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ANTIDIARRHOEAL POTENTIAL OF FRACTIONS OBTAINED FROM METHANOL EXTRACT OF *BRIDELIA ATROVIRIDIS* (EUPHORBIACEAE) LEAVES ON CASTOR OIL-INDUCED DIARRHOEA IN SWISS ALBINO MICE

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ABSTRACT

Background: Diarrhoea constitutes a major health concern, especially for children under the age of five. Orthodox drugs available for the treatment of diarrhoea are fraught with some challenges not limited to high cost, adulteration and toxic side effects, thereby making a case for alternative remedies, with several medicinal plants known to contain biologically active components that possess curative properties against diarrhoea.

Objective: The study was undertaken to evaluate the effect of fractions (ethyl acetate, n-butanol and residual aqueous) obtained from methanol extract of Bridelia atroviridis leaves on castor oil induced diarrhoea in Swiss albino mice.

Methods: Powdered plant material was extracted with absolute methanol using soxhlet apparatus and further fractionated successively with ethyl acetate, n-butanol and distilled water. In castor oil-induced diarrhoea, castor oil-induced enteropooling and electrolyte concentration tests, fasted mice divided into nine (9) groups of five mice each were administered 250 and 500 mg/kg of fractions obtained from B atroviridis leaves while standard drug (loperamide) was administered at 3 mg/kg.

Results: However, only aqueous fraction (250 and 500 mg/kg) showed significant ($p<0.05$) difference in volume of intestinal content when compared to castor oil control group.

Conclusions: This study provides scientific data on the antidiarrhoeal potential of fractions obtained from methanol extract of Bridelia atroviridis leaves on castor oil-induced diarrhoea in Swiss albino mice, hence justifying its use in traditional medicine.

Keywords: Diarrhoea, health-concern, medicinal-plant, castor oil-induced

1. INTRODUCTION

Diarrhoea constitutes a major health concern, especially for children under the age of 5 years and accounts for up to 17% mortality in hospitalized children (Mahesh *et al*., 2010). Worldwide distribution of diarrhoea accounts for more than 5-8 million deaths each year in infants and children below 5 years old especially in developing countries (Misra *et al*., 2014). Several commercial drugs such as Loperamide, diphenoxylate and racecadotril have been used to combat diarrhoea (Velázquez *et al*. 2010), but the problem posed by the high cost, adulteration and increasing toxic side effects of these synthetic drugs coupled with their inadequacy in the treatment of diarrhoea, especially in the developing countries cannot be over emphasized (Valarmathy *et al*., 2010). Drug resistance presents an ever increasing health challenge that involves all major microbial pathogens and antimicrobial drugs (Levy and Marshall, 2004). Medicinal plants contain biologically active chemical substances such as as saponins, tannins, essential oils, flavonoids, alkaloids etc; which

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possess curative properties (Sofowora, 1993). Okigbo *et al*. (2009) reported that medicinal plants have potential against diseases such as HIV/AIDS, malaria, diabetes, sickle-cell anaemia, mental disorders and microbial infections. More so, in an effort to discover new compounds, researchers screen plant extracts to detect secondary metabolites with relevant biological activities (Jeyachandran *et al*., 2009). According to the World Health Organization, 80% of the world's population use medicinal plants in the treatment of diseases and in African countries, this rate is much higher (WHO, 2001).

Medicinal plants are potential sources of antidiarrhoeal drugs, therefore many international organizations have encouraged studies on the possible ways of treating and preventing diarrhoea through traditional medical practices (Rahman *et al*., 2015). A range of medicinal plants with antidiarrhoeal properties has been widely used by the traditional healers; however, the effectiveness of many of these medicinal preparations have not been scientifically evaluated (Omodamiro and Ibeh, 2014). One of such medicinal plants is *B. atroviridis.* There is paucity of information on the antidiarrhoeal potential of *B. atroviridis*, therefore, this study was designed to evaluate the antidiarrhoeal properties of fractions obtained from methanol extract of *B. atroviridis* leaves on castor oil-induced diarrhoea in Swiss albino mice.

