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Screening of New Caledonian and Vanuatu medicinal plants for antiprotozoal activity

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Abstract

Sixty-seven extracts of 30 medicinal plants traditionally used in New Caledonia or Vanuatu by healers to treat inflammation, fever and in cicatrizing remedies were evaluated in vitro for their antiprotozoal activity against *Leishmania donovani*, *Leishmania amazonensis* and *Trypanosoma cruzi*. Among the selected plants, *Pagiantha cerifera* was the most active against both *Leishmania* species; four extracts were active against promastigotes of *Leishmania donovani* at EC_{50} values inferior to 5 μg/ml. *Garcinia pedicillata* extract had an EC_{50} value of 12.5 μg/ml against intracellular amastigotes of *Leishmania amazonensis*. Alone *Amborella trichopoda* reduced by more of 80% the trypomastigotes of *Trypanosoma cruzi* in the blood.

Keywords: New Caledonia; Vanuatu; Leishmanicidal activity; Trypanocidal activity

1. Introduction

Research on antiprotozoal drugs of medicinal plant origin is a multidisciplinary task which involves researchers and students in the fields of botanics, phytochemistry, parasitology, pharmacology and medicine. New Caledonia possesses a relatively rich flora. The quotient of the number of species of native phanerogams by surface area (density/km^2) is 0.157, which is a high figure compared to those obtained for other Pacific islands. Its high specific endemicity ratio is remarkable (near 75%) (Jaffré et al., 2001). Although parasitic diseases as leishmaniasis and trypanosomiasis are absent from New Caledonia and Vanuatu, the IRD (Institut de Recherche pour le Développement) initiated investigations to find new natural active compounds from traditional medicines of these countries. Leishmania and South American trypanosomiasis produce skin lesion (cutaneous ulcer, Carlo Romaña oedema) and our aim was to discover new lead compounds from plants used in traditional medicine for healing skin diseases. Ethnopharmacological data employed in our selection of plants are based on prior information collected amongst traditional healers. The selected plants were used in traditional medicines of New Caledonia or Vanuatu for the treatment of inflammation and fevers.

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The current study was undertaken to evaluate in vitro crude extracts of 30 plants in a screening for activity against leishmaniasis and South American trypanosomiasis (Chagas disease).

2. Materials and methods

2.1. Plant material

Thirty plants from 20 families, native from New Caledonia and Vanuatu, were selected on the basis of ethnopharmacological information or on their potential cicatrizing or antiinflammatory properties, as presumed following local medicinal knowledge. They were identified at the SNT&ST and at the Botany Laboratories, Institut de Recherche pour le Développement (IRD), Nouméa, New Caledonia. Voucher specimens are deposited at the Herbarium of IRD Centre, Nouméa. Plant materials were oven-dried at 40 °C and then ground.

2.2. Preparation of plant extracts

2.2.1. Preparation of crude ethanolic extracts

Dried powdered material (50 g) was extracted by maceration with 250 ml of ethanol (80%) or dichloromethane, for 3 h under shaking at room temperature. Total extracts were filtered and concentrated to dryness at reduced pressure.

2.2.2. Preparation with Soxhlet apparatus

Acronychia laevis (136 g, leaves), *Codiaeum peltatum* (61 g, stem bark), *Crossostylis multiflora* (102 g, leaves, 139 g stem bark) were successively extracted in Soxhlet with petroleum ether, dichloromethane and methanol. Filtered extracts were dried using a rotary evaporator under reduced pressure at 45 °C.

2.2.3. Preparation of essential oils

Extracts of *Myoporum crassifolium* heart wood were obtained by hydrodistillation (essential oil) or by Soxhlet extraction with hexane.

2.3. Biological assays

The following parasites were used in this study: *Leishmania donovani* from the department of Parasitology of the Faculty of Pharmacy of Châtenay-Malabry (Paris XI, France), *Leishmania amazonensis* from the Pasteur Institute and *Trypanosoma cruzi* from the Department of Tropical Medicine, IICS, Asuncion, Paraguay. Biological tests were performed twice and each tested concentration in triplicate.

