was reweighed to assess the difference in bowel weight expressing the mass of the bowel contents. Inhibition of intestinal content volume or mass was calculated according to the following formula [25]:

Inhibition of intestinal content mass or volume (%) = (MVt-MVe) /MVt x100

MVt: Mass or volume of intestinal contents of rats treated with distilled water (negative control)

MVe: Mass or volume of intestinal contents of rats treated with Loperamide (positive control) or extract.

2.7 Evaluation of the effect of *M. oppositifolium* on castor oil-induced fecal bowl propulsion

These effects were determined by assessing intestinal motility as decribed by Ezeigbo II et al. [26]. Rats were grouped and treated as described previously. One hour later, all the 5 groups received 2 mL/kg of castor oil One hour after administration of the cathartic agent, each rat received per os, 1mL/kg charcoal (5% activated charcoal dissolved in 1% Carboxymethylcellulose). One hour after charcoal administration, the rats were sacrificed by decapitation, sutures placed at the pylorus and caecum allowed isolation and removal of their intestine which was subsequently laid on a bench. The progression of charcoal along the intestine (from the pylorus to the cecum) was measured and expressed as the percentage of progression of stained diarrheal feces in relation to the total length of the small intestine. Inhibition of fecal bolus progression was calculated according to the following formula [47]:

Inhibition of fecal bolus progression (%) = (Dt-De) /Dt x100

Dt: distance traveled by charcoal in the intestine of negative control rats,

De: distance traveled by charcoal in the intestine of rats in the positive control and test groups.

2.8 Extraction and isolation of compounds

This extraction and isolation were carried out by the chemist Pascal Sonna in organic chemistry laboratory of the University of Yaoundé I. The powder of *M. oppositifolium* (2.500g) was cold macerated successively in dichloromethane methanol CH_2Cl_2-MeOH (1 :1) for 48 hours and then in methanol for 12 hours at room temperature. The analysis by thin layer chromatography allowed to mix the two organic fractions and to obtain 120 g of organic extract. After separation and purification by using various chromatographic methods (column chromatography, preparative thin layer chromatography, flash chromatography on silica gel G60), various components of the plant were isolated.

2.9 Statistical analysis

The results of the field studies were expressed in the form of number of participants in relation to the total number of people surveyed. The results of the *in vivo* properties of the plant were presented as histograms were expressed as mean ± Standard Error of the Mean (SEM) of several replicate tests. The comparison between the negative (castor oil) and positive (Loperamide) controls as well as with the batches of rats (treated with different doses of the extract) was done using ANOVA followed by Dunnett's posttest. Graph Pad Prism software version 5.03 (GraphPad Software, San Diego, California, USA) was used to perform the statistical analyses and the probability values $p< 0.05$ were considered statistically significant.

Repeated Chromatography on silica gel G60 followed by Sephadex LH-20

3. Results

3.1 Perception of diarrhea by traditional practitioners and patients

From the field surveys and clinical observations carried out with 5 traditional practitioners and 11 people who had stayed with these traditional practitioners, showed that, among the 5 traditional therapists of this study, all of them were above 60 years old and, only 1 had less than 10 years of practice and all were from the same village. Out of the 11 patients/relatives interviewed, 4 were between 55 and 70 years old and the other 7 were mothers accompanying their children who were less than 6 years old, that is. 64% of patients treated. Diarrhea was defined as "a breakthrough of the stomach" by increasing the frequency

of stools, generally liquid. They were traditionally classified according to the explanation of the symptoms by the respondents into simple, glairy and glairo-sanguinous diarrhea.

According to the traditional healers, the latter two were often accompanied by abdominal pain and vomiting. The etiologies of diarrhea had multiple: creeping and teething of infants, "poisoning, evil spell, witchcraft", food poisoning. Majority of traditherapists and patients continued to believe that diarrhea was also caused by bad luck and witchcraft. However, infant creeping and teething, food poisoning and poor hygiene constitute a large majority of the causes of diarrhea. The treatment of diarrhoea is essentially done with the help of several medicinal plants, including *Mallotus oppositifolium* (MO) and *Euphorbia hirta*, which were used by 4/5 therapists respectively. *Psidium goyava* (Myrtaceae) is the plant most known by patients in the treatment of diarrhea as shown in the Table 1 below and M. oppositifolium is used by the traditherapist consulted for the diarrhoeas known as simple, the mucous diarrhoeas and the glairo-sanguinous diarrhoeas as shown in the Table 2 below.

Table 1: Anti-diarrheal plants mentioned by traditional therapists (n =5).