2. MATERIALS AND METHODS

The leaves of *Bridelia atroviridis* were collected from its natural habitat in Okpokwu local government area of Benue State during the month of July, 2016. The sample was identified and authenticated at the Herbarium unit of the Department of Biological Science, Ahmadu Bello University, Zaria. A voucher number (3289) was assigned to the plant and a specimen deposited in the same Department for future reference. Methanol, *n*-butanol and ethyl acetate (all of analytical grade), Loperamide (Hovid Bhd. Malaysia) and Castor oil (Bell, Sons and Co. Ltd, Southport England) were used. Adult Swiss Albino mice (aged 6-8 weeks) of both sexes weighing (20-30 g) were obtained from the Animal House of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, A.B.U, Zaria, acclimatized to normal laboratory conditions (25±2^{o}C with a 12 hour light and dark cycle) for one week prior to study and fed standard pellet diet and water *ad libitum*.

2.1. Plant preparation

The leaves were cleansed and air-dried at room temperature for seven days before grinding to coarse powder using pestle and mortar. The powdered material of the plant (1100 grams) was then extracted in Soxhlet apparatus with 2 litres of absolute methanol as solvent. The methanol extract was concentrated to dryness at 50^{o}C using water bath. 20 grams of methanol extract was further fractionated using 1.5 litres each of distilled water, absolute ethyl acetate and *n*-butanol. The fractions were concentrated to dryness by evaporation on water bath at 50 ^{o}C. Percentage yield was calculated and the fractions were preserved in an airtight bottle under cool temperature until required.

2.2. Phytochemical Analysis

Standard procedures as described by Sofowora

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(1993), Evans (2002) and Silva *et al*., (2003) were used for identification of Phytochemicals. Test for tannins (ferric chloride test), saponins (frothing test), flavonoids (NaOH test), terpenoids and steroids (Lieberman-Burchard's test), alkaloids (Wagner's test), carbohydrates (Molisch's test), glycosides (Keller-Killiani test), and anthraquinones (Bontragers test) were carried out.

2.3. Acute toxicity test

The method of Lorke (1983) was used to determine the median Lethal Dose (LD_{50}) of each of the fractions obtained from methanol extract of *B. atroviridis*. The experiment was conducted in two phases and mice were fasted for 12 hours prior to administration of various fractions. In phase 1, nine (9) mice divided into three groups of three mice each. The extract was administered orally in three graded doses (10, 100 and 1000 mg/kg). Group I, II and III received 10, 100 and 1000 mg/kg respectively of each fraction. Mice were observed for 4 hours after administration for signs of toxicity. After 24 hours, no death was recorded, hence the second phase was initiated. In phase 2, three mice divided into three groups of one mouse each were given 1600 mg/kg, 2900 mg/kg and 5000 mg/kg, respectively, of each of the fraction. The mice were then observed for signs of toxicity for the first 4 hours and mortality after 24 hours.

Median Lethal Dose (LD_{50}) = $\sqrt{(}$(highest non-lethal dose) × (lowest lethal dose))

2.4. Castor oil-induced diarrhoea

Forty five (45) mice of both sexes were divided into nine groups of five mice each: Group I (Normal control) were given only distilled water at the dose of 10 ml/kg body weight, group II (castor oil control) received 10 ml/kg distilled water while group III received loperamide (3 mg/kg) as standard drug, group IV, V, VI, VII, VIII and IX received ethylacetate, *n*-butanol and aqueous fractions each at either 250 or 500 mg/kg. All doses were administered orally and animals fasted for 18 hours prior to the test. After 1 hour, all groups except group I were given 0.5 ml of castor oil orally. Mice were then housed individually in cages lined with white blotting paper (changed hourly) and observed for four hours, for the presence of diarrhoeal stool and the time of onset of diarrhoea was recorded. A numerical score based on stool consistency was assigned as follows: normal stool =1, semisolid stool =2 and watery stool =3. The stool frequency of each group was expressed as percent inhibition (%) of diarrhoea. The percent inhibition of defecation was calculated as follows:

