HOSTED BY
ELSEVIER
Asian Pacific Journal of Tropical Biomedicine
journal homepage: www.apjtb.com

Document heading doi:10.12980/APJTB.4.201414B214

Antidiarrhoeal and antibacterial activity of aqueous and methanolic leaves extracts of *Dissotis thollonii* Cogn. (Melastomataceae)

Ateufack Gilbert[1*], Tadjoua Tchoumbou Herve[1], Youseu Nana William[1], Sama Fonkeng Leonard[2], Kuiate Jules-Roger[2], Kamanyi Albert[1]

[1]*Department of Animal Biology, Dschang University, Laboratory of Animal Physiology and Phytopharmacology, P.O Box 67, Dschang, Cameroon*

[2]*Department of Biochemistry, Dschang University, Laboratory of Microbiology and Antimicrobial Substances, P.O Box 67, Dschang, Cameroon*

ARTICLE INFO

Article history:
Received 23 May 2014
Received in revised form 6 Jun 2014
Accepted 25 Jun 2014
Available online 9 Jul 2014

Keywords:
Antidiarrhoeal
Antibacterial
Phytochemical test
Dissotis thollonii

ABSTRACT

Objective: To investigate the phytochemical test and selected pharmacological activities (antidiarrhoeal and antibacterial activity) of the aqueous and methanolic leaves extracts of *Dissotis thollonii* Cogn. (Melastomataceae) (*D. thollonii*).

Methods: The aqueous and methanolic extracts were evaluated for their antibacterial activities on the *in vitro* growth of 2 clinical isolates (*Staphylococcus aureus* and *Shigella flexneri*), and 5 reference bacteria strains [*Escherichia coli* ATCC 8739 (*E. coli*), *E. coli* ATCC 10536, *Salmonella typhi* ATCC 6539, *Enterobacter aerogenes* ATCC 13048 and *E. coli* ATCC 11775] by determining the minimum inhibitory concentrations (MICs) and bactericidal concentrations using broth microdilution method as well as on the infectious, secretory and osmotic induced diarrhoea models in rats.

Results: The aqueous extract inhibited the *in vitro* growth of all bacteria tested (the 05 reference bacteria strains and the 02 clinical isolates), with MICs values comprised between 32 and 512 μg/mL, whereas the methanolic extract has done the same with the MICs values located between 128 and 512 μg/mL. *In vivo*, the methanolic and aqueous extracts provoqued at all doses, a significant decrease ($P<0.001$) in the bacterial load in the faeces of rats, 6 and 7 d of treatment respectively. Infected animals relatively lost weight when treated with the aqueous extract but it remained constant for those treated with the methanolic extract. The results also showed that, the aqueous and methanolic leaves extracts of *D. thollonii* had, after 6 h of observation, significantly ($P<0.001$) inhibited *in vivo*, diarrhoea induced experimentally by castor oil and magnesium sulphate, particularly by prolonging the latency time, reducing the water content of faeces, the frequency of defecation and the number of wet defecation as compared to the negative control which received distilled water and the animals having received 2.5 mg/kg of loperamide used as reference substance. The phytochemical assay revealed the presence of flavonoids, phenols and polyphenols in the leaves extracts of *D. thollonii*, which are compounds with antidiarrhoeal and antibacterial activities.

Conclusions: The leaves of *D. thollonii* thus have antibacterial and antidiarrhoeal effects, which could result from their activities on blocking the inhibiting effects of the bacterial enzymes, inhibiting the bacterial protein synthesis, allowing the rupture of the lipopolysaccharidic membrane, as well as on inhibiting prostaglandins-E_2 synthesis or increasing the hydroelectrolytic reabsorption. These results attestted the ethnopharmacological use of *D. thollonii* leaves in the treatment of diarrhoea and gastro-intestinal infections.

1. Introduction

Diarrhoeal diseases represent the second cause of infant mortality after pneumonia in developing countries. In sub-Saharan Africa, 18% per year is the infant (less than 5 years) mortality rate related to diarrhoea[1]. According to World Health Organization[2], diarrhoea is responsible for almost two million deaths of children every year which represent approximately 20% of infant mortality rate. To face with this physiopathology, various products are used, coming from chemotherapy or phytotherapy. In developing countries, the study of the plants can urgently

*Corresponding author: Ateufack Gilbert, Physio-Pharmacologist (Ph.D), Department of Animal Biology, Dschang University, Laboratory of Animalphysiology and Phytopharmacology, P.O Box 67 Dschang, Cameroon.
Tel: +23775277614
E-mail: ateufack2000@yahoo.fr

lead to the adequate resolution of the medical needs to lower cost, joint with a proven scientific effectiveness and an optimal cultural acceptability. Thus, the World Health Organizaion encourages the development of the traditional medicine which uses the medicinal plants partly[3]. Among the many exploited species, *Dissotis thollonii* (*D. thollonii*) is traditionally used in the treatment of several evils. Thus it is used by traditional healer in Senegal, on eczematous wounds and in west region of Cameroon[4]. The leaves are recommended in therapy of the gastro-intestinal disorders (obstruction, amoebiasis, diarrhoeas, vomiting, and constipation) and ulcers. The aim of this study was, therefore, to evaluate the antidiarrhoeal activities of aqueous and methanolic leaves extracts of *D. thollonii* in experimentally induced-diarrhoea in rats and the *in vitro* antibacterial activities of the same extracts over some enterobacteria strains.

