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 \textit{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 (LtV A) for corroboration of the results of the 24 extracts, it was found that 15 samples had minimum inhibitory concentration (MIC) values $> 10\mu g/ml$, eight MIC values of $100\mu g/ml$ and one MIC values of $50–100\mu g/ml$. So far, four extracts with MIC values $\leq 100\mu g/ml$ were tested for antitrypanosomal activity in mice, experimentally infected with \textit{Trypanosoma brucei brucei}. Only, the aqueous extracts of the leaves of \textit{Terminalia avicennioides} Guill. and Perr. (Combretaceae) and the stem bark of \textit{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 \textit{Terminalia avicennioides}.

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Keywords: Medicinal plants; \textit{Trypanosoma brucei brucei}; Antitrypanosomal activity; Africa

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 \textit{Trypanosoma brucei rhodesiense} in East Africa and \textit{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 \textit{Trypanosoma congolense}, \textit{Trypanosoma vivax} and \textit{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 at 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 trypanosomes in humans.
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; Adeyewumi 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, Moreh-abougu, Mandjo, Bandiangara, Kalibombo, Sido, Blendio and Siby, Kalassa, Dankorodalaba, Tienfala, Morih-abougu, Mandjo, Bandiangara, 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 solvating 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®, 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), portions 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 stabalized 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, 10⁶ cells were transferred into each well of 96-well microtitre 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 hpes 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 calf serum (Invitrogen Corporation, UK) and with 1 ml/100 ml of anticontamination cocktail according to Maser 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>
<thead>
<tr>
<th>Plant species and family</th>
<th>Traditional names in Bambara</th>
<th>Voucher numbers</th>
<th>Plant parts</th>
<th>Traditional preparations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acacia nilotica (L.) Delile (Mimosaceae)</td>
<td>Buwanan, Bognanan</td>
<td>M2a</td>
<td>Stem and root bark</td>
<td>Decoction, infusion</td>
</tr>
<tr>
<td>Afzelia africana Pers. (Leguminosae)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anogeissus leiocarpus Guill. and Perr. (Combretaceae)</td>
<td>Bencé</td>
<td>C2</td>
<td>Stem bark, leaves</td>
<td>Decoction</td>
</tr>
<tr>
<td>Balanites aegyptiaca Delile (Simaroubaceae)</td>
<td>Ntilé</td>
<td>SI</td>
<td>Roots, root bark, shoots</td>
<td>Decoction, infusion, powder</td>
</tr>
<tr>
<td>Bombax ceiba (L.) Gaertn. (Bombacaceae)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cassia sieberiana DC. (Leguminosae)</td>
<td>Sinja</td>
<td>L2</td>
<td>Twigs/leaves</td>
<td>Decoction</td>
</tr>
<tr>
<td>Ceiba pentandra (L.) Gaertn. (Bombacaceae)</td>
<td>Bency</td>
<td>C5a</td>
<td>Stem bark, root bark, leaves</td>
<td>Decoction</td>
</tr>
<tr>
<td>Cissus quadrangularis L. (Vitaceae)</td>
<td>Wujiwókóba</td>
<td>Vb</td>
<td>Twigs/leaves</td>
<td>Decoction</td>
</tr>
<tr>
<td>Diospyros mespiliformis Hochst. DC. (Ebenaceae)</td>
<td>Sounsonfing</td>
<td>E1</td>
<td>Leaves</td>
<td>Decoction</td>
</tr>
<tr>
<td>Entada africana Guill. and Perr. (Mimosaceae)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Holarrhena floribunda Durand and Schinz (Apocynaceae)</td>
<td>Bassira, Basoroy</td>
<td>A3</td>
<td>Twigs/leaves</td>
<td>Decoction</td>
</tr>
<tr>
<td>Khaya senegalensis A. Juss. (Meliaceae)</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Lawsonia alba Lam. (Lythraceae)</td>
<td>Jabi</td>
<td>L4</td>
<td>Twigs/leaves</td>
<td>Decoction</td>
</tr>
<tr>
<td>Leptadenia hastata Decne. (Asclepiadaceae)</td>
<td>Nsoyin</td>
<td>A4</td>
<td>Aerial parts</td>
<td>Decoction, infusion</td>
</tr>
<tr>
<td>Loranthus pentagonus DC. (Loranthaceae)</td>
<td>Si-ladon</td>
<td>L3</td>
<td>Whole plant</td>
<td>Decoction</td>
</tr>
<tr>
<td>Maytenus senegalensis (Lam.) Exell (Celastraceae)</td>
<td>Ngeke</td>
<td>C3</td>
<td>Twigs/leaves</td>
<td>Decoction, infusion</td>
</tr>
<tr>
<td>Mitragyna inermis Kuntze (Rubiaceae)</td>
<td>Jun</td>
<td>L4</td>
<td>Leaves, stem bark</td>
<td>Decoction, infusion</td>
</tr>
<tr>
<td>Mitragyna speciosa Korth (Rubiaceae)</td>
<td>Joro, Giorgi</td>
<td>PI</td>
<td>Roots</td>
<td>Maceration, decoction, infusion</td>
</tr>
<tr>
<td>Moringa oleifera Lam. (Moringaceae)</td>
<td>Foé</td>
<td>F1</td>
<td>Shoots</td>
<td>Infusion, decoction</td>
</tr>
<tr>
<td>Neem (Azadirachta indica) (Meliaceae)</td>
<td>Joro, Giorgi</td>
<td>PI</td>
<td>Roots</td>
<td>Maceration, decoction, infusion</td>
</tr>
<tr>
<td>Pterocarpus officinalis L. (Fabaceae)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sterculia foetida (Sterculiaceae)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Terminalia ivorensis (Combretaceae)</td>
<td>Wolofë, Wolóboum</td>
<td>C5a</td>
<td>Stem bark, root bark, leaves</td>
<td>Decoction, infusion, maceration</td>
</tr>
</tbody>
</table>

