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Antileishmanial activities associated with plants used in the Malian traditional medicine

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Abstract

Sixty-four extracts issued from twenty-one plants used in the Malian traditional medicine – several of them as antiparasitic drugs – were assayed for their antileishmanial effects against both extracellular and intracellular forms of *Leishmania major*. Seven extracts from six different plants – *Sarcocephalus latifolius*, *Zanthoxylum zanthoxyloides*, *Entada africana*, *Bobgunnia madagascarensis*, *Pseudocedrela kotschyi* and *Psorospermum guineense* – were found to be significantly active against the intracellular form of the parasite.

Keywords: Plant extracts; Mali; Antileishmanial activity; *Leishmania major*; Traditional medicine

1. Introduction

In the frame of the search for new leads against the most neglected parasitic diseases, it is of particular interest to evaluate the antileishmanial potential of some of the most frequently used drugs of the Malian traditional Pharmacopoeia.

Leishmaniasis is a parasitic disease caused by protozoal organisms of the *Leishmania* genus that are transmitted to humans by sandfly bites. Infections can vary from simple cutaneous leishmaniasis to mucocutaneous and fatal visceral leishmaniasis or kala-azar. Leishmaniasis is prevalent in 88 countries throughout the world (72 are developing countries) and affects more than 12 million people. In the majority of cases, the cutaneous leishmaniasis form heals without treatment, leaving the person immune to further infection. All other forms are extremely difficult to treat, often requiring a long course of pentavalent antimony drugs – meglumine antimoniate (Glucantime®) or sodium stibogluconate (Pentostam®) – which present severe side effects and limitations of use. Only a very limited number of drugs are available at the moment for the treatment of leishmaniasis (Croft and Coombs, 2003) and resistance to some of these drugs has recently been reported, requiring the use of more toxic drugs, such as amphotericin B. Most available drugs are costly (AmBisome®), require long treatment regimes (pentavalent antimonials) and are becoming more and more ineffective due to drug resistance issues (pentavalent antimonials in India), necessitating the discovery of new drugs. Leishmaniasis has indeed become one of the priorities of the WHO/TDR Program. Recently (2002), miltefosine or Impavido® – the only available oral treatment for visceral leishmaniasis developed by Asta Medica (now Zentaris) in cooperation with the WHO/TDR – has been registered in India.

In Mali, TM has been recognized of national interest with structures, budget and training established in order to encourage, facilitate and control its use all over the country. This includes a legal frame, a national management run by the Department of Traditional Medicine (Institut National de Recherche en Santé Publique), associations of traditional healers and the allocation of a national budget for TM. Although leishmaniasis does not

Abbreviations: TM, traditional medicine; HAT, human African trypanosomiasis; DCM, dichloromethane; MeOH, methanol; H_2O, water; WHO/TDR, World Health Organisation/Tropical Diseases Research; TP, true positive; FP, false positive; FN, false negative; TN, true negative

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Table 1
Uses of the selected plant material in the Malian and African traditional medicines

