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PHYTOCHEMICAL SCREENING AND ANTIDIARRHEAL ACTIVITY OF ETHANOLIC FRESH ROOT BARK EXTRACT OF *MONDIA WHITEI* IN ALBINO RATS

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ABSTRACT
The study was conducted to validate the anti diarrheal activity of ethanolic fresh root bark extract of *Mondia whitei* using white albino rats. We also determined the acute toxicity and phytochemical composition of the extract. The study was carried out in May, 2013. The ethanolic extract of root bark of *Mondia whitei* was evaluated for castor oil induced diarrhea, as well as Transit time and enteropooling activity in wistar Albino rats. Phytochemical screening and acute toxicity were also determined. Each treatment group of the respective doses used contained six study animals. Doses of 200, 400 and 600 mg/kg body weight were used in the respective experimental set-up groups with loperamide and atropine sulphate used as positive control while normal saline was used as negative control in all study set ups. The ethanolic fresh root bark extracts of *Mondia whitei* showed strong presence of saponins, phenols, alkaloids and tannins which are known to have antidiarrheal activity. The study revealed statistically significant ($p < 0.05$), dose-dependent anti diarrheal and enteropooling activity, but also reduced transit distance, with the dose of 600 mg/kg being highly significant ($p < 0.001$) as compared to the other treatment groups. The mean lethal dose (LD_{50}) was above 5000 mg/kg in mice. The study showed that the ethanolic fresh root bark extract of *Mondia whitei* has anti-diarrheal activity and it's safe for use, thus corroborates with its traditional use. The anti-diarrheal action may be linked partly to direct inhibitory effect of the extract on the propulsive movement of the gastrointestinal tract smooth muscle, increased electrolytes and fluid absorption and infiltration in the tissue.
Keywords: *Mondia whitei*; Phytochemical; Antidiarrheal; transit time; Enteropooling; Root Bark

INTRODUCTION

The world is endowed with rich heritage of herbal plants with medicinal value. The medicinal plants have been used for centuries to manage and treat diseases since they contain phytoagents with therapeutic values. It is estimated that about 25 % of all modern medicines are directly or indirectly derived from higher plants[1]. It's a known fact that at least two out of every 10 medicines prescribed in hospitals is derived from plants, most of them discovered through the use of indigenous medicinal plants[2]. It is also estimated that at least seven out of every 10 medicines used in the treatment of cancer have been derived from medicinal plants[3]. WHO estimates that 80 % of people living in developing countries depend exclusively on traditional medicine[4,5]. This is also true in some developed countries where the use of modern medicine is predominant[6]. Presently there is an increasing worldwide interest in herbal medicine with increase in laboratory testing of the pharmacological activities and ability to treat various ailments. Diarrhea is characterized by frequent passage of unformed, loose or watery stools, usually three or more times in 24 hours and it is the main clinical gastrointestinal disease manifestation of infectious and non-infectious agents[4,5]. Up to 75 % of disease morbidity and manifestation in African children is attributed to diarrhea with a severity that seems to depend to some extent on etiology and age[6]. It's postulated that worldwide, Rotavirus infection is responsible for the most severe forms of diarrhea, especially in children and may account for up to 40 % of cases in the developed countries and 25 % in the developing world[7]. Irrespective of the etiology, diarrhea almost always occurs when there is an imbalance between absorption and secretion activity of the intestines whereby secretion exceeds absorption[8]. It has also been reported that even minimal changes in normal intestinal fluid and electrolyte balance may result in diarrhea. It is estimated that worldwide, nine million children younger than 5 years of age die annually due to diarrheal cases[9,10]. These deaths occur mostly in rural African communities where health care facilities are inadequate and the majority of the people lack access to clean and safe water, a major vehicle for transmission of diarrheal diseases[11]. It has also been claimed that diarrhea kills more young children around the World than malaria, acquired immunodeficiency syndrome (AIDS) and tuberculosis combined[12]. In the developing world many rural communities prepare medicinal remedies from indigenous plants since they are the only readily accessible and affordable therapies for the control of diarrhea[13,14]. Uganda has an ancient heritage of traditional medicine communities in both urban and rural areas where use of extracts, decoctions/concoctions or ashes of various plant parts (roots, rhizomes, tubers, aerial parts, stem barks and leaves) as remedies for diarrhea and other illnesses is common. Plants like *Psidium Guava, Leonitis nepotifolia* and *Moringa oileferia* have been used in management of diarrhea[15,16]. *Mondia whitei* is a woody climber with a large tuberous root stock from the Periplocaceae family. It is widely distributed in tropical Africa, from Guinea through Cameroon to East Africa. The roots are used either as spices, aphrodisiacs or for the treatment of Urinary tract infection, jaundice and headache, while the Whole plant is used to treat diarrhea[17,18]. The extracts of *Mondia whitei* have shown to exhibit good antimicrobial activities against *Salmonella typhi* and *Escherichia coli* which are among the major bacteria associated with diarrhea[19]. However, there is no enough scientific documentation of its antidiarrheal activity in Uganda, even though the rural people continue to use it as a non specific remedy in the treatment of diarrhea. This study was therefore designed to evaluate the anti-diarrheal activity