Plant parts used	**Number of censored Tradi-therapists**
Psidium goyava (Myrtaceae) leaves	4 (80 %)
Aerial parts of *Euphorbia hirta* (Euphobiaceae*)*	4 (80 %)
Mallotus oppositifolium(Euphorbiaceae) leaves	4 (80 %)
Adansonia digitata (Bombacaceae) leaves	3 (60 %)
Alchornea cordifolia (Euphorbiaceae) leaves	4 (80 %)
Chromolaena odorata (Araceae) leaves	2 (40 %)
Adansonia digitata (Bombacaceae) bark	2 (40 %)

( ) : Evocation rate for use in the treatment of diarrhea.

Table 2: Distribution of tradithérapeutes according to the use of M. oppositifolium in the management of different forms of diarrhea

	Simple D.	Mucous D.	glairo-sanguinous D
Number of therapists using *M. Oppositifolium* according to the type of diarrhea (D.)	3 (60 %)	3 (60 %)	4 (80 %)

( ) favourable response rate, n = 5.

3.2 Simplified and indicative monograph of *M. oppositifolium* on diarrhea

According to traditional therapist, the plant is used in the treatment of all forms of diarrhea. The plant would also have effects on painful and inflammatory articular syndromes according to the therapist. From the primary data of this exploration, we have elaborated an

indicative monograph on the management of diarrheal syndromes. Called *Arbre de kisse kisse* in French, the Vernacular names of MO are Mchacha in Swahili [27] and Ukpo in Nigeria [28], Duchè in Maka (East Cameroon), Fanden in Bafia and Ofesse in Béti in Central Cameroon. A 3 m high shrub with tracer roots, *M. oppositifolium* is a plant with cordate, entire, serrated, flattened leaves carried by a long thin petiole of circular section. The vegetal is a perennial dicotyledonous spermaphyte found in equatorial and tropical forests and in transitional zones with savannah. Some leaves have excrescences identified as scabies. For traditionally treat diarrhea, the leaves are picked in the morning by the therapists which advises not to pick them at night or during and after the rain. Generally, a glass (25 cL) of infusate is given by oral way morning and evening until the end of the diarrhoeas, for an adult of approximately 75 kg.

3.2 Effects of *M. oppositifolium* on secretory diarrhea

Latency time before the appearance of diarrheal feces

The time to onset of diarrheal feces were 48, 240, 49, 128, and 180 minutes respectively, in rats treated with distilled water (DW) negative control, Loperamide 5 mg/kg (Lo) positive control, and decoction of *M. oppositifolium* at doses of 31.25, 62.5, and 125 mg/kg as shown in Figure 1. From that figure we can observe that *M. oppositifolium* at the doses of 62. 5 and 125 mg/kg significantly increased the diarrheal latency compared to negative group. The highest dose of extract (125 mg/kg) caused the greatest delay in onset time of castor oil-induced diarrheal stools (180 min) compared to rats treated with distilled water.

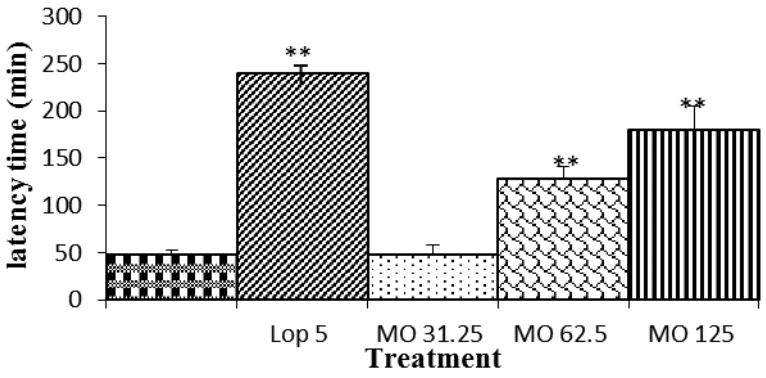

Figure 1: Latency of onset of diarrheal feces in rats treated with distilled water (DW), Lo 5 mg/kg and MO 32.25, 62.5 and 125 (MO 125) mg/kg of CP. Values are expressed as mean ± SEM, n = 5. * $p < 0.05$, ** $p < 0.01$compared to control group (DW).