Plant name [family]	Organ	TM use in Mali	Mode of preparation	TM use in other African countries associated with parasitic diseases and some of their symptoms[a]
Sarcocephalus latifolius (Smith) Bruce [Rubiaceae]	Trunk bark	Malaria	Decoction	Malaria (Ivory Coast, Sierra Leone, Togo), antihelminthic (Nigeria), fever, malaria, vermifuge (Arbonnier, 2000)
Zanthoxylum zanthoxyloides (Lam.) Zepernick and Timler [Rutaceae]	Roots	Malaria, bone pain	Maceration, infusion	HAT[b] (Ivory Coast)
Khaya senegalensis A. Juss. [Meliaceae]	Trunk bark	Malaria, stomach ache	Decoction	Leishmaniasis (Guinea-Bissau), HAT (Nigeria, Ivory Coast), malaria (Burkina Faso, Sudan), fever, malaria (Arbonnier, 2000)
Spilanthes oleracea Linn. [Oleraceae]	Flowers	Malaria	Decoction	Malaria [Neuwiger]
Entada africana Gill. and Perr. [Fabaceae]	Roots	Malaria, cough, liver ailment, hepatitis	Decoction, maceration, bath	
Boscia senegalensis Hochst. [Capparaceae]	Leaves	Antihelminthic, head ache[a]	Fumigation[a]	Malaria (East Africa), vermifuge, schistosomiasis (Arbonnier, 2000)
Boscia angustifolia A. Rich. [Capparaceae]	Trunk bark	Head ache, intestinal disorders	Decoction	Malaria (East Africa), schistosomiasis (Arbonnier, 2000)
Cassia sieberiana DC. [Fabaceae]	Roots	Stomach ache, malaria	Maceration with honey	HAT (Ivory Coast, Nigeria), malaria (Sierra Leone, Ghana), fever, schistosomiasis (Neuwinger, 1996, 2001)
Piliostigma thonningii (Schumach.) Milne-Redhead [Fabaceae]	Fruits	Inflammation	Topical application	Malaria (South Africa), antihelminthic (Nigeria), headache (Arbonnier, 2000)
Bobgunnia madagascarensis J.H. Kirkbr. and Wiersema [Fabaceae]	Roots	Liver ailment	Maceration	Malaria (Ivory Coast)
Pseudocedrela kotschyi (Schweinf.) Harms [Meliaceae]	Roots	Malaria, abdominal pain	Decoction	HAT (Nigeria), malaria (Sudan), fever, syphilis, onchocercosis (Arbonnier, 2000)
Cissus quadrangularis A. Cheval. [Vitaceae]	Stems	Intestinal worms	Decoction	Malaria (Senegal, South Africa), fever (Arbonnier, 2000)
Gardenia ternifolia Schum. and Thonn. [Rubiaceae]	Roots	Icter, sexual asthenia	Decoction, powder	Malaria, vermifuge, onchocercosis (Arbonnier, 2000)
Glinus oppositifolius Aug. DC. [Aizoaceae]	Aerial parts	Malaria	Decoction, powder	Headache (East Africa)
Securinega virosa (Willd.) Baill. [Euphorbiaceae]	Roots	Abdominal pain, sexual asthenia	Powder	Malaria, schistosomiasis (Ghana, Benin, Botswana, Nigeria, Tanzania), HAT (Uganda) vermifuge, schistosomiasis (Arbonnier, 2000)
Tamarindus indica Linn. [Fabaceae]	Roots	Sexual asthenia	Powder	HAT (Nigeria)
Prosopis africana Taub. [Fabaceae]	Roots, fruits	Sexual asthenia, wounds	Powder	HAT (Nigeria), vermifuge (Neuwinger, 1996, 2001)
Mitragyna inermis (Willd.) K. Schum. [Rubiaceae]	Leaves	Malaria	Decoction	Malaria (Ivory Coast, Ghana)
Psorospermum guineense Hochr. [Clusiaceae]	Leaves, roots	Mycoses	Topical application (ointment)	Syphilis (Tanzania)
Dichrostachys glomerata Chiov. [Fabaceae]	Fruits	Mycoses, inflammation	Topical application (ointment)	STI (Zimbabwe)
Daniellia oliveri Hutchinson and Dalziel [Fabaceae]	Leaves	Migraine	Decoction, vapour bath	Wounds (Nigeria), mosquito repellent (Guinea Bissau)

[a] Not an exhaustive list: data from our internal database in addition to citations in Arbonnier and Neuwinger when mentioned.
[b] Human African trypanosomiasis.

affect Mali, several of the selected drugs are used in the Malian folk medicine to treat parasitic diseases as malaria or symptoms that could be associated to such diseases. In addition to this, some of these plants have been reported in other African TM systems against leishmaniasis, HAT or other parasitic diseases (see Table 1). The lack of phytochemical and biological investigation, as regards antiprotozoal activities, makes these drugs suitable candidates for a targeted antileishmanial screening.

