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Phytochemical Composition and *In vivo* Antibacterial Activity of the Aqueous Leaf Extract of *Polygonum limbatum* on *Escherichia coli*-infected Guinea Pigs

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Authors' contributions

This work was carried out in collaboration between all authors. All authors have directly participated in the work and have read and approved the final version submitted.

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ABSTRACT

Background: *Polygonum limbatum* Meisn (Polygonaceae) extracts have been used in West region of Cameroon for the treatment of urogenital infections. The therapeutic efficacy of the aqueous leaf extract of *Polygonum limbatum* against urogenital infections was investigated on *Escherichia coli*-infected guinea pigs.

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Methods: The infected animals received the extract orally at 200, 500 and 700 mg/kg body weight for 15 days during which the vaginal bacterial load was measured every 72 h. The side effects of the extract were also evaluated through hematological and biochemical analyses. Phytochemical screening was done using standard methods.
Results: In general, a considerable decrease in the bacterial load was observed in the treated groups with time. On the 13th day of treatment, the doses of 700, 500 and 200 mg/kg induced 98.45%, 93.02% and 67.05% decrease in the bacterial load, respectively. After 15 days of treatment, WBC counts significantly increased in infected animals. Significant decreases were observed in the protein titers of the heart, lungs and kidneys. Serum total proteins and transaminases were significantly decreased. Urinary protein concentration significantly decreased at the dose 200 mg/kg and increased at 700 mg/kg of extract. Serum and urinary creatinine significantly decreased. Flavonoids, alkaloids, triterpenes, polyphenols, tannins and saponins were found in the extract.
Conclusion: This study demonstrated marked therapeutic effects of *P. limbatum* aqueous leaf extract against urogenital infection caused by *Escherichia coli*. Also, no critical sign of toxicity was observed in treated animals.

Keywords: Urogenital infection; plant extract; side effects; hematological and biochemical parameters.

1. INTRODUCTION

Plants have always been an important source of drugs. From aspirin to taxol, modern pharmaceutical industries have largely used secondary metabolites from plants origin for new drug research [1]. Plants and plant-based derivatives constitute an important part of the human health care system since ancient civilization because of their unquestionable medicinal properties. Ended, many plant are known for their antibacterial, antioxidant, anti-inflammatory, anti-carcinogenic, antiviral, anti-fungal, anti-abortive and anti-parasites properties [2-8]. Studies carried out by Raskin et al. [9] have shown that 30% of the commercialized drugs contain active principles isolated for the first time from medicinal plants. These plants can bring about a solution to certain diseases, mostly in developing countries where synthetic drugs are relatively expensive and not very accessible to the underprivileged social strata [10,11].

Polygonum limbatum Meisn (Polygonaceae) grows mainly in the wetlands. It is found in many countries of tropical Africa and in India [12]. In Cameroon, the plant is frequently found in the North, Far-North, North-West and West regions, along the lakes and rivers. The aqueous leaf extract of *Polygonum limbatum* Meisn has been reported to be used in folk medicine in Cameroon for the treatment of various infections, including gastrointestinal disorders, cutaneous infections and urogenital infections [13]. The *in vitro* antimicrobial activity of the methanol leaf extract of *Polygonum limbatum* against *Escherichia coli* has been demonstrated [14].

Nonspecific urogenital infections are caused by human commensalist bacteria, which become pathogenic either due to a change in their normal behavior/habitat or due to a failure in the immune system [15]. Among these bacteria are *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* [15-17]. These infections cause serious health problems especially in developing countries where they are more or less endemic. *Escherichia coli* is frequently resistant to common antibiotics [18].

In a continuation of our search for therapeutic agents from natural sources with potential for the treatment of urogenital infections [15,19,20], the *in vivo* antimicrobial activity of the aqueous leaf extract of *Polygonum limbatum* was investigated on *Escherichia coli*–infected guinea pigs. The side effects of the extract were also evaluated.

2. MATERIALS AND METHODS

2.1 Plant Material

The leaves of *Polygonum limbatum* were freshly collected during the month of June 2010 in Dschang, a locality of the High Lands of West region, Menoua division, Cameroon. The plant was authenticated by a plant taxonomist at the Cameroon National Herbarium (CNH) where a

voucher specimen was kept under the registration number 35-592/CNH.

