Effects of Oxalis barrelieri L. (Oxalidaceae) aqueous extract on diarrhea induced by Shigella dysenteriae type 1 in rats

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Abstract
Aim: Oxalis barrelieri is a medicinal plant commonly used in Cameroon, for the treatment of many diarrheal diseases. The antibacterial properties of O barrelieri aqueous extract (WOb) against Shigella dysenteriae type 1 were investigated in vitro and in vivo.

Methods: Antibacterial activity was evaluated in vitro by disc diffusion method and by macrodilution method. S dysenteriae type 1 at a dose of 1.2 × 10⁹ CFU was administrated orally to rats to induce shigellosis. For 6 consecutive days, diarrheic rats were treated with O barrelieri aqueous extract (50 and 100 mg/kg BW) or norfloxacin (20 mg/kg BW). The diarrheal stool weight and S dysenteriae type 1 density were assessed during the treatment period, and death rate recorded. Nitric oxide production in blood and in colonic homogenate and blood parameters were assessed, and the histological section of the colon was performed in the survivors.

Results: The minimal inhibitory concentration and minimal bactericidal concentration of WOb were, respectively, 6 mg/mL and 25 mg/mL. The mean minimal bactericidal concentration/mini-mal inhibitory concentration ratio for WOb against S dysenteriae type 1 was high (>4); WOb could be classified as a bacteriostatic drug. WOb significantly (P < .01) reduced bacterial density and diarrheal stool weight. WOb decreased nitric oxide production (P < .01) in the large intestine and protected the mucosa of the colon from bacterial destruction.

Conclusion: The results suggest that O barrelieri aqueous extract possesses bacteriostatic and antidiarrheal activities and reduces damages caused to intestinal mucosa barrier by pathogenic mechanisms of Shigella. This extract could be used as an alternative therapeutic for infectious diarrhea.

KEYWORDS
antibacterial susceptibility, antidiarrheal activity, Oxalis barrelieri, rat, Shigella dysenteriae type 1

1 | INTRODUCTION

In mammals, the gastrointestinal tract harbors various microbes that play an essential role in maintaining its physiological homeostasis.¹ Changes in the composition of these gut microbes can alter the intestinal barrier and the immune system,²,³ which can lead to gastrointestinal infections. Gastrointestinal infections caused by bacteria, viruses, and parasites are usually manifested by diarrhea or by inflammatory bowel diseases or gastroenteritis.⁴ Diarrhea is defined as the emission of at least 3 nonmolded or liquid stools per day.⁵,⁶ It is an alteration in normal bowel movement that leads to the increase in water and electrolyte content, volume or liquid stool frequency, and abdominal pain.⁷ An emission of 10 g/kg BW/d of feces in infants and children and 200 g/kg BW/d in an adolescent or an adult is considered abnormal.⁸ Diarrhea can be infectious or not. Noninfectious diarrhea can be due to hormones that accelerated intestine transit, to
osmotic substances and/or laxatives. Infectious diarrhea can be due to virus, bacteria, and/or parasites. In the world and particularly in developing countries, infectious diarrhea still remains one of the leading causes of infant mortality. A multicentric study from 6 Asian countries estimated *Shigella* as the causative agent in 5% of the diarrheal cases. As little as 10 to 100 *Shigella* can cause shigellosis in human, whereas 1.2 × 10⁹ *Shigella* can cause dysenteric diarrhea in rats. Shigellosis occurs worldwide, in sporadic, endemic, epidemic, and pandemic forms. Most of the cases are children <5 years of age. The annual number of shigellosis episodes throughout the world is estimated to be 164.7 million, with 69% of all episodes and 61% of all deaths attributable to shigellosis involving children <5 years of age. The economic impact of diarrhea and its treatment are of considerable importance. The annual treatment costs for diarrhea are very high and ranged from US$ 907 116 to US$ 1 851 280 for ambulatory clinical consultations and from US$ 701 833 to US$ 4 581 213 for hospitalizations. The re-emergence of *Shigella dysenteriae* type 1 (Sd1) with added resistance to ciprofloxacin, which has epidemic potential, has also been reported. Many synthetic drugs such as diphenoxylate and loperamide are available for the treatment of diarrhea, but they have toxic side effects. Therefore, the search for new, safe, more effective, and less toxic molecules has continued to be an important area of research in pharmacology. Since antiquity, diarrhea has been treated with medicinal plants in traditional medicine. *Oxalis barrelieri* L. is traditionally used by Cameroon inhabitants (Central Africa) for diarrhea care. The *O barrelieri* aqueous extract, at doses of 50 and 100 mg/kg BW, showed significant antidiarrheal activities in rats treated with castor oil. Phytochemical studies of *O barrelieri* aqueous extract revealed the presence of compounds such as phenols, terpenoids, anthocyanidins, anthraquinones, coumarins, and saponins. This work has been initiated to evaluate the antidiarrheal properties of *O barrelieri* aqueous extract on infectious diarrhea, by using Sd1–induced diarrhea in rats as a model.