2. Materials and methods

2.1. Collection and identification of plant material

The fresh leaves of *D. thollonii* were collected in April 2012 in the Bafou village, Menoua division, West Region of Cameroon. A sample was identified at the National Herbarium of Cameroon (Yaoundé) and repertoried under code 13292/SRF Cam.

2.2. Preparation of the plant material

The collected plant parts (leaves) were separated from undesirable materials. They were dried under shade, ground and stored in an airtight container prior to extraction.

2.3. Preparation of plant extract

The aqueous extract was prepared by boiling 600 g of powder in 3.6 L distilled water for 15 min as indicated by the traditional healer. The decoction was cooled during 1 h and filtered using filter paper (Whatman No. 1), and the filtrate was evaporated in a regulated drying oven at 35 °C to give 38 g of the aqueous extract corresponding to an extraction yield of 6.33% (w/w). The other portion of leaf powder (200 g) was macerated in 1.5 L of methanol for 72 h, filtered and the solvent removed from the extract under reduced pressure, using a Büchi (R-200) rotary evaporator at 65 °C. This gave 7 g of the methanol extract, corresponding to a yield of 7.00% (w/w).

2.4. Experimental animal

Wistar Albino female rats of 2 to 2.5 months and weighing on average 140 g were used for infectious induced diarrhoea model whereas both sexes (1.5 to 2 months) of the same rat strain, weighing on average 100 g, were used for the tests of diarrhoea induced by magnesium sulfate and castor oil. They were bred in the animal house of the Department of Animal Biology, University of Dschang, Cameroon, under natural room conditions. Animals were fed with a standard diet and received water *ad libitum*. Prior to experimental protocol, the rats were acclimatized for 48 h to laboratory conditions for minimizing any nonspecific stress. The studies were conducted according to standard protocols for the use of laboratory animals (Registration No. 173/CPCSEA, January 2000, India).

2.5. Chemicals and reagents

All reagents used in the study were of high purity. The chemicals included ethylic alcohol 95°, methanol, distilled water, bleach (Geochim, Cameroon), sodium chloride (Sigma Chemicals Co., UK), iodonitrotetrazolium (Sigma, St Louis), ciprofloxacin (Sinochem Ningbo, P.R., China), tetracycline, Mueller Hinton broth, Salmonella-Shigella agar, loperamide (imodium), castor oil and magnesium sulphate.

2.6. Microorganisms

The microorganisms used in this study included 2 clinical isolates [*Staphylococcus aureus* (*S. aureus*) and *Shigella flexneri* (*S. flexneri*)] and 5 bacterial strains [*Escherichia coli* ATCC 8739 (*E. coli*), *E. coli* ATCC 10536, *Salmonella typhi* ATCC 6539, *Enterobacter aerogenes* ATCC 13048 (*E. aerogenes*) and *E. coli* ATCC 11775]. The strains were maintained at 4 °C on agar plates.

2.7. Phytochemical tests

The extracts were analyzed for the presence of various phytoconstituents. The qualitative determination of these phytochemical compounds was conducted according to the method described by Wahid *et al*[5].

2.8. In vitro antibacterial activity

The *in vitro* antibacterial activity of the extract was performed by determining the minimum inhibitory concentrations (MICs) using broth microdilution method[6]. For that, 100 μL of Mueller Hinton broth was firstly introduced in 96 wells microplates. The stock solutions of extracts and ciprofloxacin (standard drug) were consequently introduced in triplicate into the first well and a serial two fold dilution was performed to obtain 12 concentrations, ranging from 1 024 to 8 μg/mL for the extracts and from 8 to 1 μg/mL for the standard drug. Then, 100 μL of the inoculum

was added into each well for a final volume of 200 μL/well. The microplates were, therefore, carefully covered with a sterile lid, packed and incubated at 37 °C for 24 h after which 20 μL of iodonitrotetrazolium (INT) was added in one of the pairs of column of well. The viable bacteria were observed through a pink colour in the presence of INT. All concentrations at which no visible colour changes were observed were considered as inhibitory concentrations and the lowest of these concentrations was considered as the MIC[7]. The bactericidal concentrations were determined by adding 50 μL aliquots of the preparations (without INT), which did not show any visible colour change after incubation during MIC assays, into 150 μL of extract-free Mueller Hinton. After 48 h of incubation at 37 °C, all extracts concentrations at which no colour changes were observed were considered as bactericidal concentrations. The smallest of these concentrations was considered as the minimal bactericidal concentration (MBC).