Table 1. Investigated medicinal plants collected in the South of Mali in November 2001.
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 μl trypanosome culture medium per well. Another 100 μl containing 10^2 trypanosomes in the logarithmic growth phase were added to each well. The final concentrations of the extracts were 100, 10, 5 and 1 μg/ml, respectively. Cultures without and with diminazene aceturate in 2- or 10-fold dilutions from 0.01 to 1 μg/ml were prepared under identical conditions as for the extracts. The plates were then incubated at 37 °C with 5% CO₂ added to the air in a humidified incubator. After 96h, they were examined finder 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 ≤ 10 μg/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 bells 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 μg/ml. Only extracts showing MIC values ≤ 10 μg/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 ≤ 100 μg/ml) in the LtVA (Table 3) were evaluated in vivo for their trypanocidal activity using the standardised mouse test (Eischer et al., 2001) with minor modifications. After intraperitoneal inoculation of each mouse with 1 × 10^3 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 milliliter of blood was calculated as log using the rapid matching method for estimating the host’s parasitaemia according to Herbert and Lumosden (1976). At higher 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 μg/ml, 22 extracts MIC values of 10 μg/ml, 98 extracts MIC values of 100 μg/ml and 43 extracts MIC > 100 μg/ml. The MIC values of the most active extracts (MIC values ≤ 10 μg/ml) are shown in Table 2. Diminazene aceturate used as a positive control had a MIC value of 0.05 μg/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 μg/ml, eight extracts a MIC value of 100 μg/ml and 15 extracts MIC values > 100 μg/ml. Diminazene aceturate used as a positive control
had MIC values in the range of 0.01–0.05 μg/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 μg/ml in LILIT have been investigated in vivo. None of these tested was able to clear the blood of infected animals from trypanosomes. However, the aqueous extracts of the leaves of *Trypanosoma brucei* and of the stem bark of *Celtis integrifolia* reduced the parasitaemia of infected animals at the dose of 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 *Trypanosoma brucei* as test organism. Therefore, it was not possible to confirm the claimed trypanocidal properties of one or more of the plant parts used in West Africa in traditional medicine to treat trypanosomes in both human and animals could be scientifically confirmed.

### 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 trypanocidal activity of MIC values ≤ 10 μg/ml. The antitrypanosomal activity of those extracts re-tested in LVA 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 μg/ml. The remaining 14 extracts (62.5%) had MIC values > 100 μg/ml.