2.2 Animals and Standard Antibiotic

Adult female guinea pigs weighing 250-350 g were used in this study. They were obtained from local breeding structures. The animals were housed in plastic cages under normal laboratory conditions (12 h light/dark cycle; 23 ± 2°C) for an acclimatization period of one week (minimum length of time necessary for those animals to adapt to their new environment). During this period, they were given food and tap water *ad libitum*. Thereafter, their food was supplemented with 100% tetracycline at 100 g/kg of food for 5 days, in order to rid them of eventual infections. Vaginal samples were taken and enriched on tryptose phosphate at 37°C for 18 h, then 50 µL of this mixture was taken and cultured on salt free Mac Conkey agar at 37°C for 24 h. The absence of colonies after the incubation period was an evidence of successful disinfection. Disinfected animals were further immunodepressed by oral administration of cyclophosphamide (Sigma-Aldrich, France) at 250 mg/kg body weight for 3 days as described by the modified method of Aviles et al. [21]. Oxytetracycline (obtained from Sigma, St. Louis, MO, USA) was used as standard drug for these *in vivo* assays.

This work was carried out with respect for the welfare of animals, as recommended by WHO [22]. All studies involving animals were conducted according to the ethical guidelines of the Committee for Control and Supervision of Experiments on Animals (Registration no. 173/CPCSEA, dated 28 January, 2000), Government of India, on the use of animals for scientific research.

2.3 Test organism and Culture Media

Based on the fact that *E. coli* is widely involved in non-specific urinogenital tract infection and is also most resistant to common antibiotics [18], an isolate of the enteropathogenic bacterium *E. coli* (*Enterobacteriaceae*) was used in this work. It was obtained from the Medical Microbiology Laboratory of Pasteur Centre, Yaoundé, Cameroon. The media used in this work were salt free Mac Conkey agar, tryptose phosphate broth (DIFCO Laboratories Ltd, United Kingdom) and Mueller Hinton agar (International Diagnostics Groups PLC, Topley House, 52 Wash Lane, Bury, Lancashire BL96 AU, UK).

2.4 Preparation of Aqueous Extract

P. limbatum leaves were air-dried at room temperature and reduced to coarse powder. 100 g of the powder was soaked in 1 L of distilled water for 3 days, stirring 2 times a day. The filtrate obtained was evaporated under reduced pressure to yield a dark green aqueous extract (15.5 g), which was kept in the freezer at -20°C until required.

2.5 Phytochemical Screening

The phytochemical screening was performed using standard methods described by Harbone [23]. The aqueous leaf extract of *Polygonum limbatum* was screened for the following classes of phytochemicals: alkaloids, flavonoids, triterpenes, steroids, polyphenols, saponins, tannins, anthocyanins and anthraquinones.

2.6 *In vivo* Antimicrobial Activity

Thirty adult female Guinea pigs were divided into six groups of five animals each (groups 1, 2, 3, 4, 5 and 6). At day 3, subsequent to immunodepression, the animals of the groups 2 to 6 were infected each by intravaginal administration of 263 µL of *E. coli* inoculum (corresponding to $7x10^8$ CFU). On day 3 following infection, samples were taken and the bacterial load was assessed. The animals of the group 1 served as neutral control (non-infected and untreated) whereas the ones of the group 2 served as negative control (infected and untreated). The animals of the group 3 were treated with oxytetracycline (40 mg/kg body weight) and served as positive control, while those of the groups 4, 5, 6 were treated with the plant extract at 200 mg/kg (the dose used by the traditional healer), 500 mg/kg body weight (i.e. 2.5 times the traditional healer's dose) and 700 mg/kg body weight (i.e. 3.5 times the traditional healer's dose) respectively. The various doses of extract and standard drug were administered orally to the corresponding animals and twice daily (morning and evening). The treatment lasted 15 days, and animals were given food and tap water *ad libitum*.

During the treatment period, vaginal samples were collected every three days [Day 1 (before treatment), day 4, day 7, day 10, day 13] to assess the level of the infection.

