Ethnopharmacology in the search for new leishmanicidal drugs

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Key words: ethnopharmacology, leishmaniasis, antileishmanial activity, Colombian plants

Introduction

Cutaneous leishmaniasis is a zoonotic disease caused by species of the protozoal parasite, *Leishmania*. The disease causes deep characteristic tropical ulcers and/or nodules, which, upon healing, often result in disfiguring permanent scars. The different forms of leishmaniasis require expensive treatments, and the currently used medicines, pentavalent antimonials and/or pentamidine salts, show toxicity together with numerous side effects. Diverse cultural groups around the word have developed extensive inventories of ethnomedical therapies to treat parasitic infections. But, compared to malaria and other major tropical diseases, only a handful of authors have investigated the leishmaniasis-related ethnomedical knowledge, and practices or catalogued the medicinal plants used for treatment of the disease.

The pacific coast of Colombia is part of the the biogeographical Chocó region which goes from Panama to the Ecuadorian coasts. The region is predominantly populated by black ethnic population and is an endemic area for malaria and cutaneous and mucocutaneous leishmaniasis. Traditional therapies against protozoal infections still play an important role among these communities.

Materials and methods

Ethnopharmacological and botanical studies

Ethnopharmacological and botanical researches were carried out in the Department of Valle, in the Occidental part of Colombia, mainly on the Pacific coast near Buenaventura. The coastal area near Buenaventura, inhabited by Afro-Colombian communities and characterized by a very hot and humid climate, is occupied by primary and secondary forest and is a endemic region for cutaneous and mucocutaneous leishmaniasis. Besides selecting plants on the basis of ethnopharmacological criteria, we collected the other species on the basis of chemotaxonomic criteria. Herbarium samples were determined by Lic. R. Gonzalez, and voucher specimens were deposited at the Herbarium of the Universidad del Valle, Cali (CUVC).

Preparation of extracts

For each part of plant, the methylene chloride extract was prepared by macerating 5 g of powdered dry plant material in stoppered flasks containing 50 ml of methylene chloride for 3 days. After extraction, the same plant material was dried and used again for thé preparation of the methanolic extract, using 50 ml of methanol in a stoppered flask for 3 days. After filtration, the solvent was evaporated under reduced pressure.

Biological assays

Leishmanicidal assays were performed in vitro on the promastigote forms of *Leishmania*. Three strains of *Leishmania* were used during these investigations: *Leishmania mexicana amazonensis* (IFLA/BR/67/PH8) responsible for the cutaneous form, *L. braziliensis braziliensis* (MHOM/BR/75/M 2903) responsible for the mucocutaneous form of the disease, and *L. donovani* infantum (MHOM/IN/PP75) responsible for the visceral form. All strains were obtained from IBBA (La Paz Bolivia).

Leishmania promastigote were cultivated at 28°C. in Schneider-Drosofila medium (Sigma S9895) supplemented with heat inactivated (56°C. for 30 min) fetal calf serum (10%). Plant extracts passed through 0.22 µm Millipore filters, were previously dissolved in saline or DMSO (with a final concentration not exceeding 0.1%) and then dissolved in the culture medium. Parasites in logarithm growth phase were dispatched in 96 flat bottom well plates at a concent

ation of 106/ml. Each well contained increasing concentration of the extract, from 10 µg/ml up to 100 µg/ml during 72 hours. The activity was determinated by evaluating the movements of the parasites with an inverted microscope and compared to control wells (without extract and with reference drugs). The movements were estimated as follow: 0 cross means that the parasite are in good conditions and the drug inactive;1 cross, the drug is poorly active; 2 crosses, the drug is active; 3 crosses, no movement is detected, the drug is very active. Pentamidine (Aldrich chemical) and ketoconazole (Janssen Pharmaceutica) were used as reference drugs. All assays were carried out in triplicate (Moretti *et al.*,1998).

Results and discussion

In table 1, we report the use of 5 plants used topically to treat cutaneous leishmaniasis on the Pacific coast of Colombia. Table 2 summarizes the results obtained with the extracts of the botanical species that showed toxicity against Leishmania spp.

4 out of the 5 species used traditionally against leishmaniasis (80%) were active in vitro at 100 µmg/ml against *Leishmania* spp. promastigotes: *Conobea scoparioides, Hygrophila guianensis, Otoba novogranatensis* and *Otoba parviflora.* On the other hand, out of the 40 other species selected on the basis of bibliographic or chemotaxonomic criteria, 5 only (12 %) showed leishmanicidal activity *in vitro*: *Tabernaemontana obliqua, Huberodendron patinoi, Protium amplium, Marila laxiflora* and *Guarea polymera.*

Hygrophila guianensis Nees (Acanthaceae), Chupador. The leaves of this herbaceous plant are used as a topical application against leishmaniasis by black and indigenous groups of Southwest Colombia (Caballero, 1995). Neither biological nor chemical data about this species could be found in the literature.

Tabernaemontana obliqua (Miers) Leeuwenb. (Apocynaceae), syn. *Bonafousia obliqua* Miers, Mierda de guagua. Various species from this genus are used in Colombia and in all the Amazonian area as antirheumatic (García Barriga, 1992; Duke and Vasquez, 1994). The genus is well-known for the presence of indole alkaloids. Neither biological nor chemical data about this species could be found in the literature.

Huberodendron patinoi Cuatrec. (Bombacaceae), Carrá. This large tree is used as a commercial source of timber on the Pacific coast of Colombia (Poyry, 1982). The species is endemic of the Chocó region. Neither biological nor chemical data about this species could be found in the literature.