3.3 Effects of *M. oppositifolium* on the mass of intestinal contents

The mass of intestinal contents was 3.9, 2.5, 3.6, 2.44, and 2.8 g, respectively for rats treated with DW, Lo and MO at the doses of 31.25, 62.5, 125 mg/kg and represented in Figure 2. The percentages of inhibition of intestinal content mass (compared to DW) were 36.8, 9.7, 38.8, and 28.9%, respectively for rats treated with Loperamide MO at doses 31.25, 62.5, and 125 mg/kg. Among the three concentrations of the extract, the 62.5 mg/kg concentration showed the greatest reduction in intestinal content mass (38.8%).

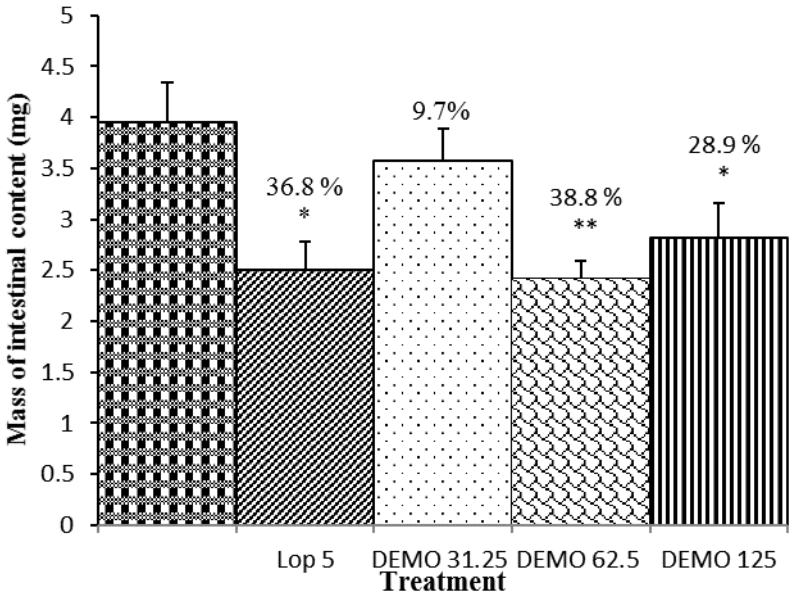

Figure 2: Mass of intestinal contents in rats treated with DW, Lo 5 mg/kg and DEMO 31.25, 62.5 and 125 mg/kg PC. Values are expressed as mean ± SEM, n = 5. * $p < 0.05$; ** $p < 0.01$ compared to control group (ED). %: rate of decrease in intestinal content mass compared to negative control.

3.4 Effects of *M. oppositifolium* on the volume of intestinal contents

The different volumes of intestinal contents are represented in figure 3. The percentages of intestinal volume reduction calculated were 52.5, 32.6, 49.8, and 37.3%, respectively for DW-treated rats, Lo and DEMO at 31.25, 62.5, and 125 mg/kg. Of the three doses of the plant extract, the 62.5 mg/kg dose showed the greatest reduction in this volume of intestinal content (49.8%).

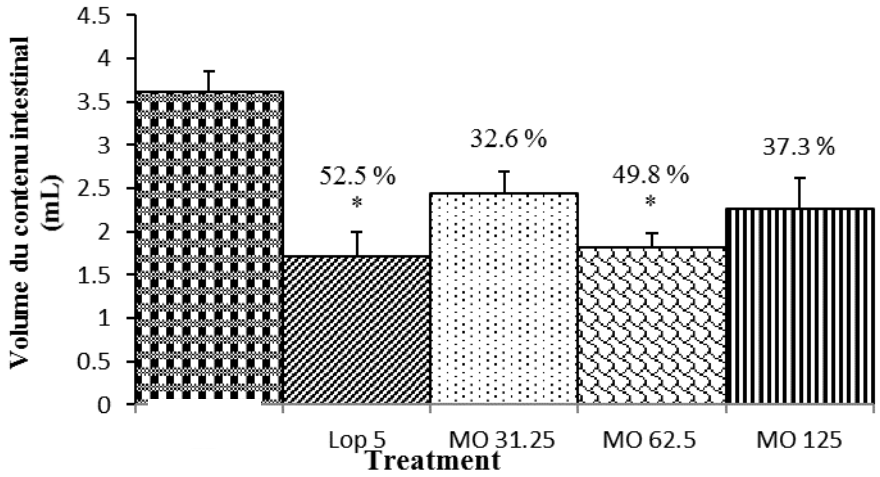

Figure 3: Volume of intestinal contents in ED, Lo 5 mg/kg and DEMO treated rats 31.25, 62.5 and 125 mg/kg of PC. Values are expressed as mean ± SEM, n= 5. *p < 0.05 compared to control group (DW). %: Rate of decrease in intestinal content volume compared to DW.

