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Research Article

Phytochemical Analysis and *In Vitro* Evaluation of Trypanocidal Efficacy of *Stachytarpheta jamaicensis*

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Abstract

A study on phytochemical analysis and *in vitro* evaluation of trypanocidal efficacy of *Stachytarpheta jamaicensis* was carried out. Chemical tests were carried out on the plant extracts using standard procedures. Methanol and aqueous extracts of *S. jamaicensis* were tested for *in vitro* anti-trypanosomal activity. *In vitro* assays were carried out in 96-well microtitre plates at effective test concentrations of 4, 2 and 0.4 mg/ml using deminazene diaceturate (Nozomil®) as the positive control. The results of Phytochemical screening revealed the presence of Alkaloids, Cardiac glycosides, Flavonoids, Saponins, Tamins, Phlobatannins and Terpenes in the leaf extract of *S. jamaicensis* but Free Antraquinones were not detected. However, qualitative screening of the root of *S. jamaicensis* revealed the presence of alkaloids, flavonoids, saponins, tamins, and terpenes. phlobatannins, cardiac glycosides and free antraquinones were not detected. *In vitro* studies showed tested extracts eliminated motility in *T. congolense* and *T. brucei* within 30minutes of exposure. Nozomil® eliminated trypanosomal motility within 20 min even at the lowest concentration tested (0.4 mg/ ml). Thus, *S. jamaicensis* contains bioactive components which exhibit a promising trypanocidal efficacy. However, in vivo evaluation of this plant may validate effective application of this plant in the treatment of trypanosomiasis.

Keywords: *Stachytarpheta jamaicensis*; Phytochemical analysis; Trypanocidal efficacy; Trypanosomiasis.

Introduction

Trypanosomes are haemoflagellate protozoa belonging to the family Trypanosomatidae. They cause African trypanosomiasis (or African sleeping sickness) and American trypanosomiasis and are transmitted by an insect vector (Tsetse fly) of the genus Glossina and reduviid bugs respectively [1]. The complex *Trypanosoma brucei* have two subspecies that are morphologically indistinguishable, causes distinct disease patterns in humans: *T. b. gambiense* causes West African sleeping sickness and *T. b. rhodesiense* causes East African sleeping sickness. (A third member of the complex, *T. b. brucei*, under normal conditions does not infect humans). The protozoan parasite, *Trypanosoma cruzi*, causes American trypanosomiasis (or Chagas' disease), a zoonotic disease that can be transmitted to humans by blood-sucking reduviid bugs [1].

Large populations of human beings and domestic animals are at risk of debilitating infections with trypanosomes in vast areas of sub-Saharan Africa where the disease is endemic. Human infections with trypanosomes are called Human African Trypanosomosis (HAT) while animal infections are known as African Animal Trypanosomosis (AAT). These infections are more prevalent in the rural areas [1,2] and are transmitted through the bite of infected tsetse flies (*Glossina spp.*). Trypanosomosis is of great significance to human health and animal production in Africa [3].

Stachytarpheta jamaicensis commonly known as Blue flower, Light blue snake weed Rat tail, Brazilian tea, verbena cimarrona, rooter comb, or blue porter weed or Snake weed is of medicinal importance in medicinal systems in various countries, pharmacological effects due to the presence of various bioactive components. In

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herbal medicine, *S. jamaicensis* itself has been recorded to demonstrate antacid, analgesic [4], anti-inflammatory [5]. There are reports of drug resistance and and toxicity of currently used trypanocidal. This study was therefore aimed at evaluating extract of *S. jamaicensis* for anti-trypanosomal activity in vitro. This plant origin may provide an alternative to chemically synthesized drugs that this parasite has become resistant.

Materials and methods

Collection and identification of plant materials

S. jamaicensis species were collected from their natural habitat in Ikot Nkim, Ibesikpo Asutan Local Government Area, Akwa Ibom State. The plant was identified by a Biosystematics/Taxonomist with a voucher number UUPH 78(b), voucher specimen was kept in Herbarium unit, Department of Pharmacognosy and Natural Medicine, University of Uyo, Uyo , Akwa Ibom State, Nigeria. Leaf and root of the plant were used for the experiment. The collected plant specimens were taken to Federal University of Agriculture, Deparment of Biological Sciences' Laboratory for extraction and further experiments.

