

***In vivo* Evaluation of Antidiarrhoeal Activity of Methanol Extract, Fractions and Isolated Compounds from *E. abyssinica* Stem Bark**

¹Gerald N. Teke, ¹Paul K. Lunga, ²Hippolyte K. Wabo, ¹Jules-Roger Kuiate and ¹Jean P. Dzoyem

¹Laboratory of Microbiology and Antimicrobial Substances, Faculty of Science,
University of Dschang, Dschang, Cameroon

²Department of Chemistry, Faculty of Science, University of Dschang, Dschang, Cameroon

Abstract: The stem bark of *E. abyssinica* is used in traditional medicine for the treatment of diarrhoea in west region of Cameroon. To substantiate this folkloric claim, the methanol extract, fractions and isolated compounds from *E. abyssinica* stem bark were investigated for their antidiarrhoeal activities in Wistar albino rats. The methanol extract of *E. abyssinica* stem bark was pre-dissolved in a mixture of methanol and water and partitioned into n-hexane, ethyl acetate and n-butanol fractions. The ethyl acetate portion was fractionated by column chromatography and the structures of isolated compounds elucidated by spectroscopic analyses in conjunction with literature data. Antidiarrhoeal activity was investigated in terms of purging index, consistency of faeces and intestinal transit in castor oil induced diarrhoea, and faecal *Shigella* load, faecal frequency, variation in bodyweight and blood chemistry in *Shigella*-induced diarrhoea model in Wistar rats. Two known compounds [(5S,6R,8aR)-5-(carboxymethyl)-3,4,4a,5,6,7,8,8a-octahydro-5,6,8a-trimethylnaphthalenecarboxylic acid (1), methyl 3,4,5-trihydroxybenzoate (2) were isolated. The methanol extract, fractions and isolated compounds showed antidiarrhoeal activities evidenced by the reduction in % respondent to diarrhoea, a delay in the period for diarrhoea induction, reduction in purging index and wateriness of faeces. Compounds 1 and 2 inhibited diarrhoeal droppings by 81.01 and 87.34%, respectively. Compared to the methanol extract, fractionation increased the shigellocidal activity with ethyl acetate fraction most active. The experimental findings show that the methanol extract, fractions and isolated compounds from *E. abyssinica* stem bark possess significant antidiarrhoeal activities justifying the traditional use of this plant in diarrhoeal treatment and may be developed as phytomedicine.

Key words: Antidiarrhoeal activity, castor oil, shigellosis, isolated compounds, *Entada abyssinica*

INTRODUCTION

Diarrhoea is characterized by increased frequency of bowel movement, wet stool and abdominal pain (Ezekwesili *et al.*, 2004). Diarrhoea, especially of infectious origin, is one of the leading causes of malnutrition and death among children in developing countries nowadays (Havagiray *et al.*, 2004). Antibiotics used as antidiarrhoeal drugs sometimes provoke adverse effects and microorganisms tend to develop resistance toward them (Soberon *et al.*, 2007). Therefore, the search for safe and more effective agents from plant origin has continued to be an important area of active research.

Entada abyssinica (Mimosaceae) is an understorey forest deciduous tree, 3-15 m high, with a flat spreading crown. It is widespread in central and eastern tropical Africa (Katende, 1995). The stem bark is grey, slightly fissured and flaking off in irregular patches. The leaves

are glabrous and round at the apex. The flowers are creamy white and sweet scented (Bekele-Tesemma and Tengnas, 1993; Beentje, 1994). Some biological activities of *Entada abyssinica* have been reported in which antiviral activity (Cos *et al.*, 2002), antibacterial activity (Yineger and Yewhalaw, 2007) and antitrypanosomal activity (Nyasse *et al.*, 2004). A number of biologically active compounds of the diterpene classes (Freiburghaus *et al.*, 1998), flavonoids and phytosterol glycosides (Asfaw *et al.*, 2000) have been isolated from *E. abyssinica*. Ethnomedically, a decoction of the stem bark is taken to treat diarrhoea, rheumatic and abdominal pains and coughs (Bekele-Tesemma and Tengnas, 1993; Beentje, 1994). In a previous study we demonstrated the antimicrobial properties of *E. abyssinica* stem bark, its fractions and compounds (Teke *et al.*, 2011). Since this plant is also used in traditional medicine to treat diarrhoea, this study was therefore designed to evaluate

the antidiarrhoeal properties of the methanol extract, fractions and compounds from the stem bark of *E. abyssinica* in castor oil- and *Shigella*-induced diarrhoea models in rats.

