International Journal of Pharmacological Research
ISSN: 2277-3312
www.ssjournals.com
Journal DOI:10.7439/ijpr

In-vitro anthelmintic activity of *Vernonia amygdalina* Del. (asteraceae) roots using adult *Haemonchus contortus* worms

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Abstract
This study was to investigate the anthelmintic activity of *Vernonia amygdalina* (Asteraceae) which is used by traditional medicine practitioners in Migori County, Kenya using adult *Haemonchus contortus* worm as a model. 50 g of ground powder of *Vernonia amygdalina* (roots) was extracted separately with 300 ml each of methanol, acetone and water. The yields of the extracts were 4.34 g, 4.67 g and 4.20 g for methanol, acetone and water respectively. The anthelmintic activity of 6.25 mg/ml, 12.5 mg/ml and 25 mg/ml concentrations of aqueous, acetone and methanol crude extracts of *Vernonia amygdalina* (roots), were compared with the effect produced by the standard reference drug albendazole with Phosphate Buffered Saline (PBS) used as a negative control. Methanolic extract gave the most active metabolite followed by water. Acetone gave the least potent extract. Death of *Haemonchus contortus* worm was determined within a period of 24 hrs. *Vernonia amygdalina* (roots) extract had mean mortality of 20-33.3% at 6.25 mg/ml; 23.3-46.7% at 12.5 mg/ml and 26.7-56.7% at 25 mg/ml. The result indicated that *Vernonia amygdalina* contains tannins, saponins and cardiac glycosides which are anthelmintic agents this justifies its traditional use in the treatment of helimnthiosis.
Keywords: *Vernonia amygdalina*, *Haemonchus contortus*, *In-vitro* anthelmintic activity, Albendazole, Migori County, Kenya

1. Introduction
Helminth infections (helminthiasis) are the most common infections in man that affect large proportions of the world's population[1]. Most diseases caused by helminths are chronic and debilitating in nature. They probably cause more morbidity, greater economic and social deprivation among humans and animals than any other parasites. Helminthiasis is endemic in regions with poor sanitation, poor family hygiene, malnutrition and crowded living condition. It has been estimated that about half of the world's population suffers from helminthiasis and the number is increasing. In the treatment of helminthiasis, anthelmintic drugs are used irrationally and recently, anthelmintics use has been found to produce toxicity in human beings[1]. High costs of conventional anthelmintics have led to limited effective control of the parasites. In some cases, wide spread use of low quality anthelmintics has enhanced development of resistance[1].The discovery of new plants containing bioactive substances that act as anthelmintics is therefore considered a breakthrough in managing helminthiases[1]. Numerous studies from various parts of the world have shown that certain species effectively reduce the degree of parasite infestation in ruminants and are promising alternatives to conventional anthelmintics[2].

The number of higher plant species on earth is estimated at 250,000-500,000, of these, only about 6% have been screened for biological activity and a reported 15% have been evaluated phytochemically[3]. There are a great number of plants with purported antiparasitic properties, which have not been reproduced under experimental conditions[4].

Several plants are used to manage helminth infections. Information on chemical composition of these plants can be generated for further advanced research work. *Vernonia amygdalina* is among the plants used by herbalists of Migori County, Kenya as a dewormer. The aim of this research study was to determine the chemical composition and *in-vitro* anthelmintic activity of *Vernonia amygdalina* extract.

2. Materials and methods

2.1. Area of study

Migori County is located in the western part of Kenya in Nyanza Province between latitude 0^0.24' South and 0^0.40' South and longitude 34^0 East and 34^0.50' East. It covers an area of 2,597 km^2 and borders Kisii, Homabay and Narok counties (figure 3). According to 2009 census, Migori County has a population of approximately 917,170 of which 34% of the population lives in the urban areas. The proposed County capital is Migori which is a cosmopolitan town. Migori County has four district hospitals, clinics and dispensaries distributed within the County. It has thirteen Divisions namely Karungu, Nyatike, Muhuru, Suba East, Suba West, Uriri, Awendo, Rongo, Mabera, Masaba, Kehancha, Kegonga and Ntimaru. The County has vibrant commercial centres which include Migori, Awendo, Rongo, Sori Karungu, Muhuru, Kehancha and Isibania (see figure 4).

