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ANTITRYPANOSOMAL ACTIVITIES OF SOME SELECTED NIGERIAN ETHNOMEDICINAL PLANTS

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Abstract

The phytochemical components and antitrypanosomal activities of four selected Nigerian plants (*Pterocarpus santalinoides* leaves, *Picrlima nitida* seeds, *Dacyroedes edulis* stem bark and *Terminalia catappa* leaves) were investigated. Phytochemistry was determined following standard methods while antitrypanosomal activity was determined via the *in vitro* assay using five different concentrations (4, 2, 1, 0.25 and 0.0625 mg/ml) of the plant extracts. Concentrations of *P. santalinoides* hydroethanol leaf extract (PSHELE) were further incubated for 60 minutes, inoculated in mice for the infectivity test and monitored for 21 days. Phytochemical studies revealed the presence of flavonoids, tannins, carbohydrate and reducing sugar in PSHELE; flavanoids, tannins, alkaloids, carbohydrate and reducing sugars in *P. nitida* hydroethanol seed extract; alkaloids, reducing sugars, steroids, tannins and saponins in *T. catappa* hydroethanol leaf extract, and alkaloids, phenols, flavonoids, tannins, cardiac glycosides and reducing sugars in *D. edulis* hydroethanol stem bark extract. *P. santalinoides* hydroethanol leaf extract exhibited strong *in vitro* antitrypanosomal activity followed by *P. nitida* hydroethanol seed extract (PNHESE) with IC₅₀ of 0.011 and 0.022 mg/ml respectively. Following infectivity test, none of the PSHELE concentrations was able to exhibit loss of infectivity following incubation for 60 minutes. Though, a concentration-dependent prolongation of the pre-patent period was observed. It was therefore concluded that PSHELE and PNHESE possess antitrypanosomal activities and could serve as a potential candidate for development of new lead against African trypanosomosis.

Keyword: Ethnomedicinal plants; Antitrypanosomal activity; *Trypanosoma brucei*; Phytochemistry; Infectivity test.

Introduction

African animal trypanosomosis (AAT) is a fatal and often debilitating disease complex caused by tsetse transmitted protozoan parasite, *Trypanosoma*. AAT persist in the sub-Saharan Africa including Nigeria and constitutes a serious setback to improved profitable livestock production in tropical Africa (Obi et al., 2019). There is no effective vaccine against trypanosomes because of the phenomenon of antigenic variation exhibited by the parasites. Thus, AAT prevention/control methods involve the use of trypanotolerant livestock breeds, tsetse control and chemotherapy, which are beset with a lot of challenges. Chemotherapy, the most promising and widely practiced AAT control strategy is confronted with problems of resistance, toxicity, high treatment costs and absence of new drugs. Thus, there is an urgent need for a new, efficacious and safe trypanocides either synthetically or from plant origins.

Some plants are believed and widely used singly or in combination with others in folkloric medicine to treat many diseases of man and animals especially in rural sub-Saharan African villages. Though, their effectiveness remains uncertain. *Pterocarpus santalinoides* also known locally as *Nturukpa* (Igbo), *Gyadar kurmi*, (Hausa) and *Gbengbe* (Yoruba) is used traditionally to treat diarrhea, malaria, elephantiasis, amoebic dysentery, stomach-ache, and fever (Odeh and Tor-Anyiin, 2013). *Dacyroedes edulis* (African pear) in combination with the leaves of other



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plants are used in folkloric medicine to treat blood-borne parasitic infections, wound, skin diseases, dysentery and fever (Ajibesin, 2011). Various parts of *Picralima nitida* and *Terminalia catappa* are either used singly or in

combination with other plants for the treatment of fever, hypertension, jaundice, and blood protozoan infections by traditional healers (Etukudo, 2003). Notwithstanding the numerous oral reports of the folkloric use of the above medicinal plants for the treatment of blood parasitic infections including trypanosomosis, paucity of data exists in scientific literature on their antitrypanosomal effects. The desire for a locally available and potent antitrypanosomal agent and need to verify the claims of traditional healers/rural villagers necessitated this study.