## 2 MATERIALS AND METHODS

### 2.1 Plant material

Whole plants of *O barrelieri* were collected in Yaoundé (Center Region of Cameroon) in September 2014 between 8 AM and 10 AM. The plant was identified at the National Herbarium of Cameroon in Yaoundé, and a voucher specimen was deposited under no. 49998 HNC. Whole plants were washed thoroughly with water, shade-dried, and ground. The powder (407 g) was macerated in distilled water (5 L) for 3 days in a percolator. After 3 days of maceration, the mixture was filtered by opening the tap of the percolator and was concentrated by vacuum distillation at 50°C to yield 128 g (31.45%) of brown extract. A stock solution (10 mg/mL) was prepared by dissolving 10 g of dry extract in distilled water to obtain 1000 mL of solution.

### 2.2 Experimental animals

Prior to the study, male and female Wistar albino rats (60-98 g), approximately 6 weeks old, were selected and allowed to acclimatize for 1 week to our laboratory environment (22°C-25°C and 12 h light/12 h of darkness). In vivo experiments on rats were performed according to the European Union guidelines on animal care (CEE Council 86/609) that was adopted by the Ministry of Scientific Research and Innovation of Cameroon. Animals housed in metabolic cages (1 animal/cage) were fed a diet consisting of carbohydrates (50%-55%), fats (15%-20%), and proteins (25%-30%).

### 2.3 Bacterial strain

Clinical isolates of Sd1 from patients with severe infections were provided by the Centre Pasteur of Yaoundé, Cameroon.

### 2.4 In vitro antibacterial susceptibility

#### 2.4.1 Disc diffusion method

Minimal inhibitory concentration (MIC) was determined with adapted E-test according to disc diffusion method. Sterile 6-mm Ø filter paper discs (Schleicher & Schul, no. 2668, Dassel, Germany) were impregnated with 50 μL of *O barrelieri* aqueous extract at various concentrations. Sd1 inoculum of 0.5 mL (5 × 10⁵ CFU) was flooded in *Salmonella Shigella* (SS) and in Mueller Hinton agar (Oxoid) and incubated for 15 minutes at 37°C. After incubation, the impregnated paper discs were placed on the plates according to decreasing extract concentrations (50 000-24 μg/mL) (Figure 1). The petri dishes were incubated at 37°C for 24 hours. After 24-hour incubation at 37°C, the growth inhibition and the MIC (first disc that did not present growth inhibition) were observed on the petri dishes. The test was performed under sterile conditions in duplicate and repeated 3 independent times.

