Scholars Academic Journal of Biosciences (SAJB) **ISSN 2321-6883 (Online)**
Sch. Acad. J. Biosci., 2017; 5(3):230-239 **ISSN 2347-9515 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI : 10.36347/sajb.2017.v05i03.018

Original Research Article

Antidiarrhoeal, Antibacterial and Toxicological Evaluation of Harungana Madagascariensis

Jean R Mba, Fidele CL Weyepe, Aristide LK Mokale, Armelle D Tchamgoue, Lauve RY Tchokouaha, Tsabang Nole, Protus A Tarkang, Donfagsitelie T Nehemie, Tchinda T Alembert, Mbita M, Dongmo B, Gabriel A Agbor[#]
Centre for Research on Medicinal Plants and Traditional Medicine, Institute of Medical Research and Medicinal Plants Studies, P.O. Box 13033, Yaoundé Cameroon.

***Corresponding author**
Gabriel A. Agbor
Email: agogae@yahoo.fr

Abstract: *Harungana madagascariensis* is a medicinal plant used for the treatment of diarrhoeal disease. The present study evaluated the antidiarrhoeal, antibacterial and safe use of hydro-ethanolic extract of *Harungana madagascariensis* at acute and subacute administration. The antidiarrhoeal activity was characterized as inhibitor of Castor oil induced diarrhea and upper gastrointestinal transit at preventive and curative administration. Prominent enteric bacteria were screened in vitro for antibacterial activity using the microdilution method. At acute administration an LD_{50} of 1650 mg/kg was obtained for *H. madagascariensis*. Meanwhile at sub-acute administration *H. madagascariensis* induced noticeable changes in transaminases activities as well as cholesterol, urea and glucose concentration which may serve as indicators of toxicity. It also had a dose related inhibitory effect on growth rate compared to control animals. In castor oil induced diarrhea, Loperamide (reference drug) had the strongest inhibitory effect followed by the 200 mg/kg dose extract. The difference between the inhibitory effect of 400 and 800 mg/kg extract were not significant. On gastrointestinal motility, Loperamide had the strongest preventive effect compared to all the doses of extract tested, while 100 mg/kg was the most effective amongst the plant extracts. However, for the curative effect the response was dose related with 400 mg/kg being more effective than 200 mg/kg and 100 mg/kg. For the antibacterial study *H. madagascariensis* extract prevented the growth of all 12 tested bacterial species. *P. aeruginosa*, *E.coli*, *S. flexneri* and *S. aureus* were the most sensitive (MIC = 62.5 μg/ml) while *P. aeruginosa* ATCC 27853 and *P. aeruginosa* ATCC 01 were the least sensitive (MIC = 500 μg/ml). *H madagascariensis* aqueous extract possess some antidiarrhoeal and antibacterial activities though it may be toxic.
Keywords: Antibacterial activity, castor oil induced diarrhea, gastrointestinal motility, *H madagascariensis*, toxicity testing.

INTRODUCTION

Diarrhoea is a major cause of mortality and morbidity with an estimated two billion cases every year globally, and that 1.9 million children below five years of age die annually, mostly in developing countries [1, 2]. The absorption and secretion of water and electrolytes throughout the gastrointestinal tract is a finely balanced, dynamic process and, when there is loss of this balance caused either by decreased absorption or increased secretion, diarrhoea results. Symptoms in humans include stomach pain, abdominal cramps, bloating, thirst, weight loss and frequent passing of loose or watery stool and it can be classified into acute (< 2 weeks), persistent (2 – 4 weeks) or chronic (> 4 weeks). Its pathophysiology defines diarrhoea to be either osmotic or secretory, with possible overlaps. Osmotic diarrhoea occurs when the small intestine cannot absorb osmotically active particles and fluid is drawn into the gut to exceed its absorptive capacity. Underlying causes include osmotic laxatives, inflammation of the intestinal lining and increased motility of the intestines. Secretory diarrhoea occurs when the bowel mucosa secretes excessive amounts of fluid, either due to activation of a specific pathway by a toxin or inherent abnormalities in the enterocytes [3].

Most cases of diarrhoea are the symptoms of an infection in the gastrointestinal tract by microbes such as bacteria, viruses and parasites. This infection can be spread through food, drinking water and unhygienic conditions. The most commonly identified causes of acute diarrhoea are the bacteria *Salmonella typhi*, *Staphyloccocus aureus*, *Campylobacter*, *Shigella flexneri* and Shiga toxin-producing *Escherichia coli*. In developing countries, enteric bacteria and parasites are

more prevalent than viruses and typically peak during the summer months. In both industrialized and developing countries, viruses are the predominant cause of acute diarrhea, particularly in the winter season, whereas parasitic agents such as *Cryptosporidium parvum, Giardia intestinalis, Entamoeba histolytica,* and *Cyclospora cayetanensis* that are not common in the developed world, are usually restricted to travelers [2, 4]. There are cases of chronic diarrhoea that are labeled "functional" because they cannot be explained by structural or biochemical abnormalities [5]. In the developed world, irritable bowel syndrome (IBS) is the most common cause of functional diarrhoea [6]. IBS is a complex of symptom, usually diagnosed by a process of elimination of other possible problems. There is cramping abdominal pain and altered bowel habit, either with diarrhoea or constipation. Inflammatory bowel disease (IBD) is another cause of chronic diarrhoea, in which the diagnosis is either ulcerative colitis or Crohn's disease, and there is often blood and pus in the stool in both conditions.

