

## Evaluation of medicinal plants from Mali for their in vitro and in vivo trypanocidal activity

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### Abstract

Water, methanol and dichloromethane extracts prepared from various parts of 40 medicinal plant species from Mali were investigated for their trypanocidal activity against *Trypanosoma brucei brucei*. Of a total of 165 extracts tested in vitro in the Low Inoculation Long Incubation Test (LILIT), 24 extracts showed a high trypanocidal activity. Using the Long-Term Viability Assay (LtVA) for corroboration of the results of the 24 extracts, it was found that 15 samples had minimum inhibitory concentration (MIC) values > 10 µg/ml, eight MIC values of 100 µg/ml and one MIC values of 50–100 µg/ml. So far, four extracts with MIC values ≤ 100 µg/ml were tested for antitrypanosomal activity in mice, experimentally infected with *Trypanosoma brucei brucei*. Only, the aqueous extracts of the leaves of *Terminalia avicennioides* Guill. and Perr. (Combretaceae) and the stem bark of *Ceiba pentandra* (L.) Gaertn. (Bombacaceae) were able to reduce the parasitaemia in animals treated at the dose of 100 mg/kg b.w. (intraperitoneally, two times daily for 3 days) and of 150 mg/kg b.w. (per os, two times daily for 3 days), respectively. The reduction of parasitaemia was, however, statistically significant ( $p=0.002$ ) only in case of treatment with *Terminalia avicennioides*.

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### 1. Introduction

Tsetse-transmitted trypanosomosis is of great significance to human health and animal production in Africa. The human disease (sleeping sickness) is caused by *Trypanosoma brucei rhodesiense* in East Africa and *Trypanosoma brucei gambiense* in West and Central Africa and is highly debilitating and invariably fatal if untreated. African animal trypanosomosis (AAT) is essentially caused by *Trypanosoma congolense*, *Trypanosoma vivax* and *Trypanosoma brucei* and is one of the most important diseases of domestic livestock in sub-Saharan Africa.

The control of human and animal trypanosomosis is based on a limited number of compounds, many of which are chemically closely related and have been in use for more than 40 years. The repeated use of trypanocidal drugs in the control of AAT has

led to the development of drug-resistant trypanosome populations. So far, resistance to one or more of the three trypanocidal drugs used in cattle has been reported in a least 13 countries in sub-Saharan Africa (Geerts and Holmes, 1998). Drug resistance in human infective trypanosomes plays a smaller role than in animals, the control of sleeping sickness being much more impeded by high toxicity and limited efficacy of the drugs used in the late-stage of the disease (Matovu et al., 2001). Because of these serious problems encountered in the control of both human and animal trypanosomosis, there is an urgent need for new trypanocidal drugs.

Plants have been, since immemorial time, among the common sources of medicaments. Most of plant-derived medicines have been developed on the basis of traditional knowledge in health care and in many cases, there is a correlation between the indications of pure substances and those of respective crude extracts used in traditional medicine (Farnsworth et al., 1985). Literature surveys and field studies showed that plants are used in traditional medicine in Africa to treat trypanosomosis in humans

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and animals (Bizimana, 1994; Freiburghaus et al., 1996; Youan et al., 1997). So far, only few of these plants have been evaluated for their trypanocidal activity (Asuzu and Chineme, 1990; Freiburghaus et al., 1996, 1997; Youan et al., 1997; Adewunmi et al., 2001). In the present work, 59 plant parts from 40 plant species collected in Mali, which are used in traditional medicine to treat trypanosomoses in both humans and animals, have been screened for their in vitro, and partly, in vivo trypanocidal activity. The aim of this study was to verify whether the claimed trypanocidal properties of these plants in traditional medicine can be scientifically confirmed. It was hoped that the trypanocidal activity of one or more plant part(s) could be proven and that, thus, this work would contribute to the acceptance of traditional medicine and to the solution of the growing problems of drug resistance of trypanosomes.