2.9. S. flexneri-induced diarrhoea

The experimental animals were firstly deparasitized through an oral administration of 10 mg/kg of tetracycline for 3 d[8]. These animals were further acclimatized in fitted cages, where they were receiving standard diet and water *ad libitum*. Furthermore, animals were left under sterile conditions during 4 d in order to maximize the complete elimination of the deparasitizing substance. At the end of this period, rats were deprived from food for 18 h, but received water *ad libitum* and then orally inoculated with a *S. flexneri* suspension, coldly prepared at the 1.0 McFarland standard scale (1.5×10^8 CFU/mL). Infected rats were set out in groups and submitted to a treatment with three test doses (500, 250 and 125 mg/kg of body weight (b.w) of plant extracts. Group 1 (neutral) was made of non-infected/non-treated rats. The third group (positive control) received ciprofloxacin at 2.5 mg/kg b.w as reference drug and Group 2 (negative control) received distilled water. All the substances (extracts plants and ciprofloxacin) were administered *per os* until complete elimination of bacterial load in faeces. These faeces were collected each day between 8 h and 9 h. The follow-up of the treatment was carried out by evaluating the load of *S. flexneri* in a culture of 0.5 g of faeces on Salmonella-Shigella agar Petri dishes, incubated at 37 °C for 24 h.

2.10. Castor oil-induced diarrhea in rats

The animals were initially screened by observing stool's aspect. Those not showing diarrheic stools were selected for the final experiment. Thus, forty-eight rats were randomly divided into eight equal group (n=6) divided into controls, standard and test groups. The negative control groups received distilled water (1 mL/100 g b.w). The positive control group received loperamide at the dose of 2.5 mg/kg orally. The test groups received aqueous and methanolic extracts of *D. thollonii* at doses of 125, 250 and 500 mg/kg orally. Each animal was placed in individual cage, the floor of which was lined with filter paper, changed every hour. Diarrhoea was induced by oral administration of 1 mL/100 g b.w castor oil to each rat, 1 h after the above treatment. During an observation period of 6 h, parameters such as the latency time, the frequency of defecation, the number of wet defecation and the water content of feces were recorded. Water content of feces was expressed in terms of percentages using the formula:

$$Wc\ (\%)=\left(\frac{Fw-Dw}{Fw}\right)\times100$$

Where, Wc=Water content of feces; Fw=Fresh weight (g); Dw=Dry weight (g).

2.11. Magnesium sulphate-induced diarrhoea in rats

A similar protocol as for castor oil-induced diarrhoea was followed. Diarrhoea was induced by oral administration of magnesium sulfate at the dose of 3 g/kg to the animals, 1 h after administration of distilled water to the negative control groups, loperamide (2.5 mg/kg) to the positive control group, the plant extracts of *D. thollonii* in doses of 125, 250 and 500 mg/kg b.w to the test groups. All the treatments were administered orally.

2.12. Statistical analysis

Values are presented as mean±SEM (standard error of the mean). The measurement data were analyzed by One-way and Two-way ANOVA, followed by Tukey-Kramer multiple comparison test if the overall differences were significant (P<0.05) for secretory and osmotic induced diarrhoea, whereas the Bonferroni post-test (P<0.05) was used for the infectious diarrhoea.

3. Results

3.1. Phytochemicals tests

Several groups of phytoconstituents compounds were highlighted in the two extracts of *D. thollonii*. The phytochemicals tests showed the presence of tannins, flavonoids, sterols, anthraquinones, phenols and polyphenols in the methanolic extract of the leaves of *D. thollonii* whereas the aqueous extract contained only two types of secondary metabolites (flavonoids and polyphenols).

3.2. Evaluation of antibacterial activity

Both plant expressed antibacterial activities on all the tested microorganisms. However, these activity were more interesting with the aqueous extract, which presented an inhibitory effect at lower concentrations (MIC between 32 and 512 μg/mL) and bactericidal activity on five bacteria (*S. flexneri* and *S. aureus* (clinical isolate); *E. aerogenes* ATCC 13048; *E. coli* ATCC 10536 and *E. coli* ATCC 11775) of the seven tested, with MBC/MIC ratio <4. Whereas methanolic extract did the same with MIC between 128 and 512 μg/mL. Results are showed in Table 1.

Table 1

Antibacterial activities (MIC, MBC and MBC/MIC ratios) of aqueous and methanolic leaves extracts of *D. thollonii*.

Test substances (μg/mL)	Parameters	St	Ec	Ea	Sa	Ec1	Sf	Ec2
Methanolic extract	MIC	128	128	256	128	512	128	128
	MBC	512	512	512	1 024	512	512	512
	MBC/MIC	4	4	2	8	1	2	4
Aqueous extract	MIC	32	64	128	512	256	128	64
	MBC	256	128	256	>1 024	256	256	256
	MBC/MIC	8	2	2	2	1	2	4
Ciprofloxacin	MIC	2.00	0.25	2.00	2.00	0.50	2.00	0.125
	MBC	2.00	0.25	2.00	2.00	0.50	2.00	0.125
	MBC/MIC	1	1	1	1	1	1	1

St: *Salmonella typhi* ATCC 6539; Ec: *E. coli* ATCC 11775; Ea: *E. aerogenes* ATCC 13048; Sa: *S. aureus*; Ec1: *E. coli* ATCC 10536; Sf: *S. flexneri*; Ec2: *E. coli* ATCC 8739.

