American Journal of Biochemistry 2012, 2(1): 1-6
DOI: 10.5923/j.ajb.20120201.01

Evaluation of Medicinal Plants from Nupeland for Their *in vivo* Antitrypanosomal Activity

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Abstract Over 25% of our common medicines contain at least some compounds obtained from plants. Nupeland located on the coastal basins of Rivers Niger and Kaduna in North Central Nigeria is greatly influenced by the savannah-forest vegetations with biodiversity rich in medicinal plants. This type of vegetation also harbours tsetsefly which are the vector of trypanosomes, thus making the area highly endemic to sleeping sickness. Trypanosomiasis is a tropical disease threatening human health and economical development and is responsible for the death of about half a million patients per year. Nupeland inhabited predominantly by Nupe tribe are traditionally noted for generations of using medicinal plants in curing human trypanosomal infections; many of which have not been scientifically proved. Natural products derived from plants offer novel possibilities to obtain new drugs that are active against trypanosomes. In furtherance to the earlier in vitro trypanocidal activity evaluation of some Nupeland medicinal plants; six medicinal plants traditionally used for treatment of sleeping sickness in Nupeland namely: *Acacia nilotica, Bombax buonopozense, Heterotis rotundifolia, Pterocarpus erinaceus, Terminalia avicennioides* and *Zanthoxylum zanthoxyloides* were investigated for in vivo antitrypanosomal activity. The solvent soluble extracts obtained by standard methods from various plant parts (stem barks and fruits) of Nupeland medicinal plants were evaluated for their in vivo antitrypanosomal activities against *Trypanosoma brucei brucei* as well as their phytochemical screening were performed by standard procedures. The extracts of *A. nilotica* (stem bark), *B. buonopozense* (stem bark), *H. rotundifolia* (whole plant), *T. avicennioides* (round fruit) and *Z. zanthoxyloides* (stem bark) were effective on trypanosomes. The extracts of *A. nilotica, B. buonopozense* and *H. rotundifolia* exhibited antitrypanosomal effects at 200, 300 and 800 mg/kg body weight respectively and cleared the parasites from circulation with prolonging survival period of up to 30 days. The stem bark extracts of *P. erinaceus, T. avicennioides* and *Z. zanthoxyloides* showed only trypanostatic effects and could not clear the parasites completely. These extracts contain metabolites that are associated antitrypanosomal effects; hence these medicinal plants may be sources of new antitrypanosomal compounds that may be active against T. b. brucei. The discovery of these potent antitrypanosomal extracts from these plants has increased their potentials to provide lead compounds for the development of new natural drugs for effective treatment of sleeping sickness. It has also justified the claim that some medicinal plants of Nupeland possess antitrypanosomal activity and could be useful in the management of trypanosomiasis. The isolation of trypanocidal compounds from these extracts will require State-Art-Instrumentation particularly for further bioactive fractionation and characterization using chromatographic and spectroscopic techniques.

Keywords Antitrypanosomal, Medicinal Plants, Nupeland, Trypanosomiasis, Sleeping Sickness

1. Introduction

African Trypanosomiasis, also known as African sleeping sickness is one of the neglected diseases in about thirty-six countries of sub-Saharan Africa threatening more than sixty million lives on daily basis[1]. Trypanosomes are the causative agent of sleeping sickness in sub-Saharan Africa[1]. The current chemotherapy of the human trypanosomiasis relies on only six drugs (Suramin, Pentamidine, Melarsoprol Eflornithine, Arsobal and Mel B), five of which were developed > 30 years ago[1]. Furthermore, these drugs display undesirable toxic side effects[1] and studies shown the emergence of drug-resistant trypanosomes[2]. Therefore, the development of cost-effective new drugs in the treatment of sleeping sickness is urgently required in order to control the disease. However, it has also been observed that natural products derived from plants offer novel possibilities to obtain new drugs that are active against trypanosomes 3]. Many investigators targeted finding new anti-trypanosomal agents to combat the trypanosomomiasis by screening extracts of African plants[4-11].