2.3.1. Antileishmanicidal assay

Leishmania donovani (MHOM/ET/L82/LV9) promastigotes were kindly provided by Pr. S.L. Croft from the WHO collection at the London School of Hygiene and Tropical Medicine. The test was performed as previously described by M'Bongo et al. (1997) and Okpekon et al. (2004). Briefly, promastigotes grown at 27 °C and cultivated in HEPES (25 mM)-buffered RPMI 1640 medium containing 10% fetal calf serum (FCS) and 50 μg/ml gentamycin. The test was performed in 96-well microtitre plates maintained at 27 °C. Promastigotes forms from a logarithmic phase culture were suspended to yield 10^6 cells/ml after haemocytometer counting. Each well was filled with 100 μl of culture medium and the plates were incubated at 27 °C for 1 h before drug addition. Each extract was dissolved in DMSO, then in medium and placed in microtitre plates in triplicate. The viability of parasites was evaluated by the tetrazolium-dye (MTT) colorimetric method. The results are expressed as the concentration inhibiting parasite growth by 50% after a 72 h incubation period. The starting concentration for screening was 100 μg/ml. Pentamidine was the reference drug.

In vitro activity against intracellular *Leishmania amazonensis* amastigotes. Female BALB/c mice aged 2–4 months were obtained from the breeding center of the Pasteur Institute. *Leishmania amazonensis* strain LV79 (MPRO/BR/1972/M1841) was propagated in BALB/c mice. *Leishmania amazonensis* amastigotes were isolated from lesions and purified as described earlier (Antoine et al., 1989). Bone marrow plugs from tibias and femurs of BALB/c mice were suspended in RPMI 1640 medium (Seromed) supplemented with 10% heat-inactivated fetal calf serum (FCS, Dutscher, Brumath, France), 50 mg/ml of streptomycin, 50 IU/ml of penicillin (culture medium) and with 15% L-929 fibroblast-conditioned medium. Cells were then distributed in bacteriologic Petri dishes (Greiner, Germany) and were incubated at 37 °C in a 5% CO_2 atmosphere. Five days later, adherent macrophages were washed with Dulbecco's phosphate buffered solution (PBS) and taken off by treatment for 20 min at 37 °C with Ca^{2+} and Mg^{2+}-free Dulbecco's PBS containing 2 mg/ml of glucose. Recovered macrophages were suspended in culture medium and they were then deposited in flat-bottom 96-well plates (Tanner, Switzerland) at a density of 4×10^4 cells/well. Twenty-four hours after replating, macrophages were infected at a multiplicity of 5 amastigotes per host cell and were incubated at 34 °C, which is the permissive temperature for the survival and multiplication of LV79 strain amastigotes. In most instances, more than 95% of the macrophages were found to be infected.

For all drugs, stock solutions were prepared in DMSO at a concentration of 500 μg/ml. Two fold serial dilutions were made from 250 μg/ml in culture medium supplemented with 0.5% DMSO final. Twenty-four hours after infection, freshly prepared drugs were added to the infected cultures in triplicate. The first final drug concentration was 25 μg/ml and the final DMSO concentration 0.1%. This DMSO concentration was proven to have no effect on control cultures.

Thirty hours after drug addition, infected cultures were examined using an inverted phase contrast Zeiss microscope (magnification of 400). Toxic effects in the macrophages were evidenced by the change in morphological features i.e. loss of

refringency, vacuolation of cytoplasm or loss of cytoplasmic material.

Leishmanicidal effects of drugs are easily detectable looking at the regression of parasitophorous vacuoles and the overall decrease in parasite number. For some drugs, a complete clearance of amastigotes was achieved. Amphotericin B, miltefosine and an alkenylquinoline, *E*-3-Quinol-2-yl-prop-2-en-1-ol (Fakhfakh et al., 2003).

2.3.2. Antitrypanosomal assay

For in vitro tests against *Trypanosoma cruzi*, Albino mice infected with *Trypanosoma cruzi* were used 7 days after infection. Blood was obtained by cardiac puncture using 3.8% sodium citrate as anticoagulant in a 7:3 blood/anticoagulant ratio. The parasitemia in infected mice ranged from 1×10^5 to 5×10^5 parasites per millilitre. The extracts were dissolved in cold DMSO to give a final concentration of 250 μg/ml.

