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Antidiarrhoeal, Antibacterial Activities and Growth Stimulatory Effect on Some Probiotic Organisms of Aqueous and Methanol Leaves Extract of *Dissothis Thollonii*

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ABSTRACT

Dissotis thollonii (D. thollonii) is traditionally used for the treatment of diarrhoea in Africa. The study aim to evaluate aqueous and methanol extracts of leaves of D. thollonii (Melastomataceae) for their antidiarrhoeal, antibacterial activities as well as probiotics growth stimulation effect. Both extracts effects were evaluated using rat animal models for in vivo assay. Antimicrobial activities were also been evaluated. D. thollonii extracts at all doses (125, 250 and 500 mg/kg) significantly inhibited the mean volume of intestinal fluid in prostaglandin E2 induced enteropooling assay, with the treatment of both extracts similarly to that of the standard antidiarrhoeal drug (loperamide), as well as the significant reduction of electrolytes concentration in the intestinal fluid. There was no inhibition, but rather growth of all probiotics tested. Extracts inhibited growth of 3 reference bacterial strains (Staphylococus aureus NCTC 6571, Bacillus subtili ATCC 23857 and Enterococus feacalis ATCC 39532) with minimum inhibitory concentrations (MIC) of 1000, 500 and 250 µg/ml, corresponding to percentage inhibition of 54.75 ± 0.47, 52.74 ± 4.40, and 50.15 ± 4.72 with methanol extract respectively. We also observed inhibition of one clinical isolate (Helicobacter pylori) with both extract at minimum inhibitory concentrations (MIC) of 2000 µg/ml, corresponding to the inhibition percentage of 54.37 ± 0.60 and 50.33 ± 0.66 respectively to the aqueous and methanolic extract. In vivo, extracts provoqued at all doses, a significant decrease (P<0.001) of the bacterial load in the faeces of rats, during all days of treatment compare to those which was treated with distilled water. Histological examination of the caecum showed at the dose of 250 and 500 mg/kg of aqueous and methanol extract respectively, no degradation of different layers compare to neutral and positive control (ciprofloxacin). These results attested the ethnopharmacological used of D. thollonii leaves in the treatment of diarrhoea and gastro-intestinal infections.

Keywords: *Dissotis thollonii*; Antidiarrhoea; Prostaglandin E2; Antimicrobial; Probiotics

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INTRODUCTION

Diarrhoea is a major health burden especially in developing countries where sanitation is inadequate. Diarrhoea has led to a significant increase in infant mortality and morbidity particularly in low income settlements [1]. Characterized by more than three loose motions of stool per day, diarrhoeal diseases have remained astronomical in spite of the efforts provided by organizations to curb with. Nowadays, there is a great interest in herbal remedies for the treatment of a number of ailments like diarrhea. These plants can be extremely useful as leads for synthetic modification and optimization of biological activity. Thus, the World Health Organizaion encourages the development of the traditional medicine which uses the medicinal plants partly [2]. Among the many exploited species, *D. thollonii* is traditionally used in the treatment of several evils. Thus it is used by traditional healer in Senegal and in west region of Cameroon on eczematous wounds [3]. The leaves are recommended in therapy of the gastrointestinal 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, the *in vitro* antibacterial activities of the same extracts over some enterobacteria strains and to determine the effect of these extracts on beneficial intestinal microflora.

MATERIALS AND METHODS

Collection and Identification of Plant Material

The fresh leaves of *D. thollonii* were collected in September 2016 in the Bafou village, Menoua division, West Region of Cameroon. A sample was identified at the National Herbarium of Cameroon (Yaoundé) by comparison with existing voucher specimen No.13292/SRF Cam.

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.

Preparation of Plant Extracts

The aqueous extract was prepared by boiling 800 g of powder in 5 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 51.6 g of the aqueous extract corresponding to an extraction yield of 6.45% (w/w). The other portion of leaf powder (900 g) was macerated in 6.75 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 63.8 g of the methanol extract, corresponding to a yield of 7.09% (w/w).

Experimental Animal

Wistar Albino female rats of 2.5 to 3 months and weighing on average 180 g were used for infectious induced diarrhoea model whereas female (2 to 2.5 months) of the same rat strain, weighing on average 140 g, were used for the tests of prostaglandin E2 induced enteropooling. They were bred in the Animal House Facility, Panjwani Center for Molecular Medicine and Drug Research (PCMD), International Center for Chemical and Biological Sciences. 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 the Protocol Number#: 2016-0024 as assigned by Institutional Animal Care and Use Committee (IACUC).

Chemicals and Reagents

All reagents used in the study were of high purity. The chemicals included isopropyl alcohol 70%, 90% and100%, xylene, hematoxylin, 0.5% eosine, methanol, deonized water, distilled water, saline solution, MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide), AlamarBlue, ciprofloxacin (Hilton Pharma, Korangi Industrial Area, Karachi, Pakistan), Doxycycline Hyclate (Pfizer, S.I.T.E, Karachi, Pakistan) , Mueller Hinton broth, Manitol Salt Agar, Tryptone Soy Agar, Brain Heart Infusion Broth, Brain Heart Infusion Agar, loperamide (Zadip Pharma NIG. LTD 488, Town planning Way, Hupeju, Lagos), Prostaglandin E2 (ZAFA Pharmaceutical Laboratory. A-46, S.I.T.E., North Karachi-Pakistan).