#### 2.4.2 Macrodilution method

This method has been adopted from NCCLS M26-A, with modifications. The Sd1 strains were adjusted to achieve a turbidity equivalent to a 0.5 McFarland (1 × 10⁶ CFU/mL) and diluted (1:1000) in brain heart infusion (Oxoid). A dilution series of the extract, ranging from 50 000 to 24 μg/mL, were prepared and then transferred to the broth in 14 tubes. A 1.0-mL extract was pipetted into tubes by twofold dilutions. Freshly grown bacteria of 1.0 mL were added to the tubes in a density of 10⁵ CFU/mL (final concentration/tube). The tubes were incubated overnight at 37°C. CFU was determined by diluting each well in tenfold dilutions. From each dilution, aliquots were transferred to agar plates and incubated.
The method described to determine the MIC was inoculated on the SS agar plate and incubated for 24 hours at 37°C. After incubation, the number of Sd1 was determined. After 6 days of treatment, all survival animals were sacrificed and their blood and their colon were collected for blood cells count using manual method and/or for NO test using the modified Griess method. Colon fragments were fixed in 10% buffered formalin for histopathological examination.

2.6 | NO dosage

Nitric oxide concentration was evaluated in serum and in colon homogenates. To obtain Griess solution, 0.25 mL of Griess 1 (0.8 g sulfanilic acid + 250 mL acetic acid 30%) was added to 0.25 mL of Griess 2 (0.05 g of α-naphthyleamine + 100 mL acetic acid 30%). A 0.5-mL serum or homogenate of the colon was added to 0.5 mL of Griess solution, and the mixture obtained was left for 20 minutes at room temperature. After 20 minutes, the optical density of each mixture was read using a spectrophotometer (T60-1611ESW) at 553 nm and recorded.

2.7 | Hematological studies

Figured elements of the blood (red blood cells [RBC], white blood cells [WBC], and platelet cells [PC]) were counted by a manual method using a light microscope (MOTIC 1820 LED: SM7432-MC1ST-RPIWFM). The hematocrit (Ht) of each rat was determined using the microhematocrit tube. Hemoglobin level (Hb) was determined by the spectrophotometric method. The blood was diluted in a Drabking solution (1/250), and the absorbance was read at 510 nm and recorded. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and MCH concentration (MCHC) were calculated, respectively, by the formulæ:

\[
MCV = \frac{Ht}{RBC} \times 10^{12}
\]

\[
MCH = \frac{Hb}{RBC} \times 10^{12}
\]

\[
MCHC = \frac{Hb}{Ht} \times 10^{12}
\]

2.8 | Histopathological investigations

Histopathological investigations were done according to methods described in the literature. For this purpose, colon fragments of each experimental rat were fixed in 10% buffered formalin in labeled flasks for histological examination. These colon fragments were embedded in paraffin wax, and 2-μm-thick sections were made with the microtome. These preparations were mounted on glass slides that were then stained with hematoxylin and eosin and examined under a standard light microscope (MOTIC 1820 LED: SM7432-MC1ST-RPIWFM).
2.9 Statistical analysis

The data are means ± standard error of the mean (X ± SEM) expressed in tables, figures, and photos. These data were analyzed by 1-way analysis of variance followed by the Dunnett t test and the Tukey test using Computer Pad InStat 3.05 (Graph Pad software, USU).

3 RESULTS

3.1 Susceptibility of Sd1 to O barrelieri aqueous extract

In vitro, the O barrelieri water extract showed an intersection point between the growth zone and the growth inhibition zone corresponding to the MIC. This MIC was about 6 mg/mL (Figure 2).

3.2 MIC and MBC values of O barrelieri water extract by the graphic method

The O barrelieri water extract (WOb) showed in vitro inhibitory activity on Shigella growth. The MIC and MBC values were 6 mg/mL and 25 mg/mL, respectively (Figure 3). The MBC/MIC ratio was 4.16.

