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ANTIDIARRHEAL ACTIVITY OF AQUEOUS EXTRACT AND SOME ISOLATED FLAVONOIDS FROM *MORINDA MORINDOIDES* (BAKER) MILNE-READH. (RUBIACEAE) LEAVES IN ANIMAL MODEL

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ABSTRACT

The present study reports the antidiarrheal properties of aqueous extract and its fractions, and some isolated flavonoids from *Morinda morindoides* leaves assessed against castor oil and magnesium sulfate-induced diarrhea, gastrointestinal motility and castor oil induced-enteropooling in Wistar rats. Results indicated that aqueous extract from *M. morindoides* leaves and its chloroform, ethylacetate, *n*-butanol and residual aqueous soluble fractions were found to be able to inhibit castor oil and magnesium sulfate induced-diarrhea in mice when tested at oral doses of 100 and 200 mg/kg bodyweight respectively at different extents. They antidiarrheal effects were characterized by significant reduction level of all diarrheal parameters. Aqueous extract produced 73.0 ± 0.4 and 74.1± 1.4% inhibition of defecation and diarrhea in castor oil test and 70.1 ± 04 and 71.8 ± 0.7% when administered at the highest oral dose of 200 mg/kg body weight. All soluble fractions showed the same effect (% inhibition > 60%) while the detannified extract showed low activity (% inhibition < 50% against defecation and diarrhea). The aqueous extract and its fractions statistically induced significant dose-dependent decrease of the propulsion of the charcoal meal passing through the gastrointestinal tract at administered oral

doses of 200 mg/kg body weight with a inhibition percentage from 46.59 to 59.32% compared to untreated group ($p < 0.01$). They also significantly inhibited castor oil induced-enterepooling in Wistar rats with a percentage inhibition greater than 60%. The isolated flavonoids rutin, quercectrin, quercetin and kaempferol-7-*O*-rahmnolysophoroside (morindaoside) only inhibit diarrhea-induced by castor-oil (50 < % inhibition < 60% of defecation and diarrhea) and were devoid with significant effect against diarrhea-induced by magnesium sulfate. They also significantly inhibited gastrointestinal motility (45.02 to 58.67%) and castor oil induced-enteropooling in treated animals by producing more than 80% inhibition. This finding clearly showed that tannins and flavonoids are partly responsible for this biological activity. These results show that all selected *M. morindoides* leaves samples possess interesting antidiarrheal properties which can partly support and justify its current use for the treatment of diarrhea in traditional medicine.

KEYWORDS: *Morinda morindoides*, Rubiaceae, leaf, aqueous extract, flavonoids, antidiarrheal activity.

INTRODUCTION

Diarrhea is the passage of liquid or watery stools three or more times per day. In developing countries, diarrhea is a major cause of morbidity and mortality for malnourished children and concerns also adults It is a symptom of simple gastro-enteritis and of most intestinal infections. It is a major problem in developing countries, but common wordwilde. Viruses are often responsible, but the severest forms of infections diarrhea are generally those due to bacteria such as *Campylobacter jej*uni, *Escherichia coli*, *Salomonella enteritidis*, *Shigella* spp, *Staphylococcus aureus*, *Vibrio cholera* and *Yersinia enterocolitica* (Hardman and Limbirg, 1996).

Thus, antibacterial treatment was considered appropriate in tropical regions for diarrhea due to invasive, but not non-invasive pathogen microorganisms and other antidiarrheal drugs should be avoided in this case since they may aggravate the condition and occasionally increase the possibility of toxic megacolon (Hardman and Limbird, 1996; Sweetman, 2002). In addition, in severe cases, oral rehydration and electrolyte imbalances are the principal risk, particularly in infants, children and frail elderly patients (Bruneton et al., 2006). For treatment, oral dehydratation therapy therefore is a concertone for patients with acute illnesses resulting in significant diarrhea and is recomanded by WHO. This is particularly

important in developing countries, where the use of such therapy save many thousands of lives every year (Bruneton et al., 2006).

Although it is well known that diarrhea can have an infectious or no infectious origin, in traditional medicine, the cause of the disease is often unknown because of the lack of a specific and precise diagnosis in traditional medicine practices. In addition, some complex factors related to the disease including the living of people in areas of poor sanitation and socio-economic status, poor life style environmental conditions and the non-avaibility of guaranteed conventional medical treatments are also the conditions with negative effects on the disease (Orozco et al., 1995; Stanley, 1996, Teke et al., 2007).

Taking account of the frequent use of some medicinal plant species in traditional medicine for the treatment of diarrhea, many scientific investigations in different pharmacological tests were performed to prove their potentiality to cure diarrhea and some of them were found to possess antidiarrheal property *in vitro* and/or *in vivo* tests at different extents in various pharmacological tests (Galvez et al., 1996; Zavala et al., 1998; Lozoya et al., 2002, Pérez et al., 2005; Mathabe et al., 2006, Roof et al., 2007; Jia et al., 2008; Cimanga et al., 2010; Nsaka et al.2012; Balekar et al., 2014; Dhakad, 2017; Cimanga et al., 2018).