Primary control efforts for non-specific diarrhoea include oral rehydration therapy, zinc/ vitamin supplementation in diet, use of probiotics, anti-motility and anti-secretory agents but these do not address the underlying causes. Therefore, therapy of specific causes requires the use of antimicrobial agents. However, some of these agents show adverse effects and the emerging resistance of causative micro-organisms to some of them remains a cause for concern. Several studies in developing countries show that multi-drug resistant organisms have been emerging over the last decades, with the general spread of resistance to commonly used drugs such as amoxicillin, co-trimoxazole, chloramphenicol [7-9]. In most cases, there is lack of modern medical facilities and population resort to the use of medicinal plants for therapy [10]. In view of searching for new therapeutic sources, there is the need for the evaluation of the activity and safety of some of the plants in common folk use.

Harungana madagascariensis Lam. ex Poir, of the Guttiferae family, is commonly known as haronga, orange-milk or dragon's blood tree. It is an evergreen shrub or tree with a much branched, heavy, spreading canopy, approximately 12 meters tall, with occasional specimens up to 27 meters. The bole is straight and cylindrical bole [11]. It is a multipurpose tree and particularly valued for its multiple medicinal uses and as a dye.

Biological claims include antimicrobial activity (leaf extract) [12], antibacterial [13], antioxidant [14] as well as antiplasmodial and anti-trichomonal [15] activities. Therefore, in light of its biological activities on some microorganisms, this study aims at evaluating its safety profile, preventive and curative antidiarrhoeal activities in castor oil-induced diarrhoea animal models, as well as its antibacterial effects on some causative agents.

MATERIALS AND METHODOLOGY

Chemicals

Loperamide (Imodium, standard reference antidiarrheal drug), castor oil (laxative agent), charcoal meal (marker diet, 10% activated charcoal in 5% gum acacia), Cholesterol, Triglyceride, Alanine amino transferase, Aspartate amino transferase, Creatinine, Urea and glucose enzymatic kits were used.

Preparation of plant material

Fresh stem bark of *Harungana madagascariensis* was harvested in the environ of Donga Mantung Division of the North West Region of Cameroon in the month of August. Plant identification and voucher specimen referencing was done at the national herbarium Yaoundé of Cameroon. The stem bark was chopped into small bits of about 2 cm. This was then dried with aid of hot air oven and latter ground to powder. The ground sample was then extracted in hydro-ethanolic (50 % ethanol) solvent for 72 hours. The extract was filtered and concentrated with the aid of a rotary evaporator and freeze dried. The freeze dried sample was now used for the study.

Phytochemical screening

Preliminary phytochemical screening was performed according to the methods described by [16].

Toxicity testing

Experimental animals

The male albino mice (20-25 g) and male Swiss albino rats (150-230 g) produced from the Laboratory of Pharmacology of the Centre for Research on Medicinal Plants and Traditional Medicine, Institute of Medical Research and Medicinal Plants Studies, Yaoundé were used for acute and sub-acute toxicity studies of *Harungana madagascariensis.* The animals were housed in wire mesh cages and fed with standard laboratory diet and had ordinary tap water *ad libitum*.

Acute toxicity studies

Albino mice were used in this study. Before the actual determination of the median lethal dose (LD_{50}), a pilot study using the 'staircase method' determined the range between the maximum nonlethal and minimum lethal doses of H madagascariensis [17]. A more reliable LD_{50} assay was performed using a three dose level (between 0.5 - 2g/kg dissolved in distilled water) within maximum nonlethal and minimum lethal dose range (6 mice in each group). Another group of mice was kept as control and received 1.0 ml of distilled water. The animals were observed for first 7 hours and then on daily bases for any toxic symptoms (change in activity such as excitation, fatigue, diarrhea, itching, curved tail, shivering, falling of hair and mortality) for three days. During this period, the number of dead mice

was counted in each group. The percentage of animals that died at each dose level was transformed to probits [17] and then LD_{50} determined by the method of Miller and Tainter [18].

Sub-acute toxicity testing

Three doses of the *Harungana* extract (500-2000mg/kg) were administered to experimental rats on a daily base for 4 weeks during which the animals were monitored for abnormal behaviour and the food and water consumption monitored daily. Another group of rats kept as control was administered distilled water throughout. At the end of 4 weeks the animals were sacrificed and blood collected into EDTA tubes and plasma separated for plasma biochemical analysis. The animals were weighed weekly and the liver, heart, kidney and spleen were isolated cleaned and weighed at the end of the experiment after sacrificing the animals. The liver sample of each experimental group was preserved in 10 % formalin for histopathological examination.

The experimental animals were grouped and treated as follows:
Group 1: Control animals
Group 2: *Harungana* (500mg/kg dose)
Group 3: *Harungana* (1000 mg/kg dose)
Group 4: *Harungana* (2000 mg/kg dose)

Histopathological examination

Liver samples were preserved in 10% formaldehyde solution. The slices of liver were processed and embedded in paraffin wax. Sections of about 4-6 microns were made and stained with hematoxylin and eosin and photographed [19, 20]

Antidiarrhoeal activity testing
Inhibition of Castor oil induced diarrhoea

The method proposed by Galvez *et al.;* [21] modified by Oben *et al.;* [22] was used to experimental needs. Adult mice fasted for 16 hours were divided into 6 groups (Group I - V) of 6 each and treated as follows:
Group 1 (diarrhoeal control): castor oil (0.4 ml).
Group 2 (standard control): Loperamide (0.25 mg/kg body weight),
Group 3: 200 mg/kg body weight *Harungana* extract,
Group 4: 400 mg/kg body weight *Harungana* extract,
Group 5: 800 mg/kg body weight *Harungana* extract,

Castor oil (0.4 ml) was administered to all groups by intubation 30 minutes after the Loperamide and plant extracts were administered. The animals were then placed in separate wired-meshed cages and the total number of stool and wet stool were counted. The percentage diarrhea inhibition was then calculated as a function of the diarrhoeal control.