## 2. Materials and methods

### 2.1. Plant collection and authentication

Using the existing knowledge at the *Département de Médecine Traditionnelle* in Bamako, Mali (Diallo et al., 1996), the investigated plants (Table 1) were collected in the southern part of Mali (Siby, Kalassa, Dankorodalaba, Tienfala, Moribabougou, Mandjo, Bandiagara, Kalibombo, Sido, Blendio and Sikasso) and authenticated by Coulibaly Djibril and Drissa Diallo in November 2001. Their voucher specimens are preserved at the Herbaria of the *Département de Médecine Traditionnelle* in Bamako, Mali and the Institute for Parasitology and International Animal Health, Freie Universität, Berlin, Germany.

### 2.2. Preparation of crude plant extracts

Collected plant parts were dried in the shade and powdered at the *Département de Médecine Traditionnelle* in Bamako. After transport to Germany, crude extracts were prepared in Berlin by consecutively extracting 20–40 g of the powdered plant material by distilled water, methanol and dichloromethane. A 10-fold quantity of solvent in relation to plant material was used for the extraction. The extraction by water was performed at room temperature for 1 h and then for 20 min. by sonification. The extract was filtered through filter paper (Schleicher and Schuell, Germany), lyophilised and stored at 4 °C until use. The extraction by methanol and dichloromethane took place under reflux. The extracts were then filtered through a filter paper and concentrated by removing the solvents on a rotary evaporator. Once removed, the solvent-free extracts were stored at room temperature until use. For in vitro tests, stock solutions were prepared by solving appropriate amounts of plant extract into 100% DMSO and stored at –20 °C. The test extract solutions were freshly prepared from the stock solutions and diluted with culture medium (see Section 2.6) to give the highest concentration of DMSO < 1%.

### 2.3. Trypanocidal drug

Commercial diminazene aceturate (Berenil<sup>®</sup>, Hoechst AG, Germany, Batch No. 507W742) was used to validate the tests

and to give reference values. Concentrations were calculated on the basis of 44.5% active ingredient of diminazene aceturate in Berenil. A stock solution of the drug was prepared in distilled water, filtered through a 0.2 µm membrane filter (Schleicher and Schuell, Germany), portioned and stored at –20 °C. For the test, the required quantity was thawed on the day of use and correspondingly diluted with culture medium.

### 2.4. Trypanosome stock

The *Trypanosoma brucei brucei* STIB 345 strain for both in vitro and in vivo studies is a derivative of the stock EATRO 1529, which was isolated in 1969 from an infected *Glossina pallidipes* in Kiboko, Kenya and cryopreserved after six passages in mice. In 1973, EATRO 1529 was stabilised after five short passages in rats and re-named as STIB 345 (Brun et al., 1979). STIB 345 is sensitive to diminazene aceturate (Kaminsky, personal communication).

### 2.5. Feeder layer cells

Fibroblast-like cells, originally isolated from 15-day-old embryos of *Microtus montanus-mice* and adapted for in vitro tests, were used for the cultivation of blood stream trypanosome forms. The cell line used originated from the Swiss Tropical Institute Basel. For in vitro testing, 10<sup>4</sup> cells were transferred into each well of 96-well microtiter plates (Greiner Bio-One, Germany).

### 2.6. Culture medium

The medium for the cultivation of bloodstream trypanosome forms was prepared according to Baltz et al. (1985) with some modifications. It consisted of Minimum Essential Medium (MEM) 25 MM hepes with Earle's salts without L-glutamine (Invitrogen Corporation, UK) supplemented with 10 ml/l non-essential amino acids (100×), 0.292 g/l L-glutamine, 1.600 g/l glucose, 0.181 g/l L-cysteine, 0.110 g/l pyruvic acid–Na–salt, 0.039 g/l thymidine and 0.068 g/l hypoxanthine. This stock medium was further supplemented with 20 ml/100 ml of horse serum (Invitrogen Corporation, UK), 5 ml/100 ml of cattle serum (Invitrogen Corporation, UK) and with 1 ml/100 ml of anticontamination cocktail according to Mäser et al. (2002). Immediately before use, this medium was supplemented with 100 µl/20 ml 2-mercaptoethanol 50 MM. The *Microtus montanus* embryonal fibroblast-like cells were cultivated in the same medium, the only difference being that the stock medium was supplemented with 10 ml/100 ml of calf bovine serum (Invitrogen Corporation, UK) instead of horse and cattle serum.

