In vitro Antitrypanosomal Activity and Phytochemical Screening of Selected Acacia Plant Species Aqueous Methanol Extract

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ABSTRACT
Trypanosomiasis has been recognized as a scourge in sub-Saharan Africa for centuries and chemotherapy of the disease still remains unsatisfactory. This study is to explore an alternative source of antitrypanosomal agents from the stem bark of four Acacia species; Acacia nilotica, Acacia sieberiana, Acacia geradii and Acacia hockii. Dried stem bark of each plant was pulverized and extracted with 98% methanol by maceration. Phytochemical screening was carried out followed by in vitro testing of extracts on the motility of Trypanosoma congolense maintained in Ringer solution. Motility assessment of trypanosome was carried out after exposure with varied concentrations of the extracts for 2 hours. Thereafter, infectivity test was carried out using albino mice. Seventy-two mice, divided into twenty-four (24) groups of three animals were each inoculated with 100µl of the mixture containing the varying extract concentrations intraperitoneally. Berenil was used as standard drug control. Establishment of infection and subsequent Parasitaemia were monitored in the animals for 60 days. The Phytochemical assay revealed the presence of anthraquinones, tannins, glycosides, cardiac glycosides and terpenes in all the extracts. Saponin was only present in Acacia nilotica and Acacia geradii. Incubation of parasites with each of the four acacia species recorded cessation in parasite motility which was concentration dependent. The highest concentration 20 mg/ml showed the highest effect within fifteen (15) minutes of incubation which was similar to the Berenil incubated control. However, lower doses (0.005 and 0.00005) mg/ml did not show difference from the non extract incubated negative control. Incubation of T. congolense with Acacia nilotica, Acacia sieberiana, Acacia geradii and Acacia hockii at 20, 10 and 1 mg/ml inhibited the ability of the parasites to establish infection in the albino mice as compared the standard control drug. The results indicate that the methanolic stem extracts of the four acacia species possess antitrypanosomal activity with potentials for the treatment of trypanosomiasis.

Key words: Acacia nilotica, Acacia hockii, Acacia geradii, Acacia sieberiana, trypanosomiasis, Trypanosoma congolense, alternative therapy.

INTRODUCTION
African Trypanosomiasis has been a severe parasitic disease in sub-Saharan African with devastating effect on both man and livestock for centuries [1]. The disease is caused by parasitic protozoa of the genus Trypanosoma transmitted by blood-sucking tsetse fly. Human African trypanosomiasis (HAT) commonly called sleeping sickness results from infections with Trypanosoma brucei rhodesiense and Trypanosoma brucei gambiense which accounts for over 95 % of the reported cases [2]. It is estimated that 60 million people in 36 African countries are at risk of developing the disease which threatens to reach epidemic proportions, followed by periods of relative disease control [1, 3, 4]. There has been a significant reduction in the number of reported cases over the last two decades as a result of concerted campaign and free treatment programmes [2]. The clinical symptoms of the early haemolymphatic phase are diverse and non-specific; they are common to several infections endemic in sub-Saharan Africa which may result in mistaken or delayed diagnosis [1]. Also the recent
identification of asymptomatic disease carriers gives huge cause for some concern. Sleeping sickness patients die within months when infected with *Trypanosoma brucei rhodesiense* the acute form; or within years when infected with *Trypanosoma brucei gambiense* the chronic form of the disease if untreated.\[1\]. Animal African trypanosomiasis (AAT) is a major constraint to the productivity of cattle and other domestic animals in tsetse-infested areas in sub – Saharan Africa[5]. The disease reduces the quality of food and milk production with significant socio-economic consequences [1]. The annual loss attributed to animal trypanosomiasis in potential crop and livestock production is valued at billion US dollars [6]. The disease renders tsetse infested regions of sub-Saharan Africa; a third of the total land mass of the African continent with good pasture and water unsuitable for agriculture and rearing of animals [1].

