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IN VITRO ANTIAMOEBIC ACTIVITY OF EXTRACTS AND FRACTIONS FROM *IPOMEA BATATAS* L. (CONVOLVULACEAE) AND *URENA LOBATA* (L.) LAM (MALVACEAE) AGAINST THE PROTOZOA *ENTAMOEBA HISTOLYTICA*, ACUTE AND SUB-ACUTE TOXICITY OF AQUEOUS EXTRACTS

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ABSTRACT

Results from the evaluation of antiamoebic activity of extracts and fractions from the two medicinal plants *Ipomea batatas* and *Urena lobata* revealed that aqueous and 80% methanol extracts from the two selected medicinal plants exerted pronounced antiameobic activity by inhibiting the growth of *Entamoeba histolyca* with minimal amoebicidal concentrations (MAC) respectively of 4.25±.01 and 3.15±0.02 µg/ml, and 3.08±0.02 and 1.28±0.00 µg/ml corresponding to inhibitory concentrations IC_{50}) respective of 2.15±0.02 and 1.75±0.00 µg/ml, and IC_{50} values respectively of 2.15±0.02 and 1.28±0.03 µg/ml. Soluble fractions from the partition of aqueous extract of *I. batatas* showed antiamoebic activity with MAC and IC_{50} values ranging respectively between 6.06±0.03 to 12.63±0.01µgml, while those from *Urena lobata* vary from 5.45±0.01 to 8.54±0.03 µg/ml. All aqueous extracts from both selected medicinal plant didn't affect the concentration levels of hematological and biochemical parameters. They had not negative action on the organ weights of treated animals since their architecture was normal compared to negative control. No death of treated animals was observed and both aqueous extracts were considered as non-toxic since their lethal doses 50 (LD_{50}) were estimated to be greater than 5000 mg/kg body weight.

KEYWORDS: *Ipomea batatas*, Convolvulaceae, *Urena lobata* , Malvaceae, leaves, acute and subacute toxicity, antiamoebic activity.

1. INTRODUCTION

Amoebiasis may have been fist recognized as a deadly disease by Hypocrate (460 to 377 B.C.), who described a patient with fever and dysentery (Alam et al., 2014). From that time, invasive amoebiasis is one the worldwide most prevalent and fatal infectious diseases and it is estimated around 40 to 50 million people infected or suffer from amoebic colitis and liver abscesses worldwide, which result in 50.000 to 100.000 deaths annually (Pnganiban et al., 2014; Nyamwamu et al., 2015; Herrera-Martínez et al., 2016; Wardana et al, 2018; Gonzales et al., 2019; Nezaratizade et al., 2021).

It ranks the third position of the list of parasitic infections causing the death worldwide after malaria and schistosomiasis (Panganiban et al., 2014; Quintanilla-Licea et al., 2014; Garbi et al., 2018). It is a common infection mainly found in developing countries and predominantely affects people or individuals living with poor socioeconomic conditions, non or poor-hygienic practices and malnutrition affecting mainly children under 5 years old, i.e. living in inadequate social conditions and sanitary systems (Pananiban et al., 2014) and is a leading cause of severe diarrhea worldwide, particularly in children below 5 years of age living in low-and middle-income countries (LMICs) (Gonzales et al., 2019).. The infection is acquired through contamined food and water with the parasite *E. histolytica* and its cysts release amoebic trophozoites that may invade intestinal mucosa and disseminate through a hematogenous route to other organs like liver where they cause amoebic abscesses, but the transmission occurs through oral and anal sex and via contamined enema apparatuses (Hernández-Carlos et al., 2016; Gonzales et al., 2019). Prevalence rates of the disease are highest in developing countries in Asia, particularly in India

subcontinent, Indonesia, Sub-Saharan Africa and tropical regions of Africa, and areas of Central and South America (Petri and Singh, 1999; Gabri et al., 2015; Nyamwamu et al., 2015). In high-income countries, infection occurs primarily among returning travelers or recent immigrants from endemic regions, homo-sexuals engaging in oral-anal sexual practices, immunosuppressed people, and institutionalized individuals (Gonzales et al., 2019).

Amoebiasis is a disease caused by the parasite *Entamoeba histolytica* belonging to the phylum protozoa and is associated with high morbidity and mortality mainly in developing countries and becomes a serious and major public health problem worldwide (Quitntanilla-Licea et al., 2014). It is the third leading cause of health problem in these countries and affects more than 10% of the world's population. If it is not treated or left untreated, this disease cause severe complications including hepatic and intestinal tissue destruction (Bharti et al., (2006)**.**