3.3 Antidiarrheal activities

Normal rats that were not inoculated with Shigella and were treated with distilled water showed no signs of diarrhea. A few hours (4 h) after Sd1 inoculum administration, the rats became calm, less mobile, curled up, and showed erect hairs. Twenty-six hours after inoculum administration, the rats emitted the first diarrheal stool and became more aggressive. During the treatment, rats recovered mobility progressively, and their aggressiveness decreased. Diarrheal stools were soft or liquid containing mucus or blood marks and attracted flies by their fetid odors. These symptoms disappeared after 3 days of treatment. During treatment, no death was recorded in all treated groups and NC. However, we recorded 100% death in DC group (Table 1). The first day of onset of diarrhea, stool weights were 3.06 ± 0.49 g, 2.78 ± 0.62 g, 2.60 ± 0.37 g, and 2.42 ± 0.37 g, respectively, for TD, Nor20, WOb50, and WOb100. These values increased in untreated group over time, decreased significantly (P < .01) from the second day in norfloxacin-treated group (Nor20) and from the third day in extract-treated groups (Figure 4). Diarrhea was eliminated by the sixth day of treatment.

After onset of diarrhea in rats, the number of Sd1 was about 1.2 × 10^9 in all groups. These values increased significantly (P < .05) Nor20, WOb50, and WOb100. These values increased in untreated group over time, decreased significantly (P < .01) from the second day in norfloxacin-treated group (Nor20) and from the third day in extract-treated groups (Figure 4). Diarrhea was eliminated by the sixth day of treatment.

After onset of diarrhea in rats, the number of Sd1 was about 1.2 × 10^9 in all groups. These values increased significantly (P < .05)
from third day in untreated group. In all treated groups, these values decreased significantly ($P < .01$) to $0.9 \times 10^4$, $59.4 \times 10^6$, and $14.1 \times 10^5$ CFU, respectively, for Nor20, WOb50, and WOb100 at the sixth day of treatment (Figure 5).

### 3.4 Effect of *O. barrelieri* on NO production

In DC rats, NO production in colon was markedly high compared to NC: $418.15 \pm 48.65 \mu M$ against $57.34 \pm 3.76 \mu M$ ($P < .01$), 2 days after the onset of diarrhea. After 6 days of treatment, NO production in colon was significantly reduced ($P < .01$) by 20 mg/kg BW norfloxacin and by 50 and 100 mg/kg BW aqueous extract of *O. barrelieri* after the sixth day of treatment (Figure 6A). The blood level of NO was higher in DC than normal rats: $132.42 \pm 17.26 \mu M$ against $60.59 \pm 6.61 \mu M$. After 6 days of therapy, neither the norfloxacin nor the *O. barrelieri* extract significantly reduced the production of NO in diarrheic rats blood (Figure 6B).

![FIGURE 5](image-url)  
*Shigella dysenteriae* A1 density (log10 transformed) in diarrheic rat stools over 6 days treatment with 50 mg/kg BW (WOb50) and 100 mg/kg BW (WOb100) *Oxalis barrelieri* water extract and norfloxacin 20 mg/kg BW (Nor20). Data are the mean ± SEM (n = 5). Significant difference: *P < .05* and **P < .01* compared with diarrheic control (DC) rats; *P < .05* and *P < .01* compared with initial values; *P < .01* compared with reference drug (Nor20). d1: diarrhea appearance and treatment start

![FIGURE 6](image-url)  
Nitric oxide (NO) production in colon (A) and blood (B) in diarrheic rats after 6 days treatment with norfloxacin 20 mg/kg BW (Nor20) and *Oxalis barrelieri* aqueous extract 50 mg/kg BW (WOb50) and 100 mg/kg BW (WOb100). Data are mean ± SEM (n = 5). Significant difference: **P < .01* compared with normal control (NC); *P < .05* and **P < .01* compared with diarrheic control (DC); *P < .01* compared with reference drug (Nor20)