3.5 Effects of *M. oppositifolium* extract on motor diarrhea

The effect of *M. oppositifolium* on the time of onset of diarrheal feces is summarized in figure 4. The extract leaves of *M. oppositifolium* showed a dose-dependent significant increase of the time of onset of diarrheal feces compared to the negative control group treated with distilled water. The delayed of the onset of castor oil-induced diarrheal stools induced by the dose of 125 mg/kg on rats (Figure 5), was almost the same as the one induced on the secretory diarrhea in Figure 2.

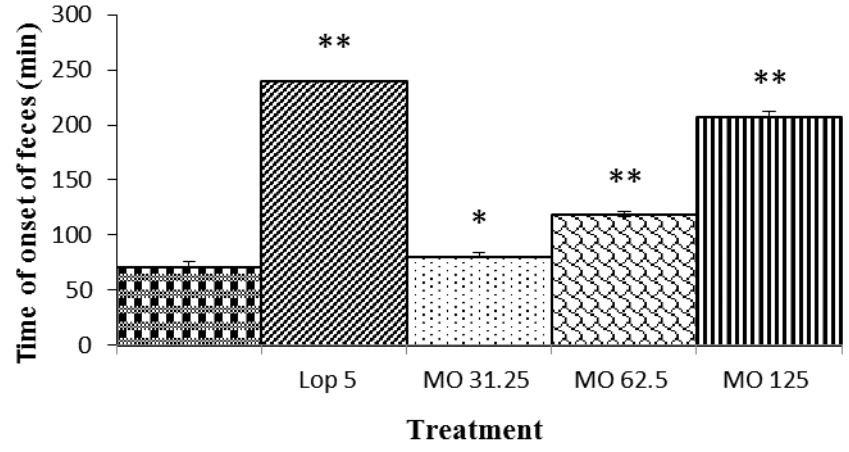

Figure 4: Time of onset of diarrheal feces in rats treated with DW, Lo 5 mg/kg and DEMO 31.25, 62.5 and 125 mg/kg PC. Values are expressed as mean ± SEM, n = 5. * p < 0.05 ** p < 0.01 compared to control group (DW).

3.6 Effects of *M. oppositifolium* on the frequency of diarrheal feces

The number of diarrheal feces excreted is shown in figure 5. The percentages of reduction in the frequency of diarrheic feces were 52.38, 22.62, 28.57, and 58.33% for Lo and the three extract doses, respectively. Only the dose 125 mg/kg showed inhibition of the number of diarrheal feces per hour greater than 50% close to the value obtained with the reference drug.

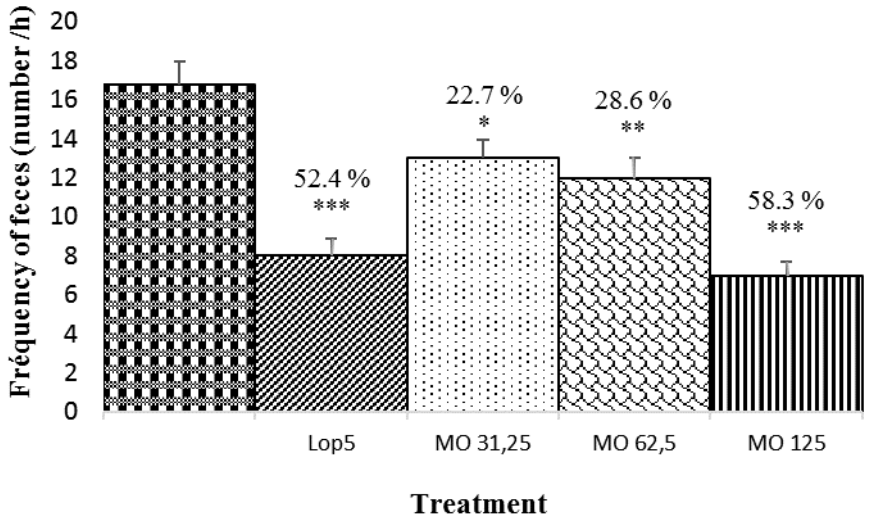

Figure 5: Frequency of faeces emitted (number/h) in rats treated with ED at Lo 5 mg/kg and MO 32.25, 62.5 and 125 mg/kg CP. Values are expressed as mean ± SEM, n = 5. *p < 0.05; ** p < 0.01; *** p < 0.001 compared to control group (DW). %: Fecal mass inhibition rate compared to negative control (DW).

