

**ANTIDIARRHEAL ACTIVITY OF EXTRACTS AND FRACTIONS OF IPOMOEA
BATATA LEAVES (CONVOLVULACEAE) IN ANIMAL MODEL*****Cimanga Kanyanga R^{1,2}, Ndala Kasanda N¹,**¹Department of Medicinal Chemistry and Pharmacognosy, Laboratory of Pharmacognosy and Phytochemistry, Faculty of Pharmaceutical Sciences, University of Kinshasa, PO.Box 212, Kinshasa XI, Democratic Republic of Congo.²Department of Pharmaceutical Sciences, Laboratory of Natural Product & Food Research and Analysis (NaturA), University of Antwerpen, Universiteitsplein 1, B-2610, Wilrijk, Antwerpen, Belgium.***Corresponding Author: Cimanga Kanyanga R**

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ABSTRACT

The present study involved the assessment of antidiarrheal activity of extracts and fractions as well as acute and sub-acute toxicity of aqueous extract from *Ipomea batatas* leaves in animal model. In castor-oil-induced diarrhea, results showed that aqueous and 80% methanol extracts, and chloroform, ethylacetate, *n*-butanol and residual aqueous soluble fractions from *I. batatas* leaves administered at the highest oral dose of 200 mg/kg body weight, delayed significant diarrhea and defecation production onset times from 134.4±0.2 to 152.2±0.2 minutes (min). They next caused marked decrease of diarrheal parameter levels wet and hard faeces to 1.2±0.3 to 3.3±0.3 and 1.2±0.3 to 2.8±0.3 respectively, and secreted intestinal fluid volume to 0.1±0.0 to 0.3±0.1 respectively. They produced 66.7±0.0 to 89.0±0.1% and 68.0±0.2 to 90.1±0.2 % inhibition of diarrhea and defecation production respectively. On the other hand, the administration of Loperamide as the reference antidiarrheal product at oral dose of 5 mg/kg body weight, produced prominent enhancement of diarrhea and defecation onset time to 157.3±0.3 min with prominent decrease of diarrheal parameter levels wet and hard faeces to 1.1±0.2 and 0.7±0.2 respectively, and secreted intestinal volume to 0.1±0.0. All compared to negative control showing low onset time of 87.2±0.3 min and high diarrheal parameter levels as wet and hard faeces to 11.2±0.4 and 9.7±0.3 respectively, associated to secreted intestinal fluid volume of 1.5±0.3. It produced 91.0±0.1 and 92.0±0.2% inhibition of diarrhea and defecation respectively. In magnesium sulphate-induced diarrhea, all sampled from *I. batatas* induced marked delaying of diarrhea and defecation onset time from 136.4±0.1 to 156.3±0.1 min with prominent reduction of diarrheal parameter levels of wet and hard faeces varying from 1.2±0.3 to 3.4 and 1.5±0.3 to 3.2±0.1 respectively, and secreted intestinal fluid volume ranged between 0.1±0.0 and 0.3±0.1. Loperamide showed remarkable effects by delaying diarrhea and defecation onset time to 160.3±0.1 min, in significantly decreasing at the same time diarrheal parameter levels of wet and hard faeces to 1.2±0.1 and 0.9±0.2 respectively followed by secreted intestinal fluid volume of 0.1±0.0 compared to negative control showing low onset time of 93.3±0.1 min, accompanied with high diarrheal parameter levels of wet and hard faeces of 10.2±0.2 and 7.7±0.5 respectively and secreted intestinal fluid volume of 2.5±0.3. In addition, Loperamide (5 mg/kg body weight) and these samples from *I. batatas* (200 mg/kg body weight) caused significant inhibition of gastro-intestinal motility and enteropooling. These reported results clearly demonstrated that Loperamide, extracts and fractions from *Ipomea batatas* possessed interesting antidiarrheal properties and can be used in traditional medicine for the treating of diarrhea in African countries where it known this medical use.

KEYWORDS: *Ipomea batatas*, leaves, Extract, fractions, diarrhea, antidiarrheal activity, gastro-intestinal motility, enteropooling.**1. INTRODUCTION**

Diarrhea is generally defined as the passage of abnormally liquid or unformed stools associated with increased frequency of defecation, and abdominal pains (Biru et al., 2016). It is the passage of three or more loose stools and characterized by increased gastro-intestinal motility and secretion and a decrease in the absorption of fluid and electrolytes (Mekonnen et al.,

2018). Diarrheal disease is one of the leading causes of preventable death in developing countries, and it mainly affects children and infants (May and Cisholm-Burns, 2020). In the intervention of diarrhea, antimotility and antisecretory agents remain as the main agents used to decrease such pathophysiologic changes (Formiga et al., 2017; Mekonnen et al., 2018).

Despite the reductions in morbidity and mortality worldwide, diarrhea still accounts for more than 2 million deaths annually and is associated with impaired physical and cognitive development in resource-limited countries (Biru *et al.*, 2016). Concerning the death, many studies had reported a variable number of death in million mainly for children under 5 years in Africa Sub-Saharan and South-East Asian regions each year (WHO, 2015; Lalid Labu *et al.*, 2015; Biru *et al.*, 2016, Derebe *et al.*, 2018, Kola-Mustapha *et al.*, 2019; de Souza Pessoa *et al.*, 2020).

Diarrheal disease is characterized by frequent defecation of faeces of low consistency, which may be due to a disturbance in the transport of water and electrolytes in the intestines. Though, there are multiple causes for diarrheal disease and include infection by bacteria, infections by other organisms (helminths and parasites) and pre-formed toxins, eating contaminated foods and that upset the digestive system, allergies and intolerances to certain foods (Celiac disease or lactose intolerance), medications, radiation therapy, malabsorption of food (poor absorption), diabetes, alcohol abuse, diseases of the intestines (such as Crohn's disease or ulcerative colitis, laxative abuse, overactive thyroid (hyperthyroidism), some cancers, surgery on your digestive system, trouble absorbing of certain nutrients, also called malabsorption. The diarrhea may also follow constipation, especially for people who have irritable bowel syndrome, unhygienic environment, infected food, contaminated drinking water with microorganisms (Kola-Mustapha *et al.*, 2019; <https://my.clevelandclinic.org/health/diseases/4108-diarrhea>, 2021, <https://www.webmed.com/digestive-disorders/digestives-diseases-diarrhea>, 2021). Its symptoms include: bloating in your belly, cramps, thin or loose stools, watery stools, an urgent feeling that you need to have a bowel movement, and throwing up and more serious symptoms including: blood or mucus in your stool, weight loss and fever. If you have watery stools more than three times a day and you're not drinking enough fluids, you could become dehydrated. This situation can be a serious problem if it is not correctly treated (<https://my.clevelandclinic.org/health/diseases/4108-diarrhea>, 2021). Clinically, diarrhoea may result from disturbed bowel functions, in which cases, there is impaired intestinal absorption, excessive intestinal secretion of water, electrolytes and a rapid bowel transit (Tadesse *et al.*, 2017).

However, diarrhea can be serious Preoccupation in certain groups of people, including young children, older adults (the elderly) and those with medical conditions. For each of these people, diarrhea can cause other health problems.

It Exit 3 types of diarrhea: **Acute diarrhea:** The most common, acute diarrhea is loose watery diarrhea that lasts one to two days. This type doesn't need treatment and it usually goes away after a few days. **Persistent diarrhea:** This type of diarrhea generally persists for

several weeks – two to four weeks. **Chronic diarrhea:** Diarrhea that lasts for more than four weeks or comes and goes regularly over a long period of time is called chronic diarrhea (Mekomen *et al.*, 2018; <https://my.clevelandclinic.org/health/diseases/4108-diarrhea>, 2021).

The experience symptoms of dehydration include: dark urine and small amounts of urine or loss of urine production, rapid heart rate, headaches, flushed, dry skin, irritability and confusion, light-headedness and dizziness, and severe nausea and vomiting, the inability to tolerate or keep anything down by mouth, dry mouth and tongue, no tears when crying, no wet diapers for three hours, sunken eyes, cheeks, sunken soft spot on top of skull and listlessness or irritability (<https://my.clevelandclinic.org/health/diseases/4108-diarrhea>, 2021, <https://www.mayaclinic.org/diseases-condition/d/dehydration/symptoms-causes/syc-20354086>).