MATERIALS AND METHODS

Drugs, chemicals and kits: The following drugs, chemicals and kits were used in the study: loperamide (Sigma Aldrich; Steinheim, Germany), diphenoxylate HCl (Sigma Aldrich), atropine sulfate (Sigma Aldrich), vegetable charcoal (Carbophos, AJC Pharma, Angoulême, France) and castor oil from a local pharmacy, chloroform (BDH Chemicals Ltd., Poole, England), creatinine kit (Jaffe-Kinetic, Germany) and triglycerides kit (IVD, SGM Italia-Roma), Tween 80 (Fisher Scientific, Loughborough, United Kingdom) and SS agar (Liofilchem).

Plant material, extraction and phytochemical screening:

The stem bark of *E. abyssinica* was collected in May 2007 in Menoua Division, West Cameroon. Botanical identification was done at the Cameroon National Herbarium where a voucher specimen was kept under the reference number 44732/HNC. The stem bark was cut into pieces, air-dried under shade and ground into powder. A mass of 375 g of powder were exhaustively extracted with 1 L of methanol. After filtration, the solvent was evaporated under reduced pressure in a rotary evaporator at 45°C to afford the methanol extract (47.50 g). The extract (32.50 g) was pre-dissolved in 100 mL of a mixture of methanol and water (1:9) and then 400 mL of n-hexane was added and shaken vigorously. After about 30 min, the n-hexane phase was collected and the process repeated thrice. Methanol was then evaporated from the polar phase and the residue treated sequentially with ethyl acetate and n-butanol. The n-hexane, ethyl acetate and n-butanol were evaporated under reduced pressure in rotary evaporator to afford 4.64, 15.78 and 2.63 g of fractions respectively. The aqueous residue (9.44 g) was obtained after drying the residual portion in the oven at 40°C for 48 h.

Fractionation and isolation of compounds from ethyl acetate fraction:

A quantity of 10.5 g of the ethyl acetate fraction was subjected to silica gel 60 (0.20-0.500 mm) flash chromatography and eluted with mixtures of n-hexane (Hex) and ethyl acetate (EtOAc) of increasing polarity (0-100%) to yield a total of 9 fractions of 200 mL each. These fractions were combined on the basis of TLC profiles into four major fractions: F1 [4.77 g, Hex/EtOAc (100:0), (80:20), (70:30)], F2 [2.48 g, Hex/EtOAc (60:40), (50:50)], F3 [1.56 g, Hex/EtOAc (40:60)] and F4 [0.63 g,

Hex/EtOAc (30:70), (20:80), (0:100)]. The antidiarrhoeal activity tested by the procedure described in Section 2.6 was present only in fraction F1. This fraction was further dissolved in a mixture of Hex and EtOAc (60:40) and a whitish compound precipitated. It was filtered and rinsed with EtOAc to afford compound 1 (100.36 mg). The filtrate (4.5 g) was subjected to further silica gel column chromatography (0.063-0.200) and eluted with Hex-EtOAc (25:75). A total of 60 column fractions (10 mL each) were collected and grouped into four sub fractions, denoted F1.1 (1-8), F1.2 (9-22), F1.3 (23-34) and F1.4 (35-60), based on their thin-layer chromatograms. Fractions F1.1 and F1.2 were of negligible quantities (2.00 mg, too small for the performance of biological assays). Fraction F1.3 (1.2 g) did not exert antidiarrhoeal activity but Fraction F1.4 (1.6 g) with antidiarrhoeal activity was further suspended in Hex-EtOAc (30:70) and a substance precipitated which was rinsed with the same system to afford compound 2 (113 mg).

Chemical analysis: Aluminium sheet pre-coated with silica gel 60 GF254 (Merck) was used for thin layer chromatography (TLC). The spots were visualized under UV light (254 and 366 nm) with a UV lamp model 52-58 mineralight and sprayed with 50% aqueous solution of H₂SO₄ followed by heating at 100°C.

IR spectra were measured with KBr disks using FT-IR-8400 S Shimadzu spectrophotometer. EI-MS were carried out on a GCT Premier CAB109 TOF mass spectrometer. 1H-, 13C-NMR and 2D-NMR (COSY 1H-1H, HMBC and HSQC) spectra were recorded in acetone-d₆ (500 MHz for 1H and 125 MHz for 13C) on a Bruker-Avance-500 MHz NMR spectrometer.