Table 1
Ethnobotanical data of studied plants

Family name	Scientific name	Voucher	Local name	Part used	Uses
Amborellaceae	*Amborella trichopoda* Baillon	PC21-1-03	No name collected		No use known
Apocynaceae	*Cerberiopsis candelabra* Vieillard var. *candelabra*	PC3310	candélabre (ncf)	Latex	Poison
Apocynaceae	*Melodinus scandens* Forster & G. Forster	JWRG6	girawa (nemi), kuimuea (xârâcuu)	Leaves	Pulmonary congestion
Apocynaceae	*Pagiantha cerifera* (Pancher & Sébert) Markgraf	PC3310	arbre à cire (ncf)	Latex, bark	Rhumatisms
Clusiaceae	*Calophyllum caledonicum* Vieillard	JWRG22	No name collected		
Clusiaceae	*Garcinia pedicillata* Seeman	DN 55	No name collected		
Cyperaceae	*Scleria scrobiculata* Nees & Meyen	JWRG25	No name collected		
Droseraceae	*Drosera neocaledonica* Raymond	PC3194 & 3229	gobe-mouche (ncf)	Whole plant	Tuberculosis, chest pains
Euphorbiaceae	*Codiaeum peltatum* (Labillardière) P.S. Green	JWRG23	Croton	Bark	Tuberculosis
Euphorbiaceae	*Fontainea pancheri* (Baillon) Heckel	DN 72	No name collected		Ichtyotoxic
Euphorbiaceae	*Glochidion billardieri* Baillon	JWRG3	Hmana (drehu)	Leaves	Cicatrization
Fabaceae	*Erythrina variegata* var. *fastigiata* Guillaumin (comb. ined)	JWRG8 & DN 68	peuplier canaque mâle (ncf) amî (iaai), ngetae (fagauvea)	Bark	Fever
Loganiaceae	*Fagraea berteriana* A. Gray	JNN 18	bois pétrole, bois tabou (ncf), mindugo (arhö)	Bark, leaves	Rhumatisms, irritations caused by *Semecarpus* spp.
Myoporaceae	*Myoporum crassifolium* Forster & G. Forster	DN93	ndji (kwênyii)	Wood essential oil	
Myristicacae	*Myristica fatua* Houttuyn	PC1046	nandai (bislama pidgin, Vanuatu)	Sap, fruit	Yaws
Myrtaceae	*Babingtonia leratii* (Schlechter) A.R. Bean	JWRG5	fausse bruyère (ncf), noku in (kwênyii)	Flowering branches	Rhumatisms
Myrtaceae	*Tristaniopsis calobuxus* Brongniart & Gris	DN 17	No name collected		
Myrtaceae	*Tristaniopsis glauca* Brongniart & Gris	DN 18	No name collected		
Myrtaceae	*Tristaniopsis yateensis* J.W. Dawson	DN 84	No name collected		
Piperaceae	*Piper methysticum* G. Forster	commercial	kava	Root	Rhumatisms
Polygonaceae	*Polygonum subsessile* R. Brown	JWRG13	No name collected		
Rhizophoraceae	*Crossostylis multiflora* Brongniart & Gris	JWRG20	hêtre noueux (ncf), chêne gris de farino (ncf), opwäro (paicï)	Bark	Ulcerations
Rubiaceae	*Gardenia urvillei* Montrouzier	JW 77	tiaré des forêts sèches (ncf), peiokwé (ajië)	Bark	Candidosis
Rutaceae	*Acronychia leavis* Forster & G. Forster	JWRG14	bolè (nengone)	Wood or leaves	Cicatrization
Rutaceae	*Murraya crenulata* (Turzaninow) Oliver	JW36	No name collected		
Rutaceae	*Zieridium melicopaefolium* Guillaumin	JWRG10	wayiü (cèmuhî)	Bark	Vulnerary
Santalaceae	*Santalum austrocaledonicum* Vieillard	JWRG7	santal (ncf), tapaka (drehu)	Leaves	Vulnerary
Sapindaceae	*Cupaniopsis glomeriflora* Radlkofer	JWRG4	chêne blanc (ncf)	Leaves	Ulcerations (external)
Smilacaceae	*Smilax orbiculata* Vieillard ex A. de Candolle	JWRG1	wênyii (ôrôê)	Leaves	Cicatrization
Urticaceae	*Cypholopus decipiens* Winckler	JWRG28	No name collected		

Table 2
Antiprotozoal activity of some medical plants from New Caledonia and Vanuatu

