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# Assessment of anti-protozoal activity of plants traditionally used in Ecuador in the treatment of leishmaniasis

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ABSTRACT

*Aim of the Study:* For the assessment of the in vitro anti-protozoal potential of plants traditionally used in Ecuador in the treatment of leishmaniasis, a combined approach based on interviews with healers as well as a literature search was carried out.

*Materials and Methods:* From three regions of Ecuador, 256 local healers called "Agents of Traditional Medicine" (ATMs) were interviewed about their knowledge of the use of plants to treat and heal the illness recognized by the ATMs as leishmaniasis. From literature sources, 14 plants were identified as being used in the treatment of leishmaniasis. Subsequently, plant material was collected from a representative selection of 39 species. A total of 140 extracts were screened in vitro against *Leishmania donovani*, *Plasmodium falciparum*, *Trypanosoma brucei rhodesiense* and *Trypanosoma cruzi*. Additionally, these extracts were evaluated for their anti-microbial activities using five gram-positive and -negative bacteria as well as *Candida albicans*.

*Results and Conclusions:* The survey resulted in 431 use-records for 145 plant-taxa used for the treatment of leishmaniasis. The 10 most frequently reported taxa accounted for 37.7% of all records. In the case of leishmaniasis, activity was observed for *Elephantopus mollis*, *Minquartia guianensis*, *Bocconia integrifolia*, *Gouania lupuloides*, *Scoparia dulcis*, an as-yet-unidentified species of *Piper* and *Brugmansia*. For the leaves of *M. guianensis* and the twigs and bark of *G. lupuloides* a good selectivity index (SI) was found. $IC_{50}$ values and the SI of active plant extracts are presented.



## 1. Introduction

Malaria, leishmaniasis, sleeping sickness and Chagas disease are life-threatening diseases that represent a risk to the majority of the world population. The most relevant of them is malaria, which caused ca. 247 million infections and ca. one million deaths in 2006 (WHO, 2009[a,b]). Leishmaniasis, caused by *Leishmania* sp., sleeping sickness (*Trypanosoma brucei rhodesiense*) and Chagas disease (*Trypanosoma cruzi*) are three neglected diseases that affect an estimated 21.3 million people among the poorest economic sectors (Hotez et al., 2007). Less than 1% of the 1393 drugs marketed between 1975 and 1999 were registered for tropical diseases, such as e.g. miltefosine against leishmaniasis (Trouiller et al., 2002; Chirac and Torreele, 2006; Eibl and Unger, 1990). Currently available drugs in the treatment of these diseases pose many disadvantages due to their strong side-effects, long treatment cycles, poor efficacy, high costs, limited availability and the occurrence of drug resistance (WHO, 2009[c]; Hotez et al., 2007).

Approximately 80% of the world population uses traditional medicine, primarily based on natural products (Macía et al., 2005) and natural products continue to play an important role in drug development programs (Strohl, 2000; Butler, 2004, 2005). Of the 14 anti-parasitic drugs approved from 1981–2006, seven are natural products or derived from natural products including artemisinin and three of its derivatives (Newman and Cragg, 2007).

Leishmaniasis is a disease that has been reported from 88 countries around the world, ranging from the tropics to the subtropics and including southern Europe. The number of humans infected has reached ca. 12 million with an annual incidence of 2 million and an additional 350 million people are estimated to be at risk. There are two main clinical forms of leishmaniasis: (i) visceral (VL) and (ii) cutaneous (CL) (WHO, 2009[d]). Although therapy of this disease has improved in recent years (Berman, 2005), it still represents an

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important health problem on a global scale. In Ecuador, VL has not yet been reported; however, the number of cases of CL has increased over the past years. Moreover, in four provinces (Orellana, Bolivar, Azuay and Esmeraldas) leishmaniasis is ranked among the 10 principal causes of morbidity (MSPE, 2007).

The present investigation aimed to identify plants with antiprotozoal potential, with a special focus on their use in the treatment of leishmaniasis. Two types of information were surveyed: that collected during interviews with healers using traditional medicine recognized here as Agents of Traditional Medicine (ATMs) and that found in the literature.

The most frequently represented plant species from the interviews, together with 14 species chosen by guidance from the literature survey were selected for an *in vitro* screening against *Plasmodium falciparum*, *Leishmania donovani*, *T. brucei rhodesiense*, *T. cruzi* and L-6 cells. Additionally, the anti-microbial and anti-fungal potentials of these plant extracts were assessed against *Staphylococcus aureus*, *S. epidermidis*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Escherichia coli* and *Candida albicans* using the agar diffusion method. In light of the screening results, the leishmaniacidal potential of the plant extracts was compared to the reported traditional use of these plants.

## 2. Methodology

### 2.1. Field program

The plant species reported to be used in the treatment of leishmaniasis were identified and collected in three regions of Ecuador (Fig. 1); Amazonian lowlands (100–500 m, province of Napo); lowlands of the Pacific coast (<500 m, province of Esmeraldas); and two communities in the Andes region (3000–3100 m, province of Bolívar).

The selection of these provinces was based on three criteria: (i) leishmaniasis is among the 10 main causes of morbidity in Bolivar and Esmeraldas (MSPE, 2007), (ii) Calvopiña et al. (2004) have reported that traditional methods are used in the treatment of leishmaniasis in about 70% of the cases in the northwestern region of Ecuador and app. 100% in the Amazon region, and (iii) to be able to compare the plants and methods used in the treatment of leishmaniasis in these three regions of Ecuador.

The method of interviewing used in the present study (addressed as type D method) was developed and evaluated by Kvist et al. (2001) for similar ethnobotanical studies performed in Peru. The method aims to determine medicinal plants used in the treatment of particular diseases and provides specific information about plants used to treat special or atypical illnesses (in our case leishmaniasis). In brief, the information is provided by people who have been educated in the use of plants for healing or treating different diseases. In the present study, the information was obtained by interviewing so-called Agents of Traditional Medicine (ATMs). ATMs are men and women recognized as healers by their communities as well as by the Department of Intercultural Health of each Provincial Division (i.e. entities which are part of the Ministry of Public Health of Ecuador, MPHE). The names and locations of the ATMs were provided by the MPHE.

Each ATM obtained a financial reward ($5–20 US dollars) for the information and the plant material delivered. The amount of money depended mainly on the effort required in the collection of the plant material. Upon arrival at a community, the MPHE's representative (if any) or local leaders were contacted and provided with information about the aims of the investigation.

The information collected included personal experience of the ATMs with regard to leishmaniasis, patients treated and healing methods. For each plant reported by the ATM, an individual questionnaire addressing the part of the plant used, preparation, common name and the mode of application was filled out.

A use-record is defined as "one specific plant taxon used alone or in a mixture in the treatment of leishmaniasis reported by the respective ATM" following a similar approach to Phillips and Gentry (1993). A high number of use-records indicates the most frequently used and widely known plant species among the ATMs and may serve to assess the potential of a particular remedy.

Similar to Kvist et al. (2006), most of the plants presented in our study were identified by the common name used by the ATMs. Common names given in De la Torre et al. (2008) allowed the identification of the scientific names of the plant-taxa investigated. Their identity was subsequently confirmed by comparing the voucher specimen with herbarium vouchers.

During a period of six months, 104 communities and five main cities were visited with a total of 256 ATMs of five ethnic groups of Ecuador interviewed. These included ATMs of the Kichwa of Amazonia in Napo, Kichwa of the Andes in Bolivar and Chachi, Épera and Afroecuadorian people in Esmeraldas. Additionally, one Awá ATM (anonymous) and one Mestizo ATM were interviewed. In Table 1, the list of plant species reported in the treatment of leishmaniasis together with relevant information about the collection is presented.

### 2.2. Selection of the plants used for the screening

#### 2.2.1. Selection based on interviews with ATMs

39 representative species were collected for *in vitro* screening. From these 39 species analyzed, 18 have at least four use-records and among them eight species were cited more frequently. The remaining 20 species have been reported in less than four use-records. Table 2 shows the plant species screened according to this source.

#### 2.2.2. Selection based on literature

Based on a search on relevant literature, 14 species were selected. The main source of literature information was "El Libro de las Plantas Útiles del Ecuador". This source cites ethnobotanical information recorded from herbarium vouchers from the six most important herbaria in Ecuador, the data base TROPICOS from Missouri Botanical Garden and selected publications. Eight species were reported for the treatment of leishmaniasis in De la Torre et al. (2008). Three of them, namely *Jacaranda glabra*, (2 use-records), *Scoparia dulcis* (4 use-records) and *Piptadenia* sp. (1 use-record), were also reported by ATMs.

The final five species were collected based on their activities either in wound-healing or against leishmaniasis. This information was obtained from different literature sources. They are *Minquartia guianensis*, *Pentagonia macrophylla*, *Smilax siphilitica*, *Prosopis juliflora* and *P. pallida*. *M. guianensis* and *Smilax* sp. were also reported by ATMs. The species collected under this selection criterion are listed in Table 3.

### 2.3. Plant collection and identification

The botanical material for these 39 species (Table 2) and voucher specimens for all species were collected at the site and during the time of visit to ATMs from August 2008–January 2009. The species of this collection were identified at the Herbario QCA of the Pontificia Universidad Católica del Ecuador (QCA-PUCE) where the vouchers are deposited.

14 species from literature were collected during August–September 2006 in the provinces of Napo, Orellana, Pichincha, Loja and Esmeraldas (Table 3). Plant identification was performed by staff of the Herbario Nacional del Ecuador (HNE) where the vouchers are deposited.

