

Original Research Article

ANTI DIARRHEA POTENTIAL AND ACUTE TOXICITY STUDIES OF METHANOLIC EXTRACT OF *CYMBOPOGON CITRATUS* AND *VERNONIA AMYGDALINA* AGAINST CASTOR OIL INDUCED DIARRHEA MODEL IN RATS.

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ABSTRACT

Diarrhea is a common cause of death in developing countries and second most common cause of infant deaths worldwide. The effect of a methanolic leaf extract of Bitter leaf (*Vernoniaamygdalina*) and Lemon grass (*Cymbopogoncitratus*) on Castor oil-induced diarrhea in male albino rats was investigated. Fifty (50) white male albino rats ranging 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 groups; **Five groups of rats with induced diarrhea were treated with 2 mg/kg loperamide, 100, 200, and 400 mg/kg *Vernoniaamygdalina* and *Cymbopogancitratus* extract, or vehicle respectively.** The cumulative frequencies of wet and formed stools were noted on the 3rd- 6th hour. It was observed that 400mg/kg body weight of the extracts reduced the fecal spot compared to 200mg/kg and 100mg/kg body weight. *Vernoniaamygdalina* and *Cymbopogancitratus* extracts were found to be non-toxic. The phytochemical screening of the methanolic extract of *Vernoniaamygdalina* and *Cymbopogancitratus* revealed the presence of some bioactive components which may be responsible for the antidiarrhea properties observed in this study. The above findings suggest that the methanolic extracts of *Vernoniaamygdalina* and *Cymbopogancitratus* contain active constituents that have antidiarrhea activities that may lead to its use in treating diarrhea.

Keywords: *Vernoniaamygdalina*, *Cymbopogancitratus*, diarrhea, castor oil, loperamide

1.0 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 315,000 deaths of preschool age children are 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 sanitation, limited access to safe drinking water as well as in adequate 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, it is a commonly use opioid antidiarrhea agent which acts by increasing colonic phasic segmenting activities through inhibition of presynaptic cholinergic nerves in the sub-mucosal and myenteric plexuses. These effects result in fecal water absorption thus reducing the frequency of defecation [9,14].*Vernoniaamygdalina* 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]. *Vernoniaamygdalina* may be effective against amoebic dysentery [18]; gastrointestinal disorders [19-20]; microbial and parasitic activities [21-22]; hepatotoxicities [23]; and cancer [24-29]. *Cymbopogoncitratus* 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]. *Cymbopogoncitratus* belongs to the Poaceae family with slender sharp edged leaves and pointed apex [31]. *Cymbopogoncitratus* 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 *Vernoniaamygdalina* and *Cymbopogoncitratus* for treatment of diarrhea. The aim of this study is to determine the effect of *Vernoniaamygdalina* and *Cymbopogoncitratus* methanolic extracts as anti diarrhea agents and to determine if they possess any toxicological effects as a proof of ascertaining their safety.

Comment [L1]: Cymbopogon or cymbopogon

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2.0 Materials and Methods

2.1 Collection, Preparation and Extraction of plant samples

Lemon grass (*Cymbopogancitratus*) leaves was obtained from Mararaba market, a suburb of Abuja, Nigeria, it was identify and authenticated by MrsTarfa D.Florence, The Head of Department, Medicinal Chemistry and Quality Control, National Institute of Pharmaceutical Research and Development, Abuja, Nigeria. Voucherspecimen number of the plant NIPRD/15/014. These leaves were dried for fifteen days at ambient laboratory temperature (20-28°C). It was milled to a finepowder using commercial blender (Blendtec), and stored in the dark at laboratory temperature in closed containers until required. The powdered sample of the leaves of *Cymbopogancitratus* were weighed using electric balance.24.1g *Cymbopogancitratus*were used for the methanolic extraction, using soxhlet apparatus. The Methanolic extract was dried invacuo using rotary evaporator.

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Bitter leaf(*Vernoniaamygdalina*) was obtained from Masaka market a suburb of Abuja, Nigeria, it was identify and authenticated by MrsTarfa D.Florence, The Head of Department, Medicinal Chemistry and Quality Control, National Institute of Pharmaceutical Research and Development, Abuja, Nigeria. Voucherspecimen number of the plant is NIPRD/09/014. These leaves were dried for fifteen days at ambient laboratory temperature (20-28°C). It was milled to a finepowder using commercial blender (Blendtec), and stored in the dark at laboratory temperature in closed containers until required. The powdered sample of *Vernoniaamygdalina*leaves were weighed using electric balance. 22.8g *Vernoniaamygdalina* were used for the methanolic extraction, using soxhlet apparatus. The Methanolic extract was dried invacuo using rotary evaporator.