2.7 Vaginal Samples Collection and Processing

For each sample collection, sterile cotton swab was introduced aseptically deep into the animals vagina to have the samples which were then placed in a sterile bottle containing 2 mL sterile tryptose phosphate. The bottle was sealed and placed in an incubator at 37℃ for 18 h, after which, a volume of 50 µL was taken from the bottle (after homogenization), diluted with sterile distilled water and cultured on salt free Mac Conkey agar (Sigma-Aldrich, France). After 24 h of incubation, pink to red colonies of identifiable *E. coli* were counted.

2.8 Urine, Blood and Organs Collection

Following vaginal samples collection, animals were subjected to 12 h fast during which their urines were collected [24], then they were anaesthetized by chloroform vapor inhalation, and dissected. The blood samples were collected by cardiac puncture and introduced into two sets of sterile plastic tubes with one containing EDTA and the other nothing. The blood collected without EDTA was allowed to stand for complete clotting and centrifuged at 3000 rpm for 15 min, and serum obtained frozen.

Organs such as heart, liver, spleen, kidneys, ovaries, uterus and the lungs were collected, weighed and their protein levels determined.

2.9 Tissue Homogenates Preparation

Homogenates of each organ was prepared at 15% (15 g of organ in 100 mL NaCl 0.9% solution) [25]. The preparation was centrifuged at 3000 rpm for 15 min and the supernatant collected and stored until required.

2.10 Hematological and Biochemical Analyses

Hematocrit measurement was done on the EDTA-collected blood. Capillary tubes were filled with blood, sealed at each end and centrifuged at 12000 rpm for 5 min on a micro-centrifuge. The percentage of red blood cells was determined using the hematimetric scale.

A Thomas chamber was used to quantify the total red blood cells (RBCs) and white blood cells (WBCs) counts on the EDTA-collected blood. The blood was pipetted up to the graduation 0.5 of Thomas pipette and completed to the graduation 10 with Marcano liquid (1:200 dilution). After homogenization, a drop of the mixture was loaded in Thomas chamber and the cells were counted under the microscope [26].

For white blood cells counting, the blood was pipetted up to the graduation 1 of Potain pipette and completed to the graduation 1 L with Lazarus liquid (1:20 dilution). After homogenization, a drop of the mixture was loaded in Thomas chamber and the cells were counted under the microscope [26].

Serum biochemical parameters such as transaminases (ALT, AST) and creatinine were estimated using commercial kits (DIALAB, Austria). Liver, spleen, heart, lungs and serum proteins were determined by the Biuret method as described by Gornall et al. [27]. The ovarian, uterine, renal and urinary proteins were determined by the method of Bradford [28].

2.11 Statistical Analysis

Statistical analyses were performed with the aid of SPSS for Windows software program. Data were expressed as mean ± standard deviation. Group comparisons were done using the Waller-Dunkan test. A p value of less than 0.05 was considered statistically significant.

3. RESULTS

3.1 Course of *Escherichia coli* Infection

The variation of the bacterial load shown by different groups of animals during the test is recorded in Table 1. On the first day, no significant difference ($p>0.05$) was noticed between the number of bacteria counted in all infected groups. However, the number of bacteria between the infected and untreated, and non-infected and untreated groups was significantly different ($p<0.05$). In general, a decrease in the number of bacteria in the test groups against an increase in the group infected and untreated was observed. The groups treated with the extract showed a reduced and dose-dependent bacterial load. On the 13^{th} day of the treatment, the doses 700, 500 and 200 mg/kg induced 98.45%, 93.02% and 67.05% decrease in the vaginal bacterial load, respectively. Oxytetracycline, an antibiotic commonly used to cure animals' bacterial infections, reduced the bacterial load to zero after 10 days, thus confirming its frequent use in veterinary medicine.

3.2 Effect of *P. limbatum* Extract and Oxytetracycline on Weight Gain

Results of body weight variation of the experimental animals are presented in Fig. 1. The group receiving the dose of 200 mg/kg showed increased weight gain up to the 5th day, whereas all the other experimental animals gained weight only during the first 4 days of treatment. This was followed by a decrease in weight gain up to the 15th day. However, the group receiving the dose of 700 mg/kg showed a slight increase in weight gain from the 8th day up to the 15th day. The group receiving oxytetracycline showed a progressive weight loss from day 1 to day 15.