The 4 major mechanisms behind the pathophysiology of diarrhoeas are **(a)** osmotic diarrhea, which is caused by increase in intraluminal osmolarity and decrease in water absorption, **(b)** secretory diarrhea, which increases the secretion of electrolytes, **(c)** deranged intestinal motility causing a decreased transit time and **(d)** inflammatory and infectious diarrhea, which is caused by disruption of the epithelium of the intestine due to bacterial, viral, or protozoal pathogens and the immune response to inflammatory conditions in the bowel (Abdela *et al.*, 2019). In the management of diarrhea, antimotility and antisecretory agents are considered to be the mainstay agents used to decrease the pathophysiologic conditions responsible for the development of diarrhea as already mentioned above (Formiga *et al.*, 2017; Mekonnen *et al.*, 2018).

The World Health Organization (WHO) and UNICEF reported that the digestive system disorders and diarrhoea is a leading killer of children, accounting for approximately 8 to 9% of all deaths among children under age 5 worldwide particularly in 2017. This translates to over 1.300 young children dying each day, or about 480.000 children each year, despite the availability of a simple treatment solution (<https://data.unicef.org/topic/cilge-health/diarrhea-disease>, 2021). Diarrheal illnesses are one of the main reasons for morbidity and mortality in developing countries and are accountable for the death of hundreds of thousands of people every year (Haque *et al.*, 2013). According to this report, sub-Saharan Africa and southern Asia are recorded as the regions that experienced the highest child death toll as a result of diarrhoea (Liu *et al.*, 2016, Abdela, 2019). Moreover, according also to the WHO and UNICEF reports, there are about 2.5 billion cases of diarrheal disease worldwide every year, and 1.9 million children below 5 year of age die from diarrhea each year, of whom most are from developing countries and 78 % occurred in these regions,

creating a tremendous economic strain on healthcare costs (WHO, 2010, 2015; Biru *et al.*, 2016; Mekonnen *et al.*, 2018). It is estimated 17.5–21 % of all deaths in children under the age of 5 years, equivalent to 1.5 million deaths per year of infants and children for instance in developing countries. Although the reported results for death are variable, they well show that the disease remains a big health problem mainly in developing countries (Biru *et al.*, 2016).

Despite the introduction and use of many synthetic antidiarrheal drugs accompanied with various side effects like dizziness, drowsiness, tiredness, or constipation, nausea, vomiting, stomach, abdominal pains, uncomfortable fullness of the stomach, abdomen, fast, irregular heartbeat, severe dizziness, fainting and body weight loss, etc. (<https://www.webmed.com/drug/2drug/drug-56333/anti-diarrhea-oral/details#;□;text=dizziness%2C%20tiredne...>, 2021) and the irrespective of great technological development in modern medicine, generally, 80% of people mainly in developing countries rely on healing practices and medicinal plants for their daily health care needs (Ekor *et al.*, 2013; Abdela, 2019). Due to the increasing side effects and development of resistance to orthodox medicine, new and safe drug sources must be discovered from plant origin. Medicinal plants are usually preferred to treat gastro-intestinal disorders, for example, dyspepsia, amoebiasis, constipation and diarrhea because they contain multiple constituents with effect-enhancing and/or side effect-neutralizing potential and, hence are considered relatively safe and non-toxic in prolonged use (Biru *et al.*, 2016; Zewdie *et al.*, 2020).

Various preventive ways or techniques for diarrhea were reported in the literature and included hygiene, sanitation, diet, medication and supplements classified as health care, breastfeeding, immunization, supplemental zinc and probiotic could be used as the simple use of remedies such as oral rehydration solution (ORS) could be taken to reduce the number of mortality (Kola-Mustapha *et al.*, 2019).

On the other hand, medicinal plants are a promising source of new antidiarrheal drugs. For this reason, the WHO has encouraged studies pertaining to the treatment and prevention of diarrheal diseases using traditional medical practices. Currently available drugs are linked with transient adverse effects and contraindications: bloating, constipation, loss of appetite, loss of body weight, stomach pains (severe) with flatulence, nausea and vomiting (<https://www.druga.com/sfx/anti-diarrheal-side-effects.html>, 2020). Drug resistance is another challenge to think about antibiotics used in the treatment of diarrhea.

The high incidence of diarrhea in developing countries coupled with limitations of currently available antidiarrheal drugs and poor healthcare coverage may

make traditional medicines as good alternative agents for the management of diarrhea.

The use of traditional medicines to combat the consequences of diarrhea has been employed by WHO in its Diarrhea Control Program (Abdela, 2019). Herbal medicines are readily available in Africa and used easily by people to treat different ailments, among diarrhoea. However, there are few scientific data to support the safety and effectiveness of some of them. In order to ensure safety, there must be a study to show safety profiles of herbs claimed to be beneficial to humans and the animals before deciding to use them (Alelign *et al.*, 2020). Acute, sub-acute and sub-chronic toxicity studies are one of the ways to assess the safety and evaluate the effects of medicinal plant extracts and many substances upon multiple exposures (Ebohon *et al.*, 2020).

There has been increased global interest in traditional medicine and there are efforts underway to monitor and regulate herbal drugs and traditional medicine (Azaizeh *et al.*, 2010). Due to reliance of the society of resource limited areas still on herbal medicine for their health care needs, WHO recommended the integrated use of folk and modern medicine for controlling of health problems (Azaizeh *et al.*, 2010, Biru *et al.*, 2016). Herbal medicines have been used for treating diarrheal diseases, and it is estimated that up to 80% of the population in developing countries depend on traditional medicines for primary healthcare as well as also in developed countries (Codier and Stenkamp, 2012, Porwal *et al.*, 2016) as already mentioned above. There are an enormous number of herbal medicines around the world that are claimed to be effective in treating diarrhea (Mekonnen *et al.*, 2018). To revalorise these medicinal plants, nowadays, several scientific investigations are undertaken to prove the acclaimed antidiarrheal properties of various medicinal herbs used empirically to treat diarrhoea and several reports have demonstrated that they are well endowed with this biological activity at different extents and in same cases, active principles are isolated and reported (Islam *et al.*, 2013; Al Harbi and El-Ashmawy, 2015; Jalilzadeh-Amin *et al.*, 2015; Konaté *et al.*, 2015; Asrie *et al.*, 2016; Prabhu *et al.*, 2017, Sadraei *et al.*, 2018, Zhao *et al.*, 2018; Teferi *et al.*, 2019).

Thus, the present investigation deals with the assessment of antidiarrhoeal activity of *Ipomoea batatas* leaves aqueous extracts and its fractions, and 80% methanol extract against castor-oil and magnesium sulphate - induced diarrhoea, gastro-intestinal motility and enteropooling in experimental rats.

2. MATERIALS AND METHODS.

2.1. Vegetal material and identification

Leaves of *Ipomoea batatas* were collected in Central Kasai, one province of Democratic Republic of Congo. The plant was authenticated in INERA (Institut National d'Études et de Recherches Agronomiques) in Departement of Biology, Faculty of Sciences, University

of Kinshasa. A voucher specimen was deposited in the herbarium of this institute and in the laboratory of Pharmacognosy and Phytochemistry of the faculty of Pharmaceutical Sciences of the same university. The

plant material was dried at room temperature and reduced to powder using an electronic blender and the resulting powder was kept in burn bottles.



Figure 1: *Ipomoea batatas* leaves, flowers and potatoes.

2.2. Preparation of extracts and fractionation

50 g of powdered leaves were mixed with 200 ml distilled water and boiled at 100°C on a hotplate for 15 minutes. After cooling and filtration on a filter paper Whatman N° 1, the filtrate was evaporated in vacuum using a rotary evaporator resulting in a dried extract named Ibl-1 (41.07 g). 15 g of Ibl-1 were dissolved in 100 ml distilled water, filtered as described above and the resulting filtrate was successively and exhaustively extracted with solvents with different polarities chloroform, ethylacetate, *n*-butanol. All fractions including the residual aqueous phase were treated as described above yielding corresponding dried extracts named as Ibl-1.1 (3.05 g), Ibl-1.2 (3.72 g), Ibl-1.3 (2.85 g), and Ibl-1.4 (5.02 g) for chloroform, ethylacetate, *n*-butanol and residual aqueous soluble fraction respectively.