3.3. S. flexneri-induced diarrhoea in rats

The aqueous and methanolic extracts of *D. thollonii* leaves, produced significant (P<0.001) decrease in the bacterial load in the feces of rats during 9 and 8 d of treatment, respectively. The doses of 250 and 500 mg/kg of aqueous and methanolic extracts respectively, exhibited a highly significant (P<0.001) effect from Day 7, when compared to negative control group and similarly as the standard drug used. Results are illustrated by Figures 1 and 2.

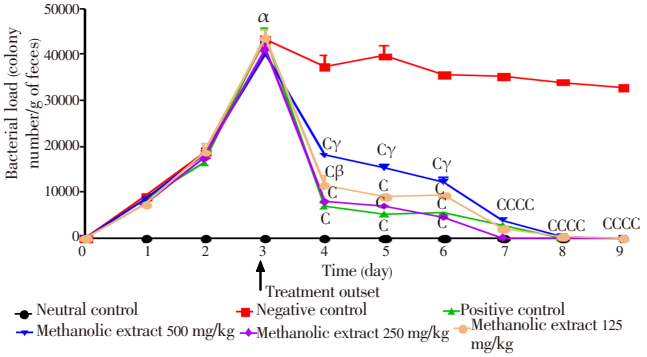

Figure 1. Evolution of the bacterial load (*S. flexneri*) in the faeces of infected-rats treated with the aqueous extract of *D. thollonii*.

C and γ: significant differences (P<0.001) compared with negative and positive control, respectively.

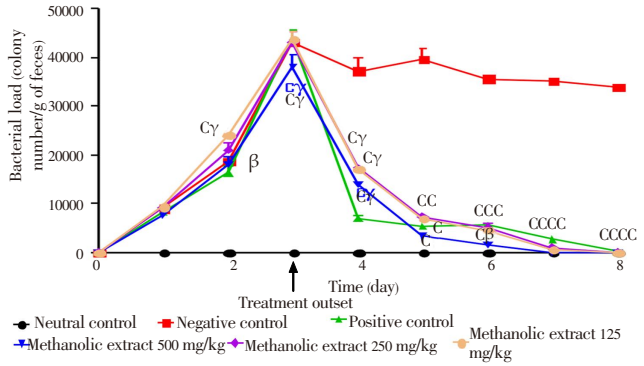

Figure 2. Evolution of the bacterial load (*S. flexneri*) in the faeces of infected-rats treated with the methanolic extract of *D. thollonii*

C and γ: significant differences (P<0.001) compared with negative and positive control, respectively.

3.4. Castor oil-induced diarrhoea in rats

Both aqueous and methanolic extracts at dose 500 mg/kg, highly significantly (P<0.001) prolonged the latency time, reduced the frequency of defecation, the number of wet defecations and the water content of feces when compared to the negative control groups. In addition, this dose of plant extracts had practically reacted like loperamide, used as standard drug. Generally, the extracts inhibited diarrhoea in a dose-dependent manner (Table 2, Figures 3-8).

Table 2

Effects of the aqueous and methanolic leaves extracts of *D. thollonii* on castor oil induced diarrhoea.

Treatment	Dose (mg/kg)	Latency time (min)	Defecation frequency	Number of wet defecations	Water content of faeces (%)
Distilled water	1 mL/100 g b.w	66.50±2.50	03.66±0.21	03.66±0.21	90.39±3.52
Standard	2.5	360.00±0.00^{c}	00.00±0.00^{c}	00.00±0.00^{c}	00.00±0.00^{c}
loperamide	125	77.17±2.73^{bγ}	02.00±0.00^{cγ}	02.00±0.00^{cγ}	84.06±2.13^{b}
Aqueous	250	183.00 ±1.80^{cγ}	01.66±0.20^{cγ}	01.66±0.20^{cγ}	73.64±1.24^{cγ}
extract	500	355.50±1.20^{c}	01.33±0.20^{cγ}	01.33±0.20^{cγ}	51.31±2.12^{cγ}
	125	56.83±0.64^{cγ}	03.50±0.22^{γ}	03.50±0.22^{γ}	85.87±1.04^{γ}
Methanolic	250	129.00±1.18^{cγ}	03.00±0.00^{γ}	03.00±0.00^{γ}	76.31±1.20^{cγ}
extract	500	170.70±1.05^{cγ}	02.33±0.20^{cγ}	02.33±0.20^{cγ}	63.96±1.26^{cγ}

Values of each column represent the mean±ESM (n=6); ^{c}P<0.001: significant differences compared to the negative control (distilled-water); ^{γ}P<0.001: significant differences compared to the positive control (loperamide).

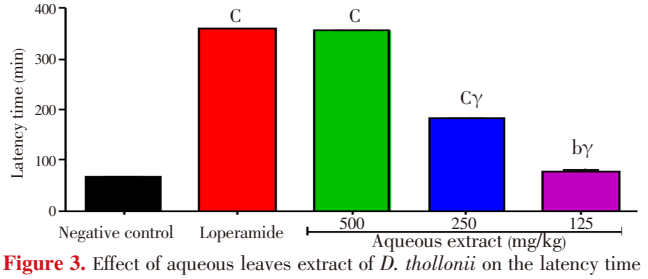

Figure 3. Effect of aqueous leaves extract of *D. thollonii* on the latency time in castor oil induced diarrhoea.