Morinda morindoides (Baker) Milne-Readh. (Rubiaceae) (Synonym: *Gaertnera morindoides* Bak. or *Morinda confusa* Hutch.) commonly called in vernacular languages as Nkonga bululu in Tshiluba, Nkongo bololo or Nkama meso in Lingala and Kikongo in Democratic Republic of Congo, is one of the most popular medicinal plants daily used in villages and towns in this country in traditional medicine near practioners or at home. Aqueous decoction of fresh leaves, which is the typical traditional remedy is used for the treatment of various illnesses among which diarrhea in children and adults (Kambu, 1990, Cimanga, 2010). The phytochemical studies of *M. morindoides* leaves have reported the presence of tannins, saponins, flavonoids, iridoids, terpenes, steroids and anthraquinones. The presence of alkaloids was doubtful. Anthocyanins and cardiotonic glycosides were not reported to be present (Kambu, 1990, Cimanga et al, 1995a,b, 1997, 2003). Previous scientific investigations on this medicinal plant part have reported some interesting biological activities related to its some traditional uses. These included the *in vitro* anticomplementary (Cimanga et al., 1995a,b, 1997, 2003), the *in vitro* an *vivo* antimalarial (Onabanjo, 1983; Tona et al., 2001; Cimanga et al., 2008), antioxidative (Cimanga et al., 1995), cardioinhibitory effect (N'Guessan et al., 2002) antiamoebic (Cimanga et al., 2006a,b), immunologic (Mankele et

al., 2006) and antispasmodic (Cimanga et al., 2010) activities. The plant part has not been yet studied for its antidiarrheal effect in animal models.

Thus, the present study deals with the *in vivo* evaluation of the antidiarrheal activity of *M. morindoides* leaves aqueous extract, its soluble fractions and some isolated flavonoids using some pharmacological tests.

2. MATERIALS AND METHODS

2.1. Plant material

Fresh leaves of *Morinda morindoides* (Baker) Milne-Readh. (Rubiaceae) were collected in Kinshasa in October 1990 and the plant was identified at the Institut d'Etudes et de Recherches en Agronomie (INERA) of the Department of Biology, Faculty of Sciences, University of Kinshasa where a voucher specimen (MN 04122004MML) was been deposited. For the present study, a new batch of plant materials was collected in September 2017. Fresh leaves were used in this study since this state of plant material is that used by practioners to prepare their remedies according to their daily practices. A part of plant materials was also dried at room temperature and reduced to powder using an electronic blender and kept in brown bottles.

Figure 1: *Morinda morindoides* (Baker) Milne-Readh. leaves and flowers. (Rubiaceae).

2.2. Reagents

Methanol (purity 99.99%), chloroform, ethylacetate (purity 99.96%), methanol (99.8%0 and *n*-butanol (purity 99% extra pure) were purchased from Acros Organic (USA). All solvents were with HPLC grade. Distilled water was used.

2.3. Preparation of extracts, fractions and isolation of flavonoids

20 g of fresh leaves were mixed with 150 ml distilled water and boiled for 30 min at 100°C on a hotplate. The mixture was cooled and filtered. The filtrate was evaporated in *vacuum* to give dried extract denoted as extract AE (12.32g, 61.60%). The detannified extract was obtained by column chromatography on polyamid CC6 (Germany) using extract AE (2.00g) eluted with methanol. The methanol solution collected without tannins was treated as described above yielding dried extract denoted as AE-0 (0.575 g) (Nsaka et al., 2012). On the other hand, an amount of extract AE (10 g) was dissolved in 200 ml distilled water, filtered and successively and exhaustively extracted with chloroform, ethylacetate and *n*-butanol. Each fraction was evaporated in *vacuum* yielding corresponding dried residues denoted as extracts A-1 (1.58, 15.80%), A-2 (2.16g, 21.60%) and A-3 (2.46g, 24.60%) respectively. The residual aqueous phase was also treated as described above yielding a dried residue denoted as extract A-4 (2.76g, 27.6%). Flavonoids were isolated from 80% methanol extract from dried plant materials (1000 g) by different chromatographic technics and identified by different conventional spectroscopic methods as previously described by Cimanga et al., (1995a, 1997).

2.4. Phytochemical screening

The phytochemical screening of the aqueous extracts and its fractions was carried out by TLC on precoated silica gel $60F_{254}$ plates (thickness layer 0.25 mm, Merck, Germany) using different mobile phases and reagents described in the literature for the detecting major phytochemical groups such as alkaloids, polyphenol compounds (flavonoids, anthraquinones, tannins), coumarins, steroids and terpenes. The froth test, HCl 0.2N and isoamylic alcohol, and Stiansy's reagent (formol and HCl conc.) were used to identify saponins, anthocyanins and tannins respectively (Harborne, 1998).

