

Full Length Research Paper

***In vitro* anthelmintic activity of stem and root barks of *Alstonia boonei* De Wild**

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***Alstonia boonei* De Wild ethanol extracts of the stem bark (ABSB) and root bark (ABRB) were evaluated for possible anthelmintic activity. Three different concentrations of each extract (50, 100 and 150 mg/ml) were evaluated for *in vitro* anthelmintic activity by determining the effects of the extracts on the paralysis and death times of *Pheretima posthuma*. Mebendazole (MBZ) 15 mg/mg was used as reference anthelmintic. ABSB and ABRB demonstrated a concentration dependent anthelmintic activity with a reduction in paralytic and death times upon increase in the concentration of the extracts. ABSB revealed better anthelmintic activity than ABRB at all concentrations tested. ABSB also revealed a significant paralytic time ($p < 0.01$) at 150 mg/ml with reference to MBZ. Phytochemical screening revealed the presence of alkaloids, tannins, flavonoids saponins and glycosides in ABSB and ABRB.**

Key words: *Alstonia boonei*, anthelmintic, *Pheretima posthuma*, death time, paralytic time.

INTRODUCTION

Medicinal plants have been used over the decades to manage helminth infections. However, scientific evaluation of some of these traditional treatments has not been conducted so as to validate their usage. With the current increase in anthelmintic resistance amongst both human and farm animals (Veracruz et al., 2011), it is imperative that the traditional usage of some of these medicinal plants be validated and novel molecules isolated in order to curb the menace. The use of medicinal plant as anthelmintic has been practised in many indigenous cultures for centuries. Studies have shown that in many developing countries, ethno medicine is still the primary treatment option for many parasitic

diseases (Tanner et al., 2011; Gazzinelli et al., 2012). *Alstonia boonei* De Wild (Apocynaceae) is an indigenous African tree mostly found in the evergreen rain forest of tropical West Africa (Gosse et al., 1999).

The plant is well known by almost all traditional healers practising along the west coast of Africa (Adotey, 2012). The bark of the plant is known to possess antirheumatic, anti-inflammatory, anthelmintic and antidiabetic properties (Hadi and Bremner, 2001). Traditionally, a cold infusion of the fresh or dried bark is used as an anthelmintic and also to expel other intestinal parasites in children (Adotey, 2012). Studies conducted on the methanol extracts of the stem bark have demonstrated potent

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anti-inflammatory, analgesic and antipyretic properties (Olajide et al., 2000). Aqueous and ethanol extracts of the stem bark have also demonstrated antimicrobial and anthelmintic activities (Adomi, 2006; Danquah et al., 2012). The stem bark which is mostly used for traditional treatment of malaria is also known to possess very potent antioxidant compounds (Akinmoladun et al., 2007).

Extensive research conducted on this plant has resulted in the identification of various bioactive compounds of diverse pharmacological activity, including; Boonein: a 9 carbon terpenoid lactone possibly known to be the precursor in indole alkaloid biogenesis (Marini-Bottelo et al., 1983). Although much research has been conducted on this plant, there is limited information on the anti-infective potentials of the various parts of the plant. Current anti-infective information available is mostly related to the stem bark of the plant. In the face of high levels of microbial resistance to antibiotics as well as other anti-infective agents, it is important that new compounds be identified and developed. This study therefore aimed to determine and compare the anthelmintic potential of the stem and root barks of *A. boonei* in order to support its traditional uses.

MATERIALS AND METHODS

Collection of plant

The stem and root bark of *A. boonei* were identified and collected in the month of March, 2015 from the Aburi Botanic Gardens in the Eastern Region of Ghana. The plant samples were authenticated by Mr. Albert Asiedu Prempeh, a botanist and curator of the garden. Voucher specimens CUC/DPS/2015/A018 and CUC/DPS/2015/A019 were deposited in the Pharmacognosy Section of the Department of Pharmaceutical Science, Central University College. The samples were washed with distilled water to get rid of debris and then air dried for 3 weeks at room temperature (25 to 28°C).

Extraction of plant

The extraction process was carried out using the method described by Adu et al. (2015) with slight modifications. The dried plant samples were milled into coarse powder using laboratory mill equipment. A quantity of 250 g of both samples was weighed and extracted using 70% v/v ethanol by cold maceration for 72 h. The supernatant and the bulk extract was then filtered using a filter paper with the aid of a vacuum pump. The extracts obtained were concentrated using a rotary evaporator (Buchi, Germany, R210) at 40°C. The concentrates were later dried in a hot air oven at 40°C to obtain the solid extract. The extracts were then stored at 4°C until needed.

Phytochemical screening

Phytochemical tests for the presence of some plant secondary metabolites were performed on the powdered plant samples for the presence of tannins, alkaloids, flavonoids and glycosides (Trease

and Evans, 2002).