Amoebic colitis appears due to *E. histolytica* infection in large intestine because of the presence of trophozoites and cysts in two cycle stages found in this protozoa. The trophozoites are found in the intestine and evolve from the cysts and invade the intestinal mucosa, spread to liver and cause amoebic liver abscesses (Sehgal et al., 1996; Nezaratizade eta al., 2021). Dysentery is any infection of the intestines, causing severe diarrhoea with blood and mucus. So, symptoms of amoebic dysentery include severe abdominal pains, diarrhea, tenderness and blood stools which can contain blood and mucus, but the disease may spread to liver and other organs resulting in death (Somalata et al., 2014; Quintanilla-Licea et al., 2014). High temperature (fever) may be another symptom, but this is not common. It may also experience loss of appetite and weight (Amebiasis explained: Symptoms, treatment, prevention (medicalnewstoday.com /article/amoebiasissymtoms, 2023)

Amoebiasis is common in tropical countries with undeveloped sanitation. It's most common in the Indian subcontinent, parts of Central and South America, Mexico, and parts of Africa. It's relatively rare in the United States. People with the greatest risk for amoebiasis include: people who have traveled to tropical locations where there's undeveloped sanitation, immigrants from tropical countries with undeveloped sanitary conditions, people who live in institutions with undeveloped sanitary conditions, such as prisons, men who have sex with other men, people with suppressed immune systems and other health conditions. While most people have no symptoms, amoebiasis can cause bloody diarrhea, colitis, and tissue destruction (https://.medicalnewstoday.com/article/amoebiasis symptoms, 2023).

The person can then spread the disease by releasing new cysts into the environment through infected feces. When symptoms occur, they tend to appear 1 to 4 weeks after ingestion of the cysts. Symptoms at this stage tend to be mild and include loss stools and stomach crampings. In a rare complication of the disease, the trophozoites may breach the intestinal walls, enter the bloodstream and travel to various internal organs. They most commonly end up in the liver, but may also infect the heart, lungs, brain or other organs. Globally, 50 million people suffer from amoebic colitis and liver abscesses resulting in 50.000 to 75.000 to100.000 death each year (Quintanilla-Licea et al., 2014; Herrera-Martínez et al., 2016; Gabri et al., 2018; Nezaratizade et al., 2021.

If trophozoites invade an internal organ, they can potentially cause: abscesses, infections, severe illness and death. If the parasite invades the lining of the intestine, it can cause amoebic dysentery. This last is a more dangerous form of amoebiasis with frequent watery and bloody stools and severe stomach crampings. Another very rare complication is fulminant necrotizing amoebic colitis, which can destroy bowel tissues and leads to bowel perforation and peritonitis. The liver is a frequent destination for the parasite, where it can cause a collection of pus called an amoebic liver abscesses. Symptoms include fever and tenderness in the upper-right part of the abdomen among other (https://www.healthline.com/health/amoebiasis symptoms, 2023).

For the treating amoebiasis, synthetic and natural products are currently used. Among synthetic drugs, Metronidazole is an effective treatment for invasive amoebiasis, but it provokes some untoward or unpleasant effects in treated patients such as headache, nausea, dry mouth, loss of appetite, diarrhoea, unpleasant smell and metallic taste or flavor as well as neurotoxicity (Bansal et al., 2004 Bautista et al., 2011; Quintanilla-lea et al., 2014; Herrera-Martínez et al., 2016; Nezaratizade et al., 2021). It is also found to kill amoeba, but is incapable to destroy the cysts, although it is considered as reference drug of choice and its resistance becoming a major public health problem is reported (Dardona and Al-Hindi, 2019). Another most commonly used drug against amoebiasis is Nitroimidazole, a combination of Tinidazole and Metronidazole and causes genotoxic effects (Marie and Petri, 2013; Nezaratizade et al., í2021). Nitazoxamide is the most recent addition and is a nitrothiazole derivative whose structure is similar to metronidazole. It has greater antiparasitic effect against various intestinal protozoal and parasitic infections compared to Metronidazole, and its effectiveness with its major metabolite Tizoxanide against both luminal and invasive forms have been demonstrated (Gonzales et al., 2019; Nezatatizade et al., 2021). Few other antiameobic drugs such as Secnidazole, Tinidazole and Ornidazole have a longer half-life and show better results against amoebic colitis caused by *E. histolytica* infection (Gonzales et al., 2019).

Besides *Entamoeba histolytica*, there is another protozoa named *Acanthamoeba castellanii* which causes also mainy infections, particularly amoebiasis. Its cysts are resistant to antimicrobial chemotherapy although several drugs such as Chlorhexidine, Amphotericin B, Pentamidine etc. are used to treat amoebiasis caused by this protozoa (Niyyati et al.; 2016). But, there are several difficulties faced by the treatment due to the toxicity of used drugs on humans, limitation of the penetration of the drug through the blood brain barrier, and the morphological modification or transformation of the trophozoites into cysts which are more though to kill (Anwar et al., 2020).This parasite seems to be treated with nanoconjugates particles such as quercetin (QT), kolavenic acid (PGEA) isolated from plant extract of *Polyalthia longifolia var pendula* and extract of crude plant methanolic extract of *Caesalpinia pulcherrima*. These plant substances are conjugated with silver nanoparticles (AgNps) to determine the effects of the natural compounds and their nanoconjugates against clinical isolate of *A. castellanii* originating keratitis patient (ATCC 50492) belonging to T4 genotype. QT, PGEA and CPLM (*Caesalpinia pulcherrima* methanol) exhibited significant amoebicidal effects while silver nanoparticles conjugation enhanced their amoebicidal activity (Anwar et al., 2020). Other medicinal plants like *Allium sativum, Arachis hypogaea, Curcuma longa Croton isabeli,, Croton pallidulus* and other, have shown antiamoebic action against this amoebae (Degerli et al., 2012, Vunda et al., 2012) and are remained promising in future for the isolation of active principles.

