Scholars Academic Journal of Pharmacy (SAJP) **ISSN 2320-4206 (Online)**
Sch. Acad. J. Pharm., 2014; 3(1):38-42 **ISSN 2347-9531 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublisher.com

Research Article

In-Vitro Trypanocidal Activity of Aqueous Stem Bark of *Haematostaphis barter*i against *Trypanosoma congolense* and *Trypanosoma brucei brucei*

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Abstract: *In* vitro trypanocidal activity of crude and fractionated aqueous stem bark of *H. barteri* against *Trypanosoma brucei brucei* and *congolense* was investigated. Varying concentrations of the crude extract were incubated with the parasites in the wells of microtitre plates. Motility of the parasites was monitored at 5 minutes interval for 1 hr. The crude extract was fractionated chromatographically using different solvents and *in* vitro trypanocidal activities of the eluents against the test organisms determined. Phytochemical screening of both the crude and fractionated extract revealed the presence of secondary metabolites that include flavonoids, tanins, alkaloids, terpenoids and carbohydrates. The crude extract had the highest *in* vitro trypanocidal activity. Motility of *T. congolens* ceased 40 minutes after incubation with the crude extract at 0.25 mg concentration. Motility of both parasites ceased 35-40 minutes after incubation with 0.5mg of the crude extract. Fractionation reduced insignificantly the *in* vitro trypanocidal activity against the parasites. Among the four fractions obtained, Methanolic fraction gave the highest trypanocidal activity. Parasite motility stopped 35-40 minutes after incubation. It is obvious that the stem bark of *H. barteri* has *in* vitro trypanocidal activity and fractionation of the crude spatially distributed the various metabolites among the solvents.
Keywords: Motility, Aqueous, *in* vitro ,trypanocidal activity, Metabolites

INTRODUCTION

African trypanosomiasis caused by protozoan parasite, is one of the major factors retarding the growth of livestock especially in sub-Saharan Africa. The huge reproductive loses in livestock due to African Animal trypanosomiasis are attributed to low foetal weights, premature births and poor lactation [1], low quality sperm in infected animals and reduced sperm motility are other featured problems [2].

Chemotherapy, the main means of controlling the disease is under threat due to parasite resistance [3] and toxicity of trypanocidal drugs [4], poor prospect for a vaccine due to antigenic variation of the parasite [5], further compounded with uncertain and unprofitable market or perhaps the localised nature of the disease. The few registered trypanocides, which have been in use over 40 years, are frequently toxic, require lengthy administration, lacks efficacy and unprofitable production. The urgent need for new, safe, effective and cheap drugs cannot be over emphasised.

Traditional use of medicinal plants in the treatment and management of diseases in developing countries is on increase. Findings in this area have provided evidence of relationship of plants and medicine. Recent reports have confirmed antitrypanosomal activities of some medicinal plants [6-11].

H. barteri is a guinea savannah plant normally grown in rocky area belonging to the family of Anacardiaceae. It is known as"blood Plum" in English. The plant has numerous medicinal uses [12].Traditional medical practitioners in the north eastern Nigeria use the plant in the treatment and management of trypanosomiasis. However, there is no any existing scientific evidence on the efficacy of the plant. This work was designed to evaluate the *in* vitro trypanocidal efficacy of *H. Barteri a*gainst *Trypanosoma brucei brucei* and *congolense*

MATERIALS AND METHODS

Plant sample

The stem bark of *H. barteri* was collected from Hong local government, Adamawa state of Nigeria. It was identified at the forestry department of Modibbo Adama University of Technology Yola, where a voucher specimen was kept.

Plant Sample Handling

Exactly 100g of the powdered stem bark was macerated in 400 ml of water and left overnight. The filtrate was evaporated to dryness on water bath at 40 ^{0}c. The concentrated extract was stored in the refrigerator at less than 10 ^{0}c until it was required.