**Table 1**
List of plant species reported to be used in the treatment of leishmaniasis by agents of traditional medicine (ATMs).

| Family | Group | | | | | No. use-records | Part used | Application | Plant origin | Voucher QCA-JSL No. |
|---|---|---|---|---|---|---|---|---|---|---|
| | Coast (E) | | | Amazon (N) | Coast (B) | | | | | |
| Genus/species | Af | Indian | | | Mes | | | | | |
| | Af | Ch | Aw | Kic | Mes | | | | | |
| Acanthaceae | | | | | | | | | | |
| *Fittonia* Coem. sp. | | | | × | | 1 | L | Tp | W | 295 |
| *Hygrophila* R.Br. sp. | | | | × | | 3 | T | Tp | W | 276 |
| *Sanchezia* Ruiz & Pav. sp. | | | | × | | 2 | L | Tp | W | 393 |
| Adoxaceae | | | | | | | | | | |
| *Sambucus nigra* L. | | | | × | | 2 | L | Tp | W | 274 |
| Amaranthaceae | | | | | | | | | | |
| *Alternanthera* Forssk. sp. | | | | × | | 2 | L, st | Tp | W | 343 |
| *Iresine diffussa* Humb.& Bonpl. | | | | × | | 1 | L | Tp | W | 324 |
| Amaryllidaceae | | | | | | | | | | |
| *Crinum* L. sp. | | | | × | | 1 | ro | Tp | W | 064 |
| Anacardiaceae | | | | | | | | | | |
| *Spondias purpurea* L. | | × | | | | 1 | b | Tp | W | 209 |
| Apocynaceae | | | | | | | | | | |
| *Tabernaemontana sananho* Ruiz & Pav. | | | | × | | 1 | b | Tp | W | 004 |
| Araceae | | | | | | | | | | |
| *Anthurium* Schott sp. | | | | × | | 2 | L | Tp | W | 014 |
| *Colocasia esculenta* (L.) Schott | | | | × | | 1 | L | Tp | W | 401 |
| *Dieffenbachia* Schott sp. | | | | × | | 1 | Ls | Tp | W | 189 |
| *Philodendron* Schott sp. 1 | | | | × | | 1 | L | Tp | W | 183 |
| *Philodendron* Schott sp. 2 | | | | × | | 1 | L | Tp | W | 301 |
| *Philodendron* Schott sp. 3 | | | | × | | 5 | L | Tp | W | 312 |
| *Rhodospatha* Poepp. sp. | | | × | | | 1 | L | Tp | W | 197 |
| *Stenospermation* Schott sp. 1 | | | | × | | 2 | L | Tp | W | 175 |
| *Stenospermation* Schott sp. 2 | | | | × | | 1 | L | Tp | W | 342 |
| *Xanthosoma* Schott sp. | | | | × | | 11 | L, Ls, SO | Tp | W | 390 |
| Asteraceae | | | | | | | | | | |
| *Acmella brachyglossa* Cass. | | | | × | | 1 | L | Tp | W | 162 |
| *Adenostemma brasilianum* (Pers.) Cass. | | | | × | | 1 | L | Tp | W | 083 |
| *Ageratum conyzoides* L. | | | | × | | 1 | L | Tp | W | 325 |
| *Bidens pilosa* L. | | | | × | | 1 | se | Tp | W | 113 |
| *Clibadium* cf. *microcephalum* S.F. Blake | | | | × | | 10 | L, st | Tp | W | 106 |
| *Conyza bonariensis* (L.) Cronquist | | | | × | | 3 | T | Tp | W | 399 |
| *Conyza* Less. sp. | | | | × | | 2 | L, st | Tp | W | 362 |
| *Elephantopus mollis* Kunth | | | | × | | 4 | T | Tp | W | 006 |
| *Eupatorium* L. sp. | | | | × | | 2 | T | Tp | W | 263 |
| *Mikania* Willd. sp. | | | | × | | 2 | L | Tp | W | 066 |
| *Piptocoma discolor* (Kunth) Pruski | | | | × | | 1 | L | Tp | W | 071 |
| *Taraxacum officinale* F.H. Wigg. | | | | | × | 1 | L | Tp | W | 196 |
| *Vernonanthura patens* (Kunth) H. Rob. | | | | × | | 3 | L, st | Tp | W | 291 |
| Begoniaceae | | | | | | | | | | |
| *Begonia* L. sp. | | | | × | | 2 | st | Tp | W | 056 |
| Bignoniaceae | | | | | | | | | | |
| *Crescentia cujete* L. | | | | × | | 1 | L | Or | W | 173 |
| *Jacaranda glabra* (DC) Bureau & K. Schum. | | | | × | | 2 | L | Tp | W | 130 |
| Bombacaceae | | | | | | | | | | |
| *Matisia cordata* Bonpl. | | | | × | | 3 | L | Tp | W | 279 |
| Caesalpiniaceae | | | | | | | | | | |
| *Bauhinia tarapotensis* Benth. | | | | × | | 5 | L, st | Tp, Or | W | 262 |
| *Cassia* L. sp. | | | | × | | 1 | L, st | Tp | W | 323 |
| Caricaceae | | | | | | | | | | |
| *Carica papaya* L. | | | | × | | 6 | b, L | Tp | Cul | 218 |
| Celtidaceae | | | | | | | | | | |
| *Trema integerrima* (Beurl.) Standl. | | | | × | | 1 | L, st | Tp | W | 316 |

Table 1 (*Continued*)

| Family / Genus/species | Group | | | | | | No. use-records | Part used | Application | Plant origin | Voucher QCA-JSL No. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Coast (E) | | | Amazon (N) | Coast (B) | | | | | | |
| | Af | Indian | | | Mes | | | | | | |
| | Af | Ch | Aw | Kic | Mes | | | | | | |
| Commelinaceae | | | | | | | | | | | |
| *Dichorisandra hexandra* (Aubl.) Standl. | | | | × | | | 10 | L, st, T | Tp | W | 155 |
| *Dichorisandra* J.C. Mikan sp.1 | | | | × | | | 1 | st | Tp | W | 282 |
| Costaceae | | | | | | | | | | | |
| *Costus* L. sp. | | | | × | | | 2 | L | Tp | W | 095 |
| Convolvulaceae | | | | | | | | | | | |
| *Ipomoea* L. sp. | | | | × | | | 1 | L | Tp | W | 012 |
| Crassulaceae | | | | | | | | | | | |
| *Bryophyllum pinnatum* (Lam.) Oken | | | | × | | | 30 | L | Tp, Or | W | 138 |
| *Bryophyllum gastonis-bonnieri* (Raym.-Hamet & H. Perrier) Lauz.-March. | | | | × | | | 2 | L | Tp | W | 035 |
| Cucurbitaceae | | | | | | | | | | | |
| *Cayaponia* Silva Manso sp. | | | | × | | | 1 | L | Tp | W | 027 |
| *Gurania spinulosa* (Poepp. & Endl.) Cogn. | | | | × | | | 2 | L | Tp | W | 063 |
| *Gurania* (Schltdl.) Cogn. sp. | | | | × | | | 8 | L | Tp | W | 177 |
| Dilleniaceae | | | | | | | | | | | |
| *Doliocarpus* Rol. sp. | | | | × | | | 2 | L | Tp | W | 018 |
| Equisetaceae | | | | | | | | | | | |
| *Equisetum bogotense* Kunth | | | | | | × | 1 | st | Tp | W | 195 |
| Euphorbiaceae | | | | | | | | | | | |
| *Acalypha arvensis* Poepp. | | | | × | | | 1 | T | Tp | W | 322 |
| *Croton lechleri* Müll. Arg. | | | | × | | | 4 | Ex | Tp | W | 076 |
| *Euphorbia* L. sp. | | | | × | | | 1 | L, st | Tp | W | 337 |
| *Manihot esculenta* Crantz | | | | × | | | 3 | L, Cg | Tp | Cul | 139 |
| Fabaceae | | | | | | | | | | | |
| *Cajanus cajan* (L.) Millsp. | | | | × | | | 1 | b | Tp | W | 334 |
| *Desmodium adscendens* (Sw.) DC. | | | × | × | | | 8 | L, st, T, fr | Tp, Or | W | 185 |
| *Lonchocarpus seorsus* (J.F. Macbr.) M. Sousa | | | | × | | | 1 | br | Tp | W | 090 |
| *Mucuna* Adans. sp. | | | | × | | | 2 | b | Tp, Or | W | 058 |
| *Myroxylon balsamum* (L.) Harms | | | | × | | | 1 | B | Tp | W | 029 |
| *Phaseolus* L. sp. | | | | × | | | 1 | L, st | Tp | W | 022 |
| Gesneriaceae | | | | | | | | | | | |
| *Drymonia turrialvae* Hanst. | | | | × | | | 5 | L | Tp | W | 060 |
| *Drymonia* Mart. sp. | | | | × | | | 1 | T | Tp | W | 011 |
| Haemodoraceae | | | | | | | | | | | |
| *Xiphidium caeruleum* Aubl. | | | | × | | | 2 | L, tw | Tp | W | 151 |
| Lamiaceae | | | | | | | | | | | |
| *Hyptis capitata* Jacq. | | | | × | | | 1 | L | Tp | W | 400 |
| *Hyptis mutabilis* (Rich.) Briq. | | | | × | | | 7 | L, T | Tp | W | 273 |
| *Hyptis pectinata* (L.) Poit. | | | | × | | | 2 | L, T | Tp | W | 302 |
| *Minthostachys* (Benth.) Spach sp. | | | | × | | | 1 | L, fr | Tp | W | 310 |
| *Ocimum campechianum* Mill. | × | | | × | | | 3 | L | Tp | W | 199 |
| *Salvia* L. sp. | | | | × | | | 1 | L | Tp | W | 406 |
| Lecythidaceae | | | | | | | | | | | |
| *Couroupita guianensis* Aubl. | | | | × | | | 2 | Fr | Tp | W | 017 |
| *Grias neuberthii* J.F. Macbr. | | | | × | | | 1 | Se | Tp | W | 065 |
| Loasaceae | | | | | | | | | | | |
| *Klaprothia fasciculata* (C. Presl) Poston | | | | × | | | 1 | L, st | Tp | W | 364 |
| Loganiaceae | | | | | | | | | | | |
| *Strychnos* L. sp. | | | | × | | | 1 | se | Tp | W | 021 |
| Loranthaceae | | | | | | | | | | | |
| *Struthanthus* Mart. sp. | | | | × | | | 1 | T | Tp | W | 104 |
| Malpighiaceae | | | | | | | | | | | |
| *Banisteriopsis caapi* (Spruce ex Griseb.) C.V. Morton | | | | × | | | 4 | L, st | Tp | Cul | 110 |

