# Phytochemical Constituents and *In Vitro* Antitrypanosomal Activity of Ethanol Seed Extract of *Buchholzia Coriacea*

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**Abstract**

The medicinal efficacy of *Buchholzia coriacea* seed extract was screened for possible anti-trypanosomal activity *in vitro*. Whole *Buchholzia coriacea* seeds were extracted with 80% ethanol (EtOH) to obtained crude extract. The flavonoid fraction was extracted from crude ethanol extract using n-butanol and purified in column chromatography eluted with methanol. The crude extract and flavonoid fraction obtained were screened for *in vitro* antitrypanosomal activity by mixing 20 µl of blood sample containing about 20-25 parasites per field with 5 µl of crude ethanol extract and flavonoid fraction separately at 20.0, 2.0 and 0.2 mg/ml producing effective concentrations of 4, 0.4 and 0.04 mg/ml, respectively. Result indicated that flavonoid fraction of *B. coriacea* exhibited complete caesation of motility of *T. b. brucei* within 60 minutes at concentrations of 4 and 0.4 mg/ml while the crude ethanol extract recorded only reduction on parasite count and sluggishness in motility as compared to untreated control. Differences in the amount of phytochemical present could probably be responsible for the observed antitrypanosomal activity. This study therefore provides scientific evidence for the traditional use of ethanol crude extract and flavonoid fraction of *Bucholzia coriacea* seed for the management of trypanosomosis.

**Keywords:** *Buchholzia coriacea*, Parasitaemia, Phytochemicals, Trypanosome

## 1.0 Introduction

Trypanosomiasis originates from a disease caused by the parasitic protozoa known as trypanosomes of the genus *Trypanosoma*. The protozoa are ubiquitous, microscopic, elongated flagellated unicellular organisms which live and multiply in the blood and other body fluids of their host. They cause sleeping sickness in humans and related diseases in domestic and wild animals alike [1]. They are associated with five well differentiated sub genera which comprise all the ten fewer well-defined species of the genus *Trypanosoma* [2]. Species of the parasite include; *Trypanosoma brucei, Trypanosoma congolense, Trypanosoma envansi, Trypanosoma cruzi, Trypanosoma vivax and Trypanosoma equiperdum.*

*Trypanosoma brucei,* a distant relative of malaria parasite, is endemic to Africa. It consists of three sub-species, two of which if not treated are fatal to humans because they affect the central nervous system where they elicit neurological problems such as coma, death and general debilitation which is referenced to as African sleeping sickness [3, 4]. *T. brucei rhodesiense* is commonly found in East Africa and southern Africa while *T. brucei gambiense* found in West Africa. *T. brucei brucei* which is not infectious to human but in cattle causes a wasting disease called Nagana [5]. This disease has been reported by Igweh and Onabanjo [6] to occur more in the northern part of Nigeria. The tsetse fly belts of sub-Sahara Africa are between $14^0$N and $20^0$S [5]. The saliva of blood sucking female tsetse flies located in Africa in a belt that stretches south of the Sahara and north of the Kalahari are resposible for transmission [5]. The rapid increase in drug resistance, drug counter-feiting, unpleasant side effects of most trypanocidal drugs, vector resistance to insecticides and high cost of chemotherapeutic and chemoprophylactic agents have brought about increased incidence of the disease and thereby necessitating increased search for efficacious therapeutic agents [7,8].

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The anti-trypanosomal activities of most plants have also been known to be as a result of the phytochemicals present in them [9]. These phytochemicals exert beneficial physiological responses and serve as efficient chemotherapeutic agent. *Buchholzia coriacea* has multiple medicinal values. The seed gave its common name (wonderful kola) because of its usage in traditional medicine. The plant is documented to possess diverse medicinal potentials including the anti-plasmodial properties [10]. Therefore, there is a need to evaluate its anti-trypanosomal potential since the seed extract of this plant have been reported to alleviate some symptoms similar to that of African trypanosomiasis.

## 2.0 Materials and Methods

### *2.1 Plant Collection and Identification*

The leaves, seed and stem bark of fresh *Buchholzia coriacea* were obtained from Kure Market and Kasuwa Gwari in Bosso Local Government Area, Minna, Nigeria. The plant was authenticated and identified by a Botanist in Biological Sciences Department, Federal University of Technology, Minna, Nigeria.

### *2.2 Test Parasite*

*Trypanosoma brucei brucei (T. b. brucei)* isolate (Federe strain) were obtained from the Department of Vector and Parasitology, Nigerian Institute for Trypanosomiasis Research, (NITR) Kaduna, Nigeria. It was originally isolated during an outbreak that occurred at Federe, Jos East Local Government Area, Plateau State, Nigeria in 1997 and cryopreserved in liquid nitrogen. The parasites were maintained in the laboratory by continuous passage in mice intraperitoneally until required.