### 2.7. Experimental animals

*Mastomys coucha-mice* of either sex, weighing 30–40 g, were supplied from the breeding colony of the Institute for Parasitology and International Animal Health of the Freie Universität Berlin. The animals were maintained on standard pellet diet and water ad libitum during the entire trial period.

Table 1  
Investigated medicinal plants collected in the South of Mali in November 2001

Plant species and family	Traditional names in Bambara	Voucher numbers	Plant parts	Traditional preparations
<i>Acacia nilotica</i> (L.) Delile (Mimosaceae)	Buwanan, Bogonan	M2a	Stem and root bark	Decoction, infusion
<i>Afzelia africana</i> Pers. (Leguminosae)	Lingué, Dankan	L1a	Stem bark	Decoction
<i>Ampelocissus grantii</i> (Baker) Planch (Vitaceae)	Kongho forokofaraka	Via	Aerial parts	Maceration
<i>Annona senegalensis</i> Pers. (Annonaceae)	Mondé-Sounsoun	A2	Stem bark, leaves	Decoction, infusion, maceration
<i>Anogeissus leiocarpus</i> Guill. and Perr. (Combretaceae)	Ngalama	C5a	Root bark, leaves	Decoction
<i>Balanites aegyptiaca</i> Delile (Simaroubaceae)	Nsègèné	SI	Roots, root bark, shoots	Decoction, infusion, powder
<i>Bauhinia reticulata</i> DC. (Leguminosae)	Niaman	Lib	Twigs/leaves	Decoction
<i>Boscia angustifolia</i> A. Rich. (Capparaceae)	Berecè	C2	Stem bark, twigs/leaves	Powder, decoction
<i>Cassia siebehana</i> DC. (Leguminosae)	Sinja	Lie	Roots	Decoction, maceration, infusion
<i>Ceiba pentandra</i> (L.) Gaertn. (Bombacaceae)	Banan	B2	Stem bark, root bark, leaves	Decoction
<i>Celtis integrifolia</i> Lam. (Urticaceae)	Kamaua, Gamyà	U1	Twigs/leaves	Decoction
<i>Cissua quadrangularis</i> L. (Vitaceae)	Wulujòlòkòba	V1b	Twigs/leaves	Decoction
<i>Cochlospermum tinctorium</i> Perr. (Bixaceae)	N'tiribara	B1	Roots	Powder, decoction, infusion
<i>Combretum glutinosum</i> Guill. and Perr. (Combretaceae)	Diangara, Tiangara	C5b	Stem bark	Decoction, infusion
<i>Combretum micranthum</i> G. Don (Combretaceae)	Ngolobè	C5c	Leaves	Decoction
<i>Diospyros mespiliformis</i> Hochst. DC. (Ebenaceae)	Sounsounfing	E1	Leaves	Decoction
<i>Entada africana</i> Guill. and Perr. (Mimosaceae)	Samanèrè	M2b	Roots	Decoction, infusion, maceration
<i>Erythrophleum guineense</i> G. Don (Leguminosae)	Tali, Teli	Lid	Leaves	Decoction
<i>Ficus iteophylla</i> Miq. (Moraceae)	Nsèrèninjè, Jatigifaga-yiri	M3	Leaves	Decoction
<i>Gardenia triacantha</i> DC. (Rubiaceae)	Burencè	R1a	Roots	Powder, maceration
<i>Guiera senegalensis</i> J.F.Gmel. (Combretaceae)	Nkunje	C5d	Roots, leaves	Decoction
<i>Holarrhenanfloribunda</i> Durand and Schinz (Apocynaceae)	Bassira, Bassoro	A3	Twigs/leaves	Decoction
<i>Khaya senegalensis</i> A. Juss. (Meliaceae)	Jala	M1a	Stem bark	Decoction, maceration
<i>Lannea microcarpa</i> Engl. and Krause (Anacardiaceae)	Npeguba	A1	Stem bark, leaves	Decoction
<i>Lawsonia alba</i> Lam. (Lythraoae)	Jabi	L4	Twigs/leaves	Infusion
<i>Leptadenia hastata</i> Decne. (Asclepiadaceae)	Nsoyin	A4	Aerial parts	Decoction, infusion
<i>Loranthus pentagonia</i> DC. (Loranthaceae)	Si-ladon	L3	Whole plant	Decoction
<i>Maytenus senegalensis</i> (Lam.) Exell (Celastraceae)	Ngeke	C3	Twigs/leaves	Decoction, infusion
<i>Mitragyna inermis</i> Kuntze (Rubiaceae)	Jun	Rib	Leaves, stem bark	Decoction, infusion
<i>Nauclea latifolia</i> Blanco (Rubiaceae)	Baro	R1c	Root bark	Decoction, infusion
<i>Opilia celtidifolia</i> Endl. Ex Walp (Olacaceae)	Korongoyin, Sogèn	O1	Twigs/leaves	Infusion, decoction, maceration
<i>Parinari curatellifolia</i> Planch. Ex Benth. (Chrysobalanaceae)	Tutu	C4	Stem bark, twigs/leaves	Decoction,
<i>Pterocarpus erinaceus</i> Lam. (Fabaceae)	Gòni, Gouéni	F1	Shoots	Infusion, decoction
<i>Securidaca longepedunculata</i> Fresen. (Polygalaceae)	Joro, Diro	PI	Roots	Maceration, decoction, infusion
<i>Spilanthes oleraceae</i> Jacq. (Asteraceae)	Farimani	A5	Flowers	Decoction
<i>Sterculia tormentosa</i> Guill. and Perr. (Sterculiaceae)	Kènyèkoro, Kungosira	S2	Roots, stem bark	Decoction, maceration
<i>Strychnos spinosa</i> Lam. (Loganiaceae)	Nkantoroni	L2	Roots	Maceration, decoction
<i>Tamarindus indica</i> L. (Caesalpiniaceae)	Ntomi	CI	Leaves	Decoction, maceration, infusion
<i>Terminalia avicennoides</i> Guill. and Perr. (Combretaceae)	Wolocè, Wòlòbugun	C5e	Stem bark, root bark, leaves	Decoction infusion, maceration
<i>Trichilia emetica</i> Vahl (Meliaceae)	Sulafinsan	M1b	Stem bark	Decoction, infusion, maceration