3.3 Effect of *P. limbatum* Extract on Relative Organs' Weight

It can be seen from Table 2 that the relative weight of the ovaries remained unchanged ($p>0.05$) in all the experimental groups compared to the neutral and negative controls. However, the lungs and uterus showed a significant ($p<0.05$) decrease of their relative weight in all test groups, compared to the neutral control. In addition, the groups treated with extract at doses of 700 mg/kg and 500 mg/kg showed a significant ($p<0.05$) decrease in relative weight of all organs, except the liver and spleen, compared to the neutral control. The same is true for the infected and untreated group and the group treated with oxytetracycline for the kidneys weight. All test groups showed no significant ($p>0.05$) variation of spleen relative weight compared to the neutral control, except for the infected and untreated group.

Generally, all the doses of extract (200, 500 and 700 mg/kg) significantly ($p<0.05$) reduced the relative weight of the heart, spleen and uterus, compared to the negative control (the infected and untreated group). The relative weight of the liver was significantly ($p<0.05$) reduced only at the dose of 700 mg/kg, whereas the relative weight of the kidneys was significantly ($p<0.05$) increased at all the doses of the extract, compared to the negative control. Oxytetracycline significantly ($p<0.05$) increased the relative weight of the lungs, liver and kidneys, whereas it significantly ($p<0.05$) decreased the relative weight of the spleen, compared to the negative control.

3.4 Effect of *P. limbatum* Extract on Hematological Parameters

As shown in the Table 3, all extract-treated groups showed no significant ($p>0.05$) difference in hematocrit compared to the neutral and negative controls. However, the group treated with oxytetracycline showed a significant ($p<0.05$) decrease in hematocrit, compared to the neutral and negative controls. All test groups showed a significantly ($p<0.05$) increase in the number of white blood cells, compared to the neutral control group and the infected and untreated group (negative control). However, a dose-dependent increase in white blood cells in the treated groups at different doses of the extract was noted. Furthermore, only the infected and untreated group showed a significant decrease in red blood cells compared to the neutral control. The group treated with oxytetracycline showed no significant ($p>0.05$) change in the red blood cells compared to the neutral control. However, the level of white blood cells and red blood cells significantly ($p<0.05$) increased at all doses of the extract and oxytetracycline, compared to the negative control.

Table 1. Evolution of the number of bacteria obtained after 18 hours of enrichment during treatment

Group and dose	Number of bacteria obtained after 18 hours of enrichment x 10^5/day				
	Day 1	Day 4	Day 7	Day 10	Day 13
Extract: 700 mg/kg	3.98 ± 0.26^{b}	1.50 ± 0.25^{b}	0.65 ± 0.23^{a}	0.93 ± 0.13^{ab}	0.08 ± 0.05^{a}
Extract: 500 mg/kg	3.94 ± 0.41^{b}	2.16 ± 0.48^{b}	1.90 ± 0.44^{b}	1.62 ± 0.36^{b}	0.36 ± 0.12^{a}
Extract (Traditional healer's dose): 200 mg /kg	3.58 ± 0.34^{b}	2.08 ± 0.47^{b}	1.70 ± 0.46^{b}	1.70 ± 0.66^{b}	1.70 ± 0.66^{a}
Oxytetracycline: 40 mg/kg	3.97 ± 0.40^{b}	0.26 ± 0.90^{a}	0.03 ± 0.01^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Infected and untreated	3.75 ± 0.29^{b}	4.65 ± 0.42^{c}	4.25 ± 1.04^{d}	5.17 ± 0.83^{c}	5.16 ± 0.25^{b}
Non-infected and untreated	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Values in the table are Mean±SD of 5 measures

a, b, c, d: Values with the same superscripts in the same column are not significantly different (p> 0.05)

3.5 Effect of *P. limbatum* Extract on Tissue Proteins

Results of the tissue protein levels of the different organs (Table 4) showed a significant and dose-dependent reduction of heart protein levels in extract-treated groups compared to the neutral control (non-infected and untreated). Also, compared to the neutral control group, lung protein level decreases when the dose increases. The groups treated with oxytetracycline, and with the extract at the dose 700 mg/kg had liver protein levels significantly ($p<0.05$) increased compared to the neutral control. As regarding spleen proteins, infected and untreated group, as all groups treated with the extract, showed a significant ($p<0.05$) increase compared to the neutral control. For the kidneys, all test groups showed a significant decrease in the protein level compared to the neutral control, except the group treated with the dose 200 mg/kg. The same is true for the group treated with oxytetracycline concerning the uterine proteins. For the ovary, the infected and untreated group as well as the one treated with oxytetracycline showed significant ($p<0.05$) decrease in protein levels compared to the neutral control.