% Inhibition = (no. of wet stool by diarrhoeal control - no. of wet stool by test) × 100% / no. of stool by diarrhoeal control.

Inhibition of upper gastrointestinal transit (preventive studies)

The method proposed by Aye-Than *et al.;* [23] was used in groups of six mice each, using a "charcoal meal" as a marker diet. The mice were given increasing doses of the plant extract in 1 ml of distilled water by intubation and 30 min later castor oil was administered, followed by the marker diet after another 30 min. The mice were sacrificed by cervical dislocation 30 min after the market diet i.e 1 h after castor oil administration. The intestinal transit of the marker diet was calculated as a percentage of the distance travelled by the charcoal meal compared to the length of the small intestine.

Inhibition of upper gastrointestinal transit (curative studies)

The method proposed by Aye-Than *et al.;* [23] was used in groups of six mice each, using a "charcoal meal" as a marker diet. The mice were given castor oil and 30 min later was administered increasing doses of the plant extract in 0.2 ml of distilled water by intubation, followed by the marker diet after another 30 min. The mice were sacrificed by cervical dislocation 30 min after the marker diet. The intestinal transit of the marker diet was calculated as a percentage of the distance travelled by the charcoal meal compared to the length of the small intestine.

Antibacterial assay
Microorganisms

Crude extract was tested against a panel of microorganisms including six bacterial strains (*Enterobacter aerogenes* ATCC 13048, *Enterococcus faecalis* ATCC 10541, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas aeruginosa* ATCC 01, *Providencia stuartii* ATCC 29916 and *Staphylococcus aureus* ATCC 25922) and six isolates (*Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Salmonella paratyphi A*, *Salmonella paratyphi B*, and *Staphylococcus aureus*). The isolates were obtained from Centre Pasteur of Yaoundé, Cameroon, while the reference strains were obtained from American Type Culture Collection (ATCC). The strains and isolates were grown at 35°C and maintained on nutrient agar. The bacterial cell suspension was prepared at 1.5×10^8 colony forming units per ml (CFU/ml) following the McFarland 0.5 turbidity standard.

Broth Microdilution Method

Minimum inhibitory concentrations (MICs) were determined using Mueller Hinton Broth (MHB) by microdilution method [24]. A twofold serial dilution of the extract (1000-0.48 µg/ml) was performed in a total

volume of 200 µl/well. A negative control (5%, v/v aqueous DMSO, medium and inoculum) and positive control (5%, v/v aqueous DMSO, medium, inoculum and water-soluble antibiotic) were included. Each well of 96-well sterile microplate received the test substance at the different concentrations and bacterial suspension (100 µl) in MHB. The plates were covered and incubated at 35°C for 18 h. Bacterial growth was monitored colorimetrically using *p*-iodo nitro tetrazolium chloride (INT). Viable bacteria change the yellow dye of *p*-iodo nitro tetrazolium violet to a pink color. MIC values are recorded as the lowest concentration of the substance that completely inhibited bacterial growth that is the solution in the well remained clear after incubation with INT. Minimum bactericidal concentrations (MBCs) were determined by plating 10 µl from each negative well and from the positive growth control on Mueller Hinton Agar. MBCs were defined as the lowest concentration yielding negative subcultures. The experiments were performed in triplicate. Ciprofloxacin at the concentration ranging between 128 and 0.0625 µg/ml served as positive control.

STATISTICAL ANALYSIS

All results were expressed as mean ± SEM for triplicate determinations. Data were subjected to one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison tests. Differences of $p < 0.05$ were considered statistically significant. GraphPad Prism 5 was used for these analyses.

RESULTS AND DISCUSSION

Phytochemical screening

In herbal medicine standardization bioactive principles identification and quantification is crucial which may depend largely on the solvent type and nature of plant part used [25]. In the present study the bark of *H madagascariensis* was extracted with water/ethanol (50:50) and the phytochemical screening revealed the presence of alkaloids, catechic tannins, phenolics, saponins, coumarins, flavonoids, triterpenes, polyuronides and polyoses. Earlier phytochemical analysis revealed the presence of same bioactive components [12, 13, 26, 27] that may possess anti-diarrhoeal activity.

Acute toxicity

The effect of acute oral administration of *Harungana* is presented in Table 2 and Figure 1. At 500mg/kg the mice tolerated the plant extract with no death registered. However, at 1000 mg/kg dose above 33 % of the experimental animals died and above 66% died when treated with 2000mg/kg extract (Table 2). Following the equation of the regression line of the probit curve, the LD_{50} was calculated to be 1650mg/kg (1.65g/kg). This shows some degree of toxicity and calls for concern. Comparing the LD_{50} of the extracts (1.65g/kg) to the value of the Organization for Economic Cooperation and Development (OECD) guidelines, which state that any LD_{50} below the limit test dose of 2000 mg/kg may be declared toxic [28, 29] the *Harungana* hydro-ethanolic extract can be labeled toxic at acute administration.

