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Short Communication

Chemical Composition, *In vivo* Antihelmintic, and Acute Toxicity Studies of *Buchholzia coriacea* Engler (Capparaceae) Seed Extract

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ABSTRACT

Parasitic illnesses are widespread in tropical Africa due to the conducive climatic and sanitary circumstances that facilitate their transmission. Given the constraints of synthetic medicine, it is necessary to revert to phytotherapy. Phytochemical analysis, antihelmintic, and acute toxicity investigations were conducted on extracts of *Buchholzia coriacea* (BC) engl. seeds. These extracts were evaluated on *Heligmosomoides bakeri*. The air-dried and pulverized seeds of *BC* were extracted with methanol by cold maceration and concentrated using a rotary evaporator. Quantitative phytochemical screenings of the crude seed powder and the methanol extract were performed. Antihelmintic activity was assessed through total worm count reduction (TWCR). *In vivo* acute toxicity studies of crude seed powder and methanol seed extracts of *BC* were performed. The phytochemical analysis revealed a significant concentration of flavonoids (13.04 ± 0.02%). Results obtained from the *in vivo* antihelmintic study revealed a reduction in total worm count from 2.50 ± 0.00, 16.75 ± 0.01, 8.59 ± 0.01, 17.97 ± 0.00, and 0.00 ± 0.00% in day 0 to 100.00 ± 0.00% in day 18 for methanol, ethyl acetate, *n*-hexane, chloroform, and diethyl ether, respectively, against *H.bakeri,* compared to abendazole, which showed TWCR from 3.75 ± 0.00% in day 0 to 100.00 ± 0.00% in day 6. The acute toxicity assessment revealed an LD_{50} value of 5000 mg/kg. The results emphasize the therapeutic possibilities of BC seeds as a natural source of bioactive compounds with antihelmintic activities. This highlights the need for more research to explore their potential use in pharmaceutical applications.

Keywords: *Buchholzia coriacea, Heligmosomoides bakeri,* Anthelmintics. Phytochemical, Acute toxicity.

Introduction

Infections with helminths are the most prevalent in humans. These infections have increased in number globally and may lead to malnutrition, anaemia, pneumonia, and eosinophilia. [1] In communities that lack adequate housing, clean water, proper sanitation, and greater access to healthcare, infection thrives and persists. [2] Antihelmintic drugs are available, and besides their side effects and resistance by helminths, they are not easily accessible in some rural communities in Nigeria and some other developing countries. In Nigeria and other parts of Africa, various plant parts have been exploited as medicine. [8, 9]. One of such medicinal plants is *Buchholzia coriacea* (BC), also known as wonderful kola because of its diverse medicinal uses. Amongst the Igbo people of South-eastern Nigeria, it is commonly known as Uke, while the Edo and Yoruba people call it Owi and Uworo, respectively. [10] BC is a member of the *Capparidaceae* family and is widely distributed in Nigeria and other parts of Africa. BC is used in traditional African medicine for a variety of medicinal purposes. [11] BC seeds are applied to the abdomen to promote dystocia. [12, 13]

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The effect of this plant on diabetes are well known. [12, 13] Prior research indicates that the seed contains beneficial phytochemicals. [14]BC has found use in ethnomedicine for the management of malaria, ulcers, hypertension, rheumatism, asthma, cough, infertility in men, migraine headaches, and psychiatric disorders, among others. [15] The pharmacological properties of BC seeds, such as antitrypanosomal, antidiabetic, anti-haemolytic, anti-apoptotic, and antioxidant, have been reported. [16] Preliminary studies on the secondary metabolites of BC seed ethanol extract by GC-MS have been reported. [17] Ajaiyeoba *et al.*[18] studied the *in vitro* antihelmintic activity of BC leaves and stems at 10–100 mg/mL concentrations with significant antihelmintic activity recorded as evidenced by the time of paralysis and death of worms. Although there have been several publications on BC's medicinal properties, there is still a lack of information about its *in vivo* antihelmintic action on *Heligmosomoides bakeri* and the acute toxicity effect of the seed extract. Hence, it is essential to evaluate the chemical composition, antihelmintic properties, and acute toxicity of the BC seed extract. This study would establish a crucial foundation for subsequent research into cultivating this plant as a medicinal plant.

Materials and Methods

Plant collection and identification

BC fruits were harvested from Nkata Alocha, Okaiuga, Umuahia, Abia State, Nigeria, on 23rd October, 2022. Identification and authentication were conducted at the Forestry Research Institute of Nigeria. The herbarium number was FHI 109920.