On the other hand, 50 g of plant material were macerated 300 ml 80% methanol for 24 h. After filtration giving a methanol macerate, the marc was exhaustively percolated with the same solvent. Macerate and percolate were combined and evaporated in vacuum yielding dried 80% methanol extract named Ibl-2 (42.85 g).

2.3. Detannification

5 g of aqueous extract Ibl-1 of *I. batata* were dissolved in 10 ml methanol, filtered on filter paper Whatman N° 1 and submitted to column chromatography (CC) on polyamid SC-6 (0.05-016 mm, 60 x 2 cm, Macherey-Nagel, Germany) eluted with methanol until the total decoloration. In these conditions, tannins remained on column fixed by the adsorbent and eluates without tannins were collected, evaporated in vacuum yielding detannified extract denoted as Ibl-1' (3.89 g) (Nsaka *et al.*, 2013).

2.4. Phytochemical screening

The identification of major phytochemical groups was carried out in tubes using different appropriate reagents described in the literature for les alkaloids, polyphenols, anthraquinones, flavonoids, coumarins, anthocyanins, catechic and gallic tannins, proanthocyanidins, polysaccharides, reducing sugar, carbohydrates and nitroso compounds, etc. (Harborne, 1998, Trease and Evans.2000, Credo *et al.*, 2018). It was executed as followed for the detection of:

- Polyphenols: - 2 ml solution + FeCl₃ 5% : burn precipitate as positive test.
: - 2 ml solution + Barton's reagent (potassium ferricyanure 1%) + FeCl₃ 8%: blue color as positive test.
- Tannins: - 2 ml solution + Stiasny's reagent (formol + H₂SO₄ conc.): burn precipitate as positive test for catechic tannins, filter and to the filtrate add FeCl₃ 5% : burn or other color precipitate as positive test for gallic tannins,
- Proanthocyanidins : 2 ml solution + vanillin 1%/H₂SO₄ 5%, in MeOH, heat for 5 minutes, brick red color as positive test,
- Anthraquinones: 2 ml de solution + Börtranger's reagent (NaOH 10% or NH₃OH 10%) : red or red-violet color as positive test.
- Flavonoids: - 2 ml de solution + Shinoda's reagent (HCl + Zn powder or chip) : red-violet or other colors as positive test.
-2 ml solution + NaOH 10% or NH₄OH 10%: yellow color as positive test.
-2 ml solution + Neu's reagent (diphenylboric ethanolamine complex acid): yellow color as positive test.
- Anthocyanins: 2ml solution + HCl 0.2N, heat appearance of red color extractible in isoamylic alcohol as positive test.

- Coumarins: 2 ml solution + NaOH 10% intense blue fluorescence under UV at 366 nm as positive test.
- Nitroso compounds: 2 ml solution + ninhydrin 2%, heat for 10 minutes, appearance of violet color as positive test.
- Cardiotoxic heterosides: 2 ml solution + Carr-Price's reagent SbCl_3 : red color as positive test.
- Alkaloids: 2 ml de solution + Dragendorff's reagent : orange precipitate as positive test,
2 ml solution + Mayer's reagent: white yellow precipitate as positive test.
- Terpenoids and steroids: 1 ml de solution + Liberman-Bucchart's reagent (acetic anhydride + H_2SO_4) : various colors as positive with the mainly common violet color.

Next, the presence of some phytochemical groups were more confirmed by TLC (Thin layer chromatography using appropriate mobile phases and reagents for the identification of alkaloids; $\text{CHCl}_3/\text{MeOH}/\text{NH}_4\text{OH}$: 8/2/0.5 with Dragendorff's reagent, flavonoids: BAW (*n*-butanol/acetic acid/water): 4/5/1 (top layer) with Neu's reagent, steroids and terpenoids: $\text{CHCl}_3/\text{MeOH}$: 9/1 with Lieberman-Bouchardt's reagent.

2.5. Evaluation of Loperamide, extracts and fractions effects on castor oil-induced diarrhoea in normal animals

The methods used were previously described by Tadesse *et al.*, (2017) and Biru *et al.*, (2018). Wistar rats weighing 140-145 g either sex were divided in 5 groups and orally administered 100 and 200 mg/kg bw of extracts and soluble fractions as followed:

- Group I (2 rats) received orally 5 ml distilled water as negative control group,
- Group II (2 rats) received orally Loperamide 2.5 mg/kg body weight (bw) as positive control group,
- Groups IIIa and b, and IVa ou b (5 rats each for each oral dose) were orally administered aqueous Ibl-1 and 80% methanol Ibl-2 respectively,
- Groups Va and b, VIa to VIIIa and b (5 rats for each oral dose) were orally administered chloroform. ethylacetate, *n*-butanol and residual aqueous soluble fractions Ibl-1.1 to -1.4,
- Group IX was administered aqueous detannified extract Ibl-1'.

One hour after pre-treatment of diarrheic animals with each sample with fixed oral doses, animals were administered 0.5 ml castor oil. The time between the administration of castor oil and the appearance time of the first diarrhea (onset time) was noted. Other diarrheic parameters such as wet and hard faeces and secreted intestinal liquid volume were recorded after 4 h of observation. Percentages inhibition of defecation and diarrhea were calculated using the following formulas:

$$\text{Inhibition of defecation} = \frac{\text{Dfc} - \text{Dfts}}{\text{Dfc}} \times 100$$

Where Dfc is the quantity of hard faeces of negative control group and Dfts the quantity of hard faeces of tested sample in respective animal,

$$\text{Inhibition of diarrhea} = \frac{\text{Dc} - \text{Dts}}{\text{Dc}} \times 100$$

Where Dc was the quantity of wet faeces of negative control and Dts the quantity of wet faeces of tested sample in respective animal.

2.6. Evaluation of extracts and fractions effects on magnesium sulphate induced-diarrhea in animals

The protocols used were previously described by Pandhare *et al.*, (2018) and Naher *et al.* (2019). In the present case, the diarrhea was induced by the administration of magnesium sulphate at oral dose of 2 g/kg bw. Animals were divided in the same groups as described above and administered the same samples at the same oral doses and by the same way. All diabetic parameters cited above were recorded at the same time. Percentages inhibition of defecation and diarrhea were calculated using the same formulas mentioned above.

2.7. Castor oil induced enteropooling or castor oil-induced fluid accumulation

The intraluminal fluid content was determined by the methods described by Dhakad *et al.*, (2017) and Tagne *et al.* (2019). Animals were left to free consumption of water *libitum* and were divided in 5 groups with 5 rats each and administered tested samples at 200 mg/kg bw as followed:

- Group I received orally 5 ml saline solution (NaCl 0.9%, 5 ml/kg bw) as negative control group,
- Group II received Loperamide (2.5 mg/kg .bw.) as positive control,
- Groups IIIa et b, IV a et b were orally administered aqueous and 80% methanol extracts Ibl-1 and -2 respectively,
- Groups Va et b to VIII a et b were orally given soluble fractions IbL-1.1 to -1.4,
- Group IX was administered detannified aqueous extract Ibl-1'.

One hour after pre-treatment with each test samples at determined oral doses, 0.5 ml of castor oil was orally administered and left for 2h. And 1 h after, all animals were sacrificed. Their abdomens were opened and small intestine from pylori to caecum was banded, dissected and carefully washed with distilled water. The washed organ was dried in hot at 50°C and weight (P1). Its content was collected and measured. The empty small intestine was weighted (P2). P1-P2 was the weight of the small intestine content. The reduction percentages of intestinal secretion were calculated using the following formula:

$$\text{Inhibition of secretion} = \frac{\text{AVCSINC} - \text{AVCSITS}}{\text{AVCSINC}}$$

where AVCSINC was the average volume content of small intestine of negative control and AVCSICTS average volume content of small intestine of rats treated with tested sample.

$$\text{Inhibition of small intestinal weight} = \frac{\text{AWSINC} - \text{AWSITS}}{\text{AWSINC}}$$

Where AWSINC is the average weight of small intestine of negative control and AWSITS the average weight of small intestine of tested sample.