### *2.3 Extraction of Buchholzia coriacea Seeds*

Fresh seeds of *B. Coriacea* were properly washed with distilled water and air dried at room temperature for two weeks to a constant weight and then pulverized to a coarse powder using mortar and pestle. The fine powder was separately kept in airtight plastic containers until they are required for further analysis. The pulverized seed (1.2 kg) was extracted using maceration by reflux method which was done in 80% ethanol for 24 hours [11]. The solution was thereafter filtered using Whatman No 1 and the filtrate was concentrated using rotary evaporator. The resulting extract was weighed and stored in refrigerator at $4^{o}$C.

### *2.4 Extraction of Flavonoid Fraction*

The method described by Jouad *et al*. [12] was used in the extraction of flavonoid fraction from the crude ethanolic extract. Hydro ethanol (EtOH) extract (64.5 g) of *B. coriacea* was dissolved in 400 ml distilled water and extracted with n- butanol (n-BuOH, 3×1L), saturated with water. After evaporation of the solvent under reduced pressure, 23 g of BuOH extract were obtained. The BuOH extract was subjected to column chromatography on Sephadex LH20 and eluted and by methanol yielding 12.4 g of crude flavonoid fraction.

### *2.5 Estimation of Phytochemical Constituents*

Standard methods were used to estimate the phytochemicals present. The total alkaloid contents was estimated by the gravimetric method of Harborne [13]; total phenolic content (TPC) by Folin-Ciocalteu method described by A.O.A.C, [14]; flavonoid contents (TFC) by **t**he aluminum chloride colorimetric method as described by Chang *et al* [15] with some modifications. Tannin was estimated as described by A.O.A.C. [14]; saponin by Spectrophotometric method of Brunner [16]; Phenol by spectrophotometric method described by Harborne [13] while glycoside, steroids, and terpene were determined using the method of Gupta *et al* [17].

### *2.6 In-vitro Antitrypanosomal Activity Test*

*In vitro* trypanocidal activity was performed according to the method described by Atawodi *et al*. [18] with slight modification. Blood (20 µl) containing about 20-25 parasites per field after dilution with PBSG, was mixed with 5 µl of extract solution of 20.0 mg/ml, 2.0 mg/ml and 0.2 mg/ml to produce effective test concentrations of 4.0 mg/ml, 0.4 mg/ml and 0.04 mg/ml, respectively. To ensure that the effect monitored was that of the extract alone, a set of control was included which contained the parasite suspended in 10% PBSG only. After 5 min incubation in closed Eppendorf tubes maintained at $37^{o}$C, small drop of test mixtures was placed on separate microscope slides and covered with cover slips and the parasites observed every 5 min for a total duration of sixty minutes. The number of parasites count was determined microscopically at X 40 magnification using the "rapid matching" method of Herbert and Lumsden [19]. Cessation or drop in motility of the parasites in extract-treated blood compared to that of parasite-loaded control blood without extract was taken as a measure of trypanocidal activity.

## 3.0 Results

### *3.1 Phytochemical Constituents of B. coriacea Seed Extract*

The plant seeds contain substantial amount of phytochemicals. The percentage composition of ethanolic extract of *B. coriacea* are alkaloid (2.17%), glycosides 2.16 %, saponins 2.57%, steroids 0.18%, tannins 5.34%, flavonoids 3.18%, terpenes 0.22% and phenol 1.86%. The results showed that tannin had the highest concentration followed by flavonoid while steroids had the least (Table 1).

**Table 1: Percentage Composition of the Phytochemicals Present in Ethanol Seed Extract of *B. coriacea***

| Phytochemicals | Percentage (%) Composition |
|---|---|
| Alkaloids | 2.17 |
| Flavonoids | 3.18 |
| Phenols | 1.86 |
| Saponins | 2.57 |
| Glycosides | 2.16 |
| Steroids | 0.18 |
| Tannins | 5.34 |
| Terpenes | 0.22 |

### *3.2 In vitro Activity Bioassay*

Incubation of the crude ethanol extract of the seed resulted to reduction in number of parasite particularly at 4 and 0.4 mg/ml concentration when compared with untreated control (Fig. 1). Incubation up to 60 minute led to sluggish movement and significant reduction in the number of parasite present. However with the flavonoid fraction, there was total cessation of the parasites at 60 minutes following incubation when compared to the untreated control. This observation was concentration dependent with the highest concentration of 4.0 mg/ml recording cessation of motility at 50 minutes of incubation (Fig. 2).