Sub-acute toxicity

The liver is one of the most important organs with diverse metabolic activities with a host of enzymes. Amongst these two enzymes (ALAT and ASAT) serve as the standard markers for hepatocellular damage with increases in activity in the serum. This is often achieved when toxins are administered to experimental animals [30-32]. *Harungana* administration induced noticeable increases in ALT and AST activity as well as cholesterol, urea and glucose concentration (Table 3). These increases were dose related and significant ($P < 0.05$) for ALT and AST and may serve as indicators for potential toxicity in the hepatic tissues and pancreatic gland. *Harungana* extract had an inhibitory effect on growth rate as measured by weight gain (Table 4). The normal control animals had the highest percentage weight gain, followed by the group of animals treated with the smallest dose (500 mg/kg). Thus the *Harungana* extract had a significant ($P < 0.05$) dose response effect on inhibition of growth rate. However, *Harungana* did not have any noticeable effect on the relative weight of organs. Creatinine and urea are end products of protein metabolism and are indicative of renal function. Their concentrations remain fairly constant under normal conditions unless renal function changes [33]. Treatment of experimental animals with *Harungana* extract did not alter creatinine and urea hence renal function was not altered. Similarly, *Harungana* extract did not alter the lipid parameter (cholesterol and triglyceride).

Histopathological examination revealed a normal liver with clear centrolobular vein and hepatocytes separated by capillary sinusoids in experimental animals treated with distilled water. A similar liver structure was observed in experimental animals treated with 500 mg/kg extract. However, at higher doses of *Harungana* hydro-ethanolic extract administration inflammation of the portal space and vacuolization of hepatic parenchyma cells were observed at 1000 mg/kg while 2000 mg/kg induced congestion of the vascular cells (Fig. 2).

Antidiarrhoeal studies

Diarrhea is characterized as an abnormal frequent expulsion of stool of low consistency which may be due to a disturbance in the transport of water and electrolytes in the intestine [34]. Castor oil (ricin oleic acid) induces changes in electrolyte, water transport, increases peristaltic activity and it's often used to induce experimental diarrhea [35-37]. The effect of castor oil has earlier been associated with

prostaglandins release, the main cause of arachidonic acid-induced diarrhea [37]. One of the most used antidiarrheal medications in Cameroon is Loperamide. Studies on loperamide showed that loperamide can effectively antagonized castor oil induced diarrhea [38], prostaglandin [39], or cholera toxin [40] by stimulating antimotility and antisecretory activities [41].

In the present study loperamide which was used as the standard drug had the strongest inhibition of castor oil induced diarrhea with an 81.81 percentage inhibition (Table 5). This was followed by the 200 mg/kg with 69.7 percentage inhibition. There was not much difference between the inhibitory effect of 400 and 800 mg/kg extract. For the preventive and curative effect of *H madagascariensis* on castor oil induced gastrointestinal motility, Loperamide had the strongest preventive effect compared to all the doses of extract tested (Table 6) meanwhile, the lowest dose of 100 mg/kg was the most effective amongst the plant extract i.e. better than 400 mg/kg. This is in agreement with the results for the castor oil induced diarrhea presented in Table 5. However, for the curative effect the response was dose related. That is the highest dose of 400 mg/kg was more effective than the 200 mg.kg and 100 mg/kg.

Antibacterial activity

MIC and MBC values of the crude extract were established and the results are shown in Table 7. All the bacteria tested were inhibited by the hydro-ethanolic extract with MIC ranging from 62.5-1000 μg/ml for all the bacteria, isolates and Gram^{-ve}; 125-1000 μg/ml for the strains and 62.5-250 μg/ml for Gram^{+ve}. *P. aeruginosa* ATCC 27853 and *P. aeruginosa* ATCC 01 were the least sensitive (MIC = 500 μg/ml). *P. aeruginosa*, *E.coli*, *S. flexneri* and *S. aureus* were the most sensitive (MIC = 62.5 μg/ml). Antibiotic exerted a higher inhibitory effect on bacterial (MIC = 0.125-128 μg/ml) than the hydro-ethanolic extract. The activity of plant extracts will be classified as significant (MIC < 100 µg/mL), moderate (100 < MIC ≤ 625 µg/mL) or weak (MIC > 625 µg/ mL) [42]. Hydro-ethanolic extract had a significant activity against *P. aeruginosa*, *E.coli*, *S. flexneri* and *S. aureus* and a moderate activity on the rest of bacteria tested. The MBC/MIC ratio activity for all the bacteria tested varied between one (1) and eight (8) for the crude extract. According to Marmonier [43], plant extract exerted two types of activities: a bacteriostatic (MBC/MIC > 4) and bactericidal activity (MBC/MIC ≤4). Hydro-ethanolic extract of *H. madagascariensis* was bactericidal against eleven of twelve bacteria tested.

Hydro-ethanolic extract of *H. madagascariensis* showed variable antibacterial activities in a dose-dependent manner against twelve bacterial strains and isolates tested. Earlier reports suggested that these broad spectra of action could be related to the presence of alkaloids, tannins, flavonoids and phenolics [44, 45]. The varying degree of sensitivity of the bacterial strains may be due to the intrinsic tolerance of the bacterial and the nature and combinations of compounds present in the extract [46]. The results of this work also revealed that the Gram^{-ve} and Gram^{+ve} bacteria had a comparable susceptibility. This may suggest that the mode of action of the extract was not related to the cell wall composition. Hydro-ethanolic extract of *H. madagascariensis* exerted a bactericidal effect against bacteria tested. However, the bactericidal action of natural substances on bacterial cells is still insufficiently clarified. Several mechanisms are involved inhibition of glycolysis and depletion of potassium, changing the morphology of the bacterial cell, precipitation of proteins and nucleic acids, inhibition of selective membrane permeability and membrane deterioration, absorption and formation of a film around the bacterial cell with inhibition of the breathing, absorption and excretion processes and inhibition of macromolecules synthesis: DNA, RNA and proteins [47]. The antibacterial activities of *H. madagascariensis* were reported [13, 46, 12, 27]. Our results showed a higher inhibitory effect than those obtained by previous investigators. These differences in susceptibility can be attributed to the difference in the geographical region, soil composition, age and part of the plant and solvent extraction [48].