Microorganisms

The microorganisms used in this study included 6 bacterial strains [*Escherichia coli* ATCC 25922 (*E. coli*), *Staphylococus aureus* NCTC 6571 (*S. aureus*), *Pseudomonas aeruginosei* NCTC 10662 (*P. aeruginosei*), *Bacillus subtili* ATCC 23857 (*B. subtili*), *Salmonella typhi* ATCC 14028 (*S. typhi*) and *Enterococus feacalis* ATCC 39532 (*E. feacalis*)], one clinical isolate (*Helicobacter pylori*) and 6 probiotics (*Lactobacillus lactis*, *Pediococcus acidilactisi*, *Lactobacillus fermentum* strain1, *Lactobacillus fermentum* strain2, *Enterococcus durans* and *pediococcus lolie* LMG-25667). These microorganism were chosen based on their clinical and pharmacological importance [4]. They were obtained from Hussain Ebrahim Jamal Research Institute of Chemistry, International Center for Chemical and Biological Sciences, Karachi, and were used for evaluating antimicrobial activity. The strains were maintained at 4°C on agar plates.

***In vitro* Antimicrobial Activity**

Alamar blue assay:

For this assay, a single colony of different strains of bacteria grown in Tryptone Soy Agar (TSA) and in BHI Agar for *Helicobacter pylori*, were inoculated in Mueller Hinton Broth (MHB) and incubated overnight at 37°C for bacterial strains and for *Helicobacter pylori*, inoculation was done in Urea Broth, then incubation during 48 h at 37°C at 10% CO_2. Fully grown turbid bacterial culture was then diluted to adjust with 0.5 Mcfarland Turbidity Index (1.5×10^8 CFU/ml). Stock solution (80 mg/ml) of different extracts was prepared in distilled water. 10 µl of extracts were placed in triplicate in the first well of sterile 96-well micro titer plate except the control wells. This gives concentration of 4000 µg/mL of extract, and a serial two fold dilutions was performed to obtain 7 concentrations, ranging from 4000 to 62.5 µg/mL for the extracts. Finally, 7 µl of bacterial suspension were then added in each well. Plates were sealed by parafilm and incubated 20 hr at 37°C. Next day, 10% AlamarBlue dye was added in each well and incubated in shaking incubator at 80 rpm, 37°C for 2-3 hrs. The color change from blue to reddish pink shows there is growth of bacteria [5]. The absorbance was read at 570 nm and 600 nm in spectrophotometer, and the % inhibition of different extract was calculated [5].

MTT assay:

In this assay, a single colony of different strains of Probiotics grown in Brain Heart Infusion Agar (BHIA) were inoculated in Brain Heart Infusion Broth (BHIB) and incubated overnight at 37°C. Fully grown turbid bacterial culture was then diluted to adjust with 0.5 Mcfarland Turbidity Index (1.5×10^8 CFU/ml). Stock solution (160 mg/ml) of different extract was prepared in distilled water, and 5 µl, 3.75 µl and 2.5 µl respectively for the concentration of 4000 µg/mL, 3000 µg/mL and 2000 µg/ml of these extracts were placed in well of flat bottom, polystyrene, sterile 96-well micro titer plate except the control wells, in final 200 µL solution. Each concentration was placed in triplicates. Finally, bacterial suspension (5×10^6 CFU/mL) was then added in each well. Plates were sealed by parafilm and incubated 18-20 hr at 37°C. Next day, 20 µL MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) dye was added in each well and incubated in shaking incubator at 80rpm, 37 °C for 2-3 hrs. The color change from yellow to black shows there is growth of bacteria. The absorbance was read at 570 nm in spectrophotometer, and the % inhibition of different extracts was calculated using the formula:

$$\% \text{ inhibition} = 100 - ((\text{test absorbance}/\text{untreated control absorbance}) \times 100)$$

***In vivo* Antidiarrhoeal Activity**

***Enterococcus feacalis*-induced diarrhea:**

The experimental animals were firstly deparasitized through an oral administration of 10 mg/kg of Doxycycline for 3 days [6]. 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 days 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 *Enteroccocus feacalis* (*E. faecalis)* suspension, coldly prepared at the 1.0 McFarland standard scale (3×10^8 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. Group 2 (negative control) received distilled water. The third group (positive control) received ciprofloxacin at 2.5 mg/kg b was reference drug and. All the substances (extracts plants and ciprofloxacin) were administered per os until complete elimination of bacterial load in faeces.

