Journal of Medical and Biomedical Sciences (2012) 1(1): 21-27

ORIGINAL ARTICLE

The Anthelmintic Activity of *Vernonia Amygdalina* (Asteraceae) and *Alstonia Boonei* De Wild (Apocynaceae)

C.A. Danquah[1], G.A. Koffuor[1], K. Annan[2] and E.C. Ketor[1]

[1]Department of Pharmacology, [2]Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, KNUST, Kumasi, Ghana

Intestinal worms affect a host of individuals resulting in malnutrition, stunted growth, intellectual retardation and cognitive deficits. The aim of this study is to investigate the antihelminthic activity of *Alstonia boonei* De Wild (Apocynaceae) and *Vernonia amygdalina* (Asteraceae) using earthworms (*Lumbricus terretris*). The worms were directly exposed to 50, 100, and 200 mg/ml of aqueous and ethanolic bark extracts of *Alstonia boonei* and leaf extract of *Vernonia amygdalina* and piperazine citrate in a petri dish and in an organ bath. The control group was exposed to distilled water. The time of paralysis and death were determined within a period of 6 h in the petri dish method whiles spontaneous movements of the worms before and after drug administration were recorded on a slow moving kymograph drum in the organ bath method. All doses of the aqueous and ethanolic extracts significantly ($P \leq 0.001$) reduced the time of paralysis and time of death compared to the vehicle treated group. The time of paralysis and time of death in the tissue bath method corresponded to that obtained by direct exposure. The extracts exhibited anthelmintic activity and thus could be an inexpensive and readily available source of anthelmintic treatment.
Journal of Medical and Biomedical Sciences (2012) 1(1), 21-27

Keywords*:* Piperazine citrate, Time of paralysis, *Lumbricus terretris,* Tannins

INTRODUCTION

Anthelmintics are drugs that act locally to expel worms from the gastro-intestinal tract or systemically to eradicate adult helminths or developmental stages that invade organs and tissues (Devi *et al.,* 2009). These medicines are used in human and other animal populations to destroy parasites that live in the body. According to World Health Organisation statistics, more than two billion people harbour parasitic worm infections (Khurana, 2010). In areas of high prevalence, simultaneous infection with more than one type of helminths is common.

Correspondence: Mr George A. Koffuor, Dept. of Pharmacology, KNUST, Kumasi, Ghana
E-mail: gkoffuor@yahoo.com

One of the problems with antihelmintics is that many of them have been used for a long time and over time parasites have developed drug resistance (Sarojini *et al.*, 2011). Most of the existing anthelmintics e.g. levamisole produce side effects such as abdominal pain, loss of appetite, nausea, vomiting, headache and diarrhea (Goodman and Gilman, 2001). Much emphasis has been placed on phytomedicine for some time now due to their outstanding advantages over synthetic drugs. Among these advantages are; least side effects, low cost and least drug resistance. Thus phytomedicine has become a good alternative to synthetic anthelmintics (Pawan, 2009). However, most phytomedicine still depends on "trial and error" basis or based on conventional wisdom. A variety of *in vivo* and *in vitro* methods has been employed to evaluate and validate anthelmintic properties of plant remedies (Pawan, 2009).

Two plants namely *Alstonia boonei* De Wild *(Apocynaceae)* commonly known as Pattern wood or stool wood and *Vernonia amygdalina (Asteraceae)* commonly known as bitter leaf are known to possess anthelmintic activity (Ghana Herbal Pharmacopoeia, 2007). *Alstonia boonei* is distributed throughout the tropics and the rain forest of West and Central Africa (Olajide, 2000). A bark extract is widely used to treat malaria, typhoid fever, gonorrhea, yaws, asthma and dysentery, and is also applied to sores, ulcers, snakebites, rheumatic pain and toothache, and as a galactagogue. A maceration of the bark is taken to treat jaundice, cough and sore throat, and is applied externally to treat skin conditions like eczema, ringworm and acne (Palla, 2005).