Table 1: Bioactive composition of hydro-ethanolic extract of *H. madagascariensis*

STRUCTURAL GROUP	RESULTS
Catecho tannins	+
Galli tannins	-
Phenolic compounds	+
Reducing substances	+
Alkaloids	+ + +
Saponins	+ +
Poly-uronides	+
Poly-oses	+
Anthocyanines	+
Triterpenes glycoside	+ + +
Flavonoid glycoside	+ + +
Coumarin	+
Steroid glycoside	-

+ = present, - = absent, +++ =abundance

Table 2: Showing dose administered mortality and Probit values

Group	N° of Mice	N° of Death	% of Death	Dose Adm (g/kg)	Probit value
1	6	0	0	0.5	1.9098
2	6	2	33.33	1	4.5684
3	6	4	66.67	2	5.4316

50 % death has a probit value of (4.5684+5.4316)/2 = 5
From Fig 1 probit of 5 has a corresponding dose of 1.649649 g/kg ~1.65 g/kg
Hence the LD_{50} of *H madagascariensis* = 1.65 g/kg

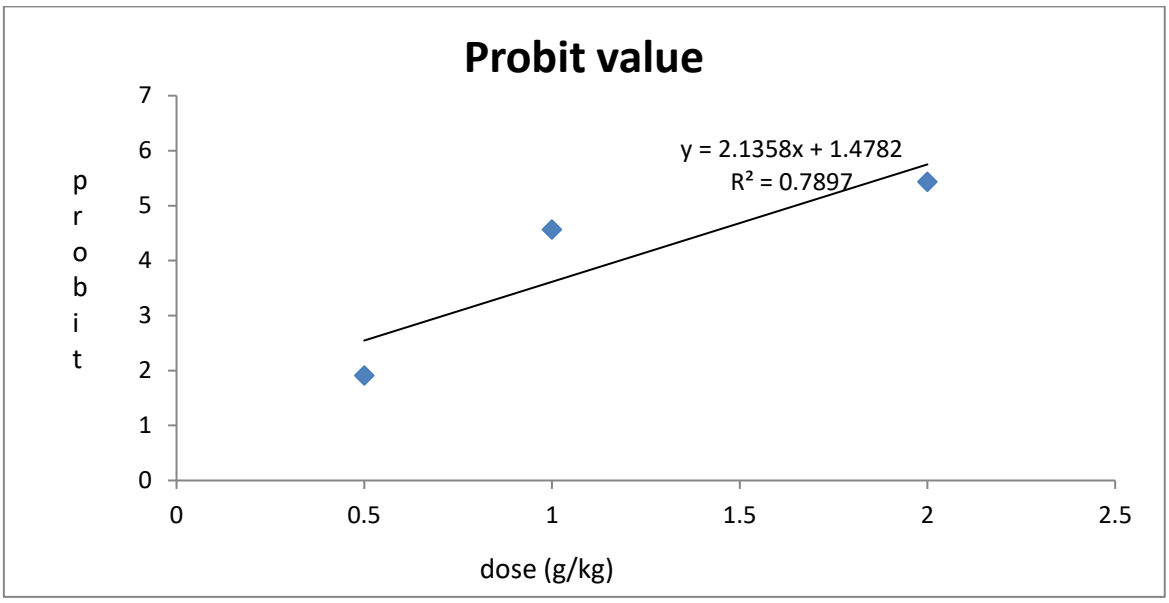

Fig 1: A plot of Probit against log dose for the determination of LD_{50}

Table 3: Effect of *H madagascariensis* on plasma Biochemical Parameters

Parameters	Group 1 (control)	Group 2 (500 mg/kg)	Group 3 (1000 mg/kg)	Group 4 (2000 mg/kg)
LT (U/L)	23.40 ± 3.71	23.26 ±6.23	28.22 ±3.30[a]	28.84 ±2.38[a]
AST (U/L)	24.43 ±1.89	28.34 ±5.90	27.65 ±7.33	31.84 ±1.30[a]
Cholesterol (mg/dl)	36.47 ±6.30	40.26 ±4.70	38.65 ±8.29	44.156 ±2.65
Triglyceride (mg/dl)	108.94 ±2.14	104.71 ±13.14	113.64 ±5.32	110 ±1.52
Creatinine (mg/dl)	0.75 ±0.18	0.78 ±0.12	0.73 ±0.12	0.83 ±0.09
Urea (mg/dl)	46.52 ±14.01	52.63 ±5.38	55.63 ±9.72	55.16 ±4.47
Glucose (mg/dl)	71.23 ±12.30	81.35 ±14.93	78.45 ±10.85	80.28 ±3.75

Data= Mean ± Standard Deviation. n = 5 rats per group; [a] significantly different compared to control

Table 4: Effect of *H madagascariensis* on relative organ weight and % weight gain (g) of experimental animals

Dose (mg/kg)	Liver	Heart	Kidney	Spleen	% weight gain
control	2.71 ±0.17	0.32 ±0.03	0.68 ±0.05	0.38 ±0.07	23.44 ±3.17
Group 1 (500 mg/kg)	2.74 ±0.12	0.35 ±0.02	0.65 ±0.03	0.35 ±0.03	15.64 ±4.74[a]
Group 2 (1000 mg/kg)	2.86 ±0.15	0.34 ±0.02	0.65 ±0.04	0.30 ±0.05	10.16 ±5.27[a]
Group 3 (2000 mg/kg)	2.5 ±0.2	0.30 ±0.04	0.57 ±0.08	0.32 ±0.06	6.56 ±1.99[a]