(5S,6R,8aR)-5-(carboxymethyl)-3,4,4a,5,6,7,8,8a-octahydro-5,6,8a-trimethylnaphthalenecarboxylic acid (1): White powder; 13C NMR (100 MHz, CDCl₃+CD₃OD): δ 14.9 (5-Me), 17.8 (C-4), 20.7 (6-Me), 21.0 (8a-Me), 25.3 (C-7), 27.0 (C-3), 29.4 (C-8), 35.2 (C-6), 37.5 (C-8a), 38.5 (C-5), 43.6 (C-1'), 44.6 (C-4a), 136.9 (C-2), 142.6 (C-1), 169.3 (1-COOH), 175.4 (C-2'); FABMS: m/z 279 ([M-H]⁺, 98), 153 (100), 151 (44), 46 (13); HRFABMS: m/z 279.1592 (calcd. for C₁₆H₂₃O₄: 279.1596).

Methyl 3,4,5-trihydroxybenzoate (2): Colourless needles; 13C NMR (100 MHz, CDCl₃): δ 51.7 (OCH₃), 109.8 (C-2/C-6), 125.2 (C-1), 148.4 (C-3/C-5), 138.9 (C-4), EIMS: m/z 184 ([M]⁺, 99), 153 (100), 125 (14), 107 (2), 79 (3); HREIMS: m/z 184.0370 (calcd. for C₈H₈O₅: 184.0372).

Animals: Wistar albino rats (170-180 g), both males and females were used for the antidiarrhoeal test and Swiss mice (20-26 g) for acute toxicity. They were housed in standard plastic cages and provided with food and water

ad libitum. The studies were conducted according to the ethical guidelines of 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.

Castor oil-induced diarrhoea in rats: The animals were starved for 18 h prior to experiments and randomly distributed into 9 groups of 10 (5 males and 5 females) per study dose of the test substances. Rats of the first three groups were administered the crude methanol extract orally (100, 200 and 400 mg kg⁻¹, respectively). Groups 4, 5, 6 and 7 received n-hexane, ethyl acetate, n-butanol and aqueous residue fractions, respectively at 80 mg kg⁻¹ body-weight. Groups 8 and 9 received loperamide and diphenoxylate HCl, respectively (reference drugs) at 2.5 mg kg⁻¹ body weight. Groups 10, 11 and 12 animals were given fraction F1, compounds 1 and 2 at 2.5 mg kg⁻¹, respectively. Group 13 received 1 mL 100⁻¹ g⁻¹ body weight of 5% ethanol/tween 80 in normal saline (negative control). Sixty minutes after drug treatment, each animal was administered castor oil orally (1 mL 100⁻¹ g⁻¹ body weight). The latent period (the time between castor oil administration and appearance of first diarrhoeic drop) was recorded. Observation for defecation continued up to 6 h on filter paper placed beneath the individual perforated rat cages. This paper was replaced every hour. The used filter paper was weighed (M₁). Finally, the filter paper was exposed in the laboratory to dry over a period of 14 h and it was reweighed (M₂). The faecal water content was calculated as (M₂-M₁) g. The percentage of rats that responded to diarrhoea, the latent period, the mean stool frequency, frequency of diarrhoeic droppings and fecal water content were recorded (Murugesan *et al.*, 2000). The purging indices (Mohd *et al.*, 2004), the percentage inhibition of diarrhoeic droppings (Ezekwesili *et al.*, 2004) were evaluated.

$$\text{Purging index} = \frac{\% \text{ respondents} \times \text{average No. of stool}}{\text{Average latent period}}$$

$$\% \text{ Inhibition of diarrhoeic droppings} = \frac{M_o - M_e}{M_o} \times 100$$

where,

M_o: Mean number of droppings caused by castor oil

M_e: Mean number of droppings caused by drug or extract

Effects of plant extracts, fractions and isolated compounds on gastrointestinal motility: Based on a previous method (Akah *et al.*, 1998), another set of rats were fasted 18 h but had free access to water. The animals