These faeces were collected each 2 days between 10 h and 11 h. The follow-up of the treatment was carried out by evaluating the load of *E. feacalis* in a culture of 1g of faeces on Manitol Salt Agar Petri dishes, incubated at 37°C for 24 h. At the end of treatment, animals were sacrificed. Liver, spleen and caecum were removed. 0.5 g of liver and all spleen were crushed by a homogenizer in 0.5 ml of saline and centrifuged at 4000 × g for 15 min at 4°C for pelleting bacterial [7]. 1 ml of saline solution were add in pellet and mixed by vortexing, then 0.25 ml was used for evaluated the *E. feacalis* load.

Histological Assessment

One part of the caecums were preserved in formalin fixative (3.7%) solution and followed by tissue dehydrated with isopropyl alcohol and xylene. Then, each sample was embedded in paraffin wax, sectioned at 6 µm in slides prior for staining. Haematoxylin and eosin 0.5% stain was used. The slides were examined under light microscope and recorded with 10×BF.

Prostaglandin-E2 Induced Enteropooling

The effect of the extracts on Prostaglandin- E2 induced enteropooling was investigated using the method described by [8] with slight modification. Six groups of female wistar rats of six (6) animals each (150-180) were fasted for 18 hours before the experiment but had access to water. Rats were treated orally with vehicle (10 mL/kg), Loperamide (2.5 mg/kg p.o.) or aqueous and methanol extracts (125, 250 and 500 mg/kg body weight). Immediately after extract administration, PGE2 (100 µg/kg) p.o. was administered. After 30 min following administration of PGE 2 each rat was sacrificed by administering excessive dose of ketamine and the whole length of the intestine from pylorus to caecum was dissected out and weigh, its content collected in measuring cylinder and volume measured, then the empty intestines was weigh and the difference between the full and empty intestines was calculated [9].

Electrolytes Concentration in Intestinal Fluid

The volume of intestinal content was centrifuged at 2000×g for 15 min at 4°C. the supernatant was collected for determination of the concentration of 23Na, 24Mg, 43Ca, 44Ca and 39K using the method describe by [10] with slight modification. 250 µl of each sample were preleved and put each in corresponding vials. A volume of 300 µL concentrated nitric acid (HNO_3) (Ultrex purity, Fisher), 200 µL concentrated hydrochloric acid (HCl) (Ultrex, Fisher), and 100 µL of non-stabilized 30% hydrogen peroxide (H_2O_2) solution (Ultrex, Fisher) was added to each vial. Deionized water was added to provide a final volume of 2.0 mL. Samples were then sealed, placed in the microwave, and digested following the program described in Table 1. After digestion, samples were homogenized with 5 ml of deonized water and sonicated at 65°C, then diluted 100 times and run in inductively coupled plasma mass spectrometry (ICP-MS) with flow rate for organ gas of 15 l/min, electron multiplier as detector and detection limits for each sample like presented in Table 2.

Table 1: Microwave digestion program for study samples

Max.Temperature (°C)	Ramp (min)	Hold (min)	Max. Pressure (PSI)	Power (W)	Stir rate
200	7:00	9:00	300	200	Medium

Table 2: ICP-MS analyze for each element

Elements	Detection limits
43 Ca	30.32
44 Ca	35.72
39 K	14.17
24 Mg	18.25
23 Na	29.15

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 prostaglandin E2 induced enteropooling and bacterial load in spleen and liver, whereas the Bonferroni post-test ($P<0.05$) was used for the infectious diarrhea (bacterial load in feaces).

RESULTS AND DISCUSSION

Evaluation of Antibacterial Activity

Both plant extracts expressed antibacterial activities on *Staphylococus aureus* NCTC 6571 and *Enterococus feacalis* ATCC 39532, whereas *Bacillus subtili* ATCC 23857 show activity only with methanol extract. However, these activity were more interesting with the aqueous extract, which presented higher inhibition on *Enterococus feacalis* ATCC 39532 (71.93% ± 0.18) and *Staphylococus aureus* NCTC 6571 (76.18% ± 4.48), all at the concentration of 4000 µg/ml, whereas methanol extract presented inhibition at the same concentration with the same microorganism at 65.47% ± 3.48 and 66.52% ± 3.07 respectively. Extracts also show good activity with *Helicobacter pylori* with the inhibition percentage of 59.14 ± 0.82 and 64.56 ± 0.78 respectively with aqueous and methanol extract at the concentration of 4000 µg/ml. Results are showed in Table 3.

Table 3: Effect of aqueous and methanol extract of *D. thollonii* against different bacteria by employing Alamar Blue assay and their % inhibition. Data are reported as the Mean ± SEM. All values were read in triplicate