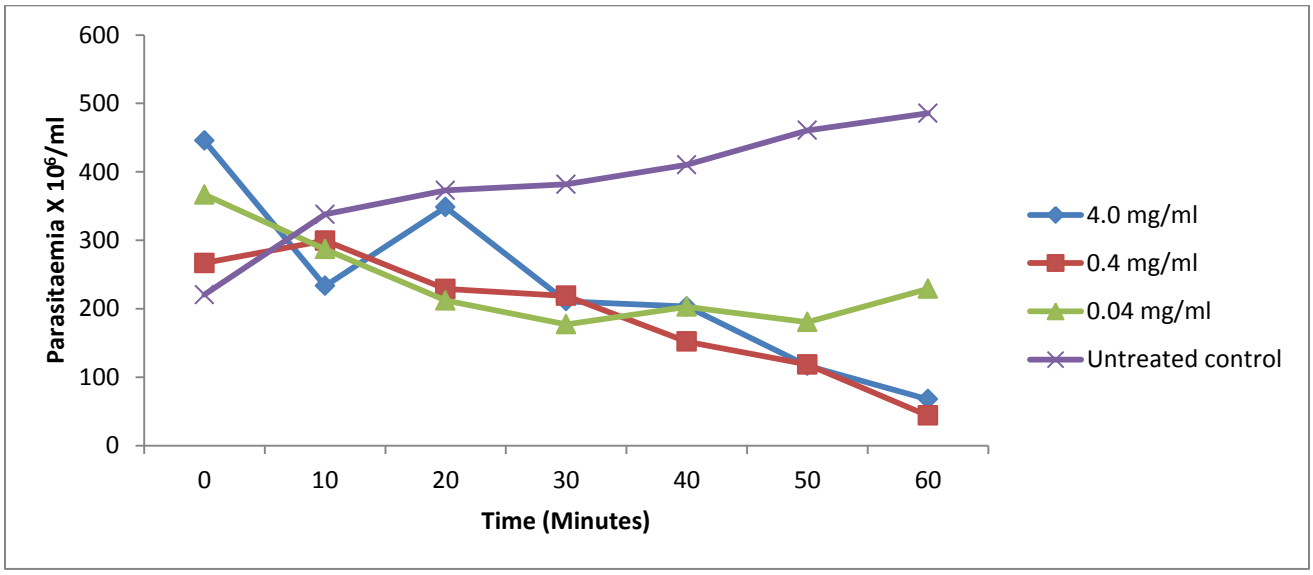


**Fig. 1: *In vitro* antitrypanosomal activity of different concentrations of crude *B. coriacea* extract**

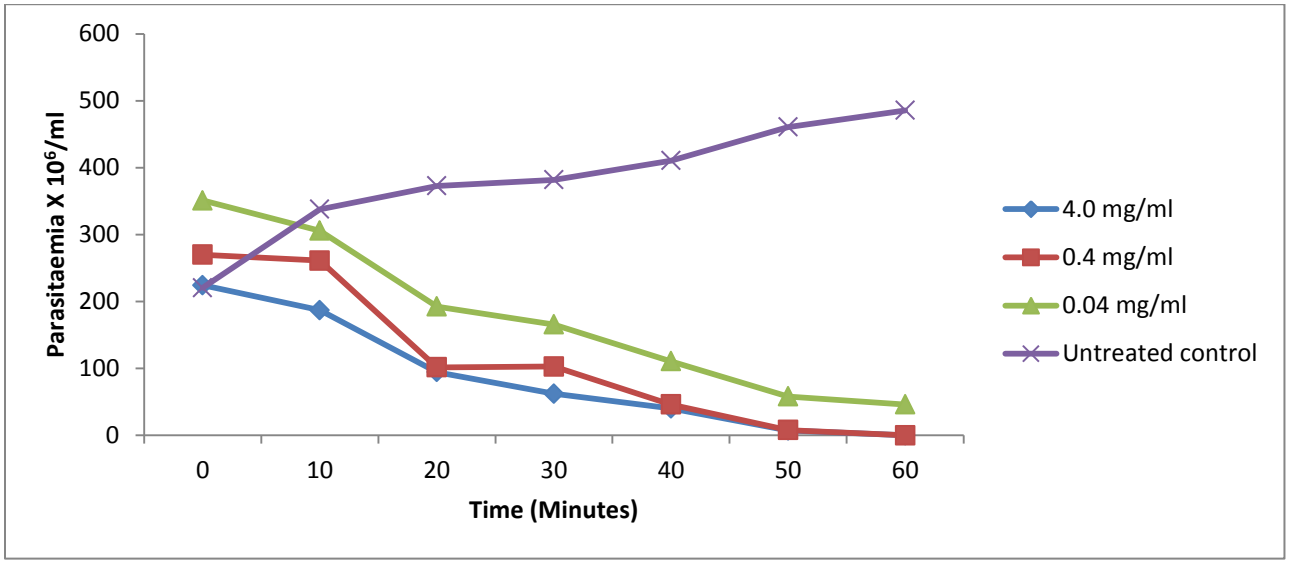


**Fig. 2: *In vitro* antitrypanosomal activity of different concentrations of total flavonoid fraction of *B. coriacea***

Table 2 summarizes the effect of the different concentrations of the crude extract and total flavonoid fraction after incubation of *T. b. brucei* with *B. coriacea* extract. The changes observed in the behavioural pattern described how effective the extract is on the trypanosome at the varied concentrations.The changes observed ranges from non-motile to lethagic.

**Table 2: I*n vitro* Behavioural Pattern of *T. b. brucei* after Incubation with the Extract and Flavonoid Fraction**

| | | Time Intervals (Minutes) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Extract** | | **0** | **10** | **20** | **30** | **40** | **50** | **60** |
| Flavonoid | 4.0 mg/ml | Motile | Slightly Motile | Lethargic | Sluggish | Non-Motile | Non-Motile | Non-Motile |
| | 0.4 mg/ml | Motile | Motile | Slightly Motile | Lethargic | Sluggish | Lethargic | Non-Motile |
| | 0.04 mg/ml | Motile | Motile | Motile | Lethargic | Lethargic | Lethargic | Non-Motile |
| Crude | 4.0 mg/ml | Motile | Motile | Motile | Slightly Motile | Lethargic | Sluggish | Sluggish |
| | 0.4 mg/ml | Motile | Motile | Motile | Motile | Slightly Motile | Sluggish | Sluggish |
| | 0.04 mg/ml | Motile | Motile | Motile | Motile | Slightly Motile | Slightly Motile | Sluggish |
| Untreated Control | Blank | Motile | Motile | Motile | Motile | Moderately Motile | Moderately Motile | Moderately Motile |

## 4.0 Discussion

The antitrypanosomal activity is dependent on the bioactive components, solvents and part of plant used [20]. All the phytochemicals tested showed appreciable amount with tannins having the highest percentage composition closely followed by the flavonoid. The types of solvent and part of plants used may also account for the variation in phytochemicals present [21]. *In vitro* experimental procedure has shown that flavonoids possess anti-inflammatory, anti-allergic, anti-viral and anticarcinogenic properties [10]. Therefore