2.5. Castor oil-induced diarrhea in mice

The methods used were previously described by (Balekar et al, 2014; Dhakad et al., 2017). Wistar rats of 145-157 g bodyweight (bw) of either sex were divided into 6 groups (6 mice for each oral dose of tested extracts and fractions). Group I orally received distilled water 5 ml/kg bw, 2 rats as negative control while group II received atropine 5 mg/kg bw, 2 rats as positive control. Groups II to IV orally received 100 and 200 mg/kg bw respectively of aqueous extract AE and detannified aqueous extract. Groups V to VIII received the same oral doses of chloroform, ethylacetate, *n*-butanol, residual aqueous soluble fractions and

respectively. Sixty minutes after treatment with each selected sample with respective oral doses, each animal was orally administered 0.5 ml castor-oil one the time between oil oral administration and appearance of the first diarrhea drops was noted (the onset time). The defecation and diarrhea were observed continued up to 4 h on pre-weight filter paper placed in individual mice cages (P1) and replaced every hour. After drying paper filters with wet faeces at 50°C for 1h, the mean water content was calculated. Other parameters such as the onset time, the mean number of defecation, the number of wet and hard weight faeces were recorded in 4 h of observation. The percentage inhibitions of diarrhea and defecation drops were calculated using the following formula:

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

where Dc is the mean number of drops caused by castor oil or magnesium sulfate (negative control group) and Ds the mean number of drops caused by the test samples (treated groups).

$$\% \text{ Inhibition of defecation} = \frac{Pc - Ps}{Pc} \times 100$$

where Pc is the mean number of defecation caused by castor oil or sulfate of magnesium 9negative control group) and Ps the number of defecation caused by test samples (treated groups).

2.6. Magnesium sulphate-induced diarrhea

The protocols used, were the same as for castor oil-induced diarrhea (Balekar et al, 2014; Dhakad et al., 2017). But in this case, diarrhea was induced by oral administration of an oral dose of magnesium sulfate (2 g/kg bw) to animals grouped in the same way as described above. 30 minutes after pretreatment with each selected sample at the oral doses of 100 or 200 mg/kg respectively, pretreated animals received 5 ml of magnesium sulfate at oral dose . The mean onset time, total number wet and hard faeces and intestinal fluid in 4 h were recorded. The percentage inhibitions of diarrhea and defecation by tested samples were calculated using the same above formula described in castor-oil experiment.

2.7. Gastrointestinal motility test

The gastrointestinal motility was evaluated according to the methods previously described by Balekar et al., (2014) and Dhakad et al., (2017). Wistar rats were fasted for 18 h in in individual cages and divided in the same groups as described above. Each animal in each

group was administered with 1 ml charcoal meal (5% deactivated charcoal in 10% aqueous tragacanth). After, groups I and II received 10% aqueous tracanth and atropine (5 mg/kg bw) as negative and positive control respectively. Groups III to VIII received 100 and 200 mg/kg of *M. morindoides* samples (aqueous extract AE, fractions and detannified extract) respectively 1 h before administration of castor oil. After 30 min, all animals were under anesthesia and killed. The distance traveled by charcoal meal from the pylorus was measured and expressed as percentage of the total length of the intestine from pylorus to caecum. The percentage inhibition transit was calculated using the following formula:

$$\% \text{ Inhibition transit} = A- B \times 100/B.$$

where A is the distance travelled by charcoal in negative groups and B the distance travelled by charcoal in treated groups.

2.8. Castor oil induced-enteropooling

The methods described by Dhakad et al., (2017) and Tadesse et al., (2017) were followed for this study. Wistar rats of either sex were fasted for 18 h with free access to food and water grouped as in castor oil-induced diarrhea test. One hour after the administration of castor oil 0.5 ml/rat, animals were sacrificed by cervical dislocation. Their abdomen was open and the whole length of the intestine from the pylorus to the caecum, was ligated, intestines dissected and carefully removed. The small intestines were weighted and the intestinal contents were collected by milking into a graduated tube to measure the volume. The empty intestines were reweighted and the difference between the two weights was calculated. The percentage of reduction of intestinal secretion and weight of intestinal content were determined by using the following formula:

$$\% \text{ Inhibition of secretion by using MVSIC} = \frac{\text{MVICC - MVICT}}{\text{MVICC}} \times 100$$

Where MVSIC is the mean volume of the small intestinal content, MVICC is the mean volume of the intestinal content of the negative control group and MVICT is the mean volume of the intestinal content of the treated animals.

$$\% \text{ Inhibition of weight by using MWSIC} = \frac{\text{MWICC - MWICT}}{\text{MWICC}} \times 100$$

Where MWSIC is the mean weight of the small intestinal content, MWICC is the mean weight of the intestinal content of the negative control group and MWICT is the mean weight of the intestinal content of treated animals.

2.11. Statistical analysis

Results are presented as mean ± S.E.M.. Significance of the differences in comparison to the negative control groups was determined with Student's t-test when *P* value ≤ 0.05.