## 2.8. In vitro evaluation of trypanocidal activity

For the determination of the in vitro trypanocidal activity of crude plant extracts, the Long Incubation Low Inoculation Test (LILIT) (Brun and Lun, 1994) was used as a screening test, whereas the Long-Term Viability Assay (LtVA) (Kaminsky et al., 1989) was subsequently used for confirmation.

### 2.8.1. LILIT

Extract dilutions were prepared in duplicate in a 96-well microtiter plate (Greiner Bio-one, Germany) in 100  $\mu$ l trypanosome culture medium per well. Another 100  $\mu$ l containing  $10^4$  trypanosomes in the logarithmic growth phase were added to each well. The final concentrations of the extracts were 100, 10, 5 and 1  $\mu$ l/ml, respectively. Cultures without and with diminazene aceturate in 2- or 10-fold dilutions from 0.01 to 1  $\mu$ g/ml were prepared under identical conditions as for the extracts. The plates were then incubated at 37 °C with 5% CO<sub>2</sub> added to the air in a humidified incubator. After 96 h, they were examined under an inverted phase microscope and the minimum inhibitory concentration (MIC) of the extracts and the control cultures was determined. The MIC was defined as the lowest concentration of plant extract at which trypanosomes had lost their normal morphology or motility. For confirmation, all extracts which showed MIC values  $\leq$  10  $\mu$ g/ml were tested twice.

### 2.8.2. LtVA

The Long-Term Viability Assay was set up in the similar way as the LILIT, the differences being that the trypanosomes were cultivated in the presence of feeder layer cells and that the plates were incubated for 10 days. Moreover, the medium and the extract/drug were changed when required according to the density of the trypanosomes. The plates were daily examined until the MIC determination on the 10th day. The plant extracts were tested at a final concentration of 1, 10, 20, 50, 100, 500 and 1000  $\mu$ g/ml. Only extracts showing MIC values  $\leq$  10  $\mu$ g/ml in LILIT were investigated in the LtVA.

