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Antiprotozoal activity of South American medicinal plants

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ABSTRACT South American medicinal plants used in the treatment of leishmaniasis, malaria and chagas diseases were studied for their active components. Methods of selection, collection and identification of plants were briefly discussed.

ABSTRAK Tumbuhan perubatan Amerika Selatan yang digunakan untuk rawatan penyakit leishmaniasis, malaria dan chagas dikaji untuk mendapatkan sebatian aktifnya. Kaedah pemilihan, pengumpulan dan pengenalan tumbuh-tumbuhan tersebut dibincangkan.

(Antiprotozoal activity, tropical diseases, ethnopharmacology, alkaloid, chemotaxonomy)

INTRODUCTION

The treatment of tropical diseases continues to overwhelm the health care services of developing countries. Although diseases such as malaria, leishmaniasis and chagas affect millions of people of these countries, they are considered as nonlucrative by the pharmaceutical industries ("Orphan drugs ", of limited values).

The French Institute of Scientific Research for the Development in Cooperation (ORSTOM) has initiated and developed original investigations of alternative compounds for treatment of leishmaniasis, malaria, and chagas diseases, jointly with South American scientific institutions [1]. This project begun 12 years ago in French Guyana and then was extended to Bolivia, Paraguay, and recently in Colombia. It is conducted through a close cooperation with the CNRS at Rheims (France) and with the University of Châtenay-Malabry in Paris.

Ethnobotanic informations in healing cutaneous and mucocutaneous leishmaniasis, collected in the areas where these diseases are endemic may be of great interest. Their collection is facilitated by its obvious clinical symptoms, which are easily identified by the native populations. Field work was carried out in two tropical foothills of the Andes: in the Alto Beni, the enquiries were conducted with the Chimane Indians, a native community which is well-adapted to the ecosystem and has a thriving medical tradition. Other enquiries were carried out in the Chapare lowlands, an Amazonian region that has been greatly disturbed by the recent influx of many settlers. Medical services are lacking in this rural environment and too costly for their inhabitants. As a result, they rely on the popular health practices and use the medicinal plants available to them.

EXPERIMENTAL

Selection, collection, and identification of plants:

Two strategies:
Ethnopharmacology: plants used by the indigenous populations to treat malaria, leishmaniasis and chagas disease.

Chemotaxonomy: plants belonging to genera and families related with those showing antiparasitary activity.

Screening of crude plant extracts and pure natural compounds to evaluate the antiparasitary activity

Malaria
In vivo test: Classical four days supressive test on mice infected with *Plasmodium berghei* or *P. vinckei.*

Leishmaniasis

In vitro tests:
Test on intracellular amastigotes. This test uses the specific methodology developed for natural products

- evaluation of cytotoxicity effect on peritoneal macrophages from mice
- Test on extracellular promastigotes: Detection of promastigote survival from *Leishmania braziliensis* strain MHOM/BR/75/M2903, *L. amazonensis* strain MHOM/GF/84/CAY H-142, and *L. chagasi* strain MHOM/BR/74/PP75.

In vivo test:

- Experimental cutaneous leishmaniasis on BALB/c mice.

Evaluation of the cytotoxic effect on non specific mammalian tumoral cells (KB).

Preliminary *in vitro* and *in vivo* tests are carried out with hydroalcoholic extracts prepared with the different organs (~ 100 g) of the selected plants.

RESULTS AND DISCUSSION

Over a two year period of time, we returned every two or three months to the same areas until we were able to collect fertile plant specimens which facilitated botanical identification. The sterile plants were tagged to permit future identification. The relative abundance of the plants was also measured in order to determine how new harvesting could be possible for a more in-depth chemical or pharmacological study.

Aqueous alcoholic extracts from the 15 species selected have been assayed for antileishmanial activities. Eight of them displayed significant *in vitro* antiparasitic activities; they are indicated in Table 1.

Table 1. Medicinal plants of the Bolivian tropical lowlands locally used against leishmaniasis.