Materials and method

Trypanosomes, trypanocides, experimental animals and plant materials

The *Trypanosoma brucei* used was a field isolate obtained from an infected dog presented at the University of Nigeria Veterinary Teaching Hospital, Nsukka and molecularly identified via nested ITS-PCR. The *Trypanosoma brucei* was thereafter maintained in mice. The ethical trypanocide used was diminazene aceturate (Diminaveto[®], V.M.D, Belgium). Forty adult male albino mice were procured from the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka and were used for the infectivity experiment. *D. edulis* stem bark, *P. nitida* seeds, *P. santalinoides* and *T. catappa* fresh leaves were collected from Obukpa in Nsukka Local Government Area of Enugu state. The plants were identified by a plant taxonomist at the Department of Plant Science and Biotechnology, University of Nigeria Nsukka and voucher specimens deposited.

Extraction of plant material, phytochemistry and in vitro antitrypanosomal assay

The stem barks of *D. edulis* were sliced into tiny chunks. The different plant parts were dried and pulverized individually. 300 g of the pulverized individual plant parts were subjected to cold maceration extraction in extraction jars using 70% ethanol for 72 hours. Whatmann[®] No.1 filter paper was used to filter the extracts and the filtrates were concentrated using hot air oven at 37⁰ C. The extracts were stored in a refrigerator until when required. The phytochemical constituents of the extract were determined following standard procedures (Trease and Evans, 2002). The *in vitro* antitrypanosomal activities of the extracts were performed in triplicates in 96 well micro-titer plates (Flow Laboratories Inc.) as described in Atawodi et al., (2003). Scoring of complete cessation or reduction of motility of the parasite in relation to the negative control was used to determine efficacy. The IC₅₀ calculator tool (<https://www.aatbio.com/tools/ic50-calculator>) was used to calculate the IC₅₀ (concentration of both the extracts and diminazene aceturate at which 50% of the trypanosomes were inhibited).

Blood incubation infectivity test

Based on the *in vitro* test results, PSHELE was selected and subjected to blood incubation infectivity test as described in Mergia et al. (2015). Parasite suspensions were incubated for one hour at different concentrations (4, 2, 1, 0.25 and 0.125 mg/ml) of *P. santalinoides* hydroethanol extract and were inoculated in mice (four mice per extract and drug concentration). The infected mice were monitored daily for 21 days parasitaemia.

Data analysis

The data generated were subjected to descriptive statistics and expressed as mean $\pm$ standard error of the mean.

Results and Discussion

The percentage yields of the extracts were 12.2%, 3.1%, 3.8%, and 4.6% w/w for *P. santalinoides*, *D. edulis*, *P. nitida* and *T. catappa* hydroethanol extracts. The phytochemical constituents of the *P. santalinoides* hydroethanol leaf extract (PSHELE) were flavonoids, tannins, carbohydrate and reducing sugar. flavanoids, tannins, alkaloids, carbohydrate and reducing sugars were present in the *P. nitida* hydroethanol seed extract (PNHESE) while alkaloids, reducing sugars, steroids, tannins and saponins were present in *T. catappa* hydroethanol leaf extract (TCHELE). The *D. edulis* hydroethanol stem bark extract (DEHESBE) contained alkaloids, phenols, flavonoids, tannins, cardiac glycosides and reducing sugars. Varying phytochemicals have been reported for some of these medicinal plants (Obi et al., 2019) and could be attributed to the parts of plant, stage of growth, age, location and solvent used for extraction.

Of the extracts tested for antitrypanosomal activity, PSHELE exhibited the strongest activity at all the concentrations tested with IC₅₀ of 0.011 mg/ml (Table 1). PNHESE also displayed good trypanocidal activity with



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IC₅₀ of 0.022 mg/ml. The standard drug, diminazene aceturate also exhibited similar activity like the PSHELE with IC₅₀ of 0.009 mg/ml. These results corroborate the use of these plants in folkloric medicine for the treatment of blood parasitic infections including African trypanosomosis. Parasite motility assessed via *in vitro* assay, is a