Compounds	Conc. µg/ml	*E. coli* ATCC 25922	*S. aureus* NCTC 6571	*Pseudomonas aeruginosei* NCTC 10662	*Bacillus subtili* ATCC 23857	*Salmonella typhi* ATCC 14028	*Enterococus feacalis* ATCC 39532	*Helicobacter Pylori*
Aqueous Extract	4000	26.93 ± 1.01	76.18 ± 4.48	<0	28.41 ± 2.06	<0	71.93 ± 0.18	59.14 ± 0.82
	2000	5.16 ± 1.05	73.87 ± 0.42	<0	17.84 ± 2.19	<0	64.91 ± 2.01	54.37 ± 0.60
	1000	<0	66.14 ± 3.81	<0	17.75 ± 0.04	<0	64.05 ± 2.51	32.98 ± 0.44
	500	<0	50.21 ± 5.99	<0	16.15 ± 0.17	<0	57.02 ± 2.37	17.10 ± 0.40
	250	<0	28.56 ± 0.62	<0	12.48 ± 1.14	<0	54.76 ± 2.69	9.60 ± 0.35
	125	<0	<0	<0	<0	<0	31.70 ± 2.52	2.30 ± 0.37
	62.5	<0	<0	<0	<0	<0	30.65 ± 5.39	<0
Methanol Extract	4000	1.92 ± 0.02	66.52 ± 3.07	<0	65.88 ±1.62	<0	65.47 ± 3.48	64.56 ± 0.78
	2000	<0	61.88 ± 5.08	<0	64.15 ± 0.29	<0	55.16 ± 2.09	50.33 ± 0.66
	1000	<0	54.75 ± 0.47	<0	55.33 ± 0.26	<0	53.80 ± 2.05	26.54 ± 0.57
	500	<0	44.86 ± 1.49	<0	52.74 ± 4.40	<0	53.41 ± 1.86	11.19 ± 0.67
	250	<0	27.42 ± 0.00	<0	46.02 ± 0.69	<0	50.15 ± 4.72	6.32 ± 0.98
	125	<0	22.79 ± 0.11	<0	44.77 ± 4.93	<0	49.45 ± 2.88	3.54 ± 0.34
	62.5	<0	5.59 ± 0.00	<0	2.24 ± 1.90	<0	46.74 ± 3.06	<0
Ofloxacin	50	93.35 ± 1.67	92.15 ± 0.71	83.12 ± 0.59	93.00 ± 0.58	94.11 ± 0.68	85.32 ± 0.66	-
Amoxicilin +Metronidazol	125	-	-	-	-	-	-	78.61 ± 0.20

Effect of Extracts on Intestinal Microflora

All extracts presented no inhibition and enhance growth of all probiotics tested at all the concentration, except the aqueous extract on *Lactobacillus lactis* sub sp. K98 which present low inhibition (34.76% ± 4.09, 36.13% ± 0.1 and 39.53% ± 4.09) respectively at the concentration of 4000 µg/mL, 3000 µg/mL and 2000 µg/mL compare to Doxycyclin (80.23% ± 0.42) at the concentration of 4 µg/mL (Table 4).

Table 4: Effect of aqueous and methanol extract of *D. thollonii* on the inhibition percentage of probiotics, data are reported as the mean ± SEM all values were read in triplicate, AEDt: aqueous extract of *D. thollonii*, MEDt: methanolic extract of *D. thollonii*

	Lactobacillus lactis sub sp. K98	*Pediococcus acidilactisi*	*Lactobacillus fermentum* strain1	*Lactobacillus fermentum* strain2	*Enterococcus durans L.O.*	*Pediococcus lolie LMG-25667*
AEDt 4000 µg/ml	34.76 ± 4.09	<0 (growth)	<0 (growth)	<0 (growth)	<0 (growth)	<0 (growth)
AEDt 3000 µg/ml	36.13 ± 0.1	6.91 ± 2.72	<0 (growth)	<0 (growth)	<0 (growth)	<0 (growth)
AEDt 2000 µg/ml	39.53 ± 4.09	14.11 ± 5.98	23.74 ± 2.76	<0 (growth)	<0 (growth)	<0 (growth)
MEDt 4000 µg/ml	7.39 ± 0.04	<0 (growth)	<0 (growth)	<0 (growth)	0.00 ± 0.00	<0 (growth)
MEDt 3000 µg/ml	7.01 ± 2.24	<0 (growth)	<0 (growth)	<0 (growth)	3.02 ± 0.03	<0 (growth)
MEDt 2000 µg/ml	14.82 ± 4.86	<0 (growth)	<0 (growth)	<0 (growth)	13.42 ± 3.17	<0 (growth)

Doxycyclin 4 µg/ml	80.23 ± 0.42	63.46 ± 0.10	57.68 ± 0.35	81.72 ± 1.91	82.13 ± 0.33	37.66 ± 3.60

Enterococcus Feacalis-Induced Diarrhoea in Rats

The aqueous and methanol extracts of *D. thollonii* leaves, produced significant (P<0.001) decrease in the bacterial load in the feces of rats during day 2 until the end of treatment. The doses of 250 and 500 mg/kg of aqueous and methanol extracts respectively, exhibited a highly significant (P<0.001) effect from Day 6 for aqueous extract and day 8 for methanol extract, when compared to negative control group and similarly as the standard drug used. Results are illustrated by Figures 1 and 2. Extracts also showed a significant inhibition (P<0.001) of bacteria load in liver and spleen at all the doses of aqueous extract, 250 and 500 mg/kg of methanol extract, when compared to negative control group (distilled water), and there is no significant difference (P>0.05) when compared to positive control group (ciprofloxacin). Results are illustrated by Figures 3 and 4.