Generally, the extract at all doses significantly ($p<0.05$) decreased the protein levels of the heart, lungs, spleen and kidneys, compared to the negative control (the infected and untreated group). The protein level of the uterus was rather significantly ($p<0.05$) increased at all doses of the extract, compared to the negative control. Oxytetracycline rather significantly increased the protein levels of the heart, lungs and uterus, and significantly ($p<0.05$) decreased the protein levels of the spleen and the kidneys, compared to the negative control.

3.6 Effect of *P. limbatum* Extract on Serum and Urinary Proteins, Creatinine and Serum Transaminases

The variation of the serum and urinary proteins and creatinine, and serum transaminases shown by different groups of animals during the experiment is recorded in Table 5. The groups treated with extracts showed a significant ($p<0.05$) dose-dependent increase of serum protein levels against a significant ($p<0.05$) dose-dependent decrease in urinary proteins compared to the neutral control. Infected and untreated group also showed a significant ($p<0.05$) change in its serum and urinary proteins, which followed the pattern of the extract-treated groups, compared to the neutral control.

As for creatinine, there was a simultaneous and significant ($p<0.05$) dose-dependent decrease in serum and urinary creatinine in all the extract-treated groups compared to the neutral control group. The same observation was made for serum and urinary levels of creatinine in oxytetracycline-treated group. Also, serum and urinary creatinine levels significantly ($p<0.05$) decreased in the infected and untreated group (negative control) compared to the non-infected and untreated (neutral control). Regarding the ALT, a decrease was noted in all test groups except the groups treated with 200 mg/kg and the oxytetracycline which had a rather significant ($p<0.05$) increase compared to the neutral control group. Concerning the AST, the decrease was significant ($p<0.05$) and dose-dependent in all groups treated with extract. A significant ($p<0.05$) decrease in AST was also observed with oxytetracycline.

The extract at 700 mg/kg significantly ($p<0.05$) decreased the protein level of the serum and significantly increased that of the urines, compared to the negative control. Also, the serum creatinine level significantly ($p<0.05$) decreased, whereas that of the urines significantly increased at the same dose of extract, compared to the negative control. The ALT activity was significantly ($p<0.05$) increased, whereas that of the AST was significantly ($p<0.05$) decreased at all doses of the extract, compared to the negative control. The levels of transaminases followed the same trends for oxytetracycline, compared to the negative control (the infected and untreated group).

3.7 Phytochemical Composition

Table 6 shows the phytochemical composition of the aqueous leaf extract of *P. limbatum.* The phytochemical screening revealed the presence of different groups of secondary metabolites, including flavonoids, alkaloids, triterpenes, polyphenols, tannins and saponins, while anthraquinones, anthocyanines and steroids were absent in the extract.