$$\% \text{ inhibition} = \frac{Mc - Md}{Md} \times 100$$

Where Mc = mean number of droppings caused by castor oil, Md = mean number of droppings caused by drug or extract (Shoba and Thomas 2001; Uddin *et al*., 2005).

2.5. Castor oil-induced enteropooling

The castor oil-induced enteropooling was carried out according to the method described by Robert *et al*. (1976) and Qnaise *et al*. (2007). Forty five (45) mice of both sexes were divided into nine groups (n=5) and fasted for 18 hours prior to the experiment. Distilled water at the dose of 10 ml/kg body weight was administered

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to group I (Normal control) while group II (castor oil control) were treated with distilled water 10 ml/kg body weight and castor oil, group III were treated with loperamide (3 mg/kg) body weight as standard drug, while group IV, V, VI, VII VIII and IX were given ethyl acetate, *n*-butanol and aqueous fractions at either 250 mg/kg or 500 mg/kg. All doses were administered orally and after one hour, all mice except group I were challenged with 0.5 ml of castor oil orally. One hour after castor oil administration, mice were sacrificed by chloroform anesthesia and the small intestine from the pylorus to the caecum excised, intestinal content was weighed and volume measured by using a graduated tube.

2.6. Determination of sodium ion (Na+) and potassium ion (K+) concentrations

The same set of animals previously used in the enteropooling test were used for determination of Na^+ and K^+ ion concentration. The effluent from the intestinal loops (serosal solution) was collected and measured in a graduated tube. This was further centrifuged at 1500 × g for 30 minutes and the supernatant was obtained and used for Na^+ and K^+ analysis (Omoboyowa *et al*., 2015) using an electrolyte analyzer that measures change in the membrane potential. The potential generated is compared with the potential of a reference electrode. Sodium and potassium ion concentrations are expressed in terms of mmol/L or mEq/L.

2.7. Statistical analysis

Results were expressed as Mean ± SD. Data collected were subjected to one-way analysis of variance (ANOVA) followed by Duncan multiple range test (DMRT) and *P* value <0.05 was considered significant using IBM Statistical Package for Social Sciences (Version 21, International Business Machines Corporation).

3. RESULTS

3.1. Percentage Yield of Fractions Obtained from *Bridelia atroviridis* Leaves

The initial extraction of the powdered plant material (about 1100 g) with methanol produced 21g of methanol extract representing a 1.91% yield. Further fractionation of the methanol extract produced 11.69 g (58%), 4.26 g (21.3%) and 3.40 g (17%) of ethyl acetate, *n*-butanol and residual aqueous fractions respectively (Figure 1).

3.2. Phytochemical Constituents of Fractions Obtained from *Bridelia atroviridis* Leaves

Preliminary phytochemical screening of fractions obtained from *B. atroviridis* leaves revealed the presence of Alkaloids, Flavonoids, Tannins, Saponins, Cardiac glycosides and Carbohydrates. Anthraquinones were not traced in all the fractions while Triterpenes and Steroids were absent in aqueous but present in ethylacetate and *n*-butanol fractions (Table 1).

3.3. Lethal Dose (LD_{50}) of Fractions Obtained from *Bridelia atroviridis* Leaves

The result for LD_{50} of fractions obtained from of *B. atroviridis* leaves (Table 2) showed no mortality or toxic reactions both at the first and second phase of the experiment upon oral administration. The oral median lethal dose of *B. atroviridis* leaves fractions is therefore greater than 5000 mg/kg in mice.