Trypanosomes

Trypanosoma brucei brucei and *Trypanosoma congolense* were obtained from Nigerian Institute of Trypanosomiasis Research, Vom, Jos,Nigeria.

Phytochemical Screening

Phytochemical components of both the crude and fractionated extracts were determined using methods of Sofowora[13] andTrease and Evans [14].

Separation of the crude extract

Exactly 30g of coarse silica gel was dissolved in a buffer saline and was packed in a column. Elution with buffer continued until the gel was well packed. The crude extract (20g) was wrapped in a Watman filter paper and placed on top of the gel in the column. The sample was eluted with ethyl acetate, benzene, methanol, acetic acid and water consecutively. The fractions were concentrated on water bath at 40 ^{0}c.

***In* vitro Screening**

Stocks of both the crude and fractionated extracts were formed with dextrose saline followed by serial dilution. Infected blood with *Trypanoma brucei brucei* and *Trypanosoma congolense* was harvested from a donor rat at peak parasitemia 10^9. Aliquots of 20 µl(0.12 mg/ml) of the extract was incubated in the wells of microtitre plates in triplicates with 40 µl of infected blood at 37 ^{0}c. For control, the 20 µl of the extract was replaced with dextrose saline. Motility of parasites was observed at every 5 minutes interval for 1 hr on a glass slide covered with slip under the microscope (at x40).

RESULTS

Table 1 presents the result of phytochemical constituents of the plant. Alkaloids, tanins, cardiac glycosides, carbohydrate, saponins and terpenoids are found in both the crude and fractionated extracts. Steroids and phlabotanin were absent. Other fractions lack some of the phytochemicals observed in the crude.

The crude extract had *in* vitro antitrypanosomal activity against both the parasites. The observed activity was dose dependant with *T.congolense* exhibiting higher susceptibility compared to the *T. brucei brucei* species (figure 1-3).

Out of the four fractions obtained during separation of the crude extract, methanolic fraction gave the highest *in* vitro activity against the parasites (figure 4 - 6). However, the *in* vitro activity was insignificantly less than that exhibited by the crude extract.

Table 1: Result of phytochemical analysis of crude and fractionated extracts of *H. barteri*

Phytochemicals	Crude Extract	Fractionated Extracts			
		Water	Acetic Acids	Ethyl Acetate	Methanol
Phlobatanin	--	--	--	--	--
Saponins	+	+	+	+	+
Flavonoids	+	+	+	+	+
Steroids	--	--	--	--	--
Tepenoids	+	+	+	+	+
Cardiac Glycoside	+	+	--	--	+
Alkaloids	+	+	--	--	+
Carbohydrates	+	+	--	--	+
Tanins	+	--	+	+	+
Anthraquinone	+	+	--	--	--

+ = Present, -- = Absent.

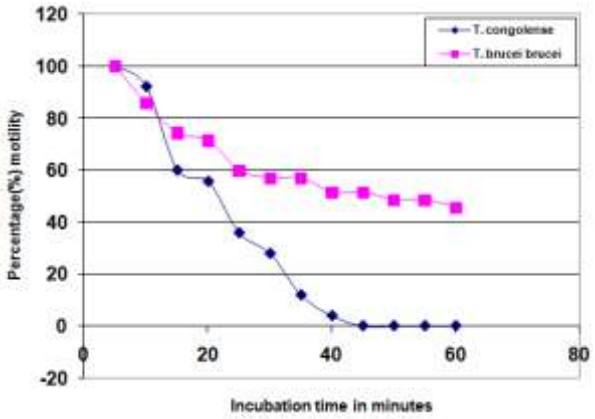

Figure 1: Profile of incubation time against motility of *T. brucei brucei* and *T. Congolense* at 0.25mg of the crude aqueous extract