2. Materials and methods

2.1. Plant material

The plant material was obtained from the traditional healers at the Marché de Médine, Bamako and Mali, also collected in the neighbourhood of Siby and Sotuba (see Table 1). The identification of the plants and their assessment of use in the Malian folk medicine have been performed by Dr. Drissa Diallo, together with the traditional healers of the Marché de Médine. Voucher specimens (numbers are provided in Table 2) are deposited at the Laboratoire de Pharmacognosie et Phytochimie, Genève, Switzerland.

2.2. Extraction and isolation

The air-dried and milled organs were successively extracted three times with DCM and three times with MeOH at room temperature (extraction ratio as g of vegetal material:mL solvent was 1:10 for all extracts). After filtration, a separated evaporation of the solvents under reduced pressure, followed by lyophilisation of the residues, gave the DCM and MeOH extracts. The H_2O extracts were obtained after extraction three times with H_2O (same extraction ratio as above), filtration and lyophilisation of the liquid extracts.

2.3. Antileishmanial assay against Leishmania major

This assay was performed as described by Mauël (1984). Survival (%) of free-living *Leishmania major* promastigotes were measured with extracts at 35 and 17.5 μg/mL. Survival (%) of intracellular *Leishmania major* were performed with RAW 264.7 parasitized mouse macrophages at the same concentrations. Assays were performed in triplicate. Amphotericin B was used as a reference compound.

2.4. Toxicity assay against macrophages

This assay was performed as described by Mauël (1984). Survival (%) of unparasitized RAW 264.7 mouse macrophages were measured after addition of extracts at 35 and 17.5 μg/mL. Assays were performed in triplicate.

3. Results and discussion

Twenty-one plants used in the Malian and/or the African traditional medicine for their antiparasitic/antiprotozoal properties or selected due to their wide use in the Malian TM (see Table 1) were submitted to a biological screening against *Leishmania major*. In order to cover the largest range of compounds contained in these drugs, it was decided to obtain the DCM, MeOH and H_2O extracts from the selected material. Sixty-four extracts issued from these plants were assayed for their antileishmanial effects against *Leishmania major* on both extracellular and intracellular forms of the parasite as well as for their toxicity against non-infected RAW macrophages. These results are presented in Table 2. In order to facilitate the reading of these results, the most active and toxic extracts are shown in bold characters.

A total of four extracts from four different plants—*Zanthoxylum zanthoxyloides* (Syn. *Fagara zanthoxyloides* L.) (DCM extract of root bark), *Entada africana* (H_2O extract of roots), *Bobgunnia madagascarensis* (DCM extract of root bark) and *Pseudocedrela kotschyi* (DCM extract of roots) were found to exhibit a marked activity (<10% of *Leishmania major* parasite survival) at the tested concentrations against the intracellular form of the parasite which is pathogenically significant for humans. Three other extracts obtained from three different plants displayed a moderate activity (between 10 and 20% of parasite survival) against the same form of the parasite: *Sarcocephalus latifolius* (DCM extract of stem bark), *Zanthoxylum zanthoxyloides* (H_2O extract of root bark) and *Psorospermum guineense* (DCM extract of root bark). For all these active extracts, the higher tested concentrations gave an expected lower percentage of parasite survival, the assay having been repeated three times for each sample of a given concentration. Of note, the extract of *Bobgunnia madagascarensis* displayed an antileishmanial activity that was on a par with that of amphotericin B, a drug currently used for treating the disease, while being significantly less toxic for the host cells. It is of interest to notice the correlation of activities between the extracellular and the intracellular assays as four out of the seven active extracts mentioned above exhibit at least a moderate activity on the promastigote form of the parasite (<20% of parasite survival), the three remaining extracts being just above this threshold with 20.4, 21.8 and 21.1% of parasite survival for the active extracts of *Zanthoxylum zanthoxyloides* (DCM and H_2O extracts of root bark) and *Pseudocedrela kotschyi* (DCM extract of roots), respectively.