Vernonia amygdalina is indigenous to tropical Africa and is found wild or cultivated all over Sub-Saharan Africa (Bosch *et al*., 2005). Bitter leaf, commonly called, is a highly appreciated vegetable in West and Central Africa and can be consumed in various dishes. In traditional medicine; Leaf decoctions are used to treat fever, malaria, diarrhoea, dysentery, hepatitis and cough, as a laxative and as a fertility inducer. It is also used as a medicine for scabies, headache and stomach-ache. Root extracts are also used as treatment against malaria and gastrointestinal disorders. One of the most common medicinal uses of *V. amygdalina* is as a treatment against intestinal worms including nematode (Fomum, 2004). Leaf and root barks extracts showed antimalarial activity against drug-sensitive *plasmodium berghei* (Abosi and Raseroka, 2003).

Development of resistance to most of the commercially available anthelmintics became a severe problem worldwide (Waller *et al*., 2004). Moreover, these drugs are unaffordable to the resource-poor individuals in developing countries, inaccessible or inadequately available as the majority of the population lives in the rural areas where these medicinal plants are readily available (Hammond, *et al*., 1997). These factors paved the way for herbal remedies as alternative anthelmintics (Fajmi, *et al.,* 2005).

Although some work has been done on the antihelmintic activity of *Vernonia amygdalina* and *Alstonia boonei* most of them were *in vivo* (Siamba *et al*., 2007; Alawa *et al*., 2010). Therefore to verify further the antihelmintic activity of these plants and instill more confidence in their use as antihelminthics, this *in vitro* protocol is being used on the common earthworm (Lumbricus terrestris).

The physiological resemblance of earthworms (*Lumbricus terrestris*) to intestinal roundworm, *Ascaris lumbricoides* (Nirma *et al*, 2007; Ashok, 2010) make this model suitable for antihelmintics studies in humans. The aim of the study therefore is to investigate the antihelminthic activity of *A. boonei* De Wild (Apocynaceae) and *V. amygdalina* (Asteraceae) using earthworms (*Lumbricus terrestris*) to affirm their use as alternatives to anthelmintic therapy.

MATERIALS AND METHODS

Plant collection

The bark of *Alstonia boonei* and the leaves of *Vernonia amygdalina* were collected from the botanical gardens of the Kwame Nkrumah University of Science and Technology (KNUST), and authenticated at the Department of Pharmacognosy, KNUST, and dried for extraction.

Preparation of Plant Extracts

Aqueous Extracts

The stem bark of *Alstonia boonei* was washed, sun dried and ground to a coarse powder. A 1,250 g quantity of the powdered drug was put into a glass container with 3000 ml of water. The mixture was boiled for 30 minutes and cooled. Filtration was done and the marc pressed. The filtrate was dried in an oven at 40^{o}C. A solid mass weighing 40 g was obtained (percentage yield: 3.2%). This was labeled aqueous *Alstonei boonei* bark extract (AQ ABE) for use in this study. The same procedure was used in the preparation of the *Vernonia amygdalina* leaf extract. A 625 g quantity of the powdered leaves yielded 100 g of dry residue (percentage yield: 16%) labeled aqueous *Vernonia amygdalina* leaf extract (AQ VLE) for use in this study.

Ethanolic Extracts

A 1000 g quantity of *Alstonia boonei* bark powder was packed into a percolator with its discharge port packed with cotton. Sufficient quantity of 70% ethanol was added to cover the drug and left for about 24 hours. The liquid was drained slowly from the bottom of the percolator (about 20 drops per minute) into a flask. The marc was pressed and this liquid was added to the percolate in the flask which was then concentrated in the Buchi Rotor Evaporator (Rotavapor R-210, Switzerland) and dried in the Gallenkamp hot air oven (Oven 300 plus series, England) at 40^{o}C. A dry mass of 56.8 g was obtained (representing a yield of 5.68%) and labeled as ethanolic *Alstonei boonei* bark extract (ET ABE) for use in this study. The same procedure was followed in the preparation of the *Vernonia amygdalina* leaf extract in which 300 g of powdered leaves was used. A dry mass of 21.1 g was obtained (representing a yield of 7.03%) and labeled ethanolic *Vernonia amygdalina* leaf extract (ET VLE) for use in this study.