Migori County experiences high temperatures of 21 degrees Celsius during the cold season and 35 degrees Celsius during the hot season. The major economic activity undertaken by most of the residents of Migori County is agriculture with the main commercial crops being sugarcane and tobacco. Other economic activities include fishing, mining and entrepreneurship.

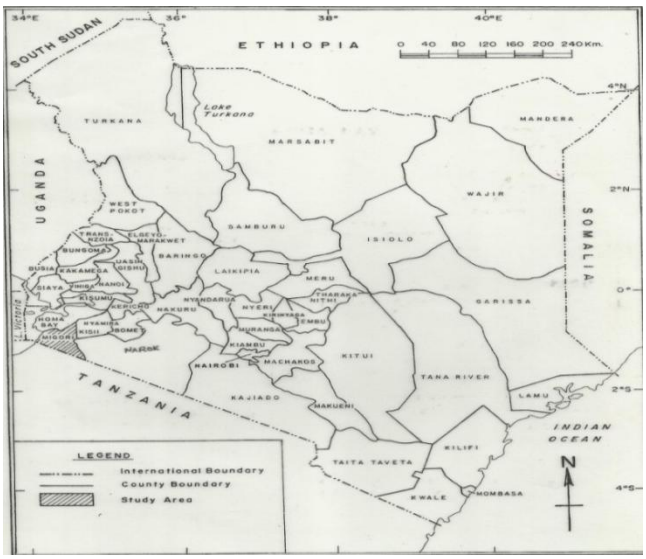

Fig 1: Map of Kenya showing the location of Migori County

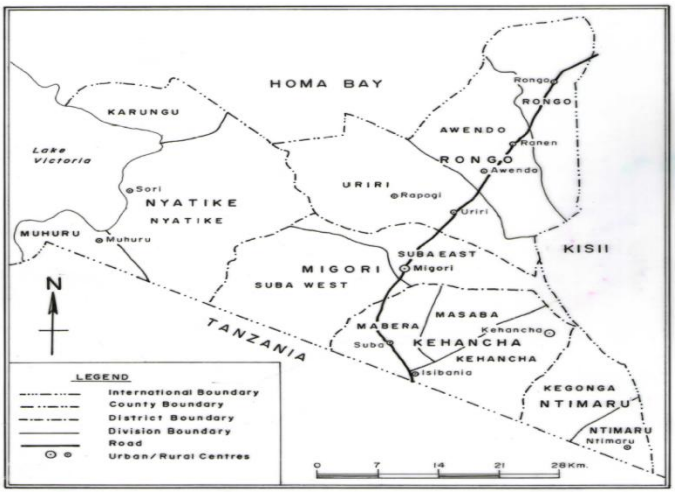

Fig 2: Map of Migori County showing thirteen Divisions

2.2 Collection of ethnobotanical data

A field survey was done prior to data collection, during which, a list of herbalists was prepared with the assistance of rural dwellers and the local authorities of Migori County. Information on the anthelmintic plants was collected for two months (August 2013 and September 2013). Identified herbalists were visited in their homes and interviewed on their knowledge of anthelmintic plants. As such, the sampling was intentionally non-random under the assumption that herbalists would provide more specific and higher quality information concerning anthelmintic plants[5].

Ethnobotanical data was collected in all the thirteen Divisions in the County. Data collection was based on open

ended interviews of the herbalists (medical practitioners). A questionnaire was used and for any additional information, complementary questions were asked[6]. Twenty six (26) herbalists between the ages 20-69 years (10 men and 16 women) were interviewed on plants used as anthelmintics. For every plant cited, vernacular name, parts used, mode of preparation and administration was recorded. Guided tours to observe and collect the plants mentioned for identification and laboratory studies were done with the help of respondents. Ethnobotanical data was compiled from field notes, herbarium sheets and available literature.