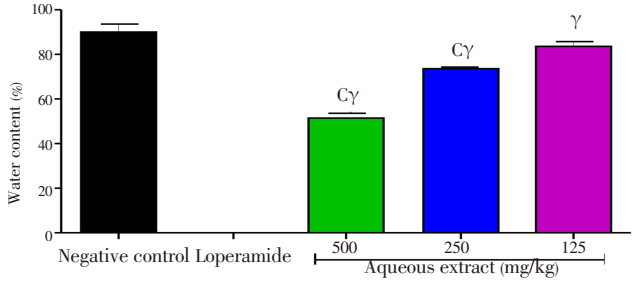

Figure 4. Effect of aqueous leaves extract of *D. thollonii* on water content of faeces in castor oil induced diarrhoea.

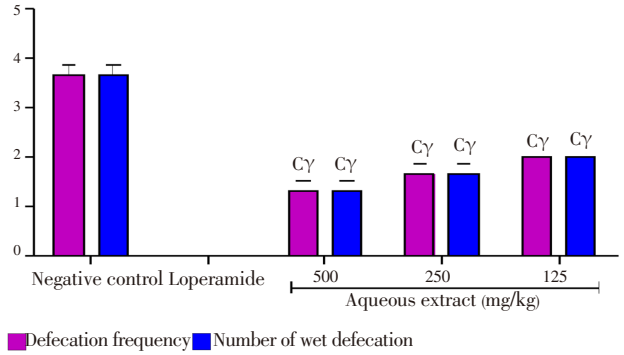

Figure 5. Effect of aqueous leaves extract of *D. thollonii* on defecation frequency and the number of wet defecation in castor oil induced diarrhoea.

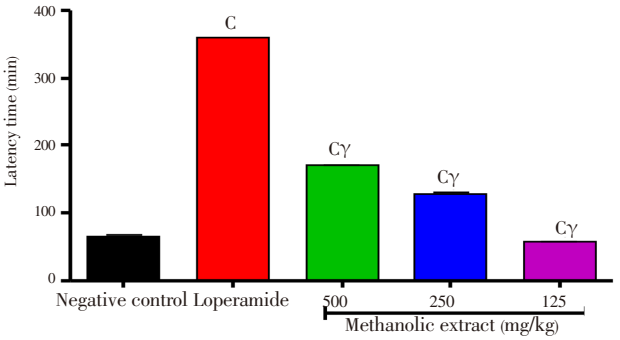

Figure 6. Effect of the methanolic leaves extract of *D. thollonii* on latency time in castor oil induced diarrhoea.

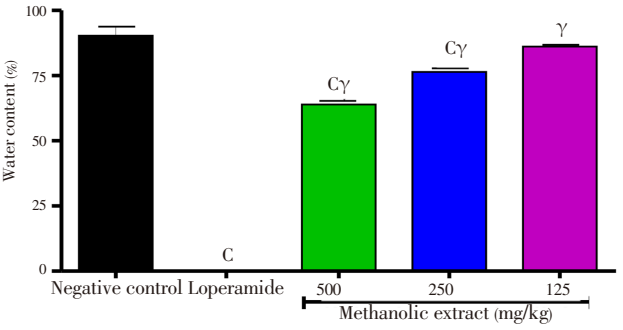

Figure 7. Effect of the methanolic leaves extract of *D. thollonii* on water content of faeces in castor oil induced diarrhoea.

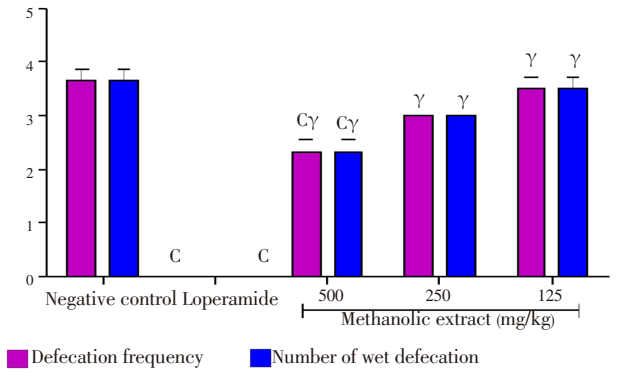

Figure 8. Effect of the methanolic leaves extract of *D. thollonii* on defecation frequency and the number of wet defecation in castor oil induced diarrhoea.

3.5. Magnesium sulphate-induced diarrhoea in rats

The treatment with standard drug (loperamide) and with all the doses of aqueous and methanolic leaves extracts of *D. thollonii*, produced a significant inhibition of osmotic diarrhoea induced by the magnesium sulfate. This was made by positively affecting all the diarrhoeal parameters evaluated, that is prolonging the diarrhoeal latency time, reducing the water content of faeces, reducing the defecation frequency and number of wet defecations, compared with negative control rats having received distilled water. Results are more pronounced in Table 3 and Figures 9-14.

Table 3

Effects of the aqueous and methanolic leaves extracts of *D. thollonii* on magnesium sulfate induced diarrhoea.