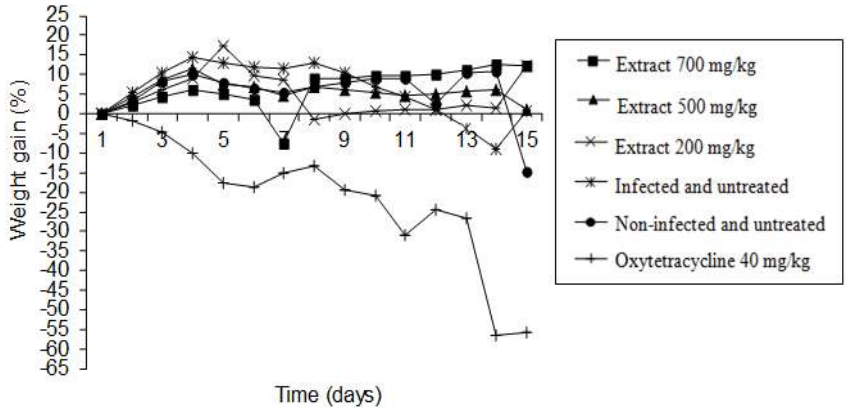

Fig. 1. Guinea pigs body weight variation as a function of dose and time

Table 2. Guinea pigs relative organ weight after 15 days of treatment

Group and dose	Relative organ weight (mg /100 g)						
	Heart	**Lungs**	**Liver**	**Spleen**	**Kidneys**	**Uterus**	**Ovaries**
Extract: 700 mg/kg	0.19± 0.01^{ab}	1.95 ± 0.13^{a}	0.49 ± 0.03 ^{b}	0.48 ± 0.03 ^{b}	0.09 ± 0.01^{a}	0.08 ± 0.02^{ab}	0.02 ± 0.01
Extract: 500 mg/kg	0.18 ±0.03^{ab}	1.81 ± 0.26^{a}	0.51± 0.07	0.42± 0.09 ^{b}	0.11 ± 0.02^{ab}	0.07 ± 0.01^{ab}	0.02 ± 0.01
Extract (Traditional healer's dose): 200 mg/kg	0.22± 0.02	2.13 ± 0.16^{a}	0.62 ± 0.04	0.44 ± 0.09 ^{b}	0.14 ± 0.04 ^{b}	0.07 ± 0.01^{ab}	0.03 ± 0.01
Oxytetracycline: 40 mg/kg	0.28±0.01^{a}	3.97±0.04^{ab}	0.96 ± 0.03^{ab}	0.48 ± 0.03 ^{b}	0.20 ± 0.01^{ab}	0.16 ± 0.02	0.03± 0.01
Infected and Untreated	0.28±0.05	1.88±0.17^{a}	0.67±0.10^{a}	3.47±1.17^{a}	0.07±0.01^{a}	0.14±0.02^{a}	0.03± 0.01
Non-infected and Untreated	0.25 ± 0.01	2.36 ± 0.12	0.58 ± 0.20	0.59 ± 0.3	0.16 ± 0.10	0.23 ± 0.10	0.04 ± 0.02

Values in the table are Mean±SD of 5 measures
Values with (a) in the same column are significantly different (p< 0.05) from the neutral control which is the non-infected and untreated group
Values with (b) in the same column are significantly different (p< 0.05) from the negative control which is the infected and untreated group

Table 3. Effect of *P. limbatum* extract on some hematological parameters

Group and Dose	Hematocrit (%)	White blood cells x 10^3/ mm^3	Red blood cells x 10^6/ mm^3
Extract: 700 mg/kg	46.50 ± 3.35	19.30 ± 4.17[ab]	5.16 ± 0.83 [b]
Extract: 500 mg/kg	43.70 ± 1.78	8.76 ± 1.86 [ab]	4.48 ± 0.44 [b]
Extract (Traditional healer's dose): 200 mg/kg	42.80 ± 0.73	7.08 ± 0.39 [ab]	4.74 ± 0.54 [b]
Oxytetracycline: 40 mg/kg	32.00 ± 0.75[ab]	8.00 ± 0.30 [ab]	4.78 ± 0.50 [b]
Infected and untreated	44.00 ± 1.79	5.40 ± 0.40	3.63 ± 0.10[a]
Non-infected and untreated	48.00 ± 1.23	5.52 ± 1.18	5.84 ± 1.22

Values in the table are Mean±SD of 5 measures
Values with (a) in the same column are significantly different (p< 0.05) from the neutral control which is the non-infected and untreated group
Values with (b) in the same column are significantly different (p< 0.05) from the negative control which is the infected and untreated group

Table 4. Effect of *P. limbatum* extract on tissue protein levels

Group and dose	Tissue protein levels (mg/g)						
	Heart	Lungs	Liver	Spleen	Kidneys	Uterus	Ovaries
Extract: 700 mg/kg	16.22±2.58 [ab]	20.74±5.67 [ab]	32.59±2.96[a]	5.14 ±0.86[a]	17.77±2.65[ab]	7.88 ± 0.34 [b]	7.28 ± 0.71
Extract: 500 mg/kg	17.00±1.87 [ab]	31.99±5.47 [ab]	25.45 ± 6.31	4.80±0.10 [ab]	22.22±0.34[ab]	7.43± 0.84 [b]	7.92 ± 0.52
Extract (Traditional healer's dose): 200 mg/kg	18.90 ±2.21 [ab]	34.36±2.90 [ab]	22.51± 3.45	4.73±0.19 [ab]	31.99±1.45[a]	6.32 ± 0.28 [b]	7.08 ± 1.39
Oxytetracy- cline: 40 mg/kg	23.63± 0.50 [b]	59.26±0.15 [ab]	29.63±0.78[a]	2.81± 0.69 [b]	29.63±1.72 [b]	6.14 ±0.08 [ab]	6.47 ± 0.10[a]
Infected and untreated	21.70 ±0.05	49.36 ± 0.17	27.65 ± 7.12	5.87 ± 0.20[a]	35.55±3.42[a]	0.89 ± 0.25[a]	6.42 ± 2.57[a]
Non-infected and untreated	22.52± 3.45	46.22± 5.75	26.06 ± 1.46	3.66 ± 0.65	29.62± 3.25	7.04 ± 0.06	8. 29 ± 0.25