Plant specimens were collected in duplicate; one specimen was used for preliminary identification in the field with the help of floras[7][8], while the other was pressed and transported to the University of Nairobi herbarium (NAI) for authentic identification by comparing with the permanently prepared herbarium collections at the NAI herbarium.

2.3 Selection of priority plant

Priority plant was selected based on the frequency report as an anthelmintic. The plant that had the highest frequency was *E. prostrata* followed by *Vernonia amygdalina*. *V. amygdalina* was chosen for this study.

2.4 Collection of *Haemonchus contortus* worms

H. contortus worms were collected from the abomasums of freshly slaughtered sheep at Burma abattoir in Nairobi. The worms were washed with distilled water then suspended in 500 ml of phosphate buffer saline (PBS) which was prepared by dissolving 0.85g of sodium chloride and 1g glucose in 1 litre of distilled water. They were then transported to the Zoology laboratory at School of Biological Sciences, Chiromo campus, University of Nairobi in an air tight can where authentication was done. They were then left for 2 hrs to acclimatize before beginning tests[9].

2.5 Preparation of the plant extract.

Vernonia amygdalina (roots) was washed with water, dried and then chopped into small pieces; this was then dried under a shade for three weeks and then ground into a powder using an electric mill[10]. It was then packed in a labeled packet. 50 g of this powder was soaked separately in 300 ml of methanol, 300 ml of acetone, and 300 ml of water in 500 ml conical flasks, covered with aluminium foil for 72 hrs and then filtered using the Whatman filter paper. The methanol and acetone extracts were each evaporated on a rotary evaporator at 60°C to obtain crude extracts which were transferred to separate marked vials which were then placed in an oven at 40°C for 2 hrs to dry the plant extracts into powder. Water extract was deep frozen, freeze dried into powder then placed in a separate marked vial. The sample vials were kept at 4ºC for further use[9].

2.5.1 Test for tannins: 0.5 mg of each of the dried powdered extract sample was boiled in 10 ml of distilled water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration[11].

2.5.2 Test for saponins: 0.5 mg of each of the dried powdered extract sample was added to 5 ml of distilled water and shaken vigorously for a stable persistent froth to occur. The froth was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion[11].

2.5.3 Test for cardiac glycosides (Keller-Killani test): 0.5 mg of each of the dried powdered extract sample was boiled in 10 ml of distilled water then 5 ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of 0.1% ferric chloride solution. This was then underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of cardiac glycosides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer[9].

Procedure was repeated for the other extracts.

2.6 *In-vitro* anthelmintic activity

This was carried out as described by Ombasa *et al* (2012) with minor modification in the extract concentrations used. 0.625 g, 1.25 g and 2.5 g of each powdered extract was dissolved in 5 ml of dimethylsulfoxide (DMSO) and made to 100 ml mark using distilled water to make 6.25 mg/ml, 12.5 mg/ml and 25mg/ml solutions[12]. Filter paper discs, 6 mm in diameter each impregnated with the above extract solutions were dried at room temperature to evaporate the DMSO. Ten (10) adult *Haemonchus contortus* worms were placed into a sterile Petri dish containing 10 ml of phosphate buffered saline (PBS). The filter paper discs containing the extracts were added and agitated. After 24 hours, the worms were removed from the Petri dish and then suspended in PBS for 30 minutes for possible recovery of their motility. Death was concluded when the worm lost their motility coupled with fading away of their body colour[13]. The number of motile (alive) and immotile (dead) worms were counted using a hand lens and recorded.

Albendazole (0.55mg/ml) was used as a reference drug (positive control). PBS was used as a negative control. Worm motility and mortality was used as the rationale for anthelmintic activity.

2.7 Statistical analysis

The results obtained for anthelmintic activity were given as mean value ± standard deviation and the data were subjected to statistical analysis using analysis of variance (ANOVA) to determine whether there were significant differences in activity of the plant extracts at different concentrations used at $p<0.05$.

3. Results and Discussion

3.1 Ethnobotany of the identified anthelmintic plants

The study identified twenty one (21) anthelmintic plants distributed among thirteen (13) families and 21 genera. The frequency of usage of the plants by the herbalists was used to pick *Vernonia amygdalina* for bioassay as given in table 1.