of ethanolic root bark extract of *Mondia whitie* in Wistar Albino rats.

MATERIALS AND METHODS

Study design

This was an experimental study in which both qualitative and quantitative data was obtained. We observed the effect of different doses of the ethanolic fresh root bark extract of *Mondia whitie* on the number or consistency of defecation, enteropooling and gastro-intestinal transit distance per unit time in castor oil induced diarrhea, in Wistar albino rats. This was compared with the effect in rats treated with Loperamide (positive control), Atropine sulphate (positive control for transit distance per unit time) and normal saline (0.9 % NaCl) which was used as negative control. Acute toxicity was evaluated in laboratory mice by determination of LD_{50} and the phytochemical composition of the extract from the plant was determined. All these activities took place in May and June of 2013.

Plant collection and processing

Fresh roots were bought from Wandegeya Market, Kampala-Uganda; packed in black polythene bags and transported to the Pharmacology and Toxicology Lab of the College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University –Kampala. Valid sample specimens were submitted for identification and authentication at Makerere University Herbarium, Department of Botany and voucher specimen deposited in the pharmacology and toxicology lab as PTRL015. The fresh root-bark was peeled off using a sharp knife and chopped into small pieces and left to dry under a shade.

Extraction process

The chopped fresh root bark pieces of the sample (425 g) were weighed using an Analytical balance (NVT1601/1) and macerated in 1.5 liters of 70 % ethanol in an amber bottle, shaken rigorously twice daily for three days. The extract was decanted, filtered using cotton wool in a Burchard funnel and further filtered using filter paper (Whatman® No1) before concentration using a rotary evaporator (CH-9230 Flawl/Schweiz, Germany) at a temperature of 55˚C to a constant volume. The extract was dried in a hot air oven (BM600) at 50˚C to obtain a semi-solid extract[14]. The dry residue was weighed and stored in the refrigerator (Labex 280, Germany) at 4˚C before the acute toxicity, phytochemical profiling and antidiarrheal activity tests were done.

The percent yield was determined using the formula

$$\text{Percentage yield: } = (w_2 \times 100)/w_1$$

Where w_1 is the weight of the fresh powdered sample before extraction and w_2 the weight of the semi-solid extracts from the macerated powder in 70 % ethanol. Therefore percentage yield = (30.64 x 100)/425 = 7.21 % w/w

Extract reconstitution

A stock solution of (200 mg/ml) for daily treatment was prepared by dissolving 4 g of the semi-solid extract in 20 ml of distilled water.

Animal selection and care

Wistar white albino rats of (80-125 g) of either sex (1:1) were used for the experiments. They were obtained from the Small Animal house of the College of Veterinary Medicine, Animal Resource and Biosecurity (COVAB), Makerere University. The rats were housed in individual separate cages under standard laboratory conditions (relative humidity 65 ± 2 %, temperature 25 ± 2˚C, 12 hours of light and 12 hours of darkness). They were fed on standard rodent pellet diet (Unga Ltd, Uganda) and tap water was provided *ad libitum*.

Drugs and chemicals

Fresh batches of the drugs and chemicals were bought for the experiments as follows: Atropine sulphate (Zhejiang Ruixin Pharmaceuticals Co., Ltd), Loperamide HCl (Remedica Ltd, Europe), Castor oil (Diarim Enterprises Ltd, Nairobi, Kenya), Normal saline (Fresanius Kabi Ltd, India) and Activated charcoal (Cyano Pharma (p) Ltd, Pologround, Indore, India).