Extraction
The plant seeds were separated from their fruits by peeling off the sheath. The fresh seeds were sliced into tiny bits and air dried at 27℃. Thereafter, they were ground into powder, weighed, and hereafter stored in appropriately marked polyethylene bags. Exactly 2 kg of the pulverized BC seed was percolated in 2.5 L of redistilled methanol (95% analytical standard) to obtain the methanol (crude) extract which was further filtered with Whatman No. 1 filter paper. At 34°C, the filtrate was concentrated using a rotary evaporator with reduced pressure.

Partitioning of the crude methanol extract
Exactly 20 g of methanol crude extract was dissolved in 150 mL of methanol. The methanol-dissolved extract was poured into a separating funnel. Exactly 150 ml of n-hexane (n-HE) was added to the funnel. The funnel was shaken gently, swirling to mix well. The mixture stood and separated into two layers, the n-hexane on top and methanol at the bottom. The layers were carefully drained each into separate containers. The same procedure was followed for the residue left after extraction with n-hexane, using ethyl acetate (EA), chloroform (CF), and diethyl ether (DE). Then each fraction was separately concentrated, under reduced pressure at 34°C by a rotary evaporator.

Quatitative phytochemical screening
Quantitative phytochemical screening was carried out on the methanol crude extract of BC seeds. Alkaloids, phenols, tannins, saponins, terpenoids, steroids, glycosides, and flavonoids were determined as described by Egbucha *et al*. [19]

Ethical consideration
Endorsed by the animal ethics committee of the Dept. of Phys. and Pharm., Michael Okpara University of Agriculture, Umudike.

Inducing mice with filariform larvae (L3) of Heligmosomoides bakeri through artificial infection.
The antihelmintic activity was assessed using the approach described by Yondo *et al*. [20] with a slight modification. A total of seventy Swiss mice, ranging in age from 5 to 6 weeks and weighing between 20 and 25 g, were utilized for the assessment of antihelmintic efficacy. The animals were assigned to cages in a random manner and given one week to adjust to their new environment. They had unrestricted access to water and food. The mice were administered orally with 100 to 110 infective larvae (L3) of *H. bakeri*, which were one week old. The larvae were suspended in a volume of 0.8 mL of water. After combining, 10 mL of a solution containing larvae was extracted from the original solution, from which 0.1 mL was then transferred onto the lid of a Petri plate. The Petri dish was positioned atop the solution to render the larvae motionless, after which they were enumerated. The solution was adjusted by either adding or removing water until it contained 13 to 15 L3 larvae. The mice were infected orally using a blunt-ended needle through gavage.

Design of the experiment
Individual mice were placed in separate cages in order to collect their faecal pellets. The faeces were thoroughly blended in a mortar, after which a strong saline solution was added. Following filtration using a sieve, the liquid remaining above the sediment was poured into two test tubes until a curved upper surface was produced. A cover slide was delicately dropped and left undisturbed for five minutes. Subsequently, the cover slide was lifted and transferred onto a slide. The eggs were enumerated using a light microscope at a magnification of four times the original size. Upon the detection of infection using qualitative microscopic tests (namely, the presence of eggs in the mice's faeces), the animals were divided into 7 groups of 10 mice each, using a random allocation method. The tested product was administered orally using a manual syringe with a 1 mL capacity and a blunt end needle.
Group 1 was treated with albendazole (400 mg/kg), a standard antihelmintic drug, and used as a positive control, while Group 2 received 4% of DMSO and served as a negative control. Groups 3, 4, 5, 6, and 7 were treated with 500 mg/kg of the ME extract, *n*-HE, EA, CF, and DE fractions, respectively. The body cavities of mice of both sexes were opened on days 0, 3, 6, 9, 12, 15, and 18 days of treatment.

Total worm count reduction (TWCR) evaluation
Following an 18-day treatment period, the body cavity was surgically accessed to extract the small intestine. The extracted organ was then transferred to spacious Petri dishes filled with 30 mL of distilled water. The organ was incised lengthwise with a pair of scissors and then threaded through the arms of a forceps. The exudates containing parasites were rinsed in water, and all recovered worms were enumerated using a dissecting microscope. The TWCR% was computed using equation 1.

$$\%\,TWCR = \frac{TWC\ control\ group - TWC\ treated\ group}{TWC\ control\ group}\ x\ 100 \qquad (1)$$