Chemotherapy, the principal control strategy of the disease is unsatisfactory[7,8]. Most of the drugs include Pentamidine, suramine, melarsoprol, DL-alpha-difluormethylornithine (DMFO) and nifurtimox for HAT and isometamidium chloride, diminazene aceturate and homidium salts for AAT show poor efficacy, serious side effects, and toxicity [9, 10]. This necessitates the search for alternative source of drugs.

Plants have been useful in treating and managing various diseases in complementary and alternative medicine and have provided humans with many medically useful compounds [11]. Acacia specie belongs to the family Fabaceae and consists of six sub-species of which three occur in Nigeria [12, 13]. *Acacia nilotica* has been reported to treat cancers and /or tumors[14, 15, 16]. The young seedless pod extract have been reported to treat ulcers while other workers have reported the antidiabetic and hypolipidemic effects of the aqueous- methanol extract of the pods[17, 18]. The root is claimed to be used for the treatment of tuberculosis[19].

The interest in *Acacia nilotica* in the search for new anti-trypanosomal agent is as a result of the various claims of its usage in alternative medicine as already stated. Anticancer drugs have been screened for trypanocidal activity, [20] and vice versa [21, 22]. The proliferating cells of cancer have some features that are similar with that of trypanosomes and plasmodium [23]. Due to the importance of both human and animal trypanosomiasis in public health, this study was conducted to evaluate the anti-trypanosomal potential of the stem extract of four acacia species namely, *Acacia nilotica, Acacia hockii, Acacia sieberiana and Acacia geradii* available in Hwol Buji of Bassa Local Government area of Jos, Plateau state, Nigeria.

**MATERIALS and METHODS**

**Plant materials**

Stem bark of four acacia plants were obtained from Hwol Buji in Bassa Local Govt area of Plateau State located at Latitude 10.01° N and longitude 008.89° E. The plants were authenticated by Azila J.J of the Department of Horticulture, Federal College of Forestry, Jos, Nigeria. Specimen Vouchers were deposited for each plant and identified as follows, *Acacia hockii* - FHJ269, *Acacia (Vachellia) nilotica* – FHJ270, *Acacia geradii* – FHJ 271 and *Acacia (Vachellia) sieberiana* – FHJ271. These were deposited in the Herbarium.

**Ringer’s Solution**

The quantity, 7.2 g of sodium chloride (NaCl), 0.37 g potassium chloride (KCl) and 0.17 g calcium chloride (CaCl₂) were weighed and dissolved in distilled water. The solution was made up to 1 litre. The pH of the solution was adjusted to 7.4. The solution was then filtered through a 0.22 µm filter paper and autoclaved before use.

**Extraction and Concentration of the Samples**

The various plant species were air-dried under shade, pulverized to fine powder and stored in plastic containers for use when required [24, 25]. Pulverized powdered material of *Acacia sieberiana* (A.s), *Acacia hockii* (A.h), *Acacia geradii* (A.g) and *Acacia nilotica* (A.n) were exhaustively macerated by cold extraction in
98 % methanol. The mixture was allowed to stand for 24 hours. The mixture was separated by decanting and filtering using a clean piece of muslin cloth and subsequently with Whatman filter paper No. 1. Additional fresh solvent was added to the residue, it was occasionally agitated and the extraction process repeated 3 times. Excess solvent was removed using freeze dryer [24, 25].

**Phytochemical Screening**

The four acacia species extracts were subjected to qualitative tests for the presence of Saponin, tannins, cardiac glycosides, anthraquinones, flavonoids, alkaloids, terpenes, glycosides and triterpenoids using the methods described by Trease and Evans, (1989) [26]. All determinations were done in triplicates.

**In vitro Studies**

Whole blood containing *Trypanosoma congoense* was collected from an albino mouse raised in the Animal House Unit of the Nigerian Institute for Trypanosomiasis Research (NITR), Vom. The number of parasites was determined microscopically at X40 magnification using the “Rapid Matching” method [27]. A suspension of trypanosome was prepared in normal saline and the concentration adjusted to 3.1620 x 10^7 parasite per/ml.