3.4. Effect of Fractions Obtained from *Bridelia atroviridis* Leaves on Stool Consistency and Onset of Diarrhoea in Mice

The time taken to induce diarrhoea in mice administered ethyl acetate *n*-butanol and residual

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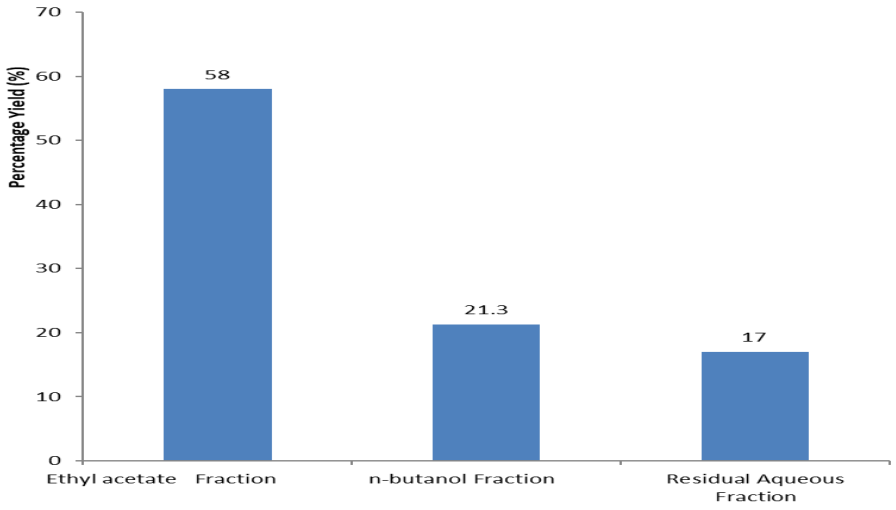

Figure 1: Percentage Yield of Fractions Obtained from *Bridelia atroviridis* Leaves

Table 1: Phytochemical Constituents of Fractions Obtained from *Bridelia atroviridis* Leaves

Phytochemicals	Fractions		
	Ethyl acetate	*n*-Butanol	Residual Aqueous
Flavonoids	+	+	+
Tannins	+	+	+
Saponins	-	+	+
Cardiac glycosides	+	+	+
Alkaloids	+	+	+
Carbohydrate	+	+	+
Steroids	+	+	
Anthraquinones	-	-	-
Triterpenes	+	+	-

Table 2: Median Lethal Dose (LD_{50}) of Fractions Obtained from *Bridelia atroviridis* Leaves

Phase	Fractions	Dose (mg/kg body weight)		
1		10	100	1000
	Ethyl acetate	No death	No death	No death
	n-Butanol	No death	No death	No death
	Residual Aqueous	No death	No death	No death
2		1600	2900	5000
	Ethyl acetate	No death	No death	No death
	n-Butanol	No death	No death	No death
	Residual Aqueous	No death	No death	No death

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aqueous fractions (250 mg/kg) of *Bridelia atroviridis* leaves was significantly ($p > 0.05$) longer than mice in the castor oil control group. The group administered loperamide (standard drug) also showed significant ($p > 0.05$) difference when compared to castor oil control group. The total faeces in all fraction treated mice was significantly ($p > 0.05$) reduced when compared to the castor oil control mice. The standard drug also presents a significant ($p > 0.05$) reduction in total faeces. Similarly, there is a significant ($p > 0.05$) decrease in total diarrhoea faeces in all treated mice when compared to the castor oil control. Data revealed that ethyl acetate (250 mg/kg), *n*-butanol (500 mg/kg) and aqueous (250 and 500 mg/kg) fractions presented the highest percentage inhibition of diarrhoea (62.83%, 50.54%, 52.70% and 51.35% respectively) compared with the castor oil control group. In addition, at a dose of 250 mg/kg body weight, the ethyl acetate fraction produced similar effect with the standard drug (62.83%). However, the aqueous and *n*-butanol fractions appeared to significantly stimulate the onset of diarrhoea at 500 mg/kg body weight respectively when compared with the castor oil control (Table 3).

