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Laxative effect of ethanol leaf extract of *Vernonia amygdalina* del. (Asteraceae) in wistar strain albino rats

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Abstract
Vernonia amygdalina Del is derived from the leaves of a small ever-green shrub found all over Africa called *Vernonia*, belonging to the family Asteraceae. It is an important medicinal plant as well as a highly nutritious vegetable plant that has diverse reported medicinal values. This study focused on the phytochemical screening and investigation of the laxative activity of ethanol leaf extract of *Vernonia amygdalina.* Fresh leaves of the plant were collected; shade-dried, ground and extracted with ethanol using reflux method. The extract was screened for the presence of phytochemicals. The acute toxicity level was evaluated using Lorke's method while laxative potential of the leaf was examined using loperamide-induced constipation in rats. The results obtained from this study showed the presence of carbohydrates, terpenoids, alkaloids, saponins and cardiac glycosides. The I.P. LD_{50} was 2154 mg/kg suggesting the plant was not toxic. The laxative effect of the extract was significant ($p<0.05$) and dose-dependent effect when administered treatment with extract doses of 125 mg/kg, 250 mg/kg and 500 mg/kg on Wistar strain albino rats respectively. The effect of the ethanol extract was had a significantly ($p<0.05$) highest laxative activity when compared to a standard drug, sodium picosulfate (a positive control). This study amply justifies the ethnomedical claim that the leaves are used as laxatives.

Keywords: Vernonia amygdalina, phytochemicals, laxative, acute toxicity, loperamide.

1. Introduction
There are a number of gastrointestinal tract (GIT) problems such as dysentery, diarrhoea, peptic ulcer, inflammatory bowel disease and constipation that are hampering the well-being of most communities in developing countries [1, 2]. Constipation is a GIT disorder that creates discomfort to normal life [1]. It is a symptom of most GIT disorders, diabetes mellitus and opiate addiction. It is defined as a delay or difficulty in defecation, present for two or more weeks and sufficient to cause significant distress to the patient [2]. Juan *et al.* [3] described constipation as a functional disorder of the GIT, characterized by persistently difficult, infrequent or incomplete defecation in the absence of any physiological abnormality. The condition is common, with a prevalence of between 4% and 20% in cross sectional community based surveys [4]. Constipation is a period of 8 weeks with at least two of the following symptoms; defecation frequency less than three times per week, faecal incontinence frequency greater than once per week, passage of large stools that clog the toilet, palpable abdominal or rectal faecal mass, stools withholding behaviour or painful defecation [5]. Constipation has a wide range of factors that can precipitate its occurrence, including lack of fiber in the diet, lack of physical activity, low fluid intake, poor bowel habits (ignoring the urge to go), and certain medications such as antidepressants, opiates, painkillers, certain antacid, iron supplements etc. [5]. Insufficient fluids/dehydration, too little fibre and high sugar diet are three of the most common causes of constipation. In the absence of enough fluids and bulk, and a large sugar diet, the peristaltic muscles become sluggish and stool becomes hard and develops roughage. These roughages can cause a rectal fissure, a painful microscopic tear in the rectum [6]. The prevalence of gastrointestinal tract disorder in Nigeria and most African countries has resulted insignificant decrease in productivity and even loss of lives especially in infants, children and the elderly. It is therefore important to investigate the GIT effect of this plant so as to find out if it would be useful in alleviating constipation at an affordable cost and thus improve the health status of mankind.