Two hundred (200) microlitre (µL) of trypanosome suspension was added to same volume of Ringer solution in seven tubes. Thereafter, 400 µL of varying concentrations of extract 2 x 10 mg/ml, 1 x 10 mg/ml, 0.1 x 10 mg/ml, 2 x 10^1 mg/ml, 5 x 10^3 mg/ml and 5x 10^5 mg/ml were dispensed into tubes 1 to 6. Tube 7 served as the negative control. The contents of the tubes were each examined immediately and subsequently at intervals of 15 minutes for 2 hours by aspirating small amounts using a Pasteur pipette onto clean slides then covered with cover slips and checking for the presence and motility of the parasites under the microscope (X40 objective lens) [28, 29, 30]

**Infectivity test**

Infectivity studies were carried out in four batches according to the number of extracts investigated. Batch five was the standard drug control. Twenty one animals in each batch were divided into 7 groups of 3 mice each. At the end of the *in vitro* studies, animals in groups 1-6 were each inoculated intraperitoneally with 100µL of the mixture containing trypanosome suspension and varying concentration of the extract in ringer solution. Group 7 served as negative control. Parasitaemia was monitored daily for sixty (60) days [27, 28, 29].

**Ethical statement**

The animals used were kept in cages and given access to food and water *ad libitum*. They were kept under the protocol of the University of Jos institutional Animal care and use (F17-00379) as well as international accepted principles for laboratory animal use and care.

**RESULTS AND DISCUSSION**

**Phytochemical Screening**

Phytochemical Screening result revealed the presence of various phytochemical constituents such as anthraquinones, tannins, glycosides, cardiac glycosides, terpenes and alkaloids in all the four acacia species investigated. Saponin was only detected in *Acacia nilotica* and *Acacia geradii* (Table 1). This finding agrees with Ogbadoyi et al. (2011) [13]. However, Kamau et al., (2016) [31] detected the additional presence of Saponin in *Acacia hockii* which was not detected in the present study. This variation suggests that Phytochemical constituents of plants may vary depending on several factors that influence the metabolism and accumulation of secondary metabolite [32]. These environmental differences include temperature, precipitation, humidity, soils, illumination, and altitude [33, 34]. Several authors have identified the presence of Saponin, tannins, cardiac glycosides in plants that showed trypanocidal activities, [35] which could also be responsible for the antitrypanosomal activity observed in this study.
Table 1: Phytochemical Constituents of Methanolic Stem Bark of *Acacia* species

| Phytochemical      | *Acacia nilotica* | *Acacia hockii* | *Acacia geradii* | *Acacia sieberiana* |
|--------------------|------------------|-----------------|-----------------|---------------------|
| Flavonoid          | -                | -               | -               | -                   |
| Alkaloid           | +                | -               | +               | +                   |
| Saponins           | +                | -               | +               | -                   |
| Tannins            | +                | +               | +               | -                   |
| Glycoside          | +                | +               | +               | +                   |
| Cardiac glycosides | +                | +               | +               | +                   |
| Terpenes           | +                | +               | +               | +                   |
| Anthraquinones     | +                | +               | +               | +                   |
| Steroids           | -                | -               | -               | -                   |
| Triterpenoids      | -                | -               | -               | -                   |

Key: + = Present; - = Absent

**In vitro Studies**

Results of the *in vitro* studies are shown in Tables 2-6. The reduction in parasite motility over time was observed to be concentration dependent for the four acacia species, *Acacia nilotica*, *Acacia hockii*, *Acacia sieberiana* and *Acacia geradii*. The higher the concentration of the extracts the less time it took to inhibit parasite motility. At a concentration of 2 x 10^{-3} mg/ml of the extracts, the parasites were completely immobilized after fifteen (15) minutes of incubation which was also to diminazene aceturate (standard drug) incubated control. However, lower doses 5 x 10^{-5} mg/ml and 5 x 10^{-3} mg/ml did not show difference from the negative control throughout the period of incubation of the parasites. Cessation in the parasite motility was taken as a measure of the anti-trypanosomal effect of the extracts. This compares with the work of earlier authors [29, 30].