3.3. Effect of Fractions Obtained from *Bridelia atroviridis* Leaves on Castor Oil-induced Enteropooling in Mice

From the results (Table 4), there was a significant ($p<0.05$) difference in weight of intestinal content of *n*-butanol (500 mg/kg), aqueous (250 and 500 mg/kg) and Loperamide treated groups when compared to castor oil control group. There was no significant ($p>0.05$) difference in volume of intestinal content in all the treated groups, except mice treated with aqueous fraction (250 and 500mg/kg) when compared to castor oil control group. Aqueous and *n*-butanol fractions administered at 500 mg/kg presented the highest percentage inhibition of intestinal weight contents (76% and 56% respectively), which was better than the standard drug (52%).

3.4. Effect of Fractions Obtained from *Bridelia atroviridis* Leaves on Serosal Fluid Electrolyte Concentration

Data indicate that there were no significant ($p > 0.05$) differences in serosal sodium and potassium ion concentrations of all treated groups compared to castor oil control group, except for aqueous fraction (250 mg/kg) with significantly ($p > 0.05$) higher serosal sodium ion concentration (Table 5).

4. DISCUSSIONS

Despite the fact that diarrhoea is preventable, it accounts for nine percent (9%) of all deaths among children under age five worldwide. Diarrhoea is usually attributed to altered motility and fluid accumulation in the intestinal lumen. Some antidiarrhoeal agents are known to reduce diarrhoea by decreasing gastrointestinal tract motility and/or the secretion (Akuodor *et al*., 2010). Several studies have validated the use of antidiarrhoeal medicinal plants by investigating the biological activity of extracts from these plants, such as antispasmodic effects, delayed intestinal transit, water absorption and the intraluminal fluid accumulation (Gutiérrez *et al*., 2007). In this study, we evaluated the acute toxicity (LD_{50}) profile, Phytochemical constituents, antidiarrhoeal potential of fractions obtained from *Bridelia atroviridis* leaves, as well as its effect on serosal electrolyte (Na^+ and K^+) concentrations.

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Table 3: Effect of Fractions Obtained from *Bridelia atroviridis* Leaves on Stool Consistency and Onset of Diarrhoea in Mice

Treatment/Dose	Onset time of diarrhoea	Total no. of faeces	Total no. of diarrhoeal faeces	% Inhibition of diarrhoea
DW (10 ml/kg)	0.00 ± 0.00^{a}	2.20 ± 0.44^{a}	0.00 ± 0.00^{a}	-
CO + DW (10 ml/kg)	104.00 ± 5.29^{d}	9.60 ± 1.14^{d}	7.40 ± 1.14^{e}	-
CO + LPM (3 mg/kg)	155.20 ± 4.76^{g}	3.60 ± 2.70^{ab}	2.20 ± 0.81^{b}	62.83
CO + EA (250 mg/kg)	170.33 ± 4.50^{h}	4.60 ± 2.50^{abc}	3.00 ± 0.81^{b}	62.83
CO + EA (500 mg/kg)	102.66 ± 0.57^{d}	6.60 ± 2.88^{c}	4.50 ± 0.57^{cd}	39.18
CO + NB (250 mg/kg)	132.66 ± 12.70^{f}	6.20 ± 2.48^{bc}	5.25 ± 0.95^{d}	29.05
CO + NB (500 mg/kg)	78.00 ± 15.13^{c}	5.00 ± 1.87^{bc}	2.50 ± 1.29^{bc}	50.54
CO + AQ (250 mg/kg)	117.33 ± 7.50^{e}	5.20 ± 1.09^{bc}	3.00 ± 1.41^{bc}	52.70
CO + AQ (500 mg/kg)	50.66 ± 5.13^{b}	6.20 ± 1.30^{bc}	3.60 ± 0.54^{bc}	51.35

