



Anti-diarrhea Potential and Acute Toxicity Studies of Methanolic Extract of *Vernonia amygdalina* and *Cymbopogon citratus* against Castor Oil Induced Diarrhea Model in Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OSO and JOS designed the study, wrote the protocol and supervised the work. Authors OSO and JOS carried out all laboratories work and performed the statistical analysis. Authors OGT and JOS managed the analyses of the study. Authors JOS, OTO and JAF wrote the first draft of the manuscript. Authors JOS and OGT managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Diarrhea is a common cause of death in developing countries and second most common cause of infant mortality worldwide. The effect of a methanolic leaf extract of Bitter leaf (*Vernonia amygdalina*) and Lemon grass (*Cymbopogon citratus*) on Castor oil-induced diarrhea was

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investigated. Fifty (50) rats between 120-227g were induced with castor oil for development of diarrhea. They were divided into two sub-groups of twenty-five (25) subsequently divided into five groups treated with 2 mg/kg loperamide, 100, 200, and 400 mg/kg respectively. The cumulative frequencies of wet and formed stools were noted on the 3rd - 6th hour. It was observed that 400 mg/kg body weight of the extracts reduced the fecal spots compared to 200 mg/kg and 100 mg/kg body weight. Plant extracts were found to be non-toxic. The phytochemical screening of the methanolic extract revealed the presence of some bioactive components which may be responsible for the anti-diarrhea properties observed in this study. The above findings suggested that the methanolic extracts contain active constituents that have anti-diarrhea activities that may lead to its use in treating diarrhea.

Keywords: Ricimoliec acid; flavonoid; loperamide; phytochemical analysis; fecal spots.

1. INTRODUCTION

Diarrhea disease is the cause of almost three million deaths mainly among children younger than five years of age [1]. Worldwide, the annual global burden of diarrhea is enormous, involving 3 to 5 billion cases and nearly 2 million deaths, accounting for almost 20% deaths in children below five years [2]. In Africa, the diarrhea-specific mortality in children has been estimated at about 106 per 1000 [3]. Available reports in Nigeria indicate that more than 315,000 deaths of preschool age children were recorded annually as a result of diarrhea disease [4,5,6]. The contribution of the various pathogens to diarrhea may differ substantially between regions depending on local meteorological, geographic and socio-economic conditions [7]. Underlying reasons for the spread of diarrhea diseases are found in poor hygiene and poor sanitation, limited access to safe drinking water as well as inadequate education of health care providers and recipients [8]. Diarrhea symptoms are characterized by increased number of loose or watery stools, fluid loss and dehydration [9,10]. Diarrhea is treated mostly with antimicrobial drugs, but this treatment is generally ineffective [11,12,13]. In this study Loperamide is used as a positive control, which acts by increasing colonic phasic segmenting activities through inhibition of pre-synaptic cholinergic nerves in the sub-mucosal and mesenteric plexuses. These effects result in fecal water absorption thus reducing the frequency of defecation [9,14]. *Vernonia amygdalina* commonly known as bitter leaf is a shrub that peaks in height around 3 meters. It grows in several parts of Africa, including the tropics and particularly South Africa, Zimbabwe and Nigeria [15-17]. *Vernonia amygdalina* may be effective against amoebic dysentery [18]; gastrointestinal disorders [19-20]; microbial and parasitic activities [21-22]; hepatotoxicities [23]; and cancer [24-29]. *Cymbopogon citratus* also

known as Lemon grass is a perennial grass of about 55 species found mostly in warm region, especially in tropical and subtropical countries [30]. *Cymbopogon citratus* belongs to the Poaceae family with slender sharp edged leaves and pointed apex [31]. *Cymbopogon citratus* is widely used in Asia as cuisines, in India as sedatives, febrifuge and immunostimulant [32,33] and in Nigeria for stomach problem and typhoid [34,35]. Since the beginning of human history, all natural plants are rich source of medicinal agents and have been in practice as folk medicine, especially in traditional medicine [36]. This study was based to explore the possibility of using herbs such as *Vernonia amygdalina* and *Cymbopogon citratus* for treatment of diarrhea. The aim of this study is to determine the effect of *Vernonia amygdalina* and *Cymbopogon citratus* methanolic extracts as anti diarrhea agents and to determine if they possess any toxicological effects as a proof to ascertain their safety.