of the first three test groups (n = 8) received the methanol extract orally at the doses 100, 200 and 400 mg kg⁻¹ body weight, respectively. Groups 4, 5, 6 and 7 received 80 mg kg⁻¹ of the n-hexane, ethyl acetate, n-butanol and aqueous residue fractions, respectively. Group 8 rats received atropine sulphate (batch EP304X) at 2.5 mg kg⁻¹ body weight via intra peritoneal route as the reference drug while groups 9 and 10 animals were given compounds 1 and 2 (2.5 mg kg⁻¹). The eleventh group (negative control) received 5% ethanol/tween 80 in normal saline (1 mL 100⁻¹ g⁻¹ b.wt.). After 50 min, all the animals were given 1 mL of vegetable charcoal meal (batch AJ0148) as a food tracer prepared at 10% in normal saline (0.9% sodium chloride). After an observation period of 40 min, each rat was sacrificed and dissected. The small intestine was removed and its total length measured (cm). The movement of charcoal from the pylorus was equally measured (cm). The intestinal charcoal transit was expressed as a percentage of the distance moved by charcoal to the total length between the pylorus and the caecum.

Shigella-induced diarrhoea: The curative effects of plant extracts were evaluated on diarrhoea induced by *Shigella flexneri* as described previously (Kamgang *et al.*, 2005). The inoculum was prepared at 9×10⁸ CFU mL⁻¹ (McFarland 3 standard). Diarrhoea provoked by *S. flexneri* in rats was further confirmed by the presence of blood in faeces and White Blood Cells (WBCs) in fresh stool preparations (Rubhana *et al.*, 2006). The rats were randomly assigned to study groups of 5 males and 5 females each. Each animal was placed in a cage whose bottom was lined with a gauge that allowed faeces to pass through in order to minimise re-infection from faecal matter. The methanol extract and fractions were suspended in 5% tween 80 in 0.9% sodium chloride and orally administered to the rats at 100 and 80 mg kg⁻¹, respectively. Ciprofloxacin (2.5 mg kg⁻¹) was used to compare plant extract activity alongside a negative control receiving only the vehicle. The evolution of treatment was assessed by examining faecal *Shigella* load of stool cultures on *Salmonella-Shigella* agar. Stool was collected 3 consecutive days before the induction of diarrhoea and 5 consecutive days after the induction of diarrhoea. For this purpose, 0.5 g of faeces was suspended in 4.5 mL sterile saline solution followed by serial dilutions. Then 200 µL of each dilution was cultured to count the number of *Shigella* colonies. At the end of the assay, all the animals were sacrificed and their blood, heart and liver collected for the determination of total proteins (Gornal *et al.*, 1949) and blood cells. Sera triglycerides and creatinine levels were assayed using standard kits.

Acute oral toxicity evaluation: Eight-week-old Swiss mice (20-26 g), breed in the Animal House of the Department of Biochemistry (Faculty of Science, University of Dschang), were randomly selected and housed in standard plastic cages. They were starved for 18 h, but had access to tap water ad libitum prior to extract treatment. The 60 animals of the experiment were distributed into five groups of 12 animals each (six males and six females). Group 1 (saline control) did not receive plant extract while groups 2-5 animals received 4, 8, 12 and 16 g kg⁻¹ of the methanol extract, respectively. Mice were observed for mortality and behavioural changes for 48 h after treatment (Emerson *et al.*, 1993).

Statistical analysis: Data were analysed by the one-way analysis of variance (ANOVA) and means were compared using Duncan test at $p < 0.05$. The experimental results were expressed as the mean \pm standard deviation.

RESULTS

Effects of extract, fractions and compounds on faecal characteristics and in castor oil-induced diarrhoea:

Compounds 1 and 2 were identified as 1,2,3,4,4a,7,8,8a-octahydro-1,2,4a-trimethyl-1,5-naphthalenedicarboxylic acid and methyl-3,4,5-trihydroxybenzoate (methyl gallate), respectively (Fig. 1). Pre-treatment of animals with methanol extract, fractions and isolated compounds from *E. abyssinica* stem bark reduced diarrhoea induced by castor oil (Table 1). The latent period was significantly prolonged ($p < 0.05$) in the plant extract-treated rats compared to the saline control rats. At 400 mg kg⁻¹, the purging indices and the inhibition percentage of diarrhoeic droppings for the methanol extract were comparable to those of the reference drugs at 2.5 mg kg⁻¹. The ethyl acetate fraction (80 mg kg⁻¹) was the most active fraction while the aqueous residue fraction

displayed the least antidiarrhoeal activity. Compounds 1 and 2 inhibited diarrhoeal droppings by 81.01 and 87.34%, respectively, similar to diphenoxylate HCl (84.81%), but greater than loperamide (72.65%).