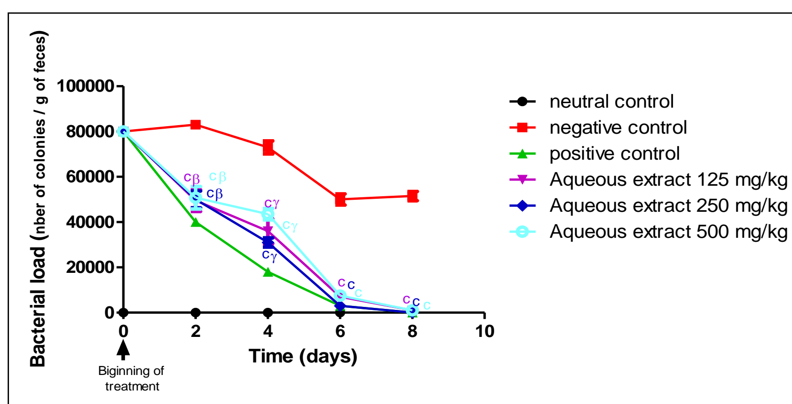

Figure 1: Variation of the bacterial load (*Enterococcus feacalis*) in the feces of infected rats treated with the aqueous extract of *D. thollonii*. Each point represent the mean ± SEM (n=6). β: significant differences (P<0.01) compared with positive control (ciprofloxacin), C and γ: significant differences (P<0.001) compared with negative (distilled water) and positive control (ciprofloxacin), respectively

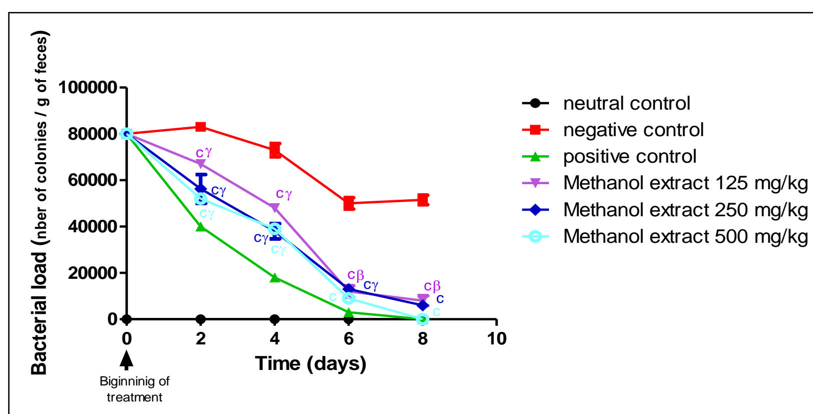

Figure 2: Variation of the bacterial load (*Enterococcus feacalis*) in the feces of infected rats treated with the methanol extract of *D. thollonii*. Each point represent the mean ± SEM (n = 6). β: significant differences (P<0.01) compared with positive control (ciprofloxacin), C and γ: significant differences (P<0.001) compared with negative (distilled water) and positive control (ciprofloxacin), respectively

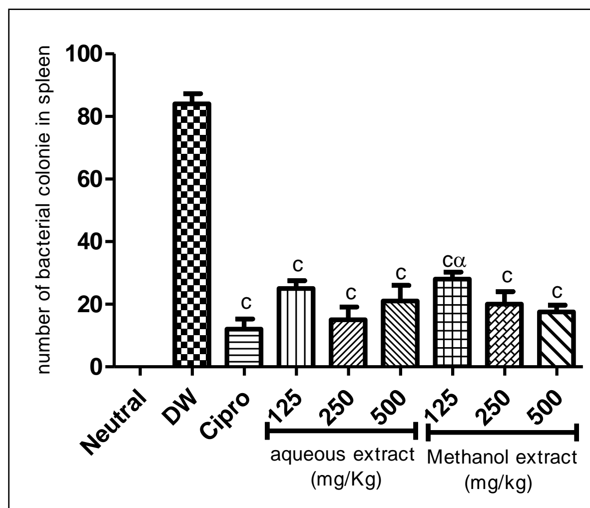

Figure 3: Number of bacterial colonie (*Enterococcus feacalis*) in the spleen of infected rats treated with the aqueous and methanol extract of *D. thollonii.* Each point represent the mean ± SEM (n = 6). α: significant differences (P<0.05) compared with positive control (ciprofloxacin), C : significant differences (P<0.001) compared with negative control (distilled water)