3.7 Effects of *M. oppositifolium* on diarrheal fecal masses per hour

Figure 6 expresses the effect of the plant extract on the mass of diarrheal feces. The percentages of decrease in diarrheal stool mass were 70, 37, 36 and 56% for Loperamide, and the three doses of extracts respectively. The 125 mg/kg dose showed the greatest reduction in hourly mass of diarrheal feces induced by castor oil 0.5 mL/kg and compared to the negative control.

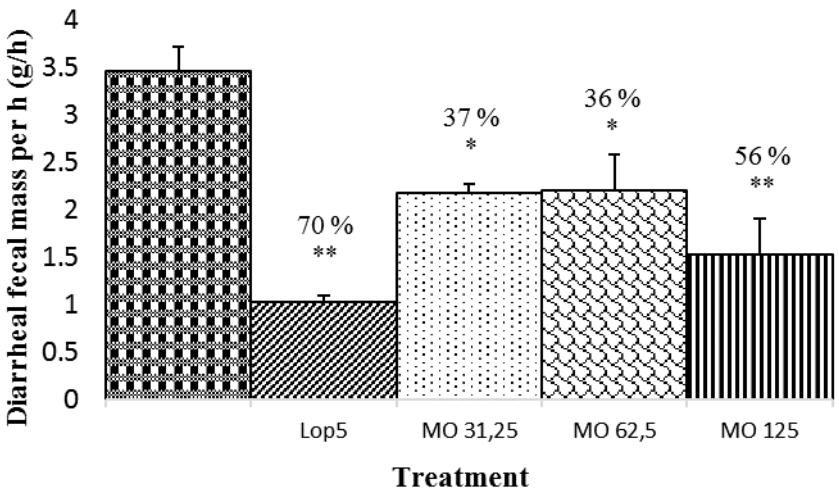

Figure 6: Mass of feces emitted per hour in rats treated with DW at Lo 5 mg/kg and DEMO 32.25, 62.5 and 125 mg/kg CP. Values are expressed as mean ± SEM, n = 5. * $p < 0.05$; ** $p < 0.01$ compared to control group (DW). %: Fecal inhibition rate compared to negative control.

3.8 Effects of *M. oppositifolium* on the progress of activated charcoal-stained fecal bolus

Progress of the stained fecal bolus along the digestive tract of diarrheic rats is presented in figure 7 a and b (expressed as percentage of distance traveled). It was 91.51% for rats treated with distilled water, 78.95% for rats treated with Loperamide 5mg/kg, and 81.82, 76.08, and 79.93% for animals treated with the different doses of aqueous extract of *M. oppositifolium* (31.25, 62.5, and 125 mg/kg), respectively. With a percentage of 71.9 %, the extract dose of 125 mg/kg caused the greatest reduction in castor oil-induced diarrheal stools.

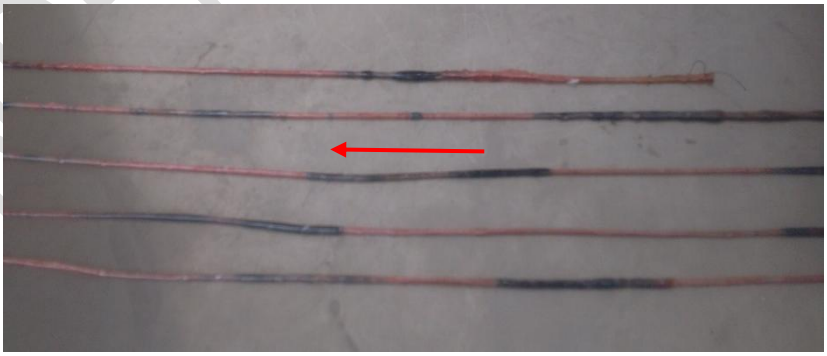

Figure 7 a: Progression of the fecal bolus along some isolated digestive tracts of rats.