Laxatives, purgatives or aperients are substances that loose stool and increase bowel movements. They are used to treat and/or prevent constipation. A sufficiently high dose of a laxative can cause diarrhoea [7]. Laxatives may be administered orally or rectally. The goal is for one to have soft but formed stool each day. Laxatives are widely prescribed drugs to treat constipation [8]. Laxative medications and enemas may be recommended for people who have made diet and lifestyle changes and are still constipating. Numerous over-the-counter laxatives are available for treating the symptoms of chronic constipation and are generally regarded as second-line therapy to lifestyle, which are non-pharmacologic interventions. [3] Where non pharmacological therapy fails to manage constipation, laxatives, suppositories and enemas are available options [9]. However, the uses of laxatives have associated side effects and may not be available for the common man. Therefore, medicinal plants may be an alternative with probably fewer side effects and also cost effective [9]. Plant materials have been seen as a valuable source of medicinal agents' right from ancient times. Medicinal plants have the potential for treating diseases with fewer side effects when compared to synthetic drugs. [10] Most plants contain a variety of phytopharmaceuticals, which have important application in the field of agriculture, human and veterinary medicine [10]. These have played a major role in developing novel drug for the treatment and prevention of diseases. Therefore, it is very important to have sufficient knowledge regarding herbs commonly used in our communities not only because of their wide spread usage, but also because they have the potentials to cause toxic reaction or interact with other drugs [10] Symptoms of GI and Mortality Diseases are pain, heartburn, abdominal distension, nausea, vomiting, bloating, constipation and diarrhea [11] Laxatives, purgatives or aperients are substances that can lose stool and increase bowel movements. They are used to treat and/or prevent constipation. A sufficiently high dose of a laxative can cause diarrhoea [7]. Laxatives may be administered orally or rectally. The goal is for one to have soft but formed stool each day. Laxatives are widely prescribed drug to treat constipation [8]. *Vernonia amygdalina* is one of such plants generally used in most communities in Nigeria for making soup and as blood supplements. It is a member of the daisy family, is a shrub or a small tree of 2-5 m high with petiolate leaf of about 6 mm in diameter and elliptic in shape. The leaves are green with a characteristic odour and a bitter taste. It grows under a range of ecological zones in Africa and produces a large mass of forage and is drought tolerant [12]. The plant *V. amygdalina* is commonly used in Nigeria as food supplements and for medicinal purposes. The leaf decoctions are used to treat fever, malaria, diarrhoea, dysentery, hepatitis and cough, as well as a laxative and fertility inducer. They are also used as medicine in the management of scabies, headache and stomachache. The roots extracts are also used for the treatment of malaria and gastrointestinal disorders. In Nigeria, the leaves are placed on a wound as a substitute for iodine. One of the most common medicinal uses of *V. amygdalina* is as a treatment of intestinal worms including nematodes [13]. *V. amygdalina* is well known as a medicinal plant with several uses attributed to it scientific studies on the plant proves its potentials as; Anti-malarial, Anti-plasmodial, Analgesic, Anti-pyretic, Anti-Inflammatory, Antifungal, Antiparasitic, Anti-viral, Anti-coagulant and Anti-thrombic, Activity [14], Anti-helmintic Activity [11], Anti-cancer Activity [15], Anti-oxidant and Anti-diabetic Activity [16], Anti-fertility Activity [17] and Anti-allergic Activity [18]. In spite of the number of scientific investigations that has been undertaken to validate the local use of this plant, there seems to be no study on the laxative activity of the leaves of the plant, despite the folklore claims of the use of this plant for the aforementioned purpose. The present study was aimed at examining the laxative activity of the ethanol leaf.

Fig 1: *Vernonia amygadalina* (Del.) plant in its natural habitat

Material and methods

Plant collection and identification

The *V. amygdalina* leaf was collected from Polo, in Maiduguri and was identified by Professor S. S Sanusi, a Plant Taxonomist of the Department of Biological Sciences, Faculty of Science, University of Maiduguri, Borno State) at which the voucher specimen number was assigned (UM/FPH/01C/001/001) and deposited in the Herbarium Department of Pharmacognosy, Faculty of Pharmacy, University of Maiduguri, Maiduguri.

Processing and extraction of plant material

The fresh leaves of *V. amygdalina* were shade-dried at room temperature for 14 days. The dried leaves were pulverized using wooden mortar and pestle to a powdered form for ease extraction of active compounds. The powdered plant material (200g) was soxhlet-extracted using 90% ethanol for 6 hrs at the Postgraduate laboratory, Department of Pure and Applied Chemistry, Faculty of Science University of Maiduguri. The extract obtained was evaporated to dryness using rotary evaporator at 40^{o}C and stored until required for use.

Experimental animals and acclimatization

Fifty-three (53) adult albino rats of both sexes weighing 90-180 g were used for both the acute toxicity (LD_{50}) study and the laxative effect. The rats were brought from the Animal House section of the Faculty of Pharmacy and Department of Biochemistry, University of Maiduguri, Borno State. The animals were maintained in standard wire meshed plastic cages in the Animal House of Physiology and Biochemistry Laboratory, Faculty of Veterinary Medicine. The animals were kept in plastic cages at standard condition of temperature; light and humidity for a period of two weeks to allow them acclimatize to laboratory condition. They were allowed free access to drinking water and standard livestock feed (Grand Cereals and Oil Mils Ltd.) Bukuru, Jos, Plateau State, Nigeria and were handled according to the International Guiding Principle for Biomedical Research Invoving Animals [19].