It is remarkable that the activity of the 24 extracts tested in both tests was weaker (up to 100-fold) in LVA than in LILIT. Thus, the methanol extract of the stem bark of *Afzelia africana* and the aqueous extract of the stem bark of *Aeschynomene indica* which had in LILIT MIC values of 1 and 5 μg/ml, respectively, had MIC values of 100 μg/ml in LVA. Other extracts which had MIC values of 10 μg/ml in LILIT had MIC values of ≥ 100 μg/ml in LVA. 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 LVA 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 μg/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 LVA 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 *Trypanosoma brucei*) 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

### 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*.

<table>
<thead>
<tr>
<th>Plant species/kind of extract</th>
<th>Plant part(s)</th>
<th>MIC (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acacia nilotica</em></td>
<td><em>H</em>&lt;sub&gt;2&lt;/sub&gt;<em>O</em>-extract</td>
<td>Stem bark 100</td>
</tr>
<tr>
<td><em>Afzelia africana</em></td>
<td><em>H</em>&lt;sub&gt;2&lt;/sub&gt;<em>O</em>-extract</td>
<td>Stem bark 100</td>
</tr>
<tr>
<td><em>Anogeissus leiocarpus</em></td>
<td><em>H</em>&lt;sub&gt;2&lt;/sub&gt;<em>O</em>-extract</td>
<td>Leaves 100</td>
</tr>
<tr>
<td><em>Combretum micranthum</em></td>
<td><em>H</em>&lt;sub&gt;2&lt;/sub&gt;<em>O</em>-extract</td>
<td>Leaves 100</td>
</tr>
<tr>
<td><em>Celtis integrifolia</em></td>
<td><em>H</em>&lt;sub&gt;2&lt;/sub&gt;<em>O</em>-extract</td>
<td>Roots 100</td>
</tr>
<tr>
<td><em>Ceiba pentandra</em></td>
<td><em>H</em>&lt;sub&gt;2&lt;/sub&gt;<em>O</em>-extract</td>
<td>Leaves 100</td>
</tr>
<tr>
<td><em>Diminazene aceturate</em></td>
<td>-</td>
<td>0.01–0.05</td>
</tr>
<tr>
<td><em>Hevea brasiliensis</em></td>
<td><em>H</em>&lt;sub&gt;2&lt;/sub&gt;<em>O</em>-extract</td>
<td>Leaves 100</td>
</tr>
<tr>
<td><em>Lunasia chinensis</em></td>
<td><em>H</em>&lt;sub&gt;2&lt;/sub&gt;<em>O</em>-extract</td>
<td>Leaves 100</td>
</tr>
<tr>
<td><em>Lycopus europaeus</em></td>
<td><em>H</em>&lt;sub&gt;2&lt;/sub&gt;<em>O</em>-extract</td>
<td>Leaves 100</td>
</tr>
<tr>
<td><em>Moringa oleifera</em></td>
<td><em>H</em>&lt;sub&gt;2&lt;/sub&gt;<em>O</em>-extract</td>
<td>Leaves 100</td>
</tr>
<tr>
<td><em>Nauclea latifolia</em></td>
<td><em>H</em>&lt;sub&gt;2&lt;/sub&gt;<em>O</em>-extract</td>
<td>Leaves 100</td>
</tr>
<tr>
<td><em>Parkinsonia aculeata</em></td>
<td><em>H</em>&lt;sub&gt;2&lt;/sub&gt;<em>O</em>-extract</td>
<td>Leaves 100</td>
</tr>
<tr>
<td><em>Psidium guajava</em></td>
<td><em>H</em>&lt;sub&gt;2&lt;/sub&gt;<em>O</em>-extract</td>
<td>Leaves 100</td>
</tr>
<tr>
<td><em>Strophanthus gratus</em></td>
<td><em>H</em>&lt;sub&gt;2&lt;/sub&gt;<em>O</em>-extract</td>
<td>Leaves 100</td>
</tr>
<tr>
<td><em>Terminalia avicennioides</em></td>
<td><em>H</em>&lt;sub&gt;2&lt;/sub&gt;<em>O</em>-extract</td>
<td>Leaves 100</td>
</tr>
<tr>
<td><em>Trypanosoma brucei</em></td>
<td><em>H</em>&lt;sub&gt;2&lt;/sub&gt;<em>O</em>-extract</td>
<td>Leaves 100</td>
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<tr>
<td><em>Vigna unguiculata</em></td>
<td><em>H</em>&lt;sub&gt;2&lt;/sub&gt;<em>O</em>-extract</td>
<td>Leaves 100</td>
</tr>
<tr>
<td><em>Withania somnifera</em></td>
<td><em>H</em>&lt;sub&gt;2&lt;/sub&gt;<em>O</em>-extract</td>
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<tr>
<td><em>Ziziphus mucronata</em></td>
<td><em>H</em>&lt;sub&gt;2&lt;/sub&gt;<em>O</em>-extract</td>
<td>Leaves 100</td>
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</tbody>
</table>