Family name	Scientific name	Part used	Extract	*Leishmania donovani* (EC 50 μg/ml)	*Trypanosoma cruzi* (% reduction) at 250 μg/ml	*Leishmania amazonensis* (IC 50 μg/ml)	*Cytotoxicity* upon macrophages
Amborellaceae	*Amborella trichopoda* Baillon	Seeds	MeOH	>10	0	>25	>25
		Bark	CH_2Cl_2	>10	12.1 ± 1.5	>25	>25
		Leaves	CH_2Cl_2	>10	80.4 ± 5.3	>25	>25
Apocynaceae	*Cerberiopsis candelabra* Vieillard var. *candelabra*	Leaves	CH_2Cl_2	>10	20.7 ± 1.2	>25	>25
			MeOH	>10	43.0 ± 2.7	>25	>25
		Fruits	CH_2Cl_2	>10	70.1 ± 4.8	>25	>25
			MeOH	>10	28.9 ± 3.2	>25	>25
Apocynaceae	*Melodinus scandens* Forster & G. Forster	Leaves	EtOH	>10	44.5 ± 3.3	>25	>25
Apocynaceae	*Pagiantha cerifera* (Pancher & Sébert) Markgraf	Fruits	CH_2Cl_2	>10	71.8 ± 5.7	25	25
		Leaves	CH_2Cl_2	5	24.4 ± 1.9	12.5	>25
Clusiaceae	*Calophyllum caledonicum* Vieillard	Bark	CH_2Cl_2	>10	37.8 ± 3.1	>25	>25
		Leaves	CH_2Cl_2	>10	11.2 ± 0.9	>25	>25
Clusiaceae	*Garcinia pedicillata* Seeman	Leaves	CH_2Cl_2	>10	20.7 ± 1.1	12.5	12.5
Cyperaceae	*Scleria cf. polycarpa* Boeckeler	Aerial parts	CH_2Cl_2	>10	0	>25	>25
Droseraceae	*Drosera neocaledonica* Raymond	Whole plant	MeOH	6.5	50.9 ± 4.1	>25	>25
Euphorbiaceae	*Codiaeum peltatum* (Labillardière) P.S. Green	Bark	CH_2Cl_2	5	64.1 ± 4.7	25	25
Euphorbiaceae	*Fontainea pancheri* (Baillon) Heckel	Leaves	CH_2Cl_2	>10	60.8 ± 5.4	>25	>25
Euphorbiaceae	*Glochidion billardieri* Baillon	Leaves	EtOH	>10	0	25	25
		Bark	EtOH	>10	71.4 ± 5.3	25	25
Fabaceae	*Erythrina variegata* var. *fastigiata* Guillaumin (comb.ined.)	Bark	EtOH	>10	76.3 ± 6.0	25	25
Loganiaceae	*Fagraea berteriana* A. Gray	Leaves	MeOH	>10	NT	NT	NT
			CH_2Cl_2	>10	60.0 ± 5.7	>25	>25
		Bark	MeOH	>10	NT	NT	NT
			CH_2Cl_2	>10	14.6 ± 1.2	>25	>25
		Flowers	Hexane	>10	65.6 ± 5.8	>25	>25
Myoporaceae	*Myoporum crassifolium* Forster & G. Forster	Wood	Hexane	>10	28.9 ± 3.2	25	25
			Hydrodistillate	>10	14.4 ± 1.7	25	25
Myristicacae	*Myristica fatua* Houttuyn	Fruits	CH_2Cl_2	9	59.3 ± 5.9	25	>25
			MeOH	>10	63.0 ± 6.1	>25	>25
Myrtaceae	*Babingtonia leratii* (Schlechter) A.R. Bean	Leaves	EtOH	>10	42.8 ± 4.9	25	25
Myrtaceae	*Tristaniopsis callobuxus* Brongniart & Gris	Leaves	MeOH	>10	25.6 ± 3.2	>25	>25
			Petroleum Et	>10	29.1 ± 3.4	>25	>25
		Bark	MeOH	>10	37.6 ± 4.3	>25	>25
			Petroleum Et	>10	26.4 ± 2.9	>25	>25
		Root	MeOH	>10	67.3 ± 6.4	>25	>25
			Petroleum Et	>10	NT	NT	NT