Medicinal plant are still invaluable source of safe, less toxic, lower price, available and reliable natural resources of drugs all over the world (Gabri et al., 2015). Thus, many medicinal plants are currently used in different traditional medicine worldwide to treat amoebiasis and the consumers found some reliefs and more of them are scientifically investigated to prove their effectiveness against *E. histolytica in vitro* and *in vivo* tests (Tona et al., 2000, Cimanga et al., 2006a, b; Gabri et al., 2018; Nyamamu et al.; 2015; Herrera-Martínez et al.; 2016; Elizondo-Luévano et al., 2018; Vardana et al., 2018)

2. MATERIALS AND METHODS

2.1. Plant materials

Fresh leaves of *Ipomea batatas* and *Urena lobata* were collected in Grand-Kasai in Democratic Republic of Congo. They were identified in INERA (Institut d'Etudes et Recherches Agronomiques) at Department of Biology, Faculty of Sciences, University of Kinshasa where their voucher specimens were deposited in the herbarium. The plant materials were dried in a hot at 50°C for 3 days and dried materials were reduced to powder using an electronic blender, and the resulting powder were kept in brown bottles hermetically closed before use to avoid contamination.

2.2. Preparation of aqueous extract and its fractionation

50 g of each powdered leaves were macerated in 200 ml distilled water for 24 h. After the recuperation of macerate by filtration on filter paper Wathman N° 1, the marc was exhaustively percolated with the same solvent. Macerate and percolate were combined and evaporated in *vacuo* to give respective dried extracts denoted as Ipa-1.1 (46.56 g) for *Ipomea batatas* and Ula-1 (47.25 g) for *Urena lobata*. 20 g of each extract were dissolved in 200 ml distilled water and filtered. Each filtrate was extracted with solvents of different polarities like chloroform, ethylacetate and *n*-butanol. All solvents were treated as described above yielding corresponding dried extracts denoted as Ipa-1.1 (2.56g), Ipa-1.2 (3.65g) Ipa-1.3 (3.05g) and Ipa-1-4 (6.12g) for *Ipomea batatas* and Ula-1.1 (2.65 g) Ula-1.2 (3.62 g), Ula-1.3 (3.11 g) and Ula-1.4 (6.35 g) for *Urena lobata* corresponding respectively to chloroform, ethylacetate, *n*-butanol and residual aqueous fractions.

(A) (B)

Figure 1: *Ipomea batatas* (A): leaves, stem and flowers *Urena lobata* (B): leaves, stem and flowers.

3. Antiamoebic testing

In vitro antiamoebic testing. *Entamoeba histolytica* strain used in this investigation is a laboratory strain isolated from patients with acute amoebic dysentery, and kindly provided by of the Institute of Tropical Medicine, Faculty of Medicine, University of Kinshasa. The parasite was grown and cultured in sterile tubes containing 9 ml of a diphasic medium (medium N of Pasteur Institute) called Dobbel and Laidlaw's medium. The mixture was stirred and incubated for one week at 37°C in a hot. The daily examination and counting of amoebae through a microscope and with the aid of Neubauer's cell respectively, were made in order to monitor parasitic growth and to detect possible contamination. Uncontaminated tubes containing an average number of amoebae of 2.5 million/ml culture medium were selected as test tubes.

For sample testing, 1 ml of test samples with known test concentration (0.1 to 500 µg/ml) was added to a series of test tubes containg 1 ml of the parasite suspension. The tubes were filled up with sterile cotton and the mixture was stirred. Each test was performed in duplicate. Two sets of controls were carried out. One control was *Entamoeba histolytica* cultured in medium without extract as negative control, and the second consisted with Metronidazole (0.05 mg/ml) in 1 ml of the parasite suspension. All tubes were plugged with sterile cotton and incubated at 37° C for one week. The counting of dead and living amoebae was done daily through a microscope with the aid of Neubauer's cell. The test was considered as positive if vegetative and cystic forms were not microscopically observed (Tona el aI., 1998, Cimanga et al., 2006a, b, c). The minimum amoebicidal concentrations (MAC) ranging from 0.01 to 500 ug/ml and inhibitory concentrations 50 (IC_{50}, µg/ml) were determined for each tested sample.