Species	Family	Region/ Community (1)	Biological Actives Constituents	Ref.
Peschiera van heurckii L.Allorge	Apocynaceae	Chapare/Gambas	bis-indole alkaloids conodurine, demethylconodurine	2
Dictyoloma peruviana Planch.	Rutaceae	Chapare/Gambas	quinolones alkaloids	3
Potomorphe peltata L.	Piperaceae	Chapare, Beni/Gambas	?	
Bocconia frutescens H. & B. *and B. sp.*	Papaveraceae		alkaloids: sanguinarine chelerythrine	
Pera benensis Rusby	Euphorbiaceae	Beni/Chimanes	quinones: plumbagine biplumbagine	7,8
Galipea longiflora Kr.	Rutaceae	Beni/Chimanes	quinolines alkaloids	4,5,6
Ampelocera edentulata Kulm	Ulmaceae	Beni/Chimanes	tetralones	9

(1): Chapare and Beni : two tropical foothills of the Bolivian Andes
Gambas: old settler of the tropical lowlands
Chimanes: a native indian community

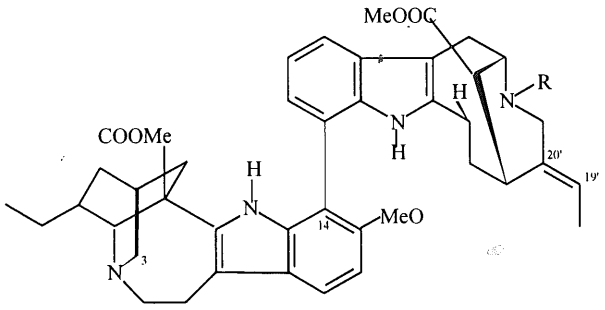
Several compounds with antipararasitic activities have been already isolated and identifed from these species.

They belong to two major chemical groups:

- Antileishmanial alkaloids

Bisindole alkaloids from *Peschiera van heurkii*, Apocynaceae

Fractionation of the alkaloid active fraction yielded 20 indole and bisindole alkaloids. Fifteen of them are known compounds. The strongest leishmanicidal activities were observed with the dimer alkaloids conodurine **1** and N-demethyl-conodurine (= gabunine) **2**. These compounds are also known to be cytotoxic. Interestingly, our results showed that they displayed a weak toxicity towards macrophage host cells, associated with a strong activity against the intracellular parasite cells [2].



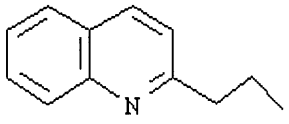
1 R= Me: conodurine
2 R= H : N-demethyl conodurine

Quinolinone alkaloids from *Dictyoloma peruviana* Planch., Rutaceae

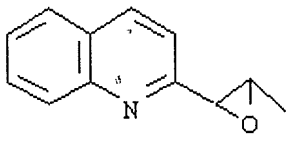
The stem-bark of *Dictyoloma peruviana* Planch. yielded two new piperidino [1,2a] 4 – quinolinones: dictyolomide A **3** and dictyolomide B **4**. These compounds induced complete lysis of *Leishmania amazonencis* at 10 mg/ml and at 100 mg/ml for other species or strains [3].

2 - substituted quinoline alkaloids from *Galipea longiflora* Krause, Rutaceae

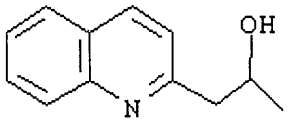
Probably the most interesting results were obtained with 2-substitued quinoline alkaloids **5** - **8** isolated from *Galipea longiflora*.. Compounds like chimanine D possess good *in vitro* activities on various strains of parasites. Their are also active in BALB/c mice infected with *Leishmania donovani, L. amazonenis* and *L. venezuelensis* [4, 5, 6]. Natural compound and synthesised derivatives are more active than Glucantime (International patent).



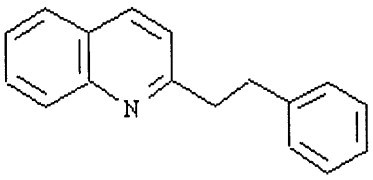
5 2-n-propylquinoline



6 chimanine



7 2-(2hydroxypropyl) quinoline

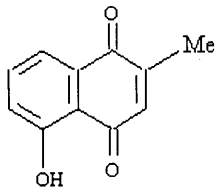


8 2-styrilquinoline

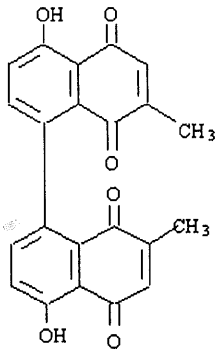
Antileishmanial Quinones

Plumbagine **9** and biplumbagine **10** - **11**, from *Pera benensis* Rusby, Euphorbiacae.