If the intracellular test is considered as a standard, the extracellular assay can be considered as a rather specific (98.0%) but quite insensitive (57.1%) predictive model if the threshold for positive results is set at 20% of parasite survival for both assays (see Table 3). A threshold set at 25% of parasite survival permits to increase significantly the sensitivity (87.5%) without altering the specificity (98.0%) of the predictive model (see Table 4). Although such observations should be confirmed on a larger sample size, these result seems to indicate that the extracellular form of the parasite – much easier to cultivate and to prepare for an assay purpose than the intracellular one – could be used as a first rapid screening assay in order to identify hits among a high number of extracts with a reasonably good level of confidence. One should however be aware of the limitations of such an assay as some antileishmanial compounds are known to act through macrophage activation or after concentration and metabolization in the macrophage (FN in the extracellular assay). Thus, the intracellular assay should remain the golden standard to evaluate

Table 2
Antileishmanial activities and macrophagic toxicity of the tested plant extracts

Plant name	No	Organ	Place of collection	Extract	*Leishmania major*				Macrophages[a]	
					Promastigote form[b]		Amastigote form[c]			
Sarcocephalus latifolius	2000036	Stem bark	M	DCM	**15.0 ± 0.6**	20.5 ± 1.3	**18.5 ± 2.1**	54.1 ± 2.9	87.6 ± 1.3	96.5 ± 1.7
				MeOH	**16.7 ± 1.4**	30.3 ± 2.1	31.6 ± 2.7	68.9 ± 1.8	94.3 ± 2.6	95.0 ± 3.2
				H_2O	92.3 ± 0.6	96.4 ± 1.3	84.6 ± 2.8	97.1 ± 1.2	96.1 ± 3.5	97.3 ± 2.4
		Wood	M	DCM	29.9 ± 1.2	44.1 ± 1.0	45.3 ± 3.6	43.2 ± 2.4	95.3 ± 2.9	94.5 ± 3.0
				MeOH	25.1 ± 2.5	38.1 ± 3.3	25.0 ± 1.4	33.8 ± 0.9	96.7 ± 1.8	97.8 ± 1.6
				H_2O	94.5 ± 1.1	95.2 ± 2.0	56.8 ± 3.2	87.7 ± 2.5	88.2 ± 3.3	95.1 ± 2.0
Zanthoxylum zanthoxyloides	2000022	Root bark	M	DCM	20.4 ± 2.3	39.8 ± 3.6	**0.9 ± 0.3**	**6.0 ± 1.4**	86.7 ± 2.0	97.0 ± 1.4