4. Acute an sub-acute toxicity evaluation

The **oral acute** toxicity study of aqueous extracts of I. *batatas* and *U. lobata* leaves were evaluated according to Organization for Economic Co-operation and Development (OECD) guideline 423 on BALB/c mice (20–30 g), using the highest test dose of 5000 mg/kg bw administered once to 10 Wistar rats weighting 140 to 150 g bw from respectively *I. batatas* and *U. lobata* leaves. All the animals were kept at overnight fasting before to every experiment with free access to water. Before doses administration, the body weight of each animal was determined and the dose was calculated according to the body weight. The animals were observed for any toxic effect for first 4 h after the treatment period. Further, animals were investigated for a period of 3 days for any toxic effect. Behavioral changes and other parameters such as body weight, urinations, food intake, water intake, respiration, convulsion, tremor, temperature, constipations, changes in eye and skin colors, etc. were observed and investigated.

The **sub-acute** toxicity study was done according to the instructions of the Organization for Economic Cooperation and Development (OECD: 407).The number of Wistar rats with the same body weight as in acute toxicity were used The animals were randomly divided into four groups. Group I (Control) received orally 5 ml distilled water/kg bw. Group IIa and b, IIIa and b and IVa a b (**a** pour *I. batatas* and **b** pour *U. lobata*) I. received separately daily and orally respectively the doses of 500, 1000 and 5000 mg/kg bw of aqueous extract Iba-1 from *I. batatas* and Ula-1 from *U. lobata* leaves. All doses were administered daily for 28 days by oral gavaging. After the first dose, animals were observed for mortality, signs of toxicity, and behavioral changes (aggression, unusual vocalization, agitation, sedation and somnolence, convulsions, tremors, ataxia, catatonia, paralysis, fasciculation, prostration and unusual locomotion, abnormality in food consumption, and asphyxia) for the first 4 h, and finally periodically up to 48 h.

5. Hematological and biochemical examination

On 29th day, all the animals were sacrificed by an anesthesia (diethyl ether) after an overnight fasting (6 h). The blood sample was collected into test tube with and without ethylene diamine tetra acetic acid as an anticoagulant respectively for biochemical and hematological parameters. The blood without the ethylene diamine tetra acetic acid (EDTA) was used to evaluate biochemical parameters, allowed to clot after centrifugation at 2 500 rpm/min for 15 min to obtain serum and stored at -20 °C until assayed for biochemical estimation and without EDTA for hematological parameters analysis. After collecting the blood, all the vital organs such as liver, kidney, heart, pancreas and small intestine were separated, weighed each organ on electronic balance and relative organ body weight of both test treated groups were determined and compared to control group. The relative organ weight (ROW) of each organ was calculated (Abotsi et al., 2011; Kifayatullah et al., 2015).

4. RESULTS AND DISCUSSION

4.1. Antiamoebic activity of the two selected medicinal plants *I. batatas* and *U. lobata* leaves

E. histolytica was cultured in Dobbel and Laidlaw's medium and tested against extracts and fractions from *I. batatas* and *U. lobata* leaves. Following criteria were resounded and adopted: MAC, $IC_50 \leq 10$ µg/ml: pronounced activity, $10 < MAC, IC_{50} \leq 20$ µg/ml: good activity, $20 < MAC, IC_{50} \leq 30$ µg/ml: moderated activity, $30 < MAC, IC_{50} \leq 40$ µg/ml: weak activity, $40 < MAC, IC_{50} \leq 50$µg/ml: very weak activity, MAC, $IC_{50} > 50$ µg/ml: inactive.

Reported results in the present study revealed that aqueous Iba-1 and 80% methanol Iba-2 extracts exhibited pronounced antiamoebic activity with minimal amoebicidal concentrations (MAC) and inhibitory concentrations 50 (IC_{50}) values respectively of 4.25 and

2.54±0.00, and 2.15±0.02 and 1.28±0.03 µg/ml showing that the second extract showed higher activity compared to the first one. Moreover, soluble fractions from *I. batatas* produced also the same biological effects. Indeed, chloroform soluble fraction Iba-1.1 rich in steroids and terpenoids showed good antiamoebic activity with MAC value of 10.25±0.03 µg/ml and its activity was considered as pronounced since its produced an IC_{50} value of 5.66±0.03 µg/ml. Ethlyacetate soluble fraction Ila-1.2 rich in flavonoids exhibited pronounced activity against *E. histolytica* by inhibiting its growth with MAC and IC_{50} values respectively of 6.23±0.00 and 3.65±0.03 µg/ml while *n*-butanol Iba-1.3 soluble fraction rich in saponins exerted the same effect by inhibiting the growth of the tested parasite with MAC and IC_{50} values of 12.65±0.01 and 6.38±0.00 µg/ml respectively as good and pronounced effect in regarding its MAC and IC_{50} value. Residual aqueous Ib-1.4 soluble fraction displayed pronounced antiamoebic activity with MAC and IC_{50} values of 5.45±0.01 and 2.71±0.03 µg/ml.

Table 1: Antiamoebic activity of extracts and fractions from *I. batatas* and *U. lobata* leaves.