Table 1 (*Continued*)

| Family | Group | | | | | No. use-records | Part used | Application | Plant origin | Voucher QCA-JSL No. |
|---|---|---|---|---|---|---|---|---|---|---|
| | Coast (E) | | | Amazon (N) | Coast (B) | | | | | |
| Genus/species | Af | Indian | | | Mes | | | | | |
| | Af | Ch | Aw | Kic | Mes | | | | | |
| Malvaceae | | | | | | | | | | |
| *Abutilon* Mill. sp. | | | | × | | 2 | L | Tp | W | 315 |
| *Hibiscus rosa-sinensis* L. | | | | × | | 1 | L | Tp | W | 328 |
| *Hibiscus* L. sp. | | | | × | | 1 | L | Tp | W | 311 |
| Marantaceae | | | | | | | | | | |
| *Ischnosiphon* Körn. sp. | | | | × | | 1 | L | Tp | W | 010 |
| Melastomataceae | | | | | | | | | | |
| *Adelobotrys* DC sp. | | | | × | | 1 | T | Tp | W | 361 |
| *Clidemia allardii* Wurdack | | | | × | | 1 | L | Tp | W | 329 |
| *Miconia* Ruiz & Pav. sp. 1 | | | | × | | 1 | L | Tp | W | 152 |
| *Miconia* Ruiz & Pav. sp. 2 | | | | × | | 1 | L | Tp | W | 020 |
| *Tococa guianensis* Aubl. | | | | × | | 1 | L | Tp | W | 091 |
| Meliaceae | | | | | | | | | | |
| *Cedrela odorata* L. | | × | | × | | 8 | b, L | Tp, Or | W | 207 |
| Meteoriaceae | | | | | | | | | | |
| *Meteoridium* (C. Müll.) sp. | | | | × | | 1 | T | Tp | W | 042 |
| Mimosaceae | | | | | | | | | | |
| *Inga edulis* Mart. | | | | × | | 1 | L | Tp | Cul | 108 |
| *Inga oerstediana* Benth. | | × | | | | 3 | b, L | Tp | W | 202 |
| *Piptadenia* Benth. sp. | | | | × | | 1 | L | Tp | W | 257 |
| Moraceae | | | | | | | | | | |
| *Artocarpus altilis* (Parkinson) Fosberg | | | | × | | 6 | L | Tp | Cul | 269 |
| Musaceae | | | | | | | | | | |
| *Musa acuminata* Colla | | | | × | | 1 | Cg | Tp | Cul | 073 |
| *Musa* × *paradisiaca* L. | | | | × | | 1 | Fr(br) | Tp | Cul | 085 |
| Myrtaceae | | | | | | | | | | |
| *Psidium guajava* L. | | × | | × | | 7 | b, L | Tp | Cul | 280 |
| Olacaceae | | | | | | | | | | |
| *Minquartia guianensis* Aubl. | | | | × | | 3 | b, L | Tp | W | 019 |
| Onagraceae | | | | | | | | | | |
| *Ludwigia* L. sp. | | | | × | | 2 | L, st | Tp | W | 345 |
| Phyllanthaceae | | | | | | | | | | |
| *Phyllanthus attenuatus* Miq. | | | | × | | 1 | L | Tp | W | 052 |
| Piperaceae | | | | | | | | | | |
| *Piper barbatum* Kunth | | | | × | | 1 | L | Tp | W | 331 |
| *Piper hispidum* Sw. | | | | × | | 1 | L | Tp | W | 255 |
| *Piper musteum* Trel. | | | | × | | 1 | L | Tp | W | 159 |
| *Piper peltatum* L. | | | | × | | 12 | L | Tp | W | 398 |
| *Piper* L. sp. 1 | | | | × | | 1 | Cg | Tp | W | 003 |
| *Piper* L. sp. 2 | | | | × | | 1 | L | Tp | W | 215 |
| Poaceae | | | | | | | | | | |
| *Pharus* P. Browne sp. | | | | × | | 1 | L | Sahu | W | 157 |
| *Zea mays* L. | | | | × | | 3 | L, fr(br), fl(sty) | Tp | Cul | 168 |
| Polygonaceae | | | | | | | | | | |
| *Rumex pulcher* L. | | | | × | | 1 | L | Tp | W | 115 |
| Rhamnaceae | | | | | | | | | | |
| *Gouania lupuloides* (L.) Urb. | | | | × | | 2 | b | Tp | W | 055 |
| Rosaceae | | | | | | | | | | |
| *Prunus* L. sp. | | | | × | | 1 | L, st | Tp | W | 250 |
| Rubiaceae | | | | | | | | | | |
| *Borreria laevis* (Lam.) Griseb. | | | | × | | 1 | L, st | Tp | W | 347 |
| *Coussarea* Aubl. sp. | | | | × | | 2 | b | Tp | W | 062 |

Table 1 (*Continued*)

| Family | Group | | | | | No. use-records | Part used | Application | Plant origin | Voucher QCA-JSL No. |
|---|---|---|---|---|---|---|---|---|---|---|
| | Coast (E) | | | Amazon (N) | Coast (B) | | | | | |
| Genus/species | Af | Indian | | | Mes | | | | | |
| | Af | Ch | Aw | Kic | Mes | | | | | |
| *Hamelia* Jacq. sp. | | | | × | | 1 | L | Tp | W | 044 |
| *Psychotria* L. sp. 1 | | | | × | | 2 | L | Tp | W | 013 |
| *Psychotria* L. sp. 2 | | | | × | | 7 | L, st | Tp | W | 229 |
| *Psychotria* L. sp. 3 | | | | × | | 1 | L | Tp | W | 339 |
| *Rudgea bracteata* J.H. Kirkbr. | | | | × | | 2 | L | Tp | W | 153 |
| Rutaceae | | | | | | | | | | |
| *Citrus* L. sp. 1 | | | | × | | 1 | se | Tp | Cul | 089 |
| *Citrus* L. sp. 2 | | × | | | | 2 | fr | Tp | Cul | 200 |
| *Ruta graveolens* L. | | | | | × | 1 | L, fl | Tp | Cul | 194 |
| Sapotaceae | | | | | | | | | | |
| *Chrysophyllum* L. sp. | | | | × | | 2 | se | Tp | W | 039 |
| *Pouteria torta* subsp. *tuberculata* (Sleumer) T.D. Penn. | | | | × | | 2 | L | Tp | W | 150 |
| Scrophulariaceae | | | | | | | | | | |
| *Scoparia dulcis* L. | | | | × | | 4 | L, T | Tp | W | 395 |
| Smilacaceae | | | | | | | | | | |
| *Smilax* L. sp. | | | | × | | 2 | T | Tp | W | 096 |
| Solanaceae | | | | | | | | | | |
| *Brugmansia* Pers. sp.1 | | × | | × | | 13 | L, fl | Tp | W | 319 |
| *Brugmansia* Pers. sp.2 | | | | × | | 1 | L | Tp | W | 373 |
| *Brunfelsia grandiflora* D. Don | | | | × | | 7 | L, Cg | Tp | Cul | 050 |
| *Capsicum* L. sp. | | | | × | | 1 | L | Tp | Cul | 026 |
| *Cestrum racemosum* Ruiz & Pav. | | | | × | | 3 | L | Tp | W | 001 |
| *Markea* Rich. sp. | | | | × | | 1 | L | Tp | W | 288 |
| *Nicotiana tabacum* L. | | | | × | | 40 | L, tw | Tp | Cul | 107 |
| *Solanum mammosum* L. | | | | × | | 1 | L | Tp | W | 333 |
| *Solanum nigrum* L. | | | | × | | 1 | T | Tp | W | 212 |
| *Solanum* L. sp. 1 | | | | × | | 2 | L | Tp | W | 141 |
| *Solanum* L. sp. 2 | | | | × | | 1 | fr | Tp | W | 372 |
| *Witheringia solanacea* L'Hér. | | | | × | | 19 | L, st, T | Tp | W | 088 |
| Sterculiaceae | | | | | | | | | | |
| *Theobroma cacao* L. | | | | × | | 2 | se(ari) | Tp | Cul | 053 |
| Thelypteridaceae (Pteridophyta) | | | | | | | | | | |
| *Thelypteris* Schmidel sp. | | | | × | | 1 | L | Tp | W | 341 |
| Theophrastaceae | | | | | | | | | | |
| *Clavija weberbaueri* Mez | | | | × | | 2 | L | Tp | W | 016 |
| Urticaceae | | | | | | | | | | |
| *Urtica dioica* L. | | | | × | | 1 | L | Tp | W | 338 |
| *Urera laciniata* Goudot | | | | × | | 6 | L, st | Tp | W | 266 |
| Verbenaceae | | | | | | | | | | |
| *Duranta* L. sp. | | | | × | | 1 | L | Tp | W | 314 |
| *Lantana camara* L. | | | | × | | 1 | L | Tp | W | 309 |
| *Lantana* L. sp. | | | | × | | 1 | L, st | Tp | W | 359 |
| *Verbena litoralis* Kunth | | | | × | | 10 | L, st, T | Tp, Or | W | 111 |
| Violaceae | | | | | | | | | | |
| *Leonia* Ruiz & Pav. sp. | | | | × | | 2 | L | Tp | W | 134 |
| Zingiberaceae | | | | | | | | | | |
| *Zingiber officinale* Roscoe | | | | × | | 4 | SO | Tp | W | 224 |
| Total use-registers | | | | | | 432 | | | | |

Abbreviations N: Napo; E: Esmeraldas; B: Bolivar; Af: Afroecuadorian; Ch: Chachi; Awa: Awa; Kic: Kichwa; Mes: Mestizo; b: bark; L: leaves; Ls: leave stalk; Cg: young leaves; tw: twigs; st: stems; fl: flowers; fl(sty): flower styles; fr: fruit; fr(br): bracts; se: seeds; SO: storage organs; ro: roots; Ex: exudates; T: whole plant; Tp: topical application; Or: Oral application; Sahu: sahumerio/incensing; W: wild; Cul: cultivated.