				MeOH	40.8 ± 1.3	45.7 ± 3.8	30.5 ± 3.3	34.1 ± 2.5	92.3 ± 4.5	97.8 ± 2.9
				H_2O	21.8 ± 1.7	33.7 ± 0.9	**14.5 ± 0.4**	**17.7 ± 0.5**	**65.9 ± 5.2**	**69.2 ± 3.7**
Khaya senegalensis	2000080	Stem bark	M	DCM	97.9 ± 1.2	95.1 ± 2.6	n.t.	n.t.	n.t.	n.t.
				MeOH	93.4 ± 3.3	84.6 ± 4.2	n.t.	n.t.	n.t.	n.t.
Spilanthes oleracea	2001004	Blossoms	So	DCM	92.1 ± 1.3	95.2 ± 2.3	32.6 ± 2.1	30.7 ± 1.9	**80.1 ± 4.3**	93.4 ± 3.1
				MeOH	52.5 ± 3.0	54.8 ± 1.7	72.5 ± 3.2	93.1 ± 1.2	97.2 ± 3.1	96.1 ± 2.8
				H_2O	68.3 ± 4.1	81.2 ± 3.5	84.4 ± 2.3	98.6 ± 0.7	91.7 ± 2.2	99.4 ± 1.5
Entada africana	2002002	Roots	M	DCM	72.6 ± 3.4	74.0 ± 4.2	46.9 ± 3.8	82.3 ± 2.4	96.6 ± 2.8	95.1 ± 3.4
				MeOH	79.7 ± 3.8	78.1 ± 4.4	81.3 ± 2.7	90.6 ± 1.5	90.8 ± 3.0	93.2 ± 2.7
				H_2O	**15.3 ± 1.4**	22.5 ± 0.6	**5.0 ± 0.9**	**7.3 ± 1.1**	**79.9 ± 4.1**	**82.3 ± 3.2**
Boscia senegalensis	96123	Leaves	M	DCM	54.4 ± 3.7	60.9 ± 3.5	43.8 ± 1.9	48.1 ± 2.4	93.7 ± 3.9	97.2 ± 2.6
				MeOH	47.3 ± 2.1	43.5 ± 2.2	82.7 ± 1.8	92.4 ± 2.6	89.8 ± 3.7	95.6 ± 1.5
		Fruit bark		MeOH	55.0 ± 1.1	64.3 ± 3.2	91.3 ± 1.2	96.5 ± 2.1	99.3 ± 1.2	97.6 ± 1.9
Boscia angustifolia	2000030	Bark	M	DCM	96.9 ± 2.1	91.0 ± 4.6	20.8 ± 2.5	48.3 ± 3.5	94.9 ± 2.5	99.1 ± 0.8
				MeOH	73.1 ± 3.3	71.8 ± 4.1	89.7 ± 0.9	97.2 ± 1.3	85.7 ± 3.0	90.2 ± 3.5
Cassia sieberiana	2000024	Root bark	M	DCM	56.2 ± 2.8	62.6 ± 3.9	52.3 ± 2.6	65.7 ± 3.2	90.2 ± 3.3	96.0 ± 2.2
				MeOH	59.3 ± 1.8	68.5 ± 2.1	71.6 ± 1.4	83.4 ± 2.6	95.3 ± 2.2	98.3 ± 0.9
				H_2O	48.2 ± 0.5	53.1 ± 2.3	24.5 ± 2.2	36.1 ± 1.7	**71.8 ± 3.0**	**80.4 ± 2.5**
Piliostigma thonningii	2000026	Leaves	M	DCM	32.0 ± 2.2	50.8 ± 3.7	68.1 ± 4.5	83.9 ± 3.2	93.6 ± 4.1	96.3 ± 2.9
				MeOH	34.9 ± 1.7	45.1 ± 2.8	90.7 ± 2.4	97.2 ± 1.1	97.7 ± 1.6	99.3 ± 1.2
				H_2O	49.6 ± 1.4	77.5 ± 2.6	88.3 ± 3.1	92.8 ± 2.7	95.4 ± 3.5	97.1 ± 2.0
Bobgunnia madagascarensis	97012	Root bark	Si	DCM	**0.2 ± 0.0**	**0.6 ± 0.1**	**0.4 ± 0.1**	**3.6 ± 0.3**	**75.3 ± 1.2**	95.4 ± 1.5
Pseudocedrela kotschyi	2000038	Roots	M	DCM	21.1 ± 1.5	28.2 ± 2.2	**5.6 ± 1.2**	**9.2 ± 0.8**	90.6 ± 1.4	96.2 ± 1.9