Extracts and fractions	Minimal amoebicidal concentrations (µg/ml)	Inhibitory concentrations 50 (IC_{50}, µg/ml)
Ipomea batatas		
Iba-1	4.25±0.01	2.15±0.02
Iba-1.1	10.25±0.03	5.66±0.03
Iba-1.2	6.23±0.00	3.65±0.03
Iba-1.3	12.65±0.01	6.38±0.00
Iba-1.4	6.06±0.03	3.04±0.02
Iba-2	3.08±0.02	1.53±0.00
Urena lobata		
Ula-1	3.15±0.02	1.76±0.00
Ula-1.1	8.15±0.01	4.73±0.01
Ula-1.2	5.75±0.02	2.82±0.03
Ula-1.3	8.54±0.03	4.17±0.02
Ula-1.4	5.45±0.01	2.71±0.03
Ula-2	2.54±0.00	1.28±0.03
Metronidazole	0.05±0.01	0.03±0.00

With regards on samples from *Urena lobatata*. It was observed that aqueous Ula-1 and 80% methanol Ula-2 extracts exerted pronounced antiamoebic activity with MAC and IC_{50} values respectively of 3.15±0.02 and 2.54±0.00, and 1.76±0.00 and 1.28±0.03 µg/ml suggesting that 80% methanol Ula-2 extract showed higher activity compared to aqueous Ula-1 extract. Its soluble fractions acted the same manner in inhibiting the growth of the protozoa *E. histolytica* with different magnitudes. In fact, chloroform Ula-1.1 soluble fraction showed pronounced antiamoebic activity with MAC and IC_{50} values of 8.15±0.01 and 4.73±0.01 µg/ml while ethylacetate Ula-1.2 soluble fraction had the same level of activity by inhibiting *E. histolytica* growth with MAC and IC_{50} values of 5.75±0.02 and 2.82±0.03 µg/ml. n-butanol Ula-1.4 and residual aqueous Ula-1.4 displayed also pronounced activity with MAC values respectively of 8.54±0.03 and 5.45±0.01 µg/ml and IC_{50} values respectively of 4.17±0.02 and 6.39±0.01 µg/ml.

4.2. Effects of acute and sub-acute toxicity exerted by aqueous extracts Iba-1 and Ula-1 from the two selected medicinal plants *I. batatas* and *U. lobata* leaves

Acute toxicity: The acute toxic effect of aqueous extracts was determined as per the OECD guideline 423, by administration once, the highest oral dose of 5000 mg/kg bw. No treatment related toxic symptom or mortality were observed after oral administration of this highest oral dose. The general behavioral of the extract treated animals and control group was observed first for short period of 4 h followed by long period 3 days. After this monitory period, it did not display any drug related changes in behavior, breathing, skin effects, water and food consumption, impairment in food intake and temperature. Therefore, administered aqueous extracts from the two selected studied medicinal plants, seemed to be safe and devoid of any visible toxic effect at a dose level of 5000 mg/kg bw, and the lethal dose 50 (LD_{50}) was estimated be >4 000 mg/kg. However, there were no sign of sedation, lethargy and drowsiness after the administration of plant extracts at dose of 5000 mg/kg bw compared to control group. The parameters observed for acute toxicity study after the administration of the test plant extracts were compared with normal or negative group and presented in (Table 2).

The sub-acute toxic study of the tested plant extract was determined as per OECD guideline 407. All the tested group animals treated with plant extracts at all administered oral doses of 500, 1 000 and 5000 mg/kg bw daily survived throughout the 28 days. No clinical toxicity signs were observed in the both plant extracts treated groups compared to the control group. All observations done in acute toxicity were the same observed in sub-acute toxicity and are valid. No death of treated animals was observed after 28 days of

observation i.e. all animals survived in good state and gained body weight as illustrated in Figure 2.

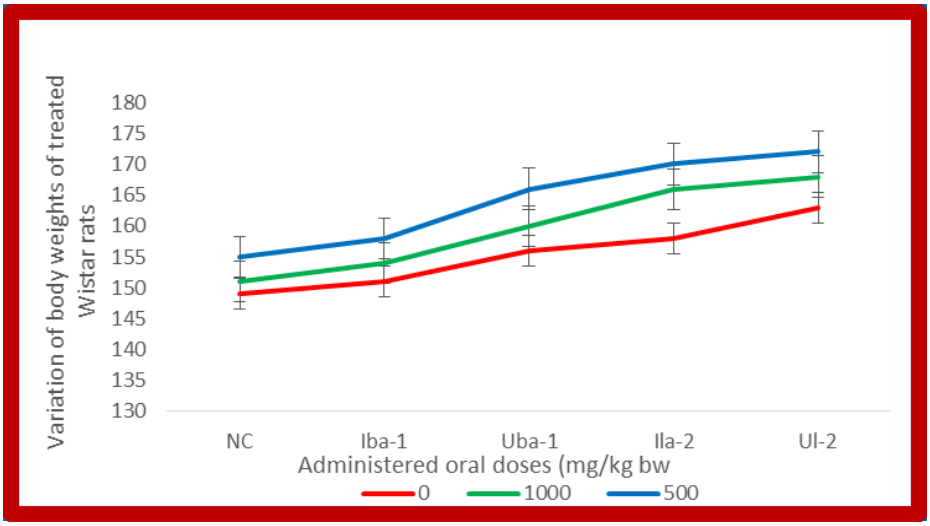

Figure 2: Variation of body weights OD treated Wistar rats with aqueous Ila-1 and Ula-1 of *Ipomea batatas* and *Urena lobata* leaves respectively.