Treatment	Dose (mg/kg)	Latency time (min)	Defection frequency	Number of wet defecations	Water content of faeces (%)
Distilled water	1 mL/100 g b.w	135.90±0.34	04.14±0.14	04.14±0.14	97.61±0.12
Standard	2.5	356.70±0.60^{c}	01.00±0.00^{c}	01.00±0.00^{c}	40.69±2.14^{c}
loperamide	125	151.70±0.56^{cγ}	03.00±0.00^{cγ}	03.00±0.00^{cγ}	79.97±1.00^{cγ}
Aqueous extract	250	209.30±0.49^{cγ}	02.50±0.19^{cγ}	02.38±0.19^{cγ}	63.99±1.26^{cγ}
	500	243.00±1.32^{cγ}	02.12±0.12^{cγ}	01.87±0.12^{cγ}	54.95±1.24^{cγ}
Methanolic extract	125	216.70±0.64^{cγ}	02.57±0.20^{cγ}	02.57±0.20^{cγ}	80.86±0.74^{cγ}
	250	234.10±1.93^{cγ}	02.28±0.18^{cγ}	02.28±0.18^{cγ}	71.04±0.73^{cγ}
	500	257.30±2.14^{cγ}	01.85±0.14^{cβ}	01.85±0.14^{cβ}	65.71±1.02^{cγ}

Values of each column represent the mean±ESM (n=6); ^{c}P<0.001: significant differences compared to the negative control (distilled-water); ^{β}P<0.01; ^{γ}P<0.001: significant differences compared to the positive control (loperamide).

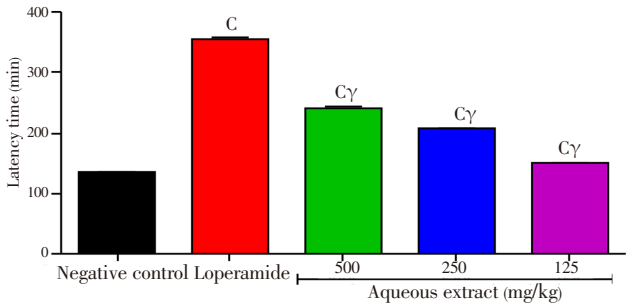

Figure 9. Effect of the aqueous leaves extract of *D. thollonii* on latency time in magnesium sulfate induced diarrhoea.

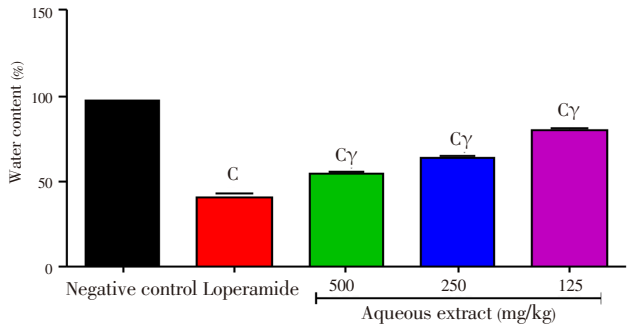

Figure 10. Effect of the aqueous leaves extract of *D. thollonii* on water content of faeces in magnesium sulfate induced diarrhoea.

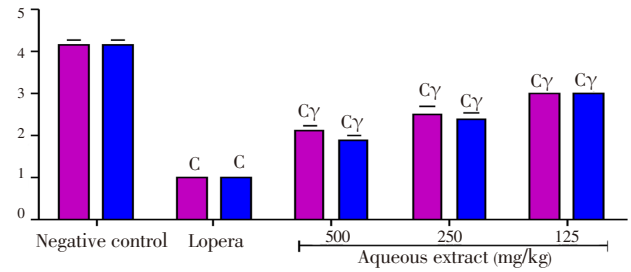

Figure 11. Effect of the aqueous leaves extract of *D. thollonii* on defecation frequency and the number of wet defecations in magnesium sulfate induced diarrhoea.

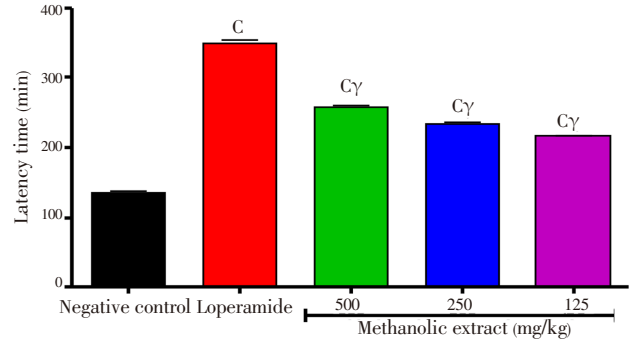

Figure 12. Effect of the methanolic leaves extract of *D. thollonii* on latency time in magnesium-sulfate induced diarrhoea.

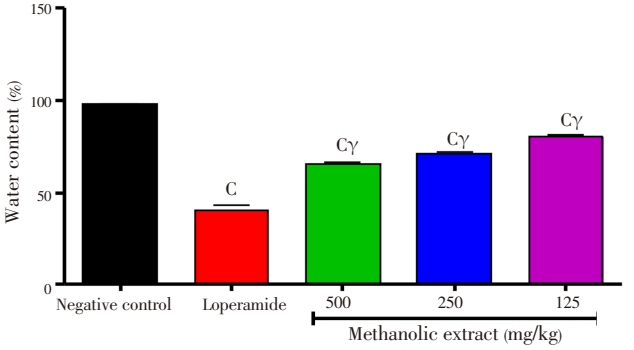

Figure 13. Effect of the methanolic leaves extract of *D. thollonii* on water content of faeces in magnesium-sulfate induced diarrhoea.