Nigeria is naturally endowed with both savannah and tropical rainforest vegetations. These diverse flora offer a wide spectrum of unique medicinal plants. In Nigeria, the indigenous people are exploiting a variety of herbs for ef-

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Published online at http://journal.sapub.org/ajb

fective curing of various ailments[12]. In Nigeria, several ethnobotanical studies of Nigerian plants used in the traditional management of trypanosomiasis indicated both significant in vitro /in vivo antitrypanosomal activity[13-32].

Notable among these plants studied within the Nigerian biosphere, extracts of *Afrormosia laxiflora, Anogeissus leiocarpus, Annona senegalensis, Cochlospermum planchonii, Khaya senegalensis, Piliostigma reticulatum, Prosopis africana, Securidaca longepedunculata* and *Terminalia avicennioides* distinctively exhibited the significant trypanocidal activity. In few cases, metabolites responsible for the associated activity have been isolated and potent bioactive compounds were reported[33]. This clearly suggests that tropical plants could be a very promising source of new generations of trypanocidal agents. Therefore, the foregoing observations, spur our group to investigate some plants used in the management of sleeping sickness by the indigenous Nupe speaking people of Niger State, Nigeria.

Nupeland located on the coastal basins of Rivers Niger and Kaduna, North Central Nigeria greatly influenced the savannah-forest vegetations of the area. Naturally, this type of vegetation is endemic to sleeping sickness. Nupeland inhabited predominantly by Nupe tribe are agrarian in nature and practice farming with traditional medicine. It is noteworthy that Nupe tribe is traditionally noted for centuries of using medicinal plants in the management of human trypanosomal infections among the inhabitants of Nupeland[34]. The following plants namingly: *Acacia nilotica*, (Nupe: Gabaruwa; Hausa: Bagaruwa); *Bombax buonopozense* (Nupe: Kutukpachi; Igho: Akpe; Yoruba: Ogbolo); *Heterotis rotundifolia* (Nupe: Edingi-bata; Yoruba: Ajagunmorasin), *Pterocarpus erinaceus* (Nupe: Zanchi; Hausa: Madobiya; Igho: Aze egu; Yoruba: Apepe); *Terminalia avicennioides* (Nupe: Kpace; Hausa: Baushe; Igho: Edo; Yoruba: Igi odan); and *Zanthoxylum zanthoxyloides* (Nupe: Kosonkori; Hausa: Fasakuwari ; Yoruba: Ata) are listed as medicinal plants used for the treatment of trypanosomiasis in Nupeland. It is against this background that in vivo antitrypanosomal activity evaluation of six medicinal plants from Nupeland of North Central Nigeria primarily to justify or otherwise the efficacy of these medicinal plants used in the management of human trypanosomal infections among the Nupe tribe was carried out.

2. Methodology

The plant materials used namingly: (Acacia nilotica (stem bark) *Bombax buonopozense* (stem bark), *Heterotis rotundifolia* (whole plant), *Pterocarpus erinaceus* (stem bark), *Terminalia avicennioides* (round fruit), *Zanthoxylum zanthoxyloides* (stem bark) were collected from a forest near Emitete village along Bida-Doko Road in Lavun local Government area of Niger State, Nigeria as described by the traditional healers. They were botanically identified by Mal Muazzim Ibrahim of the Herbarium Unit of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development, Garki – Abuja, Nigeria where voucher specimens were deposited. Extract preparation, phytochemical screening and therapeutic monitoring of the extracts were performed as earlier reported[35] and the extracts of the six Nigerian medicinal plants were evaluated for antitrypanosomal activity against T. b. brucei infected mice[35]. The mice were grouped into A, B, C, E, F, H, G and with groups A, B, C, E, F and H containing three (3) mice each in a group and were treated with various doses of 100 – 500 mg/kg body weight of the extract.