Effects of methanol extract, fractions and compounds on gastrointestinal motility in rats:

The methanol extract (100, 200 and 400 mg kg⁻¹), fractions (80 mg kg⁻¹) and isolated compounds (2.5 mg kg⁻¹) retarded intestinal charcoal meal propulsion (% transit) in rats compared to the saline control for an observation period of 40 min. The methanol extract at 100 mg kg⁻¹ produced the least inhibitory effect (6.92%) on intestinal transit. The inhibitory intestinal motility effects of compounds 1 and 2 (58.72 and 61.74%, respectively) were comparable to that of the standard drug, atropine sulphate (57.9%) as depicted in Table 2.

Effects of the methanol extract and fractions on faecal

Shigella load: The methanol extract and fractions from *E. abyssinica* reduced significantly rat shigellosis in the

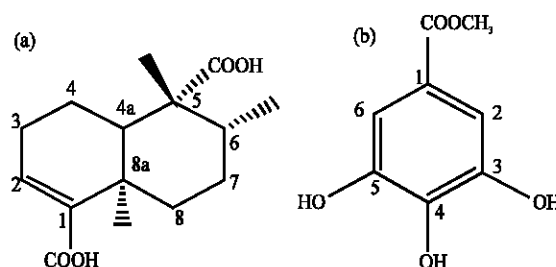


Fig. 1(a-b): Chemical structures of compounds isolated from *Entada abyssinica* ethyl acetate fraction. 1: (5S,6R,8aR)-5-(carboxymethyl)-3,4,4a,5,6,7,8,8a-octahydro-5,6,8a-trimethylnaphthalenedicarboxylic acid, 2: methyl 3,4,5-trihydroxybenzoate

Table 1: Effects of methanol extract, fractions and isolated compounds from *E. abyssinica* on faecal characteristics in rats

Faecal characteristics								
Test material	Dose (mg kg ⁻¹)	Respondent (%)	Latent period (min)	Total stool frequency	Frequency of diarrhoeic droppings	Purging index	Faecal water content (g)	Inhibition of diarrhoeic droppings (%)
Saline control	0.0	100.00	60.80 \pm 6.620	10.20 \pm 1.31	7.90 \pm 0.73*	16.67	3.22 \pm 0.55*	0.00
Methanol extract	100.0	100.00	134.30 \pm 19.36*	5.50 \pm 1.35*	2.50 \pm 0.52*	4.10	1.68 \pm 0.23*	68.35
	200.0	100.00	245.30 \pm 36.27*	5.50 \pm 1.77*	2.50 \pm 0.84*	2.24	1.17 \pm 0.06*	68.35
	400.0	50.00	314.14 \pm 26.71*	5.14 \pm 0.69*	1.85 \pm 0.69*	0.81	1.10 \pm 0.06*	76.58
n-hexane fraction	80.0	60.00	247.50 \pm 33.72*	4.50 \pm 1.22*	2.66 \pm 0.51*	1.09	1.23 \pm 0.03*	66.32
Ethyl acetate fraction	80.0	50.00	358.60 \pm 34.20*	2.80 \pm 0.83*	1.20 \pm 0.44*	0.39	0.78 \pm 0.08*	84.81
n-butanol fraction	80.0	50.00	206.80 \pm 52.91*	2.40 \pm 1.14*	1.60 \pm 0.54*	0.58	1.32 \pm 0.06*	79.74
Aqueous residue fraction	80.0	70.00	167.66 \pm 22.55*	6.00 \pm 1.26*	3.00 \pm 0.89*	2.50	1.28 \pm 0.06*	62.02
Diphenoxylate HCl	2.5	50.00	361.00 \pm 29.66*	3.40 \pm 0.54*	1.20 \pm 0.44*	0.47	0.68 \pm 0.06*	84.81
Loperamide	2.5	60.00	337.16 \pm 26.53*	3.83 \pm 0.75*	2.16 \pm 0.75*	0.68	1.05 \pm 0.25*	72.65
Compound 1	2.5	66.66	125.75 \pm 9.750*	3.50 \pm 0.18*	1.50 \pm 0.17*	1.85	1.22 \pm 0.09*	81.01
Compound 2	2.5	50.00	223.33 \pm 12.77*	3.00 \pm 0.00*	1.00 \pm 0.00*	0.67	0.70 \pm 0.02*	87.34
Fraction F1	2.5	50.00	273.33 \pm 7.940*	3.00 \pm 1.15*	1.33 \pm 0.57*	0.55	1.30 \pm 0.10*	87.34

