

# Phytochemical and Antitrypanosomal Studies of Different Solvents Extracts of *Boswellia dalzielii*

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## Abstract

Solvent extracts of the various parts of *Boswellia dalzielii* (leaves, stem bark and root bark) were screened *in vitro* and *in vivo* for antitrypanosomal activity against *Trypanosoma brucei brucei*. Methanolic extract of the leaves, stem bark and root bark of *Boswellia dalzielii* at 20 mg/ml caused motility to cease after 35, 20 and 20 minutes, respectively while at 10 mg/ml, motility ceased at 50, 30 and 20 minutes respectively. Chloroform extract of root bark of the plant caused motility to cease after 40 minutes at concentration of 20 mg/ml. *In vivo* activity of methanol extract of the plant on *T. brucei brucei* infected mice showed consistent parasitemic suppressions at 300 mg/kg with methanolic extract of the leaves displaying highest activity. Reagent-based chemical analysis conducted on the extract revealed the presence of saponins, alkaloids, flavonoids, tannins, resins, steroids and triterpenes. These findings suggest that methanolic extract of *Boswellia dalzielii* leaves is more effective than other parts of the plant, and hence deserve further evaluation in the therapy of trypanosomiasis.

**Keywords:** Phytochemical, Antitrypanosomal, Solvents extracts, *Boswellia dalzielii*, *T. brucei brucei*

## 1. Introduction

Trypanosomiasis is a potentially fatal disease of human and domestic animals in tropical Africa and South America (Fairlamb, 1982). It has undergone a dramatic and devastating resurgence in recent years (Smith et al., 1998) especially in Sub-Saharan Africa (Welburn *et al.*, 2001). Atawodi (2005) noted that the significance of trypanosomiasis to human health, nutrition and economy is enormous, thereby necessitating continuous research for better ways of eliminating the disease. Unfortunately, the scarcity of compounds, the high incidence of side effects, and the emergence of resistance strains have rendered existing chemotherapy, inadequate (Atouguia & Costa, 1999). Therefore, there is need to explore other agents, especially of plant origin for new generations of anti-trypanocidal agents that are more effective, less toxic, and readily available at cheaper prices.

From early times, plants and plant products have been the primary source of food, shelter and transport materials, clothing, fragrances, flavors and ingredients of medicinal substances for mankind. There have been reports that a vast majority of the population particularly those living in villages largely depend on herbal medicines (Gupta, 1994). According to Sülsen *et al.* (2007), natural products are important sources of new drugs because their

derivatives are extremely useful as lead structures for synthetic modification and optimization of bioactivity.

*Boswellia dalzielii* Hutch (family: Burseraceae), a tree of the Savanna forest of Nigeria has long been known for its medicinal purposes. The bark of *Boswellia* species yields a whitish gum resin, olibanum or frankincense, which has been extensively evaluated for its medicinal and therapeutic potentials. This spurred interest in other parts of the plant. But, aside from report of Atawodi (2005) which indicated that *Boswellia dalzielii* had antitrypanosomal activity *in vitro* there are hardly other reports on the antitrypanosomal properties of the plant. Hence this work was undertaken to evaluate the *in vivo* antitrypanosomal effects of extract of the plant as well as identify the major classes of phytochemicals present in its methanolic extract.

## 2. Materials and Methods

### 2.1 Plant material

Fresh parts of the plant were collected randomly from Shika and Samaru, Zaria, Kaduna State, Nigeria. The Herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, confirmed the taxonomic identity of the plant, and a specimen with voucher Nr 1042 was preserved. Fresh plant material were rapidly washed under running tap water, air dried until brittle and then homogenized to fine powder and stored in airtight bottles.

#### 2.1.1 Preparation of Plant Extract

Fifty grams (50 g) each of the pounded dried plant materials were weighed into different conical flasks and sequentially extracted with 250 ml each of petroleum ether, chloroform, methanol and water, using the mechanical shaker. The extracts were dried *in vacuo* with the exception of the aqueous extract, which was evaporated on the water bath at 50°C. All extracts were then stored in the refrigerator at 4°C until required.