Acute toxicity test

The methods previously described[20-24] were adopted using thirteen Swiss albino mice (16-24 g). In the first phase, three increasing doses (2500 – 5000 mg/kg) of the ethanolic fresh root bark extract were administered orally using gavage tube (size 4) to three groups each containing three mice. In the second phase, more specific doses were administered to four groups each containing one mouse. The median lethal dose (LD_{50}) value was determined as the geometric mean of the highest non-lethal dose and the lowest lethal dose of which there is 1/1 and 0/1 survival.

Ethical issues

The study animals were handled according OECD and NIH guidelines on experimental animal care, use and handling[24,25]. The study was approved by the Student Research Review Committee of the College of Veterinary Medicine, Animal Resources and Biosecurity of Makerere University.

Statistical analysis

The results are expressed as mean ± standard error of the mean (SEM). While One Way Analysis of Variance (ANOVA) was employed for differences within and between treatment groups; the Dunnet multiple comparison post- hoc test was used for mean differences with normal control. 95 % level of significance ($p \leq 0.05$) was used for statistical significance.

RESULTS

Phytochemical screening

The phytochemical screening results showed that the 70 % ethanolic extract of *Mondia whitei* had high concentration of saponins, catechol tannins, basic alkaloids and phenols with moderate presence of anthranol glycosides. However flavonoids and terpenoids were weakly present while reducing sugars were absent (Table 1).

Table1: Result of selected phytochemicals of ethanolic root bark extract of *Mondia whitei*

Phytochemical parameters	Results	Color change
Saponins	+++	1 cm layer foam
Tannins (catechol)	+++	Green blackish coloration
Flavanoids	+	Red coloration
Alkaloids	+++	Yellowish white precipitate
Anthranal glycosides	++	Pink coloration
Phenols	+++	Bluish black coloration
Terpenoids	+	Faint emerald green
Reducing sugars	-	Absence of red coloration

Key: +Weakly present, ++moderately present, +++Strongly present, -Absent

Acute toxicity

The LD_{50} was above 5000 mg/kg while no signs of toxicity and mortality observed in the various treatment groups with 7 days observation post single dose exposure. Doses above 5000 mg/kg in plant experiments are considered to be experimentally safe according to EPA and OECD toxic substances classification[24]. The study was carried out as recommended by[20-23].

Effects of ethanolic root bark extract of *Mondia whitei* in castor oil induced diarrhea

The ethanolic stem bark extract of *Mondia whitie* showed a dose response inhibition in castor oil induced diarrhea with the highest dose (600 mg/kg) showing better activity with $p < 0.01$ and $p < 0.001$ compared with both negative and positive control (Table 2).

Table 2: Effect of ethanolic root bark extract of *Mondia whitei* on wet fecal counts on castor oil induced diarrhea in Wistar albino rats (n = 6, mean ± SEM, p < 0.05)

Treatment groups	1 h	2 h	3 h	4 h
Negative control	4.00 ± 0.58	3.000 ± 1.265	2.833 ± 0.4014	2.500 ± 0.56
Positive control	0.0 ± 0.0***	1.667 ± 0.5164	1.000 ± 0.2582**	0.33 ± 0.21***
Group 1 (200 mg/kg)	0.1667 ± 0.1667***	1.833 ± 1.472	1.667 ± 0.3333	0.83 ± 0.31**
Group 2 (400 mg/kg)	0.3333 ± 0.2108***	1.833 ± 0.9832	0.8333 ± 0.3073***	0.50 ± 0.22***
Group 3 (600 mg/kg)	0.0 ± 0.0***	0.6667 ± 0.8165**	0.6667 ± 0.3333***	0.33 ± 0.21***

Values are mean fecal-counts ± SEM, n = 6, p < 0.05, *p < 0.05 significant;**p < 0.01 very significant; and ***p < 0.001 extremely significant as compared to both control groups.

Effects of ethanolic fresh root bark extract of *Mondia whitei* on the weight of wet fecal droppings

The ethanolic fresh root bark extract of *Mondia whitei* showed a dose-dependent statistical significant ($p = 0.001$) reduction in the weight of wet fecal droppings among the treatment groups, with the extract-groups showing same level of significance (Table 3).