Collection of Worms
Earthworms *(Lumbricus terrestris)* of lengths 6-12 cm were obtained from the damp, cool, and covered area of the gardens of the Faculty of Horticulture (KNUST). The worms were transferred into a glass bottle with some quantity of the soil from which they were taken. The worms were identified and authenticated at the Department of Biological Science, KNUST, Kumasi, Ghana.

Experimental design
Phytochemical screening
Phytochemical screening was conducted on AQ ABE, AQ VLE, ET ABE and ET VLE to ascertain the presence of phytochemicals as described by Wagner and Bladt, (1996) and Harborne, (1998). The tannin content was determined according to the method of Glasl (1983) using pyrogallol (99.5% HPLC) as reference compound.

Gross Motility and Mortality Studies
This method was carried out as described by Ajaiyeoba *et al.,* (2001) and Iqbal *et al.,* (2001) with some modifications. Fifty (50) ml quantities of suspensions (concentrations: 50 mg/ml, 100 mg/ml and 200 mg/ml) prepared from weighed quantities of AQ ABE, AQ VLE, ET ABE and ET VLE were poured into labelled petri-dishes. Five worms were put into each petri-dish and compared with that of piperazine citrate (50, 100, and 200 mg/kg); the reference anthelmintic and the control (distilled water). Time for paralysis and death were determined as described in Table 1. The experiment was terminated after 6 hours.

Tissue-bath Studies
The tissue-bath experiment was designed as described by Goodwin, 1958 with some modifications by researchers. Worms were placed in prepared fine nylon stockings (18 cm long and 0.6-0.8 cm wide) and suspended in a 40 ml capacity glass tube of length 40 cm and width 6 cm containing modified Tyrode solution maintained at 37 ^{o}C in a Havard research apparatus (Harvard Apparatus Ltd, Kent, UK). The open end of the stockings was closed behind the worm with the use of a rubber band. The band was attached to a sinker made from a piece of heavy metal. The closed anterior end was tied to a piece of thread (which lowered the worm into the tube containing Tyrode) and attached to the frontal writing lever of Havard apparatus. Oxygen was bubbled through the tyrode solution. With sufficient counterweight on the lever, the worm was kept upright in the tube. Enough time was allowed for spontaneous movement of the worm in the bath to be stable (seen as a uniform baseline recording on the Harvard kymograph) before administration of 20 ml of 200 mg/ml of AQ ABE, AQ VLE, ET ABE and ET VLE or 5 ml of 150 mg/ml piperazine citrate (doses were selected from preceding experiment). In the control, the worms were suspended in modified Tyrode solution. Time for paralysis and death (seen as a decrease in spontaneous movement and no movement respectively) of the worm as recorded on a slow moving Harvard kymograph drum was noted. Termination time for the experiment was 3 hours. The procedure was repeated five times for each treatment group.

Table 1: A guide to ascertain paralysis and death of experimental worms

Parameter	Description
Paralysis	Marked decrease in vigorous wriggling movement of the worm indicates paralysis
	If the worm revived in physiological solution (Tyrode)
Death	Evoked pin prick response: Slow movement of the worm after being pricked with pin indicates paralysis. No movement indicates death.
	Confirmed by dipping the worm in warm water at 50 ^{o}C and shaken vigorously. No response indicates death

Statistical Analysis
Data is presented as mean ± SD (N=5). Analysis of the effects between doses in treatment groups and the control and between the aqueous and ethanolic extracts was conducted by two-way ANOVA followed by Bonferroni's post hoc test. GraphPad Prism Version 5.0 for Windows (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses. $P \leq 0.05$ was considered statistically significant in all analysis.

RESULTS

Preliminary Phytochemical Screening
Results from phytochemical screening indicate the presences of alkaloids, tannins, and glycosides in all extracts (Table 2).