2.8. Evaluation of gastrointestinal motility by charcoal meal

Selected Wistar rats were first fasted for 18 h, and had free access to water. After the grouping in five groups as described above (castor oil test), they were orally administered a single dose of 200 mg/kg bw of the aqueous extract Ibl-1 from *I. batatas* leaves. After 1 h, each animal was administered 0.5 ml of castor oil and 1 ml of 5% activated charcoal suspension for intestinal motility test. Next, all animals were sacrificed 30 min after administration of castor oil and dissected. The small intestine from pylorus to caecum, was removed and its length was measured. 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 (Shoba and Thomas, 2014; Biru *et al.*, 2016; Abdela *et al.*, 2019). The inhibition of travelled distance by charcoal meal or peristaltic index was calculated using the following formula:

$$\text{Inhibition of travelled distance by charcoal meal or Peristaltic index} = \frac{\text{TDAC}}{\text{TLSI}} \times 100$$

TDAC od PI = Travelled distance by active charcoal or peristaltic index,

TLSI = Total length of small intestine of treated rats.

$$\% \text{ Inhibition} = \frac{\text{PINc} - \text{PITS}}{\text{PINc}} \times 100$$

Where PINC was the peristaltic index in negative control and PITS the peristaltic index in tested sample.

4. RESULTS AND DISCUSSION

4.1. Phytochemical screening

Results from the phytochemical study revealed the presence of alkaloids, flavonoids, tanins catechic and gallic, and proanthocyanidins), polyphenols, saponins, steroids, terpenoids, reducing sugars, carbohydrates and polysaccharides. Anthraquinones, anthocyanins,

Table 1: Phytochemical screening.

| Phytochemical group | Results | Phytochemical groups | Results |
|-------------------------|---------|----------------------|---------|
| Alkaloids | ++ | Polysaccharides | ++ |
| Anthocyanins | - | Tannins | +++ |
| | | Catechic | +++ |
| | | Gallic | +++ |
| | | Proanthocyanidins | +++ |
| Anthraquinones | _ | Steroids | +++ |
| Saponins | ++ | Terpenoids | +++ |
| Cardiotonic heterosides | _ | Reducing sugar | +++ |
| Coumarins | _ | Polyphenols | +++ |
| Flavonoids | ++ | Carbohydrates | +++ |

cardiotonic heterosides and coumarins were not detected in our experimental conditions. Our results are in good agreement with Luo *et al.*, (2005) for the presence of flavonoids whose some were isolated such as tiliroside, astragaline, rhamnocitrin, rhamnetin and kaempferol, Ling-Yuz *et al.*, (2009) for the presence of steroids, terpenes and flavonoids among which some were isolated like tetracosane, myristic acid, beta-sitosterol, beta-carotene, daucosterol and quercetin, Yin *et al.*, (2008) for the presence of polyphenols among which citrusin, caffeic acid, 3,4-di-O-caffeoylquinic acid, 1,2,3,4-tetrahydro-beta-carboline-3-carboxylic acid were isolated and Panda and Sonkamble, (2012) and Hossain, (2019) for carbohydrates.

4.2. Effects of extracts and fractions from *I. batatas* leaves on castor oil-induced diarrhoea

Castor oil (CO) had been widely used for induction of diarrhoea in anti-diarrheal activity studies because it

released ricinoleic acid, a metabolite that caused diarrhoea, upon metabolism in the gut. Ricinoleic acid initiated diarrhoea via mechanisms such as irritation of gastro-intestinal (GI) mucosa, leading to the release of prostaglandin which stimulated gastrointestinal motility (GIM) and electrolytes secretion, reducing electrolytes absorption from the intestine and colon, which were similar to the pathophysiologic processes resulting in diarrhoea (Mekonnen *et al.*, 2018).

The use of CO as diarrhoea inducer had been well documented (Shiferie and Shibeshi, 2013, Sisay *et al.*, 2017). When administered orally, it produced irritant laxative effect mediated by its active metabolite ricinoleic acid released by the action of intestinal lipases and induced thus diarrhoea. Ricinoleic acid produced local irritation and inflammation of the intestinal mucosa, causing the release of prostaglandins that eventually increased gastrointestinal motility, net secretion of water

and electrolytes (Rajat et al., 2013; Sisay et al., 2017). CO induced diarrheal model was designed to assess the potential of a test substance in its overall antidiarrheal activities. The onset time for defecation and diarrhea, the frequency and weight of fecal outputs as well as the secreted volume intestinal fluid were determined as the main diarrheal parameters (Sisay et al., 2017). Diarrhea induced by castor oil resulted from the action of ricinoleic acid which caused the irritation and inflammation of the intestinal mucosa leading to prostaglandins (PGE₂) release. The released PGE₂ stimulated GI and secretion of water and electrolytes (Rajat et al, 2013; Wansi et al., 2014), thus inducing an increase in the peristalsis and an intestinal hyper secretion of fluid. The inhibition of prostaglandins biosynthesis prolonged the time of induction of diarrhea by castor oil (Wansi et al., 2014).

For good interpretation of understanding of the reported results, following criteria were taken account: 80 ≤ % inhibition Dia or Def = 100; pronounced antidiarrheal activity, 70 ≤ % inhibition Dia or Def ≤ 80; good activity, 60 ≤ % inhibition Dia or Def ≤ 70; moderate activity, 50 ≤ % inhibition Dia or Def < 60; weak activity, 50 ≤ % inhibition Dia or Def ≤ 40 µg/ml: very weak activity, inhibition Dia or Def < 40% inactive.

In this test, it was observed that the oral administration of castor oil at dose of 5 mg/kg body weight (bw) provoked copious and abundant diarrhoea in treated normal rats characterized by an appearance times (onset time) of diarrhoea and defecation at 85.2±0.3 minutes and an enhancement of diarrheic parameter levels wet and hard

faeces at 11.2±0.4 and 9.07±0.3 respectively, and secreted volume of intestinal fluid of 7.5±0.3. This state well showed that these treated animals were in diarrheic state and need to be treated.

Firstly, the administration of Loperamide as reference antidiarrhoeal product at oral dose of 2.5 mg/kg body weight (bw) induced significantly increase of the appearance time (onset time) of diarrhoea and defecation at 157.3 minutes accompanied with markedly decrease of diarrheic parameter levels wet and hard faeces to 1.1±0.1 and 0.7±0.2 respectively, and secreted volume intestinal fluid to 0.1±0.0 compared to negative control presenting a low onset time of 85.3±0.3 minutes and significant increase of diarrheic parameter levels wet and hard faeces of 11.2±0.4 and 9.7±0.3, and secreted volume of intestinal fluid of 7.5±0.3.

Secondly, the oral administration of aqueous extract Ibl-1 and 80% methanol extract Ibl-2 at oral doses of 100 and 200 mg/kg bw caused prominent enhancement of onset time for diarrhea and defecation production as well as all diarrheic parameter levels in dose-dependent manner (Table 2). When administered at the highest oral dose of 200 mg/kg bw, both extracts caused significant increase of onset time for diarrhea and defecation production to 148.3±0.2 and 152±0.2 minutes respectively. They at the same time carried significant reduction of all diarrheic parameter levels wet and hard faeces to 1.9±0.1 and 1.2±0.3, and 1.7±0.1 and 0.9±0.2, and

Table 2: Effects of extracts and fractions from *I. batatas* leaves on castor oil-induced diarrhoea in animals.