Ethical approval
All experiments were conducted in accordance with the National Institute of Health Guidelines for the Care and use of Laboratory Animals (NIH Publications No.80-23) as revised in 1996.

Preliminary qualitative phytochemical screening
The screening was done in accordance with the standard protocol as describe by Evans [20] The extract was screened for the presence of alkaloids, tannins, flavonoids, saponins, anthraquinones, terpenoids, cardiac glycosides, and carbohydrate.

Pharmacological studies
Acute toxicity (ld_{50}) study
The acute toxicity of the ethanol leaf extract of *V. amygdalina* was determined using a standard conventional procedure described by. Lork [21]. In this study, intraperitoneal route of administration was considered. The test comprised 2 phases which include:
Phase 1: The rats were divided into three groups of three rats each for intraperitoneal administration, they were then treated with the ethanol leaf extract of *Vernonia amygdalina* at doses of 10 mg/kg, 100 mg/kg and 1000 mg/kg body weight intraperitoneally and were observed for signs of acute toxicity and mortalities for 24 hours, in which no death occurred and guaranteed for phase 2 trials.
Phase 2: Three dose levels and three rats were used based on the result of phase 1 after 24 hours for the intraperitoneal route. They were given the dose of the *Vernonia amygdalina* extract at 1600 mg/kg, 2900 mg/kg and 5000 mg/kg respectively. The rats were then observed for 24 hours for signs of toxicity and death. The LD_{50} was calculated as the geometric mean of the lowest dose that caused death suing the formula:

$$LD_{50} = \sqrt{a \times b}$$

Where
A=least dose that killed the animal, 1/1 and
B= highest dose that did not kill the animal, 0/1.

Laxative activity study of ethanol leaf extract of *vernonia amygdalina* in rats
The method described by Capasso *et al*., [22] was adopted for this study with little modification. The rats fasted for 12 hrs before the experiment were individually placed in cages lined with clean filter paper. They were divided into five (5) groups. The first group acting as the negative control was administered normal saline (5 mL/kg, p. o.), second group received sodium picosulfate (5 mg/kg, p.o), which served as the positive control. Groups 3, 4 and 5 received 125, 250 and 500 mg/kg per os of the *Vernonia amygdalina* extract. The faces production (total number of normal as well as wet faces) in all five groups was monitored for 6 h. The wet faces of each rat were counted and recorded at the end of the experiment. Laxative Activity Study of Ethanol Leaf Extract of *Vernonia amygdalina* on Loperamide- Induced Constipation in Rats This study was carried out based on protocol described by Takahura *et al*. [23] as adopted by Méité *et al*. [24] was employed. Rats were placed individually in cages lined with clean filter paper, allowed to fast for 18 hours and were divided into five (5) groups of five rats each. The first group received normal saline of 5 mL/kg, p.o and served as a negative control. The second group received per *o.s* the standard drug sodium picosulfate (5 mg/kg. doses of 125, 250 and 500 mg/kg, *p.o*. of the ethanol extract of *Vernonia amygdalina* were administered to the remaining three groups of rats. All the rats received Loperamide (5 mg/kg, *p.o*.) by gavage after 1 hr. The total number of normal as well as wet faeces in all five groups was monitored for 8 h. The content of the intestine was collected by milking and the weights of the empty intestine and the content were also taken. The percentage (%) fluid accumulation was calculated as:

$$\% \text{ Fluid Accumulation} = \frac{\text{weiht of content(g)}}{\text{Weight intestine and content (g)}} \times 100$$

Statistical analysis: The data generated during the study were expressed as Mean $\pm$ Standard Error of the Mean (SEM) and analysed by one way analysis of variance (ANOVA) followed by Newman- Keul's test using GraphPad Instat version 5.01(2007). The $p<0.05$ was considered significant [25].

Results and discussion
The ethanol extract profile of vernonia amygdalina
The weight, colour, texture and the percentage yield of the ethanol leaf extract of *Vernonia amygdalina* from reflux extraction are presented in Table 1. The weight of the extract was 49.87 g, the colour of the extract was dark green, and its texture was gummy while percentage yield was 24.94 $^{w}/_{w}$.