General appearance and behavioral observations revealed the normality of body weight, digestion, eye color, food intake, general physique, locomotion and rate respiration and didn't known any modification even little. Total absence of asphyxia, catatonia, coma,, convulsions, death, drowsiness, diarrhea, gastro-intestinal disorders, paralysis and sedation were noted. These observations suggested that the treated animals were healthy.

Table 2: General appearance and behavioral observations of acute toxicity study for control and treated groups.

Observation	Control group	500 mg/kg	1000 mg/kg	5000 mg/kg
Asphysia	Absent	Absent	Absent	Absent
Body weights	Normal	Normal	Normal	Normal
Death	Absent	Absent	Absent	Absent
Drowsiness	Absent	Absent	Absent	Absent
Catatonia	Absent	Absent	Absent	Absent
Change color in skins	Absent	Absent	Absent	Absent
Coma	Absent	Absent	Absent	Absent
Convulsions	Absent	Absent	Absent	Absent
Diarrhoea	Absent	Absent	Absent	Absent
Digestion	Normal	Normal	Normal	Normal
Eye colors	Normal	Normal	Normal	Normal
Food intake	Normal	Normal	Normal	Normal
Gastro-intestinal disorders	Absent	Absent	Absent	Absent
General physique	Normal	Normal	Normal	Normal
Paralysis	Absent	Absent	Absent	Absent
Rate of respiration	Normal	Normal	Normal	Normal
Locomotion	Normal	Normal	Normal	Normal
Sedation	Absent	Absent	Absent	Absent

4.3. Effect of plant extract on relative organ body weight

There was significant difference in average organs and relative organ weights between control and extracts treated groups at all administered oral doses in sub-acute toxicity ($p < 0.05$) of

Table 3: Average organ weights (g) of oral administration of aqueous extracts of *I. batatas* Wistar rats.

Organs	Relative organ weights		
	Negative control	Ila-1 and Ula-1 : 1000 mg/kg extract	Ila-1 and Ula-1 : 5000 mg/kg extract
Liver	3.520 ± 0.01	3.543 ± 0.01	3.588 ± 0.03
Kidney	1.340 ± 0.01	1.345 ± 0.03	1.350 ± 0.02

Pancreas	0.534 ± 0.01	0.552 ± 0.03	0.557 ± 0.00
Heart	0.642 ± 0.002	0.648 ± 0.02	0.652± 0.01
Intestine	2.593 ± 0.01	2.588 ± 0.02	2.602 ± 0.02

Table 4: Average organ weights (g)of oral administration of aqueous extracts of *U. lobata* Wistar rats.

Organs	Relative organ weights		
	Negative control	Ila-1 and Ula-1 : 1000 mg/kg extract	Ila-1 and Ula-1 : 5000 mg/kg extract
Liver	3.532 ± 0.00	3.545 ± 0.02	3.590 ± 0.03
Kidney	1.3404± 0.02	1.348 ± 0.00	1.357 ± 0.01
Pancreas	0.535 ± 0.01	0.558 ± 0.02	0.560 ± 0.02
Heart	0.641 ± 0.002	0.646 ± 0.01	0.654± 0.02
Intestine	2.592± 0.01	2.60± 0.00	2.602 ± 0.02

1000 and 5000 mg/kg bw from respectively *I. batatas* and *U. lobata* leaves (Tables 3 and 4) . All vital organs of treated animals gained body weights compared to negative control. The results revealed that, the vital organs such as liver, kidney, heart, pancreas and small intestine were not adversely affected or altered through the treatment by the two aqueous administered extracts from respectively *I. batatas* and *U. lobata* leaves suggesting their safety.

2.6. Effect of plant extract on hematological parameters

Red blood cell count, hematocrit, mean cell volume, hemoglobin, white blood cell count, hemoglobin, monocytes, neutrophils, lymphocytes and platelets of the control and plant treated groups were determined and compared with control group using an automatic haemotology analyzer (Sysmex K21, Tokyo, Japan). All evaluated hematological parameters in treated Wistar rats at the highest oral dose of 5000 mg/kg bw showed significant difference compared to negative control ($p < 0.05$).

The administrations of *I. batatas* and *U. lobata* extracts exerted significant ($P < 0.05$) increase of HGB concentrations respectively 17.4±0.1 and 17.9±0.1 g/dl, RBC respectively to 8.7±0.2 and 8.9±0.2 due probably by the consumption of iron by treated, and WBC to 17.4±0.0 anf 18..9±0.3 compared with negative control respectively. Elevated hemoglobin didn't usually cause symptoms, but it can cause complications, including blood clots. A high hemoglobin level is often an indicator of diseases, including polycythemia vera, cancers, heart diseases, lung diseases, and kidney or liver diseases.(https://www.veryhealth.com/high-hemoglobin-5211560#~:a-test=Elevated/cohemoglobin.in%20doesn't%20ususaaly%20caus..., 2023.