Table 2: Motility Assessment of *Acacia nilotica* Extract on *Trypanosoma congoense*

| Concentration (mg/ml) | 0 | 15 | 30 | 45 | 60 | 75 | 90 | 105 | 120 |
|-----------------------|---|----|----|----|----|----|----|-----|-----|
| 20                    | + | -  | -  | -  | -  | -  | -  | -   | -   |
| 10                    | + | -  | -  | -  | -  | -  | -  | -   | -   |
| 1                     | + | -  | -  | -  | -  | -  | -  | -   | -   |
| 0.2                   | + | +  | +  | +  | +  | +  | +  | +   | -   |
| 0.005                 | + | +  | +  | +  | +  | +  | +  | +   | +   |
| 0.00005               | + | +  | +  | +  | +  | +  | +  | +   | +   |
| Control               | + | +  | +  | +  | +  | +  | +  | +   | +   |

Key: + = motility; - = No motility
Table 3: Motility Assessment of *Acacia sieberiana* Extract on *Trypanosoma congolense*

| Concentration (mg/ml) | 0  | 15 | 30 | 45 | 60 | 75 | 90 | 105 | 120 |
|-----------------------|----|----|----|----|----|----|----|-----|-----|
| 20                    | -  | -  | -  | -  | -  | -  | -  | -   | -   |
| 10                    | -  | -  | -  | -  | -  | -  | -  | -   | -   |
| 1                     | +  | +  | +  | +  | +  | -  | -  | -   | -   |
| 0.2                   | +  | +  | +  | +  | +  | +  | -  | +   | -   |
| 0.005                 | +  | +  | +  | +  | +  | +  | +  | +   | +   |
| 0.00005               | +  | +  | +  | +  | +  | +  | +  | +   | +   |
| Control               | +  | +  | +  | +  | +  | +  | +  | +   | +   |

Key: + = motility; - = No motility

Table 4: Motility Assessment of *Acacia geradii* Extract on *Trypanosoma congolense*

| Concentration (mg/ml) | 0  | 15 | 30 | 45 | 60 | 75 | 90 | 105 | 120 |
|-----------------------|----|----|----|----|----|----|----|-----|-----|
| 20                    | -  | -  | -  | -  | -  | -  | -  | -   | -   |
| 10                    | -  | -  | -  | -  | -  | -  | -  | -   | -   |
| 1                     | -  | -  | -  | -  | -  | -  | +  | -   | -   |
| 0.2                   | +  | +  | +  | +  | -  | -  | +  | +   | +   |
| 0.005                 | +  | +  | +  | +  | +  | +  | +  | +   | +   |
| 0.00005               | +  | +  | +  | +  | +  | +  | +  | +   | +   |
| Control               | +  | +  | +  | +  | +  | +  | +  | +   | +   |

Key: + = motility; - = No motility
Table 5: Motility assessment of Acacia hockii Extract on Trypanosoma congolense

| Conc (mg/ml) | Motility Rate (Minutes) |
|-------------|-------------------------|
| 0           | 15 30 45 60 75 90 105 120 |
| 20          | -   -   -   -   -   -   -   - |
| 10          | -   -   -   -   -   -   -   - |
| 1           | +   +   +   +   +   +   +   +   + |
| 0.2         | +   +   +   +   +   +   +   +   + |
| 0.005       | +   +   +   +   +   +   +   +   + |
| 0.00005     | +   +   +   +   +   +   +   +   + |
| Control     | +   +   +   +   +   +   +   +   + |