Myrtaceae	*Tristaniopsis glauca* Brongniart & Gris	Leaves	MeOH	>10	40.8 ± 5.1	>25	>25
			Petroleum Et	>10	0	>25	>25
		Bark	MeOH	>10	NT	NT	Nt
			Petroleum Et	>10	30.3 ± 2.5	>25	>25
Myrtaceae	*Tristaniopsis yateensis* J.W. Dawson	Leaves	Petroleum Et	>10	31.7 ± 3.2	25	25
			MeOH	>10	NT	NT	NT
		Bark	Petroleum Et	>10	36.8 ± 3.4	>25	>25
			MeOH	>10	26.0 ± 2.1	>25	>25
Piperaceae	*Piper methysticum* G. Forster	Bark	EtOH	>10	40.9 ± 3.9	>25	>25
		Leaves	EtOH	>10	0	>25	>25
		Methysticin		>10	60.4 ± 6.8	>25	>25
		Yangonin		>10	57.3 ± 6.1	>25	>25
		Kavain		>10	66.8 ± 6.1	>25	>25
		Dihydrokavain	>10	0	>25	>25	
Polygonaceae	*Polygonum subsessile* R. Brown	Bark	CH_2Cl_2	>10	35.7 ± 3.9	>25	>25
		Leaves	CH_2Cl_2	>10	0	>25	>25
Rhizophoraceae	*Crossostylis multiflora* Brongniart & Gris	Leaves	CH_2Cl_2	5.5	34.2 ± 2.8	>25	>25
		Bark	CH_2Cl_2	>10	41.3 ± 5.7	>25	>25
Rubiaceae	*Gardenia urvillei* Montrouzier	Flowers	Hexane	4.5	34.9 ± 2.7	>25	>25
			CH_2Cl_2	>10	22.1 ± 1.9	>25	>25
			EtOH	>10	44.0 ± 5.4	>25	>25
Rutaceae	*Acronychia leavis* Forster & G. Forster	Leaves	CH_2Cl_2	>10	32.5 ± 4.1	>25	>25
Rutaceae	*Murraya crenulata* (Turczanimow) Oliver	Wood	Hydrodistillate	>10	37.4 ± 4.8	>25	>25
			Hexane	>10	0	>25	>25
Rutaceae	*Zieridium melicopaefolium* Guillaumin	Bark	EtOH	3.5	48.8 ± 5.9	>25	>25
Santalaceae	*Santalum austrocaledonicum* Vieillard	Bark	EtOH	>10	42.2 ± 3.9	25	25
		Leaves	EtOH	>10	0	25	25
Sapindaceae	*Cupaniopsis glomeriflora* Radlkofer	Leaves	EtOH	3.3	38.7 ± 5.0	>25	>25
Smilacaceae	*Smilax orbiculata* Vieillard ex A. de Candolle	Leaves	EtOH	>10	71.3 ± 7.9	25	25
Urticaceae	*Cypholopus decipiens* Winckler	Leaves	CH_2Cl_2	>10	59.4 ± 6.4	25	25
		Bark	CH_2Cl_2	>10	34.9 ± 4.5	>25	>25
Reference drugs							
	Amphotericin B					0.06	25
	Miltefosine			1.5		3.12	12,5
	Alkylquinoline: *E*-3-Quinol-2-yl-prop-2-en-1-ol					0.78	7.8
	Violet gentiane				100		

NT: not tested.

Aliquots (10 μl) of each extract at different concentrations (4, 20, 40, 100, 250 μg/ml) in triplicate were mixed in microtiter plates with 100 μl infected blood containing different parasite concentrations (1×10^5 and 1×10^6 parasites/ml). Infected blood and infected blood containing gentian violet at a 250 μg/ml concentration were used as control. The plates were shaken for 10 min at room temperature and kept at 4 °C for 24 h. Each solution was examined microscopically at 400×, placing a 5 μl-sample on a slide and covering it with a 22 mm × 22 mm coverglass for parasite counting. The activity of each extract (% of parasite reduction) was compared with the standard drug gentian violet at the concentration of 250 μg/ml (Rojas de Arias et al., 1994).