### 2.4. Preparation of extracts

Shade-dried, powdered plant material (each 1–5 g) was extracted twice using ultrasound (Brandsonic 220, INULA) subsequently with dichloromethane and methanol (each 5 min). Several samples reported in Table 3 were first extracted with hexane (details specified in Table 3).

The dried extracts were used to prepare stock solutions of c. 10 mg/mL in DMSO for use in *in vitro* assays. The exact concentration of extracts tested is detailed in each protocol. The

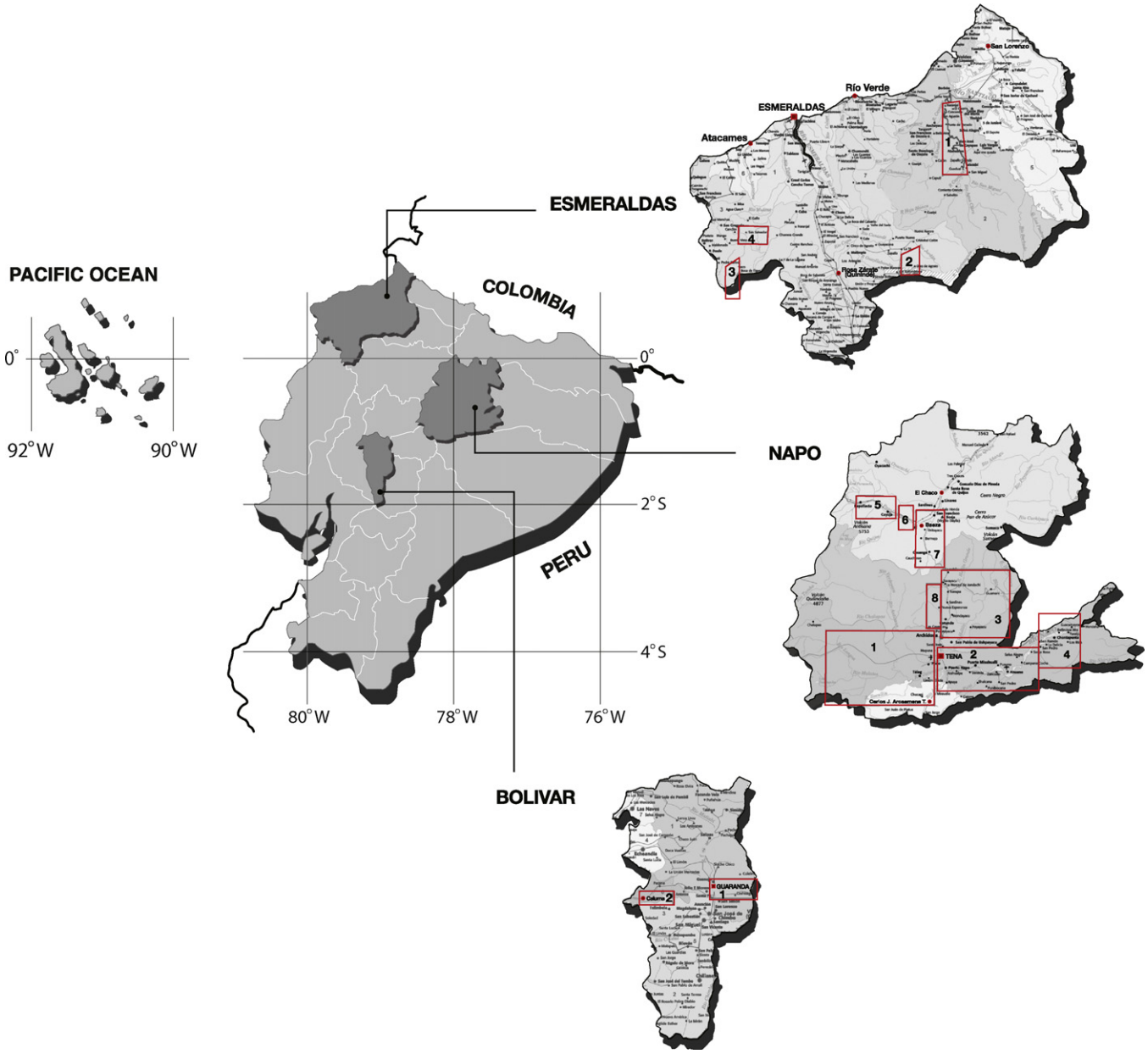


**Fig. 1.** Map of Ecuador showing the locations of the areas of study. Ethnic groups found in the areas studied (squares): Esmeraldas 1) Chachi, Awa, Épera, Afroecuatoriano, 2–4) Chachi. Napo 1–8) Kichwa of Amazonia. Bolivar 1) Kichwa of the Andes, 2) Mestizo.

extracts and DMSO solutions were stored at −20 °C prior to the analysis.

### 2.5. Biological assays

#### 2.5.1. In vitro activity against P. falciparum

*In vitro* activity against erythrocytic stages of *P. falciparum* was determined using a $^{3}$H-hypoxanthine incorporation assay (Desjardins et al., 1979; Matile and Pink, 1990), using the chloroquine and pyrimethamine resistant K1 strain that originated from Thailand (Thaithong et al. 1983) and the standard drug chloroquine (Sigma C6628). Extracts were dissolved in DMSO at 10 mg/mL and added to parasite cultures incubated in RPMI 1640 medium without hypoxanthine, supplemented with HEPES (5.94 g/L), $NaHCO_3$ (2.1 g/L), neomycin (100 U/mL), AlbumaxR (5 g/L) and washed human red cells A+ at 2.5% haematocrit (0.3% parasitaemia). Serial drug dilutions of seven 2-fold dilution steps covering a range from 5 to 0.156 μg/mL were prepared. The 96-well plates were incubated in a humidified atmosphere at 37 °C; 4% $CO_2$, 3% $O_2$, 93% $N_2$. After 48 h 50 μL of $^{3}$H-hypoxanthine (=0.5 μCi) was added to each well of the plate. The plates were incubated for a further 24 h under the same conditions. The plates were then harvested with a Betaplate™ cell harvester (Wallac, Zurich, Switzerland), and the red blood cells transferred onto a glass fibre filter then washed with distilled water. The dried filters were inserted into a plastic foil with 10 mL of scintillation fluid, and counted in a Betaplate™ liquid scintillation counter (Wallac, Zurich, Switzerland). $IC_{50}$ values were calculated from sigmoidal inhibition curves using Microsoft Excel. Each sample was tested in two independent assays. A third assay was performed if the calculated $IC_{50}$ values differed more than by a factor of 2. As positive control chloroquine at 0.07 μg/mL was used.

#### 2.5.2. In vitro activity against L. donovani

50 μL of SM medium (Cunningham, 1977) at pH 5.4 supplemented with 10% heat-inactivated FBS were added to each well of a 96-well microtiter plate (Costar, USA). Serial drug dilutions were

**Table 2**
Plant species collected during interviewing process arranged by the number of use-records by agents of traditional medicine (ATMs).