| Groups | OD | Onset time | WF | HF | SIFV | % IDia | % IDef |
|---------------|-----|------------|----------|---------|---------|----------|----------|
| NC:CO I | 5 | 87.2±0.3 | 11.2±0.4 | 9.7±0.3 | 1.5±0.3 | 0 | 0 |
| Loper II | 2.5 | 157.3±0.2 | 1.1±0.1 | 0.7±0.2 | 0.1±0.0 | 91.0±0.2 | 92.7±0.2 |
| Ibl-1 IIIa | 100 | 140.3±0.1 | 2.1±0.3 | 1.7±0.1 | 0.3±0.3 | 81.2±0.0 | 82.4±0.1 |
| IIIb | 200 | 144.6±0.4 | 1.9±0.3 | 1.5±0.2 | 0.2±0.0 | 83.0±0.3 | 84.5±0.2 |
| Ibl-2 IVa | 100 | 148.3±0.2 | 1.5±0.1 | 1.1±0.1 | 0.2±0.0 | 86.6±0.2 | 88.6±0.1 |
| IVb | 200 | 152.2±0.2 | 1.2±0.3 | 0.9±0.2 | 0.1±0.0 | 89.2±0.1 | 90.7±0.2 |
| Ibl-1.1 Va | 100 | 120.4±0.3 | 3.7±0.3 | 3.1±0.2 | 0.5±0.2 | 66.7±0.0 | 68.0±0.2 |
| Vb | 200 | 134.4±0.2 | 3.3±0.2 | 2.8±0.3 | 0.3±0.1 | 70.5±0.1 | 71.1±0.0 |
| Ibl-1.2 VIa | 100 | 135.6±0.3 | 2.5±0.2 | 2.1±0.3 | 0.4±0.3 | 77.6±0.3 | 78.3±0.0 |
| VIb | 200 | 140.7±0.2 | 2.1±0.4 | 1.8±0.3 | 0.1±0.0 | 81.2±0.1 | 81.4±0.1 |
| Ibl-1.3 VIIa | 100 | 128.2±0.2 | 3.2±0.6 | 2.9±0.2 | 0.2±0.0 | 71.4±0.3 | 70.1±0.0 |
| VIIb | 200 | 136.2±0.3 | 2.7±0.0 | 2.3±0.4 | 0.2±0.7 | 75.8±0.0 | 76.2±0.2 |
| Ibl-1.4 VIIIa | 100 | 132.4±0.4 | 2.8±0.2 | 2.4±0.5 | 0.2±0.3 | 75.0±0.0 | 75.2±0.3 |
| VIIIb | 200 | 138.8±0.3 | 2.6±0.1 | 2.2±0.1 | 0.2±0.6 | 76.7±0.1 | 77.3±0.0 |
| Ibl-1' IX | 200 | 115.4±0.5 | 6.8±0.3 | 5.8±0.5 | 0.8±0.5 | 39.2±0.3 | 40.2±0.1 |

OD: oral dose, NC: negative control, CO; castor oil, Loper : Loperamide, % Dia and % Def: Ibl-1 and -2: aqueous and 80% methanol extract, Ibl-1.1 to -1.4: chloroform, ethylacetate *n*-butanol and residual aqueous soluble fractions respectively form the partition of aqueous extract Ibl-1.

Secreted volume of intestinal volume of 0.2±0.0 and 0.1±0.0 respectively compared to negative control with low onset time and high levels of diarrheic parameters (Table 2).

Figure 2 showed the percentage reduction of diarrhea and defecation by Loperamide, aqueous extract Ibl-1,

80% methanol extract Ibl-2 and aqueous detannified Ibl-1' extract administered at the highest oral dose of 200 mg/kg bw. Loperamide administered to diarrheal rats at oral dose of 5 mg/kg bw carried percentage reductions of 90.17 and 92.78% of diarrhea and defecation respectively. At the highest oral dose of 200 mg/kg bw to treated diarrheal rats, aqueous extract Ibl-1 and 80% methanol Ibl-2 caused percentage reductions of 83.03

and 84.53%, and 89.28 and 92.78% respectively. The inhibition capacity of diarrhea and defecation by Ibl-1 seem to be the same while for Ibl-2, it more inhibited defecation than diarrhea. Aqueous detannified extract acted the same manner by producing percentage reduction of diarrhea and defecation by 39.28 and 40.20% with the same level on these two parameters. Its activity was low compared to its parent extract Ibl-1.

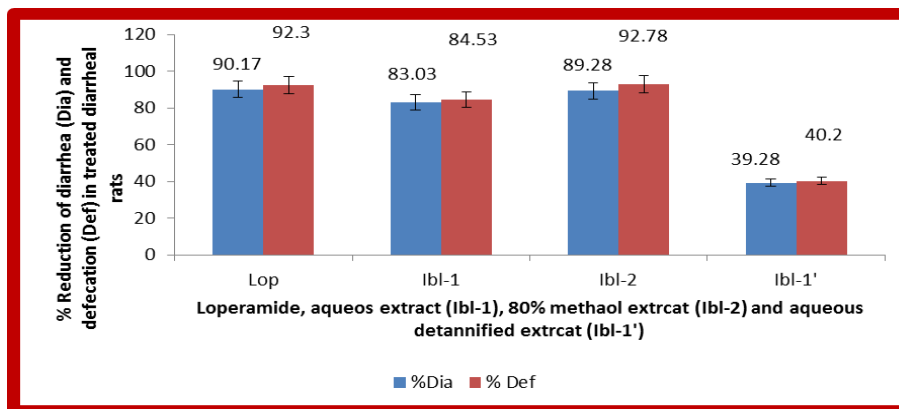


Figure 3: Percentage reductions of diarrhea and defecation by Loperamide, aqueous extract Ibl-1, 80% methanol extract Ibl-2 and aqueous detannified extract Ibl-1' in castor oil-induced diarrhea.

At the same highest oral dose, soluble fractions acted the same manner as the parent aqueous extract. They carried markedly enhancement of onset time and reduction of diarrheic parameter levels at different magnitudes (Table 2). At the highest oral dose of 200 mg/kg bw, they provoked an increase of onset time from 134.4±0.2 to 140.7±0.2 minutes with significant decrease of diarrheic parameter levels for which the values varied between 2.1±0.3 and 3.3±0.2 for wet faeces, 1.8±0.3 to 2.8±0.3 for hard faeces, and 0.2±0.1 to 0.1±0.01 for secreted volume of intestinal volume compared to negative control showing a low onset time and high diarrheic parameter levels (Table 2). In accordance with their activity level together with aqueous Ibl-1 and 80% methanol Ibl-2 extracts, the decreasing order of activity can be given as Ibl-2 > Ibl-1 > Ibl-12 > Ibl-1.4 > Ibl-3 > Ibl-1.1. In addition their activity was also related to the nature of components that they contained i.e ethylacetate

Ibl-1.2 was rich in flavonoids, residual aqueous Ibl-1.4 was rich in phenolic compounds other than flavonoids, *n*-butanol Ibl-1.3 was rich in saponins and chloroform Ibl-1.1 contained steroids and terpenoids as major constituents.

Figure 3 indicated the percentage reductions of diarrhea and defecation production by soluble fractions Ibl-1.1 to Ibl-1.4. Administered at the highest oral dose of 200 mg/kg bw, these soluble fractions reduced markedly diarrhea and defecation production in producing 70.53 to 81.25 and 71.13 to 81.44% respectively. Ethylacetate Ibl-1.2 was the most active presenting 81.25 and 81.44% of both diarrheal parameters respectively. It was followed by residual aqueous Ibl-1.4 with 76.78 and 77.32%, *n*-butanol Ibl-1.3 with 75.90 and 76.28% and chloroform Ibl-1.1 with 70.53 and 71.13% reductions of diarrhea and defecation production respectively.

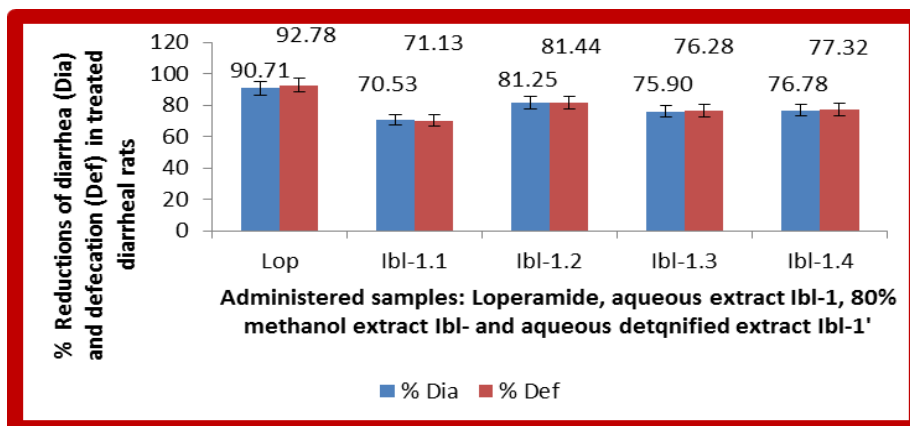


Figure 3: Percentage reductions of diarrhea and defecation by Loperamide, soluble fractions Ibl-1.1 to -1.4 in castor oil-induced diarrhea.