Experimental organism

Adult Indian earthworms (*Pheretima posthuma*) which have anatomical resemblance to human intestinal roundworms were obtained from the nursery of a vegetable farm near Central University College, Accra, Ghana. Normal saline solution (0.9%) was used to wash the worms to remove all debris.

In vitro anthelmintic activity evaluation

The experiment was performed with slight modifications to the method described by Bhawar et al. (2009). Earthworms of lengths 3.0 to 6.0 cm were employed. Extract concentrations of 50, 100 and 150 mg/ml were prepared using distilled water. Mebendazole (MBZ) at a concentration of 15 mg/ml was used as the reference standard. Normal saline solution (0.9%) was used as a negative control.

Experimental procedure

Five earthworms were placed in each Petri dish into which the various extracts concentrations and reference drug were added. Observations were made for the time taken by the various extract concentrations to cause paralysis and death of the individual worms. Paralysis was observed when no movement was seen in the worms unless when shaken vigorously. Death was denoted by a lost in motility of the worms even upon pricking with a pin and placement in 50°C warm water, coupled with a fading away of body colour. Solutions within which worms demonstrated vigorous motility and life after ≥ 360 min of exposure were classified as not exerting anthelmintic activity (Na= No activity).

Statistical analysis

Results were presented as mean \pm standard deviation (N=5). Analysis was done using GraphPad prism version 5 (GraphPad Software, San Diego, CA, USA) by two way analysis of variance (ANOVA) followed by bonferonni post-test analysis which recognises $*p<0.05$, $**p<0.01$, and $***p<0.001$ as statistically significant.

RESULTS

Preliminary phytochemical screening

Results from the phytochemical screening revealed the presence of tannins, alkaloids, glycosides and flavonoids in both *Alstonia boonei* stem bark (ABSB) and *Alstonia boonei* root bark (ABRB) (Table 1).

Anthelmintic activity

The extracts demonstrated some level of anthelmintic activity at the concentrations tested. ABSB demonstrated more potent activity than ABRB in a concentration dependent manner with shorter paralysis and death times as compared to ABRB (Tables 2 and 3).

Table 1. Phytochemical screening.

Phytochemicals	RESULTS	
	ABRB	ABSB
Alkaloids	+	+
Tannins	+	+
Saponins	+	+
Glycosides	+	+
Flavonoids	+	+

(+) = Present; (-) = Absent; ABSB: *Alstonia boonei* stem bark; ABRB: *Alstonia boonei* root bark.

Table 2. Paralysis time of ABSB and ABRB against *P. posthuma*.

Extract concentration (mg/ml)	Time (min)		
	ABSB	ABRB	0.9% saline
50	55.00 ± 3.43	Na	Na
100	22.00 ± 1.21*	127.00 ± 5.16	Na
150	17.00 ± 2.10**	93.00 ± 2.04	Na
MBZ 15 mg/ml	27.00 ± 2.23	-	-

ABSB: *Alstonia boonei* stem bark; ABRB: *Alstonia boonei* root bark; MBZ: mebendazole; Na: No activity; * $p < 0.05$; ** $p < 0.01$, values are mean ± SD (N=5)

Table 3. Death time of ABSB and ABRB against *P. posthuma*.

Extract concentration (mg/ml)	Time (min)		
	ABSB	ABRB	0.9% saline
50	Na	Na	Na
100	175.00 ± 4.52	Na	Na
150	100.00 ± 2.47	151.00 ± 2.27	Na
MBZ 15 mg/ml	102.00 ± 2.18	-	-

ABSB: *Alstonia boonei* stem bark; ABRB: *Alstonia boonei* root bark; MBZ: mebendazole; Na: No activity; values are mean ± SD (N=5)

DISCUSSION

The results from the anthelmintic bioassay revealed that both extracts ABSB and ABRB have anthelmintic activity. These results correlate with research conducted by Danquah et al. (2012) reporting the possible anthelmintic potentials of the stem bark extracts of *A. boonei*. The ability of plants to exhibit anthelmintic activity has largely been attributed to the presence of tannins. Tannins are believed to exert anthelmintic activity by interfering with the energy generation of the helminth parasite by uncoupling oxidative phosphorylation or by binding to free proteins in the gastrointestinal tract of the helminth. This eventually results in death of the parasite (Danquah et al., 2012; Adu et al., 2015; Olusegun-Joseph et al., 2012). The results from the phytochemical screening

revealed the presence of tannins in both ABSB and ABRB; this could have been responsible for the anthelmintic activity.

Studies conducted by Mute (2009) also reported the role of alkaloids in anthelmintic activity by causing paralysis of worms through their action on the central nervous system of the helminth. This therefore implies that the presence of alkaloids in the extract also contributed to their anthelmintic activity.