Data= Mean ± Standard Deviation. n = 5 rats per group; a significantly different compared to control

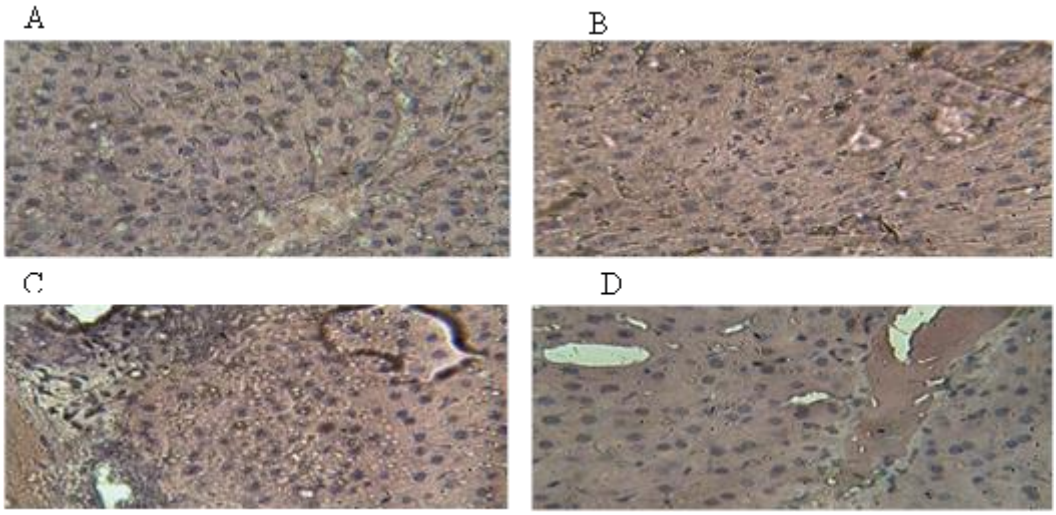

Fig: 2 Histopathology of rats' liver after subacute administration of *H. madagascariensis* extract

A= normal animal administered distilled water; presents centrolobular vein with hepatocytes separated by capillary sinusoids. B= Animals administered 500 mg/kg extract; presents normal liver. C= animals treated with 1000mg/kg extract presents inflammation of the portal space and vacuolization of hepatic parenchyma cells. D= animals treated with 2000 mg/kg presents congestion of the vascular cells.

Table 5: Effect of *H madagascariensis* on Castor oil induced diarrhea in mice

Treatment	Inhibition (%)	*n*
Group 1 : Castor oil (0.4 ml)	-	6
Group 2 : Loperamide (0.25 mg/kg)	81.81	6
Group 3 : Extract (200 mg/kg)	69.70	6
Group 4 : Extract (400 mg/kg)	54.55	6
Group 5 : Extract (800 mg/kg)	57.52	6

Data= Mean ± Standard Deviation. n = 6 mice per group.

Table 6: Preventive and curative effect of *H madagascariensis* on gastrointestinal motility.

Treatments	% transit curative	% transit preventive
Group 1 : Distilled water (0.4ml)	10.23± 2.11	13.5± 0.91
Group 2 : Castor oil (0.4ml)	90.80± 5.98[a]	84.11 ±16.92[a]
Group 3 : loperamide (0.25mg/kg)	29.23± 8.01[abc]	24.06± 8.22[abc]
Group 4 : extract (100mg/kg)	79.01± 8.23[ab]	45.33± 5.00[ab]
Group 5 : extract (200mg/kg)	64.23± 8.05[ab]	55.82 ±6.78[ab]
Group 6 : extract (400mg/kg)	52.03± 13.73[ab]	48.06± 8.31[ab]

Data= Mean ± Standard Deviation. n = 6 mice per group. [a] significantly different compared to Group 1; [b] significantly different compared to Group 2 (castor oil control); [c] significantly different compared to the rest of the groups.

Table 7: MIC and MBC (µg/ml) of the hydro-ethanolic extract of *H. madagascariensis*

Bacteria	Parameters	Substance	Antibiotic reference drug
		Crude Extract	Ciprofloxacin
Gram-negative			
E. coli	MIC	62.5	1
	MBC	62.5	1
	MBC/MIC	1	1
E. aerogenes ATCC13048	MIC	250	32
	MBC	250	32
	MBC/MIC	1	1
P. stuartii ATCC 29916	MIC	250	128
	MBC	500	128
	MBC/MIC	2	1
P. aeruginosa ATCC 27853	MIC	500	1
	MBC	1000	16
	MBC/MIC	2	16
P. aeruginosa ATCC 01	MIC	500	1
	MBC	1000	32
	MBC/MIC	2	32
P. aeruginosa	MIC	62.5	1
	MBC	250	16
	MBC/MIC	4	16
S. flexneri	MIC	62.5	0.25
	MBC	62.5	1
	MBC/MIC	1	4
S. paratyphi A	MIC	125	0.125
	MBC	250	0.5
	MBC/MIC	2	4
S. paratyphi B	MIC	125	0.5
	MBC	1000	2
	MBC/MIC	8	4
Gram-positive			
E. faecalis ATCC 10541	MIC	125	4
	MBC	125	16
	MBC/MIC	1	4
S. aureus ATCC 25922	MIC	125	8
	MBC	250	16
	MBC/MIC	2	2
S. aureus	MIC	62.5	8
	MBC	250	8
	MBC/MIC	4	1

CONCLUSION

Harungana madagascariensis hydro-ethanolic extract possess some antidiarrhoeal activity against physiological diarrhea. However, this effect is not good enough compared to the standard drug (Loperamide) already in the market. Overall Loperamide (0.25 mg/kg) was approximately twice as strong as the most effective dose of *Harungana* extract. *Harungana* as an anti-diarrhea agent, will be problematic because it possesses a lethal dose of 1.65g/kg which makes it toxic at acute administration. The sub-acute toxicity revealed modification of the activities of alanine amino transferase, aspartate amino transferase and concentrations of some plasma biochemical parameters such as cholesterol, urea and glucose. Sub-acute administration also prevented growth rate measured as weight gain.