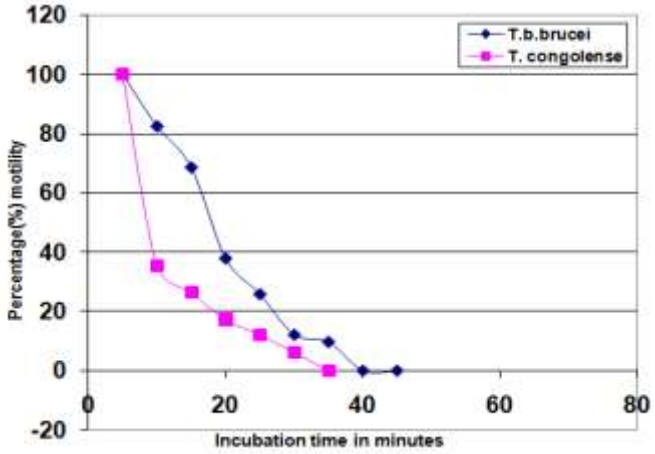

Fig. 2: Profile of incubation time against motility of *T. brucei brucei* and *T. congolense* at 0.5mg of the crude aqueous extract

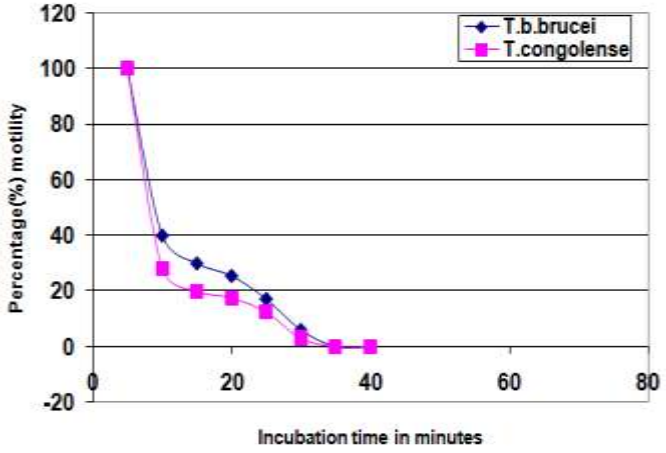

Fig. 3: Profile of incubation time against motility of *T. brucei brucei* and *T. congolense* at 1 mg of the crude aqueous extract

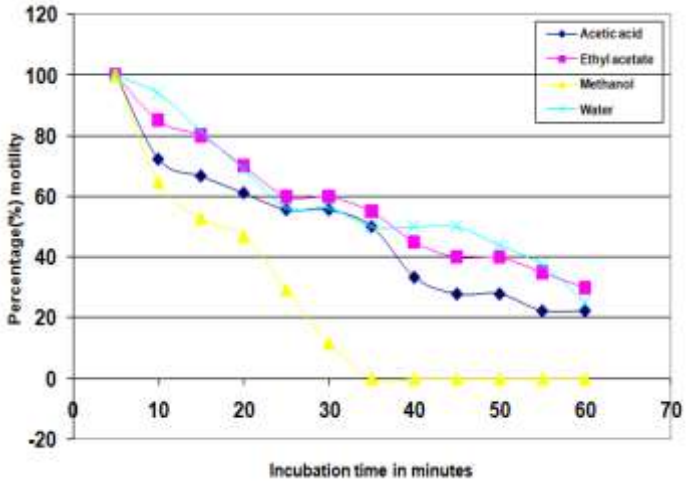

Fig. 4: Profile of incubation time against motility of *T. Brucei brucei* at 1mg of different fractions of the extract