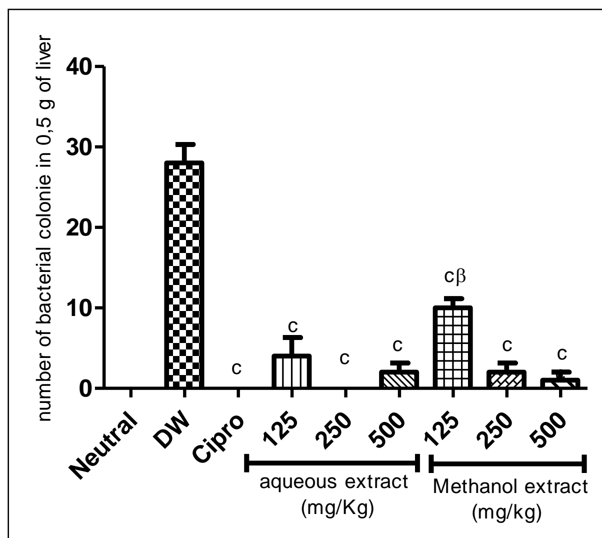

Figure 4: Number of bacterial colonie (*Enterococcus feacalis*) in 0.5 g of liver of infected rats treated with the aqueous and methanol extract of *D. thollonii*. Each point represent the mean ± SEM (n = 6). β: significant differences (P<0.01) compared with positive control (ciprofloxacin), C : significant differences (P<0.001) compared with negative control (distilled water)

Histological Assessment in Caecum

Histological examination of the caecum removed from animals that were treated with distilled water showed higher degradation of different layers (mucosa, submucosa and muscularis), while those who received aqueous and methanol extract at the dose of 250 and 500 mg/kg respectively appear intact with all the different layers compare to neutral and positive control (ciprofloxacin). However, 125 mg/kg of aqueous and methanol extract show higher degradation of mucosa, while aqueous extract 500 mg/kg show light degradation of this layer (Figure 5).

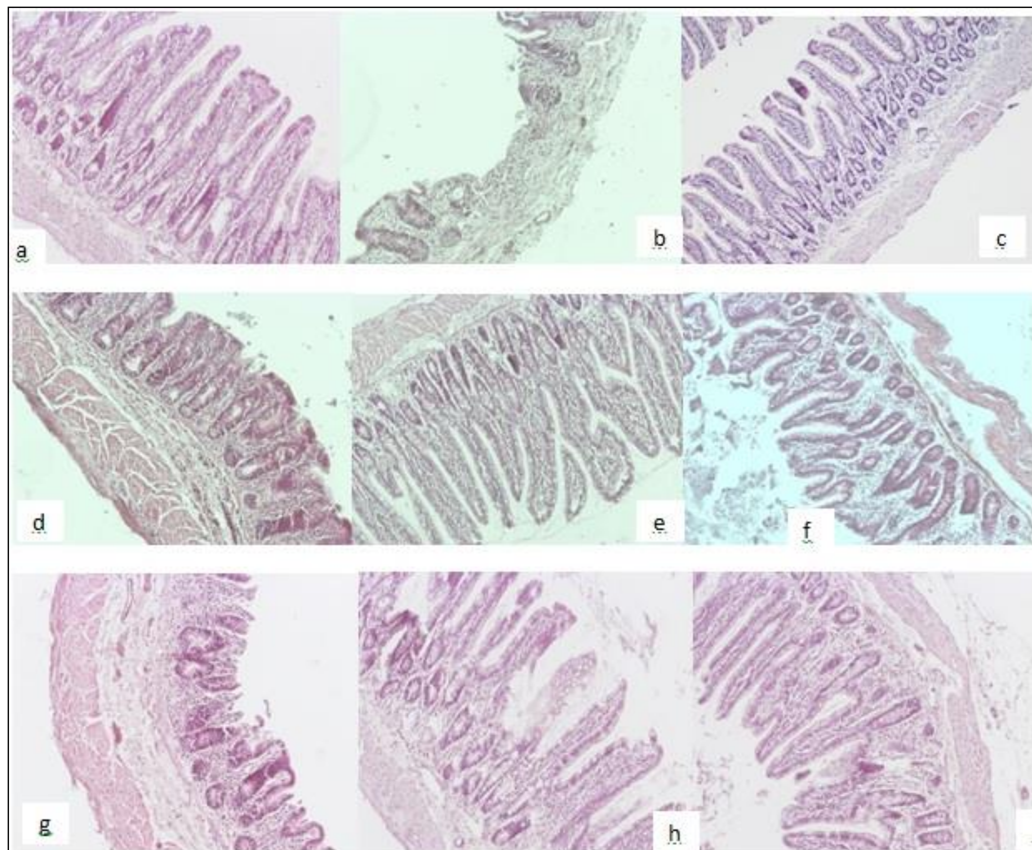

Figure 5: Histological analysis of caecum. a: section of a neutral control rat: no degradation are seen and the layers are normal. b: negative control (distilled water): there is denaturation of all layers. c: histological section of a positive control (ciprofloxacin) : no degradation are seen and the different layers are normal. d: treated with aqueous extract (125 mg/kg): there is higher degradation of vilis. e: treated with aqueous extract (250 mg/kg): all the different layers are present and normal f: treated with aqueous extract (500 mg/kg): there is light degradation of vilis and non-regularity on muscularis. g: treated with methanol extract (125 mg/kg): there is higher degradation of mucosa. h: treated with methanol extract (250 mg/kg): all layers are present but, there is low degradation of villis. i: rat treated with methanol extract (500 mg/kg): there is no degradation of different layers

Effect of the Extracts on Prostaglandin- E2 Induced Enteropooling in Rats

The aqueous and methanol extracts at doses of 250 and 500 mg/kg significantly inhibited prostaglandin E2-induced enteropooling in terms of volume of intestinal fluid and in a manner comparable to that of the standard antidiarrhoeal drug – loperamide (2.5 mg/kg b.w). There is no significant different ($P>0.05$) between all the treated groups when compare to untreated group in terms of mass of intestinal content (Table 5).