Values are means \pm standard deviation, n = 10, Compound 1: (5S,6R,8aR)-5-(carboxymethyl)-3,4,4a,5,6,7,8,8a-octahydro-5,6,8a-trimethylnaphthalenedicarboxylic acid, Compound 2: Methyl 3,4,5-trihydroxybenzoate. *Values in a column are significantly different from the saline control, Duncan ($p < 0.05$)

Table 2: Effects of methanol extract, fractions and isolated compounds from *E. abyssinica* on intestinal charcoal transit in rats

Test substances	Dose (mg kg ⁻¹)	Intestinal charcoal transit (%)	% inhibition of charcoal transit
Saline control	0.0	73.07±2.13	0.00
Reference substance			
Atropine sulphate	2.5	31.06±3.69*	57.49
Isolated compounds			
1 ^a	2.5	30.16±2.63*	58.72
2	2.5	27.95±1.75*	61.74
Plant extract and fractions			
Fraction F1	2.5	31.50±2.25*	56.89
<i>n</i> -hexane fraction	80.0	56.33±4.03*	22.90
Ethyl acetate fraction	80.0	39.33±2.06*	46.17
<i>n</i> -butanol fraction	80.0	41.48±2.89*	43.23
Aqueous residue fraction	80.0	54.33±3.93*	25.64
Methanol extract	100.0	68.01±1.77*	6.92
	200.0	59.94±2.04*	17.96
	400.0	53.10±3.23*	27.32

Values are means±standard deviation, n = 8; *Values in the same column are significantly different from the saline control, Duncan (p<0.05), Compound 1: ^a1,2,3,4,4a,7,8,8a-octahydro-1,2,4a-trimethyl-1,5-naphthalenedicarboxylic acid and methyl-3,4,5-trihydroxybenzoate

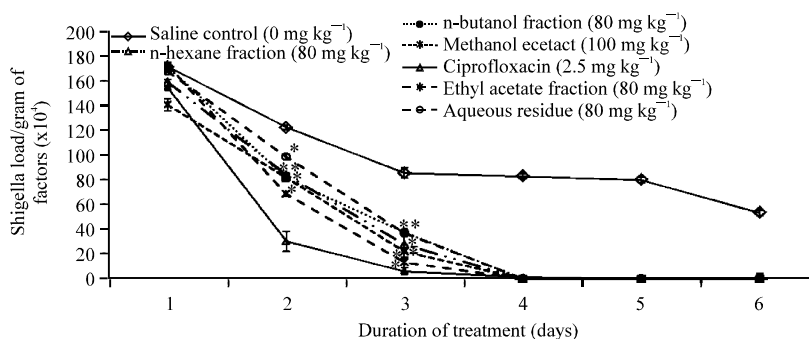


Fig. 2: Evolution of faecal *Shigella* load in rats following treatment with test samples from *E. abyssinica*. The values are means±standard deviation, n = 10, *Values are significantly different from the saline control by Duncan multiple range test at p<0.05

infected animals with duration of treatment (Fig. 2). The evolution of the total colony counts of *S. flexneri* per gram of faeces in all the plant extract-treated animals decreased significant (p<0.05) by day 2 of treatment with respect to the control saline. Although *S. flexneri* was most susceptible to the standard drugs, the latter and the methanol extract and fractions of *E. abyssinica* stem bark completely eradicated rat shigellosis by the fourth day of treatment.

Effects of treatment on body weight variation in *Shigella* induced-diarrhoeic rats: After *Shigella*-diarrhoea induction, the variation in body weight increased with duration of treatment. The saline control rats instead lost weight after day 1 of treatment. But from day 2 up to day 6, the body weight increased gradually but remained less than those of the other test groups (Fig. 3).