Although they had the same capacity of reduction, significant difference ($p < 0.05$) was observed between Ibl-1.2 compared to remaining soluble fractions, Ibl-4 compared to Ibl-1.1 and Ibl-1.3 and Ibl-1.3 compared to Ibl-1.

Table 3 showed different scores recorded by extracts and fractions in castor oil-induced diarrhoea in Wistar rats. As the criteria were dependent to each group of researchers., for good interpretation, following criteria were adopted: score; 0-2: pronounced activity, 3-5; good

activity, 6-8: moderate activity: 9-10: low activity, > 10: inactive. Thus, Loperamide as reference antidiarrhoeal product at oral dose of 2.5 mg/kg bw, aqueous Ibl-1 and 80% methanol Ibl-2 extracts at oral dose of 200 mg/kg bw, presented scores of 0, 2 and 1 respectively as pronounced antidiarrhoeal activity. Ethylacetate Ibl-1.2 and residual aqueous Ibl-1.4 soluble fractions had a common score of 2 as also a pronounced antidiarrhoeal activity. Chloroform Ibl-1.1 and *n*-butanol Ibl-1.3 soluble fractions had also the same score of.

Table 3: Scores of extracts and fractions from *I. lobota* in castor oil-induced diarrhoea in Wistar rats.

| Groups | Oral doses (mg/kg bw) | Diarrhoea | score | Total score |
|---------------|-----------------------|-----------|-------|-------------|
| NC : CO I | 5 ml DW | 6 | 0 | 10 |
| Loper II | 5 | 0 | 0 | 0 |
| Ibl-1 IIIa | 100 | 0 | 2 | 2 |
| IIIb | 200 | 0 | 2 | 1 |
| Ibl-2 IVa | 100 | 0 | 2 | 2 |
| IVb | 200 | 0 | 1 | 1 |
| Ibl-1.1 Va | 100 | 3 | 2 | 8 |
| Vb | 200 | 2 | 1 | 5 |
| Ibl-1.2 VIa | 100 | 1 | 1 | 3 |
| VIb | 200 | 0 | 2 | 2 |
| Ibl-1.3 VIIa | 100 | 2 | 1 | 5 |
| VIIb | 200 | 1 | 2 | 4 |
| Ibl-1.4 VIIIa | 100 | 1 | 1 | 3 |
| VIIIb | 200 | 0 | 2 | 2 |
| Ibl-1' IX | 200 | 3 | 2 | 8 |

See Table 2, DW: distilled water

5 as good antidiarrheal activity while the detannified extract Ibl-1' showed high score of 8 as weak antidiarrheal activity compared to its parent aqueous extract Ibl-1 (Table 3).

4.3. Effects of extracts and fractions from *I. batatas* leaves on magnesium-sulphate induced diarrhoea in normal rats

Magnesium sulphate had been reported to induce diarrhea by increasing the volume of intestinal content through the prevention of reabsorption of water and electrolytes. It caused increased loss of intestinal content due to the reduction in reabsorption of water and cholecystokinin release from the duodenal mucosa. The increase of the secretion and motility of small intestine, prevented the reabsorption of sodium chloride and water, and induced diarrhea (Iman *et al.*, 2012). It had been also demonstrated that it promoted the release of cholecystokinin from the duodenal mucosa, which increased the secretion and motility of small intestine and thereby prevented the reabsorption of sodium chloride (NaCl) and water (Verma *et al.*, 2011; <https://www.rechargetate.net/figure/Mechanism-of-castor-oil-and-magnesium-sulfate-induced-diarrhea-castor-oil-induc...>, 2020). This cholecystokinin acted by rising the secretions and motility of small intestine and

also by inhibiting the reabsorption of sodium chloride and water as already mentioned above. MgSO₄ also induced diarrhea through its osmotic properties preventing the reabsorption of water, leading to an increase in the volume of the intestinal content (Degu *et al.*, 2016; Naher *et al.*, 2019).

In the present test, the oral administration of magnesium sulphate in normal rats induced copious and abundant diarrhoea appearing at onset time of 90.2±0.1 minutes with high levels of diarrheic parameters wet and hard faeces to 10.2±0.2 and 8.7±0.5, and secreted volume of intestinal volume to 5.5±0.3 respectively and did not show inhibition of diarrhoea and defecation as in CO-induced diarrhea. Next, Loperamide used as reference antidiarrhoeal drug administered at oral dose of 2.5 mg/kg bw, prominently delayed diarrhea and defecation onset time to 160.3±0.2 accompanied with low diarrheic parameter levels wet and hard faeces to 1.2±0.1 and 0.9±0.2, and secreted volume of intestinal fluid to 0.3±0.1 respectively. Otherwise, aqueous Ibl-1 and 80% methanol Ibl-2 extracts administered at oral doses of 100 and 200 mg/kg bw caused significant increase of diarrhea and defecation onset time and markedly decrease of all diarrheic parameter levels in dose dependent manner (Table 4).

Table 4: Effects of extracts and fractions from *Ipomoea batatas* leaves on magnesium sulphate -induced diarrhoea in animals.

| Groups | OD | Onset time | WF | HF | SIFV | % IDia | % IDef |
|----------------|-----|------------|----------|----------|----------|----------|----------|
| NC: SM I | 5 | 93.2±0.1 | 10.2±0.2 | 7.7±0.5 | 2.5±0.3 | 0 | 0 |
| Loper II | 2.5 | 160.3±0.2 | 1.2 ±0.1 | 0.9 ±0.2 | 0.1±0.1 | 88.2±0.0 | 89.6±0.2 |
| Ibl-1 IIIa | 100 | 144.3±0.1 | 2.5 ±0.3 | 2.3 ±0.1 | 0.3± 0.0 | 75.5±0.3 | 73.5±0.1 |
| IIIb | 200 | 150.6±0.2 | 2.1 ±0.3 | 1.9 ±0.2 | 0.2 ±0.1 | 80.4±0.0 | 78.1±0.3 |
| Ibl-2 IVa | 100 | 150.2±0.2 | 2.0±0.1 | 1.7±0.1 | 0.2±0.1 | 79.4±0.0 | 80.4±0.3 |
| IVb | 200 | 156.3±0.0 | 1.8±0.2 | 1.5±0.3 | 0.1±0.0 | 82.3±0.0 | 82.7±0.2 |
| Ibl-1.1 IVa | 100 | 129.4±0.1 | 3.9 ±0.3 | 3.4 ±0.2 | 0.5 ±0.2 | 64.7±0.3 | 61.0±0.2 |
| IVb | 200 | 136.4±0.2 | 3.4 ±0.1 | 3.2 ±0.1 | 0.3 ±0.1 | 66.7±0.0 | 63.2±0.1 |
| Ibl-1.2 Va | 100 | 140.6±0.0 | 2.7 ±0.2 | 2.4 ±0.3 | 0.4 ±0.0 | 73.5±0.3 | 72.4±0.1 |
| Vb | 200 | 148.7±0.1 | 2.4 ±0.1 | 2.0 ±0.1 | 0.2 ±0.0 | 79.5±0.3 | 77.0±0.1 |
| Ibl-1.3 VIa | 100 | 136.2±0.2 | 3.1 ±0.0 | 2.9 ±0.2 | 0.5 ±0.0 | 69.6±0.1 | 66.7±0.3 |
| VIb | 200 | 142.2±0.0 | 2.9 ±0.2 | 2.6 ±0.4 | 0.4 ±0.0 | 72.5±0.0 | 70.1±0.2 |
| ILs-bs-1.4VIIa | 100 | 139.4±0.3 | 2.8±0.0 | 2.5 ±0.2 | 0.4 ±0.1 | 71.5±0.3 | 71.2±0.0 |
| VIIb | 200 | 145.1±0.2 | 2.7±0.1 | 2.5 ±0.2 | 0.2± 0.0 | 78.4±0.1 | 71.2±0.3 |
| Ibl-1' IX | 200 | 118.4±0.2 | 6.8± 0.1 | 6.0 ±0.4 | 0.1 ±0.0 | 33.4±0.0 | 31.0±0.0 |

See Tale 2, NC; negative control, SM: magnesium sulphate

Indeed, at the highest oral dose of 200 mg/kg bw, both administered extracts in treated diarrheic rats carried marked onset time to 150.6±0.2 and 156.3± 0.0 minutes respectively followed by marked reductions of diarrheic parameters levels to 2.1±0.1 and 1.8±0.2, and 1.9±0.2 and 1.5±0.3 and secreted volume of intestinal fluid to 0.2±0.0 to 0.1±0.0 respectively. All compared to negative control showing low diarrhea and defecation onset time of 90.2±0.1 and high Diarrheic parameter levels of 10.2±0.2 and 8.7±0.5 for wet and hard faeces respectively, and secreted volume of intestinal fluid of 5.5±0.3.