Table 1: Anthelmintic plants identified during the study.

Botanical name	Vernacular name	Family	Habit	Parts used	Mode of preparation	Number of Independent Reports (IR)	Ranking
Bidens pilosa VOO 017/2013	Anyiego	Asteraceae	Herb	Whole	Decoction	7	16
Tamarindus indica VOO 014/2013	Chwaa	Leguminosae subfam. Ceasalpinioideae	Tree	Bark	Concoction	15	10
Combretum collinum VOO 015/2013	Keyo	Combretaceae	Tree	Roots	Decoction	6	17
Solanecio mannii VOO 004/2013	Maroo	Asteraceae	Shrub	Leaves	Infusion	21	5
Leonotis nepetifolia VOO 005/2013	Nyanyodhi	Lamiaceae	Herb	Leaves	Decoction	5	18
Sclerocarya birrea VOO 010/2013	Ng'ong'o	Anacardiaceae	Tree	Bark	Decoction	11	13
Albizia coriaria VOO 006/2013	Ober	Leguminosae subfam. Mimosoideae	Tree	Leaves	Infusion	20	6
Euclea divinorum VOO 012/2013	Ochol	Ebenaceae	Tree	Roots	Decoction	8	15
Aloe secundiflora VOO 019/2013	Ogaka	Aloaceae	Herb	Leaves, roots	Decoction	17	8
Plectranthus barbatus VOO 011/2013	Okita	Lamiaceae	Shrub	Leaves	Decoction	24	3
Rotheca myricoides VOO OO2/2013	Okwero	Verbenaceae	Herb	Roots	Infusion	16	9
Ximenia americana VOO 008/2013	Olemo	Olacaceae	Tree	Roots	Decoction	12	12
Vernonia amygdalina VOO 003/2013	Oluswa	Asteraceae	Tree	Leaves, roots	Infusion	25	2
Hypitis suaveolens VOO 021/2013	Oluwo ndara	Lamiaceae	Herb	Whole	Decoction	1	21
Erythrina abyssinica VOO 009/2013	Orembe	Leguminosae subfam. Papilionoideae	Tree	Bark	Decoction	10	14
Eclipta prostrata VOO 020/2013	Osieko	Asteraceae	Herb	Whole plant	Infusion	26	1
Cucumis aculeatus VOO 018/2013	Otangle	Cucurbitaceae	Herb	Fruits	Decoction	23	4
Harrisonia abyssinica VOO 013/2013	Pedo	Simaroubaceae	Tree	Roots	Infusion	4	19
Carica papaya VOO 007/2013	Poipoi	Caricaceae	Tree	Roots	Decoction	18	7
Searsia natalensis VOO 016/2013	Sangla	Anacardiaceae	Tree	Roots	Decoction	2	20
Kigelia africana VOO 001/2013	Yago	Bignoniaceae	Tree	Bark	Concoction	14	11

Vernonia amygdalina is a shrub or a small tree 2-8 m; bark pale grey; twigs tomentose. Leaves lanceolate to obovate-lanceolate, up to 15 cm long, 5 cm broad, finely glandular and pubescent beneath. Flower heads white, sweet scented, 8mm in diameter, phyllaries 3-4 mm long with dark tips[8][14]. Either the leaves or the roots is made into an infusion and drunk.

Fig 3: *Vernonia amygdalina* (Asteraceae)

Each of the crude plant extract obtained was weighed to determine their yield. Percentage yield was then calculated as follows:

Percentage yield=Quantity of Extract /Quantity of plant material X 100

The results are given in table 2.

Table 2: Yield and percentage yield of crude plant extracts

Plant species	Methanol extract		Acetone extract		Water extract		
	Yield (grams)	**Percentage yield (%)**	**Yield (grams)**	**Percentage yield (%)**	**Yield (grams)**	**Percentage yield (%)**	**Average yield (grams)**
V. amygdalina (roots)	4.34	8.68	4.67	9.34	4.20	8.40	4.40

The decreasing order of extract yield was acetone, methanol and water.