| Family | Genus/species | Part of the plant | | Application | No. use-records | Extract | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Yield (%) | | No. |
| | | Tested | Used | | | DCM | MeOH | |
| Solanaceae | *Nicotiana tabacum* | L | L/r | Tp | 40 | 2.7 | 8.3 | 2 |
| Crassulaceae | *Bryophyllum pinnatum* | L | L | Tp, Or | 30 | 2.5 | 2.9 | 2 |
| Solanaceae | *Witheringia solanacea* | L | Tw/T | Tp | 19 | 3.4 | 13.6 | 2 |
| Solanaceae | *Brugmansia* sp. 1 | L | L/fl | Tp | 13 | 3.5 | 8.9 | 2 |
| Solanaceae | *Brugmansia* sp. 2 | L | L | Tp | | 3.2 | 10.4 | 2 |
| Piperaceae | *Piper peltatum* | L | L | Tp | 12 | 8.9 | 8.9 | 2 |
| Araceae | *Xanthosoma* sp. | L | L/ls/SO | Tp | 11 | 2.9 | 3.5 | 2 |
| Asteraceae | *Clibadium* cf. *microcephalum* | L | tw | Tp | 10 | 5.7 | 11.7 | 2 |
| Verbenaceae | *Verbena litoralis* | L | tw/T | Tp, Or | 10 | 2.7 | 15.3 | 2 |
| Cucurbitaceae | *Gurania* sp. | L | L | Tp | 8 | 0.6 | 3.4 | 2 |
| Meliaceae | *Cedrela odorata* | b | L/b | Tp, Or | 8 | 0.6 | 3.2 | 2 |
| Myrtaceae | *Psidium guajava* | b | L/b | Tp | 7 | 0.5 | 7.1 | 2 |
| Solanaceae | *Brunfelsia grandiflora* | L | L | Tp | 7 | 4.2 | 15.7 | 2 |
| Rubiaceae | *Psychotria* sp. 2 | L | tw | Tp | 7 | 1.4 | 7.2 | 2 |
| Caricaceae | *Carica papaya* | L | L/b | Tp | 6 | 1.5 | 5.8 | 2 |
| Araceae | *Philodendron* sp. 3 | L | L | Tp | 5 | 1.2 | 3.7 | 2 |
| Asteraceae | *Elephantopus mollis* | L | T | Tp | 4 | 4.9 | 3.8 | 2 |
| Malpighiaceae | *Banisteriopsis caapi* | L | tw | Tp | 4 | 1.7 | 5.4 | 2 |
| Zingiberaceae | *Zingiber officinale* | SO | SO | Tp | 4 | 4.5 | 2.6 | 2 |
| Lamiaceae | *Ocimum campechianum* | L | L | Tp | 3 | 3.3 | 5.0 | 2 |
| Mimosaceae | *Inga oerstediana* | ib | L/b | Tp | 3 | 0.2 | 1.3 | 2 |
| | | L | | | | 0.4 | 5.8 | 2 |
| | | b | | | | 0.4 | 4.8 | 2 |
| Crassulaceae | *Kalanchoe* cf. *gastonis-bonnieri* | L | L | Tp | 2 | 5.0 | 3.3 | 2 |
| Dilleniaceae | *Doliocarpus* sp. | L | L | Tp | 2 | 1.0 | 3.7 | 2 |
| Fabaceae | *Mucuna* sp. | tw | b | Tp, Or | 2 | 0.7 | 2.0 | 2 |
| Rhamnaceae | *Gouania lupuloides* | b/Tw | b | Tp | 2 | 0.8 | 2.9 | 2 |
| Solanaceae | *Solanum* sp. 1 | L | L | Tp | 2 | 2.8 | 8.1 | 2 |
| Violaceae | *Leonia* sp. | tw | L | Tp | 2 | 2.6 | 5.0 | 2 |
| Anacardiaceae | *Spondias purpurea* | b | b | Tp | 1 | 0.3 | 5.9 | 2 |
| Araceae | *Dieffenbachia* sp. | tw | Ls | Tp | 1 | 3.3 | 25.2 | 2 |
| Araceae | *Philodendron* sp.1 | L | L | Tp | 1 | 1.7 | 4.6 | 2 |
| Asteraceae | *Bidens pilosa* | L/fl/tw | se | Tp | 1 | 5.2 | 5.7 | 2 |
| Asteraceae | *Taraxacum officinale* | L | L | Tp | 1 | 2.3 | 6.4 | 2 |
| Equisetaceae | *Equisetum bogotense* | st | st | Tp | 1 | 0.9 | 3.8 | 2 |
| Fabaceae | *Phaseolus* sp. | L | tw | Tp | 1 | 3.2 | 9.8 | 2 |
| Loranthaceae | *Struthanthus* sp. | tw | T | Tp | 1 | 6.5 | 9.9 | 2 |
| Mimosaceae | *Inga edulis* | L | L | Tp | 1 | 0.8 | 10.0 | 2 |
| Piperaceae | *Piper* sp. 2 | L | L | Tp | 1 | 2.9 | 7.1 | 2 |
| Polygonaceae | *Rumex pulcher* | L | L | Tp | 1 | 5.6 | 15.8 | 2 |
| Rutaceae | *Ruta graveolens* | L/fl | L/fl | Tp | 1 | 2.8 | 7.0 | 2 |
| Total extracts screened | | | | | | | | 82 |

Abbreviations b: bark; L: leaves; Ls: leaf stalks; fl: flowers; tw: twigs; ib: inner bark; SO: storage organs; T: whole plant; se: seeds; st: stems; Tp: Topical application; Or: Oral application; DCM: dichloromethane extract; MeOH: methanol extract.

prepared covering a range from 30 to 0.041 μg/mL. Then, $10^5$ axenically grown *L. donovani* amastigotes (strain MHOM/ET/67/L82) in 50 μL medium were added to each well and the plate was incubated at 37 °C under a 5% $CO_2$ atmosphere for 72 h. 10 μL of resazurin solution (12.5 mg resazurin dissolved in 100 mL distilled water) were then added to each well and incubation continued for a further 2–4 h. The plate was then read in a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wavelength of 536 nm and emission wavelength of 588 nm (Räz et al., 1997). Fluorescence development was measured and expressed as percentage of the control. Data were transferred into the graphic programme Softmax Pro (Molecular Devices Cooperation, Sunnyvale, CA, USA) which calculated $IC_{50}$ values from the sigmoidal inhibition curves. Each sample was tested in two independent assays. A third assay was performed if the calculated $IC_{50}$ values differed more than by a factor of 2. As positive control miltefosine at 0.104 μg/mL was used.

### 2.5.3. In vitro activity against T. brucei rhodesiense

Minimum essential medium with Earle's salts (50 μL) supplemented with 0.2 mM 2-mercaptoethanol, 1 mM Na-pyruvate and 15% heat-inactivated horse serum was added to each well of a 96-well microtiter plate. Serial drug dilutions were prepared covering a range from 90 to 0.123 μg/mL. Then, $10^4$ bloodstream forms of *T. b. rhodesiense* STIB 900 in 50 μL were added to each well and the plate was incubated at 37 °C under a 5% $CO_2$ atmosphere for 72 h. 10 μL of resazurin solution (12.5 mg resazurin dissolved in 100 mL distilled water) were then added to each well and incubation continued for a further 2–4 h. Plate reading and data processing was performed as described for *L. donovani* assay. As positive control melarsoprol at 0.004 μg/mL was used.

### 2.5.4. In vitro activity against T. cruzi

Rat skeletal myoblasts (L-6 cells) were seeded in 96-well microtiter plates at 2000 cells/well in 100Il RPMI 1640 medium with 10% FBS (fetal bovine serum) and 2 mM L-glutamine. After 24 h the medium was removed and replaced by 100 μL per well containing 5000 trypomastigote forms of *T. cruzi* Tulahuen strain C2C4 containing the β-galactosidase (LacZ) gene. 48 h later the medium was removed from the wells and replaced by 100 μL fresh medium with or without a serial drug dilution. Seven 3-fold dilu-

**Table 3**
Plant species selected based on literature research and screened *in vitro*.

| Family | Gener/species | Part of the plant | | Collection site | Voucher HNE No. | Extract | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Yield (%) | | | No. |
| | | Tested | Literat. | | | HX | DCM | MeOH | |
| Araceae | *Syngonium podophyllum* Schott. | L | n.s.[a] | N | 0223310 | 0.6 | 2.3 | 3.1 | 3 |
| Bignoniceae | *Jacaranda glabra* (DC) Bureau & K. Schum. | b | L[a] | N | 0227457 | 0.2 | 0.3 | 0.9 | 3 |
| | | L | | | | 0.4 | 1.6 | 4.2 | 3 |
| Euphorbiaceae | *Croton menthodorus* Benth. | br | lx[a] | L(1) | 0223312 | 0.8 | 1.6 | 4.9 | 3 |
| | | L/fl/tw | | | | 2.3 | 1.9 | 6.3 | 3 |
| Mimosaceae | *Piptadenia* cf. *anolidurus* Barneby | L | – | N | 0227456 | 0.5 | 1.9 | 5.8 | 3 |
| Mimosaceae | *Piptadenia* cf. *pteroclata* Benth. | b | n.s.[a] | O | 0227458 | 0.2 | 0.6 | 5.1 | 3 |
| Mimosaceae | *Prosopis juliflora* (SW.) DC | b | – | L(1) | 0022316 | 0.2 | 3.0 | 4.1 | 3 |
| Mimosaceae | *Prosopis pallida* (Humb. & Bonpl. ex Willd.) Kunth | b | – | L(2) | 0223315 | 0.2 | 1.6 | 3.7 | 3 |
| Myrsinaceae | *Cybianthus sprucei* (Hook. f.) A. | L | L[a] | N | 0227460 | 0.5 | 0.4 | 3.4 | 3 |
| Olacaceae | *Minquartia guianensis* Aubl. | b | – | N | 0227459 | – | 14.0 | 7.5 | 2 |
| | | L | | | | 1.1 | 2.1 | 3.0 | 3 |
| Papaveraceae | *Bocconia integrifolia* Bonpl. | b | lx[a] | P(1) | 0223311 | 0.4 | 0.4 | 0.9 | 3 |
| | | L | | | | – | 5.7 | 7.1 | 2 |
| Rubiaceae | *Pentagonia* cf. *macrophylla* Benth. | b | – | N | 0223318 | 0.2 | 0.3 | 0.7 | 3 |
| | | L | | | | 0.5 | 1.9 | 3.8 | 3 |
| Sapindaceae | *Cupania cinerea* Poepp. | b | – | P(2) | 0223317 | 0.4 | 1.0 | 5.3 | 3 |
| Scrophulariaceae | *Scoparia dulcis* L. | T | L[a] | E | 0223313 | 0.8 | 2.3 | 3.1 | 3 |
| Smilacaceae | *Smilax* aff. *siphilitica* Humb. & Bonpl. Ex. Willd. | li | – | N | 0223314 | 0.2 | 0.5 | 2.6 | 3 |
| | L | | | | | 0.8 | 1.6 | 3.3 | 3 |
| Number of extracts screened | | | | | | | | | 58 |

Sites of collection: Pichincha: P(1) refers to Cantón Quito. Vía vieja a Nono. P(2) refers to Cantón Quito. Parroquia Pacto. Quebrada cerca del río Pillallí. Loja: L(1) refers to Cantón Catacocha. Parroquia el Sauce. L (2) refers to Cantón Catamayo. Sector Guayabal. Napo: N refers to Cantón Tena. Parroquia Misahuallí. Estación Científica Jatun Sacha. Orellana: O refers to Parque Nacional Yasuní. Esmeraldas: E refers to Cantón Quinindé. Parroquia Malintia. Recinto Naranjal. Near by the Canandé River. Abbreviations b: bark; L: leaves; fl: flowers; tw: twigs; br: branches; T: whole plant; li: liana; lx: latex; n.s.: not specified; HX: n-hexane extract; DCM: dichloromethane extract; MeOH: methanol extract.

