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ORIGINAL PAPER

The living library of The Cotapata National Park in Bolivia: an example of application of Bolivian law on the access to genetic resources

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Abstract Developing countries with a rich biodiversity want to control the use of this natural patrimony, especially in the research of natural compounds of pharmaceutical interest. Here we present the organization of six permanent plots in a mountain tropical forest on the east side of the Andean Cordillera in Bolivia, and their role in the discovery of plants with antiplasmodial or antileishmanial activities. Permanent plots are widely used in ecological survey, but rarely in bioprospecting. This set-up allows Bolivian authorities to control the bioprospecting, and facilitates further chemical studies on the bioactive plants.

Keywords Protected area · Bolivia · Mountain forest · Natural products · Antiplasmodial · Antileishmanial · Bioprospecting

Introduction

Developing countries with a rich biodiversity want to take advantage of this natural patrimony to boost their development. The discovery of new bioactive natural compounds in

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their biodiversity could be a new source of income for them (Balick and Mendelsohn 1992; Svarstad et al. 2000), though the evidence is equivocal (Firn 2003). Half of the drugs on the market today come from natural sources, mostly plants or microorganisms (Cordell 2000).

The ethnopharmacological survey is the most popular way to select plants with biological activities. However medicinal plants do not represent the entire flora of a high biodiversity area. Moreover plants coming from an ethnopharmacological study can only be used with the permission of the local community which is the depositary of the traditional knowledge, and which can refuse to give it (Lewis et al. 1999).

The aim of our work is to emphasize the knowledge and the value of some protected areas of high biodiversity, with the agreement of the governments. This work respects the Bolivian Environmental Law No. 1333 of 1992 and the Rio de Janeiro Convention on Biological Diversity firmed in 1992 (http://www.cbd.int/convention/convention.shtml), signed by the Bolivian government and ratified as “ley de la Republica No. 1580” of July 25, 1994. The regulatory laws and rules for biodiversity conservation in Bolivia are young. Regulations concerning genetic resources are based on the Decision 391 of the “Agreement of Cartagena”: Common Regime on Access to Genetic Resources of the Andean Community of Nations, formalized by the “Supreme Decree 24676” of June 1997 (Gaceta oficial 1997). The process of their application is still weak. No more than nine petitions for access to genetic resources have been submitted between 1996 and 2003 to the Bolivian governmental authorities so far two access contracts has been signed (Ministry for Sustainable Development 2004).

Here we describe the organization of plots that contain species of the trees found in a selected area of the mountain forest. The area has been chosen to mimics the originality of the flora for the study that aims at finding plants with antiparasitic activity.

The mountain forests are amongst the least known and most threatened in the tropics (Carrizosa 1990; Dodson and Gentry 1991; Henderson et al. 1991). Due to their geomorphologic and climatic complexity, they present several biotopes with high endemic flora (Gentry 1995). Altitude plays an important role in the composition of these ecosystems. Biodiversity lower on the mountain is richer than in higher forests. The mountain forests in Bolivia where the study was carried out are below the Royal Cordillera and are composed of very rich ecosystems with a high degree of biodiversity; making them of great interest for conservation (Moraes 2000).

Methodology

Organization of the plots

This study takes place in the highest portion of the east side of the Andean Cordillera in Bolivia, in a region called the “Yungas”. A national park (Parque Nacional y Área Natural de Manejo Integrado Cotapata PN-ANMI) was created in 1993. This park covers an area of nearly 60.000 ha (67°43′–68°02′ W and 16°05′–16°20′ S). Altitude ranges between 1,000 and 5,900 m, and the area is accessible by road: the lower part of the park is located 90 km NE from La Paz, and can be reached by car in 2.5 h. Climatic conditions vary according to the altitude, from areas with daily frost to hot tropical parts with an annual rainfall ranging from 1,000 mm to over 5,000 mm, and with a marked dry season between April and September (Ribera 1995; Ribera and Liberman 2006; Schawe et al. 2006).

In 1998, the Ecology Institute of the La Paz University (UMSA) installed a permanent research station (Ecological Station of Tunquini), which is used for climatologic, zoological and botanical surveys (Bach et al. 2003; Deem et al. 2004; Krömer et al. 2005).

In 2001, the DGB (General Authority on Biodiversity, Ministry for Sustainable Development, Bolivia) and the SERNAP (Protected Areas Department) gave us permission to conduct research in the National Park of Cotapata to valorize plant diversity. The resulting study deals with the concept of the pharmacological value of the flora of this park, especially for malaria and leishmaniasis. Permission for this study was granted in 2001 (note DGB/CITES No. 1314/01, the 17th of September 2001) and it was renewed in 2006 for three more years (note DGBAP/UVS No. 988/06, the 14th of September 2006).