A significant increase for RBC and WBC after administration of aqueous extracts of *I. batatas* and *U. lobata* was also observed. Clinically, meaningful increment of RBC concentration was observed, although it did not reach statistical significance compared with the control. White blood cells were a part of the human immune system that protects the body from various infections. These cells circulated through the human bloodstream and tissues to respond to injury or illness by attacking any unknown organisms that enter the body (https://my.clevelanclinic.org:health/body/21871-white-blood-cells, 2023).

The extracts of *I. batatas* revealed a significant decrease in platelet counts compared with the negative control ($p < 0.05$). For this, when the skin is injured or broken, platelets clump together and form clots to stop the bleeding. When there was not enough platelets in the blood supply, the body can't form clots. A low platelet count is called thrombocytopenia. This condition can range from mild to severe, depending on its underlying cause. Some people with thrombocytopenia may not experience any symptoms, and for more severe cases, uncontrollable bleeding can result in death. Thrombocytopenia can be caused by a range of factors such as pregnancy, medical conditions such as leukemia, or certain medications (such as blood thinners). As a result, there are multiple treatment options for thrombocytopenia which may differ depending on the root cause of the condition. control ($p < 0.05$) (Tables 5) (https://www.bing.com/search?qoconsequnces+of+desease+of.boteletewid=8551a80abcd4989a96144088&aqs=edge1. 2023. On the other hand the administration of aqueous extract of *U. lobata* provoked significant increase of platelets compared to negative In this case, thrombocytosis or a high platelet count, is diagnosed when platelet levels were greater than 450,000 platelets per microliter of blood. Many times, a high platelet count didn't cause any symptoms or mean something serious was going on. However, having a high platelet count can lead to clotting problems and, in some cases, may be associated with serious conditions, like cancer (Tables 6) (https///www.verywellhealth.com/when-to-wrong-about-high-plaquetel.count-5186732, 2023).

Red blood cells (RBC), white blood cells (WBC), neutrophils, eosinophils, lymphocytes monocytes and segmented leucocytes also knew significant increase compared to negative control ($p < 0.05$), effect due to the oral administered of aqueous extracts of *I. batatas* and U. *lobata* leaves at the highest oral dose of 50000 mg/bw (Tables 5 and 6).

Table 5: Effect of aqueous extract Iba-1 of *I. batatas* on hematological parameters.

Parameters	Negative control	Ila-1: 5000 mg/kg bw	Reference values
RBC ($x10_6$ μL^{-1})	8.7±0.2	9.2±0.1	7.6-1.029
WBC (x 10^3 μL^{-1})	17.9±0.3	17.3±0.0	6.6-20.5
Hematocrit (g/dl)	45.0±	47.1±0.2	40.7-50
Hemoglobin (g/dl)	16.5±0.2	17.4±0.1	15-18.2
Platelets (x 103 μL^{-1})	1371.0±0.2	1258.2±0.3	995-1713
Neutrophils (%)	22.2±0.3	24.0±0.1	3-4.7
Basophils (%)	0.0±0.0	0.0±0.0	0.0±0.0
Eosinophils (%)	1.2±0.1	1.6±0.4	0-2
Lymphocytes (%)	87.2±0.2	90.8±0.3	28.8-94
Monocytes (%)	3.2±0.1	3.5±0.0	0.-4
Segmented leucocytes (%)	16.5±0.3	20.7±0.2	-

Table 6: Effect of aqueous extract Iba-1 of *U. lobata* on hematological parameters.

Parameters	Negative control	Ula-1: 5000 mg/kg bw	Reference values
RBC ($x10^6$ μL^{-1})	8.9±0.2	9.6±0.1	7.6-1.029
WBC (x 10^3 μL^{-1})	18.9±0.3	18.7±0.0	6.6-20.5
Hematocrit (g/dl)	47.0±0.3	47.8±0.2	40.7-50
Hemoglobin (g/dl)	17.5±0.0	17.9±0.1	15-18.2
Platelets (x 103 μL^{-1})	1471.0±0.2	1486.3±0.3	995-1713
Neutrophils (%)	23.2±0.3	24.1±0.1	3-4.7
Basophils (%)	0.0±0.0	0.0±0.0	0.0±0.0
Eosinophils (%)	1.6±0.1	1.8±0.4	0-2
Lymphocytes (%)	89.5±0.2	93.0±0.3	28.8-94
Monocytes (%)	3.5±0.1	3.7±0.0	0.-4
Segmented leucocytes (%)	17.5±0.6	22.7±0.2	-

2.7. Effect of plant extract on serum biochemical parameters

The biochemical analysis were done on serum after centrifugation of collected blood and the following parameters like aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase, high density lipoproteins, total bilirubins (T-BIL), total proteins, albumins, urea and creatinine level were determined for both control and extracts treated groups. All analyses were determined on using clinical chemistry analyzer (Vital Scientific, Netherlands For biochemical parameters, it was observed that the oral administration of both aqueous Ia-1 and Ula-1 from respectively *I. batatas* and *U. lobata* leaves cause significant decease or reduction of glucose level of treated animals comapred to negative control ($p < 0.05$) (Table 9). This effect may be due to the hypoglycemic effect that possessed both extracts which can be exploited for the treatment in future the diabetes type 2.