Figure 5 reported the percentage reductions of Loperamide (Lop), aqueous extract Ibl-1, 80% methanol

extract Ibl-2 and detannified aqueous extract Ibl-1' on magnesium sulphate-induced diarrhea. Administered at oral dose of 2.5 mg/kg bw, Loperamide reduced diarrhea and defecation to 88.23 and 80.52 %. Aqueous extract Ibl-1, 80% methanol extract Ibl-2 and aqueous detannified Ibl-1 extract produced 79.41 and 75.32%, 80.04 and 78.00% and 33.33 and 33.07% reductions of both diarrheal parameters respectively. Significant difference was deduced between Loper compared to Ibl-1, Ibl-2 and Ibl-1' ($p < 0.05$), the same effect was also observed between Ibl-2 compared to Ibl-1 and Ibl-1', Ibl-1 compared to Ibl-1' (Fig. 5).

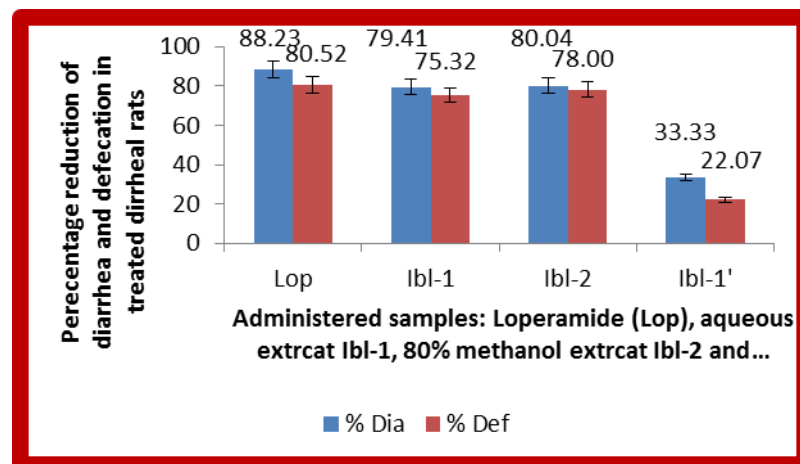


Figure 5: Percentage reductions of Loperamide (Loper), aqueous extract Ibl-1, 80% methanol extract Ibl-2 and aqueous detannified aqueous extract Ibl-1' in magnesium sulphate-induced diarrhea.

At this point, all soluble fractions also caused prominent delaying of onset times from 136.4±0.2 to 148.7±0.1 min, reduced diarrheal parameter levels to 2.4±0.1 to 3.4±0.1 and 2.0±0.1 to 3.2±0.1 for wet and hard faeces, and secreted volume of intestinal liquid of 0.3±0.1 to 0.2±0.0 respectively. Ethylacetate Ibl-1.2 soluble fraction

was the most active followed by residual aqueous Ibl-1.4, *n*-butanol Ibl-1.3 and chloroform Ibl-1.1 soluble fractions (Table 4).

Figure 6 indicated the percentage reductions of soluble fractions in magnesium sulphate-induced diarrhea.

Administered at the highest oral dose of 200 mg/kg bw, they carry percentage reductions of 66.66 to 76.47% against diarrhoea and 58.44 to 74.02% against defecation production. Ethylacetate Ibl-1.2 soluble fraction was the most active, followed by residual aqueous, *n*-butanol and chloroform soluble fractions (Fig.6).

The reduction effects of these samples on magnesium sulphate induced-diarrhoea in animals could be due to the increased absorption of water and sodium chloride from the gastrointestinal tract and by counteracting the increase in electrolytes secretion. Our observations were in perfect accord with Iman *et al.*, (2012) and Naher *et al.* (2019) who had also found the same effects for methanolic extract of *Barringtonia acutangula* leaves and seeds extracts and *Cordyline fruticosa* leaves extract respectively.

Scores from magnesium sulphate induced-diarrhoea produced by extracts and fractions from *I. batatas* leaves were presented in Table 5. The fixed criteria for the interpretation and good understanding of these score reported in castor oil test were also followed here. Results showed that aqueous Ibl-1 and 80% methanol Ibl-2 extracts exhibited scores of 2 and 1 respectively as pronounced activity while Loperamide showed a score of 1 as the same level of activity. Ethylacetate Ibl-1.2 and residual aqueous Ibl-1.4 soluble fraction showed a common score of 3 while chloroform Ibl-1.1 and *n*-butanol Ibl-1.3 had a score of 5 as good activity respectively. The detannified aqueous extract Ibl-1' showed high score of 8 as low activity compared in both tests to parent aqueous extract Ibl-1 with low score of 1 or 2 according to the case.

Table 5: Scores of extracts and fractions from *I. batatas* on magnesium sulphate induced-diarrhoea in animals.

| Groupes | Oral doses (mg/kg bw) | Diarrhoea | score | Score total |
|---------------|-----------------------|-----------|-------|-------------|
| NC: DW I | 5 ml water | 6 | 0 | 12 |
| Loper II | 5 | 0 | 1 | 1 |
| Ibl-1 IIIa | 100 | 1 | 1 | 3 |
| IIIb | 200 | 0 | 2 | 2 |
| Ibl-2 IVa | 100 | 0 | 2 | 2 |
| IVb | 200 | 0 | 1 | 1 |
| Ibl-1.1 Va | 100 | 1 | 4 | 6 |
| Vb | 200 | 2 | 1 | 5 |
| Ibl-1.2. VIa | 100 | 1 | 1 | 3 |
| VIb | 200 | 1 | 1 | 3 |
| Ibl-1.3 VIIa | 100 | 3 | 1 | 7 |
| VIIb | 200 | 2 | 1 | 5 |
| Ibl-1.4 VIIIa | 100 | 2 | 1 | 5 |
| VIIIb | 200 | 1 | 1 | 3 |
| ILbsb-1' IX | 200 | 2 | 4 | 8 |

See Table 2

These different scores also reflected their anti-diarrhoeal levels as pronounced, good, moderate or inactive related to their presented percentage inhibitions of diarrhoea and defecation mainly at the administered highest oral dose of 200 mg/kg bw as reported in both anti-diarrheal tests.

Finally, the oral administration of aqueous Ibl-1 and 80% methanol Ibl-2 at the same doses of 100 and 200 mg/kg bw to treated diarrheic animals in two successive times, leded finally complete stopping of castor oil and magnesium sulphate-induced diarrhoea and defecation production after more than 2.05 and 1.35 h, 2.40 and 2.10 h respectively.