Extracts of *V. amygdalina* (roots) was screened for tannins, saponins and cardiac glycosides using standard procedures[9]. The results are given in table 3.

Table 3: Phytochemical screening for each crude extracts for secondary metabolites.

Solvent	**Methanol**			**Acetone**			**Distilled water**		
Secondary metabolites screened	Tannins	Saponins	Cardiac glycosides	Tannins	Saponins	Cardiac glycosides	Tannins	Saponins	Cardiac glycosides
(Plant species) *V. amygdalina* (roots)	+	+	+	+	+	+	+	+	+

Key: + = Present, - = Absent

All the *V. amygdalina* (roots) extracts tested positive for saponins, tannins and cardiac glycosides.

Each of the solvent crude plant extract at concentrations of 6.25 mg/ml, 12.5 mg/ml and 25mg/ml was tested in triplicate for anthelmintic potential. Mean mortality at various concentrations were calculated as represented in table 4.

Table 4: Mean mortality ± SD of the extract concentrations used.

Plant species	**Extract**	**Mean mortality ± SD**		
		6.25 mg/ml	**12.5 mg/ml**	**25 mg/ml**
Vernonia amygdalina (roots)	Acetone	2.00±0.000	2.33±0.577	2.67±0.577
	Methanol	3.33±0.577	4.67±0.577	5.67±0.577
	Aqueous	2.00±0.000	2.67±0.577	3.33±0.577
Albendazole	0.55mg/ml	10.00±0.000	10.00±0.000	10.00±0.000
PBS	10 ml	0.00±0.000	0.00±0.000	0.00±0.000

Mean mortality of solvent extracts in decreasing order was methanol, water and acetone. *Vernonia amygdalina* root extract had a mean mortality of 20-33.3% at 6.25 mg/ml; 23.3-46.7% at 12.5 mg/ml and 26.7-56.7% at 25 mg/ml. Albendazole showed 100% mortality while PBS showed no mortality.

There was no significant difference in the worm mortality caused by acetone and aqueous extract of *V. amygdalina* roots at 6.25 mg/ml at $p<0.05$. Secondary metabolites of plant origin have been found to have both *in-vivo* and *in-vitro* anthelmintic activity[15].

Tannins, saponins and cardiac glycosides are the phytochemicals purported to have anthelmintic effect. Tannins are known to produce anthelmintic activity by binding to glycoprotein on the cuticle of the parasite. They hinder energy production in helminth parasites by uncoupling oxidative phosphorylation, which can cause death[16]. Though the exact mechanism of saponins against gastrointestinal nematodes is not very well known[9], they are known to produce inhibitory effect on inflammation[17] an activity which prevents inflammatory effects normally caused by the gastro intestinal worms to the host. Tannins have also been reported to be useful in the treatment of inflamed or ulcerated tissues[17]. Albendazole works by interference with the polymerization of microtubule[18]. The drug binds to the protein tubulin of the *H. contortus* hence causing death by starvation[9]. Cases of cardiac glycosides human poisoning have been reported[19]; therefore in its low concentrations in plant materials, when ingested by human, it can contribute to the killing of the gastrointestinal worms through its toxic effect.

4. Conclusions

The traditional use of *Vernonia amygdalina* as an anthelmintic by herbalists of Migori County, Kenya has been established in this study. This plant therefore is a potential for anthelmintic drug development.

Acknowlegements

I would like to acknowledge the University of Nairobi for giving me a scholarship for this research study. Secondly, I acknowledge my supervisors Prof. John Kokwaro Dr. Bethwell Owuor and Dr. Amir Yusuf for their invaluable support during this research work. Much appreciation also goes to the administrators and traditional medicine practitioners (TMPs) of Migori County, Mr. Patrick Mutiso, Ms. Margaret Kaigongi and Ms. Jackline Cherotich for their support during the study.

References

[1] Piyush Y, Rupali S. A review on anthelmintic drugs and their future scope. *International Journal of Pharmacy and Pharmaceutical Science* 2011; 3(3): 17-21.

