



Research Article

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PHYTOCHEMICAL SCREENING AND PURGATIVE ACTIVITY OF ETHANOLIC EXTRACTS OF *VERNONIA AMYGDALINA* DEL. LEAF

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ABSTRACT

Vernonia amygdalina has diverse ethno-medical uses including constipation. The aim of this study was to determine the phytochemical component and purgative effect of ethanolic extract of *Vernonia amygdalina* leaf on rabbit jejunum. Leaves of *Vernonia amygdalina* were collected, dried, ground and extracted using 95% ethanol. Isolated tissue of rabbit jejunum was challenged with acetylcholine as standard and different strength of the extract at dose ranges of 10 mg to 160 mg in a 50 ml capacity organ bath. The preliminary phytochemical analysis revealed the presence of alkaloids, tannins, phlobotannins, saponins, and anthraquinones. Alkaloids, tannins, and saponins appeared in high quantities, while steroids and flavonoids were absent. The extract at concentrations of 0.2, 0.4, 0.8, 1.6 and 3.2mg/ml produce contractile responses of 3.0, 7.0, 10.2, 15.3 and 15.0 mm respectively which were dose depended. Atropine was able to block the contraction exerted by the extract. These suggest that the extract may be acting on the muscarinic receptors (M₃) which are present on the intestine. This study amply justifies the ethno medical claim that the leaves are used as purgatives.

Keywords: *Vernonia amygdalina*, Purgative, Ethanol

INTRODUCTION

The revival of interest in the use of African medicinal plants by many developing countries and World Health Organization has led to intensified efforts to explore the numerous plants with medicinal importance. Research has been geared toward finding scientific evidence for the claims of African herb by traditional healers^{1,2}. For many years, natural plant materials have been seen as a valuable source of medicinal agents with proven potential for treating diseases with lesser side effects compared to the synthetic drug agent³. *Vernonia amygdalina* is one among many plants that have multiple medicinal values used in different parts of Africa. It is a shrub that grows under a wide range of ecological zones in Nigeria. The leaves are used for human consumption and washed before eating to get rid of the bitterness. It is also used instead of hops to make beer in Nigeria. It is also found in homes and villages as fence⁴. The plant has many medicinal uses which include antibacterial, antidiabetic, antihelmintic, antioxidants, antimalarial and analgesic activity⁵. In some part of Nigeria the fresh leaves has been used as an appetizer, abortifacient and purgative enemas⁶. Many herbalist recommend aqueous extract for their patients as treatment for emesis, loss of appetite and dysentery. In Northern part of Nigeria, the plants have been used for various gastrointestinal problems^{5, 6}. As a result of its ethno-medical usage it becomes necessary to evaluate the purgative effect of the plant *Vernonia amygdalina*.

MATERIALS AND METHODS

Plant Material

The leaves of the plant *Vernonia amygdalina* was obtained from a cultivated source from Vunoklang, Girei Local Government Area of Adamawa State, Nigeria. The leaves were collected in November, 2011 and were identified by Professor Sanusi O. Sanusi of Department of Biological Science, University of Maiduguri at which the voucher specimen number was assigned (26590) and deposited in the herbarium.

Extraction of *Vernonia amygdalina* leaf

The leaves of the plant were collected, air-dried at room temperature for 14 days, homogenized using a wooden pestle and mortar, grounded and finally sieved to obtain a finely divided powder. Three hundred and fifty grammes (350 g) of the powder was soaked in 1 liter of ethanol and thoroughly shaken. This was allowed to stand for 24 hours with intermittent shaking. Filtration was done to remove the coarse particles and fine particles were removed using Whitman filter paper in Buchner funnel. The sample was concentrated using rotary evaporator at 50°C to recover the solvent. The weight of the ethanol extract was 15.1 g, giving a percentage yield of 4.31%.

Preparation of stock solutions of extract and standard

One gram of the extract was weighed and dissolved in 10 ml of distilled water to get the stock solution of 100 mg/ml at which a serial dilution was made using dilution factor of 1:10 to get stock concentrations of 10 mg/ml, 1 mg/ml and 0.1 mg/ml. One gram each of acetylcholine and atropine was weighed and dissolved in 10 ml of distilled water to get a stock concentration of 100 mg/ml at which serial dilutions was done using dilution factor of

1:10 to get stock solutions that was used for the in vitro experiments.