According to Ribera (1995) the park has five main types of vegetation, changing according to the altitude. We prepared six plots in two types of forests that host the tree population of cloud forest and the mountain Yungas forest.

The cloud forest (2,300–3,500 m) has relatively small, twisted trees (10–15 m), and a canopy covered with numerous epiphytes. Some trees can reach 30 m of height. The lower part consists of lianas and bamboos. Two plots were prepared in this biotope at different altitude, so they contain different species.

In the mountain Yungas forest (1,200–2,300 m) trees are higher (15–20 m), and few trees reach more than 30 m. Four plots were set up in this biotope. Plots have been chose for their different sun exposures, in order to maximize the diversity of the species.

These six plots (20 × 20 m) were organized between October 2001 and October 2002. A phenologic monitoring was carried out on a monthly basis between 2002 and 2005, in order to spot fertile species and identify them. In every parcel, each individual with a DBH >8 cm was marked, and a voucher specimen was deposited in the Bolivian National Herbarium (LPB). During the second part of this project, samples of bark and leaves were collected from each individual identified in the various species, in order to analyse their chemical and pharmaceutical properties. These small samples (100 g of dry organ) were collected without endangering the life of the individual trees.

Plots are located as indicated in Table 1.

Determination of antiparasitic activity

Plants were collected, air dried and ground. For each resulting powder, 10 g were then left overnight in 100 ml of ethanol, and filtered. Ethanol was removed under reduced pressure and the antiplasmodial and antileishmanial activity of the extracts was evaluated.

Plasmodium falciparum was cultured according to the method described by Trager and Jensen (1976) with modifications (Benoit et al. 1995). Cultures were synchronized by 5% D-sorbitol lysis (Merck, Darmstadt, Germany) (Benoit et al. 1995). FcB1-Columbia was considered as a chloroquine-resistant strain. In vitro antimalarial activity was evaluated by [3H]-hypoxanthine (ICN, France) incorporation as described by Desjardins et al. (1979).

Table 1 Localization of the plots in the park of Cotapata

	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6
Altitude (m)	1580	1660	1720	2000	2365	2730
Geographical coordinates	16°11.600′ S 67°52.164′ W	16°11.646′ S 67°52.051′ W	16°11.520′ S 67°52.292′ W	16°12.815′ S 67°53.274′ W	16°11.635′ S 67°53.187′ W	16°11.968′ S 67°53.523′ W
Exposure	South East	South East	South West	North West	North West	North West
Type of vegetation	Lower Yungas forest	Lower Yungas forest	Lower Yungas forest	Moist upper Yungas forest	Cloud forest	Cloud forest

Incubation time between parasite culture and the drugs was 48 h. The IC_{50} of the reference drug chloroquine was 145 nM. Extracts with an $IC_{50} < 10$ µg/ml were considered active against *P. falciparum*.

Promastigotes of *Leishmania amazonensis, Leishmania brazilensis* and *Leishmania donovani* were exposed to different concentrations (10–100 µg/ml) of each extract for 72 h. Effect of extracts was compared with effect observed in parasites cultured in presence of amphotericin B and pentamidine (reference drugs for *Leishmania* species) as described in Osorio et al. (2007).

The antileishmanial activity against axenic amastigote (*Leishmania amazonensis*) was determined after 72 h incubation by a colorimetric method based on the reduction of tetrazolium salt (MTT, Sigma) (Sereno and Lemesre 1997a, b). The IC_{50} of the reference drug Amphotericine B was 300 nM. Extracts with an $IC_{50} < 50$ µg/ml were considered active against *Leishmania*.

Results and discussion

Supplementary Table 2 shows the altitudinal distribution of families, species and individuals. A total of 498 trees were marked in six plots. Species were identified for 442 individuals (89%), and genera for 49 individuals (10%). A total of 106 different species were identified among 31 families.

Rubiaceae, Melastomataceae and Euphorbiaceae were all present in five plots. The analysis of the number of individuals in each parcel shows that some species and families are well represented. At 1,580 m, two species of the Rubiaceae family, *Guettarda sabiceoides* and *Psychotria tinctoria*, are extremely well represented. At 1,660 m, the best represented families were Melastomataceae (*Meriania* aff. *axinioides, Miconia punctata*), and Rubiaceae (*Faramea candelabrum, Bathysa obovata, Elaeagia* cf. *marieae*). At 1,720 m, the best represented families are Lauraceae, Chloranthaceae (*Hedyosmum racemosum*), and Euphorbiaceae (*Alchornea triplinerva*). At 2,000 m, forests are dominated by Lauraceae, of the genus *Nectandra* (*N. cissiflora, N. turbacensis*), Rubiaceae (*Psychotria conephoroides*) and Melastomataceae (*Miconia affinis*). At 2,365 m, the main families are Melastomataceae (*Tibouchina bicolor*), Clethraceae (*Clethra revoluta*) and Saxifragaceae (s.l.) (*Escallonia paniculata*). Finally, at 2,730 m, the main families are the tree ferns (*Cyathea delgadii)*, Podocarpaceae (*Podocarpus rusbyi*) and Cunoniaceae (*Weinmannia microphylla*).