Values are means ± SD of five animals in each group. Values in the same column with different letter superscripts are significantly different $p<0.05$. Key: CO=Castor oil DW= Distilled water LPM=Loperamide EA=Ethyl acetate fraction NB=*n*-Butanol fraction AQ=Aqueous fraction. Volume of castor oil administered per mice =0.5 ml

Table 4: Effect of Fractions Obtained from *Bridelia atroviridis* Leaves on Castor Oil-

Treatment/Dose	Volume of intestinal content (ml)	Weight of intestinal content (g)	% Inhibition of intestinal content
DW (10 ml/kg)	0.07 ± 0.00^{a}	0.09 ± 0.03^{a}	-
CO + DW (10 ml/kg)	0.28 ± 0.13^{c}	0.50 ± 0.24^{c}	-
CO + LPM (3 mg/kg)	0.19 ± 0.15^{bc}	0.24 ± 0.16^{ab}	52
CO + EA (250 mg/kg)	0.20 ± 0.07^{bc}	0.32 ± 0.08^{bc}	36
CO + EA (500 mg/kg)	0.23 ± 0.04^{c}	0.38 ± 0.23^{bc}	24
CO + NB (250 mg/kg)	0.18 ± 0.07^{abc}	0.38 ± 0.10^{bc}	24
CO + NB (500 mg/kg)	0.19 ± 0.05^{abc}	0.22 ± 0.08^{ab}	56
CO + AQ (250 mg/kg)	0.10 ± 0.06^{ab}	0.26 ± 0.05^{ab}	48
CO + AQ (500 mg/kg)	0.07 ± 0.01^{a}	0.12 ± 0.04^{a}	76

Values are means ± SD of five animals in each group. Values in the same column with different letter superscripts are significantly different $p<0.05$. CO=Castor oil DW= Distilled water LPM=Loperamide EA=Ethyl acetate fraction NB=*n*-Butanol fraction AQ=Aqueous fraction. Volume of castor oil administered per mice =0.5 ml

Table 5: Effect of Fractions Obtained from *Bridelia atroviridis* Leaves on Serosal Fluid Electrolyte Concentration

Treatment/Dose	Sodium ion (Na^{+}) concentration (mmol/L)	Potassium ion (K^{+}) concentration (mmol/L)
DW (10 ml/kg)	145.78 ± 5.44^{a}	1.98 ± 0.98^{a}
CO + DW (10 ml/kg)	369.60 ± 41.16^{bc}	23.12 ± 7.07^{b}
CO + LPM (3 mg/kg)	357.40 ± 20.63^{bc}	20.14 ± 8.39^{b}
CO + EA (250 mg/kg)	373.80 ± 17.68^{bc}	15.92 ± 3.97^{b}
CO + EA (500 mg/kg)	387.00 ± 17.47^{cd}	16.06 ± 3.71^{b}
CO + NB (250 mg/kg)	369.20 ± 29.58^{bc}	22.06 ± 6.60^{b}
CO + NB (500 mg/kg)	351.60 ± 16.62^{b}	18.16 ± 4.71^{b}
CO + AQ (250 mg/kg)	407.40 ± 20.76^{d}	21.22 ± 3.26^{b}
CO + AQ (500 mg/kg)	373.00 ± 16.17^{bc}	16.42 ± 2.98^{b}

Values are means ± SD of five animals in each group. Values in the same column with different letter superscripts are significantly different $p<0.05$. CO=Castor oil DW= Distilled water LPM=Loperamide EA=Ethyl acetate fraction NB=*n*-Butanol fraction AQ=Aqueous fraction. Volume of castor oil administered per mice =0.5 ml

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Acute toxicity studies provide a short term assessment and evaluation of potential hazard test substance or consequences of single dose of a test substance, and is better presented as LD_{50}, which is the dose that kills 50% of a test population after specified test duration (Arome *et al.*, 2013). Hence an LD_{50} value ≥ 5000 mg/kg are considered relatively safe for oral use (Lorke, 1983). The LD_{50} profile of the methanol fractions of *B. atroviridis* leaves was greater than 5000 mg/kg body weight and there was neither mortality nor impaired behavior during the observation period, suggesting that the fractions are nontoxic orally and may be considered relatively safe for oral use in traditional management of diarrhoea.