## 2.9. In vivo evaluation of trypanocidal activity

Due to insufficient quantities of the extracts, only four of the nine extracts found to be active (MIC values  $\leq$  100  $\mu$ g/ml) in the LtVA (Table 3) were evaluated in vivo for their trypanocidal activity using the standardised mouse test (Eisler et al., 2001) with minor modifications. After intraperitoneal inoculation of each mouse with  $1 \times 10^5$  trypanosomes (*Trypanosoma brucei* STIB 345) treatment was administered *p.o.* or *i.p.* 24 h later. Control and treatment groups consisted of six animals each. The average treatment dose was 100 or 150 mg/kg b.w., two times daily, for 3 days. All extracts were freshly prepared in distilled water and administered at the correct dose, at a v/w ratio of 0.1 ml/10 g of mouse weight. Mice were checked daily during 5 days after the first treatment to estimate the number of trypanosomes in their tail blood in a wet blood film. The absolute number of parasites per millilitre of blood was calculated as log using the rapid matching method for estimating the host's parasitaemia according to Herbert and Lumsden (1976). At higher

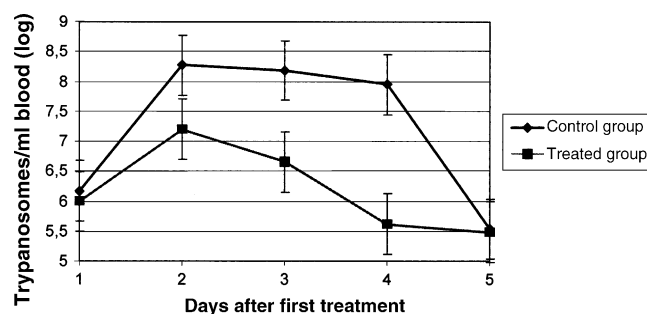


Fig. 1. Effect of *Terminalia avicennioides* on parasitaemia in mice infected with *Trypanosoma brucei brucei* (STIB 345).

levels of infection, this was achieved by matching microscopic fields of a wet blood film against charts and, when fewer parasites were present, by counting the number of trypanosomes in 5, 10 or 20 such microscope fields. For the assessment of the antitrypanosomal effect of the extracts, the level of parasitaemia (expressed as log of the absolute number of parasites per millilitre of blood) in treated animals was compared with that in control animals (Fig. 1).

## 2.10. In vitro and in vivo toxicity

As the growth inhibition of the trypanosomes in vitro could have been caused by general toxicity of the extracts, the feeder layer cells of the LtVA were used to evaluate their general toxicity. The cells were observed under an inverted phase microscope for eventual damages during the whole test period of 10 days. The general toxicity of the extracts in mice was evaluated by observing clinical signs and, partly, by pathological examinations.

## 2.11. Statistical analysis

To assess the therapeutic effects, the parasitological data of treated and control animals were statistically analysed using the Mann–Whitney test. *p*-Values  $<$  0.05 were considered as significant, those  $>$  0.05 as not significant.

## 3. Results

### 3.1. Results of in vitro evaluation of trypanocidal activity

A total of 165 extracts of 59 plant parts from 40 plant species (Table 1) was examined in LILIT. Two extracts showed MIC values of 1 or of 5  $\mu$ g/ml, 22 extracts MIC values of 10  $\mu$ g/ml, 98 extracts MIC values of 100  $\mu$ g/ml and 43 extracts MIC  $>$  100  $\mu$ g/ml. The MIC values of the most active extracts (MIC values  $\leq$  10  $\mu$ g/ml) are shown in Table 2. Diminazene aceturate used as a positive control had a MIC value of 0.05  $\mu$ g/ml.