Key:

D = Death of mice

A – Infected but treated with 100mg/kg of the extract.

B – Infected but treated with 200mg/kg of the extract.

C – Infected but treated with 300mg/kg of the extract.

E – Infected but treated with 500mg/kg of the extract.

F – Not infected but treated with 500mg/kgof the extract.

G – Infected but treated with 3.5mg/kg of Berenil.

H – Infected but not treated.

Average parasitemia level of the mice were checked at interval of two days and recorded.

3. Results

Phytochemical substances from the extract of six Nigerian medicinal plants

The phytochemical tests revealed that metabolites such as saponins, tannins, terpenes and carbohydrate have been identified in the parts of the six plant species tested[36-38] (Table 1).

Table 1. Phytochemical analysis of six crude plant extracts

Test	A. n	B. b	D. r	P. e	T. a	Z. z
Alkaloids	-	+	-	+	+	-
Tannins	+	+	+	+	+	+
Glycosides	+	-	-	+	+	+
Saponins	+	+	+	+	+	+
Terpenes	+	+	+	+	+	+
Sterols	+	-	+	+	+	-
Resins	-	+	-	-	-	+
Carbohydrates	+	+	+	+	+	+
Balsam	-	+	-	-	-	+
Flavonoids	+	-	+	+	+	+
Anthraquinones	+	-	+	-	-	-

Key + = present, - = absent, A. n = *A. nilotica*, B. b = *B. buonopozense*, D. r = *D. rotundifolia*, P. e = *P. erinaceus*, T. a = *T. avicennioides*, Z. z = *Z. zanthoxyloides*

Natural products such as alkaloids, terpenes, quinones, and polyphenols have shown potent growth inhibition of T. cruzi[39]. Triterpenoids and sterols from the plants are reported to possess antitrypanosomal activity[40]. Alkaloids are found in extract of B. buonopozense only. The antitrypanosomal activities of alkaloids like actinodaphine, dicentine, cassythine isolated from Cassytha filiformis[41] and several other alkaloids[42] displayed significant in vitro antitrypanosomal activity. The DNA intercalation in combination with portion biosynthesis inhibition is reported to be

the mechanism of action responsible fro the observed trypanosomal effect of the active alkaloids[42]. Flavonoids were found in the extracts of A. nilotica (stem bark), *T. avicennioides* (round fruit) and *Z. zanthoxyloides* (stem bark). The trypanocidal activity of several flavonoids such as quercetagetin[3]; hispidulin and santin[43] have been previously reported. An azaanthraquinone earlier isolated from *A. nilotica* was found to be responsible for the antitrypanosomal effect[44].

In vivo antitrypanosomal activity of the crude plant extracts tested

Investigation of antitrypanosomal activity of traditionally used plants has been a major area of contemporary research focus[3]. In our present studies we investigated the therapeutic potentials of the crude extract of six Nigerian medicinal plants which are the plants prominently used in Nupe ethnomedicine[34] by using *in vivo* screening for antitrypanosomal activity against *Trypanosoma brucei brucei*, which is the etiological agent for sleeping sickness, one of the most serious protozoan diseases in Africa. Therefore, the plant extracts were percolated under laboratory conditions, and concentrated *in vacuo* with a rotary evaporator. The acute toxicity of the extracts were determined alongside with the appropriate dosages used for the trypanocidal efficacy of the six extracts determined as shown in Tables 2-7.