				MeOH	39.6 ± 2.3	47.7 ± 3.1	84.2 ± 3.7	91.8 ± 2.4	98.2 ± 1.7	97.1 ± 2.0
				H_2O	47.3 ± 1.9	48.6 ± 2.5	30.2 ± 1.8	73.5 ± 2.6	95.5 ± 2.4	97.0 ± 1.8
Cissus quadrangularis	2000004	Aerial part	M	DCM	66.7 ± 3.4	62.3 ± 4.1	95.6 ± 2.3	98.1 ± 1.7	93.1 ± 2.2	94.5 ± 1.3
				MeOH	96.8 ± 1.9	98.3 ± 2.1	99.2 ± 0.6	99.8 ± 0.4	99.0 ± 1.3	98.6 ± 1.9
				H_2O	97.7 ± 0.8	96.8 ± 1.7	48.9 ± 3.0	76.0 ± 4.2	**67.5 ± 3.4**	**82.1 ± 3.2**
Gardenia ternifolia	2000007	Root bark	M	DCM	98.7 ± 2.5	99.1 ± 1.3	66.6 ± 4.3	93.2 ± 2.5	96.9 ± 2.8	98.7 ± 1.6
				MeOH	97.2 ± 2.1	98.4 ± 1.6	99.0 ± 0.8	99.7 ± 1.3	89.9 ± 3.7	94.1 ± 2.7
				H_2O	90.3 ± 3.5	93.2 ± 3.1	37.5 ± 2.2	49.6 ± 2.7	**80.1 ± 3.5**	**84.4 ± 3.3**
Glinus oppositifolius	96122	Leaves	M	DCM	61.8 ± 2.4	78.4 ± 3.6	34.7 ± 1.6	36.9 ± 1.8	95.4 ± 2.6	98.3 ± 2.1
Securinega virosa	2000013	Stem bark	M	DCM	90.7 ± 0.6	80.8 ± 2.1	85.6 ± 2.8	90.4 ± 3.3	98.6 ± 2.0	97.4 ± 2.8
				MeOH	85.9 ± 3.4	97.6 ± 1.9	98.7 ± 1.3	99.2 ± 0.9	96.3 ± 2.4	98.5 ± 1.9
				H_2O	99.6 ± 2.0	98.4 ± 2.3	85.1 ± 2.7	95.7 ± 3.1	98.1 ± 0.7	97.6 ± 1.2
Tamarindus indica	2000015	Root bark	M	DCM	87.1 ± 1.5	89.1 ± 2.6	91.3 ± 1.8	99.6 ± 0.5	**75.2 ± 3.6**	90.1 ± 2.5
				MeOH	77.3 ± 2.9	93.4 ± 1.7	96.9 ± 2.5	98.0 ± 1.6	98.7 ± 1.2	96.3 ± 1.8
				H_2O	78.7 ± 3.1	76.1 ± 2.2	68.2 ± 1.9	65.3 ± 3.3	**83.5 ± 3.1**	**84.9 ± 2.7**
Prosopis africana	2000010	Root bark	M	DCM	77.9 ± 3.6	72.6 ± 3.0	47.3 ± 1.2	55.6 ± 2.0	94.8 ± 2.3	95.5 ± 4.6
				MeOH	84.9 ± 2.8	85.5 ± 2.1	99.4 ± 0.7	98.2 ± 1.9	98.1 ± 1.6	96.3 ± 2.5
				H_2O	87.3 ± 2.1	92.6 ± 3.3	26.2 ± 1.0	30.7 ± 0.8	**79.7 ± 3.5**	92.6 ± 2.7
Mitragyna inermis	96125	Leaves	M	DCM	78.0 ± 1.7	95.4 ± 2.6	n.t.	n.t.	n.t.	n.t.
				MeOH	30.1 ± 1.1	33.7 ± 1.8	66.3 ± 2.5	87.1 ± 3.2	95.0 ± 2.1	98.2 ± 0.9
Psorospermum guineense	2000003	Leaves	M	DCM	68.8 ± 2.4	65.6 ± 3.0	78.8 ± 2.6	89.2 ± 3.7	91.3 ± 2.6	97.9 ± 1.5
				MeOH	88.5 ± 2.5	97.4 ± 1.8	89.1 ± 2.4	96.8 ± 1.5	98.5 ± 1.3	96.1 ± 2.4
				H_2O	81.9 ± 3.6	95.2 ± 2.7	99.3 ± 1.2	99.8 ± 0.9	97.1 ± 2.2	98.0 ± 1.9