Table 9: Effects of aqueous extract Ia-1 from *I. batatas* on les biochemical parameters biochemical.

Parameters	**Negative control**	**Ia-1:5000 mg/kg bw**	**Uba-1: 5000 mg/kg bw**
Albumins (g/dL)	3.70 ± 0.0 3	3.60 ± 0.04	3.60±0.04
ALT (IU)	176.50±0.01	177.7±0.02	177.7±0.02
AST (IU)	51.70±0.02	50.51±0.05	50.53±0.03
Direct bilirubins (mg/dL)	0.20 ± 0.00	0.21± 0.0	0.20±0.01
Cholesterol totals (mg/dL)	100.70 ± 0.02	102.7 ±0.03	104.0±0.02
Glucose (mg/dL	244.1 ± 0.02	238.81 ± 0.04	289.02±0.02
LDL (mg/dL)	38.50±0.01	64.3±0.03	66.60±0.04
HDL (m/mL)	64.30±0.03	66.60±0.03	68.3±0.00
PAL (mg:dL)	144.40±00.3	145.80±0.00	145.80±0.02
Triglycerides (mg/dL)	44.10 ± 0.00	44.3 0± 0.05	46.3±0.01
Total bilirubins (mg/dL)	0.50 ± 0.01	0.50 ± 0.0.4	53.45±0.02
Total Protein totals (g/dL)	7.80 ± 0.01	8.40 ± 0.01	43.50±0.02
Urea (mmo/L)	5.40±0.03	6.30±0.03	6.20±0.03
Uric acid (mmol/L)	5.80±0.01	6.0±0.05	6.40±0.03

ALT: alanine transaminase, AST: aspartate transaminase, LDL: low density lipoproteins, HDL: high density lipoproteins, PAL: phosphate alkalin.

Cholesterol and LDL cholesterol knew significant decrease while HDL cholesterol knew significant increase. This observation suggested that the administered extract can prevent cardiovascular diseases. Low-density lipoprotein (LDL**)** was the "bad," unhealthy kind of cholesterol. LDL cholesterol can build up in your arteries and form fatty, waxy deposits called plaques. High-density lipoprotein (HDL**)** is the "good," healthy kind of cholesterol. It transports excess cholesterol out of the arteries to the liver, which removes it from the body (https://healthline.com /health/cholesterol/effects-on-body, 2023).

Many medicinal plants extracts for which antiamoebic activity was scientifically and chemically studied leading to the isolation of active principles. This was the case of *Carica papaya* seeds for which the activity was attributed to an alkaloid carpasemine (Etkin et al.,19822), *Euphotbia hirta* whole plant attributed to a substance E structurally similar to choline (Krishina-Rao and Ganapaty, 1983), (-)-epicatechin, (-)-epigallocatechin and catechin from some medicinal plant species (Calzada et al, 1999), alkaloids cryptolepine, hydroxycryptolepine, quindoline, cryptoquidoline, bisryptocryptolepine from *Cryptolepis sanguinolenta* (Cimanga et al. 2018) and other, iridoids specioside, minecoside, verminoside from *Kigella pinnata* (Bharti et al., 2006) and iridoids acethylgaertneroside, gaertneric acid, gaertneroside, epoxymethoxygaertnerodise, methoxygaertneroside from *Morinda morindoides* leaves (Cimanga et al., 2006a), and other, series of flavonoids quercetin and its diglycosides, Kampferol and its diglycosides, luteolin and its glycosides, apigenin and its glycosides, chrysin and its glycoside from *Morinda morindoides* leaves (Cimanga et al., 2006b) et other.

CONCLUSION

This is the first report of the antiameobic activity of two medicinal plant *Ipomea batatas* and *Urena lobata* leaves, used as natural antidiarrhoeal agents in traditional medicine. Reported results indicated that aqueous and 80% methanol extracts as well as soluble fractions from the partition of each aqueous extract, exerted good antiamoebic activity at different extents. Both aqueous extracts had not visible noxious or deleterious effects on haemotological and biochemical parameters and didn't alter organ weights and the form these organs. They were considered to be devoid with toxic effect and no mortality of treated animals was observed leading to the estimation its lethal dose 50 (LD_{50}) greater than 5000 mg/kg body weight. Thus, the results can partly support and justify the use of both medicinal plant leaves in aqueous macerate form to treat diarrhea in traditional medicine in Democratic Republic of Congo and other African countries and in the world where they knew the same medical purpose.

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