For confirmation, the trypanocidal activities of the most active extracts in LILIT were re-evaluated in LtVA. The MIC values of the most active extracts in this test are shown in Table 3. One extract showed MIC values in the range 50–100  $\mu$ g/ml, eight extracts a MIC value of 100  $\mu$ g/ml and 15 extracts MIC values  $>$  100  $\mu$ g/ml. Diminazene aceturate used as a positive control

Table 2  
Minimum inhibitory concentration (MIC) values of the most active plant extracts from Mali as assessed in the Low Inoculation Long Incubation Test (LILIT) against *Trypanosoma brucei brucei* STIB 345

Plant species/kind of extract	Plant part(s)	MIC ( $\mu\text{g/ml}$ )
<i>Acacia nilotica</i> , H <sub>2</sub> O-extract	Stem bark	5
<i>Azelia africana</i> , CH <sub>3</sub> OH-extract	Stem bark	1
<i>Ampelocissus grantii</i> , H <sub>2</sub> O-extract	Aerial parts	10
<i>Ampelocissus grantii</i> , CH <sub>3</sub> OH-extract	Aerial parts	10
<i>Anogeissus leiocarpus</i> , CH <sub>3</sub> OH-extract	Root bark	10
<i>Boscia angustifolia</i> , H <sub>2</sub> O-extract	Twigs/leaves	10
<i>Boscia angustifolia</i> , CH <sub>3</sub> OH-extract	Twigs/leaves	10
<i>Boscia angustifolia</i> , CH <sub>2</sub> Cl <sub>2</sub> -extract	Twigs/leaves	10
<i>Ceiba pentandra</i> , H <sub>2</sub> O-extract	Stem bark	10
<i>Celtis integrifolia</i> , H <sub>2</sub> O-extract	Twigs/leaves	10
<i>Celtis integrifolia</i> , CH <sub>3</sub> OH-extract	Twigs/leaves	10
<i>Cissus quadrangularis</i> , CH <sub>3</sub> OH-extract	Twigs/leaves	10
<i>Cissus quadrangularis</i> , CH <sub>2</sub> Cl <sub>2</sub> -extract	Twigs/leaves	10
<i>Combretum micranthum</i> , H <sub>2</sub> O-extract	Leaves	10
<i>Gardenia triacantha</i> , CH <sub>3</sub> OH-extract	Roots	10
<i>Lawsonia alba</i> , CH <sub>3</sub> OH-extract	Twigs/leaves	10
<i>Nauclea latifolia</i> , CH <sub>3</sub> OH-extract	Root bark	10
<i>Nauclea latifolia</i> , CH <sub>2</sub> Cl <sub>2</sub> -extract	Root bark	10
<i>Spilanthes oleraceae</i> , CH <sub>3</sub> OH-extract	Flowers	10
<i>Terminalia avicennioides</i> , H <sub>2</sub> O-extract	Leaves	10
<i>Terminalia avicennioides</i> , CH <sub>3</sub> OH-extract	Leaves	10
<i>Terminalia avicennioides</i> , CH <sub>2</sub> Cl <sub>2</sub> -extract	Leaves	10
<i>Terminalia avicennioides</i> , H <sub>2</sub> O-extract	Root bark	10
<i>Terminalia avicennioides</i> , CH <sub>2</sub> Cl <sub>2</sub> -extract	Root bark	10
Diminazene aceturate	–	0.05

had MIC values in the range of 0.01–0.05  $\mu\text{g/ml}$ . With regard to toxicity, no damages of the feeder layer cells were observed during the test period of 10 days.

### 3.2. Results of in vivo evaluation of trypanocidal activity

So far, only four extracts with MIC values in the range of 50–100  $\mu\text{g/ml}$  in LtVA have been investigated in vivo. None of those tested was able to clear the blood of infected animals from trypanosomes. However, the aqueous extracts of the leaves of *Terminalia avicennioides* and of the stem bark of *Ceiba pentandra* reduced the parasitaemia of infected animals at the dose of

Table 3  
Minimum inhibitory concentration (MIC) values of the most active plant extracts from Mali as determined in the Long-Term Viability Assay (LtVA) against *Trypanosoma brucei brucei* STIB 345