The induction of diarrhoea using castor oil has been documented in several scientific work (Shoba and Thomas, 2001; Karthik *et al*., 2011; Rahman *et al*., 2015). Castor oil induces diarrhoea due to its active component ricinoleic acid (Ammon and Thomas, 1974) derived from the hydrolysis of triglyceride in the small intestine by pancreatic lipase (Xiao *et al*., 2014). It increases intestinal peristalsis leading to changes in electrolyte permeability of the mucosal membrane (Akuodor *et al*., 2010) causing local irritation and inflammation of the intestinal mucosa resulting in the release of prostaglandins ($PGE_2\alpha$) (Nguelefack *et al*., 2014; Xiao *et al*., 2014) known to stimulate gastrointestinal motility and secretion of water and electrolytes (Rajat *et al*., 2013). Therefore, increase in volume of intestinal content and number of stool in the castor oil control groups is an indication of diarrhoea induction in this study.

Plant extracts containing tannins, flavonoids, alkaloids, saponins and steroids have been reported to possess anti-diarrhoeal activity (Balaji *et al.,* 2012; Mohammed *et al*., 2013; Shemsu *et al*., 2013). In this study, methanol fractions of *B. atroviridis* leaves revealed the presence of Tannins, flavonoids, saponins, glycosides, steroids, triterpenes and carbohydrate.

Phytochemicals such as tannins present in plant extracts are likely to denature intestinal proteins, resulting in reduction of intestinal secretion and creating more resistance (Tripathi, 2003). Studies have shown that tannins act on the gut wall to inhibit intestinal motility and secretion making the intestinal mucus resistant by forming the protein tannate (Kumar *et al*., 2010), while flavonoids have been reported to inhibit prostaglandins and autacoids release resulting in reduction of motility and secretion induced by castor oil (Veiga *et al.,* 2001). Terpenoids, for example, abietic corrosive and steroids, such as, phytosterols have been reported to hinder generation of prostaglandin E_2 (Fernandez *et al*., 2001; Awad *et al.,* 2004) known to play a vital role in the incitement of intestinal secretions (Bern *et al*., 1989).

In this study, all fractions at the given doses were able to inhibit castor oil-induced diarrhoea evidenced by the significant ($p < 0.05$) reduction in the number of diarrhoea faeces and total faeces. Therefore, the noteworthy antidiarrhoeal property seen may likely be ascribed to the presence of these phytochemicals in the fractions. The antidiarrhoeal effect of these fractions may also be attributed to the presence of triterpenes, tannins and flavonoids which are shown to promote water and electrolyte absorption in the colon (Palombo, 2006). It is also

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possible that these fractions possess anti-inflammatory activity and may have inhibited prostaglandin synthesis. Prostaglandins of the E series are good diarrheogenic agents (Rahman *et al*., 2013; Sumi *et al*., 2015), therefore, inhibitors of prostaglandin biosynthesis are considered to delay castor oil-induced diarrhoea (Tunaru *et al*., 2012). Interestingly, the effect of ethyl acetate (250 mg/kg) is comparable to that of loperamide at the given dose. Similar outcome has been demonstrated in other scientific researches where ethyl acetate and aqueous fractions of different plants reduce the frequency of diarrhoea stool in experimental animal model (Akindele, 2006; Meite *et al*., 2009; Singh *et al*., 2013).