Protium amplium Cuatrec. (Burseraceae), Anime. Several species from this genus are sources of balsamic resinous latex used in Latin America against tumors and heavy colds (Pernet, 1972; Schultes and Raffauf, 1990). The resin essential oil of several *Protium* species, mainly constituted of monoterpenes and phenylpropanoids, show anti-inflammatory-related activity (Siani *et al.*, 1999). Neither biological nor chemical data about P. amplum could be found in the literature.

Marila laxiflora Rusby (Clusiaceae), Aceitillo. The genus *Marila* is distributed in the tropics of Central and South America and the West Indies. The roots of various species of this genus are used against dysentery by the Siona Indians of South Colombia (Schultes and Raffauf, 1990). Recently, antifungal xanthones were isolated from the roots of this species (Ioset *et al.*, 1998).

Guarea polymera Little (Meliaceae), syn. *Guarea chalde* Cuatrec., Chalde. This medium-large tree is used as a commercial source of timber on the Pacific coast of Colombia (Poyry, 1982). Neither biological nor chemical data about this species could be found in the literature.

Otoba novogranatensis Moldenke (Myristicaceae), syn. *Dialyanthera otoba* (Humb. & Bonpl.) Warb., Otobo. The genus *Otoba* comprises about ten species of shrubs to tall trees native to upland areas from Costa Rica to the western Amazon and Venezuela (Schultes and Raffauf, 1990; Gentry, 1993). Neither biological nor chemical data about this species could be found in the literature.

Otoba parviflora (Markgr.) A.H. Gentry (Myristicaceae), syn. *Dialyanthera parvifolia* Markgr., Otobo. The Waorani Indians from the Ecuadorian Amazon crush the bark and the red resin and rub it on the skin for treating infections caused by mites and fungi (Schultes and Raffauf, 1990). Farnesyl-homogentisic acid derivatives have been isolated from the seeds of the species (Ferreira *et al.*, 1995).

Conobea scoparioides (Cham. & Schltdl.) Benth. (Scrophulariaceae), Hierba de sapo. This aromatic herb or low shrub is also used in the Chocó region as anticonceptive (García Barriga, 1992). The aerial parts of the plant show cell adhesion inhibition *in vitro*, and contain cucurbitacin E and monoterpenes (Musza *et al.*, 1994; Alpande de Morais *et al.*, 1972).

Acknowledgment

The authors wish to thank the informants from the Pacific area, R.T. Gonzalez for professional support, G. Ruiz and E. Balanza for technical assistance, COLCIENCIAS (project N°1115-05-353-96), the International Foundation for Sciences, the Ministère Français des Affaires Etrangères and ECOS-Nord (action n°CF99A01) for financial support.

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Table I. Plant species used for leishmaniasis in Western Colombia

Family	Scientific name	Local name	Part used	Voucher N°
Acanthaceae	*Hygrophila guianensis* Nees	Chupador	AP	BW147
Moraceae	*Castilla elastica* Sessé	Caucho negro	L	BW120
Myristicaceae	*Otoba novogranatensis* Moldenke	Otobo	RE	BW099
Myristicaceae	*Otoba parviflora* (Markgr.) A.H. Gentry	Otobo	RE	BW070
Scrophulariaceae	*Conobea scoparioides* (Cham. & Schltdl.) Benth.	Hierba desapo	AP	BW109

AP : aerial part ; L : leaves ; R : root ; RE : resin-like bark exudate

Table II. *In vitro* leishmanicidal activity of plant extracts

Family	Scientific name	Part [a]	E [b]	Leishmanicidal activity [c]			V [d]
				La	Lb	Ld	
Acanthaceae	*Hygrophila guianensis* Nees	AP	D	++	0	++	BW147
Apocynaceae	*Tabernaemontana obliqua* (Miers) Leeuwenb.	L	M	++	+	++	BW119
Burseraceae	*Protium amplum* Cuatrec.	FR	D	++	++	++	BW092
Clusiaceae	*Marila laxiflora* Rusby	L	D	+++	+++	+++	BW137
Meliaceae	*Guarea polymera* Little	L	D	+++	+++	+++	BW066
Meliaceae	*Guarea polymera* Little	L	M	+++	+++	+++	BW066
Meliaceae	*Guarea polymera* Little	B	D	+++	+++	+++	BW066
Myristicaceae	*Otoba novogranatensis* Moldenke	L	D	+++	+++	+++	BW099
Myristicaceae	*Otoba novogranatensis* Moldenke	L	M	+++	+++	+++	BW099
Myristicaceae	*Otoba novogranatensis* Moldenke	FR	D	+++	+++	+++	BW099
Myristicaceae	*Otoba novogranatensis* Moldenke	FR	M	+++	+++	+++	BW099
Myristicaceae	*Otoba parviflora* (Markgr.) A.H. Gentry	B	D	+++	+++	+++	BW070
Scrophulariaceae	*Conobea scoparioides* (Cham. & Schltdl.) Benth.	L	D	+++	+++	+++	BW109

(a) B: bark ; FR: fruits ; L: leaves ; AP: aerial parts ; S: seeds
(b) D: methylene chloride extract ; M: methanol extract
(c) La: promastigotes of *Leishmania mexicana amazonensis* (IFLA/BR/67/PH8) ;
Lb: promastigotes of *L. braziliensis braziliensis* (MHOM/BR/75/M 2903) ;
Ld: promastigotes of *L. donovani infantum* (MHOM/IN/PP75).
For La, Lb and Ld: 0 means that the drug is inactive, + that the drug is poorly active, ++ that the drug is active and +++ that the drug is very active at 100 µg/ml of extract
(d) V: voucher number.