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Determination of the percentage yield of the crude extract

Percentage yield(%)= $\frac{\text{Weight of the plant extract}}{\text{Weight of the dried plant sample used}} \times 100$

Weight of the dried plant sample used

2.2 Phytochemical screening

The methanolic extracts of *Vernoniaamygdalina*and *Cymbopogancitratus* leaves were screened in the presence of phytochemical compounds as described by Evans [37]

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Test for alkaloids

1.0ml of the methanolic extracts, few drops of dragendorf reagent were added. An orange colour developed, indicating the presence of alkaloid.

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Test for Flavonoids

1.0ml of the methanolic extract was heated with 10ml of ethyl acetate over a steam bath for 3 minutes. The mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids

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Test for Tannins

Ferric chloride test:-1.0ml of the methanolic extract was diluted with 4.0ml of distilled water(in ratio 1:4) and few drops of 10% ferric chloride solution were added. Blue or green precipitate or colouration formed, indicating the presence of tannins.

Test for saponin

To 0.5ml of the methanolic extract, 5.0ml distilled water was added vigorously for 2 minutes. Then few drops of olive oil were added. Formation of emulsion showed the presence of saponin.

Test for steroids

Two ml of acetic anhydride was added to 0.5ml of the methanolic extracts with 2 ml H_2SO_4 . The colour changed from violet to blue indicating the presence of steroids.

Test for terpenoids (Salkowski test)

0.5ml of the methanolic extracts was mixed in 2 ml of chloroform and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the inner face was formed indicating the presence of terpenoids.

Test for Anthraquinones:

0.5ml of the methanolic extract was boiled with 10ml H_2SO_4 and filtered while hot. The filtrate was shaken with 5ml of chloroform. The chloroform layer was pipette into another test tube and 1ml of dilute ammonia was added. The resulting solution was observed for a greenish-yellow for *Vernonia amygdalina* and *Cymbopogon citratus* extracts.

Test for Cardiac Glycolyses(Keller-Killani Test):

1ml of the methanolic extracts was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1ml of concentrated sulphuric acid. A brown ring at the interface indicated deoxysugar characteristics of cardenolides.

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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; the animals were allowed to acclimatized for 21 days prior to the commencement to the experiment. All the rats were given water and pelleted standard laboratory feed, *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

Table 1: Experimental design

GROUP	DESCRIPTION	TREATMENT (<i>Cymbopogancitratus</i>)
A	Control	Normal saline
B	Standard control	Administered with 2mg/kg body weight of loperamide
C	Treatment group	Administratedwith1 ml castor oil induced Diarrhea and treated with 100mg/kg body weight extract for 6 hours
D	Treatment group	Administratedwith1 ml castor oil induced Diarrhea and treated with 200mg/kg body weight extract for 6 hours
E	Treatment group	Administratedwith1 ml castor oil induced Diarrhea and treated

		with 400mg/kg body weight extract for 6 hours
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Table 2: Experimental design

GROUP	DESCRIPTION	TREATMENT (<i>Vernoniaamygdalina</i>)
A	Control	Normal saline
B	Standard control	Administered with 2mg/kg body weight of loperamide
C	Treatment group	Administratedwith1 ml castor oil induced Diarrhea and treated with 100mg/kg body weight extract for 6 hours
D	Treatment group	Administratedwith1 ml castor oil induced Diarrhea and treated with 200mg/kg body weight extract for 6 hours
E	Treatment group	Administratedwith1 ml castor oil induced Diarrhea and treated with 400mg/kg body weight extract for 6 hours

2.4.1Infection 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 for each sub group. Rats in the first group received 10ml/kg (i.p) normal saline, the second group received loperamide 2mg/kg (i.p), third, fourth and fifth group group received 100mg/kg, 200mg/kg and 400mg/kg methanol extract of *Vernoniaamygdalina* and *Cymbopogancitrates* (i.p). After 30 minutes of administration of extract, castor oil 1ml/mouse was administered intragastrically. The animals were placed on 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].

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2.4.2. Acute Toxicity Study

Acute toxicity study was carried out according to the modified method described by Lorke (1983) [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 10mg/kg, 100mg/kg and 1000mg/kg of *Cymbopogon citratus* and *Vernonia amygdalina* 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 200mg/kg, 400mg/kg, 800mg/kg and 1600mg/kg was 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.

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Statistical analysis

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

3.0 Results

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The extracts of *Cymbopogon citratus* and *Vernonia amygdalina* (100, 200 and 400mg/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 400mg/kg. (Table 5 and 6) 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 extract yielded 10.3%w/w and 11%w/w of methanolic extract of *Cymbopogon citratus* and *Vernonia amygdalina* respectively. Phytochemical tests showed that both extract tested positive to Tannins, saponins, flavonoids, glycosides (Table 3 and 4).