Preparation of methanolic and aqueous extracts

Leaves and root of the plant species were used for the study. Plant were washed thoroughly using distilled water, to remove sand and other foreign materials and air dried on laboratory bench for two weeks. Methanolic and aqueous solvent was used for the extraction using cold maceration technique as described by Evans [6,7].

Test organisms

Trypanosoma brucei brucei and *Trypanosoma congolense* were obtained from stabilates maintained at the Nigerian Institute for Trypanosomiasis Research (NITR), Vom, Plateau State and Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University (ABU), Zaria respectively. The parasites were maintained in the laboratory by continuous passage in rats until required. Passage was considered necessary when parasitamea was at the range of 16 – 32 parasites per field (usually 4 - 5 days post infection in *Trypanosoma brucei brucei* and 7 - 9 days in *Trypanosoma congolense*). In passaging, 1 X 103 parasites were introduced 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).

Determination of Parasitaemia

Parasitaemia was monitored in blood obtained from the tail pre-sterilize with methylated spirit. The number of parasites was determined microscopically at X40 magnification using the "Rapid Matching" method of Herbert and Lumsden (1976) [8]. Briefly, the method involved 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 were obtained by matching with the table of Herbert and Lumsden (1976) and were converted to antilog to provide absolute number of trypanosomes per ml of blood.

In vitro test for trypanocidal activity

Assessment of *in vitro* trypanocidal activity was performed in triplicates in 96 well micro titer plates (Flow laboratories Inc., McLean, Virginia 22101, USA). 20 µl of blood containing about 20-25 parasites per field obtained as described under "determination of parasitaemia" was mixed with 5 µl of extract solution of 20.0 mg/ml, 10.0 mg/ml and 2.0 mg/ml to produce effective test concentrations of 4 mg/ml, 2 mg/ml and 0.4 mg/ml, respectively. To ensure that the effect monitored was that of the extract alone, a set of control was included which contain the parasite suspended in 10% DMSO only. For reference, tests were also performed with the same concentrations of Nozomil® (445 mg diminazene diaceurate+ 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^{o}C, about 2 µl of test mixtures were placed on separate microscope slides and covered with cover slips and the parasites were observed every 5 min for a total duration of sixty minutes. It should be noted that under this *in vitro* system adopted, parasites survived for about 4 h when no extract is 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 trypanocidal activity.

Results and discussion

Phytochemical Screening Results of S. jamaicensis

Phytochemical screening results of *S. jamaicensis* are presented in Table 1. The Screening of the root and leaf of *S. jamaicensis* revealed the presence of various phytochemical components. Different parts of *S. jamaicensis* gave different phytochemical screening result (Table 1).

Alkaloids, cardiac glycosides, flavonoids, saponins, tamins, phlobatannins and terpenes were detected in the leaf extract of *S. jamaicensis* but free antraquinones were not detected (Table 1). However, qualitative screening of the root of *S. jamaicensis* revealed the presence of alkaloids, flavonoids, saponins, tamins, and terpenes. phlobatannins, cardiac glycosides and free antraquinones were not detected in the root of *S. jamaicensis* (Table 1).

Table 1. Phytochemical constituents of the leaf and root of *Starchytapheta jamaicensis*

Plant Metabolite/ Phyto Constituent	Test	L	R
Alkaloids	Dragedroffs	+	+
Anthraquinones	Borntrager's	-	-
Combined Anthraquinones			
	Borntrager's	-	-
Cardiac glycosides	Salkowski's	+	-
Flavonoids	Shinoda's reduction test	+	+
Saponins	Froth	+	+
	Sodium bicarbonate	+	+
Tannins	Ferric chloride	+	+
Phlobatannins	Hydrochloric acid test	+	-
Terpens	Liebermann Burchard	+	+
Carbohydrates	Moliscoh's test	+	+

Key: + = Detected, - = Not detected, L = Leaf, R = Root

Efficacy of methanolic leaf and root extracts S. *jamaicensis* on *T. b. brucei* and *T. congolense* viability i*n-vitro*

Table 2 and 3 shows the efficacy of aqueous (Aq) and Methanolic leaf and root extracts of **S**. *jamaicensis* on the viability of *T. b. brucei* and *T. congolense in-vitro*. For all the preparations (Aq. Leaf and root extracts of *S. jamaicensis*, Methanolic leaf and root extracts of *S. jamaicensis*, there was a significant increase in efficacy with increasing concentration of the extracts and the duration of incubation of the parasite with the extracts. All the extractions eliminated motility in *T. congolense* and *T. brucei* within 30minutes of exposure. Diminal^{R} eliminated trypanosomal motility within 20 min even at the lowest concentration tested (0.4 mg/ ml). The effect was such that after 10 - 20 min of incubation, no motility was visible with extracts concentrations of 4.0 and 2.0 mg/ml.