3. RESULTS AND DISCUSSION

3.1. Effects of *M. morindoides* samples against castor oil-induced diarrhea in Wistar rats

In respect of onset opious diarrhea induced by castor-oil in untreated Wistar rats, it is well known that the active constituent of this oil from *Ricinus comminus* L. (Euphorbiaceae) is ricinoleic acid liberated from the action of lipases on the oil. Ricinoleic acid produces irritation and inflammatory effects on the intestinal mucosa leading to the release of prostaglandins. This condition induces an ion increasing in permeability of the mucosal cells and change in electrolytes transport which results in a decrease of Na^+ and K^+ absorption, stimulating thus the peristaltic activity and causes diarrhea (Galvez et al., 1996).

Considering the severity of diarrhea observed in the untreated group, a significant dose-dependent reduction in castor oil-induced diarrhea in Wistar rats was demonstrated after oral administration of *M. morindoides* aqueous extract AE and its soluble fractions (A-1 to A-4) as well as the detannified aqueous extract at different extents. Their effect was characterized by their increase or decrease level of all diarrheal parameters according to the case compared to the negative control groups (Table 1). The protection values showed were situated between 53.96±0.05 and 73.01±0.04% inhibition of defecation and 56.36±0.11 to 74.46±0.14% inhibition of diarrhea respectively, and produced by all *M. morindoides* leaves samples when tested at the highest oral dose of 200 mg/kg bw. At this highest oral dose, the aqueous extract AE reached 73.0 ± 0.4 and 74.4 ± 1.4% inhibition of defecation and diarrhea respectively as a result of the increasing of onset time and the decrease of total number of hard faeces, total number of wet faeces and intestinal fluid excreted in treated animals compared to untreated group (Table 1). The ethylacetate soluble fractions (A-2) showed a higher antidiarrheal activity than the others soluble fractions ($p < 0.01$). It produced more than 69% inhibition of defecation and diarrhea at the highest oral dose of 200 mg/kg bw compared to other fractions (53 to 60% inhibition). (Table 1).

Table 1: Effects of *M. morindoides* leaves samples on castor oil-induced diarrhea in mice.

Sample codes	D	OT	TNWF	TNHF	IFV	% IDE	% IDIA
Negative control	5 ml water	64.3 ± 1.7	12.6 ± 1.4	9.4 ± 0.3	3.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
AE	100	105.3 ± 1.2	3.6 ± 1.5	2.6 ± 0.3	1.0± 0.5	71.42 ± 0.26	72.34 ± 0.06
	200	115.6 ± 3.6	3.4 ± 2.2	2.4 ± 1.8	1.0 ± 0.6	73.01 ± 0.04	74.46 ± 0.14
AE-0	200	96.4 ± 1.2	7.6 ± 0.2	5.8 ± 0.6	1.8 ± 0.1	38.30 ± 0.12	39.68 ± 0.03
AE-1	100	117.4 ± 2.3	6.1 ± 0.4	4.2 ± 0.3	1.7 ± 0.1	51.58 ± 0.06	55.32 ± 0.03
	200	125.3 ± 1.7	5.8 ± 0.4	4.1 ± 2.3	1.7 ± 0.7	53.96 ± 0.05	56.38± 0.11
AE-2	100	118.0 ± 2.3	4.1 ± 1.4	3.2 ± 0.2	2.4 ± 0.1	67.46 ± 0.02	65.95 ± 0.31
	200	126.2 ± 2.1	3.8 ± 0.2	2.9 ± 0.1	2.1 ± 0.2	69.84 ± 0.05	69.14 ± 0.08
AE-3	100	124.4 ± 2.3	4.5 ± 1.6	3.4 ± 0.7	1.1 ± 0.9	64.28± 0.03	63.83± 0.01
	200	132.6 ± 3.1	4.2 ± 0.4	3.1 ± 0.7	1.1 ± 0.2	66.70 ± 0.14	67.02 ± 0.07
AE-4	100	117.4 ± 2.7	5.1 ± 2.3	4.1 ± 3.6	3.1 ± 0.3	59.52 ± 0.08	56.38 ± 0.04
	200	128.6 ± 3.0	4.8 ± 2.1	3.8 ± 0.4	1.0 ± 0.4	61.90 ± 0.09	59.57 ± 0.11
Morindaoside	50	114.2 ± 1.2	4.2 ± 0.4	3.4 ± 0.2	0.8 ± 0.1	66.67 ± 0.13	63.82 ± 0.04
Quercetin	50	101.2 ± 3.5	1.3 ± 0.7	1.2 ± 0.4	0.1 ± 0.3	92.06 ± 0.04	87.23 ± 0.06
Quercectrin	50	112.3 ± 2.3	4.7 ± 0.7	4.2 ± 0.2	2.0 ± 0.4	62.70 ± 0.12	55.32 ± 0.25
Rutin	50	110.7 ± 1.8	4.1 ± 2.2	3.3 ± 2.2	1.1 ±0.4	67.46 ±1.02	64.89 ± 0.05
Atropine	2.5	194.3 ± 0.2	0.2 ± 0.1	0.1 ± 0.2	0.2 ± 0.1	98.41 ± 0.5	98.89 ± 1.2

