

Full Length Research Paper

Preliminary studies on the *in vitro* antitrypanosomal activity of aqueous and methanolic crude extracts of stem bark of *Nauclea latifolia* on *Trypanosoma congolense*

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In Nigeria, herbal treatment of various types of diseases is very common using medicinal plants. In the present study, preliminary investigation on the *In vitro* antitrypanosomal activity of methanolic and aqueous extracts of stem bark extracts of *Nauclea latifolia* was evaluated on *Trypanosoma congolense*. Blood obtained from a highly infected mice with *T. congolense* (10⁷) was incubated with methanolic, and aqueous extract at 20 mg, 10 mg and 5 mg/ml and Diminivet^R (diminazene aceturate) at 10 mg, 200 and 50 µg/ml in a 96 well microtiter plate. The results revealed that methanolic and aqueous extracts had activity at 20 mg/ml. Phytochemical screening of the methanolic and aqueous extract of the bark showed that they both had flavonoids, saponnin, terpenoids and tannin. The aqueous and methanolic extract appears to show some potential activity against *T. congolense*.

Key words: *Nauclea latifolia*, Trypanocidal, *Trypanosoma congolense*

INTRODUCTION

Trypanosomiasis continues to cause morbidity and mortality on a large scale in tropical countries including Nigeria. This disease afflicts both human and livestock causing great losses. World Health Organization reports that 66 million people in 36 African countries are afflicted and 3 million cattle die as a result each year (WHO, 2000; Kristjanson et al., 1999). The rate at which the parasite has developed resistance to currently used trypanocidal drugs (Ndung'u et al., 1999; McDermott et al., 2000; Anene et al., 2001; Maikai et al., 2007) makes it imperative to search for newer more effective, cheaper and environmentally friendly drugs/agents. World Health Organization reports that 80% of world population relies on herbal medicines as their primary source of health-care. Millions of Africans rely on these herbal medicines for their primary health care (McCaleb, 2000). It is estimated that some 20,000 species of higher plants are used medicinally throughout the world (Tagboto and Townson, 2001). Plants have provided the basis for

traditional treatment for different types of diseases and still offer an enormous potential source of new chemotherapeutic agents. The potentials of compounds derived from plants has been reported (Nwude and Ibrahim, 1980; Secoy and Smith, 1983; Phillipson and Wright, 1991; Freiburghans et al., 1996; ITDG and IIRR, 1996; Nok et al., 1996; Adewummi et al., 2001; Atawodi et al., 2003; Hoet et al., 2004; Nok, 2005). Herein we report the *in vitro* activity of *Nauclea latifolia* which have been reported to be antimalarial.

MATERIALS AND METHODS

Collection of plant material

Fresh stem bark of *N. latifolia* was collected from Afaka village 35km to Kaduna (11° 10' N, 7° 38' E). The plant was identified at the herbarium unit of Department of Biological Sciences, Ahmadu Bello University Zaria where voucher specimen number was deposited.

Plant extraction

The fresh stem bark of *N. latifolia* was dried at room temperature for three weeks before crushing into powder. The powdered material (200

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Table 1. Phytochemical screening of *Nauclea latifolia*

Extract	Alkaloids	Anthraquinones	Cardiac glycosides	Flavonoids	Pylobatannins	Saponnins	Tannins	Terpenoids
Crude methanolic extract (ME)	+++	-	++	++	++	++	+++	+++
Aqueous extract (AE)	+	+	-	+++	-	++	++	++

+++ - highly present, ++ - moderately present, + - faintly present, - absent

Table 2. *In vitro* effect of crude aqueous and methanolic stem bark extracts of *Nauclea latifolia* on *Trypanosoma congolense*

Plant name	Family name	Plant parts screened	Extract used	Time taken to Immobilize parasites	Inhibitory concentration (IC) (mg/ml)	Activity on <i>T. congolense</i>
<i>Nauclea latifolia</i>	Rubiacea	Stem bark	Methanol	65min.	20	+
		Diminivet ^R Control (DMSO)	Aqueous	72min.	20	+
			-	48min.	10	+
			-	-	-	Parasites very active even after 2 hours

+ active against *T. congolense*

- not active against *T. congolense*

- IC- concentration at which no trypanosome with a normal morphology/or motility was found when compared to the controls

g) was soaked in 1000 ml distilled water/methanol for 24 h. The extract was filtered and concentrated in a hot air oven set at 35°C for three days. The dried extract was weighed and then stored in air tight container and kept at 4°C until needed.

Animals

Swiss albino mice (20 – 32 g) aged 8 -12 weeks, bred in the College of Agriculture and Animal Science, Ahmadu Bello University, Mando Road Kaduna were used for the study. They were kept in clean plastic cages in a 12 h light /dark cycle with litter changed every week. They were fed with mice cubes specially prepared by ECWA feed Jos Plateau State. They were watered *ad libitum*.

Parasites

Trypanosoma congolense (Nasarawa strain), were obtained from Nigeria Institute for Trypanosomiasis

Research Vom, Plateau State and passage into four mice. The parasites were maintained by serial passages into other mice.