Table 3: Effect of the ethanolic fresh root bark extract of *Mondia whitei* on Mean weight fecal droppings ± SEM in the treatment groups

Treatment group	Mean weight (g) wet fecal droppings ± SEM (n = 6)	95 % of CI (Lower-upper)	P-value
Normal control	2.02 ± 0.34	1.15 to 2.88	-
Positive control	1.02 ± 0.25**	0.37 to 1.67	P = 0.01
Group 1 (200 mg/kg)	0.53 ± 0.04***	0.42 to 0.64	P = 0.001
Group 2 (400 mg/kg)	0.52 ± 0.02***	0.47 to 0.56	P = 0.001
Group 3 (600 mg/kg)	0.48 ± 0.09***	0.25 to 0.72	P = 0.001

Dunnet multiple comparison test used for comparison among the treatment groups with the negative control, n = 6, P < 0.05, **p < 0.01, ***p < 0.001, SEM - standard error of mean

Effects of ethanolic root bark extract of *Mondia whitei* on castor oil induced gastrointestinal transit distance per unit time

The ethanolic root bark extract of *Mondia whitie* showed a dose dependent decrease in the propulsion of charcoal meal through the gastrointestinal tract, with 600 mg/kg ($p < 0.01$) as compared with both negative and positive ($p < 0.05$). However, there was no significant reduction in propulsive charcoal meal movement with doses of 200 and 400 mg/kg ($p > 0.05$) of the ethanolic root bark extract as compared to both control groups (Table 4).

Table 4: Effects of ethanolic root bark extract of *Mondia whitei* on castor oil induced gastrointestinal transit distance per unit time (30 minutes)

Treatment groups (n = 6)	Mean distance ± SEM [cm]	95 % CI Difference	P-Value
Negative control (Normal saline)	76.83 ± 2.798	69.64 - 84.03	P = 0.05
Positive control (5 mg/kg Atropine SO_4)	60.00 ± 6.094	44.34 - 75.66*	P < 0.05
Group 1 (200 mg/kg of extract)	64.33 ± 4.137	53.70 - 74.97	P > 0.05
Group 2 (400 mg/kg of extract)	63.50 ± 3.041	55.68 - 71.32	P > 0.05
Group 3 (600 mg/kg of extract)	54.33 ± 4.047	43.93 - 64.74**	P < 0.01

Values are mean distance [cm] ± SEM, n = 6, p < 0.05, *p < 0.05 and **p < 0.01 significant as compared to both control groups

Effects of ethanolic root bark extract of *Mondia whitei* on castor oil induced enteropooling (intestinal fluid accumulation)

The ethanolic root bark extract of *Mondia whitie* on castor oil induced enteropooling (intestinal fluid accumulation) showed a significant ($p < 0.05$) dose response anti-enteropooling activity when compared with both control groups. This was significant ($p < 0.01$ and $p < 0.001$) among all doses of 200, 400 and 600 mg/kg respectively (Table 5).

Table 5: **Effects of ethanolic root bark extract of *Mondia whitei* on castor oil induced enteropooling (intestinal fluid accumulation)**

Treatment groups (n = 6)	Mean ± SEM (ml)	95 % CI Difference	P-Value
Negative control (Normal saline)	2.717 ± 0.1014	2.456 - 2.977	P = 0.05
Positive control (Misoprostil)	2.150 ± 0.07188	1.965 - 2.335	P < 0.001
Group 1 (200 mg/kg of extract)	2.233 ± 0.08433	2.017 - 2.450	P < 0.01
Group 2 (400 mg/kg of extract)	1.717 ± 0.08724	1.492 - 1.941	P < 0.001
Group 3 (600 mg/kg of extract)	0.6667 ± 0.08819	0.4400 - 0.8934	P < 0.001

Values are mean volume [ml] ± SEM, n = 6, $p < 0.05$, *$p < 0.05$ significant, **$p < 0.01$very significant and ***$p < 0.001$ extremely significant as compared to both control groups

