Ethnopharmacology in the search for new leishmanicidal drugs

Weniger B.[1], Estrada A.[2], Aragón R.[2], Deharo E.[3], Arango G. J.[4], Lobstein A.[1], Anton R.[1]

1. Laboratoire de Pharmacognosie, Faculté de Pharmacie, Université Louis Pasteur de Strasbourg B.P. 24 67401 Illkirch Cedex (France) Email: weniger@pharma.u-strasbg.fr
2. Dep. de Química, Universidad del Valle A.A. 25360 Cali (Colombia) Email: raularan@quimica.univalle.edu.co
3. Institut de Recherche pour le Développement (I.R.D.) CP 9214 La Paz (Bolivia) Email: Plantibba@megalink.com
4. Facultad de Química farmacéutica, Universidad de Antioquia Medellín (Colombia) Email: qjarango@quimbaya.udea.edu.co

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

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

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 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

References

ALPANDE DE MORAIS A., MOURAO J.C., GOTTLIEB O.R., LEAO DA SILVA M., MARX M.C., MAIA J.G.S., MAGALHAES M.T (1972) Thymol-containing amazonian essential oils, *Anais da Academia Brasileira de Ciençias*, 44: 315-318.

CABALLERO R. (1995) *La etnobotánica en las comunidades negras e indígenas del delta del rio Patia*, Quito, Ediciones Abya-Yala.

DUKE J.A., VASQUEZ R. (1994) *Amazonian ethnobotanical dictionary*, Boca Raton, CRC Press.

FERREIRA A.G, FERNANDES J.B., VIEIRA P.C., GOTTLIEB O.R. (1995) Further farnesyl-homogentisic acid derivatives from *Otoba parvifolia*, *Phytochemistry*, 40: 1723-1728.

GARCIA BARRIGA H. (1992) *Flora Medicinal de Colombia*, Bogota, Tercer Mundo Editores.

GENTRY A.H. (1993) *A field guide to the families and genera of woody plants of northwest South America*, Washington DC, Conservation International.

IOSET J.-R., MARSTON A., GUPTA M.P., HOSTETTMANN K. (1998) Antifungal xanthones from the roots of *Marila laxiflora*, *Pharmaceutical Biology*, 36:103-106.

MORETTI C., SAUVAIN M., LAVAUD C., MASSIOT G., BRAVO J.A., MUÑOZ V. (1998) A novel antiprotozoal aminosteroid from Saracha punctata, *Journal of Natural Products*, 61: 1390-1393.

MUSZA L.I., SPEIGHT P., MC ELHNEY S., BARROW C.J., GILLIUM A.M., COOPER R., KILLAR L.M. (1994) Cucurbitacins, cell adhesion inhibitors from *Conobea scoparioides*, *Journal of Natural Products*, 57: 1498-1502.

PERNET R. (1972) Phytochemistry of the Burseraceae, *Lloydia*, 35: 280-287.

POYRY J. (1982) Estudios generales del sector maderero en el Litoral Pacífico Colombiano, *Cespedesia*, 11: 7-67.

SCHULTES R.E., RAFFAUF R.F. (1990) *The healing forest: medicinal and toxic plants of the Northwest Amazonia*, Portland, Dioscorides Press.

SIANI A.C, RAMOS M.F.S., MENEZES-DE-LIMA JR. O., RIBEIRO-DOS-SANTOS R., FERNANDEZ-FERREIRA E., SOARES R.O.A., ROSAS E.C., SUSUNAGA G.S., GUIMARÃES A.C., ZOGHBI M.G.B., HENRIQUES M.G.M.O. (1999) Evaluation of anti-inflammatory-related activity of essential oils from the leaves and resin of species of *Protium*, *Journal of Ethnopharmacology*, 66: 57-69.

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

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.