[a] De la Torre et al., 2007.

tions were used covering a range from 90 to 0.123 μg/mL. After 96 h of incubation the plates were inspected under an inverted microscope to assure growth of the controls and sterility. Then, the substrate CPRG/Nonidet (50 μL) was added to all wells. A color reaction developed within 2–6 h and was read photometrically at 540 nm. $IC_{50}$ values were calculated using Softmax Pro (Molecular Devices Cooperation, Sunnyvale, CA, USA). Each sample was tested in two independent assays. A third assay was performed if the calculated $IC_{50}$ values differed more than by a factor of 2. As positive control benznidazole at 0.435 μg/mL was used.

### 2.5.5. Cytotoxicity against L-6 cells

The rat skeletal myoblast cell line (L-6 cells) was used to assess cytotoxicity. The cells were grown in RPMI 1640 medium supplement with 1% L-glutamine (200 nM) and 10% FBS at 37 °C in 5% $CO_2$ in air. The assay was performed in 96-well microtiter plates, each well receiving 100 μL of culture medium with ca. $4 \times 10^4$ cells. After 24 h, the medium was removed from all wells and serial drug dilutions were prepared covering a range from 90 to 0.123 μg/mL. After 72 h of incubation, the plates were inspected under an inverted microscope to assure growth of the controls and sterile conditions. Then, 10 μL of Alamar blue (12.5 mg resazurin dissolved in 100 mL distilled water) was added to each well and the plates were incubated for another 2 h, then the plates were evaluated as described for *L. donovani* assay. As positive control podophyllotoxin at 0.005 μg/mL was used.

### 2.5.6. Anti-microbial and anti-fungal activity

To determine the anti-microbial potential of the extracts, the agar diffusion method was employed (Rios et al., 1988; Frei et al., 1998). The test organisms assayed were: *S. aureus* ((*Sa*), ATCC 29-213, gram+) and *Staphylococcus epidermidis* (*Se*, isolated strain, gram+), *P. aeruginosa* (*Pa*, ATCC 27853, gram−), *B. cereus* (*Bc*, ATCC 11278, gram+), *E. coli* (*Ec*, ATCC 25922, gram−) and *C. albicans* (*Ca*, ATCC 90028, yeast). The microorganisms were obtained from the Institute of Hygiene, Microbiology and Environmental Medicine at the Medical University of Graz.

In brief, dilutions of overnight microorganism cultures (in exponential growing phase) were prepared in liquid agar (Mueller–Hinton agar for all the bacteria and malt-extract agar, Oxoid, for *Ca*) to reach an optimum concentration (ca. $10^4$–$10^6$/mL). 5 mL of this seed agar were poured over 15 mL growth agar previously placed on sterile Petri-dishes (QTV 500, Sterilin). Crude extracts dissolved in DMSO (100 μg) were applied onto paper disks (Rotilabo Testblättchen 6 mm, Roth) and placed onto the agar surface. All bacteria were incubated at 37 °C for ca. 15 h and the inhibition zone was evaluated. *Ca* required longer incubation time and was evaluated after 24 or 40 h. For positive controls, gentamicin (Sigma), chloramphenicol (Aldrich), tetracycline hydrochloride (Sigma–Aldrich) and miconazole nitrate (Sigma) were used. DMSO (Merck) was applied as negative control.

**Table 4**
$IC_{50}$ values (protozoa and L6-cells) and SIs for the Species/Genera of plant extracts which showed activity against one or more protozoa tested. Extracts were considered active if the percentage of inhibition was more than 80% at 4.8 and 9.7 μg/mL, and 50% for 0.8 and 1.6 μg/mL, respectively, depending on the source of information about the species (for explanation see Section 3). Active extracts were submitted for the determination of their $IC_{50}$.

| Family | Part of the plant | Extract | $IC_{50}$ (μg/mL) | | | | $IC_{50}$ (μg/mL) L-6 cell | SI ($IC_{50}$ L-6 cells/$IC_{50}$ parasite) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Genera/Species | | | *T. b. rhod.* | *T. cruzi* | *L. don.* | *P. falc* | | *T.b.rhod* | *T. cruzi* | *L.don* | *P. falc* |
| Araceae | | | | | | | | | | | |
| *Syngonium podophyllum* | L | HX | | | | 4.3 | 53.7 | | | | 12.6 |
| | | DCM | 5.9 | | | 3.7 | 31.9 | 5.4 | | | 8.5 |
| Asteraceae | | | | | | | | | | | |
| *Clibadium* cf. *microcephalum* | L | DCM | 0.4 | | | | 4.4 | 11.3 | | | |
| *Elephantopus mollis* | L | DCM | 0.04 | | 0.6 | 2.2 | 2.8 | 70.7 | | 4.8 | 1.2 |
| Bignoniaceae | | | | | | | | | | | |
| *Jacaranda glabra* | b | DCM | | | | 3.7 | 69.1 | | | | 18.6 |
| | L | DCM | 5.9 | | | 2.3 | 21.6 | 3.6 | | | 9.4 |
| | | MeOH | 5.3 | | | 4.6 | 23.9 | 4.5 | | | 5.2 |
| Euphorbiaceae | | | | | | | | | | | |
| *Croton menthodorus* | br | MeOH | 16.3 | | | | >70 | nt | | | |
| Fabaceae | | | | | | | | | | | |
| *Mucuna* sp. | br | DCM | 4.2 | | | | 68.6 | 16.4 | | | |
| Mimosaceae | | | | | | | | | | | |
| *Inga oerstediana* | b | DCM | | | | 3.3 | 44.8 | | | | 13.5 |
| *Piptadenia* cf. *anolidurus* | L | DCM | 12.2 | | | 2.7 | 36.0 | 2.9 | | | 13.5 |
| *Prosopis juliflora* | b | DCM | | | | 2.1 | 19.6 | | | | 9.3 |
| Myrtaceae | | | | | | | | | | | |
| *Psidium guajava* | b | DCM | | | | 2.7 | 49.5 | | | | 18.3 |
| Olacaceae | | | | | | | | | | | |
| *Minquartia guianensis* | b | DCM | | | 2.8 | | 29.2 | | | 10.5 | |
| | | MeOH | | | | 1.3 | 39.4 | | | | 30.9 |
| | L | HX | 3.2 | | | | 22.0 | 6.8 | | | |
| | | DCM | 3.0 | | 0.3 | 3.5 | 11.5 | 3.8 | | 41.2 | 3.3 |
| Papaveraceae | | | | | | | | | | | |
| *Bocconia integrifolia* | b | HX | 1.9 | | 1.8 | 2.1 | 16.6 | 8.6 | | 9.4 | 8 |
| | | DCM | 0.3 | 2.9 | 0.5 | 2.8 | 3.9 | 11.6 | 1.4 | 7.5 | 1.4 |
| | | MeOH | 0.3 | 4.4 | 0.7 | 1.2 | 4.7 | 14.5 | 1.1 | 7 | 4 |
| | L | DCM | 7.0 | | | 3.2 | 48.0 | 6.9 | | | 15.2 |
| | | MeOH | 11.9 | | | | >70 | nt | | | |
| Piperaceae | | | | | | | | | | | |
| *Piper* sp. 2 | L | DCM | | | 2.2 | 3.4 | 27.4 | | | 12.3 | 8.1 |
| Rhamnaceae | | | | | | | | | | | |
| *Gouania lupuloides* | b/br | DCM | | | 1.9 | 2.8 | 40.8 | | | 21 | 14.7 |
| | | MeOH | | | 2.9 | 4.3 | >70 | | | nt | nt |
| Rutaceae | | | | | | | | | | | |
| *Ruta graveolens* | L/fl | DCM | 2.5 | | | | 24.3 | 9.7 | | | |
| Sapindaceae | | | | | | | | | | | |
| *Cupania cinerea* | b | HX | 0.7 | | 7.6 | 2.9 | 16.6 | 23.7 | | 2.2 | 5.7 |
| | | DCM | 1.1 | | | 3.1 | 31.0 | 28.2 | | | 10 |
| Scrophulariaceae | | | | | | | | | | | |
| *Scoparia dulcis* | T | DCM | 7.3 | | 1.8 | 2.8 | 10.4 | 1.4 | | 5.9 | 3.7 |
| Smilacaceae | | | | | | | | | | | |
| *Smilax* aff. *siphilitica* | L | DCM | 11.0 | | | 3.6 | 33.0 | 3 | | | 9.2 |
| Solanaceae | | | | | | | | | | | |
| *Brugmansia* sp. 1[*] | L | DCM | | | 3.0 | 2.0 | 10.9 | | | 3.7 | 5.5 |
| *Brugmansia* sp. 2[**] | L | DCM | | | | 3.4 | 24.5 | | | | 7.1 |
| Zingiberaceae | | | | | | | | | | | |
| *Zingiber officinale* | SO | DCM | 0.8 | | | | 46.0 | 56.9 | | | |
| Melarsoprol[a] | | | 0.004 | | | | | | | | |
| Benznidazole[a] | | | | 0.435 | | | | | | | |
| Miltefosine[a] | | | | | 0.104 | | | | | | |
| Chloroquine[a] | | | | | | 0.07 | | | | | |
| Podophyllotoxin[a] | | | | | | | 0.005 | | | | |

The *in vitro* $IC_{50}$ determination assays were run in duplicate on independent assays. The factor of the two independent assays was <1.5. nt = non-toxic as $IC_{50}$ on L6-cell >70 μg/mL.