### 2.2 Test Organisms and Animals

*Trypanosoma brucei brucei* was obtained from stabulates maintained at the National Institute for Trypanosomiasis Research (NITR) Vom, Plateau State, Nigeria. The parasite was maintained in the laboratory by continuous passage in rats until required. Passage was considered necessary when parasitemia was in the range of 16 – 32 parasites per field (usually 3 - 5 days post infection). In passaging,  $1 \times 10^3$  parasites were introduced intraperitoneally or intramuscularly into rats in 0.1 - 0.2 ml blood/PBS solution. For several passages, approximately 80% blood solution (v/v) was obtained by cardiac puncture into 1ml syringe containing 0.2 ml EDTA (1% w/v). About 0.1 - 0.2 ml of the blood collected as described above or blood (diluted with PBS to contain approximately  $1 \times 10^3$  parasite/ml) was injected into healthy rats acclimatized under laboratory condition for at least two weeks.

Apparently healthy male albino mice of 3-4 weeks of age, weighing between 20-25g, were used for *in vivo* studies. The mice were purchased from the Department of Pharmacology, Ahmadu Bello University, Zaria, Nigeria. The mice were maintained on a commercial mice chow and water *ad libitum*. They were housed in groups of three (3) and given two weeks to adapt to the new environment of the laboratory before being used in the experiment.

The volume of extract to be administered was calculated based on the body weight; the course of parasitemia was followed, and treatment commenced when parasitemia was 1-2 organism per fields, by intramuscular injection.

#### 2.3 *In vitro* test for antitrypanosomal effects

Exactly 10 mg of different solvent extracts of the plant were weighed into Eppendorf tubes and first dissolved in 100  $\mu$ l of 10% dimethylsulfoxide (DMSO) in PBS. Phosphate buffered saline (400  $\mu$ l) was then added to produce extract solutions of 20.0 mg/ml (stock). Another extract concentration (10.0 mg/ml) was prepared from the first extract solution by appropriate dilution with PBS. Aqueous extracts were dissolved directly in 500  $\mu$ l PBS. Extract solutions were prepared just before use.

Assessment of *in vitro* anti-trypanosomal activity was performed in triplicates in 96 wells micro titer plates (Flow laboratories Inc., McLean, Virginia 22101, USA). Twenty (20)  $\mu$ l of blood containing about 20 - 25 parasites per field obtained as described under "determination of parasitemia" was mixed with 5  $\mu$ l of extract solution of 20.0 mg/ml and 10.0 mg/ml to produce effective test concentrations of 4 mg/ml and 2 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% DMSO only. For reference, tests were also performed with the same concentrations of a standard drug, Diminal<sup>R</sup> (445mg diminazene diaceturate+ 555 mg phenazone/g, Eagle Chemical Company LTD, Ikeja, Nigeria) - a commercial trypanocidal drug.

After 5 min incubation in closed Eppendorf tubes maintained at 37°C, about 2 µl of test mixtures were placed on separate microscope slides and covered with cover slips and the parasites observed every five minutes for a total duration of sixty minutes. It should be noted that under this *in vitro* system adopted, parasites survived for about 4h when no extract was present. 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 antitrypanosomal activity. The shorter the time of cessation of motility of the parasite, the more active the extract was considered to be (Atawodi *et al.*, 2003).

#### 2.4 *In vivo* Evaluation of Anti-trypanosomal Activity

Twenty-four (40) albino mice were infected by intraperitoneal inoculation with  $10^3$  *Trypanosoma b brucei* cells. They were divided into eight (8) groups of five (5) animals each and the parasitemia levels were monitored daily. At day seven (7) post-infection, the rats in different groups were intraperitoneally administered with different extracts (300 mg/kg; twice daily); one group served as a positive control i.e. infected but not treated while another served as a negative control i.e. not infected and not treated. Preliminary experiments had indicated 300 mg/kg as the average dose that combines antitrypanosomal efficacy with low toxicologic potential.

#### 2.5 Determination of Parasitemia

Parasitemia was monitored in blood obtained from the rat tail, pre-sterilized with methylated spirit. The number of parasites was determined microscopically at x 400 magnification using the “Rapid Matching” method of Herbert and Lumsden (1976). Briefly, the method involves microscopic counting of parasites per field in pure blood or blood appropriately diluted with buffered phosphate saline (PBS, pH 7.2). Logarithm values of these counts obtained by matching with the table of Herbert and Lumsden (1976) is converted to antilog to provide absolute number of trypanosomes per ml of blood.