Table 3: Phytochemical screening of methanolic extracts of *Vernonia amygdalina*

Phytochemical constituents	Inference
Tannins	+
Saponins	+
Flavanoids	+
Alkaloid	+
Glycosides	+

Comment [L19]: flavonoids

Cardiac glycosides	+
Phlabatannins	+
Steroids	-
Anthraquinones	ND

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

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Table 4: Phytochemical screening of methanolic extracts of *Cymbopogon citratus*

Phytochemical constituents	Inference
Tannins	+
Saponins	+
Flavonoids	+
Alkaloids	+
Glycosides	+
Anthraquinones	+

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

Table 5: Number of fecal spot in experimental rats (*Vernonia amygdalina*)

GROUP	DESCRIPTION	NUMBER OF FETAL SPOT
A	Control	5.1±1.2 ^{a*}
B	Treatment with 2mg/kg body weight Loperamide	1.0±0.5 ^b
C	Treatment with 100mg/kg body weight of <i>Vernonia amygdalina</i>	2.8±0.8 ^{d,b}
D	Treatment with 200mg/kg body weight of <i>Vernonia amygdalina</i>	1.5±0.9 ^{e,b}
E	Treatment with 400mg/kg body weight of <i>Vernonia amygdalina</i>	0.5±0.3 ^{t,b}

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

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Table 6: Number of fecal spot in experimental rats (*Cymbopogon citratus*)

GROUP	DESCRIPTION	NUMBER OF FETAL SPOT
A	Control	5.1±1.2 ^{a*}
B	Infected and treated with anti diarrhea drug (Loperamide)	0.5±0.3 ^b
C	Infected and treated with 100mg/kg body weight of <i>Cymbopogon citratus</i>	2.2±1.3 ^c
D	Infected and treated with 200mg/kg body weight of <i>Cymbopogon citratus</i>	1.4±1.1 ^{d,c}
E	Infected and treated with 400mg/kg body weight of <i>Cymbopogon citratus</i>	0.6±0.5 ^{e,b,d}

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

4.0 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 stimulates peristaltic activity in the small intestine, leading to change in the electrolyte permeability of the intestinal mucosa. Ricinoleic extracts contain flavonoids which modify the production of cyclo-oxygenase (COX-1 and 2) and lipoxygenase (LOX) [42] which are essential in prostaglandin biosynthesis. Ricinoleic acid action also stimulates the release of endogenous prostaglandin [43] which contributes to the pathophysiological functions in the gastrointestinal tract [44]. This release of prostaglandins is a major cause of rachidonic-induced diarrhea [45] 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, it is a commonly use opioid antidiarrhea agent which acts by increasing colonic phasic segmenting activities through inhibition of presynaptic cholinergic nerves in the sub-mucosal and myenteric plexuses. These effects result in fecal water absorption thus reducing the frequency of defecation [46]. The biologically-active compounds of *Vernonia amygdalina* are saponins and alkaloids [47]; terpenes, steroids, coumarines, flavonoids, phenolic acids, lignans, xanthenes and anthraquinones [48-51];

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edotides [52]; tannis and [53]; sesquiterpene lactone [54,56]. In this study, qualitative phytochemical analysis was carried out on methanolic extracts of *Vernonia amygdalina* (Tables 3) revealed the presence of some important bio-active components such as tannins, alkaloids, carbohydrates, flavonoids, glycosides, cardiac glycosides. Qualitative phytochemical analysis was carried out on *Cymbopogon citratus* (Tables 4). Methanolic leaf extract of *Cymbopogon citratus* exposed the presence of some important bio-active components such as tannins, alkaloids, carbohydrates, flavonoids, glycosides, cardiac glycosides. 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-diarrheal activity of medicinal plants may be due to alkaloids, saponins, tannins, sterols and reducing sugar [56]. In the present study, on the 3rd -6th hours after the castor oil challenge, the cages were inspected for the presence of characteristic diarrhea droppings fecal spots was 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 5 and 6). It was observed in this study that at 400 mg/kg body weight of methanolic leaf extract of *Cymbopogon citratus* and *Vernonia amygdalina*, there was drastic 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.

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5.0 CONCLUSION

The methanolic extract of *Vernonia amygdalina* and *Cymbopogon citratus* neutralized the ricinoleic acid responsible diarrhea inducing property of castor oil under the normal treatment period of diarrhea. Therefore, it can be concluded that *Vernonia amygdalina* and *Cymbopogon citratus* may possess anti-diarrhea properties for effective treatment of diarrhea.

6.0 Acknowledgement

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