The results obtained however revealed that the anthelmintic activity was concentration dependent for both extracts with the higher concentrations demonstrating better anthelmintic activities (Tables 2 and 3). ABSB demonstrated better anthelmintic activity than ABRB. ABSB also demonstrated significant paralytic times ($p < 0.05$) at 100 mg/ml and ($p < 0.01$) at 150 mg/ml

with reference to MBZ. This could therefore imply that the stem bark of the plant possesses more bioactive compounds responsible for anthelmintic activity than the root bark, hence the higher activity. These findings support the folkloric use of *A. boonei* as an anthelmintic agent.

Conclusion

Ethanol extracts of stem and root barks of *A. boonei* possess anthelmintic properties. The stem bark exerts better anthelmintic activity than the root bark.

Conflict of interest

The authors have not declared any conflicts of interests.

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REFERENCES

- Adotey JPK, Adukpo GE, Boahen YO, Armah FA (2012). A Review of the Ethnobotany and Pharmacological importance of *Alstonia boonei* de Wild. ISRN Pharmacol. 2012:587160.
- Adomi PO (2006). Antibacterial activity of aqueous and ethanol extracts of the stem bark of *Alstonia boonei* and *Morinda lucida*. Sci. Res. Essays 1(2):050-053.
- Adu F, Apenteng JA, Akanwariwiak WG, Sam GH, Mintah DN, Bortsie EB (2015). Antioxidant and *in vitro* anthelmintic potentials of methanol extracts of barks and leaves of *Voacanga africana* and *Rauwolfia vomitoria*. Afr. J. Microbiol. Res. 9(35):1984-1988.
- Akinmoladun AC, Ibukun EO, Afor E, Akinrinlola BL, Onibon TR, Akinboboye AO, Obuotor EM, Farombi EO (2007). Chemical constituents and antioxidant activity of *Alstonia boonei*. Afr. J. Biotechnol. 6(10):1197-1201.
- Bhawar GB, Bhalke RD, Lodha KR, Karmase BC, Londhe CD (2009). Phytochemical investigation and *in vitro* anthelmintic activity of *Bauhinia racemosa* (L) (Leguminaceae). Pharmacology 1:300-303. <http://pharmacologyonline.silae.it/files/archives/2009/vol1/030.Bhavar.pdf>
- Danquah CA, Koffuor GA, Annan K, Ketor EC (2012). The anthelmintic activity of *Vernonia amygdalina* (Asteraceae) and *Alstonia boonei* DeWild (Apocynaceae). J. Med. Biomed. Sci. 1(1):21-27.
- Evans WC (2002). Trease and Evans Pharmacognosy. Saunders W.B publishers. 15th Edition. P 137.
- Gazzinelli A, Correa-Oliveira R, Yang G-J, Boatman BA, Kloos H (2012). A Research Agenda for Helminth Diseases of Humans: Social Ecology, Environmental Determinants, and Health Systems. PLoS Negl. Trop. Dis. 6(4):e1603.
- Gosse BK, Bryson TA, Gokou T (1999). Study of triterpenoids from *Alstonia boonei*. Soc. Ouest-Africaine Chim. 5(8):123-128.
- Hadi S, Bremner JB (2001). Initial studies on alkaloids from Lombok medicinal plants. Molecules 6(2):117-129.
- Marini-Bettolo GB, Nicoletti M, Messana I, Patamia M, Galeffi C, Oguakwa JU, Portalone G, Vaciago A (1983). Boobein, A new C-9 terpenoid lactone from *Alstonia boonei*: A possible precursor in the indole alkaloid biogenesis. Tetrahedron 39(2):323-329.
- Mute V (2009). Anthelmintic effect of *Tamarind indica* linn leaves juice extract on *Pheretima posthuma*. Int. J. Pharm. Res. Dev. 7:1-6.
- Olajide OA, Awe SO, Makinde JM, Ekhelar AI, Olusola A, Morebise O, Okpako DT (2000). J. Ethnopharmacol. 71(1-2):179-186.
- Olusegun-Joseph TS, Ofodile LN, Oguntoku T (2013). *In-vitro* evaluation of anthelmintic activity of crude extracts of leaves of *Dalbergiella welwitschii*. Int. J. Pharm. Pharm Sci. 5(1):32-33.
- Tanner S, Chuquimia-Choque ME, Huanca T, McDade TW, Leonard WR, Reyes-Garcia V (2011). The effects of local medicinal knowledge and hygiene on helminth infections in an Amazonian society. Soc. Sci. Med. 72(5):701-709.
- Vercruyse J, Albonico M, Behnke J M, Kotze AC, Prichard RK, McCarthy JS, Montresor A, Levecke B (2011). Is anthelmintic resistance a concern for the control of human soil-transmitted helminths? Int. J. Parasitol. Drugs Drug Resist. 1(1):14-27.