Table 2. Effect of *H. rotundifolia* on parasite count in mice infected with *T. b. brucei*

Post treatment days	A	B	C	E	F	G	H
2	0	0	0	0	0	0	0
4	0	0	0	0	0	0	50
6	5	0	0	0	0	0	100
8	10	0	0	0	0	0	200
10	50	5	0	0	0	0	D
12	80	50	0	0	0	0	
14	100	70	5	0	0	0	

Table 3. Effect of *A. nilotica* on parasite count in mice infected with *T. b. brucei*

Post treatment days	A	B	C	E	F	G	H
2	0	0	0	0	0	0	0
4	0	0	0	0	0	0	60
6	5	0	0	0	0	0	100
8	20	10	0	0	0	0	D
10	30	15	0	0	0	0	
12	50	20	0	0	0	0	
14	70	25	0	0	0	0	

Table 4. Effect of *B. buonopozense* on parasite count in mice infected with *T. b. brucei*

Post treatment days	A	B	C	E	F	G	H
2	0	0	0	0	0	0	0
4	8	0	0	D	D	0	5
6	100	0	0			0	100
8	50	0	0			0	150
10	50	0	0			D	200
12	D	0	0				D
14		20	0				

Table 5. Effect of *T. avicennioides* on parasite count in mice infected with *T. b. brucei*

Post treatment days	A	B	C	E	F	G	H
2	0	0	0	0	0	0	0
4	0	0	10	25	0	0	10
6	10	50	60	60	0	5	150
8	50	D	100	90	0	0	D
10	100		100	90	0	0	
12	D		150	D	0	0	
14			D		D	0	

Table 6. Effect of *Z. zanthoxyloides* on parasite count in mice infected with *T. b. brucei*

Post treatment days	A	B	C	E	F	G	H
2	0	0	0	0	0	0	0
4	0	0	0	0	0	0	80
6	0	0	0	D	D	8	90
8	80	80	65			0	100
10	100	100	100			0	D
12	100	110	100			0	
14	120	D	D			0	

Table 7. Effect of *P. erinaceaus* on parasite count in mice infected with *T. b. brucei*

Post treatment days	A	B	C	E	F	G	H
2	0	0	0	0	0	0	0
4	2	1	0	0	0	0	40
6	5	2	0	0	0	0	D
8	11	5	0	0	0	0	
10	20	10	5	0	0	0	
12	100	50	10	8	0	0	
14	D	D	D	10	0	0	

This result indicates that oral administration showed inhibition of parasitemia. Reports show that 50% deparasitation is an indication of significant activity[45]. Some researchers have shown that treatment failure is possible in cases of massive parasitemia at the time of the therapeutic intervention[46]. It is possible that the early treatments with lower concentrations of the crude extract may have favoured treatment failure. It may be that blood parasitemia could be completely eliminated if the treatment is initiated at high dose of crude extract of *H. rotundifolia*. This result confirms that the crude extract of *H. rotundifolia* contains active compounds against T. b. brucei.

The crude extract of the stem bark of *Z. zanthoxyloides* had no trypanocidal effect, even though its root bark extract was reported to be active[19]. The failure of the crude extract of the stem bark of *Z. zanthoxyloides* to show any trypanocidal action depicts that the anti-trypanosomes are lacking in the stem bark as opposed to the claims by the healers which according to the existing literature is used in Nupe ethnomedicine in the management of sleeping sickness in Nupeland[34]. However, we have also observed that the crude extract of the stem bark of *Z. zanthoxyloides* was found to exhibit trypanostatic activity. Similar to the early reports of the trypanostatic effect of ethanolic extract of the stem bark of *Faidherbia albida* and it is also effective in the management of anaemia induced by T. b. brucei in rats[47]. Trypanostatic effect is said to suppress the activity of the parasite there by sustaining the life of the mice when compared to

the control group. It is worthy to be mention that some plants have already been investigated for their antitrypanosomal activity in other studies. For instance; water, methanol and dichloromethane extracts of the leaves of *T. avicennioides* tested, but only methanolic extracts were active on *T. b. brucei*[6]. Stem bark extracts of the same plant species has shown *in vitro* effect against *T. b. brucei*[33].

In our present studies, the round fruit extract of *T. avicennioides* tested for its in vivo antitrypanosomal activity gave a result that are efficaciously comparable to those of previous investigators who showed that extracts of *T. avicennioides* were active in vitro on *T. b. brucei*[6,33]. *T. avicennioides* (round fruit) and *Z. zanthoxyloides* (stem bark) were effective on trypanosomes trypanostatically only.