Plant species/kind of extract	Plant part(s)	MIC ( $\mu\text{g/ml}$ )
Diminazene aceturate	–	0.01–0.05
<i>Terminalia avicennioides</i> , H <sub>2</sub> O-extract <sup>a</sup>	Leaves	50–100
<i>Acacia nilotica</i> , H <sub>2</sub> O-extract <sup>a</sup>	Stem bark	100
<i>Azelia africana</i> , CH <sub>3</sub> OH-extract	Stem bark	100
<i>Anogeissus leiocarpus</i> , CH <sub>3</sub> OH-extract <sup>a</sup>	Root bark	100
<i>Ceiba pentandra</i> , H <sub>2</sub> O-extract <sup>a</sup>	Stem bark	100
<i>Celtis integrifolia</i> , H <sub>2</sub> O-extract	Twigs/leaves	100
<i>Combretum micranthum</i> , H <sub>2</sub> O-extract	Leaves	100
<i>Lawsonia alba</i> , CH <sub>3</sub> OH-extract	Twigs/leaves	100
<i>Terminalia avicennioides</i> , H <sub>2</sub> O-extract	Root bark	100

<sup>a</sup> Plant extracts evaluated in *Mastomys coucha*-mice for their trypanocidal activity using *Trypanosoma brucei brucei* STIB 345 as test organism.

100 mg/kg b.w. (i.p.) for 3 days and 150 mg/kg b.w. (p.o.) for 3 days, respectively. When applying the Mann–Whitney test, the difference between treated and control animals was significant ( $p=0.002$ ) only in case of treatment with *Terminalia avicennioides* (Fig. 1). Furthermore, it seemed that the extract of the latter plant had a narrow therapeutic index, as its administration at the single dose of 200 mg/kg b.w. (i.p.) was lethal for mice within 5–6 days. Pathological examinations of dead mice treated with 200 mg/kg b.w. (i.p.) showed several necrotic areas in liver and kidneys.

## 4. Discussion

The aim of the present work was to verify whether the claimed trypanocidal properties of one or more of the plant parts used in West Africa in traditional medicine to treat trypanosomes in both humans and animals could be scientifically confirmed.

The LILIT results showed that 24 (14.5%) of the 165 tested extracts (Table 2) had a high antitrypanosomal activity of MIC values  $\leq 10 \mu\text{g/ml}$ . The antitrypanosomal activity of those extracts re-tested in LtVA could only partly be confirmed. Thus, only 9 (37.5%) of the 24 tested extracts (Table 3) showed antitrypanosomal activity with MIC values in the range of 50–100  $\mu\text{g/ml}$ . The remaining 14 extracts (62.5%) had MIC values  $> 100 \mu\text{g/ml}$ .

It is remarkable that the activity of the 24 extracts tested in both tests was weaker (up to 100-fold) in LtVA than in LILIT. Thus, the methanol extract of the stem bark of *Azelia africana* and the aqueous extract of the stem bark of *Acacia nilotica* which had in LILIT MIC values of 1 and 5  $\mu\text{g/ml}$ , respectively, had MIC values of 100  $\mu\text{g/ml}$  in LtVA. Other extracts which had MIC values of 10  $\mu\text{g/ml}$  in LILIT had MIC values of  $\geq 100 \mu\text{g/ml}$  in LtVA. It seems that the two test systems are not comparable. Indeed, they differ not only in the duration but also in the complexity as feeder layer cells are used in LtVA and not in LILIT. Possibly, the feeder layer cells could enhance the resistance of trypanosomes to extracts. However, this is not the case for diminazene aceturate, as we have in both bioassays almost similar MIC values ranging from 0.01 to 0.05  $\mu\text{g/ml}$ .

The results of the observation of the feeder layer cells showed that the general toxicity of the tested extracts was low. Therefore, the growth inhibition of trypanosomes in LtVA was not due to general toxicity of these extracts.

The results of in vivo studies showed that none of the four extracts tested was able to clear the blood of infected animals from trypanosomes and that only one of those extracts (the aqueous extract of the leaves of *Terminalia avicennioides*) tested was able to significantly reduce ( $p=0.002$ ) the parasitaemia in infected mice (Fig. 1). However, the administration of this extract at the dose of 200 mg/kg (i.p., once) was lethal to mice. All the same, it is thought that the particular extract has a promising potential, warranting an identification of the active ingredient, possibly with lower toxicity but with higher activity.