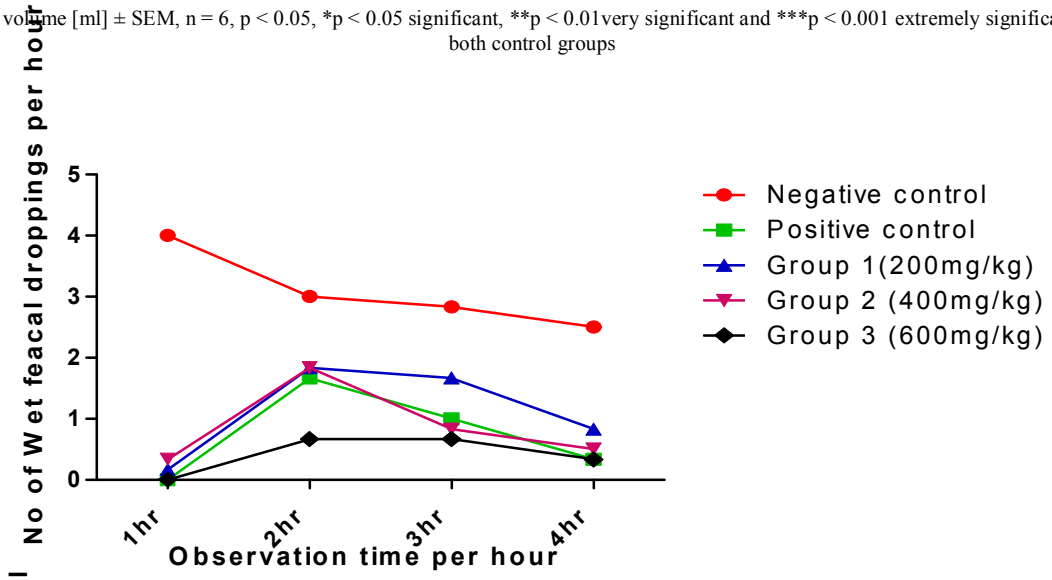

Figure 1: Mean wet fecal inhibition within 4 hour of observation at given time points

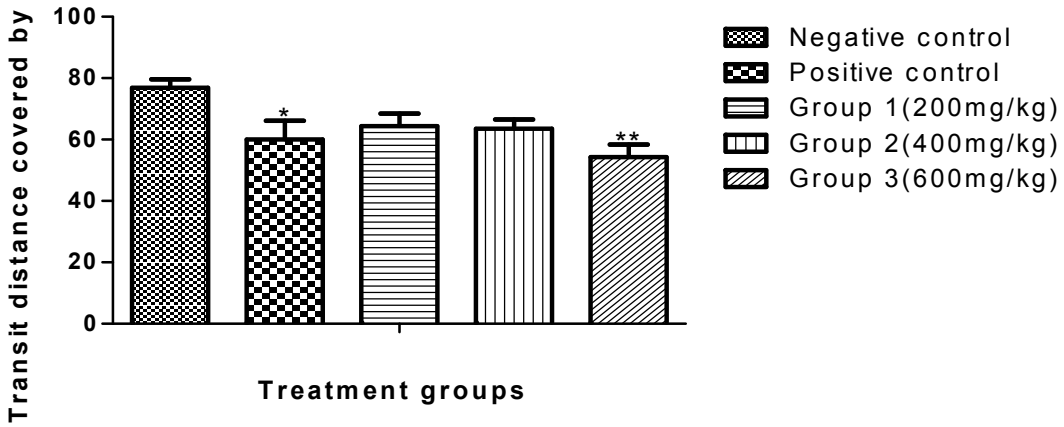

Figure 2: Mean transit distance covered by the charcoal meal with the various treatment groups