Effects of treatment on some haematological and biochemical parameters in *Shigella*-induced diarrhoea in rats: The red blood cells and white blood cells levels of

animals at the end of *Shigella*-induced diarrhoea assay revealed a significant decrease (p<0.05) in the saline control, *n*-hexane and residue fractions from those treated with the other test substances. The serum creatinine levels were significantly lower (p<0.05) in the plant extract and/or drug-treated animals compared to saline control (40.75±9.85 $\mu\text{mol L}^{-1}$). The methanol extract (21.45±2.52 $\mu\text{mol L}^{-1}$), ethyl acetate fraction (21.45±2.52 $\mu\text{mol L}^{-1}$) and ciprofloxacin (18.43±4.59 $\mu\text{mol L}^{-1}$) had comparable effects. Contrary, the sera triglycerides increased significantly in the extract-treated animals (highest in ethyl acetate, 1.76±0.16 mmol L⁻¹) as in the reference substance (1.54±0.25 mmol L⁻¹) compared to the saline control (0.68±0.16 mmol L⁻¹). The protein levels recorded in the serum, heart and liver of the test animals equally displayed a significant increase (p<0.05) in the extract-treated groups with respect to those of the saline control (Table 3).

Effects of single oral dose of methanol extract on mice: From the acute toxicity study, the methanol extract

Table 3: Effects of methanol extract and fractions from *E. abyssinica* stem on some biochemical parameters in rats

Treatment	Dose (Mg kg ⁻¹)	Biochemical parameters							
		Serum							Hepatic proteins (mg g ⁻¹)
		RBC (mm ⁻³)	WBC (mm ⁻³)	Haematocrit (%)	Creatinine (μmol L ⁻¹)	Triglyceride (mmolL ⁻¹)	Proteins (mg g ⁻¹)	Cardiac proteins (mg g ⁻¹)	
Saline control	0.0	0055940±2865	0416±75	29.9±1.79	40.75±9.85	0.68±0.16	28.73±3.54	34.34±8.25	95.46±19.20
Reference substance									
Ciprofloxacin	2.5	091770±5986*	850±63*	37.4±2.59*	18.43±4.59*	1.54±0.25*	40.92±1.80	68.66±4.83*	177.85±8.73*
Plant extract and fractions									
Methanol extract	100.0	83020±15743*	776±197*	37.6±4.06*	21.45±2.52*	1.62±0.06*	40.82±2.33*	65.02±9.98*	159.03±43.65*
n-hexane fraction	80.0	0062730±6622	0450±132	36.2±4.02*	33.24±4.53*	1.40±0.09*	40.45±3.33*	61.99±16.8*	166.01±37.69*
Ethyl acetate fraction	80.0	87320±17991*	726±133*	38.3±1.94*	21.45±2.52*	1.76±0.16*	40.94±3.53*	73.97±10.79*	176.47±41.52*
n-butanol fraction	80.0	072590±8238*	560±149*	35.4±3.47*	24.66±2.55*	1.53±0.17*	38.68±3.49*	60.13±9.92*	140.38±29.80*
Aqueous residue fraction	80.0	0062000±9470	0462±131	34.1±1.96*	32.17±3.09*	1.55±0.22*	39.98±2.87*	55.86±4.03*	129.94±26.49*

Values are means±standard deviation, n = 10, *Values in the same column are significantly different from the saline control, Duncan (p<0.05)

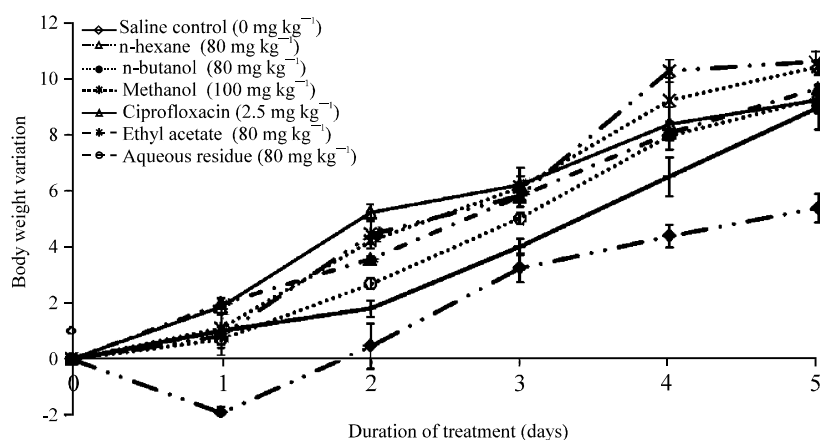


Fig. 3: Evolution of body weight variation in rats with *Shigella diarrhoea* on treatment. The values are means±standard deviation, n = 10, *Values are significantly different from the saline control by Duncan multiple range text at p<0.05

provoked deaths of mice at 8 g kg⁻¹ (4/12), 12 g kg⁻¹ (9/12) and 16 g kg⁻¹ (12/12) within 48 h observation period. The lethal dose-50 (LD₅₀) of this extract was evaluated as 9.83 g kg⁻¹ b.wt.