AE: aqueous extract (decoction 20%), AE-0: detannified extract from A, AE-1, AE-2, AE-3 and AE-4 chloroform, ethylacetate, *n*-butanol and residual aqueous phase from the partition of extract AE, D: doses (mg/kg bw) OT: onset time (minutes), TNWF: total number of wet faeces in 4h, TNHF: total number of hard faeces in 4 h, IFV: intestinal fluid volume in 4h, IDE: inhibition of defecation, IDIA; inhibition of diarrhea.

The high activity was found with aqueous extract AE (Table 1). Particularly, the ethyl acetate fraction rich in flavonoids produced 69.84 ± 0.05 and 69.14 ± 0.08% inhibition of defecation and diarrhea at the highest oral dose of 200 mg/kg body weight. In other respects, in a previous study, it was shown that ethylacetate extract from *M. morindoides* leaves collected in Daola (central west region of Ivory Coast produced 67% inhibition of diarrhea at oral dose of 1000 mg/kg bw (Miete et al., 2009). This weak activity compared to our ethylacetate fraction (69.84±0.05) tested at the highest oral dose of 200 mg/kg bw, was significant, but the difference in activity may be due not only to the nature of tested samples (extract versus fraction), but also to the origin of the plant material influencing the amount of active constituents.

On the other hand, it is well known that castor-oil causes motility and secretory diarrhea involving the association of dual effects on gastrointestinal motility, water and electrolytes transport characterized by their reduction and absorption across the intestinal mucosal (Roof et al., 2007). Other mechanisms of action of castor oil as diarrheic agent include its inhibition of $Na^+ K^+$ ATPase activity reducing thus hormonal fluid absorption, activation of adenylate cyclase or mucosal AMP-mediated active secretion (Capasso et al., 1994), platelet activating factor (Pinto et al., 1992) and stimulation of prostaglandin formation (Bayad et al., 2001). Also, inhibitors of prostaglandins biosynthesis are known to delay diarrhea provoked by castor-oil. In addition, the production of diarrhea by castor oil is due to its active constituent ricinoleic acid through its hypersecretory effect (Ammon et al., 174; Gaginella et al., 1975). These conditions suggested that *M. morindoides* samples would reduce diarrhea either by antisecretory mechanism and by increasing reabsorption of electrolytes and water, or by inhibiting induced intestinal accumulation of fluid and inhibition of prostaglandin synthesis.

Flavonoids quercetin, quercectrin, rutin and morindaoside were tested at an oral dose of 50 mg/kg bw and were found to be effective against castor oil induced- diarrhea with an inhibitor effect on defecation and diarrhea from 62 to 93% and 55 to 88% respectively compared to negative control. These effects occurred when an increase of the onset time and a decrease of number of wet and hard faeces and intestinal fluid volume were observed in treated animals compared to negative control (Table 1). The most active flavonoid was quercetin. To our knowledge, this is the first time to report the antidiarrheal activity of a flavonoid derivative of kaempferol.

For these four tested flavonols, a structure-antidiarrheal activity could be made. Thus, results indicated that the activity decreased with glycosylation in C-3 position (quercetin compared to quercectrin and rutin), while it increased with the number and nature of sugar groups in the same position (rutin compared to quercectrin) as the activity is dependent on the nature of the aglycone, the nature and position of sugar group (Kaempferol-7-*O*-rhamnosylsophoroside: morindaoside compared to quercetin-3-*O*-rhamnosylglucoside: quercectrin or quercetin-3-O-rutinoside: rutin). In this last case, kaempferol diglycoside showed high activity than quercetin diglycoside related to the reasons evoked above. Particularly, quercectrin was also previously reported to inhibit lactose-induced chronic diarrhea in rats (Galvez al., 1995) as a sign of its antidiarrheal activity. Quercectrin and rutin are both hydrolyzed in the gut by

intestinal bacteria yielding quercetin as active antidiarrheal agent and this step was found to be essential for the antidiarrheal effect of both flavonoid diglycoside derivatives.

The antidiarrheal activity of flavonoids is associated to their ability to inhibit intestinal motility and hydroelectrolytic secretion (Pinto et al., 1992, Rao et al., 1997). Our results are only qualitatively in good agreement with those previously reported and more confirming the antidiarrheal activity of quercetin and its glycoside derivatives in castor-oil model as previously reported (Galvez et al., 1993, 1996; Di Carlo et al., 1993, 1994; Cimanga et al., 2010).