### Table 2 Blood cells rate in diarrheic rats after 6 days treatment with *Oxalis barrelieri* aqueous extract 50 mg/kg BW (WOb50), 100 mg/kg BW (WOb100), and norfloxacin 20 mg/kg BW (Nor20)

| Group       | NC       | DC       | Nor 20 mg/kg | WOb 50 mg/kg | WOb 100 mg/kg |
|-------------|----------|----------|--------------|--------------|---------------|
| WBC $\times 10^3$/mm$^3$ | 10.7 ± 0.5 | 6.5 ± 0.4** | 10.4 ± 0.4b | 6.8 ± 0.3** | 5.4 ± 0.2**   |
| Hb, g/dL    | 17.6 ± 0.7 | 14.6 ± 0.7* | 16.2 ± 0.5  | 13.9 ± 0.7** | 17.1 ± 0.2    |
| Ht, %       | 49.6 ± 2.4 | 41.8 ± 2.8** | 46.3 ± 1.9  | 40.5 ± 2.9** | 51.0 ± 0.7    |
| RBC $\times 10^6$/mm$^3$ | 8.0 ± 0.3 | 7.1 ± 0.2*  | 7.6 ± 0.1   | 7.1 ± 0.3*   | 7.7 ± 0.3     |
| PC $\times 10^9$/mm$^3$ | 84.6 ± 5.4 | 68.1 ± 2.0* | 80.9 ± 1.4  | 86.7 ± 3.3   | 86.2 ± 3.1    |
| MCV, $\mu m^3$ | 61.7 ± 1.2 | 58.9 ± 3.5  | 60.6 ± 2.6  | 57.0 ± 2.2   | 66.7 ± 2.1    |
| MCH, pg     | 21.9 ± 0.0 | 20.6 ± 0.8  | 21.3 ± 0.8  | 19.6 ± 0.7   | 22.3 ± 0.9    |
| MCHC, pg/dL | 35.6 ± 0.8 | 35.2 ± 0.8  | 35.2 ± 0.5  | 34.5 ± 1.0   | 33.5 ± 0.4    |

Abbreviations: DC, diarrheic control; Hb, hemoglobin; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; NC, normal control; PC, platelet cells; RBC, red blood cells; WBC, white blood cells.

Data are the mean ± SEM (n = 5 per group). Significant difference: *P < .05*, **P < .01 compared with NC. bP < .01 compared with DC.
3.5 | Blood parameters

Red blood cells, Ht, PC, MCV, MCH, and MCHC did not significantly change in norfloxacin-treated diarrheic rats compared to NC. However, in *O barrelieri* water extract treated, only the WBC and hemoglobin decreased significantly (P < .01) compared to NC rats: 6.55 ± 0.38 × 10⁷/mm³ and 10.39 ± 0.39 × 10⁷/mm³ for WBC and 14.63 ± 0.67 g/dL and 17.65 ± 0.73 g/dL for hemoglobin, respectively (Table 2).

3.6 | Effects of *O barrelieri* water extract on the microhistology of colon in diarrheal rats

The control rat colon (Figure 7A) showed normal mucosa. In untreated diarrheal rats, colon histology showed destruction of the mucosa (Figure 7B). However, in rats treated with norfloxacin 20 mg/kg (Figure 7C), with 50 mg/kg (Figure 7D) or 100 mg/kg (Figure 7E) *O barrelieri* aqueous extract, the microhistology of the colon showed a normal mucosa.

4 | DISCUSSION

The purpose of this study was to provide scientific support for the traditional use of *O barrelieri* extract to treat diarrhea. Antidiarrheic effects of *O barrelieri* aqueous extract were investigated using the antibacterial activity in vitro on *Sd1* and the activity on shigellosis induced in rats.