Loperamide is a synthetic peripherally acting opiate, used primarily to treat diarrhoea (Heel *et al*., 1978). It acts on the μ-opioid receptors in the myenteric plexus of the large intestine (Sandhu *et al*., 1981) by decreasing its activity which further decreases the tone of the longitudinal and circular smooth muscles of the intestinal wall (Katzung, 2004.). This increases the amount of time substances stay in the intestine thereby allowing for more water absorption from feaces (Hopman *et al*., 1990). A study by Karim and Adaikan, (1977) demonstrated that the effect of loperamide is due to its anti-motility and anti-secretory properties. Thus, the fractions of *B. atroviridis* used in the present study may have mediated their effects through similar mechanisms.

The accumulation of fluid in the small intestine (Enteropooling), represents the sum of fluid being excreted from the blood into the intestinal lumen, and to a lesser extent, the portion of fluid already in the lumen but whose absorption is inhibited (Robert *et al*., 1976). In the castor oil-induced enteropooling test, treatment of mice based on different doses of aqueous fraction of *B. atroviridis* produced a significantly ($p > 0.05$) dose-dependent decrease in the intestinal fluid accumulation. Castor oil-induced diarrhoea and fluid accumulation have been shown to be attenuated by many plant extracts such as *Strychnos potatorum* (Biswas *et al*., 2002), *Ixora coccinea* (Maniyar *et al.,* 2010), or isolated molecules like Ternatin (Rao *et al*., 1997) and Piperine (Bajad *et al*., 2001). The aqueous (250 and 500 mg/kg) and *n*-butanol (500 mg/kg) fractions produced a significant ($p > 0.05$) reduction in the weight of intestinal content when compared with castor oil control group, hence aqueous fraction exhibited the best effect on this model.

Results from studies on electrolyte transport demonstrated the ability of extracts to cause absorptive efflux of potassium and sodium ions from the serosal solution to varying extent, and also antagonize the ion transport alteration effects of castor oil on K^+ and Na^+ fluxes (Omoboyowa *et al*., 2015). In this study, there was no significant ($p > 0.05$) difference in serosal sodium and potassium ion concentrations of all treated groups compared to castor oil control group, except for the aqueous fraction (250 mg/kg) with significantly ($p > 0.05$) higher serosal sodium ion concentration. This fraction might have increased the secretion of water and Na^+ into the intestinal lumen. Based on this outcome, the fractions of *Bridelia atroviridis* may have also exerted their antidiarrhoeal activities via other mechanisms of absorptive influence on the gastrointestinal tract rather than by absorptive efflux of electrolytes.

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The result of this study demonstrate that fractions (ethyl acetate, *n*-butanol and aqueous) obtained from *B. atroviridis* leaves may be considered relatively safe for oral use and possesses antidiarrhoeal activity against castor oil-induced diarrhoea and intestinal secretion, especially ethyl acetate at 250 mg/kg. Further work could be done on the purification, isolation and identification of the components responsible for the antidiarrhoeal activity of these fractions and elucidate the possible mechanism of action in these fractions.

CONCLUSIONS

The recovery yield of fractions of *B. atroviridis* leaves showed that in all, ethyl acetate had the highest percentage yield (58%) while the phytochemical screening showed the presence of tannins, flavonoids, alkaloids, carbohydrates and cardiac glycosides. Median lethal dose (LD_{50}) of all the fractions obtained from methanol extract of *B. atroviridis* was found to be above 5000 mg/kg body weight in mice, this suggest that the leaves of *B. atroviridis* were nontoxic and may be considered relatively safe for oral use in traditional management of diarrhoea. The inhibition of castor oil-induced diarrhoea is significantly ($P<0.05$) higher in the ethyl acetate (250 mg/kg) fraction and found to be comparable to loperamide. Finally, Aqueous fractions of *B. atroviridis* produced a significant ($P<0.05$) dose-dependent decrease in castor oil-induced intestinal fluid accumulation.

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