#### 2.6 Reagent-based Analysis of Phytochemical Classes

The freshly prepared extract was subjected to standard reagent-based phytochemical analyses for different constituents, including alkaloids, flavonoids, tannins, saponins, resins, steroids and triterpenes (Trease & Evans, 1983).

### 3. Results

The petroleum ether, chloroform, methanol and aqueous extracts of different parts of *Boswellia dalzielii* were analyzed for their phytochemical contents, as well as their *in vitro* and *in vivo* antitrypanosomal activity against *T. brucei brucei* at concentration of 20 mg/ml and 10 mg/ml. Complete elimination of motility of parasite when compared with control was taken as indices of antitrypanosomal effects.

Table 2 shows that chloroform and petroleum ether extracts of the *Boswellia dalzielii* (leaves, stem bark and root bark) did not eliminate motility at the different concentrations tested, while methanol extracts (leaves, stem bark and root bark) eliminated motility in *T. b brucei* within 60 minutes at the different concentrations. Aqueous extract of leaves showed very slight motility at the different concentrations. Although, chloroform extract of the root bark seemingly eliminated motility after 40 minutes for 20 mg/ml concentration, yet it could only show very slight motility after 55 minutes for 10 mg/ml concentration (this paragraph should be deleted and replaced with the proceeding ones in red).

Table 2 shows that petroleum ether extracts did not eliminate parasite motility as the leaves and root bark extracts were still very motile after 55 minutes and remained so; however, the stem bark was found to be moderately motile at 40 minutes period. The leaves and stem bark of chloroform extracts remained very motile at both concentrations; although, there was moderate motility for the root bark at 10mg/ml concentration but motility was completely eliminated at 20mg/ml concentration after 40 minutes. The stem bark and the root bark of aqueous extracts at 10mg/ml concentration were still very motile after 55 minutes while the leave extract had moderate motility only at 50 minutes period. At 20mg/ml concentration, the parasites showed moderate motility at 50, 35, and 55 minutes for leaves, stem bark and root bark respectively. However, the methanol extracts of all parts (leaves, stem bark and root bark) eliminated motility in *T. b brucei* within 60 minutes; although the time it took for that to occur vary from one part of the plant to another and from one concentration to another.

From the *in vivo* studies, methanolic extract of leaves, stem bark and root bark have antitrypanosomal activity through intramuscular administration (Figure 1). Figure 1 shows changes in parasitemia following treatment with methanolic extract of different parts of the plant (300 mg/kg). The graph shows that the root bark and the leaves extract continually reduced the parasitemia level, while the stem bark extract seemed to have resulted in relapse after day 8.

#### 4. Discussion

The methanol extract unlike the petroleum ether, chloroform and aqueous extract contains alkaloids, flavonoids, tannins while its root bark contains resins in addition (Table 1). This may account for its ability to eliminate motility in *T. b. brucei* within 60 minutes at the different concentrations (Table 2). This may also account for the very slight motility of aqueous extract of leaves at the different concentrations.

Previous workers (Freiburghaus *et al.*, 1997) have shown that the mean MIC value of common trypanocidal drugs is 10.7 mg/ml and that agent with MIC value between 5 – 20 mg/ml could be regarded as very active. In this study, *Boswellia dalzielii* was found to be active at 10 and 20 mg/ml, which is comparable to the value reported for standard trypanocidal drug.

The fact that the plant showed differential activity between extracts and between parts is confirmation of earlier report (Atawodi *et al.*, 2003), that any statement on a plants trypanocidal activity should be taken within the context of the plant part and the solvent extract tested.

It is difficult to speculate the mechanism by which these extracts exhibit their antitrypanosomal activity since the active ingredient(s) were not isolated. However, accumulated evidence (Sepulveda-Boza & Cassels, 1996) suggest that many natural products exhibit their antitrypanosomal activity by virtue of their interference with the redox balance of the parasites acting either on the respiratory chain or on the cellular defenses against oxidative stress. This is because natural products possess structures capable of generating radicals that may cause peroxidative damage to trypanothione reductase that is very sensitive to alterations in redox balance. It is also known that some agents act by binding with the kinetoplast DNA of the parasite. The antitrypanosomal principles of the plant tested in this study is unknown, until further studies are carried out.