Key: + = motility; - = No motility

Table 6: Motility Assessment of Diminazene aceturate (standard drug) on Trypanosoma congolense

| Conc (mg/ml) | Motility Rate (Minutes) |
|-------------|-------------------------|
| 0           | 15 30 45 60 75 90 105 120 |
| 20          | +   -   -   -   -   -   -   - |
| 10          | +   +   -   -   -   -   -   - |
| 1           | +   +   +   +   +   +   +   +   + |
| 0.2         | +   +   +   +   +   +   +   +   + |
| 0.005       | +   +   +   +   +   +   +   +   + |
| 0.00005     | +   +   +   +   +   +   +   +   + |
| Control     | +   +   +   +   +   +   +   +   + |

Key: + = motility; - = No motility

The infectivity studies

Figures 1-5 shows the infectivity profile of Trypanosoma congolense to mice after incubation with the various concentrations of the acacia species under investigation. Incubation of T. congolense with Acacia nilotica, Acacia sieberiana, Acacia geradii and Acacia hockii at 2 x10 mg/ml, 1 x 10 mg/ml, 0.1x10 mg/ml and 2 x 10^{-1} mg/mL inhibited the ability of the parasite to infect the albino mice; this observation was similar to the standard drug control. However, the ability to
initiate parasitaemia and lethal infection in the mammalian host was retained in the lower concentration, $5 \times 10^{-3}$ mg/ml and $5 \times 10^{-5}$ mg/mL.

The present study has shown that the four acacia species has antitrypanosomal effect \textit{in vitro}. This is evident in the ability of the extract to immobilize the trypanosomes and render them un-infective to mice at certain concentration. This may suggest that the acacia species in one way or another, may have blocked respiration and glycolysis and perhaps cell division in the trypanosome [29]. This is evident from the observation that trypanosomes that were not completely immobilized after the incubation period (120 minutes) were not able to initiate infection in the experimental host, when compared with the control groups which initiated infections. The \textit{in vitro} activity of some drugs normally suggests some potentially vulnerable loci of chemotherapeutic attack. The two parameters, motility and infectivity, employed in the present \textit{in vitro} study serve as useful preliminary guide as to whether an inhibitor acts on energy – producing reactions, on macromolecular synthesis, or on both [28, 29].

![Figure 1: Parasitaemia Profile of \textit{Trypanosoma congoense} in Mice Post Incubation with \textit{Acacia hockii}, \textit{Acacia geradii}, \textit{Acacia sieberiana}, \textit{Acacia nilotica} & Diminazene aceturate](image-url)
Figure 2: Parasitaemia Profile of *Trypanosoma congolense* in Mice Post Incubation with *Acacia hockii, Acacia geradii, Acacia sieberiana, Acacia nilotica* & Diminazene aceturate

Figure 3: Parasitaemia Profile of *Trypanosoma congolense* in Mice Post Incubation with *Acacia hockii, Acacia geradii, Acacia sieberiana, Acacia nilotica* & Diminazene aceturate
Figure 4: Parasitaemia Profile of *Trypanosoma conglobense* in Mice Post Incubation with *Acacia hockii*, *Acacia geradii*, *Acacia sieberiana*, *Acacia nilotica* & Diminazene aceturate

Figure 5: Parasitaemia Profile of *Trypanosoma conglobense* in Mice Post Incubation with *Acacia hockii*, *Acacia geradii*, *Acacia sieberiana*, *Acacia nilotica* & Diminazene aceturate
CONCLUSION
The results of our findings indicated that the stem bark aqueous methanol extracts of *Acacia nilotica*, *Acacia sieberiana*, *Acacia hockii* and *Acacia geradii* caused *in vitro* cessation of motility in *T. congolense*. These observation couple with the presence of secondary metabolites in these plants might be responsible for the observed antitrypanosomal activity and thus could serve as potential for the management of African trypanosomiasis.

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