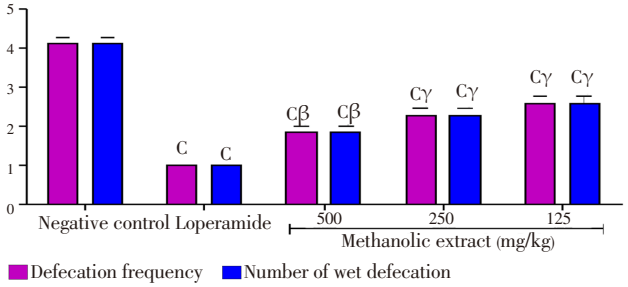

Figure 14. Effect of the methanolic leaves extract of *D. thollonii* on defecation frequency and the number of wet defecation in magnesium-sulfate induced diarrhoea.

4. Discussion

The results of antimicrobial activities, revealed that the two extracts were active against the the 2 clinical isolates tested and 5 bacterial strains. Nevertheless, aqueous extract presented a greater activity against the bacterial strains and isolates as compared to the methanolic extract. This could be due to secondary metabolites (polyphenols and the flavonoids) present in various extracts, of which several pharmacological and antibacterial activities were proved[9-12]. It was also demonstrated that these secondary metabolites were equipped with good antibacterial molecule sites, which caused the rupture of the lipopolysaccharidic membrane of the Gram-negative bacteria like *E. coli*, thus facilitating the restoration of protein channels-like structures to support the flow of antimicrobial compounds towards intra bacterial target sites[13,14]. In another hand, this activity could be done either by inhibiting the bacterial protein synthesis, or by blocking the inhibiting effects of the bacterial enzymes[15]. It should be noted that the active ingredients which act on the wall of the bacterium must find suitable receptors for their fixation and action, whereas those which act inside the cell must not only cross the membrane, but they must also find target in the cell[16]. The installation of the diarrhoea, associated to the febrility observed at Day 3 in infected rats, could be explained by the intestinal invasion of *S. flexneri*, coupled with the action of verotoxin which have led to a generalized tiredness of the organism. This bacterium would have thus penetrated in the epithelial cells of the mucous membrane, where it would have quickly multiplied and provoked the formation of abscesses and ulcerations, leading to the modification of intestinal reabsorption mechanisms[17]. The reduction in the bacterial load observed from the 2nd day in all infected animals faeces, treated with selected dose extracts (500, 250 and 125 mg/kg) could be due to the same presence of polyphenols and the flavonoids which have acted through the bactericidal mechanisms described above, as demonstrated by Yala *et al*[18]. The ricinoleic acid, released after digestion by intestinal lipases of the castor oil stimulates the peristaltic activity of the small intestine, thus causing a change of the permeability of the electrolytes of intestinal mucus[19]. The aqueous and methanolic leaves extract of *D. thollonii* at different doses could have acted according to anti secretary and spasmolytic mechanisms, through the inhibition of prostaglandins-E_2 biosynthesis and by increasing the reabsorption of electrolytes (Na^+, K^+, Cl^-). Unfortunately, both leaves plant extracts at dose 500 mg/kg could have also acted through the mechanisms comparable to that of loperamide which is known to inhibit the release of the inflammatory mediators, antagonized the histaminic activity when being fixed at the type-1 histaminic receptor (H_1), thus preventing the excessive contractions of the intestinal smooth muscle. In a healthy person, an excess of magnesium can lead to osmotic diarrhoea associated to distension of the intestine. Magnesium sulfate induced diarrhoea by increasing the secretion of electrolytes which create an osmotic imbalance. The antidiarrhoeal properties of flavonoids rise from their ability to inhibit the intestinal motility and the hydroelectrolytic secretions[20]. The antidiarrhoeal activity of the aqueous and methanolic extracts against the osmotic diarrhoea, induced by magnesium sulfate could be attributed to their antisecretory actions, coupled with the re-establishment of intestinal osmotic balance. It is thus noticed that the significant prolongment of the latency time, the significant reduction of defecation frequency, the reduction of water content of faeces and number of wet defecations demonstrated the efficacy of leaves extracts of *D. thollonii*, as effective

antidiarrhoeal agents.

In conclusion, this study showed that *D. thollonii* leaves extracts possessed antibacterial and antidiarrhoeal activities in rats, mediated firstly through bacteriostatic or bactericidal pathway and secondly through the re-establishment of intestinal balance. The study thus provided pharmacological basis to the use of the plant in intestinal disorders like diarrhoea and suggests using other experimental species, in order to illustrate an understandable conclusion which can be allowed to a human approach.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Moszynski P. Diarrhoeal diseases still kill more than 1.5m children under 5 each year. *BMJ* 2007; **335**: 1227.