Acute toxicity screening
The acute toxicity evaluation of the BC was carried out in according to the methods used by Akomas *et al*.[21] Forty-five mice were allocated into 9 groups of 5 mice each, with each group receiving a specific dosage of the extract. Groups 1, 2, 3, 4, and 5 were administered 1000, 1500, 2000, 2500, and 3000 mg/kg body weight of the ME extract, respectively, while groups 6, 7, 8, and 9 were administered 3500, 4000, 4500, and 5000 mg/kg body weight of the ME extract, respectively. After treatment, animals in all groups were placed under watch for the manifestations of toxicity signs and deaths within a 24-hour period and a further 7 days. The documented fatalities were utilised to assess the LD_{50} value of the extract. Karber's formula, written in equation 2, was applied.

$$LD_{50} = LD_{100} - \frac{\Sigma DD\ x\ MD}{N} \qquad (2)$$

Where;
LD_{50} = The dose that produced mortality of 50%.
$LD1_{00}$ = The dose that produced mortality of 100%.
ΣDD x MD = Aggregate of the products of dosage variation and average mortality

N = No. of animals within each category

Results and Discussion

Phytochemical screening of BC seeds.
Table 1 shows the quantitative phytochemical screening results of BC crude seed powder and methanol extract. The results obtained from the crude seed powder showed that flavonoids (5.30 ± 0.05%) recorded the highest percentage composition, followed by alkaloids (3.96 ± 0.01 %). The percentage composition of saponins, phenols, phytates, glycosides, hydrogen cyanide, and steroids was minute. In the methanol extract, flavonoids (13.04 ± 0.02%) registered the highest presence, followed by phytates (3.01 ± 0.01 %), terpenoids (2.01 ± 0.01%), and alkaloids (1.45 ± 0.01%), while the other phytochemicals were present in minute quantities.
The phytochemicals detected have well-established pharmacological and therapeutic properties. Alkaloids are plant-based chemicals that have medical uses like lowering blood pressure, fighting cancer, lowering blood sugar, stopping seizures, killing protozoa, killing microbes, boosting the immune system, and relieving pain. [22] Alkaloids also possess neuroprotective properties against neurodegenerative diseases.[23] Further, alkaloids improve immune functions, nutrition, and physical performance. [22, 23] BC could be used in the treatment and prevention of infections, relief of pain, and fever, as well as in the prevention and management of neurodegenerative diseases. The use of BC in traditional medicine to relieve chest and wrist pain, and in the treatment of psychiatric disorders in ethnomedicine could be attributed to the presence of alkaloids in the plant.

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Table 1: Quantitative phytochemical constituents of the crude seed powder and methanol seed extract of BC

Phytochemicals	Composition (%)	
	Crude powder	Methanol extract
Alkaloids	3.96 ± 0.01	1.45 ± 0.01
Flavonoids	5.30 ± 0.05	13.04 ±0.02
Cardiac glycosides	0.12 ± 0.01	0.10 ± 0.02
Hydrogen cyanide	0.10 ± 0.02	0.07 ± 0.01
Steroids	0.02 ± 0.00*	0.01 ± 0.00*
Saponins	0.99 ± 0.01	0.99 ± 0.01
Tannin	0.02 ± 0.00	0.09 ± 0.01
Terpenoids	0.05 ± 0.00	2.01 ± 0.01
Phenol	0.85 ± 0.05	0.67 ± 0.00
Phytate	0.69 ± 0.00	3.01 ± 0.01

Values represent mean ± SEM of triplicate determination

Flavonoids, which are polyphenolic compounds with antiallergic, antioxidant, anticancer vasodilator, anti-inflammatory, antimicrobial, and immune-stimulating activities [24] were present at high concentrations in BC seeds. Consumption of foods rich in dietary flavonoids may protect against and lower the risk of cardiovascular diseases, cancer, inflammation, stroke, obesity, and high blood pressure. [25] Flavonoids also act as neuroprotectants against neurodegenerative diseases and help modulate cell signalling and gene expression related to disease development. [25] Flavonoids protect the body against neurodegenerative diseases through their antioxidant properties as free radical scavengers. [26] Consumption of BC seeds may help reduce the risk of cardiovascular and neurodegenerative diseases. The high quantity of flavonoids in BC seeds may be responsible for the use of the plant seed in ethnomedicine against diabetes, hypertension, and cancer, as well as its reported antidiabetic properties.

Cardiac glycosides are used for the prevention and treatment of chronic heart failure, edema, arrhythmias, as well as ventricular rate control in people with atrial fibrillation. Cardiac glycosides, by acting on the cellular sodium-potassium ATPase, increase the output force of the heart and decrease its rate of contractions. [27] This experimental results showed that BC seeds contains cardiac glycosides, its consumption may assist in the prevention and treatment of heart failure.