Table 2 (*Continued*)

Plant name	No	Organ	Place of collection	Extract	*Leishmania major*				Macrophages[a]	
					Promastigote form[b]		Amastigote form[c]			
		Root bark	M	DCM	**3.5 ± 0.3**	**12.8 ± 0.9**	**14.0 ± 0.6**	25.2 ± 0.1	**80.2 ± 4.0**	92.1 ± 3.3
				MeOH	44.7 ± 1.1	49.8 ± 2.2	99.1 ± 1.7	99.9 ± 1.2	95.3 ± 3.1	94.2 ± 2.7
				H_2O	42.7 ± 1.9	49.4 ± 2.0	56.0 ± 2.3	62.3 ± 3.1	**81.0 ± 4.4**	**83.6 ± 3.9**
Dichrostachys glomerata	2000032	Fruits	M	DCM	45.0 ± 1.6	50.5 ± 1.5	43.8 ± 2.6	49.7 ± 2.4	96.8 ± 3.0	99.3 ± 0.5
				MeOH	71.1 ± 0.4	82.3 ± 1.3	87.2 ± 1.9	95.3 ± 2.5	98.4 ± 1.5	96.7 ± 2.8
Daniellia oliveri	2000005	Bark	M	DCM	34.6 ± 0.6	55.8 ± 1.5	24.9 ± 1.1	50.0 ± 2.3	97.8 ± 2.9	97.5 ± 1.2
				MeOH	49.1 ± 2.8	66.8 ± 1.6	90.1 ± 1.7	97.4 ± 2.2	93.2 ± 2.1	96.7 ± 3.0
		Leaves		DCM	38.3 ± 1.2	55.9 ± 2.0	n.t.	n.t.	n.t.	n.t.
				MeOH	57.4 ± 2.7	89.1 ± 1.8	81.4 ± 2.5	99.1 ± 0.7	98.1 ± 1.6	96.9 ± 2.2
Amphotericin B					**1.1 ± 0.2**	**1.7 ± 0.3**	**0.6 ± 0.1**	**1.5 ± 0.2**	**60.2 ± 2.1**	**74.6 ± 2.7**

n.t., Not tested; M, Marché de Médine, Bamako; So, Sotuba; Si, Siby.

[a] Survival (%) of macrophages exposed to 35 μg/mL (left column) and 17.5 μg/mL (right column) of the indicated extracts.

[b] Survival (%) of extracellular *Leishmania major* exposed to 35 μg/ mL (left column) and 17.5 μg/ mL (right column) of the indicated extracts.

[c] Survival (%) of intracellular *Leishmania major* after exposure of parasitized macrophages to 35 μg/ mL (left column) and 17.5 μg/mL (right column) of the indicated extracts.

Table 3
Comparison of *Leishmania major* intracellular and extracellular assays for a threshold set at 20% of parasite survival

A. Threshold: 20% of parasite survival		Intracellular assay	
		+	−
Extracellular assay	+	4 (TP)	1 (FP)
	−	3 (FN)	49 (TN)
		7 (TP + FN)	50 (FP + TN)
		Sensitivity = TP/(TP + FN)	Specificity = TN/(FP + TN)
		4/(4 + 3) = 57.1%	49/(49 + 1) = 98.0%

TP, true positive; TN, true negative; FP, false positive; FN, false negative.

the in vivo antileishmanial potential of an extract or of a pure compound.

Among the tested drugs, *Khaya senegalensis* (Abreu et al., 1999) and *Pseudrocedrela kotschyi* (El Tahir et al., 1998) were the only ones that have been reported elsewhere for their in vitro antileishmanial activities. However, the MeOH extract from the bark of *Khaya senegalensis* and the MeOH extract from the stem of *Pseudrocedrela kotschyi* were only assayed against the extracellular form of *Leishmania donovani*. In addition to these results and in agreement with our experimental observations, *Khaya senegalensis* failed to exhibit any significant antileishmanial activity against *Leishmania major* at concentrations higher than 100 μg/mL (El Tahir et al., 1998). In another study (Kayser and Abreu, 2001), two antileishmanial dimeric proanthocyanidins, catechin-(4α,6)-catechin and catechin-(4α,8)-catechin, have also been isolated and chemically characterized from the crude bark MeOH extract of *Khaya senegalensis*. These compounds were shown to act as indirect immunostimulating principles (EC_{50} = 3.85 and 3.98 μg/mL, respectively) as they were inactive against promastigotes of *Leishmania donovani* (EC_{50} > 25.0 μg/mL) but exhibited significant effects when tested against the amastigotes of the same species (Kayser and Abreu, 2001). MeOH and DCM extracts from *Khaya senegalensis* stem bark were however found to be inactive against both intracellular and extracellular forms of *Leishmania major*. Khalid et al. (1998) have reported the moderate in vitro antiprotozoal activities of the limonoids 2,6-dihydroxyfissinolide and fissinolide against the promastigote form *Leishmania major*. The discrepancy of the activity results obtained on *Khaya senegalensis* could be explained by differences of sensitivity inherent to