[*] Collected in Napo.

[**] Collected in Esmeraldas.

[a] Positive control.

**Table 5**
Plant species of which extracts showed activity on the microorganisms tested and $IC_{50}$ on L6-cells.

| Family | Part of the plant | Extract | Microorganism (diffusion area) | | | | | | $IC_{50}$ (μg/mL) L-6 cells |
|---|---|---|---|---|---|---|---|---|---|
| Species | | | *Bc* | *Ca* | *Ec* | *Pa* | *Sa* | *Se* | |
| Anacardiaceae | | | | | | | | | |
| *Spondias purpurea* | b | MeOH | d | – | d? | d? | d | d | 35.5 |
| Asteraceae | | | | | | | | | |
| *Clibadium* cf. *microcephalum* | L | DCM | d | – | – | – | – | – | 4.4 |
| *Elephantopus mollis* | L | DCM | ++ | ++ | – | – | d | d? | 2.8 |
| Loranthaceae | | | | | | | | | |
| *Struthanthus* sp. | br | MeOH | – | – | – | – | – | + | >70 |
| Meliaceae | | | | | | | | | |
| *Cedrela odorata* | b | MeOH | + | – | – | d? | d | d? | 69.8 |
| Mimosaceae | | | | | | | | | |
| *Inga oerstediana* | b | MeOH | + | – | – | d? | d | d | 36.3 |
| *Piptadenia* cf. *pteroclada* | b | MeOH | + | – | – | d? | d | d | 32.4 |
| Myrtaceae | | | | | | | | | |
| *Psidium guajava* | b | DCM | – | – | – | d? | – | – | 49.5 |
| | | MeOH | d | – | d? | – | d | d | 34.8 |
| Olacaceae | | | | | | | | | |
| *Minquartia guianensis* | b | DCM | +++ | – | – | – | +++ | ++ | 29.2 |
| | | MeOH | + | – | – | – | d | ++ | 39.4 |
| | L | DCM | ++ | – | – | – | + | ++ | 11.5 |
| | | MeOH | + | – | – | – | d | + | 36.2 |
| Papaveraceae | | | | | | | | | |
| *Bocconia integrifolia* | b | DCM | +++ | – | – | – | + | + | 3.9 |
| | | MeOH | +++ | d | – | – | ++ | ++ | 4.7 |
| Rhamnaceae | | | | | | | | | |
| *Gouania lupuloides* | b | MeOH | + | – | – | – | – | – | >70 |
| Rubiaceae | | | | | | | | | |
| *Pentagonia* cf. *macrophylla* | b | MeOH | d | – | – | – | – | – | 51.54 |
| Sapindaceae | | | | | | | | | |
| *Cupania cinerea* | b | HX | d | – | – | – | – | – | 16.6 |
| | | DCM | + | – | – | – | – | – | 31.0 |
| | | MeOH | + | – | – | – | – | – | 51.0 |
| Positive control | | | +++ (a) | +++ (c) | +++ (a) | +++ (d) | +++ (e) | +++ (b) | |
| Negative control | | | – | – | – | – | – | – | |
| Podophyllotoxin | | | | | | | | | 0.005 |

The samples were tested at a concentration of 100 μg and those displaying activity were tested in duplicate. The plant extracts showing inhibition zones on the agar plate at 100 μg were also analyzed at 200 μg. Results: inhibition zone +: <2 mm clear area, ++: 2–3.9 mm, +++: >4 mm, d = diffuse at 100 μg concentration and clear inhibition zone at a concentration of 200 μg, –: not active, d? = diffusion at 100 μg as well at 200 μg. Chloramphenicol 5 μg (a) and 10 μg (b), miconazole 0.5 μg (c), tetracycline 0.1 μg (d), and gentamicin 2.5 μg (e). *In vitro* $IC_{50}$ determination assays on L6-cells were run in duplicate in an independent assay. The factor of the two independent assays was <1.5. nt = non-toxic as $IC_{50}$ on L6-cell >70 μg/mL.

## 3. Results

Anti-protozoal activity was assessed at 4.8 and 0.8 μg/mL for the plants collected based on interviewing (Table 2) and at 9.7 and 1.6 μg/mL for plants selected from literature (Table 3). The extracts were considered active if the percentage of inhibition was more than 80% at the higher concentration and more than 50% at the lower concentration. Active extracts were submitted for the determination of their $IC_{50}$ (Table 4).

The focus of this study was to determine the anti-protozoal potential of plants used traditionally in the treatment of cutaneous leishmaniasis in three different regions of Ecuador. Studies including the present article revealed that interviewed people do not have a clear conception of leishmaniasis (i.e. the mode of transmission, the vector and the life cycle of the parasite) (Vázquez et al., 1991; Isaza et al., 1999). Therefore, answers from the ATMs with regard to the treatment of leishmaniasis refer also to the treatment of obvious cutaneous problems such as ulcers. Consequently, the most common skin-related pathogens *S. aureus*, *S. epidermidis* and *C. albicans* were selected as well for the screening. *P. aeruginosa* and *B. cereus* associated with traumatic wounds were also chosen. To get a more complementary picture of anti-microbial activities, *E. coli* was chosen as a gram negative bacterium (Murray et al., 1995; Brenner et al., 1989). We are aware of the limitation of the agar diffusion assay, especially in regard to non-polar compounds (Ríos et al., 1988), and it is not intended to draw any broader conclusions about the anti-microbial potential of the plant extracts. Screening results are shown in Table 5.

To determine the general cytotoxicity of the plant extracts as opposed to a specific anti-parasitic activity, the $IC_{50}$ against a different cell line (i.e. L-6 cells) was evaluated (Tables 4 and 5).

The selectivity index (SI = $IC_{50}$ cytotoxicity (L-6 cells)/$IC_{50}$ parasite) for active extracts is given in Table 4. A SI < 10 usually indicates that the activity might be due to general cytotoxicity.

## 4. Discussion

### 4.1. Field results

Ecuador is a culturally diverse country occupied by 18 ethnic groups assembled in five main categories: Mestizo 77% (mixture of races), Indigenous 7%, Afroecuadorian 5%, white 10% and others <1% (INEC, 2001).

In Napo, more than 50% of the population is indigenous and most of these belong to the Kichwas (INEC, 2001). Among the Amazon Kichwa population, the ATM's medical practice is well recognized and respected. According to Espinel (2005), various cultural factors have contributed to the conservation of these medical practices, e.g. the mutually respectful and friendly patient–ATM relationship, a strong sense of tradition and the transmission of knowledge that allows patients to understand the healing procedures. There are various types of ATMs. Espinel (2005) reported a complete description of the different hierarchies of the Kichwa ATMs (e.g. shamans, masseurs, midwives, healers). With regard to the treatment of leishmaniasis and other illnesses considered severe or difficult to treat, ATMs frequently recommend practices that accompany the medication. Among these are diets avoiding certain food (e.g. pork, fat, spicy food, alcohol), ingestion of hallucinogenic beverages, sexual abstinence and the practice of mystic and religious rites. Similar practices have been reported from Colombia (Vázquez et al., 1991). Additionally, aspects of Christian religion are integrated within the healing process by ATMs; e.g. healing is done in the name of Jesus or God. Of the 214 ATMs from the Amazon Kichwas interviewed and representing 62.4% of ATMs recognized by MPHE in Napo, 76.6% have treated leishmaniasis while 5.6% do not know the disease.

In Bolivar, ca. 24% of the population is indigenous and belonging to the Kichwa (INEC, 2001). According to Knapp (1991), the Andean Kichwas are the largest ethnic group in Ecuador and the only American–Indian group that occupies the Andes. De la Torre et al. (2006) pointed out that there are several ethnobotanical studies in almost every community of the Andean region, however, they basically consist of compilation of plants and do not evaluate their biological activities (*in vivo* or *in vitro*). Use-records were not reported from the ATMs of the Kichwa living at high altitudes in regard to the treatment of leishmaniasis. When asked about leishmaniasis, these ATMs referred to the ones living at low altitudes.[1] In Bolivar, 12 Kichwa ATMs (MPHE recognizes 61 ATMs) and one Mestizo ATM were interviewed. Of these, four were familiar with wounds characteristic of leishmaniasis. The ATM Mestizo who reported use-records was from the coastal part of Bolivar (low lands).

In Esmeraldas, ca. 3% of the population is indigenous and ca. 24% is Afroecuadorian. The most representative indigenous ethnic group in Esmeraldas are the Chachi, also recognized as the Cayapas (INEC, 2001). Traditional economic activities of the Chachi are agriculture, hunting and fishing. However, today the direct exploitation of timber occupies the first position, endangering their ecosystem and traditions (De la Torre et al., 2008). Healing procedures by *Mirukos* (ATM in Chapalachi, the language of the Chachi) are limited to the treatment of magical-religious illnesses and fever, flu and cough (Ventura et al., 1997). Out of 54 Chachi ATMs recognized by the MPHE, 20 were interviewed.