The most interesting results are those of *A. nilotica*, *B. buonopozense* and *H. rotundifolia* extracts which showed the antitrypanosomal effects at 200 and 300 mg/kg body weights. Doses were able to clear the parasites from circulation within 6 and 7 days of treatment respectively with prolonging survival period of up to 30 days. While the extracts of *T. avicennioides* and *Z. zanthoxyloides* showed trypanostatic effects and could not clear the parasites completely.

From the phytochemical studies, the type of compounds which could be responsible for the antitrypanosomal activities are associated with the metabolites and the results obtained showed that the crude extract of *Z. zanthoxyloides* contain some metabolites that suppresses the activity of the trypanosomes rather than killing the parasites completely.

The stem bark extract of *P. erinaceaus* exhibit mild antitrypanosomal activities and could be more effective in the treatment of trypanosomiasis if the extract is purified further since the parasitemia level increases and the mice were not able to survive for a long period (Table 7). Despite that the mice were not able to survive for a long time, the infected mice treated with extract still live longer than those infected but not treated which only lived for just six days.

This study has provided evidence that *T. avicennioides* and *Z. zanthoxyloides* extracts exhibits trypanostatic effect which is often associated with reduction in anemia and promote weight gain in experimental African trypanosomiasis[48]. Anaemia is the most outstanding clinical and laboratory feature of African trypanosomiasis[6] and also the primary cause of death[49]. The stem bark of *Z. zanthoxyloides* is used in treating sickle cell anaemia and analgesic[34]. Trypanostatic effect of the plant extracts were explained with corresponding increase in PCV which prolong the lifespan of treated animals by reducing the parasite load or neutralizing the toxic metabolites produced by trypanosomes[13]. Based on these results and the fact that plant part extracts are traditionally used in the treatment of African Trypanosomiasis. Nevertheless, the activity values for the active extracts were high compared to the values obtained for commonly used trypanocidal drugs diminazene aceturate. However, since the crude extracts have a very complex composition, purification might lead to pure compounds with highly increased activity. Such differences between our results and those of other authors may be due to the known variation in the chemical composition of plants according to the geographical area and the time or season of collection[33].

In our in vivo studies, with mice infected with T. b. brucei; the methanol extracts of round fruit of T. avicennioides and stem barks of *A. nilotica, B. buonopozense, P. erinaceus* and *Z. zanthoxyloides* were investigated, but only *A. nilotica, B. buonopozense* and *P. erinaceus* were found to be significantly active. The antitrypanosomal activity of six crude extracts of medicinal plants from Nupeland determined by in vivo using T. b. brucei, only *A. nilotica* and *B. buonopozense* were the most promising ones. The results of the present study confirmed that the use of medicinal plants in folk medicine contributes significantly to primary health care, and that natural products are potential sources of new drugs for the treatment of important tropical diseases caused by trypanosomes. The high activity values obtained for these plants render them candidates for the isolation of anti- trypanosomal compounds which could develop into new lead structures for drug development. Therefore, the trypanocidal effects of extracts will require further isolation of trypanocidal compounds from these extracts which requires State-Art-Instrumentation particularly for further bioactive fractionation and characterization using chromatographic and spectroscopic techniques.

4. Conclusions

Many plant species are used in traditional Nigeria medicine to alleviate symptoms of trypanosomiasis, and several interesting extracts and chemical constituents have originated for further inquiry following *in vitro* and *in vivo* antitrypanocidal activity evaluation. However, much work remains to be done on the systematic assessment of antitrypanosomal efficacy of local plants against trypanosomes.

ACKNOWLEDGEMENTS

We thank the the traditional healer consulted, Baba Alhassan Bangbara Bida who supplies the ethnobotanical information of these plants. We are also grateful to Mal Muazzim Ibrahim of the Herbarium Unit of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development, Garki – Abuja, Nigeria for identifying the plant species.

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