Phytochemical screening of ethanol leaf extract of *vernonia amygdalina*
The phytochemical screening of the extract revealed the presence of cardiac glycosides, alkaloids, terpenoids, saponins, carbohydrates and flavonoids and are presented in Table 2. The bitter taste of the leaf extract of *Vernonia amygdalina* usually associated with the plant could be due to the presence of some non-nutritional factors such as alkaloids, saponins, tannins and glycosides found in the plant. In an earlier study conducted by Omenka *et al.* [26] it was reported that the ethanolic extract of *Vernonia amygdalina* contained phlobotannins and anthraquinones which is contradicted by this study which these phytochemicals were not detected. The variation in the environmental factors such as temperature, rainfall, time of collection and age difference might be responsible. However, the phytochemicals in this study are in conformity with the findings of Ekam *et al.* [27] Flavonoids as detected in this study have been reported to play a role in analgesic activity primarily by targeting prostaglandins [28]. Cardiac glycosides have strong activity on the heart and some have been used in the treatment of congestive heart failure (CHF). Cardiac glycosides may also have pesticidal properties [29]. Tannins also constitute the active principles of plant-based medicines. According to literature, the tannins containing plants are used as astringents against diarrhoea and have ant-inflammatory properties [30]. Alkaloids also found in *Vernonia amygdalina* extract are produced by many plants and are responsible for antimicrobial, antifungal, antimalarial and antidepressant activities. [10] Terpenes (a phytochemical in this plant) are also in many plants and are known to have anti-inflammatory and anti-convulsant properties [30].

Table 1: The Weight, Colour, Texture and Percentage Yield of the Ethanol Leaf Extract of *Vernonia amygdalina* Del.

Parameter	Ethanol Extract
Weight	49.87 g
Colour	Dark green
Texture	Gummy
% yield (w/w)	24.94

Weight of the ground *V. amygdalina* leaf = 200 g
Weight of the extract=49.87 g

$$\% \text{ Yield} = \frac{49.87\text{ g}}{200\text{ g}} \times 100 = 24.94 \text{ w/w}$$

Table 2: Phytochemical screening of ethanol leaf Extract of *Vernonia amygdalina* Del.

S/N	Test	Result	Observation
1	Test for Carbohydrate		
	General Test-Molish's Test	+	Dull
	Test for Free reducing sugar (Fehling's Test)	+	Red
	Test for Combined reducing sugar	+	Red
	Test for Ketoses	-	Green
	Test for Pentoses	-	Green
2	Soluble starch	-	
3	Anthraquinones	-	
	Combined anthraquinones Test	-	
4	Cardiac glycosides		
	Salkowski's Test	-	
	Lieberman-Burchard's Test	+	Violet
5	Terpenoids	+	Pink to Violet
6	Flavonoids		
	Shinoid's Test	-	Buff
	Ferric Chloride Test	-	
	Lead acetate Test	-	
7	Saponins		
	Frothing Test	+	Frothing
8	Tannins		
	Ferric Chloride Test	-	
	Lead acetate Test	-	Buff
9	Phlobotannins	-	
10	Alkaloids		
	Dragendorff's Test	+	Dark-brown
	Mayer's Test	+	

KEYS: + = Present
- + Absent

Acute Toxicity Studies on Ethanol leaf extract of *Vernonia amygdalina* Del.

The result obtained for LD_{50} is shown in Table 3. The I.P (LD_{50}) of 2154 mg/kg of the ethanol leaf extract of *Vernonia amygdalina* was high indicating that the plant is probably not toxic and may not cause harm when consumed orally.

Table 3: Intraperitoneal Acute Toxicity Test of the Ethanol Leaf Extract of *Vernonia amygdalina*

Phase	No. of rats	Route	Dose (mg/kg)	Clinical sign	Mortality
1	3	Intraperitoneal	10	None	0/3
1	3	Intraperitoneal	100	None	0/3
1	3	Intraperitoneal	1000	None	0/3
2	1	Intraperitoneal	1600	None	0/1
2	1	Intraperitoneal	2900	Sedation	1/1
2	1	Intraperitoneal	5000	Sedation	1/1

$LD_{50} = \sqrt{a \times b} = \sqrt{2900 \times 1600} = 2154$ mg/kg

Where
a = least dose that killed the animal, 1/1 and
b = highest dose that did not kill the animal, 0/1.

Laxative effect of ethanol leaf extract of *vernonia amygdalina* in wistar strain albino rats

The result of this study showed a significant dose dependent increase in stool output in rats treated with ethanol leaf extract doses 125, 250 and 500 mg/kg *p.o* of *V. amygdalina* with mean defaecation of 3.070 ± 1.109, 4.750 ± 1.031 and 5.750 ± 1.181g respectively. The effect of the ethanol extract was had a significantly (p<0.05) highest laxative activity when compared to the standard drug, sodium picosulfate (a positive control) with a mean defaecation ±SEM of 5.080 ±1.11.