Values in the table are Mean±SD of 5 measures
Values with (a) in the same column are significantly different (p< 0.05) from the neutral control which is the non-infected and untreated group
Values with (b) in the same column are significantly different (p< 0.05) from the negative control which is the infected and untreated group

Table 5. Effect of *P. limbatum* extract on serum and urinary creatinine and proteins, and serum transaminases

Group and dose	Protein levels		Creatinine (mg/dl)		Transaminases (IU/L)	
	Serum (mg/ml)	Urines (µg/ml)	Serum	Urines	ALT	AST
Extract: 700 mg/kg	0.80±0.05 [ab]	35.25±5.20 [ab]	1.06±0.11 [ab]	28.96±8.15 [ab]	85.74±7.56 [b]	78.08±7.83 [ab]
Extract: 500 mg/kg	1.58±0.31 [ab]	16.00 ±4.25[a]	2.20±0.48 [ab]	4.80±2.26 [ab]	89.66 ± 3.81[b]	98.30±13.42 [ab]
Extract (Traditional healer's dose): 200 mg/kg	1.67±0.60[a]	13.20±6.30[a]	5.90±2.51 [ab]	3.20±0.72 [ab]	120.00±10.54 [ab]	200.85±17.63[a]
Oxytetracycline: 40 mg/kg	1.71±0.06 [ab]	22.00±1.15	0.08±0.01 [ab]	2.40±0.03 [ab]	146.75±6.40 [ab]	71.60±2.24 [ab]
Infected and untreated	1.15±0.07[a]	17.33±3.96[a]	1.60 ±0.02[a]	14.40±0.50[a]	70.72±2.58[a]	209.51±8.11[a]
Non-infected and untreated	1.28±0.22	25.40±1.86	17.24 ±7.70	35.93 ±2.67	91.93±4.13	378.53±5.48

Values in the table are Mean±SD of 5 measures
Values with (a) in the same column are significantly different (p< 0.05) from the neutral control which is the non-infected and untreated group
Values with (b) in the same column are significantly different (p< 0.05) from the negative control which is the infected and untreated group

4. DISCUSSION

In vivo studies revealed a significant dose-dependent decrease of bacterial load in the animals treated with different doses of *P. limbatum* extract. Moreover, the results of the present work showed that the active ingredient of the extract was not inactivated during the absorption process.

As far as the toxicity of the *P. limbatum* extract is concerned, no critical sign of side effect was observed on the treated animals at low/therapeutic doses as shown by the hematological and biochemical parameters.

In particular, creatinine, which is the catabolic product of creatine, a molecule of major importance for energy production in muscles and which is synthesized in the kidney and liver [29], has been used as an indicator of kidney damage. In most cases, the increase in serum appears only in cases of severe kidney dysfunction, but can also occur in case of a significant increase of muscles mass or intense physical activity [30]. However, it has been shown that the increase in serum creatinine can be an indicator of kidney injury only if it is coupled with a significant decrease in urinary creatinine [31]. The lower rate of urinary creatinine observed in animals treated with *P. limbatum* extract suggests at the first sight a renal dysfunction. However, the fact that it is coupled with a decrease in serum creatinine leads us to suggest that the extract acted at the level of creatinine production, by decreasing the muscular activity in experimental animals or their muscle mass. Besides, serum and urinary creatinine levels significantly decreased in the infected and untreated group (negative control) compared to the non-infected and untreated (neutral control), suggesting that the infection also influenced the creatinine production in the muscle. However, at the dose of 700 mg/kg, the serum creatinine level significantly decreased, whereas that of the urines significantly increased at the same dose of extract, compared to the negative control, suggesting a normal functioning of the kidneys.