Hydrogen cyanide, a known toxic and poisonous chemical, was detected in the seeds of BC at a very low concentration, which may not be harmful. HCN is highly toxic to mice. The LD_{50} for HCN in mice is about 3.7 mg/kg.[35] That is, 3.7 mg of HCN per kilogram of body weight is lethal to kill 50% of mice exposed to it. In BC seed, the HCN concentration was lower than 3.7 mg/kg; hence, it was not lethal to the mice. Steroids, which are hormones that regulate the development and function of the sexual organs, serve as treatments for diseases like allergies, arthritis, and those resulting from hormone deficiencies or abnormal production. [28] Although present at very low concentrations in BC seeds, steroids could contribute to the use of the plant seed in the management of fertility problems in traditional medicine.

The seeds of BC also contained saponins. Saponins lower serum cholesterol by forming insoluble complexes with cholesterol and by forming large aggregates with bile salt in the intestine, thus preventing its reabsorption. [29] Saponins also possess antiparasitic, haemolytic, anti-inflammatory, antiallergic, antitumor, antiviral, antifungal, and antioxidant activities.[29] Furthermore, saponins have beneficial effects on bone health and stimulation of the immune system. Saponins could play a contributing role in the use of BC seeds in ethnomedicine for the treatment of worm infections and the reported anti-hypercholesterolaemia activity.

Tannins possess antiparasitic, antibacterial, antiviral, and antitumor activities.[30] Tannins are used for treating diarrhoea, dysentery, fatigue, skin ulcers and sore throat.[30] Tannins may play a contributory role in the use of BC for the treatment of diarrhoea, rheumatism, ulcers and bleeding in traditional medicine.

Terpenoids possess anti-inflammatory, antioxidant, antiallergic, anti-arthritic, anti-cancer, antibacterial, and antiviral activities.[35] Terpenoids mitigate against ailments like arthritis, cancer, diabetes, ageing, and allergic diseases through their anti-inflammatory and antioxidant properties. The presence of terpenoids in the seeds of BC may contribute to the reported anti-inflammatory, antioxidant, and antimicrobial properties.

Phenolics contribute positively to human health. Their antioxidant properties act as protective agents against free radical-mediated disease processes. [31] Phytate and its byproducts of metabolism exhibit anti-inflammatory, antioxidant, and antidiabetic activities. [32] Consumption of phytate-containing foods has been reported to help regulate hyperglycemia, improve cardiovascular health, prevent kidney stones, and lower the risk of cancer and diabetes. [32] However, it hinders the body's ability to absorb some minerals. [37] The reported pharmacological and ethnomedicinal properties of BC may be attributed to the presence of the quantified phytochemicals. Thus, consumption of BC seeds could confer the medicinal and health benefits of these phytochemicals.

Antihelmintic activity

The *in vivo* antihelmintic activity of the methanol, ethyl acetate, n-hexane, chloroform, and diethyl ether extracts of BC seeds against Swiss mice infected with *H. bakeri* is shown in Table 2.

Table 2: Antihelmintic activity (% motility reduction) of the extracts

Days	AB	ME	EA	n-HE	CF	DE
	(400 mg/kg)		(500 mg/kg)			
0	3.76 ± 0.00	2.50 ± 0.00	16.75 ± 0.01	8.59 ± 0.01	17.97 ± 0.00	0.00 ± 0.00
3	67.96 ± 0.01	45.96 ± 0.01	38.27 ± 0.00	55.63 ± 0.01	41.34 ± 0.02	33.22 ± 0.00
6	97.96 ± 0.01	68.15 ± 0.00	50.48 ± 0.02	76.65 ± 0.00	54.13 ± 0.02	52.33 ± 0.02
9	100.00 ± 0.00	75.91 ± 0.01	60.20 ± 0.00	84.37 ± 0.02	73.60 ± 0.00	63.15 ± 0.00
12	100.00 ± 0.03	87.45 ± 0.00	71.71 ± 0.02	88.12 ± 0.00	86.67 ± 0.01	68.03 ± 0.01
15	100.00 ± 0.00	91.35 ± 0.01	86.17 ± 0.00	95.45 ± 0.03	90.44 ± 0.00	74.97 ± 0.01
18	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00

ME = Methanol, EA = Ethyl acetate, HE = hexane, CF= Chloroform, DE = Diethyl ether, AB= albendazole