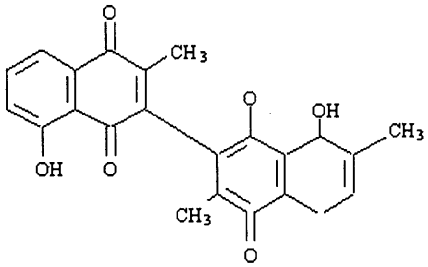
Plumbagin was found to be active *in vitro*. It was *in vivo* as potent as Glucantime at 5 mg/kg/14D by subcutaneous route [7,8].



9 plumbagine



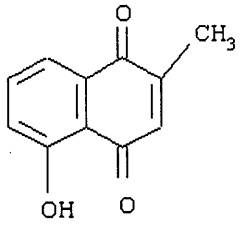
10 8,8'-biplumbagine



11 3,3'-biplumbagine

Tetralone **12** from *Ampelocera edentula*, Ulmaceae

This compound demonstrated significant activities *in vitro* and *in vivo* [9]. However the therapeutic value of this class of product is limited for its high cytotoxicity.



12 4-hydroxy-1-tetralone

Anti malarial natural products

In 1992, we came back to the studies of the antimalarial activity of natural products. We left the studies of the quassinoids bitter principles of the Simaroubaceae, for their toxicity and their too large spectrum of activities [11].

During our ethnobotanical field work in the tropical forest of Chapare province, we had the opportunity to collect samples of a small shrub which was later identified by the taxonomist Dr.Mc. Kee as *Cuatresia forsteriana*; it was the first time this species was found in Bolivia. Petroleum and alcoholic extracts of the leaves exhibited significant *in vivo* activities.

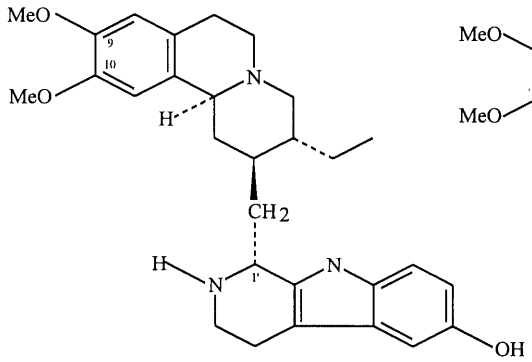
Jacaranone **13**, another quinone isolated from *Jacaranda copaia* Bignoniaceae collected in French Guyana showed *in vitro* activities on promastigotes of *Leishmania amazonensis* with an IC 90 = 0.02 mM . It was also active *in vivo* at 0.41mM / kg / 1D, but displayed also cutaneous toxicity in mice [10].

From the petroleum extracts, we isolated the n-hentriacontanol as the active compound. This fatty alcohol markedly reduced the virulence of experimentally induced *Plasmodium vinckei* infection [12]. The mode of action of this simple C31 linear alcohol and others fatty molecules are currently investigated. C18 fatty acids are active *in vitro* on *Plasmodium yoelli nigeriensis* while n-hentriacontanol is not active [13]. Their antimalarial activities are not due to lipid peroxidation, and they do not act by facilitating the transfer of iron. These fatty molecules have probably a specific target inside the parasite.

From the alcoholic extract, we isolated several steroidal withanolides **14**. Their complete structural elucidation is in progress. *Pogonopus tubulosus* (D.C.) Schumann - Rubiaceae, a tree growing in the southern South American subtropical rain forest, provide one of the numerous drugs called "falsa quina" in South America, which is used against malaria.

Three alkaloids were isolated: tubulosine **15**, psychotrine **16** and cephaeline **17**. Tubulosine showed a good *in vitro* antiplasmodial activity. Psychotrine was less active. Tubulosine was also active *in vivo* on the *P. vinckei* and *P. berghei* strains, at a lower concentration than its lethal dose. Mortality appeared when we increased the doses.

The results presented here lend support to the traditional common use of this plant as antimalarial. However tubulosine, the principal antimalarial compound isolated from this species presents unfortunately little interest in the curative treatment of malaria because of its toxicity [14].