Effect of Extracts on Electrolytes Concentration in Intestinal Fluid

The aqueous and methanol extracts show a reduction of concentration of all electrolytes at all the doses compared to negative control (distilled water). At the doses of 250 and 500 mg/kg of aqueous extract and 500 mg/kg of methanol extract, we observed higher reduction of the concentration of 23Na compared to those treated by loperamide. This higher reduction of concentration is also observed with 24 Mg at the doses of 500 mg/kg of aqueous extract, 125 and 250 mg/kg of methanol extract compare to those who were treated by loperamide. Both extracts at the doses of 250 and 500 mg/kg reduced the concentration of 39 K comparably to positive control (loperamide). The same observations are made with 43Ca at the doses of 250 and 500 mg/kg of methanol extract (Table 6).

Table 5: Effect of aqueous and methanolic extracts of *D. thollonii* on prostaglandin E2- induced intestinal fluid accumulation in rat. Data are reported as the mean ± S.E.M. for n = 6 per group. ANOVA one way followed by Tureky's test ;). [a]p <0.05; [c]p <0.001: significant differences from the negative control (distille water). AEDt : Aqueous Extract of *D. thollonii*, MEDt : Methanol Extract of *D. thollonii*

	Parameters					
Treatments	Weight full intestine	Weight empty intestine	Masse intestinal content	Mean % of inhibition	Vol. intestinal fluid	Mean % of inhibition
Distilled water	5.62 ± 0.48	4.42 ± 0.53	1.20 ± 0.38	-	1.54 ± 0.57	-
Loperam 2.5 mg/kg	5.46 ± 0.54	4.68 ± 0.62	0.72 ± 0.51	40	0.78 ± 0.61c	49.35
AEDt 125 mg/kg	6.10 ± 0.88	5.14 ± 0.67	1.00 ± 0.59	16.67	0.96 ± 0.69a	37.66
AEDt 250 mg/kg	5.24 ± 0.93	4.70 ± 0.93	0.52 ± 0.33	56.67	0.54 ± 0.37c	64.94
AEDt 500 mg/kg	5.28 ± 0.74	4.46 ± 0.72	0.70 ± 0.35	41.67	0.82 ± 0.62c	46.75
MEDt 125mg/kg	6.00 ± 0.87	4.80 ± 0.61	1.00 ± 0.56	16.67	1.20 ± 0.72a	22.08
MEDt 250 mg/kg	5.84 ± 0.63	4.82 ± 0.50	0.78 ± 0.33	35	1.02 ± 0.47c	33.77
MEDt 500 mg/kg	5.80 ± 0.79	4.90 ± 0.70	0.68 ± 0.44	43.33	0.90 ± 0.56 c	41.56

Table 6: concentrations of elements (electrolytes) in intestinal fluid of rats treated with aqueous and methanol extract of *Dissotis thollonii* (mg/ml). Data are reported as the mean ± S.E.M. for n = 6 per group. All samples were run in triplicate on ICP-MS. AEDt : Aqueous Extract of *D. thollonii*, MEDt : Methanol Extract of *D. thollonii*

	Elements				
Sample	23 Na	24Mg	39K	43 Ca	44 Ca
Distilled water	2.111 ± 0.037	0.267 ± 0.046	1.078 ± 0.020	0.298 ± 0.110	0.295 ± 0.110
Loperamide (2.5 mg/kg)	1.114 ± 0.032	0.128 ± 0.032	0.491 ± 0.008	0.167 ± 0.000	0.172 ± 0.011
AEDt 125 mg/kg	2.027 ± 0.241	0.198 ± 0.008	0.946 ± 0.173	0.172 ± 0.003	0.137 ± 0.002
AEDt 250 mg/kg	0.429 ± 0.147	0.141 ± 0.006	0.369 ± 0.051	0.210 ± 0.005	0.155 ± 0.004
AEDt 500 mg/kg	0.445 ± 0.007	0.034 ± 0.017	0.317 ± 0.003	0.205 ± 0.003	0.157 ± 0.003
MEDt 125 mg/kg	2.101 ± 0.051	0.018 ± 0.007	0.896 ± 0.032	0.174 ± 0.099	0.142 ± 0.093
MEDt 250 mg/kg	1.624 ± 0.102	0.007 ± 0.000	0.331 ± 0.059	0.163 ± 0.004	0.121 ± 0.009
MEDt 500 mg/kg	0.359 ± 0.012	0.168 ± 0.048	0.104 ± 0.044	0.114 ± 0.019	0.066 ± 0.017