In terms of relative organ weight, an atrophy of the heart, kidneys, lungs and uterus was observed in all groups treated with the extract, associated to a decrease of total proteins rate in these organs. In general, a decrease of total tissue protein levels is a sign of damage to these tissues [32]. Organs atrophy observed in this work could be indicative of after-effects following the infection, as the same observation was made for the infected and untreated group. In addition, the decrease of tissue protein rates coupled with enhancement of serum protein levels suggests and supports the cytotoxic action of the extract on those tissues that could be exerted by altering the permeability of the cell membrane or by inducing cell necrosis in the organs. On the other hand, the absence of effect observed on the spleen and ovary lets us think that the extract has no effect on these organs. However, the increase in liver protein level at the highest dose of the extract (700 mg/kg) could be a means of facing the toxicity of the extract, since the liver is a detoxification organ. It is worth noting that at the dose of 500 mg/kg, there was a non-significant decrease in the liver protein level, compared to neutral control (non-infected and untreated).

Regarding urinary proteins, there is a significant decrease of proteinuria at doses ≤ 500 mg/kg and increase at doses > 500 mg/kg. These results suggest that the extract may have a protective effect on the kidneys [33] at doses ≤ 500 mg/kg. However, more work should be done to ascertain this possible nephroprotective effect. The increase of proteinuria at high doses could be a sign of kidney injury.

Anemia is the relative reduction in red blood cells that could lead to the decreased ability to carry oxygen [34]. Anemia is usually measured by three factors: hematocrit, hemoglobin and red blood cell count [23,24]. In our case, the hematocrit values of the treated groups varied between 32 and 38%, which were still within the normal range. However, the dose-dependent increase in the number of white blood cells could be explained by the fact that the extract would have stimulated the immune system to produce more white blood cells to fight the pathogen. This suggests that the extract may have a stimulating effect on leucopoiesis [29].

Table 6. Phytochemical composition of *Polygonum limbatum* aqueous leaf extract

Class of compounds	Flav	Alka	Trit	Polyph	Anthra	Antho	Ster	Tann	Sapo
Occurrence	+	+	+	+	-	-	-	+	+

Flav: flavonoids; Alka: Alkaloids; Trit: Triterpenes; Polyph: Polyphenols; Anthra: Anthraquinones; Antho: Anthocyanines; Ster: Steroids; Tann: Tannins; Sapo: Saponins; + :Present; - :Absent

Transaminases (ALT, AST) have been linked to hepatocellular injury [35,36]. Determination of ALT activity is relatively a sensitive indicator of hepatic damage and release of ALT from the cytosol can occur, secondary to cellular necrosis or as a result of cellular injury with membrane damage [36,37]. The ALT activity is localized in the liver, and is specific to parenchymal disease [25,38], whereas the activity of AST is present in several tissues such as the heart, brain, kidney and liver [24]. At the dose of 500 mg/kg, there was a significant decrease in AST activity and a non-significant decrease in ALT activity, compared to the neutral control. These trends of decreases in AST and ALT were also observed with the highest dose of the extract (700 mg/kg). The decrease in serum transaminases (ALT and AST) coupled with the decrease in the liver protein level at 500 mg/kg suggests that the extract may rather have a protective effect on the liver at this dose. *P. limbatum* aqueous leaf extract was found in this study to contain flavonoids and tannins, which are among the polyphenols. This could justify the possible hepatoprotective effect suggested in this study. In fact, polyphenols have been found to have strengthening and protective activities on liver cells [39,40]. However, more work should be done to ascertain this possible hepatoprotective effect.

5. CONCLUSION

The body of data generated in these *in vivo* studies would therefore indicate the efficacy of the extract of *P. limbatum* against urogenital tract infections caused by *Escherichia coli.* Interestingly, on the basis of the results in the present work, there was no critical sign of toxicity observed on the treated animals at low/therapeutic doses.

ETHIC APPROVAL

This research proposal was approved be the Scientific Postgraduate School board of the University of Dschang, which is up to now consider as our institutional ethic committee for animals uses in scientific research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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