Parasitemia

This was monitored in the inoculated mice by daily obtaining blood from pre sterilized tail using the method of (Herbert and Lumsden, 1976).

Phytochemical screening of extracts

The aqueous/methanolic stem bark extract was screened as described by (Odebiyi and Sofowora, 1990). *In vitro* trypanocidal activity of crude aqueous and methanolic extracts of *Nauclea latifolia*

Aliquots of 20 µl of the extract solution prepared at 10, 5, and 2.5 mg/ml in (0.5% DMSO) concentrations were incubated with 100 µl of parasitized blood in 96 well

microtiter plates (Flow laboratories Inc. Mclean, Virginia 22101, USA) at 37°C. For the control, 20 µl of (0.5% DMSO) was incubated with 100µl of parasitized blood in microtiter plate. Reference test was done with Diminavet^R at the same concentration with the extract. The titer wells were examined at 5 min interval under (X400) objective for cessation in motility /change of morphology of the parasites in comparison to the control.

Statistical analysis

The results were expressed as means ± SEM. Level of significance was assessed by student's t test and ANOVA.

RESULTS

Table 1 shows the result of the phytochemical screening, the methanolic extract had alkaloids,

tannins and terpenoids (highly present); saponnins, glycosides and flavonoids (moderately present) while anthraquinones (absent), aqueous extracts, revealed flavonoids (highly present), while saponnins, tannins, and terpenoids were moderately present and glycosides absent. The percentage yield was 1.2; extract was yellowish in color with a pH 6 - 7. At concentration of 20 mg/ml the parasites were completely immobilised within 65 – 75 min of incubation with aqueous and methanolic extracts, while the standard drug Diminivet[®] immobilize / eliminated the parasite within 48 min of incubation at concentration of 10 mg/ml (Table 2). The control consisting of the parasites incubated with 0.5% Dimethyl sulfoxide (DMSO) showed the presence of very active parasites at the end of 2 h.

DISCUSSION

African plant species have been reported to possess trypanocidal activity (Freiburghaus et al., 1996; Hoet et al., 2004). Aqueous and methanolic extracts of stem bark of *N. latifolia* showed *in vitro* activity against *T. congolense* at 20 mg/ml, when compared to the control that had parasites still active after 2 h of observation. Madubunyi (1995) reported the root extract of *N. latifolia* to have trypanocidal properties on *Trypanosoma brucei*. Atawodi et al., (2003) reported that complete elimination of motility or reduction in motility of parasites when compared to the control could be taken as indices of trypanocidal effects. The result obtained is similar with that reported (Freiburghaus et al., 1996; Adewumi et al., 2001; Atawodi et al., 2003; Wurochekke and Nok, 2002; Hoet et al., 2004; Nok, 2005) that some plants had promising activity against trypanosomes. Though the concentration of the extracts were higher (20 mg/ml) than the concentration of the trypanocide (Diminivet[®]) which was 10 mg/ml. It could be explained that the extracts are still crude and made of complex composition of chemicals while, the standard drug is of a purer compound. Further purification of the extracts could lead to isolation of purer compounds with increased activity like the standard drug. The mechanism by which the extracts eliminate/immobilize the parasites is not immediately known at this stage of the work. Sepulveda – Boza and Cassels, (1996) suggested that many natural products exhibited their trypanocidal 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. Natural products are thought to possess structures capable of generating radicals that may cause peroxidative damage to trypanothione reductase that is very sensitive to alterations in redox balance. Plants are known to contain secondary metabolites (active principle) these include alkaloids, saponnins, terpenoids, tannins, flavonoids and glycosides; these elicit physiological response. Phytochemical screening revealed the extracts to contain alkaloids, flavonoid, tannins, cardiac glycol-

sides, and others. The result agrees with that reported (Hotellier and Delaveau, 1975; Onyeyili, 2001) that stem bark of *N. latifolia* had bitter principles, tannins, flavonoids and alkaloids. Photochemicals in contrast to synthetic pharmaceuticals based upon single chemicals, may exert their effects through the additive or synergistic action of several chemical compounds acting at a single or multiple target sites associated with a physiological process (Kaufmann et al., 1999; Tyler, 1999). Some literatures have reported that flavonoids had antitrypanosomal activity (Tarus et al., 2002) while Nok (2002) reported anzaanthraquinone to have activity against *T. congolense*. The aqueous and methanolic extracts have shown the presence of some of these phytochemicals, at this stage of the work which is preliminary; we can not say which of the phytochemicals could be responsible. Currently we are carrying out fractionation studies of the extract and also carrying out the *in vivo* experiment to confirm its activity.

Conclusion

Aqueous and methanolic extracts of *N. latifolia* stem bark appears to possess antitrypanosomal activity. This supports its medicinal usage in treating malaria, which is a protozoa parasite. The plant is easily found in most parts of Northern Nigeria, which partly explain its wide medicinal usage.

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