REFERENCE

1. World Health Organization. Diarrheal Disease: Fact Sheet. WHO, Geneva, Switzerland. 2009. http://www.who.int/ media Centre/factsheets/fs330/en/index.html.

2. World Gastroenterology Organization. Acute diarrhoea in adults and children: a global perspective. WGO, Milwaukee, Wisconsin, US. 2012. Information published online, accessed 24 October 2016.
3. Whyte LA, Jenkins HR. Pathophysiology of diarrhoea. Paediatrics and child health. 2012 Oct 31; 22(10):443-7.
4. Barr W, Smith A. Acute diarrhea. American family physician. 2014 Feb 1; 89(3).
5. Dellon ES, Ringel Y. Treatment of functional diarrhea. Current treatment options in gastroenterology. 2006 Jul 1; 9(4):331-42.
6. Juckett G, Trivedi R. Evaluation of chronic diarrhea. American family physician. 2011 Nov 15; 84(10).
7. Okeke IN, Laxminarayan R, Bhutta ZA, Duse AG, Jenkins P, O'Brien TF, Pablos-Mendez A, Klugman KP. Antimicrobial resistance in developing countries. Part I: recent trends and current status. The Lancet infectious diseases. 2005 Aug 31; 5(8):481-93.
8. Bercion R, Njuimo SP, Boudjeka PM, Manirakiza A. Distribution and antibiotic susceptibility of Shigella isolates in Bangui, Central African Republic. Tropical Medicine & International Health. 2008 Apr 1; 13(4):468-71.
9. Raji MA, Jamal W, Ojemhen O, Rotimi VO. Point-surveillance of antibiotic résistance in Enterobacteriaceae isolâtes from patients in a Lagos Teaching Hospital, Nigeria. Journal of infection and public health. 2013 Dec 31;6(6):431-7.
10. Kouitcheu ML, Penlap BV, Kouam J, Ngadjui BT, Fomum ZT, Etoa FX. Evaluations of antidiarrhoeal activity of the stem bark of Cylicodiscus gabunensis (Mimosaceae). African Journal of Biotechnology. 2006; 5(11).
11. Burkill HM. The useful plants of west tropical Africa. Edition 2. Vol. 1: families AD. Kew, Royal Botanic Gardens. 1985.
12. Kengni F, Tala DS, Djimeli MN, Fodouop SP, Kodjio N, Magnifouet HN, Gatsing D. In vitro antimicrobial activity of Harungana madagascriensis and Euphorbia prostrata extracts against some pathogenic Salmonella sp. International Journal of Biological and Chemical Sciences. 2013; 7(3):1106-18.
13. Afieroho OE, Izontimi SS, Okoroafor DO, Caleb B. Antibacterial and phytochemical evaluation of *Harungana madagascariensis* L. (Hypericaceae) seeds. Int. Res. J. of Pharm 2012; 3(11): 75-77.
14. Antia BS, Ita BN, Udo UE. Nutrient Composition and In Vitro Antioxidant Properties of Harungana madagascariensis Stembark Extracts. Journal of Medicinal Food 2015; 18(5):609-14.
15. Iwalewa EO, Omisore NO, Adewunmi CO, Gbolade AA, Ademowo OG, Nneji C, Agboola OI, Daniyan OM. Anti-protozoan activities of Harungana madagascariensis stem bark extract on trichomonads and malaria. Journal of ethnopharmacology. 2008 May 22; 117(3):507-11.
16. Bruneton J. Pharmacognosie Phytochimie plantes médicinales. 3ème édition, Tec & Doc, Paris. 1999; 623.
17. Ghosh MN. In Statistical Analysis, Fundamentals of Experimental Pharmacology, 2nd ed, Calcutta: Scientific Book Agency, 1984; 153-158, 189.
18. Miller LC, Tainter M. Estimation of the ED50 and Its Error by Means of Logarithmic-Probit Graph Paper.∗. Proceedings of the society for Experimental Biology and Medicine. 1944 Nov; 57(2):261-4.
19. Feng Y, Siu KY, Ye X, Wang N, Yuen MF, Leung CH, Tong Y, Kobayashi S. Hepatoprotective effects of berberine on carbon tetrachloride-induced acute hepatotoxicity in rats. Chinese medicine. 2010 Sep 18; 5(1):33.
20. Jeong SC, Kim SM, Jeong YT, Song CH. Hepatoprotective effect of water extract from Chrysanthemum indicum L. flower. Chinese medicine. 2013 Apr 4; 8(1):7.
21. Galvez J, Zarzuelo A, Crespo ME, Lorente MD, Ocete MA, Jimenez J. Antidiarrhoeic activity of Euphorbia hirta extract and isolation of an active flavonoid constituent. Planta medica. 1993 Aug; 59(04):333-6.
22. Oben JE, Assi SE, Agbor GA, Musoro DF. Effect of Eremomastax speciosa on experimental diarrhoea. Afr J Trad CAM. 2006, 3: 95-100.
23. Than A, Kulkarni HJ, Hmone W, Tha SJ. Anti-diarrhoeal efficacy of some Burmese indigenous drug formulations in experimental diarrhoeal test models. International Journal of Crude Drug Research. 1989 Jan 1; 27(4):195-200.
24. Salie F, Eagles PF, Leng HM. Preliminary antimicrobial screening of four South African Asteraceae species. Journal of ethnopharmacology. 1996 May 1;52(1):27-33.
25. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: a review. Internationale pharmaceutica science. 2011 Mar; 1(1):98-106.
26. Eze FN, Osamede JA. Quantitative Phytochemical Evaluation of Stem Bark Extracts of Harungana madagascariensis. British Journal of Pharmaceutical Research. 2014 Sep 1; 4(17):2068.
27. Roger T, Pierre-Marie M, Igor VK, Patrick VD. Phytochemical screening and antibacterial activity of medicinal plants used to treat typhoid fever in Bamboutos division, West Cameroon.