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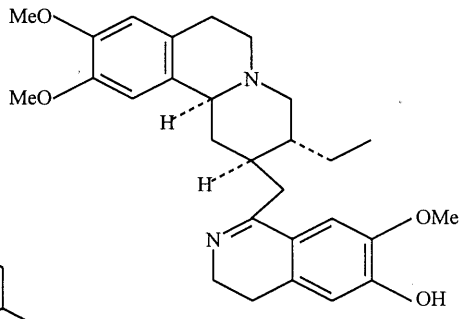

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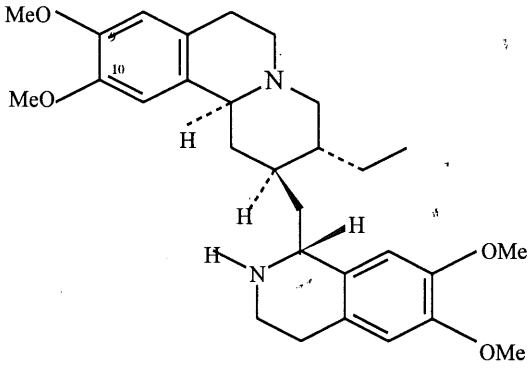

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CONCLUSION

In conclusion, the following points are worth being emphasized:

There is a lack of a simple, rapid drug-evaluation system that is universally applicable to the various leishmania species/strains. For this reason, it was necessary to develop two *in vitro* screens: bioassay on promastigote forms and mouse peritoneal macrophages infected by amastigote forms of the parasite. Although extracts of *Potomorphe peltata*, one of the more popular medicinal plant, showed *in vivo* activity, the lack of positive response with the two *in vitro* systems did not allow us for the moment a bioassay directed fractionation and purification of the active compounds.

Current research focus is on the preparation of orally active derivatives which is the only way to treat inhabitants of regions where medical services are

lacking. The demonstration that a natural product has activity against a great number of protozoal parasites is, in itself, of limited value unless the substance shows a favorable therapy: toxicity value; quassinoids are good examples of lack of specific activities.

REFERENCES

1 Moretti, C., Debitus, C., Fournet, A., Sauvain, M., Bourdy, G. and Laurent, D. (1993) *Ann. Soc. Belg. Méd. Trop.* **73**: 169-178.

2 Muñoz, V., Moretti, C., Sauvain, M., Caron, A., Porzel, A., Massiot, G. and Lemen-Olivier, L. (1994) *Planta Med.* **60**: 4455-4559.

3 Lavaud, C., Massiot, G., Vasquez, C., Moretti, C., Sauvain, M. and Balderrama, L. (1995) *Phytochemistry* **40**: 317-320.

4 Fournet, A., Angelo-Barrios A., Muñoz V., Hocquemiller R., Bruneton J., Richomme, P. and Gantier J.C. (1993) *Antimicrob. Agents and Chemother.* **37**: 859-863.

5 Fournet, A., Hocquemiller, R., Roblot, F., Cavé A., Richomme P. and Bruneton J. (1993) *J. Nat.Prod.* **56**: 1547-1552.

6 Munos, M. H., Mayrargue J., Fournet A., Gantier, J.C., Hocquemiller R.and Moskowitz, H. (1994) *Chem. Pharm. Bull.* **42**: 1914-1916.

7 Fournet, A., Angelo, A., Munoz, V., Roblot F., Hocquemiller, R. and Cave, A. (1992) *J. Ethnopharm.* **37**: 159-164.

8 Fournet, A., Angelo-Barrios, A., Munoz, V., Hocquemiller, R. and Cave, A. (1992) *Trop. Med. and Parasitol.* **43**: 219-222.

9 Fournet, A., Barrios, A.B., Muñoz, V., Hocquemiller, R., Roblotand, F. and Cave, A. (1994) *Planta Med.* **60**: 8-12.

10 Sauvain, M., Dedet, J.P., Kunesch, N., Poisson, J., Gantier, J.C., Gayral, P. and Kunesch, G. (1993) *Phytotherapy Research* **7**: 167-171.

11 Moretti, C., Deharo, E., Sauvain, M., Jardel, C., Timon David, P. and Gasquet, M. (1994) *J. of Ethnopharm.* **43**: 57-61.

12 Deharo, E., Sauvain, M., Moretti, C., Richard, B., Ruiz, E. and Massiot, G. (1992) *Ann. Parasitol.* **76**: 126-127.

13 Krugliak, M., Deharo, E., Shalmiev, G., Sauvain, M., Moretti, C., and Ginsgurg H. (1995) *Exper. Parasitol.* **81**: 97-105.

14 Sauvain, M., Moretti, C., Bravo, J., Callapa, J., Muñoz, V., Ruiz, E., Richard, B. and Lemen-Olivier, L. (1994) *Phytotherapy Research* (in press).