In vitro effect of ethanol leaf extract of *Vernonia amygdalina* on pregnant rat uterus

A white male California rabbit weighing 3.1 kg was starved for 24 hours prior to use, but it was allowed to take water. The animal was euthanized by a sharp blow on the base of the skull. A mid-line incision was made on the abdomen to expose the stomach and the intestine. A piece of the intestine (2-3 cm) was cut (10 cm away from the stomach). The piece of the gut was freed from fat and other attachment before being preserved in aerated thyroid solution at 37°C. The thyroid solution was aerated with 95% oxygen and 5% carbon dioxide. A white cotton thread in a needle was passed through both ends of the jejunum to form a loop and a knot. Care was taken not to close the lumen of the jejunum. The free end of the thread was attached to plasticine in the lever to record responses on the microdynamometer. The other end of the gut was attached to the aerator pipe and anchored to the base of the tissue, both containing thyroid solution at 37°C. The preparation was left to equilibrate for 30 minutes in a 50 ml capacity organ bath. The following drugs were tested on the rabbit jejunum; Acetylcholine (0.1mg/ml), *V. amygdalina* extract (100mg/ml) and Atropine (0.1mg/ml). Graded volumes of the drugs were administered in the following order; 0.1, 0.2, 0.4, 0.8, and 1.6 ml. The organ bath was washed thrice after each dose before adding the next dose level. Zero point two milliliters (0.2 ml) of 0.1mg/ml atropine was administered concurrently with acetylcholine and the extract in same manner above. The

responses recorded on the microdynamometer were measured in millimeters against the respective final organ bath concentration.

Phytochemical Analysis of the Leaf Extracts

The preliminary phytochemical analysis of the leaf extract was conducted based on the standard method of Trease and Evans⁷ and Sofowora⁸.

Table 1: Qualitative phytochemistry of the ethanol extract of *V. amygdalina* leaf

Phytoconstituents	Results
Tannins	+
Flavonoids	-
Phlobatannins	+
Alkaloids	+
Saponins	+
Cardiac glycosides	+
Anthraquinones	+
Steroids	-

- = absent; + = present

Table 2: Quantitative Phytochemistry of the leaf extract of *V. amygdalina*

Phytoconstituents	Methods	Results
Tannins	Soluble	+++
	Condensed	+++
Phlobatannins	Ferric chloride test	++
Alkaloids	Wagner's test	+++
	Dragendorff's test	+++
Saponins	Frothing test	+++
	Haemolysis test	+++
Cardiac glycosides	Salkowski test	++
	Keller-Killiani test	++
Anthraquinones	Borntrager's test	+

+ = Present in low concentration, ++ = Present in moderate concentration, +++ = Present in high concentration

Table 3: Effects of graded doses of acetylcholine (100 µg/ml) on spontaneous contraction of isolated rabbit jejunum in a 50 ml capacity organ bath

Vol. of Ach. administered (ml)	Final bath concentration (ng/ml)	Log of final bath concentration	Response (mm)	Percentage Response (%)
0.1	200.00	2.30	16.00	53.33
0.2	400.00	2.60	21.00	70.00
0.4	800.00	2.90	24.00	80.00
0.8	1600.00	3.20	30.00	100.00
1.6	3600.00	3.51	29.00	96.67

Table 4: Effects of graded doses of ethanol extract of *V. amygdalina* leaf (100 mg/ml) on spontaneous contraction of isolated rabbit jejunum in a 50 ml capacity organ bath

Vol. of extract administered (ml)	Final bath conc. (µg/ml)	Log of final bath concentration	Response (mm)	Percentage Response (%)
0.1	200.00	2.30	3.00	19.61
0.2	400.00	2.60	7.00	45.75
0.4	800.00	2.90	10.20	66.67
0.8	1600.00	3.20	15.30	100.00
1.6	3200.00	3.51	15.00	98.03

Table 5: Effects of interactive studies of acetylcholine and ethanol extract of *V. amygdalina* leaf with atropine

Drugs	Final organ bath conc.	Response (mm)
Ach + Atropine	20 ng/ml	0
	40 ng/ml	0
	80 ng/ml	0
	160 ng/ml	5.2
	320 ng/ml	13.3
Extract + Atropine*	200 µg/ml	0
	400 µg/ml	0
	800 µg/ml	0
	1600 µg/ml	0
	3200 µg/ml	0