DISCUSSION

Diarrhoea is a consequence of innumerable pathologic conditions. Usually diarrhoea is caused by altered motility and fluid accumulation in the intestine lumen, which can be occasioned by an increased secretion of electrolytes (secretory diarrhoea), an enhanced ingestion of osmotic substances (osmotic diarrhoea), or the presence of a virulent microorganism (infectious diarrhoea) (Hughes *et al.*, 1982; Field, 2003; Brijesh *et al.*, 2006). For this reason, in the present study, antidiarrhoeal properties of the methanol extract, fractions and isolated compounds from *E. abyssinica* stem bark were examined using various parameters, intestinal motility and *in vivo* antibacterial activity.

The action of castor oil as a diarrhoea inductor has been largely studied and its active component is ricinoleic acid, which produces an irritating and inflammatory action on the intestinal mucosa leading to the release of prostaglandins. This condition increases the permeability of mucosal cells and provokes changes in electrolyte transport, thus, causing diarrhoea (Mohd *et al.*, 2004).

In the evaluation of intestinal transit, atropine sulphate was used as standard drug. Atropine is known to inhibit intestinal transit probably due to its anticholinergic effect (Mascolo *et al.*, 1993). The extracts and compounds 1 and 2 also appeared to act on all parts of the intestine. Thus, they reduced the intestinal propulsive movement in the charcoal meal treated model. Compound 1, a rare tetranorditerpene from *E. abyssinica*, was previously isolated from *Detarium microcarpum* (Aquino *et al.*, 1992). Compound 2 is a known medicinally important substance that was previously isolated from *Entada africana* (Han *et al.*, 2004), *Acer ginnala* (Jang-Gi *et al.*, 2009) and *Galla rhois* and has been

reported to possess antimicrobial activities (Rohini *et al.*, 2005) and anti-asthmatic effects (Zeng *et al.*, 2010). The activities of these compounds against castor oil-induced diarrhoea are being reported herein for the first time. Antidiarrhoeal activity and antidysenteric properties of medicinal plants were found to be due to the presence of tannins, alkaloids, saponins, flavonoids, steroids and or terpenoids (Havagiray *et al.*, 2004). These classes of compounds previously identified in our extract (Teke *et al.*, 2011) could be responsible for the biological activities of *E. abyssinica* extracts.

The *in vivo* antishigella activity of the methanol extract and fractions of *E. abyssinica* is not surprising since we have previously demonstrated the *in vitro* antimicrobial properties of *E. abyssinica* (Teke *et al.*, 2011). It is evident that the decrease in fecal *Shigella* load in the treated animals with duration of treatment resulted from the antimicrobial activity of the chemical components detected in the extracts of this plant (Teke *et al.*, 2011).

Protein synthesis was favoured in treated animals after *Shigella*-diarrhoea induction. Diarrhoea is known to reduce the rate of protein synthesis (Sato *et al.*, 2007). Also, a loss of blood and serum protein through inflamed intestine has been reported in cases of shigellosis (Hunter *et al.*, 2001). The low protein level and low blood cell counts observed in the saline control rats could be related to the losses in faeces. This situation can provoke a decrease in body weight as was observed in the saline-treated rats. Diarrhoea especially of infectious origin is reported to provoke weight loss and retard growth rate (Scrimshaw, 1977). The concomitant increase in sera creatinine levels and reduction in triglyceride levels have been reported in diarrhoeal cases (Scrimshaw, 1977; Sato *et al.*, 2007). Interestingly, the extracts act by reversing the diarrhoeal condition; reducing the sera creatinine level while increasing the triglyceride level. This reinforces the curative antidiarrhoeal properties of this plant.

The LD₅₀ of 9.83 g kg⁻¹ bodyweight recorded in the acute toxicity assay suggests that major adverse health effects following therapy with the methanol extract from *E. abyssinica* stem bark would not be expected (Lu, 1992) but this must be confirmed by further toxicological studies, including sub-acute and chronic toxicities.

CONCLUSION

The results of this study provide clear evidence that the methanol extract, fractions and isolated compounds from *E. abyssinica* stem bark possess antidiarrhoeal activities, advocating the use of this plant in the treatment

of diarrhoeal ailment. Being a potent plant for the development of phytomedicines, further pharmacological and toxicological studies will be necessary.

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