The antitrypanosomal activity in mice was only checked during the first 5 days, since this was the period where a proper evaluation was possible. Indeed, we had observed that from the fourth or fifth day after the first treatment, the parasitaemia in

infected *Mastomys coucha*-mice mostly disappeared, independently of treatment, only to reappear in some cases after some weeks in a weaker, more chronic form.

The comparison of the activity of the tested extracts with commercial diminazene aceturate in our in vitro and in vivo studies shows that the classical drug is much more active than the extracts. For instance, the activity of the most active extracts in LILIT and LtVA was up to 200- and 10,000-fold, respectively, weaker than in diminazene aceturate. In mice, the activity of the aqueous extract of *Terminalia avicennioides* was at least five-fold weaker, since the curative dose of diminazene aceturate for drug sensitive trypanosome populations in mice is estimated to be below 20 mg/kg b.w. (Eisler et al., 2001). These differences between the activity of the extracts and diminazene aceturate were not surprising, as the crude extracts consist probably of a mixture of many substances, the substance(s) with possible antitrypanosomal activity being present only in a small concentration. The isolation of the active ingredients should help to prepare extracts/drugs with higher concentrations of active ingredients.

As to the extracts, which showed no or little activity in our in vitro systems, we cannot definitely state that they do not possess trypanocidal activity. Indeed, the selection of extracts to be tested in LtVA was arbitrary, including in the test only extracts which showed in LILIT MIC values  $\leq 10 \mu\text{g/ml}$ . Through this arbitrary selection, extracts with MIC  $> 10 \mu\text{g/ml}$  in LILIT possibly containing strong active principle(s) in low concentration(s) might have been excluded from further investigation. It is possible as well that the three extracts which were inactive in mice might exhibit their trypanocidal activity only when tested in humans or target animals, such as cattle.

The comparison of our in vitro and in vivo results is in conformity with work from other authors. Trials by Freiburghaus et al. (1996) revealed a high antitrypanosomal activity where 15% of 178 extracts tested in LILIT had MIC values of  $\leq 19 \mu\text{g/ml}$ . They were, however, less active than the commercially used trypanocidal drugs Suramin and Melarsoprol. All 15 extracts tested in LILIT by Hoet et al. (2004) also showed a high trypanocidal activity with MIC of  $\leq 19 \mu\text{g/ml}$ , but they were all toxic to mammalian cells. Like in our in vivo studies, other authors also reported that it was not possible to completely eliminate the trypanosomes from the blood of infected animals. The drugs only affected the prolongation of the life of the infected experimental animals (Asuzu and Chineme, 1990).

Due to differences in the study protocols, it is difficult to compare results between the different studies. For instance, the trypanocidal activity was expressed by some authors, like in our study, by MIC values and by other authors by inhibitory concentrations (e.g. IC<sub>50</sub>) (Freiburghaus et al., 1997). The duration of the incubation of the extracts with trypanosomes differed as well. For example, we tested the methanol extracts of the stem bark of *Khaya senegalensis* and of the roots of *Securidaca longepedunculata* for 4 days, whereas Atawodi et al. (2003) tested the same extracts for 4 h. We could find no antitrypanosomal activity in both extracts, but Atawodi et al. (2003) found them strongly antitrypanosomal. Therefore, the protocols for the evaluation of trypanocidal activity of crude plants extracts and their active

principles should be standardised to allow comparisons of the results from different studies.

In conclusion, the present work demonstrates that the medicinal plants used in traditional medicine in Africa against trypanosomoses in both humans and animals may offer some potential for new trypanocidal drug preparations. Thus, it can contribute to the acceptance of traditional medicine and to the solution of the considerable problems caused by African trypanosomosis. However, the extracts obtained from these plants have a weak activity in comparison with conventional trypanocides, as has been observed in other studies. This problem could be overcome by isolating and by preparing extracts with high concentrations of the active ingredients.

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