* = Final organ bath of atropine is 40ng/ml; 0 = No Contraction

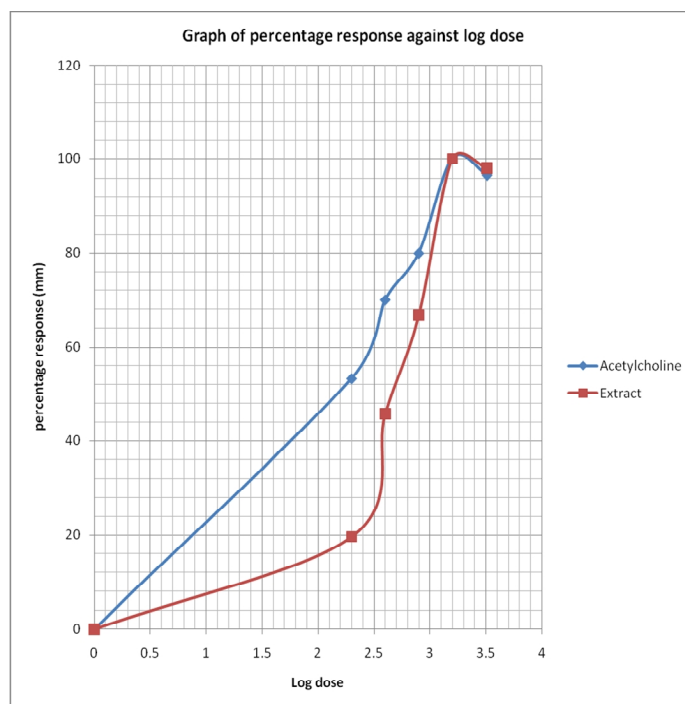


Figure 1: Effects of graded doses of acetylcholine and ethanol extract on rabbit jejunum

RESULT AND DISCUSSION

The result obtained from the phytochemistry showed that the ethanol extract of *V. amygdalina* contained tannins, phlobatannins, alkaloids, saponins, cardiac glycosides and anthraquinones. Flavonoids and steroids were not detected in the ethanol leaf extract (Table 1). Quantitatively, phytochemistry revealed the presence of tannins, alkaloids and saponins in high concentration, whereas phlobatannins and cardiac glycosides were seen in moderate concentration in the ethanol leaf extract of. However, anthraquinone was found in low concentration in the ethanol leaf extract (Table 2). The bitter taste usually associated with the plant could be due to the presence of non nutritional factors such as alkaloids, saponins, tannins, and glycosides⁹. These results contradict an earlier study done by Omenka *et al*¹⁰ which reported that ethanolic extract contains phlobatannins and anthraquinones. The variation in the environmental factors such as temperature and rainfall especially at the time of collection may be likely explanation for the difference. However, the result is in conformity with an earlier work by Ekam *et al*¹¹ in which the ethanolic extract of *Vernonia amygdalina* contained all the secondary metabolites that were tested and were present in this study. The presence of alkaloid, saponin, and tannin in relative higher quantities agrees with the report of Ugwoke *et al*¹² in which similar observation was made. The response elicited by the ethanol extract of *Vernonia amygdalina* on the rabbit jejunum which mimics that of acetylcholine may be attributable to one or more of these secondary metabolites detected. The results from the in vitro experiment on rabbit jejunum showed a depolarizing (contractile) response to the reference drug acetylcholine which was dose depended as it is shown in

Table 3. This was expected since acetylcholine increases the tone and amplitude of contraction of the stomach and intestine via muscarinic receptor¹³. The ethanol leaf extract showed similar effect as the reference drug and its response was also dose dependent. The increase in amplitude of the rabbit jejunum by the extract which is similar to that of acetylcholine may be viewed both as acting via muscarinic receptors^{14, 15}. The relative potencies of acetylcholine and the extract obtained on the same tissue can be describe by determining the effective concentrations that gives 50% biological response (EC_{50})¹³. Figure I showed the EC_{50} for acetylcholine and extract extrapolated on the graph as 162ng/ml and 501 μ g/ml respectively. From these results it is evident that acetylcholine is relatively more potent than the extract. The wide margin in potency between the extract and acetylcholine might be because of the crude nature of the extract. Table 5 showed how atropine antagonizes the contractile activity of acetylcholine and the extract. This showed that atropine blocks the muscarinic receptor (M_3) on the jejunum which result in blocking the effect of acetylcholine and the extract. However, at high doses of acetylcholine (160 ng/ml and 320 ng/ml) the antagonistic effect of atropine was blocked. This agrees with the report of Rang *et al*¹⁶ in which atropine blocks the effect of acetylcholine through a reversible competitive antagonism at M_3 receptors. When atropine was co-administered with the extract, it blocked the effect of the extract at all the dose levels used (Table 5). This showed that the extract may act via the same path as acetylcholine i.e. via the M_3 receptors that are predominantly located on the gastrointestinal muscles. Acetylcholine stimulates M_3 receptors through Gq protein. This is responsible for the stimulation of phospholipase C activity which catalyses the

formation of two intracellular messengers; Inositol 1,3,5 triphosphate (IP₃) and Diacylglycerol (DAG). These lead to release of intracellular Ca²⁺ and the activation of phosphokinase C and finally the contraction of the jejunum¹⁶.

CONCLUSION

The ethonol extract of the *Vernonia amygdalina* leaf contains secondary metabolites of pharmacological importance and may be responsible for a dose dependent contraction of the rabbit jejunum which was blocked by atropine. The blockade signifies that the extract might be acting via the M₃ receptors on the intestine. These justify its ethno medical claim that the leaves are used as purgatives.

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