2. MATERIALS AND METHODS

2.1 Collection, Preparation and Extraction of Plant Samples

Bitter leaf (*Vernonia amygdalina*) leaves and Lemon grass (*Cymbopogon citratus*) were obtained from Mararaba market, a suburb of Abuja, Nigeria, identify and authenticated by Mrs Florence D. Tarfa, The Head of Department, Medicinal Chemistry and Quality Control, National Institute of Pharmaceutical Research and Development, Abuja, Nigeria. Voucher specimen numbers of the plants are NIPRD/15/014 and NIPRD/09/014. These leaves were dried for fifteen days at 20-28°C. It was milled to a fine powder using commercial blender (Blendtec). 22.8 g *Vernonia amygdalina* and 24.1 g *Cymbopogon citratus* were used for the methanolic extraction, using soxhlet apparatus.

The Methanolic extract was dried in vacuo using rotary evaporator.

Determination of the percentage yield of the crude extract

Percentage yield (%) =

$$\frac{\text{Weight of the plant extract}}{\text{Weight of the dried plant sample used}} \times 100$$

2.2 Phytochemical Screening

The methanolic extracts of *Vernonia amygdalina* and *Cymbopogon citratus* leaves were screened in the presence of phytochemical compounds as described by Evans [37].

2.3 Experimental Animals

Rats were purchased from the animal house of the Bingham University. The animals were housed in steel cages and kept at room temperature; and were allowed to acclimatize for 21 days prior to the commencement of the experiment. All the rats were given water and pelleted feed (Vital feed, manufactured by Vital feed company), *ad libitum*. Food was withheld for 24 hours prior to each experiment. All authors hereby declare that "Principles of laboratory animal care" (NIH Publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee of the Bingham University, Karu, Nigeria. All authors hereby declare that all experiments have been examined and approved by appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

2.4 Experimental Design

2.4.1 Induction of diarrhea

The rats were fasted 12 hours prior to the commencement of the experiments and were randomly divided into five groups of five rats each. Rats in the first group received 10 ml/kg (i.p) normal saline, the second group received loperamide 2 mg/kg (i.p), third, fourth and fifth groups received 100 mg/kg, 200 mg/kg and 400 mg/kg methanol extract of *Vernonia amygdalina* and *Cymbopogon citratus* (i.p). After 30 minutes of administration of extract, castor oil 1 ml/rat

was administered intragastrically. (Table 1) The animals were placed in individual cages over clean white filter paper. Three hours after the administration of oil, the cages were inspected (by an observer unaware of the particular treatment) for the presence of characteristic diarrhea droppings. Their absence was recorded as a protection from diarrhea, and the percentage protection calculated. [38,39].

2.4.2 Acute toxicity study

Acute toxicity study was carried out according to the modified method described by Lorke [40]. A batch of 15 mice in each study was used. In first phase, rats were divided into four groups of three rats each with doses of 10 mg/kg, 100 mg/kg and 1000 mg/kg of *Vernonia amygdalina* and *Cymbopogon citratus* methanolic leaf extract administered intraperitoneally, the last group received normal saline as the control. No death was recorded after 48 hours. In the second phase, more specific doses of 200 mg/kg, 400 mg/kg, 800 mg/kg and 1600 mg/kg were administered to four groups each containing one rat. The median lethal dose (LD50) was determined as the geometric mean of the highest non lethal dose and lowest lethal dose of which there is 0/3 and 0/1 survival.

2.4 Statistical Analysis

The results on castor oil – induced diarrhea were analyzed using t-test and were regarded as significant when $P < 0.05$.

3. RESULTS

The extracts of *Vernonia amygdalina* and *Cymbopogon citratus* (100, 200 and 400 mg/kg) and loperamide gave significant protection ($P < 0.05$) on rats against castor oil-induced diarrhea when compared with the control. Highest protection was observed at 400 mg/kg. (Table 3) The animals were observed for forty-eight (48) hours for signs of toxicity, but no mortality was recorded in any of the three (3) groups thus indicating that the plant was not acutely toxic. The extraction process of the extracts yielded 10.3% w/w and 11% w/w of methanolic extracts of *Cymbopogon citratus* and *Vernonia amygdalina* respectively. Phytochemical tests showed that both extract tested positive to Tannins, saponins, flavonoids, glycosides (Table 2).