The plot organization of 2001 is still in use. The amount of dead trees has been of 6% in 5 years (32 out of 499), which typical of Amazonian biotopes. Tree falls are caused by the weight of lianas and epiphytes, and by the high seasonal rainfalls in areas with steep slopes.

For the 106 taxa identified at species level, a bibliographic survey was performed on their chemical and biological properties. We searched in the following databases: Chemical Abstracts (http://www.cas.org/), Medline (http://www.ncbi.nlm.nih.gov/entrez/), Dictionary of Natural Products on CD-Rom (http://www.ramex.com/cr/cr-dict0.html), finding no publications for 81 taxa. There are no more than three publications for 19 taxa. The most analysed taxa are *Alchornea pubescens* (4 publications), *Dendropanax arboreus* (4 publications), *Tapirira guianensis* (5 publications) and *Virola sebifera* (17 publications). Therefore the chemical composition and the pharmacological properties of the species listed here deserve further investigations. The most studied concerning chemistry and pharmacology cited in the Supplementary Table 2 are not endemic of the Tunquini biotope. In the majority they are widely distributed in the mountain forest of the Andes and some are extending to low land in Mesoamerica and Amazonian basin (http://www.mobot.mobot.org/W3T/Search/vast.html). Some of them have extended medicinal uses in different countries (Duke and Vasquez 1994).

Several organs of the taxa identified for each species level were extracted, and the extracts were tested for their activities on two parasites: one of the genus *Leishmania*, responsible for leishmaniosis, and *Plasmodium falciparum*, a parasite responsible for malaria.

For leishmaniasis, a first screening on promastigote forms of the parasite gave seven active taxa. After assessing bibliographical information on the species and the genera, facilities of recollection and abundance of the resource, three taxa were selected for further studies and recollected. These three taxa were extracted and extracts were tested on the amastigote form of the parasite. One taxon remained active, and is currently under study to identify the active compounds (Acebey et al. 2005).

For malaria, the first screening gave seven active taxa. Among them, *Cinchona pubescens* is well known to contain quinine, a major drug used against malaria, and two other species (*Alchornea pearcei, Alchornea triplinervia*), belong to genera already studied for their antiplasmodial properties (Banzouzi et al. 2002). Three taxa remain unstudied as far as their antiplasmodial properties are concerned, and deserve further investigation to identify new antiplasmodial natural compounds.

This first set-up preparation of permanent plots to facilitate bioprospecting in the Yungas could be greatly improved by using larger portions of plots, and therefore obtaining a higher degree of biodiversity (Kessler 2001).

The creation of this "permanent tree-library" or "living library" showed its educational value for scientists and authorities. Researchers have a permanent access to the resource, and can conduct studies on the field or in laboratories. The authorities can control the use of genetic resources. The access permission has been renewed regularly in the last 6 years.

However in order to obtain the maximum benefits for the scientific and academic communities, this type of research needs stability and the knowledge of the concerned authorities to control and sustain the process. Our project is one of the first, where the Bolivian government had agreed to permit access to natural resources for scientific purpose. This gives new opportunities for foreign research institutes, who have to sign an agreement of cooperation with a national scientific institution and together have to present a proposal for research to the director of biodiversity of the ministry. Both documents have to be signed by this authority. Generally it is rather easy to get the permit for collecting plant specimens for taxonomic and phylogenetic DNA analysis, if Bolivian co-researcher or students are involved. LPB handle a big exchange program for herbarium specimens with several institutions around the world. Obviously none taxonomic research and especially genetic resources research will need to be approved by the national adviser committee. One of the positive aspects of our work was the use of the same scientific language of the people in charge of the administrative regulation and the researchers. This results from a significant effort of the San Andres University of La Paz (UMSA) to participate in all the crucial meetings to elaborate the national laws to protect and use the Bolivian biodiversity. In general, research on tropical biodiversity always demands an effort in terms of time and resources on taxonomic identification. Limiting factors are missing flowering and fruiting specimens and updated taxonomic treatments for the Bolivian and Andean flora. Only few people around the world are working on the taxonomy of neotropical plants, so more botanists must be trained to recognize and name the multitude of unknown species, a big challenge for science before these biodiversity will be lost for ever, not only for young botanists in developing countries but also in the industrial countries, where are closing more and more museums with important collections of natural history or are not renewing the positions of retired curators.

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