The effects of 400 mg/kg AB, 500 mg/kg ME extract, 500 mg/kg EA, 500 mg/kg n-HE, 500 mg/kg CF, and 500 mg/kg DE fractions on the motility of helminthes were each assessed for a period of 18 days. At day zero, the percentage total worm reduction (% TWCR) was 3.76 ± 0.00, 2.50 ± 0.00, 16.75 ± 0.01, 8.59 ± 0.01, 17.97 ± 0.00, and 0.00 ± 0.00% for AB, ME extract, EA, *n*-HE, CF, and DE fractions, respectively. At day 3, the *n*-HE fraction recorded the highest activity with a reduction in worm motility to 55.63 ± 0.01%, followed by the

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ME extract (45.96 ± 0.01%), CF (41.34 ± 0.02%), EA (38.27 ± 0.00%) and DE (33.22 ± 0.00%) fractions, in comparison to AB (a standard anthelmintic drug) with a 67.96 ± 0.01% reduction in worm motility. At day 9, the anthelmintic activity against *H.bakeri* ± 0.00% for the AB, n-HE fraction, ME extract, CF, DE, and EA fractions, respectively. At day 15, 95.45 ± 0.03, 91.35 ± 0.01, 90.44 ± 0.00, 86.17 ± 0.00, and 74.97 ± 0.01% reduction in worm motility were recorded for the *n*-HE fraction, ME extract, CF, EA, and DE fractions respectively. At day 18, all the fractions achieved 100.00 ± 0.00% reduction in worm motility. CF fraction exhibited similar anthelmintic activity as the crude ME extract, and a higher activity than DE and EA fractions, while its anthelmintic activity was lower than that of the n-HE fraction. This investigation revealed that all the fractions exhibited significant anthelmintic activity against *H. bakeri*. The ability of the seed extract and fractions of BC to achieve a significant reduction in worm motility indicates the presence of anthelmintic agents in the plant seed. This anthelmintic activity against *H.bakeri* should be harnessed in the formulation of anthelmintic drugs. This investigation therefore authenticated the use of BC seeds in traditional medicine against worm infection, being rich in anthelmintic agents. The mouse host-parasite model employed was well suited for efficiently assessing the anthelminthic impact of BC within a relatively brief timeframe. The plant's reduction rate suggests that it is a promising candidate for anthelmintic purposes.[33, 34]

Acute toxicity (LD_{50})

Table 3 shows the acute toxicity of ME seed extract of BC. Results obtained from the acute toxicity study of the ME extract of BC on adult mice of both sexes revealed that no mortality was recorded upon the administration of doses ranging from 1000 to 5000 mg/kg body weight.

Table 3: Acute toxicity evaluation of ME seed extract of BC

Group	Dose (mg/kg)	No. of deaths	Mortality (%)	Dose Difference (DD)	Mean Death (MD)	DD x MD
1	1000	0	0	500	0	0
2	1500	0	0	500	0	0
3	2000	0	0	500	0	0
4	2500	0	0	500	0	0
5	3000	0	0	500	0	0
6	3500	0	0	500	0	0
7	4000	0	0	500	0	0
8	4500	0	0	500	0	0
9	5000	0	0	-	-	-

$LD_{50} = LD_{100} - \sum DD \times MD/5 = 5000 - 0/5 = 5000$ mg/kg

No toxicity behaviours or mortality were observed during the acute toxicity study period of 24 hours and a further 7 days. Based on these reports, BC seeds may be relatively safe since no acute toxicity or mortality was recorded, although more investigations are required. The number of deaths in each group was used to calculate the LD_{50} value in the application of Karber's formula and was found to be 5000 mg/kg. ME extract of BC did not cause mortality or any symptom of acute toxicity in mice even at quite a high dose, thus indicating its promising safety profile. Therefore, it is possible to use it safely during further research, probably in therapeutic applications. Absence of toxic behaviours indicated good tolerance by mice, thus pointing to its non-toxic nature. The absence of adverse effects in this wide dose range of 1000 to 5000 mg/kg is significant and underlines the high toxicity threshold for the extract, which promises well for possible human applications. Though acute toxicity studies are reassuring, sub-chronic and chronic toxicity studies are imperative to establish long-term safety. Besides, studies on the effect of the extract on various organs and systems can provide all-round safety data.

Conclusion

This study investigated the phytochemical composition, *in vivo* antihelmintic properties, and acute toxicity of BC seeds. These findings validate the historic use BC seed in herbal medicine and justify the need for additional research on its potential clinical uses. Future research should prioritise investigating the fundamental mechanisms responsible for the medicinal effects of the plant and undertaking clinical trials to confirm its effectiveness and safety in humans. Furthermore, further research is needed to investigate potential synergistic effects when combining conventional medications and to establish appropriate delivery methods.

Conflict of Interest

All authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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