Antihelmintic Activity
After 6 hours in the petri-dish, worms in the control group were still active (no paralysis); there were no deaths. Piperazine citrate and all the extracts however caused paralysis in the worms between 3.68 ± 0.90 to 59.9 ± 8.3 minutes of exposure which were very signifi-

cant ($P \leq 0.001$) compared to the control (Table 3). The paralyzing effect was dose-dependent. Again, piperazine citrate and the extracts dose-dependently and very significantly ($P \leq 0.001$) caused death in the worms between 17.4 ± 2.3 and 158.4 ± 16.4 minutes in comparison to the control (Table 4).

Table 2: Results from phytochemical screening of the aqueous and ethanolic extracts of *A. boonei* De Wild (Apocynaceae) and *V. amygdalina* (Asteraceae)

Constituent	AQ ABE	AQ VLE	ET ABE	ET VLE
Alkaloids	+	+	+	+
Tannins	+	+	+	+
Flavonoids	-	+	-	+
Glycosides	+	+	+	+
Steroids	-	-	-	-

While worms in the control group saw no paralysis or death when suspended in the tissue bath, again piperazine citrate and all the extracts significantly caused paralysis of the worms between 8.73 ± 2.38 to 16.52 ± 4.63 minutes of exposure and death between 21.5 ± 3.10 to 27.8 ± 8.62 minutes of exposure (Table 5).

DISCUSSION

In this study, anthelmintic assay was performed on adult earthworms (*Lumbricus terrestris*) due to its physiological resemblance with the intestinal roundworm parasite of human beings (Thorn *et al.,* 1977; Vigar, 1984). The experimental results indicate that the extracts of *Vernonia amygdalina* and *Alstonia boonei* have potent anthelmintic activity (the short duration of action is worth noting).

From phytochemical screening all the extracts have alkaloids, tannins, and glycosides which have been associated with antihelminthic activity (Sarojini *et al.,* 2011). Alkaloids in the aerial parts of *Cissampelos capensis* (Menispermaceae) and *Macleaya microcarpa* (Maxim) Fedde are known to be responsible for their anthelminthic activity (Ayers *et al.,* 2007; Wang *et al.,* 2010). 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

Table 3: The time (min) taken for paralysis of the earthworms (*Lumbricus terrestris*) on exposure to AQ ABE, AQ VLE, ET ABE, ET VLE and piperazine citrate at doses of 50, 100, and 200 mg/kg in a petri-dish

Dose (mg/ml)	Control	AQ ABE	AQ VLE	ET ABE	ET VLE	Piperazine citrate
Vehicle	$> 360 \pm 0.00$					
50		43.50 ± 7.67 ***	59.94 ± 8.25 ***	34.89 ± 2.48 ***	33.18 ± 12.41 ***	9.62 ± 1.32***
100		28.22 ± 2.63 ***	9.61 ± 2.10 ***	10.03 ± 0.86 ***	8.72 ± 2.99 ***	5.75 ± 1.66 ***
200		12.23 ± 0.67 ***	4.05 ± 1.06 ***	4.78 ± 0.39***	3.56 ± 0.37 ***	3.68 ± 0.90 ***

Values are means ± SD. (N=5). *** $P \leq 0.001$; compared to control group (Two-way ANOVA followed by Bonferroni's *post hoc* test).

Table 4: The time (min) taken for death of the earthworms (*Lumbricus terrestris*) on exposure to AQ ABE, AQ VLE, ET ABE, ET VLE and piperazine citrate at doses of 50, 100, and 200 mg/kg in a petri-dish

Dose (mg/ml)	Control	AQ ABE	AQ VLE	ET ABE	ET VLE	Piperazine citrate
Vehicle	> 360 ± 0.00					
50		158.4 ± 16.42 ***	76.65 ± 12.73 ***	120.5 ± 4.3 ***	37.46 ± 13.55 ***	37.72 ± 6.2***
100		39.22 ± 4.70 ***	19.64 ± 5.27 ***	17.47 ± 1.96 ***	11.82 ± 3.45 ***	22.82 ± 4.36 ***
200		21.77 ± 2.08 ***	8.14 ± 2.15 ***	9.0 ± 0.59***	4.48 ± 0.39 ***	17.37 ± 2.27 ***

Values are means ± SD. (N=5). *** $P \leq 0.001$; compared to control group (Two-way ANOVA followed by Bonferroni's *post hoc* test).