any plant with anti-carcinogenic properties may serve as a good source of trypanocide. Eflornithine currently in use to treat sleeping sickness is known to have some level of anticancer activities [10]. Infections with African trypanosomes are known to cause immunosuppresion and thus the extract of *B. coriacea* can be useful for the management of the disease since earlier report has shown the plant extract to possess immunomodulatory activity which probably suggests its therapeutic usefulness [22].

Strong indications have made it quite doubtless that trypanosome infection leads to many pathological conditions of medical importance. Earlier research has shown that different trypanosomes have different sensitivities to anti-trypanosomal agents which may be associated with bioavailability and toxicity at the concentrations tested [20]. The cessation or drop in motility of trypanosomes may serve as a measure of anti-trypanosomal potential of a plant extract. According to Freiburghaus *et al* [21], parasite motility constitutes a relatively reliable indicator of viability of most zooflagellate parasite. From the result obtained, the flavonoid fraction obtained appeared to be more active against *T.b.brucei* when compared with the crude extract. This therefore gave a clear indication that the presence of other phytochemicals in *B. coriacea* seed extract could inhibit their direct effect on the parasites. The reduced parasitaemia and motility of parasites at all the concentrations of the crude extract tested gave indication that the various phytochemicals when isolated and tested separately can provide a better anti-trypanosomal effect compared to the whole extract.

## Conclusion

Incubation of flavonoid fraction of *B. coriacea* with *T. b. brucei* infected blood resulted to complete caesation of motility while the crude ethanol extract recorded only reduction on parasite count and sluggishness in motility. *Bucholzia coriacea* flavonoid fraction is therefore trypanocidal while the crude ethanol extract is trypanostatic. Thus, *Bucholzia coriacea* seeds have great potentials which need to be exploited fully in the management of African trypanosomosis.

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