4.4. Effect on gastro-intestinal motility in Wistar rats

In the model of charcoal meal test, Loperamide (2.5 mg/kg bw), aqueous Ibl-1 and 80% methanol, Ibl-2 extracts as well as soluble fractions Ibl-1.1 to -1.4, at oral dose of 200 mg/kg bw, showed significant decrease in the movement of charcoal meal from pylorus to caecum

when compared to control group ($p < 0.05$). Indeed, Loperamide at oral dose of 2.5 mg/kg bw showed $57.22 \pm 0.00\%$ while aqueous Ibl-1 and 80% methanol Ibl-2 extract presented 47.72 ± 0.03 and $49.64 \pm 0.02\%$ inhibitions of charcoal meal movement respectively. In addition, these data revealed that the percentage reductions of gastrointestinal transit of charcoal meal were significant and were 30.61 ± 0.02 to $41.41 \pm 0.00\%$ for soluble fractions. The most active soluble fraction was ethylacetate Ibl-1.2 with $41.41 \pm 0.00\%$ inhibition of gastro-intestinal motility, followed by residual aqueous Ibl-1.4 with $37.75 \pm 0.03\%$, *n*-butanol Ibl-1.3 with $33.64 \pm 0.02\%$ and chloroform Ibl-1.1 with $30.61 \pm 0.02\%$ inhibition of GI. The detannified aqueous extract Ibl-1' presented low inhibition of GI with a percentage of $22.05 \pm 0.04\%$ compared to the parent extract Ibl-1 (Table 6). In general, these samples showed significant reduction of charcoal meal movement ($p < 0.05$) compared to negative control (Table 6).

Table 6: Effects of extracts and fraction on gastro-intestinal motility in treated animals.

| Groups | Treatment OD (mg/kg bw) | TIL (cm) | DTCM (cm) | % Inhibition |
|---------------|-------------------------|------------|------------|--------------|
| NC: CO I | 5 ml | 55.80±0.02 | 46.58±0.03 | - |
| Loperamide II | 2.5 | 56.90±0.03 | 32.56±0.3 | 57.22±0.00 |
| Ibl-1 III | 200 | 55.80±0.01 | 26.63±0.01 | 47.72±0.03 |
| Ibl-2 IV | 200 | 56.87±0.04 | 28.23±0.00 | 49.64±0.02 |
| Ibl-1.1 V | 200 | 55.10±0.02 | 16.87±0.02 | 30.61±0.02 |
| Ibl-1.2 VI | 200 | 55.20±0.04 | 22.86±0.02 | 41.41±0.00 |
| Ibl-1.3 VII | 200 | 54.25±0.01 | 18.25±0.03 | 33.64±0.02 |
| Ibl-1.4 VIII | 200 | 54.78±0.01 | 20.68±0.01 | 37.75±0.03 |
| Ibl-1' IX | 200 | 56.05±0.02 | 12.36±0.04 | 22.05±0.04 |

See Table 2, TIL: total intestinal length, DTCM: distance travelled by charcoal meal, NC: negative control, CO: castor oil, OD: oral doses

4.5. Effects on castor oil-induced enteropooling

In this test, Loperamide at the oral dose of 2.5 mg/kg bw, aqueous Ibl-1 and 80% methanol Ibl-2 extracts at the highest oral dose of 200 mg/kg bw, showed significant percentage reductions in both the average volume of content of small intestine (AVCSI) and average weight of small intestine content AWSIC compared to control. The percentage inhibitions of AVCSI and AWSIC were 87.67±0.04 and 85.00±0.02% % for Loperamide

respectively, 80.82 and 77.00±.01% for aqueous Ibl-1 extract and 82.20 and 78.46±0.03% % for 80% methanol Ibl-2 extract which significant difference ($p < 0.05$).

Soluble fractions also caused significant inhibitions of both parameters with percentage inhibitions from 71.23 to 75.35% for AWSIC and from 69.23 to 72.07 for AVSIC with

Table 7: Effects extracts and fractions from *I. batatas* leaves on accumulation fluid induced by castor oil in animals (enteropooling test).

| Groups | AWSIC (g) | % Inhibition | AVSIC (ml) | % Inhibition of AWSIC | % Inhibition of AVIC |
|--------------|-----------|--------------|------------|-----------------------|----------------------|
| NC: CO I | 0.73±0.02 | 0 | 0.65±0.07 | 0 | 0 |
| Loper II | 0.11±0.02 | 87.67±0.04 | 0.10±0.04 | 85.00±.0.02 | 84.61±0.00 |
| Ibl-1 III | 0.14±0.04 | 80.82±0.02 | 0.15±0.01 | 77.00±0.01 | 77.00±0.04 |
| Ibl-2 IV | 0.13±0.03 | 82.20±0.04 | 0.14±0.02 | 78.46±0.03 | 78.46±0.01 |
| Ibl-1.1 V | 0.21±0.02 | 71.23±0.01 | 0.20±0.01 | 69.23±0.00 | 69.23±0.02 |
| Ibl-1.2 VI | 0.18±0.01 | 75.35±0.03 | 0.18±0.02 | 72.07±0.00 | 72.30±0.04 |
| Ibl-1.3 VII | 0.21±0.02 | 71.23±0.00 | 0.20±0.02 | 69.23±0.03 | 69.23±0.01 |
| Ibl-1.4 VIII | 0.20±0.02 | 72.60±0.03 | 0.19±0.00 | 70.77±0.02 | 70.77±0.01 |
| Ibl-1' IX | 0.46±0.11 | 37.00±0.00 | 0.38±0.02 | 41.53±0.01 | 41.53±0.03 |

See Table 2, AWSIC; average weight of small intestine content, AVSIC: average volume of small intestine content.

ethylacetate Ibl-1.2 as the most active soluble fraction (75.35 and 72.07% respectively) for AWSIC and AVSIC respectively), followed by residual aqueous Ibl-1.4 (72.60 and 70.77% respectively), *n*- butanol Ibl-1.3 and chloroform (71.23 and 69.23% respectively) for AWSIC and AVIC respectively having the capacity of reducing these parameters at interesting levels.

In all evaluated biological activities, 80% methanol Ibl-2 extract exhibited high activities compared to aqueous extract Ibl-1 and its soluble fractions Ibl-1.1 to -1.4. This organic solvent can be considered as the best which can be used in further for the isolation of active constituents in high amounts. On the other hand, the detannified aqueous extract Ibl-1' displayed low activities compared to the parent extract aqueous extract Ibl-1. This finding clearly demonstrated the important role played by tannins in the manifestation of all evaluated activities

and tannins can be considered as one of the responsible active principles for the antidiarrhoeal activity of the studied plant part. This observation was in good agreement with Labu et al., (2015); Barbara de Servi, (2017) and Cimanga et al., (2019). All evaluated biological activities in the present study generally indicated that the activities showed by all samples from *I. batatas* leaves were low compared to Loperamide.

Again, tannins and phenolics present in the plant extracts were reported to inhibit release of autacoids and prostaglandins, thereby inhibit motility and secretion induced by castor oil and magnesium sulfate-induced diarrhea and exerted thus, their antidiarrhoeal activity. This activity as also promoted by antispasmodic activity recognized to tannins and phenols (Sih et al., 2018; Cimanga et al., 2019). The anti-diarrheal activity of flavonoids was attributed to their ability to inhibit hydroelectrolytic secretions and intestinal motility (Holowacz et al., 2016). Percentage of faecal output of *I. batatas* samples was also reduced at all different tested oral doses producing better effect compared to negative

group (Tadesse *et al.*, 2017). The antidiarrheal activity showed by samples from *I. batatas* leaves can be due to the presence of some phytochemical groups like alkaloids, flavonoids, steroids, terpenes, tannins and polysaccharides as evidenced by phytochemical screening since they were previously reported to exhibit this activity at different extents (Mandal *et al.*, 2010; Kabir *et al.*, 2015; Holowac *et al.*, 2016; Tadesse *et al.*, 2017; Derebe *et al.*, 2018).

5. CONCLUSION

The present study had reported for the first time de the antidiarrhoeal activity of aqueous extract and its soluble fractions as well as 80% methanol extract form *Ipomoea batatas* leaves in animal model. Results indicated that all tested samples were able to inhibit castor oil and magnesium sulphate -induced diarrhoea in normal animals. They also inhibit gastro-intestinal motility and significantly reduce castor-oil effects induced-fluid accumulation. These reported results constituted a solid scientific base supporting and justifying the use of *I. batatas* leaves for the treating of diarrhea in traditional medicine particularly in Democratic Republic of Congo and in some extents, in other African countries where it known the same medical purpose.

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