In vitro antimicrobial study showed an inhibitory activity of extract against *Sd1* growth. Minimal inhibitory concentration and MBC values of *O barrelieri* aqueous extract were high compared to norfloxacin MIC value (0.5 to >256 μg/L), although they are closer to those reported for other plant extracts, eg, 0.8 mg/mL with the methanol extract of

![Figure 7](https://example.com/figure7)
Picralima nitida, 1.17 mg/mL with aqueous extract of Mallotus oppositifolium, and 3.5 mg/mL with aqueous ethanol extract of Euphorbia prostrata ait. These results are probably due to the fact that this crude extract might contain less active compounds against this bacterial strain. For aqueous extract of O. barrelieri, the ratio MBC/MIC was higher than 4, and this could thus indicate a bacteriostatic activity. This bacteriostatic property could be confirmed by the number of Sd1 counted in the stool. The bacterial population decreased moderately in WOb-treated rats compared to norfloxacin-treated rats. Diarrheal rats developed soft stools, glary or mucus stools, and feaces with a fetid odor that evidenced the presence of pus that are typical signs of infectious or "invasive" diarrhea.

In untreated diarrheic rats, destruction of the colonic mucosa and significant NO production were due to the pathogenic effects of Shigella. However, the protection of the colonic mucosa and the significant decrease in NO production in the colon and in the blood appear to be a direct result of treatment with the extract. The pathogenesis of Shigella is multifactorial and includes the production of shiga-toxin and its ability to penetrate and destroy host tissues that largely induces an inflammatory response. Shigella would penetrate the intestinal epithelial barrier through M cells that cover the lymphoid follicles and reach the basolateral layer of the intestine where it can invade. Shigella, once in the cells, multiplies rapidly and spreads to adjacent cells. As a result of this pathogenesis, severe tissue damage in sigmoid colon and rectum, responsible for the severe dysenteric syndrome, occurs. High levels of NO in DC rats might result from the lipopolysaccharides or the enterotoxins (shiga toxin) produced by Sd1, which is very often implicated in the inflammation associated with diarrhea. Bacterial lipopolysaccharides or Sd1 enterotoxins induce the expression of the inducible NO synthase gene in different inflammatory and tissue cells for the production of NO. High bacterial load (high concentration of enterotoxins) would be responsible for the high NO level in the colon and in the blood. The extract reduced the bacterial load, causing a decrease in enterotoxins, which would lead to a decrease in NO production. O. barrelieri aqueous extract contains anthocyanidins with antioxidant properties. Reduction of oxidative stress by this compound would result in decreased NO and damage to the intestinal mucosal barrier, leading to a reduction in diarrhea.

Various physiological and pathophysiological responses in the human body are regulated by NO molecules. It regulates blood circulation, platelet function, host defense system, and neurotransmission in the central nervous system and peripheral nerves. Blood parameters revealed low values of RBC and WBC in DC rats. Shigellosis induces anemia by loss of blood (RBC and WBC) in the feces. Nitric oxide also promotes the migration of leukocytes to the inflammatory focus. The production of IL-1β and the recruitment of neutrophils through the intestinal epithelial layer induced by Shigella are responsible for the apoptosis of macrophages that could lead to a decrease of the total number of white blood cells in blood circulation. Rats treated with 50 mg/kg O. barrelieri extract exhibited low levels of WBC, RBC, Ht, and Hb. This could be explained by the ineffectiveness of this dose on Shigella or by the high level of NO that would favor the migration of leukocytes to the infected site.

O. barrelieri extract was found to be bacteriostatic, inhibited bacterial growth in vivo, and reduced NO production. Furthermore, O. barrelieri prevented the destruction of colonic mucosa and death in infected rats. These results attest the antishigellosis properties of O. barrelieri extract. These multiple results would support the use by traditional healers, of the decoction of O. barrelieri as an antidiarrheal drug. In prospect, future studies performing detailed chemical profiles of the extract will be needed to get a clear and detailed description of the major compounds of the extract and the nature of the chemicals underlying the reported antidiarrheic effect.

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CONFLICT OF INTEREST

None declared.

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