3. Results and discussion

Sixty-three extracts (methanol, ethanol, dichloromethane, petroleum ether, hexane, hydrodistillate) of 30 plants belonging to 20 botanical families were screened for their antiprotozoal activity against two species of *Leishmania* ssp. (*Leishmania amazonensis* and *Leishmania donovani*) and *Trypanosoma cruzi*. We added four kavalactones isolated from *Piper methysticum* (methysticin, yangonin, kavain and dihydrokavain) and three reference drugs amphotericin B (Fungizon®), miltefosine and violet gentian. The selections of plants based on the collected ethnobotanical data are presented in Table 1. Seven plants are used in traditional remedies (*Glochidion billardei*, *Fagraea berteriana*, *Crossostylis multiflora*, *Gardenia urvillei*, *Acronychia laevis*, *Cupaniopsis glomeriflora* and *Smilax orbiculata*). The in vitro antiprotozoal and cytotoxic activities (EC_{50} values) of extracts are shown in Table 2. Among the 67 samples investigated in this study, 9 plants showed variable levels of activity against one or more of the test organisms. Nevertheless the activity degrees of the extracts were generally far removed from the reference drugs.

Extracts from *Pagiantha cerifera*, *Codiaeum peltatum*, *Gardenia urvillei*, *Zieridium melicopaefolium* and *Cupanopsis glomerifera* exhibited the strongest activity against *Leishmania donovani* (EC_{50} below 5 μg/ml). Alone the leaves dichloromethane extract of *Pagiantha cerifera* displayed activity against the promastigote forms of *Leishmania donovani* and the intracellular amastigotes of *Leishmania amazonensis*. This activity is probably linked to presence of indole alkaloids (Van Beek et al., 1984; Bert et al., 1989). Various indole alkaloids have been previously found to be active against *Leishmania donovani* (Iwu et al., 1994). For the other part, the activity of the hexanic flowers extracts could be linked to a possible presence of triterpenes as described from *Gardenia saxatilis* (Suksamrarn et al., 2003). For *Codiaeum peltatum* and *Cupaniopsis glomerifera* no data in relation with their phytochemical composition is available. Three extracts from *Crossostylis multiflora*, *Drosera neocaledonica* and *Myristica fatua* showed only moderate activity against *Leishmania donovani* (EC_{50} ranging between 5 and 10 μg/ml). From the genera *Drosera*, various naphthoquinones were isolated (Budzianowski, 2000), the presence of naphthoquinones known for their activity against *Leishmania amazonensis* (Fournet et al., 1992) could explain the leishmanicidal activity of this plant.

Against the intracellular amastigotes of *Leishmania amazonensis*, the most active extracts were *Pagiantha cerifera* and *Garcinia pedicillata* with an IC_{50} value of 12.5 μg/ml, but with a cytotoxicity to macrophages observed in the case of *Garcinia pedicillata* (12.5 mg/ml). Recently, cancer chemopreventive agents were isolated from two *Garcinia* species collected in New caledonia (Ito et al., 2001). Fourteen extracts had IC_{50} value of 25 μg/ml, this activity may be due to their cytotoxicity macrophages.

Against the bloodstream forms of *Trypanosoma cruzi*, alone the *Amborella trichopoda* leaves extract lead to a reduction by 80% of parasites in the blood; this activity is not far from that of the reference drug, violet gentian (100%). Five extracts had some trypanocidal activity reducing by 70% the number of parasites in the blood, *Glochidion billardieri*, *Erythrina variegata* var. *fastigiata*, *Smilax orbiculata* and two Apocynaceae, *Cerberiopsis candelabra* and *Pagiantha cerifera*.

4. Conclusion

In this first screening for antiprotozoal activity with 30 medicinal plants collected in New Caledonia and Vanuatu, some plants presented interesting in vitro activities for visceral leishmaniasis. Against Chagas disease, alone *Amborella trichopoda* had an interesting activity against the trypomastigotes in the blood. Among the plants tested in this study, only *Pagiantha cerifera* was previously studied, yielding phytochemicals like indole alkaloids (Harmouche et al., 1976; Bert et al., 1989). Finally, these essays were the first attempts to search antiprotozoal activities in endemic or native species of the floras of New Caledonia and Vanuatu. In these leishmania- and tryponosoma-free countries, no direct links with related pathologies and treatments could be expected from traditional medicine. However, we tried to focus on some species employed in remedies against fevers and/or inflammation. No systematic treatment was done of the whole group of species locally administered in antipyretic and anti-inflammatory aims; it seems however that a plant having such reputations in traditional medicine may be worth to be selected in priority, especially if it is endemic or still original, respectively in terms of botany and chemistry. This approach could be a good way to a source of new drugs to treat protozoal diseases.

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