* Plant extracts evaluated in *Mastomys coucha-mice* for their trypanocidal activity using *Trypanosoma brucei* as test organism.
infected Mastomys coucha-mice mostly disappeared, indepen-
dently 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 acetate 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 LIVA was up to 200- and 10,000-fold, respectively,
weaker than in diminazene acetate. In mice, the activity of the
aqueous extract of Terminalia avicennioides was at least five-
fold weaker, since the curative dose of diminazene acetate for
drug sensitive trypanosomes 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 acetate
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 con-
centrations. 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 the extracts to be
tested in LILIT was arbitrary, including in the test only extracts which
showed in LILIT MIC values ≤ 10 µg/mL. Through this arbitrary
selection, extracts with MIC > 10 µg/mL in LILIT possibly con-
taining 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 con-
formity with work from other authors. Trials by Freibergaus et
al. (1996) revealed a high antiparasomal activity where 15%
fold weaker, since the curative dose of diminazene acetate for
drug sensitive trypanosomes populations in mice is estimated to
be below 20 mg/kg b.w. (Eisler et al., 2001). These differences
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Due to differences in the study protocols, it is difficult to com-
pare 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 concen-
trations (e.g. IC50) (Freibergaus 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 longependice-
ulata 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 medici-
 nal plants used in traditional medicine in Africa against try-
panosomes 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 try-
panosomosis. However, the extracts obtained from these plants
have a weak activity in comparison with conventional try-
panocides, 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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References
Atawodi, S.E., Bulus, T., Ibrahim, S., Ameh, D.A., Nok, A.J., Mamman, M.,
of some Nigerian savannah plants. African Journal of Biotechnology 2,
317–321.
Baltz, T., Baltz, D., Gisou, C.H., Crockett, J., 1985. Cultivation in a semi-
defined medium of animal infective forms of Trypanosoma brucei, T.
equiperdum, T. evansi, T. rhodesiense and T. gambiense. The EMBO
Journal 4, 1273–1277.
Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH (Ed.),
and Trypanosoma equiperdum isolates. Veterinary Parasitology 52, 37–46.
Brun, R., Jemsi, L., Tanzer, M., Schenker, M., Schell, K.-F., 1979. Cult-
viation of vertebrate infective forms derived from metacyclic forms of
Dallo, D., Paulson, B.S., Hovem, B., 1996. Production of traditional
medicine: preparations accepted as medicines in Mali. In: Hostettmann,
K., Ghignaungya, F., Mailard, M., Wolfenden, J.-L. (Eds.), Chemistry,
Biological and Pharmacological Properties of African Medicinal Plants.
Eisler, M.C., Brun, R., Bauer, B., Clausen, P.-H., Deloguau, V., Holmes,
PH, Irembeho, A., Machii, N., Mbwanbo, H., McDermott, J., Mehltz,
D., Murilla, G., Ndingi, J.M., Peregine, A.S., Sudal, I., Sinyangwe, L.,
Goetz, S., 2001. Standardised tests in mice and cattle for the detection
of drug resistance in tsetse-transmitted trypanosomes of African domestic
cattle. Veterinary Parasitology 97, 171–182.
Medicinal plants in therapy. Bulletin of the World Health Organization
63, 965–981.