Table 5: The time (min) taken for paralysis, and death of the earthworms (*Lumbricus terrestris*) on exposure to AQ ABE, AQ VLE, ET ABE, ET VLE and piperazine citrate at a dose of 100 mg/kg in the tissue bath.

Treatment groups	Time for Paralysis (min)	Time for Death (min)
Control	> 360 ± 0.00	> 360 ± 0.00
AQ ABE	16.52 ± 4.63 ***	27.8 ± 8.62 ***
AQ VLE	14.18 ± 2.84 ***	25.16 ± 6.09 ***
ET ABE	15.88 ± 4.47 ***	21.4 ± 5.00 ***
ET VLE	13.91 ± 2.92 ***	24.32 ± 4.88 ***
Piperazine citrate	8.73 ± 2.38 ***	21.5 ± 3.10 ***

Values are means ± SD. (N=5). *** $P \leq 0.001$; compared to control group (Two-way ANOVA followed by Bonferroni's *post hoc* test). Termination time for the experiment was 6 hours.

oxidative phosphorylation (Martin, 1997).

Piperazine a known anthelmintic is GABA mimetic. By increasing chloride ion conductance of worm muscle membrane, it produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis (Martin, 1985; Sutar *et al.,* 2010). Comparing anthelmintic activity of *Vernonia amygdalina* and *Alstonia boonei* to piperazine, the extracts may contain constituents that could probably have weak GABA-mimetic effect similar to piperazine citrate.

CONCLUSIONS

Antihelmintic effects of the extracts could ease the economic burden on antihelmintic therapy. The bark extract of *Alstonia boonei* and the leaf extract of *Vernonia amygdalina* have antihelmintic activity. These findings may partly explain some of the folklore use of these plants in the treatment of worm infestations.

ACKNOWLEDGEMENT

The Authors wish to express their gratitude to Mr. Thomas Ansah of the Department of Pharmacology, KNUST, Kumasi for his technical assistance.

REFERENCES

Abosi AO, Raseroka BH (2003). *In vivo* antimalarial activity of *Vernonia amygdalina. Br. J Biomed Sci., 60(2): 89-91*

Ajaiyeoba EO, Onocha PA, Olarenwaju OT (2001). *In vitro* anthelmintic properties of *Buchholzia coriaceae* and *Gynandropsis gynandra* extract. *Pharm Biol., 39: 217-20.*

Alawa CBI, Adamu AM, Gefu JO, Ajanusi OJ, Abdu PA, Chiezey NP, (2010). In vivo efficacy of *Vernonia amygdalina* (compositae) against natural helminth infection in Bunaji (Bos indicus) calves. Pak Vet J, 30(4): 215-218.

Ashok-Kumar BS, Lakshman K, Jayaveera KN, Nandeesh R, Manoj B, Ranganayakulu D (2010). Comparative *in vitro* anthelmintic activity of three plants from the Amaranthaceae family. *Arch Biol Sci*., *62* (*1*): *185-189*

Ayers S, Zink DL, Mohn K, Powell JS, Brown CM, Murphy T, Brand R, Pretorius S, Stevenson D, Thompson D, Singh SB (2007). Anthelmintic activity of aporphine alkaloids from Cissampelos capensis. *Planta Med.*, 3(3):296-7.

Bosch CH, Borus DJ, Siemonsma JS (2005). *Vegetables of Tropical Africa. Conclusions and Recommendations Based on PROTA 2: 'Vegetables'*. PROTA Foundation, Wageningen, Netherlands.

Devi K, Indumathy S, Rathinambal V, Uma S, Kavimani S, Balu V (2009). Anthelmintic Activity of *Asta Churna. International Journal of Health Research, 2(1): 101-103*

Fomum FU (2004).*Vernonia amygdalina* Delile In: Grubben GJH, Denton OA (Editors). PROTA 2: Vegetables/Légumes. [CD-Rom].PROTA, Wageningen, Netherlands.

Ghana Herbal Pharmacopeia (2007). Science and Technology Policy Research Institute, Accra, Ghana.