*In vivo* assays usually provide relatively reliable information of determining the extracts sensitivity on trypanosome isolates and the use of laboratory mice has the advantage of being relatively inexpensive with respect to cost of animals, housing and maintenance. However, mouse assays are considered to provide only a broad indication of the level of sensitivity of a trypanosome population (Sones *et al.*, 1988). Therefore, this result only suggests that *Boswellia dalzielii* has potential to provide therapeutic agents for treatment of African trypanosomiasis, and must be subjected to further evaluation before any definite statement can be made on its suitability as antitrypanosomal agent.

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Table 1. Phytochemical constituents of leaves, stem bark and root bark of *Boswellia dalzielii*

Constituents	Petroleum ether extract			Chloroform extract			Methanolic extract			Aqueous extract		
	Leaves	Stem bark	Root bark	leaves	Stem bark	Root bark	leaves	Stem bark	Root bark	leaves	Stem bark	Root bark
Alkaloids	0	0	0	0	0	0	++	++	++	0	0	0
Flavonoids	0	0	0	0	0	0	+++	+++	+++	+++	+++	++
Saponins	0	0	0	0	0	0	0	0	0	+++	++	+
Tannins	0	+	0	0	0	0	++	++	+++	++	++	+
Resins	+++	+	++	+++	+	++	0	0	+++	0	0	0
Steroids and triterpenes	+++	++	+++	+++	++	+	0	0	0	0	0	0

Very reactive, +++; Reactive, ++; Slightly reactive, +; No reaction, 0

Table 2. Effect of different concentrations of different solvent extracts of *Boswellia dalzielii* on motility of *Trypanosoma brucei brucei*

Time (min) after which motility ceased, reduced drastically *or reduced slightly** with different effective concentration of extract (mg/ml)									
Solvent/Concentration									
	Petroleum ether		Chloroform		Methanol		Aqueous		
Parts of Plant	20(mg/ml)	10(mg/ml)	20(mg/ml)	10(mg/ml)	20(mg/ml)	10(mg/ml)	20(mg/ml)	10(mg/ml)	
Leaves	55**	55**	55**	55**	35	50	50*	50*	
Stem bark	40*	40*	55**	55**	20	30	35*	55**	
Root bark	55**	55**	40	55*	20	20	55*	55**	

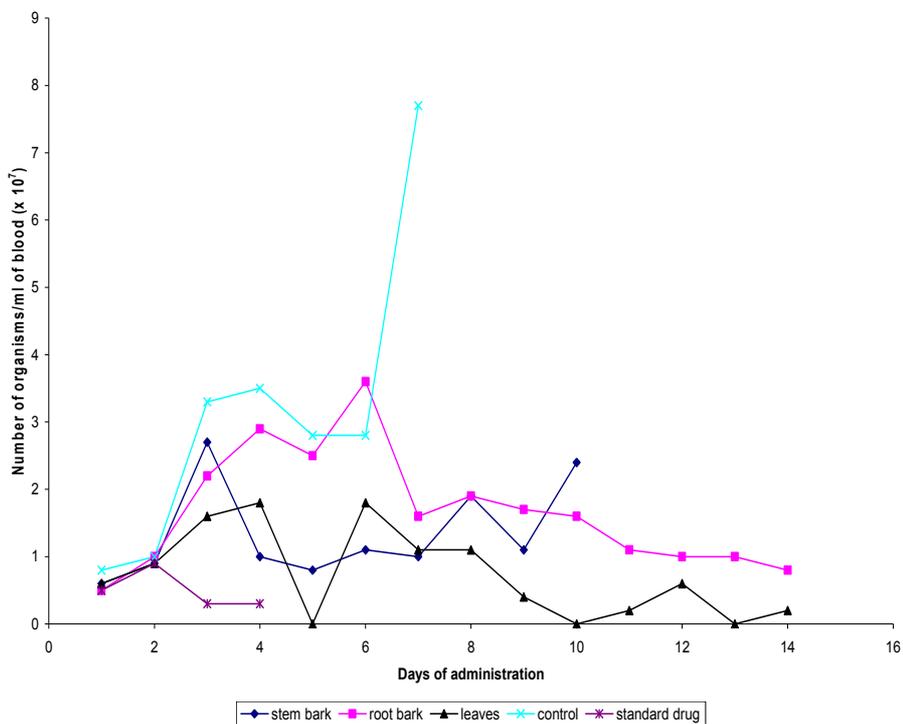


Figure 1. Changes in Parasitemia following treatment with methanolic extract of different parts of *Boswellia dalzielii* through intramuscular administration (300 mg/kg b.w.)