The major causative agents of diarrhea in human beings include various enteropathogens like, *Escherichia coli, Staphylococus aureus*, and *Enterococus feacalis*. This physiopathology is characterized by the frequent passage of liquid faeces and it involves both an increase in the motility of the gastrointestinal tract, along with increased secretion and decreased absorption of fluid, and thus a loss of electrolytes (particularly sodium) and water [11]. For the treatment and management of diarrhea in developing countries, several plant extracts are studied for this purpose. Methanol and aqueous leaves extract of *D. thollonii* that have not been studied so far, were evaluated for their antimicrobial activities *in vitro*, antidiarrhoeal potential against infectious diarrhoea induced by *Enterococcus feacalis* and non-infectious diarrhoea induced by prostaglandin-E2 in rat. The results of antimicrobial activities revealed that aqueous and methanol extracts presented no inhibition in all probiotics tested, while these extracts have inhibited pathogenic bacteria like *Staphylococus aureus* NCTC 6571 (MIC: 500 and 1000 µg/ml respectively) and *Enterococus feacalis* ATCC 39532 (MIC: 250 µg/ml). Secondary metabolites groups that have previously been described in this plant extract (tannins, flavonoids, sterols, anthraquinones, phenols and polyphenols) [6] are known to possess antimicrobial properties. This activity could be done by inhibiting the bacterial protein synthesis, or by blocking the inhibiting effects of the bacterial enzymes. 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 [6]. The census of pathogens revealed that *E. coli,* entero- invasive (EIEC) and entero-hemorragic (EHEC), *Salmonella, Shigella, Vibrio cholerae* are the major bacterial pathogens most often responsible for pandemic and epidemic diarrheal infectious diseases in developing countries [12]. However, like *Shigella flexneri, Enterococcus feacalis* is also known to secrete verotoxins which helps it to colonize the colon mucosa and get inside the epithelial cells, inducing inflammation and degeneration of the *Lamina propria* [13]. This could be explaining the installation of the diarrhoea, associated to the febrility observed at Day 0 in infected rats. Consequently, the desquamation and ulceration of the mucosa leads to the loss of blood and mucus in the intestinal lumen, which in turns hinders reabsorption. 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 would have facilitating the restoration of protein channels-like structures to support the flow of antimicrobial compounds towards intra bacterial target sites [14] as well as bactericidal mechanisms described above, as demonstrated by [15]. All these bactericidal mechanisms of the extract due to their secondary metabolites explain the good appearance of different layers of the histological section of caecum of rat treated with aqueous and methanol extract at the dose of 250 and 500 mg/kg respectively. So, these extracts, while destroying the bacteria, would have prevented the release of verotoxins, Thereby promoting inhibition of irritation and degeneration of the *Lamina propria.* In the human intestine the extensive metabolism of flavonoids has been attributed to the action of intestinal microflora [16]. So it was appropriate to conduct an experiment for evaluating the efficacy of the leaves extract of *D. thollonii* in promoting growth of some probiotics.

Clinical effects of some probiotic strains are observed in human's intestine. These include, for example, immunomodulation, modulation of intestinal flora, prevention of diarrhoeas, and lowering of fecal enzyme activities. To have an impact on the colonic flora it is important for probiotic strains to show antagonism against pathogenic bacteria via antimicrobial substance production or competitive exclusion. Enormous research efforts have focused on bacteriocin research. Although probiotic strains may produce bacteriocins, their role in the pathogen inhibition *in vivo* can only be limited, since traditional bacteriocins have an inhibitory effect only against closely related species [17]. However, low molecular weight metabolites (such as hydrogen peroxide, lactic and acetic acid, and other aroma compounds) and secondary metabolites (polyphenols and the flavonoids) may be more important in the inhibition of pathogenic bacteria and the potentialisation of some probiotics [18]. These confirm the results obtain in our study which shows an increase in growth with the dose. The extracts also significantly inhibited the PGE2 induced intestinal fluid accumulation (enteropooling). It has been shown that E type of prostaglandins cause diarrhoea in experimental animals as well as human beings by inhibiting the absorption of Na^+, Cl^- and glucose which causes the accumulation of intestinal fluid and leads to diarrhea [19,20]. Their mechanism has been associated with dual effects on gastrointestinal motility as well as on water and electrolyte transport [20,21]. Both extracts significantly reduced prostaglandin E2-induced enteropooling as evident from the higher reduction in the volume of the intestinal contents in a manner comparable to the reference drug, (loperamide) [22]. Loperamide has been reported to decrease colon flow rate and attenuate transit in the small intestine [23]. It is possible that the aqueous and methanol extracts of *D. thollonii* produced their antidiarrhoeal action by reduction in the synthesis or action of PGE2. Elsewhere, the anti-diarrheal properties of some medicinal plants have been attributed to their phytochemical constituents like tannins and some flavonoids [24], which have been shown to possess anti-diarrheal activity attributable to their ability to inhibit intestinal motility and hydro-electrolytic secretion.

CONCLUSION

The results of this investigation revealed that both extracts of *D. thollonii* contains pharmacologically active substance(s) with anti-diarrhoeal and antibacterial properties. These properties confirm the use of the plant leaves as an anti-diarrhoeal and antibacterial drug by traditional healers in Cameroon.

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