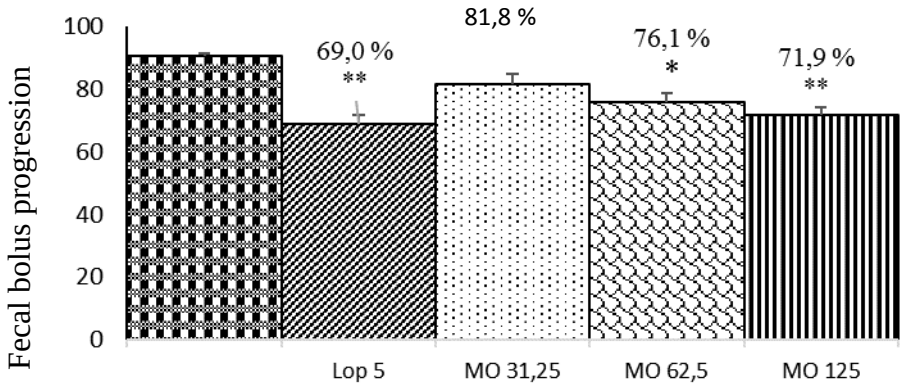

Figure 7 b: Progression of the fecal bolus (expressed as % distance traveled relative to the total length of the digestive tract) in rats treated with DW at Lo 5 mg/kg and DEMO 32.25, 62.5and 125 mg/kg PC. Values are expressed as mean ± SEM, n = 5. * $p < 0.05$; ** $p < 0.01$ compared to control group (DW).

3.9. Isolation of some of *M. oppositifolium* compounds

Sterols

Sterols crystallize in hexane-ethyl acetate mixture (9:1) as white needles. It was identified to a sterol mixture by comparison with a reference sample available in the organic chemistry laboratory. This sample initially analyzed by Gas Chromatography reported the presence of -sitosterol, stigmasterol, stigmastanol and campesterol.

β-sitosterol glucoside

β-sitosterol glucoside precipitates in ethyl acetate as whitish crystals. It has been identified to -D-glucopyranoside of -sitosterol by comparison to an authentic sample available in the organic chemistry laboratory of Yaounde I University.

Quercetin

This compound isolated from *M. oppositifolium* responds positively to the phenol test and appears as a yellow powder in the 2/3 hexane-ethyl acetate mixture. It melts between 320-321°C. All these data, in conformity with the presence of fragment ions at m/z 153 and 109 on the mass spectrum allows us to propose two substructures a and b as follows:

All these spectral data compared to those of the literature allow us to assign the following structure which is that of quercetin or 5,7,3',4'-tetrahydroxyflavonol described by Wagner et al in 1976 [29].

Diosmetin

This compound crystallizes as yellowish flakes in hexane - ethyl acetate mixture and melts between 260 - 261°C. It responds positively to the flavonoid test. Its electron impact mass spectrum shows the molecular ion M+ at m/z 300 compatible with the molecular formula $C_{16}H_{12}O_6$.

Dihydroquercetin

This molecule presents as a greenish-yellow powder in the hexane - ethyl acetate system (1:1). It melts between 301 - 303°C. It is soluble in acetone and responds positively to phenol and flavonoid tests.

Analysis of the 1H NMR spectrum of the fraction shows that it is a mixture of two products. All these data allow us to propose the structure of taxifolin or (2R,3R) -5,7,3',4'-tetrahydroxy-dihydroflavonol.

Quercitrin

Quercitrin is obtained as a beige powder in the mixture hexane-ethyl acetate (30:70). It melts between 232 - 234°C and responds positively to the Shinoda test characteristic of flavonoids. The whole of these data in comparison with those of the literature allowed us to attribute the structure of the quercetin or quercetin-3-O--L-rhamnose or Acanthophorin B described by J.H. Lee et al. [30].

Quercetin-7-O--glucose

This compound isolated from *M. oppositifolium* responds positively to flavonoid and phenol tests. It crystallizes in the hexane-ethyl acetate system (1: 4) and solubilizes in methanol. All these data in comparison with those of the literature allowed us to attribute to MO6 the structure 108 which is that of quercetin-7-O--glucose, described by Chari et al. [31].

4. Discussion

Mallotus oppositifolium is used in the equatorial, tropical sub-region of Africa and in Cameroon in the traditional treatment of several ailments, including pain, inflammation, infectious and diarrheal diseases [32, 28]. In our previous studies, we showed the inhibitory activities of this plant *in vitro* on the contractions of rat ileum fragments stimulated by Acetylcholine or Potassium Chloride, and *in vivo* on experimental induction of diarrhea by magnesium sulfate in rats [32]. In the current study, we proposed to determine its antidiarrheal properties by evaluating its effects on characteristic parameters of diarrhea

induced by castor oil, after an exploration of its local use (in Bafia, centre region of Cameroon) as an antidiarrheal.

According to ethnopharmacological exploratory surveys, diarrhea is still a major health problem in the populations especially among children who are mostly represented among the patients. Large-scale investigations in other localities will allow us to confirm these data throughout the country in order to compare them with those of the WHO [14]. The majority of traditional therapists consulted were above 60 years old and have been practicing for more than 10 years, which shows their notoriety, credibility and the trust that patients or relatives have in their practices and some are called "Doctor" as in Zambia [33]. They are therefore an alternative in primary health care [34].