The phytochemical investigation carried out in this study involving assays for akaloids, anthraquinones (free and combined), cardiac glycosides, flavonoids, saponins, tannins, phlobatannins and terpenes, the corresponding results obtained corroborated with those in literature. Results obtained from the screening of the leaf and root extracts of *S. jamaicencis* agrees with the reports of Idu *et al*. (2007) [9], Joshi *et al.* (2013) [10] and Ramakrishnan and Sivaranjani (2013) [11].

Table 2. Efficacy of extracts of S. *jamaicensis* on *T. congolense* viability *in-vitro*

Plant	Plant Part	Extract	Doses (mg/ml)	Exposure Time		
				After 10 min	After 20 min	After 30 min
S. jamaicensis	Leaf	Methanol	4.0	*	**	**
			2.0	*	**	**
			0.4	*	**	**
		Aqueous	4.0	*	**	**
			2.0	*	**	**
			0.4	-	-	*
S. jamaicensis	Root	Methanol	4.0	*	**	**
			2.0	*	**	**
			0.4	*	**	**
		Aqueous	4.0	*	**	**
			2.0	*	**	**
			0.4	-	*	*
Deminazene				*	**	**

*Key: - = reduced mobility, * = Partial elimination, ** =Complete elimination*

Table 3. Efficacy of extracts of S. *jamaicensis* on *T. brucei* viability *in-vitro*

Plant	Plant Part	Extract	Doses (mg/ml)	Exposure Time		
				After 10 min	After 20 min	After 30 min
S. jamaicensis	Leaf	Methanol	4.0	*	**	**
			2.0	*	**	**
			0.4	*	**	**
		Aqueous	4.0	*	**	**
			2.0	*	**	**
			0.4	-	*	**
S. jamaicensis	Root	Methanol	4.0	*	**	**
			2.0	*	**	**
			0.4	-	*	**
		Aqueous	4.0	*	**	**
			2.0	*	**	**
			0.4	-	*	**
Deminazene				*	**	**

*Key: * = Partial elimination, ** =Complete elimination, - = reduced mobility*

S. jamaicencis exhibited great and successful trypanocidal effecacy *in vitro*. This is not really uncanny though, earlier research of Nok *et al*. (1993) has clearly demonstrated that medicinal plants of different families could possess potent trypanocidal activity [12]. Sepulveda-Boza and Cassels (1996) documented natural products with trypanocidal activity a part of phytochemical classes [13]. Both root and leaf of the plant were seen to exhibit good trypanocidal ability. This could be attributed to this plant containing Alkaloids, Flavonoids, Phenolics and/or Terpenes. Anti-trypanosomal activities have been seen to be effective in plants with these bioactive components. For example quercetin and kaempferol major bioactive components of Flavonoid have been revealed in

the root of *S. jamaicencis* [9]. Plants with this Phytochemicals have been reported to show a good trypanocidal activity even with alkaloids compound [14]. The promising anti-trypanosoml activity observed in this experiment is also corroborated with the findings of Nok *et al*. (1993) who reported that plant with certain phytochemicals could exhibit effective anti-trypanosomal properties. In line with other findings, *S. jamaicensis* vis-à-vis other reports [12] clearly validate the relatively promising trypanocidal activity of *S. jamaicensis.*

Many reports suggest that many natural products exhibit their trypanocidal activity by their interference with the redox balance of the parasites acting either on the respiratory chain or on the cellular defenses against oxidative stress [13]. Many plants have been reported to possess structures capable of producing radicals that may cause peroxidative damage to trypanothione reductase that is very sensitive to alterations in redox balance.

Conclusions

Stachytarpheta jamaicensis contains bioactive components which exhibits a promising trypanocidal efficacy. However, *in vivo* evaluation of this plant may validate effective application of the plant in the treatment of trypanosomiasis.

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Conflict of interest

Authors have declared no competing of interests.

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