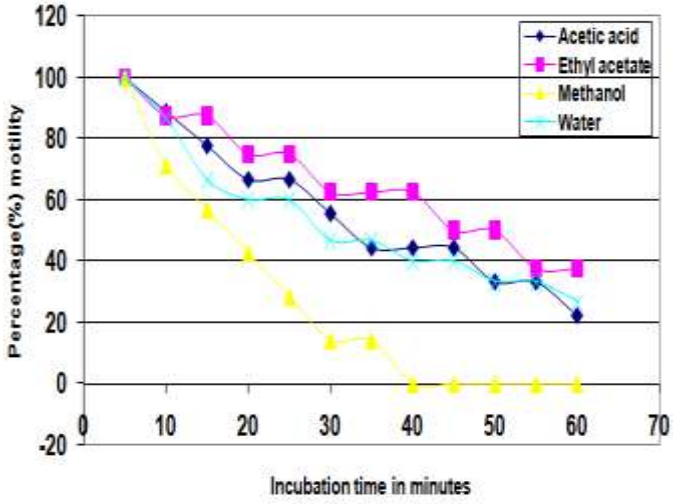

Fig. 5: Profile of incubation time against motility of *T. brucei brucei* at 0.5mg of different fractionsof the extract

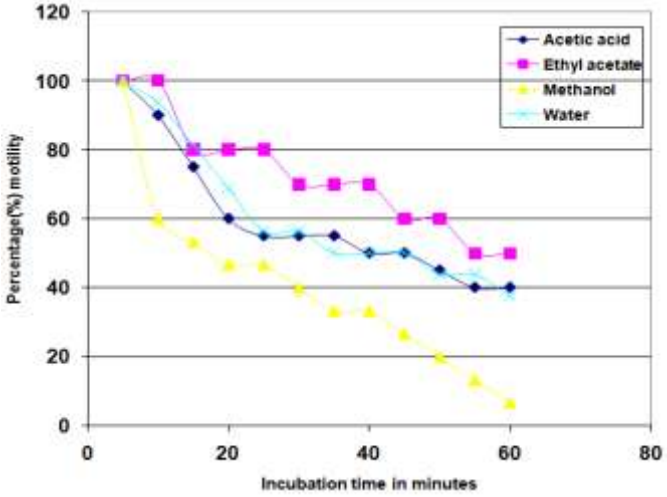

Fig. 6: Profile of incubation time against motility of *T. brucei brucei* at 0.25mg of different fractionsof the extract

DISCUSSION

Medicinal plants are those plants that contain potential phytochemical constituents used in the treatment and management of diseases. Crude extracts of medicinal plants contain several secondary metabolites with either synergistic or antagonistic effects. Separating the different constituents of the crude extract will therefore increase or decrease the efficacy of the plant.

Result of this study revealed the presence of potential secondary metabolites in the crude extract which include tanins, saponins, and cardiac glycosides. However, when the crude extract was chromatographically eluted with different solvents the constituents were separated based on their solubility.

The crude extract had *in* vitro activity against both parasites. The activity was more on *Trypanosoma congolense* suggesting that the parasite is more susceptible to the crude extract. Different susceptibility of parasites to trypanocides have been reported; *T. gambeinse* and *T. rhodiense* have different susceptibility for eflornithin a commercial drug against human trypanosomiasis[15].

Even though, all the different fractions obtained from the crude had *in* vitro trypanocidal activity against both parasites, methanolic fraction had the highest activity. The methanolic fraction contains the same secondary metabolites as that of the crude except for the absence of anthraquinones. This could be the reason why we observed insignificant decrease in the *in* vitro trypanocidal effect of the methanolic fraction compared to that of the crude. It is also suggesting that the activity of the secondary metabolites against the parasites is synergistic. Majorie[16] reported that combination of secondary metabolites enhances activity.

Bioactive screening in vitro remains a useful method for selection of plants and bioassay guided fractionation for the isolation and identification of active principle. Many medicinal plants have been found to possess *in* vitro trypanocidal activities[7, 10]. Parasite motility is

considered to be relatively reliable indicator of viability of most zoo flagellate parasites [17]. Cessation in the motility of both parasites in the present *in* vitro study clearly suggests the potentiality of both the crude and fractionated extracts of *H. barteri.*

We conclude that crude aqueous extract of *H.barteri* has potential trypanocidal activity and separation of the crude extract slightly reduced the *in* vitro activity. We are currently working on the *in vivo* activity of the plant in order to provide detailed trypanocidal activity of the plant.

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