3.2. Effects of *M. morindoides* leaves against magnesium-sulfate induced diarrhea in Wistar rats

Magnesium sulfate acts by the osmotic properties preventing reabsorption of water ions, leading to increment the volume of the intestinal content. This salt also promotes the liberation of cholecystokinin from the duodenal mucosa, which increases the In magnesium sulphate test induced diarrhea, animals in control groups produced copious diarrhea after administration an oral dose of 2 g/kg bw of magnesium sulfate.

In the present investigation, when diarrhea was induced by magnesium sulfate in Wistar rats, the oral administration of aqueous extract from *M. morindoides* leaves and its soluble fractions significantly increased the onset time and reduced other diarrheal parameters, finally resulting in the reduction of exerted diarrhea and defecation in treated animals compared to negative control (Table 2). At the highest oral dose of 200 mg/kg bw, aqueous extract AE produced 78.64± 0.04 of defecation and 77.61±0.07% of diarrhea. All soluble fraction had the same effect by causing a percentage inhibition of defecation from 66.62 ±0.01 to 73.78±0.04 and that of diarrhea from 64.92± 0.03 to 72.38±0.06% with the ethylacetate soluble fraction rich in flavonoids as the most active soluble fraction (Table 2).

At a tested oral dose of 50 mg/kg bw, all tested flavonoids showed no significant effect when diarrhea was provoked by this cathartic agent (magnesium sulfate) and our results well corroborated with other studies (Galvez at al., 1993, 1996).

In both antidiarrheal tests, at the highest oral dose of 200 mg/kg body weight, the detannified aqueous extract (AE-0) produced less than 50% inhibition of defecation and diarrhea induced by castor oil and magnesium sulfate respectively (Tables 1 and 2). Its activity was lower than

that of the parent extract (A) suggesting that tannins are partly implicated and play an important role in the manifestation of the observed activity. In addition, it as can be considerd as resposnsible for this evaluated biological activity. In general, the antidiarrheal activity of *M. morindoides* leaf samples was less than that showed by Atropine (100% inhibition of diarrhea at an oral dose of 5 mg/kg bw) used as an antidiarrheal reference product.

Table 2: Effects of *M. morindoides* leaf samples on magnesium sulfate-induced diarrhea in mice.

Sample codes	Doses	OT	TNWF	TNHF in	IFV	% IDE	% IDIA
NC water	10 ml	01.3±0.2	13.4±1.3	10.3±0.7	3.1±0.1	0	0
AE	100	132.4±0.6	3.5 ± 3.6	2.6 ±4.1	1.5±0.2	74.75±0.02	73.88±0.05
	200	139.6±1.3	3.0 ± 2.3	2.2 ±2.6	1.4±0.1	78.64±0.04	77.61±0.07
AE-0	100	102.6±0.6	8.6 ± 1.3	6.3 ± 1.5	2.3±0.2	38.83±0.11	35.82±0.13
	200	124.3±1.3	7.4 ± 0.6	5.5 ± 0.3	2.4±0.1	46.60±0.07	44.77±0.06
AE-1	100	136.3±1.4	5.1 ± 3.2	3.8 ± 1.2	1.3±0.0	63.10±0.04	61.94±0.05
	200	141.4±0.5	4.7 ± 1.3	3.5 ± 1.4	2.2±0.0	66.02±0.01	64.92±0.03
AE-2	100	146.6±1.7	4.4 ± 3.1	3.2 ± 2.6	2.1±0.2	68.93±0.12	67.16±1.3
	200	152.3±0.3	3.7 ± 0.8	2.7 ± 2.2	2.5±0.0	73.78±0.04	72.38±0.06
AE-3	100	112.3±1.4	4.8 ± 0.2	3.3 ± 0.4	1.3±0.1	67.96±0.01	64.18±0.07
	200	125.6±2.3	4.5 ± 0.6	3.3 ± 1.3	2.2±0.2	67.96±0.8	66.41±0.01
AE-4	100	131.1±1.3	3.9 ± 0.6	2.9 ± 1.2	2.4±0.0	7184 ±1.01	70.89±1.06
	200	138.3±0.2	3.7 ± 0.2	2.8 ± 1.7	2.7±0.2	72.81±0.08	72.38±0.04
Morindaoside	50	121.3±0.1	13.0±1.3	12.7±1.7	0.3±0.0	-	-
Quercetin	50	125.3±0.3	13.6±0.2	12.5±1.1	1.1±0.0	-	-
Quercectrin	50	118.3±1.2	14.2±1.5	12.5±0.5	1.7±0.0	-	-
Rutin	50	116.7±0.5	13.9±1.2	12.4±1.3	1.5±0.1	-	-
Atropine	2.5	194.3±0.2	0.3 ±0.1	0.1 ±0.2	0.1±0.0	99.03	97.76

See Table 1.

In general, aqueous extract AE and its fractions (A-1 to A-4), like the standard antidiarrheal agent Atropine statistically produced significant inhibition of the frequency of defecation and diarrhea in treated animals compared to untreated groups in both models ($p < 0.01$) and these *M. morindoides* had atropine-like effect.