Glasl H (1983). Zur Photometrie in der Drogenstandiserung. *DAZ*, 123:1979-1987.

Goodman LS, Gilman A (2001). The Pharmacological basis of Therapeutics.10th Edition, Mcgraw Hill Medical Publishing Division, New York, p1121

Goodwin LG, (1958). A method for recording the effects of anthelmintics on the movements of Ascaris lumbricoides *Brit. J. Pharmnacol., 13:197.*

Hammond DA, Feilding D, Bishop SC (1997*).* Prospects for plant anthelmintic in tropical veterinary medicine. *Vet Res Com; 21:213-228.*

Harborne JB (1998). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, 3rd edition, Chapman and Hall, London, p 302.

Iqbal Z, Nadeem QK, Khan MN, Akhtar MS, Waraich FN (2001). *In vitro* anthelmintic activity of *Allium sativum, Zingiber officinale, Cucurbita mexicana* and *Ficus religiosa. Int J Agric Biol., 3:454–7.*

Khurana A (2010). What Are Anthelmintic Drugs and How They Work? Available at: http://ezinearticles.com/?What-Are-Anthelmintic-Drugs-and-How-They-Work?&id=4191153. Accessed on July 23, 2011.

Martin RJ (1985). Gamma-Aminobutyric acid- and piperazine-activated single-channel currents from *Ascaris suum* body muscle. *Br. J. Pharmacol., 84*, 445 –461.

Martin RJ (1997). Mode of action of anthelmintic drugs. *Vet J., 154: 11-34.*

Nirmal SA, Malwadkar G, Laware RB (2007). Anthelmintic activity of *Pongamia glabra* Songlanakarin. *J Sci Technol., 29:755-757.*

Olajide OA, Awe SO, Makinde JM, Ekhelar AI (2000). Studies on the anti-inflammatory, antipyretic and analgesic properties of *Alstonia boonei* stembark. *J Ethnopharmacol., 71(1-2): 179-86*.

Palla F, (2005). *Alstonia boonei* De Wild. [Internet] Record from Protabase. Louppe D, Oteng-Amoako AA, Brink M (Editors). PROTA (Plant Resources of Tropical Africa / Ressourcesvégétales de l'Afriquetropicale), Wageningen, Netherlands.<http://database.prota.org/search.htm>. Accessed 22 July, 2011.

Pawan VK, (2009). Pharmacological Evaluation and Validation of Plant based Anthelmintics. Pura. Jammu (J & K).

Sarojini N, Manjari SA, Kanti CC, (2011). Phytochemical screening and anthelminthic activity study of *Saraca indica* leave extract. *International Research Journal of Pharmacy,* 2(5): 194-197

Siamba DN, Okitoi LO, Watai MK, Wachira AM, Lukibisi FB, Mukisira EA (2007). Efficacy of *Tephrosia vogelli* and *Vernonia amygdalina* as anthelmintics against *Ascaridia galli* in indigenous chicken. Livestock Research for Rural Development. Volume 19, Article #176.

Sutar N, Garai R, Sharma US, Sharma UK, Jaiswal A (2010). Anthelmintic activity of *Platycladus orientalis* leaves extract. *International Journal of Parasitology Research*, (2)2: 1-3.

Thorn GW, Adams RD, Braunwald E, Isselbacher KJ, Petersdrof RG (1977). Harrison's Principles of Internal Medicine. McgrawHill Co., New York, pp. 1088-1089.

Vigar Z (1984). Atlas of Medical Parasitology, 2nd Edition. P.G. Publishing House, Singapore, pp. 216-217.

Wagner H, Bladt S (1996). Plant Drug Analysis: A Thin Layer Chromatography. 2nd edition, Springer Verlag, New York.

Wang GX, Zhou Z, Jiang DX, Han J, Wang JF, Zhao LW, Li J (2010). *In vivo* anthelmintic activity of five alkaloids from Macleaya microcarpa (Maxim) Fedde against Dactylogyrus intermedius in Carassius auratus. *Vet Parasitol.,* 171(3-4):305-13.

ISSN 2026-6294