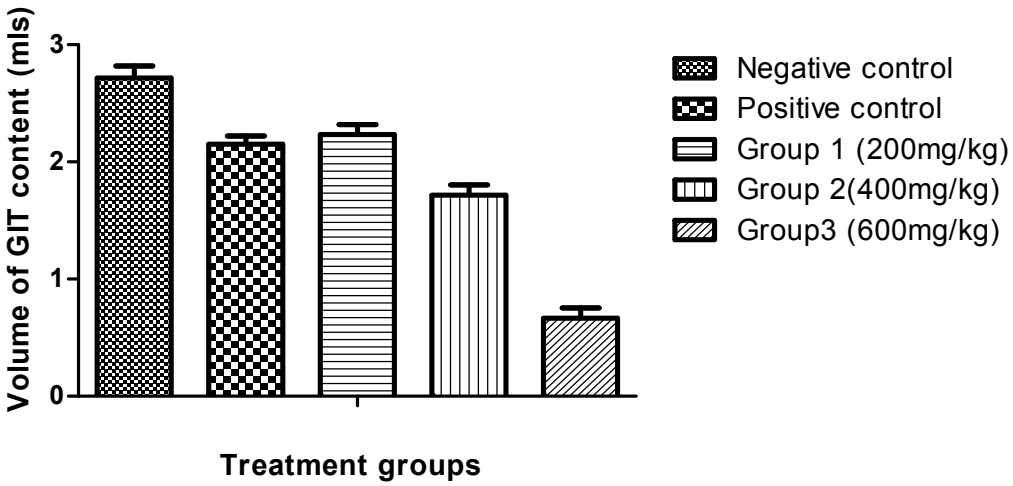

Figure 3: Mean volumes of gastrointestinal contents determined with various treatment groups

DISCUSSION

Diarrhea is a clinical condition usually characterized by reduced reabsorption, increased secretion of intestinal fluids and inflammatory changes in the intestinal mucosal lining[26]. This results into increased accumulation of fluids or semi-solid material in the lumen of both small and large intestines, triggering increased frequency of the defecation reflex, yielding a watery diarrhea via the anus. The severity of this clinical presentation is dependent on the primary causative agent of the disease. In the treatment of diarrhea, therapy is aimed at reducing the secretion of fluids, increasing reabsorption process and eliminating the cause of intestinal irritation or inflammation. In this study, we demonstrated the potential of *Mondia whitei* fresh root bark extract to reduce the severity of diarrhea by affecting the number or consistency of defecation, entero-pooling and gastrointestinal transit distance. The extract showed a dose dependent ability to inhibit diarrhea, with the highest dose of 600 mg/kg showing statistically significant ($p < 0.001$) activity when compared to the positive control (loperamide). The extract also displayed a dose-dependent reduction in the mean weight of wet fecal droppings among the various treatment groups used in the study with statistically significant reduction ($p < 0.001$) activity when compared to the positive control ($p < 0.01$). The gastrointestinal transit distance (cm) per unit time (30 minutes) was also found to reduce with increase in the dose of the extract administered. However, the significant reduction is only registered with the highest dose of 600 mg/kg ($P < 0.01$). This means that the amount of fluid reabsorbed from the intestinal luminal contents also increases with a proportionate increase in the dose of the extract administered. This phenomenon, eventually results into reduced severity of the diarrhea. All this, was further supported by the observed reduction in entero-pooling (intestinal fluid accumulation), as the dose of *Mondia whitei* root bark extract is increased, with an extremely significant comparison between the highest dose of 600 mg/kg and the positive control ($P < 0.001$). These findings could be attributed to the presence of a high concentration of saponins, catechol tannins, basic alkaloids and phenols in the extract of *Mondia whitei*. The phytochemical screening of ethanolic fresh root bark extract of *Mondia whitei* showed strong presence of Tannins, saponins, phenols and alkaloids with moderate presence of anthranal glycosides and weak presence of flavonoids and terpenoids with no reducing sugars. This agrees with studies done by[27-29]. Previous research studies have demonstrated antidiarrheal activity of tannins, flavonoids, saponins, terpenoids, phenols and alkaloids[30-32]. Tannins are known to evoke antidiarhoel effects by precipitating proteins or the electrolytes and reducing peristaltic movement and intestinal secretion[33,34]. Flavonoids have the ability to inhibit intestinal motility and hydro-electrolytic secretion which are known to be altered in this intestinal condition[35,36]. Therefore these phytochemical agents could be responsible for the significant Anti-diarrheal activity witnessed in this study. The acute toxicity study showed that the LD_{50} value is above 5000 mg/kg, which according to OECD, WHO and EPA guidelines for classification of toxic substances implies that this ethanolic extract can therefore be categorized under experimentally safe substances for use[35,36]. This explains the continuous traditional use of *Mondia whitei* in control of diarrhea in the African communities and the world at large with no severe adverse effects[37-39].

CONCLUSION

From the results obtained, it can be concluded that the ethanolic fresh root bark extract of *Mondia whitei* has an inhibitory action on gastrointestinal motility and secretion; although the mechanism of this inhibition remains unclear if it is via nitric oxide. Further tests are needed to be done to confirm the mechanism and to determine the individual active component responsible for the antidiarrheal activity of the extract.

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