Table 1. Experimental design

Group	Description	Treatment (meth extract of <i>Vernonia amygdalina</i> and <i>Cymbopogon citratus</i>)
A	Control	Normal saline
B	Standard control	Administered with 2 mg/kg body weight of loperamide
C	Treatment group	Administered with 1 ml castor oil induced Diarrhea and treated with 100 mg/kg body weight extract for 6 hours
D	Treatment group	Administered with 1 ml castor oil induced Diarrhea and treated with 200 mg/kg body weight extract for 6 hours
E	Treatment group	Administered with 1 ml castor oil induced Diarrhea and treated with 400 mg/kg body weight extract for 6 hours

Table 2. Phytochemical screening

Phytochemical constituents	Inference (<i>Vernonia amygdalina</i>)	Inference (<i>Cymbopogon citratus</i>)
Tannins	+	+
Saponins	+	+
Flavonoids	+	+
Alkaloid	+	+
Glycosides	+	+
Cardiac glycosides	+	-
Phlobatannins	+	-
Steroids	-	-
Anthraquinones	ND	+

Present = +, Absent = -, ND = Not Detected

4. DISCUSSION

Diarrhea was induced in the experimental rats by the administration of single dose of 1 ml castor oil. The castor oil model was chosen for these pharmacological studies, because of its chemical properties. The chemistry of castor oil is centered on its high content of ricinoleic acid, a product of ricinoleate which is reported to be responsible for the diarrhea inducing property of castor oil [41]. Ricinoleic acid action also stimulates the release of endogenous prostaglandin [42] which contributes to the pathophysiological functions in the gastrointestinal tract [43]. This release of prostaglandins is a major cause of arachidonic-induced diarrhea [44] which is characterized by an increase in intestinal transit time and an increase in wet faeces. In this study Loperamide is used as a positive control, which acts by increasing colonic phasic segmenting activities through inhibition of pre-synaptic cholinergic nerves in the sub-mucosal and myenteric plexuses. These effects result in fecal water absorption thus reducing the frequency of defecation [45]. The biologically-active compounds of *Vernonia amygdalina* are saponins and alkaloids [46]; terpenes, steroids,

coumarines, flavonoids, phenolic acids, lignans, xanthenes and anthraquinones [47-51]; edotides [52]; tannis and [53]; sesquiterpene lactone [54]. Qualitative phytochemical analysis was carried out on methanolic extracts of the plants, *Vernonia amygdalina* revealed the presence of some important bio-active components such as tannins, alkaloids, carbohydrates, flavonoids, glycosides, cardiac glycosides while *Cymbopogon citratus* exposed the presence of some important bio-active components such as tannins, alkaloids, carbohydrates, flavonoids, glycosides, cardiac glycosides (Table 2). Some of these components may be responsible for the anti-diarrhea properties [45]. Therefore, it is most likely that these constituents either single or in combination, contribute to the anti-diarrhea activities of the extracts. Flavonoids also possess anti-oxidant activity which is presumed to be responsible for inhibitory effects on several enzymes including those involved in arachidonic acid metabolism. Early studies have also reported that anti-diarrhea activity of medicinal plants may be due to alkaloids, saponins, tannins, sterols and reducing sugar [52]. In the present study, during the period between the 3rd and 6th hours after the castor oil challenge, the cages were inspected for the presence of characteristic diarrhea droppings and fecal spots were observed. Methanol extracts of both plants at 100 mg/kg, 200 mg/kg and 400 mg/kg body weight was administered orally to the animals which reduced the fecal spots (Table 3). It was observed that the 400 mg/kg body weight of methanolic leaf extracts of *Cymbopogon citratus* and *Vernonia amygdalina* was more effective in the reduction of fecal spots compared to the 200 mg/kg and 100 mg/kg body weight respectively. This result indicates that more of these extracts can be taken for effective treatment of diarrhea since *Cymbopogon citratus* and *Vernonia amygdalina* were confirmed not to be toxic.

Table 3. Number of fecal spots in experimental rats

Group	Description	Number of fetal spots (<i>Vernonia amygdalina</i> methanolic extracts)	Number of fetal spots (<i>Cymbopogon citratus</i> methanolic extracts)
A	Control	5.1±1.2 ^{a*}	5.1±1.2 ^{a*}
B	Treatment with 2 mg/kg body weight loperamide	1.0±0.5 ^b	0.5±0.3 ^b
C	Treatment with 100 mg/kg body weight of extracts	2.8±0.8 ^{d,b}	2.2±1.3 ^c
D	Treatment with 200 mg/kg body weight of extracts	1.5±0.9 ^{e,b}	1.4±1.1 ^{d,c}
E	Treatment with 400 mg/kg body weight of extracts	0.5±0.3 ^{f,b}	0.6±0.5 ^{e,b,d}

Values are expressed as mean ± standard deviation, *Superscripts that are different indicate significant differences

5. CONCLUSION

Vernonia amygdalina and *Cymbopogon citratus* may possess anti-diarrhea properties for effective treatment of diarrhea. The extract would therefore be effective in the management of diarrhea

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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