dependable marker of trypanosome viability and complete elimination or reduction of trypanosome motility compared to the control is considered an index of trypanocidal activity (Atawodi et al., 2003). The exact mechanism employed by the extracts to cease or reduce the motility of trypanosomes is not fully understood. However, the antitrypanosomal properties exhibited by PSHELE and PNHESE could be credited to its phytochemical constituents which exert their effects either through the additive or synergistic action of several chemical compounds acting at a single or multiple target sites [Obi et al., 2019]. Tasdemir et al. (2006) reported the potent *in vitro* antitrypanosomal activity of flavonoids, a constituent of PSHELE and PNHESE. The interference of the extract with the oxidative redox balance of trypanosomes acting either on the cellular defenses against oxidative stress or the respiratory chain (Atawodi et al., 2003) could also have contributed to the observed antitrypanosomal activities. Motility of trypanosomes is dependent on the parasite's mitochondrial and cellular respiration and inhibition of respiration compromises all energy dependent process (Mergia et al., 2015).

Following blood incubation infectivity test, mice groups that received different concentrations of the PSHELE became parasitaemic between days 5 and 19 post infection. However, only one mouse from the 4 mg/ml PSHELE concentration was parasitaemic on day 19 PI (Table 2) despite the pronounced cessation of motility recorded in the *in vitro* study. The pre-patent period was observed to be concentration-dependent with higher extract concentration having longer PPP. Mice groups that received 2 and 4 mg/ml of the standard drug were aparasitaemic throughout the experimental period. The infectivity results suggest that cessation of trypanosome motility in the *in vitro* assay does not always imply death of parasites. The ability of the PSHELE-incubated trypanosomes to establish and induce parasitaemia in infected mice suggests that PSHELE has trypanostatic and not trypanolytic activity, which appears to be concentration-dependent. The observed concentration-dependent prolongation of pre-patent period could be ascribed to the antitrypanosomal potential of PSHELE in addition to the abrogation of some vital trypanosome metabolic process during the incubation period by PSHELE.

Conclusion

This study shows that some ethnomedicinal plants such as PSHELE and PNHESE possess *in vitro* antitrypanosomal activity and could be a potential source of new drugs against African animal trypanosomosis.

Recommendation

Due to the absence of new drugs against African trypanosomosis, the authors recommend continuous screening of the ethnomedicinal plants used in folkloric medicine for antitrypanosomal activities.

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Table 1: *In vitro* antitypanosomal effect of some Nigerian ethnomedicinal plants

Treatments	4 mg/ml	2 mg/ml	1 mg/ml	0.25 mg/ml	0.125 mg/ml	0.0625 mg/ml	IC ₅₀ mg/ml
PSHELE	*	*	*	*	*	**	0.011
DEHESBE	*	**	NE	NE	NE	NE	0.072
PNHESE	*	*	**	**	NE	NE	0.022
TCHELE	*	**	**	NE	NE	NE	0.201
Positive control (DA)	*	*	*	*	*	*	0.009
Negative control (PBSG)	NE	NE	NE	NE	NE	NE	

KEY: *Ceased motility, **Drastically reduced motility, **PSHELE** – *P. santalinoides* hydroethanol leaf extract, **DEHESBE** - *D. edulis* hydroethanol stem bark extract acetate, **PNHESE** – *P. nitida* hydroethanol seed extract, **TCHELE** – *T. catappa* hydroethanol leaf extract, **DA** – Diminazine acetate, **PBSG** - Phosphate buffered saline glucose, **NE** - No effect, **IC** – Inhibition concentration.

	Test concentration	Number of parasitaemic mice	Infection interval in days (Mean ± SEM)
PSHELE	4 mg/ml	1/4	19
	2 mg/ml	2/4	17
	1 mg/ml	4/4	15±2.45
	0.25 mg/ml	4/4	12.5±2.06
	0.125 mg/ml	4/4	9.5±2.06
Positive control (DA)	4 mg/ml	0/4	0
	2 mg/ml	0/4	0
	1 mg/ml	1/4	18
	0.25 mg/ml	3/4	12.3±0.67
	0.125 mg/ml	3/4	10.33±1.33
Negative control (PBSG)		4/4	7.25±0.63

Table 2: Effect of *P. santalinoides* hydroethanol leaf extract (PSHELE) on blood incubation infectivity test

KEY: **PSHELE** – *P. santalinoides* hydroethanol leaf extract, **DA** – Diminazine acetate, **PBSG** - Phosphate buffered saline glucose, **SEM** – Standard error of the mean.