3.4. On gastrointestinal motility

Aqueous extract AE and its soluble fractions from *M. morinodides* leaves samples showed significant decrease of the motility against gastrointestinal motility of charcoal meal movement as shown in Table 3.

Table 3: Effects of aqueous extract AE and its fractions, and some isolated flavonoids from *M. morindoides* on gastrointestinal motility.

Sample codes	TTT (dose: mg/kg bw)	IL (cm)	DTCM (cm)	% IT
Negative control	2 ml 10% AT	89.2 ± 0.3	86.30 ± 0.02	0
A	100	85.7 ± 0.4	40.31 ± 0.03	53.29± 0.30
	200	80.4 ± 0.1	39.40 ± 0.06	54.34 ± 0.42
AE-0	200	79.8 ± 1.2	45.23 ± 0.14	47.58 ± 1.45
AE-1	100	76.4 ± 1.3	50.66 ± 0.07	41.29. ±1.02
	200	74.3 ± 1.2	46.11 ± 0.02	46.57 ± 1.11
AE-2	100	81.4 ± 0.4	39.37 ± 0.01	54.38 0± 0.02
	200	78.5 ± 0.7	35.70 ± 0.02	58.63 ± 0.04
AE-3	100	76.5 ± 0.4	37.82 ± 0.12	56.17± 0.07
	200	72.6 ± 0.2	35.10 ± 0.06	59.32 ± 0.05
AE-4	100	82.5 ± 0.1	42.11 ± 0.07	51.20 ± 0.12
	200	84.1 ±1.2	40.74 ± 0.03	52.79 ± 0.01
A-0	200	87.24±0.2	53.03.02±0.4	38.55±0.01
Quercetin	50	83.7 ± 0.1	35.66 ± 0.05	58.67 ± 0.06
Rutin	50	82.5 ± 0.2	45.10 ± 0.15	47.74 ± 1.07
Quercectrin	50	83.6 ± 0.7	47.44 ± 1.04	45.02 ± 0.12
Morindaoside	50	82.1 ± 0.6	42.73 ± 0.02	50.48 ± 0.08
Atropine	5	92.3±0.07	32.80 ± 0.05	62.00 ± 00.5

See Table 1, TTT; treatment, AT; aqueous tragacanth, IL: intestinal length, DTCM: distance travelled by charcoal meal, IT: intestinal transit.

A previous study showed that activated charcoal avidly absorbs drugs and chemicals on the surface of charcoal meal particles preventing absorption (Levy, 1982). By this test, it was demonstrated that samples from *M. morindoides* leaves inhibited in dose-dependent manner peristaltic movements of charcoal since there suppressed its propulsion and increased the absorption of water and electrolytes as also previously described for other medicinal plant extracts (Ukwami et al., 2012, Balekar et al., 2014, Dakhar et al., 2017).

Aqueous extract AE, its fractions and isolated flavonoids exhibited significant antidiarrheal activity by decreasing the gastrointestinal motility. They statistically induced significant dose-dependent decrease of the propulsion of the charcoal meal passing through the gastrointestinal tract at all administered oral doses (100 and 200 mg/kg bw respectively) compared to untreated group ($p < 0.01$). The decrease percentage of charcoal movement produced by all samples from *M. morindoides* leaves is higher than 50% at all administered oral doses, excepted hat of detannified aqueous extract which was 38.55±0.01% at the highest oral dose of 200 mg/kg bw.

Aqueous extract AE suppressed by 57.7 ± 0.4% intestinal transit followed by the ethyl acetate (A-2) and *n*-butanol (A3) with 55.7 ± 0.4 and 59.3 ± 0.7% inhibition of intestinal transit respectively At the highest oral dose of 200 mg/kg body weight. The activity of other renaining soluble fractions was also appreciable. The isolated flavonoids also significantly decreased gastrointestinal motility from 45 to 59% inhibition transit with the highest effect observed with quercetin (58.67 ± 0.06%) at oral dose of 50 mg/kg bw. Atropine used as a reference product reduced the motility of charcoal to a greater extents (62.00 ± 0.05% intestinal transit) compared to tested *M. morindoides* leaf samples ($P < 0.01$) (Table 3).

3.5. Castor oil induced-enteropooling

In the castor oil induced-enteropooling test, at the highest tested oral dose of 200 mg/kg bw, aqueous extract AE and its soluble fractions AE-1 to AE-4 were found to be able to significantly ($p < 0.05$) reduced the mean weight of small intestine content (MWSIC), the mean volume of small intestine content (MVSIC) and the intraluminal fluid accumulation compared to negative control group (Table 4).

Maximal inhibition of MWSIC and MVSIC was observed with aqueous extract AE (75.35±0.02 and 76.92±0.03% inhibition respectively) followed by soluble fractions A-2 (73.84 ±0.01 and 72.60±0.05% inhibition respectively) and A-4 (70.76±0.04 and 68.48±0.03% inhibition respectively).