[2] World Health Organization. More research needed into childhood diarrhoea. Geneva: World Health Organization; 2009. [Online] Available from: http://www.who.int/mediacentre/news/releases/2009/diarrhoea_research_20090310/en/ [Accessed on 7th January 2014]

[3] Fischer A. Primary immunodeficiency diseases: an experimental model for molecular medicine. *Lancet* 2001; **357**: 1863-1869.

[4] Maluma V. Les Antherotoma, Dissotis (inl. Heterotis) Melastomataceae endémiques d'Afrique centrale; 2005. [Online] Avaiable from: http://www.orchid-africa.net/PDF/TAXONOMANIA%2015.pdf [Accessed on 7th Jan 2014]. French.

[5] Mulla WA, Chopade AR, Bhise SB, Burade KB, Khanwelkar CC. Evaluation of antidiarrheal and *in vitro* antiprotozoal activities of extracts of leaves of *Alocasia indica*. *Pharm Biol* 2011; **49**(4): 354-361.

[6] Newton SM, Lau C, Gurcha SS, Besra GS, Wright CW. The evaluation of forty-three plant species for *in vitro* antimycobacterial activities; isolation of active constituents from *Psoralea corylifolia* and *Sanguinaria canadensis*. *J Ethnopharmacol* 2002; **79**: 57-67.

[7] Sankar MM, Gopinath K, Singla R, Singh S. *In-vitro* antimycobacterial drug susceptibility testing of non-tubercular mycobacteria by tetrazolium microplate assay. *Ann Clin Microbiol Antimicrob* 2008; **7**: 15.

[8] Ricicová M, Kucharikova S, Tournu H, Hendrix J, Bujddakova H, Van Eldere J, et al. *Candida albicans* biofilm formation in a new *in vivo* rat model. *Microbiology* 2010; **156**: 909-919.

[9] Gatsing D, Adoga GI. Antisalmonellal activity and phytochemical screening of various parts of *Cassia petersiana* Bolle (Cesulpiniaceae). *Res J Microbiol* 2007; **2**: 876-880.

[10] Kuete V. Potential of Cameroonian plants and derived products against microbial infections: a review. *Planta Med* 2010; **76**: 1479-1491.

[11] Jantan I, Jumuddin FA, Saputri FC, Rahman K. Inhibitory effects of the extracts of *Garcinia* species on human low-density lipoprotein peroxidation and platelet aggregation in relation to their total phenolic contents. *J Med Plant Res* 2011; **5**: 2699-2709.

[12] Sharma SK, Singh AP. Antimicrobial investigations on rhizomes of *Cyperus rotundus* Linn. *Der Pharmacia Lettre* 2011; **3**(3): 427-431.

[13] Panda SK, Dutta SK, Bastia AK. Antibacterial activity of *Croton roxburghii* Balak. against the enteric pathogens. *J Adv Pharm Technol Res* 2010; **1**: 419-422.

[14] Zakaria ZA, Zakaria ML, Amom Z, Desa MN. Antimicrobial activity of the aqueous extract of selected Malaysian herbs. *Af J Microbiol Res* 2011; **5**(30): 5379-5383.

[15] Lavigne JP. Effets des antibiotiques et mécanismes de résistance; 2009. [Online] Avaiable from: http://www.med.univ-montp1.fr/enseignement/cycle_1/PCEM2/mod-base/MB7_Bio_Med/Ressources_locales/BACTERIO/B6-ATB_et_resistance.pdf [Accessed on 7th January 2014]. French.

[16] Kuiate JR. [Biological and chemical caracterisation of dermatophytes and medicinal plants with antifungal effects in two localities of the west region of Cameroon] [dissertation]. Cameroon: Université de Yaoundé I; 2005. French.

[17] Verhaegen J. Les Entérobactéries. *Uniken Kongo* 2012; 25-27. [Online] Avaiable from: https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0CBwQFjAA&url=http%3A%2F%2Fwww.kuleuven.be%2Fvesaliusonline%2FUNIKEN%2520KONGO.doc&ei=8pe7U8DoCJWD8gXjiIC4BA&usg=AFQjCNGf1W-K8EvYwB0r_L64Ku5uBZJ3fQ&sig2=3cYr4Eu6IDJykl8GXm2yow&bvm=bv.70138588,d.bGQ&cad=rjt [Accessed on 7th January 2014]. French.

[18] Yala D, Merad AS, Mohamedi D, Korich MNO. [Classification of the mode of action of antibiotics]. *Médecine du Maghreb* 2001; **91**: 1-7. [Online] Available from: http://mas.stephanie.free.fr/microbiologie_bio2/atb.pdf [Accessed on 7th January 2014]. French.

[19] Hari Rao G, Lakshmi P. Evaluation of antidiarrhoeal activity of extract from leaves of *Aegle marmelos*. *J Appl Pharm Sci* 2012; **2**(2): 75-78.

[20] Rajabhau SS, Biradar KV, Chiude BV, Shambhulingayya HM, Goud V. *In-vivo* antidiarrhoeal activity of ethanolic extract of *Delonix regia* flowers in experimental induced diarrhoea in Wistar Albino rats. *Int J Res Pharm Chem* 2011; **1**(3): 442-446.