The effect of ethanol leaf extract of *vernonia amygdalia* on loperamide-induced constipation in wistar strain albino rats

The result of this study showed that Group A, normal saline (negative control) had a mean weight of 0.083 ± 0.005 g and a fluid accumulation of 02.85% while Groups treated with extract doses of 125, 250 and 500 mg/kg had Mean ± SEM of 0.230 ± 0.057 g, 0.408 ± 0.072 g and 0.768 ± 0.050 g with fluid accumulation of 08.46%, 11.36% 13.53% respectively, while Sodium picosulfate, 5 mg/kg (positive control) had a Mean ± SEM of 0.213 ± 0.121 g with fluid accumulation of 04.96%. The result showed a dose dependent increase in percentage intestinal content and was statically significant (p<0.05) when compared to Group E (positive control).

The response elicited by the ethanol extract of *Vernonia amygdalina* on the GIT of the Wister strain albino rats which mimicked that of stimulants/irritants may be attributable to one or more of the secondary metabolites detected in the plant. The results from the *in-vitro* experiment on the Wister strain albino rats showed enhanced stooling response to the reference drug, loperamide, which was dose dependent. This was expected since loperamide acts on receptors along the small intestine to decrease circular and longitudinal muscle activity. It causes constipation by slowing intestinal transit and increasing contact time, and perhaps also by directly inhibiting fluid and electrolyte secretion and/or stimulating salt and water absorption [31] The ethanol leaf extract showed a reverse effect to that of loperamide by increasing circular and longitudinal muscle activity having a higher number of stools and its response was dose dependent [32]. Sodium picosulfate is a member of the polyphenolic group of stimulant laxatives. It pharmacologically exerts its laxative action by the accumulation of fluid in the intestinal loop of the body, thereby increasing the bulk of the stools and stimulating the gastrointestinal motility. Also, loperamide abolishes diarrhoea by acting on intestinal motility and consequently reducing the water and stools entering the colon [33]. The laxative activity of the ethanol extract is comparable to sodium picosulphate, indicating a mechanism of action similar to it, thereby overcoming the loperamide-induced constipation. Thus proves the folklore claims of this plant as a potential laxative drug. The results of ethanol leaf extract of *Vernonia amygdalina* showed in Table 5 probably indicate a higher blockage of M_3 contraction ability and this probably indicates that the ethanol leaf extract of *Vernonia amygdalina* exhibits a laxative effect.

Table 4: The Laxative Effect of Ethanol Leaf Extract of *Vernonia amygdalina*

Group	Drug Dose (mg/kg)	Mean±SEMof Defaecation (No. of stools) in 6 hours
Normal Saline	(5 ml)	0.250 ± 0.250
Extract	125	3.070 ± 1.109[a]
Extract	250	4.750± 1.031[b]
Extract	500	5.750 ± 1.181[b]
Sodium picosulfate	(5 mg/kg)*	5.080 ±2.11[c]

= Standard laxative drug; n=4 (Number of rats in each group); SEM= Standard Error of the Mean; Mean ± SEM Based on 5 observation

within column, mean with different letters are statiscally significant ($p<0.05$) when compared to group E (positive control).

Table 5: The Effect of the Ethanol Leaf Extract of *Vernonia amygdalina* on Loperamide-induced Constipation in Rat

Group	Extract/Drug Dose (mg/kg)	Weight of Feaces (g)	% Fluid Accumulation
Normal saline	5	0.083 ± 0.005^{a}	02.85
Extract	125	0.230 ± 0.057^{b}	08.46
Extract	250	0.408 ± 0.072^{b}	11.36
Extract	500	0.768 ± 0.050^{b}	13.53
Sodium picosulfate	5	$0.21\ 3 \pm 0.121$	04.96

N=4 (Number of rats in each group); SEM=Standard Error of the Mean; Mean ± SEM Based on 5 observations within column, mean with different letters are statistically significant ($p<0.05$) when compared to group E (positive control).

Conclusion

The ethanol leaf extract of the *Vernonia amygdalina* contained secondary metabolites of pharmacological importance which may be responsible for a dose-dependent inhibition of contraction of the GIT. This justifies the ethnomedical claim of the leaves as a laxative.

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