Table 4: Effects of aqueous extract AE of *M. morindoides* leaves, its fractions and some isolated flavonoids on castor oil induced-enteropooling of Wistar rats.

Groups	TTT (200 mg/bw)	MWSIC(g)	%Inhibition	MVSIC	%Inhibition
I	Castor oil	0.73±0.02	-	0.65±0.07	-
II	Atropine	0.16±0.02	78.08±0.04	0.13±0.04	80.00±.002
III	AE	0.18±0.04	75.34±0.02	0.15±0.12	76.92±0.03
IV	AE-1	0.27±0.02	63.01±0.04	0.22±0.04	66.15±0.06
VI	AE-2	0.20±0.16	72.60±0.05	0.17±0.17	73.84±0.01
VI	AE-3	0.25±0.01	65.75±0.07	0.20±0.10	69.23±0.02
VII	AE-4	0.23±0.12	68.49±0.03	0.19±0.14	70.76±0.04
VII	AE-0	0.38±0.01	0.48±0.01	0.40±0.03	38.00±0.03
Quercetin	50	0.09±0.02	87.67±0.03	0.07±0.02	89.23±0.04
Rutin	50	0.11±0.04	84.93±0.06	0.09±0.04	86.15±0.02
Quercectrin	50	0.13±0.05	82.19±0.06	0.10±0.07	84.61±0.04
Morindaoside	50	0.10±0.01	86.30±0.01	0.08±0.02	87.69±0.05
Atropine		0.05±0.01	93.15±0.03	0.07±0.04	89.23±0.06

A-1 and A-3 soluble fractions also showed good inhibition of both intestine parameters more than 63% with A-3 as the most active sample compared to the first one. All selected flavonoids produced more than 85% inhibition of MVISC and MWSIC with quercetin as the most active flavonoid (Table 4). The detannified aqueous extract only showed 38.00±0.03 inhibition of enterpooling induced by castor oil.

The remarkable antidiarrheal effect of aqueous extract of *M. morindoides*, its soluble fractions and tested flavonoids demonstrated their efficacy in diarrheal conditions. These samples can be considered as alternative natural remedies for the treatment of diarrhea.

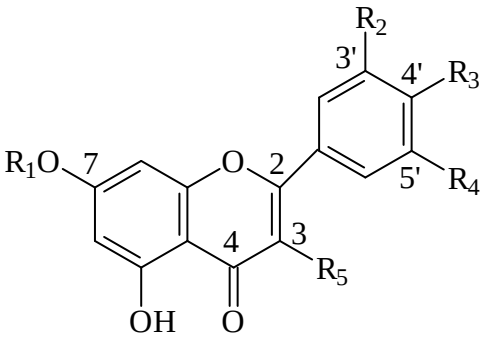
In general, it is well reported that the antidiarrheal activity of some medicinal plants is due to the presence of tannins, saponins, coumarins, flavonoids, alkaloids, steroids and terpenoids (Yakubu and Salimon, 2015; Paredes et al. 2016). Tannins and tannic acid act as antidiarrheal agents by the denaturation of proteins in the intestinal mucosa forming protein tannates which make the intestinal mucosa more resistant to chemical alterations. They reduce the peristaltic movements and intestinal secretion.

Flavonoids act as antidiarrheal agents by their ability to inhibit intestinal motility and hydroelectrolytic secretions and antispasmodic effects (Galvez et al, 1996; Cimanga et al., 2010) Steroids and triterpenes are useful for the treatment of diarrhea and may increase intestinal absorption of Na^+ and water (Tadesse et al., 2017). Thus, the antiadiarrheal activity of aqueous extract of *M. morindoides* leaves and its soluble fractions, reported in the present study, may be due to the presence of these phytochemical groups identified in this plant part as evidenced by phytochemical screening results, which in part, can react in synergistic manner for the manifestation of this biological activity. And partly, this evaluated biological activity is due to the presence of tannins and flavonoids as demonstrated by the present reported results.

4. CONCLUSION

Results from this study clearly demonstrate that samples from *M. morindoides* leaves possess a capacity to reduce diarrhea induced by castor-oil and magnesium sulfate in mice, as well gastrointestinal motility of charcoal meal and castor oil- induced-enteropooling showing thus their antidiarrheal activity. Flavonoids and tannins as shown in the present study, contribute in part to the observed activities and can be considered partly as active antidiarrheal

principles. The use of this medicinal plant part in traditional medicine for the treatment diarrhea seems to be supported and justified by these reported pharmacological properties.



	R_1	R_2	R_3	R_4	R_5
Quercetin	H	OH	OH	H	OH
Quercectrin	H	OH	OH	H	-*O*-Rha
Rutin	H	OH	OH	H	-*O*-Rut
Morindaoside	H	OH	H	H	-O-Rha-soph

Rha: rhamnoside, Rut: rutinoside, Rha-soph: rhamosylsophoroside

Figure 2: Structures of antidiarroheal flavonoids isolated from *Morinda morindoides* leaves.

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