[2] Wahab ARRL, Suleiman SF. In-vivo anthelmintic activity of Neem plant (*Azadirachta indica*) extract against third-stage *Haemonhus contortus* larvae from goats. *Global Veterinarian* 2001; 7(1): 22-26.

[3] Daniel SF, Norman RF, The value of plants used in Traditional Medicine for Drug Discovery. *Environmental perspectives* 2001; 109(1): 69-75.

[4] Athanasiadou S, Githiori J, Kyriazakis I. Medicinal plants for helminth parasite control: facts and fiction. *Animal* 2007; 1(9): 1392–1400.

[5] Luiz RSG, Reinaldo FPL, Ulysses PA. Knowledge and use of medicinal plants. Local specialists in a region of Atlantic forest in the state of Pernambuco (Northeastern Brazil). *Journal of Ethnobiology and Ethnomedicine* 2005; 1: 9.

[6] Owuor BO. Ethnobotanical and Phytochemical study of Herbal remedies of Migori District, Kenya. M.Sc. University of Nairobi; 1999.

[7] Agnew ADQ. Upland Kenya Wild flowers and ferns. 2nd Ed. Kenya: East African Natural History Society, Nairobi; 2013.

[8] Beentje HJ. Kenya Trees, Shrubs and Lianas. National Museums of Kenya, Nairobi; 1994.

[9] Ombasa O, Karem PG, Rukunga G, Mbaria J, Keriko JM, Njonge FK, *et al*. *In-vitro* Anthelmintic effects of two Kenyan plant extracts against *Haemonchus contortus* Adult worm. *International Journal Pharmacological Research* 2012; 2(3): 113-116.

[10] Egwaikhide PA, Gimba CE. Analysis of the Phytochemical Content and Anti microbial Activity of *Plectranthus glandulosis* Whole Plant. *Middle East Journal of Scientific Research* 2007; 2(3-4): 135-138.

[11] Mariita RM, Ogol CKPO, Oguge NO, Okemo PO. Methanol Extract of Three Medicinal Plants from Samburu in Northern Kenya Show Significant Antimycobacterial, Antibacterial and Antifungal Properties. *Research Journal of Medicinal Plant* 2011; *5: 4-64.*

[12] Chinnaperumal K, Abdul AR, Gandhi E, Asokan B, Abdul AZ. Anthelmintic activity of botanical extracts against sheep gastrointestinal nematodes *Haemonchus contortus*. *Parasitology Research* 2011; 109: 37–45.

[13] Kumar A, Chanda I, Singh AK. Extraction and Evaluation of Pharmacological Activity of saponin extract of *Plumeria rubra* leaves. *Pharmacologyonline* 2009; 1: 969-974.

[14] Kokwaro JO Johns T. Luo Biological Dictionary. East African Educational Publishers Ltd; 1998.

[15] Githori JB, Athanasiadou S, Thimsborg SM. Use of plants in novel approaches in the control of gastrointestinal

helminthes in livestock with emphasis on small ruminants. *Veterinary parasitology* 2006 July 31; 139(4):308-320.

[16] Danquah CA, Koffuor G.A, Annan K, Ketor EC. The Anthelmintic Activity of *Vernonia Amygdalina* (Asteraceae) and *Alstonia Boonei* De Wild (Apocynaceae). *Journal of Medical and Biomedical Sciences* 2012; *1(1): 21-27.*

[17] Mohd YM, Manik S, Saxena RC, Mohd IM, Abrar HM, Showkat HB. Phytochemical Screening and Spectroscopic Determination of Total Phenolic and Flavonoid Contents of *Eclipta alba* Linn. *Journal of Natural Products and Plant Resources* 2013; 3 (2): 86-91.

[18] Harder A. Chemotherapeutic approaches to nematodes: current knowledge and outlook. *Parasitology Research* 2002; 88: 272 - 277.

[19] Eddleston M, Ariaratnam CA, Sjöström L, Jayalath S, Rajakanthan K, Rajapakse S, *et al*. Acute yellow oleander (*Thevetia peruviana*) poisoning: cardiac arrhythmias, Electrolyte disturbances and serum cardiac glycoside concentrations on presentation to hospital. *Heart* 2000; 83: 301–306.