Traditherapists defined diarrhea, almost in the same way as a "belly breakthrough" that is., an increase in the evacuation of intestinal contents, translated by an acceleration of gastrointestinal transit with frequent liquid stools. The notion of soft stools, which was unknown to them, shows that the treatment of these affections does not begin until the appearance of liquid stools. This would delay the treatment and therefore the cure of this disease traditionally. The diarrheal diseases were classified according to the characterization and description of the stools by the traditional healers as "simple" diarrhea, mucusy diarrhea and glairo-sanguinous diarrhea. While the classification of the last two is clear, the classification of simple diarrhea seems to confuse liquid and bloody-liquid diarrhea according to their characterization based on the appearance of the stool [35]. According to their explanations of the symptoms, it would be respectively on the physiological level motor or secretory diarrhoea that may be infectious or not, responsible for infant mortality [36,37].

According to the respondents, the etiologies of diarrhea relied on divinatory diagnosis in general. Most of respondents continued to believe that these ailments were also caused by witchcraft or evil spell. This could lead to inappropriate treatment and mismanagement. *Mallotus oppositifolium* was cited by the traditherapists in the treatment of diarrheal diseases. But the plants that were most cited in the traditional treatment of diarrhea were *Alchornea cordiflora* and *Psidium goyava*. But M.oppositifolium is more widely used in the sub-region for the treatment of diarrhea and the plant has been the subject of several studies [18,19,20,23] . According to the traditherapists interviewed, the leaves of *M. oppositifolium* which were once used exclusively for the treatment of joint pain, were gradually introduced into the anti-diarrheal pharmacopoeia of this locality as a result of discussions between traditherapists from different regions. They were thus used in infusion by maceration in hot water or in decoction, administered *per os* and the posologies were often function of the mode

of preparation of the extract. This could condition the extraction of bioactive metabolites against diarrhoea because the plant would be more effective against diarrhoea in the form of decoction or when the leaves were heated on fire before maceration in water. This form is most commonly used among the Makas tribe in eastern of Cameroon where the leaves are heated on the ashes of a wood fire [32]. The recommended morning harvest of the plant is probably due to the nocturnal elaboration of additional bioactive metabolites that would make the plant harvested in the morning more effective. Whereas picked in the evening, the plant would have less metabolites developed during photosynthesis which is more important during the day [20]. The "washout" of the plant during or after rain, mentioned by these therapists would be due to the dilution of these metabolites by rainwater. The traditional use of *M. oppositifolium* in the treatment of diarrhea is expanding throughout the equatorial and tropical region of Africa [28]. This shows that the plant could be used on a large scale in the region.

Secretory diarrhea is characterized by excessive loss of fluids and electrolytes secondary to stimulation of their secretion or inhibition of their intestinal absorption, resulting in soft to liquid stools. This loss of fluids can be induced by hormones, microbial toxins, neurotransmitters and castor oil which increase the volume, mass and frequency of diarrheal feces. The increase of fluid loss by castor oil is thought to be due to ricinoleic acid which stimulates intestinal peristaltic activity by altering its permeability to electrolytes (Sodium, Potassium) by inhibiting Na+/k+ ATPase pump activity [38]. *M. oppositifolium* was evaluated in vivo on the latency time to the onset of induced diarrheal stools, and castor oil-induced gastrointestinal fluid emptying. *M. oppositifolium* decoction significantly prolonged the latency time to the onset of diarrheal stools. It also significantly reduced in a dose-dependent manner, the mass and volume of luminal contents. The percentages of inhibition corresponding to this decrease were also dose-dependent. This inhibition of the intestinal contents could be explained by the probable regression of the peristaltic propulsion movements of the gastrointestinal smooth muscles, under the effect of the plant extract. This regression would induce the reabsorption of luminal fluids transiting along the gastrointestinal tract. Like Loperamide, *M. oppositifolium* would exert its antisecretory activity by increasing the hydroelectrolytic flow from the lumen to the plasma pole of enterocytes while inhibiting the reverse flow [39]. The plant would thus act by inhibiting gastrointestinal emptying through antisecretory and/or absorptive mechanisms.