Table 4
Comparison of *Leishmania major* intracellular and extracellular assays for a threshold set at 25% of parasite survival

B. Threshold: 25% of parasite survival		Intracellular assay	
		+	−
Extracellular assay	+	7 (TP)	1 (FP)
	−	1 (FN)	50 (TN)
		8 (TP + FN)	50 (FP + TN)
		Sensitivity = TP/(TP + FN)	Specificity = TN/(FP + TN)
		7/(7 + 1) = 87.5%	50/(50 + 1) = 98.0%

TP, true positive; TN, true negative; FP, false positive; FN, false negative.

the tested *Leishmania* species and strains. These results show that it is necessary to confirm antileishmanial activities detected in a crude extract by submitting its isolated active compounds to a widely accepted and validated in vitro model such as the one described by Neal and Croft (1984).

Among the active extracts, the root bark DCM extract of *Bobgunnia madagascarensis* and of *Psorospermum guineense* as well as the H_2O extract of the roots of *Zanthoxylum zanthoxyloides* and of *Entada africana* were shown to be slightly toxic in the RAW 264.7 murine macrophage model (see Table 2). Studies of the active extracts are underway to identify the compounds responsible for the antileishmanial properties of the active extracts.

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References

Abreu, P.M., Martins, E.S., Kayser, O., Bindseil, K.U., Siems, K., Seemann, A., Frevert, J., 1999. Antimicrobial, antitumor and antileishmania screening of medicinal plants from Guinea-Bissau. Phytomedicine 6, 187–195.

Arbonnier, M., 2000. Arbres Arbustes et lianes des zones sèches d'Afrique de l'Ouest. La librairie du Cirad. Montpellier et Muséum national d'histoire naturelle, Paris.

Croft, S.L., Coombs, G.H., 2003. Leishmaniasis-current chemotherapy and recent advances in the search for novel drugs. Trends in Parasitology 19, 502–508.

El Tahir, A., Ibrahim, A.M., Satti, G.M.H., Theander, T.G., Kharazmi, A., Khalid, S.A., 1998. The potential antileishmanial activity of some Sudanese medicinal plants. Phytotherapy Research 12, 576–579.

Kayser, O., Abreu, P.M., 2001. Antileishmanial and immunostimulating activities of two dimeric proanthocyanidins from *Khaya senegalensis*. Pharmaceutical Biology 39, 284–288.

Khalid, S.A., Friedrichsen, G.M., Kharazmi, A., Theander, T.G., Olsen, C.E., Brogger Christensen, S., 1998. Limonoids from *Khaya senegalensis*. Phytochemistry 49, 1769–1772.

Mauël, J., 1984. Intracellular parasite killing induced by electron carriers, I. Effect of electron carriers on intracellular Leishmania spp. in macrophages from different genetic backgrounds. Molecular and Biochemical Parasitology 13, 83–96.

Neal, R.A., Croft, S.L., 1984. An in vitro system for determining the activity of compounds against the intracellular amastigote form of *Leishmania donovani*. Journal of Antimicrobial Chemotherapy 14, 463–475.

Neuwinger, H.D., 1996. African Ethnobotany. Poisons and Drugs. Chapman & Hall, Weinheim, Germany.

Neuwinger, H.D., 2001. African Traditional Medicine. Medpharm Scientific Publishers, Stuttgart, Germany.