28. Louis and Wallace CD, Wallace HA. In principles and method of toxicology, 4th edition, Taylor and Francis, Philadelphia, 2001: 871-873.
29. Joshi CS, Priya ES, Venkataraman S. Acute and subacute toxicity studies on the polyherbal antidiabetic formulation Diakyur in experimental animal models. Journal of health science. 2007; 53(2):245-9.
30. Nadeem M, Dandiya PC, Pasha KV, Imran M, Balani DK, Vohora SB. Hepatoprotective activity of Solanum nigrum fruits. Fitoterapia. 1997; 68(3):245-51.
31. Singh B, Saxena A, Chandan BK, Anand KK, Suri OP, Suri KA, Satti NK. Hepatoprotective activity of verbenalin on experimental liver damage in rodents. Fitoterapia. 1998; 69(2):135-40.
32. Agbor GA, Oben JE, Brahim BO, Ngogang JY. Toxicity study of Hibiscus cannabinus. Journal of the Cameroon Academy of Sciences. 2004;4(1):27-32.
33. Whitby, LG, Smith, AF, Beckett, G.J. Lecture notes on Clinical Chemistry. 4th ed. Blackwell Scientific Publications, Oxford, 1988; 153-154.
34. Lutterodt GD. Inhibition of Microlax-induced experimental diarrhoea with narcotic-like extracts of Psidium guajava leaf in rats. Journal of Ethnopharmacology. 1992 Sep 30; 37(2):151-7.
35. Agbor GA, Léopold T, Jeanne NY. The antidiarrhoeal activity of Alchornea cordifolia leaf extract. Phytotherapy research. 2004 Nov 1; 18(11):873-6.
36. Capasso FN, Mascolo N, Romano V. Laxatives and the production of autacoids by rat colon. Journal of pharmacy and pharmacology. 1986 Aug 1; 38(8):627-9.
37. Luderer JR, Demers LM, Nomides CT, Hayes Jr AH. Mechanism of action of castor oil: a biochemical link to the prostaglandins. Advances in prostaglandin and thromboxane research. 1979 Dec; 8:1633-5.
38. Niemegeers CJ, Lenaerts FM, Janssen PA. Loperamide (R 18 553), a novel type of antidiarrheal agent. Part 1: in vivo oral pharmacology and acute toxicity. Comparison with morphine, codeine, diphenoxylate and difenoxine. Arzneimittel-Forschung. 1974 Oct; 24(10):1633.
39. Karim SM, Adaikan PG. The effect of loperamide on prostaglandin induced diarrhoea in rat and man. Prostaglandins. 1977 Feb 1; 13(2):321-31.
40. Farack UM, Kautz U, Loeschke K. Loperamide reduces the intestinal secretion but not the mucosal cAMP accumulation induced by choleratoxin. Naunyn-Schmiedeberg's archives of pharmacology. 1981 Jun 1; 317(2):178-9.
41. Coupar IM. Opioid action on the intestine: the importance of the intestinal mucosa. Life sciences. 1987 Aug 24; 41(8):917-25.
42. Kuete V. Potential of Cameroonian plants and derived products against microbial infections: a review. Planta Medica. 2010 Oct; 76(14):1479-91.
43. Marmonier AA. Introduction aux techniques d'étude des antibiotiques. Bactériologie Médicale, technique usuelles. 1990:227-36.
44. Mabhiza D, Chitemerere T, Mukanganyama S. Antibacterial Properties of Alkaloid Extracts from Callistemon citrinus and Vernonia adoensis against Staphylococcus aureus and Pseudomonas aeruginosa. International journal of medicinal chemistry. 2016 Jan 20; 2016.
45. Hajji H, Talbaoui A, Elalaoui FEF, Bakri EAY, Aneb M, Elaissami A. *In vitro* evaluation of antibacterial action of *Carraluma europeae* on *Rhodoccocus equi* extracts. J. Chem. Pharmaceut. Res. 2016; 8(5): 943-952.
46. Yaouba A, Tchikoua R, Tatsadjieu NL. Antibacterial effect of plant extracts against some pathogenic bacteria. Int. J. Natural Products 2012; 1(4): 83-87.
47. Lakhdar L, Hmamouchi M, Rida S, Ennibi O. Antibacterial activity of essential oils against periodontal pathogens: a qualitative systematic review. Odontostomatol Trop. 2012 Dec; 35(Pt 170):38-46.
48. Mokale KAL, Ngono NRA, Koanga MML, Mouokeu RS, Ebelle ERM, Tchinda TA, Kuiate JR, Biyiti L, Amvam Zollo PH. Antibacterial and antioxidant properties of methanolic extract of the leaves of *Peucedanum zenkeri* (Apiaceae). Sciences, Technologies & Developpement 2012; 13(1): 36-47.