Motor diarrhea is characterized by an acceleration of gastrointestinal transit by endocrine or nervous stimulation at the origin of action potentials that will trigger contractions of smooth muscle fibers [40]. These contractions or spasms are characterized in vitro by an

increase in their tone, amplitude and frequency [22] leading to motor diarrhea [41]. These diarrheas are reflected in vivo in this study by a change in stool onset time, mass and frequency by castor oil. *M. oppositifolium* significantly prolonged stool onset time and decreased the mass and frequency of diarrheal feces emitted per hour in rats in a dose-dependent manner. Inhibition of intestinal progression of the stained fecal bolus in rats was dose-dependent on the positive control (loperamide) and aqueous extract compared to the negative controls (given distilled water). Analogous opiates, loperamide can also bind to opioid receptors in the intestinal wall. This results in inhibition of ACh and prostaglandin release from myenteric neurons, reducing peristaltic propulsion [42]. Like loperamide, this suggest that the plant act by inhibiting calcium channels and increasing segmental contractions instead of longitudinal ones and strengthen the tone of the anal sphincter [43]. *M. oppositifolium* could also inhibit peristaltic propulsion as we found in our previous *in vitro* studies [22]. The plant would thus slowdown *in vivo*, the intestinal transit accelerated by castor oil by inhibiting intestinal peristalsis. The plant decoction would thus have antispasmodic effects on the contractile activity of the intestine induced by the ricinoleic acid of castor oil in rats, giving it anti-diarrheal properties. These anti-diarrheal properties of the plant would be due to the presence of compounds active on diarrhea such as tannins [44,45], alkaloids, saponins, flavonoids, steroids and/or terpenoids [46,47].

Qualitative and quantitative phytochemical studies of *M. oppositifolium* revealed the presence of bioactive metabolites such as flavonols, tannins, flavonoids, alkaloids, anthraquinones, terpenes [18,19]. The antibacterial properties of these metabolites are well known [48,49]. Chemical fractionation of the plant's leaves has allowed the isolation of molecules with anti-diarrheal activity: sterols (β sitosterol, stigmasterol, stigmastanol, campesterol), whose properties are well known. Quercitrin and quercetin for instance possess antifungal activity against fungi such as C.albicans, C. glabrata, C. krusei, C. parapsilosis and C. tropicalis [50, 51, 52].

The presence of quercetin in *M. oppositifoluim* extracts suggests that it may also be one of the metabolites involved in its antispasmodic effect. This effect might be due to a blockade of voltage-dependent calcium channels in the rat ileum [53,54]. Like any flavonoid, diosmetin has antioxidant and anti-inflammatory effects [55,56] and may therefore confer on the plant its properties against oxidative stress-related diarrhea [21]. The glucoside of β-sitosterol is anti-inflammatory [57] antihelminthic, anti-mutagenic [58], antibacterial against Escherichia coli, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumonia* [59] and antifungal against *Aspergillus niger* and Candida albicans [60].

The presence of these molecules in the aqueous extracts of *M. oppositifolium* would confer to the plant antidiarrheal properties against infectious glairy or glairo-sanguineous diarrheas. These activities would be due to the heterogeneous set of elicited antimicrobial phytochemical metabolites contained in the plant that act synergistically against pathogens [61]. The heterogenicity of these secondary metabolites generally enhances their synergistic antimicrobial effects [62,63]. The effects of *M. oppositifolium* on secretory and motor diarrhea would be related to the presence of these metabolites revealed by qualitative and quantitative phytochemical analyses.

5. Conclusion

The ethnopharmacological approach and the in *vivo* evaluation of the effects of *M. oppositifolium* on secretory and motor diarrhoea showed promising potential use the categoryof 2 of improved traditional medicine for the treatment of diarrhoeal syndromes. Indeed, the plant is commonly used in the Centre Region of Cameroon (Bafia District) for the treatment of diarrhea. The decoction extract of this plant is used in the treatment of motor, glaireous and glairo-sanglantes diarrheas. According to phytochemical analysis, this plant contains bioactive compounds such as alkaloids, flavonoids, phenols, flavonols, tannins, anthraquinones, some of which give it antimicrobial, antispasmodic, antioxidant and antisecretory properties. *In vivo*, studies of the plant extract exhibited an anti-diarrheic effect on castor oil induced diarrhea notably by decreasing the mass, volume and number of diarrheal stools and delayed their onset time; it also inhibited fluid secretion and fecal progression in the rat intestine. *Mallotus oppositifolium* therefore acts positively on secretory and motor diarrhea. This could be explained by the presence of several secondary metabolites, some of which were revealed by chemical chromatographic fractionation. These biologically active phytochemicals, which may be valuable therapeutic indexes, act individually or synergistically to exert therapeutic effects or activities inherent to the anti-diarrheal properties of *Mallotus oppositifolium*.

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