The Épera are an indigenous group native from Colombia, who migrated to Esmeraldas 70 years ago. Thus their knowledge on Ecuadorian flora is limited (Petroecuador, 2003). In the case of the present investigation, it was found that *Jaipanas* (ATM in the Épera language) know about wounds caused by leishmaniasis, but use plants native from Colombia for their treatment. Apparently, the healing practices performed by Épera ATMs are restricted to the intake of hallucinogenic beverages and magical rituals. The Épera's first choice to treat illness is to apply modern medicine (e.g. speak to conventional doctors) and as their second choice, contact Jaipanas (Petroecuador, 2003). MPHE recognizes eight ATMs of Épera origin of which four were interviewed in the present investigation. According to De la Torre et al. (2008) there are no ethnobotanical investigations about the Épera in Ecuador.

The Awá, also known as Kuaiuer or Coiaquer, are represented by ca. 3000 people in Ecuador and ca. 10,000 in Colombia. Their social structure is based on family groups whose leader (elder man) is in charge of medical and religious practices (De la Torre et al., 2008). One of the 17 Awá ATMs recognized by the MPHE was interviewed.

The Afroecuadorian ATMs, mainly women, are the first choice for treating illness among Afroecuadorian people. This could be because Afroecuadorian ATMs diagnose, treat and heal physical and so-called spiritual illnesses also by using western medicine (e.g. iodine, menthol and other drugs). In addition, an important characteristic of the healing process is the strong influence of the Christian religion (Petroecuador, 2003). Interestingly, Chachi and other inhabitants from the area of investigation pointed out that Afroecuadorian ATMs could have more experience in the treatment of leishmaniasis. The MPHE does not have the information about the number and identity of the Afroecuadorian ATMs. In Esmeraldas, out of the 29 ATMs interviewed, 75% know about leishmaniasis wounds and 28% use plants in the treatment. According to Ventura et al. (1997), the reputation of the ATMs has notably decreased in Esmeraldas. The main reasons are access to modern medicine (e.g. through health care centres from the MPHE, the protestant or catholic churches), contact with cities and markets, and the recognition of new social values and knowledge. The lack of ethnobotanical and ethnopharmacological studies, land conflicts, ecological deterioration, a generally low level of education among the people, low agricultural production and high migration contribute as well to a renunciation of the traditional methods represented by ATMs.

Our study revealed a general concern expressed by the ATMs about the fact that the younger generation shows little or no interest in learning the healing methods of ATMs. In Napo ca. 23% of the ATMs are between 20 and 39 years old, while the remaining ATMs are much older (Espinel, 2005). Moreover, these numbers might not truly reflect the real situation. Already in 1963, Schultes remarked on the fact that as a consequence of "civilization", ancient cultures were losing their knowledge in regard to the use of medicinal plants. Although the present investigation does not focus on the traditional transfer of medicinal knowledge, it became apparent during the interviews that the situation of the ethnic groups studied is alarming and additional efforts should focus on preserving ancestral knowledge before it will vanish.

A total of 146 plant species in 61 families were reported in the present study in the treatment of leishmaniasis. The seven families with the greatest number of representatives are Asteraceae with 13 species reported, Solanaceae (12), Araceae (10), Rubiaceae (7), Fabaceae (6), Piperaceae (6) and Lamiaceae (6) accounting for 41.7% of the taxa reported. Within the Catalogue of the Vascular Plants of Ecuador, these families are found among the 11 most species-rich families with Asteraceae at position 2, Rubiaceae (4), Piperaceae (7), Araceae (8), Solanaceae (9) and Fabaceae (11) excluding Lamiaceae (28) (Jørgensen and León-Yánez, 1999). With the exception of one pteridophyte and one bryophyte, all the taxa reported belong to angiosperms. Nearly 85% of the species reported in the treatment of leishmaniasis among the ATMs are native to Ecuador (including ca. 14% endemic species) while 15% are introduced.

Different parts of the plant, including latex in some cases, are reported to be used in the treatment of leishmaniasis, but more than half of the 431 use-registers reported the use of leaves. The application is predominantly topical with the exception of *Crescentia cujete* (1/1), *Bauhinia tarapotensis* (1/5), *Bryophyllum pinnatum* (1/30), *Desmodium adscendens* (2/8), *Mucuna* sp. (2/2), *Cedrela odorata* (2/8)

[1] It would be interesting to interview ATMs living at lower altitudes, especially in Chanchán and Alausi, between 2300 and 2700 m in the province of Chimborazo (Kato et al., 2005), and in the valleys of Huigra or Paute, between 1200 and 2500 m in the province of Cañar (Calvopiña et al., 2006) where the prevalence of Andean cutaneous leishmaniasis is high.

**Table 6**
Traditional methods to prepare plants employed in the treatment of leishmaniasis.

| Mode of preparation | Use-registers | |
|---|---|---|
| | Number | Percentage |
| Ground in the mortar/pestle cold | 181 | 41.9 |
| Ground in the mortar/pestle maito[a] | 124 | 28.7 |
| Decoction | 54 | 12.5 |
| Ground in the mortar/pestle warm | 22 | 5.1 |
| Roasted over fire | 21 | 4.9 |
| Dried over the fire | 11 | 2.5 |
| Grated | 10 | 2.3 |
| Directly applied | 8 | 1.9 |
| Roasted in fire in maito[a] | 1 | 0.2 |

[a] *maito* is a local expression that refers to a way of preparing food. The preparation is wrapped in a banana leaf and warmed in the fire.

and *Verbena litoralis* (1/10), which are taken orally. About half of the use-registers list the use of ground or crushed plants which are usually applied cold, except in the province of Napo where the remedy is applied warm. According to Weigel and Armijos (2001), the warm application shows a similarity to the western medical treatment, which uses localized heat (skin temperature between 40 and 41 °C) to accelerate the wound-healing process (Table 6).

### 4.2. Anti-leishmanial activity

Seven plant species showed *in vitro* activity against axenic amastigotes of *L. donovani*: *Elephantopus mollis*, *M. guianensis*, *Bocconia integrifolia*, *Piper* sp. 2, *Gouania lupuloides*, *S. dulcis* and *Brugmansia* sp.1 (most probably *B. suaveolens*). Of these, the activities of the DCM and MeOH extracts of *G. lupuloides* and the MeOH leaf extract of *M. guianensis* are of special interest because of their selectivity indexes. Among these plants, only three are within the 26 most-reported taxa: *E. mollis*, *S. dulcis* and *Brugmansia* sp.1. For the remaining taxa, either less than four use-records were reported or they were selected based on literature. *G. lupuloides* showed only two use-records.

The 26 most frequently reported taxa represent 60% of the use-records. *Nicotiana tabacum* is at the top of the list with 40 use-records and at the bottom are *Banisteriopsis caapi*, *Croton lechleri*, *E. mollis*, *S. dulcis* and *Zingiber officinale* each with only four use-records. The rest of the plants could be considered as "noise" or infrequently reported plant-taxa. Nevertheless, they were also evaluated *in vitro*. Although a limited geographical distribution and/or scarcity may limit the use of ethnobotanically and phytochemically interesting species (Kvist et al., 2006), in the case of a rare illness it could also mean that the ATMs are not sure what plant treatment is the best and thus the knowledge cannot be considered as reliable information.

### 4.3. Anti-plasmodial, anti-trypanosomal and microbial activities

The following 16 plant species showed *in vitro* activity against the *P. falciparum* K1 strain: Syngonium podophyllum, *E. mollis*, *J. glabra*, *Inga oerstediana*, *Piptadenia* cf. *anolidurus*, *P. juliflora*, *Psidium guajava*, *M. guianensis*, *B. integrifolia*, *Piper* sp. 2, *G. lupuloides*, *Cupania cinerea*, *S. dulcis*, *Smilax* aff. *siphilitica*, *Brugmansia* sp. 1 and *Brugmansia* sp. 2. Of these, the MeOH extracts of *G. lupuloides* and the bark of *M. guianensis* showed good selectivity indexes.

14 plant species displayed *in vitro* activity against *T. brucei rhodesiense*, however, only extracts of eight had $IC_{50}$ values of <5 μg/mL. These were *Clibadium* cf. *microcephalum*, *E. mollis*, *Mucuna* sp., *M. guianensis*, *B. integrifolia*, *Ruta graveolens*, *C. cinerea* and *Z. officinale*. Of these, the DCM extracts of *Z. officinale* and *C. cinerea* and the hexane extract *C. cinerea* were interesting because of their selectivity indexes. The extract of *E. mollis* showed a general cytotoxicity, but possessed high selectivity (SI of >70) against *T. brucei rhodesiense*.

Only the bark of *B. integrifolia* showed *in vitro* activity against *T. cruzi*, however, this extract was also generally cytotoxic towards all the targets and had a low selectivity index.

Five of the species inactive against protozoa showed some degree of anti-microbial activity: *Spondias purpurea*, *Struthanthus* sp., *C. odorata*, *Piptadenia* cf. *pteroclada* and *Pentagonia* cf. *macrophylla*. Of these, only *C. odorata* was reported in more than one use-register. For *C. odorata* (eight use-registers), medicinal uses reported from several ethnic groups in Ecuador, particularly among the Amazon Kichwa, include the treatment of wounds, digestive problems, cramps, flu, fever and pain (De la Torre et al., 2008). *C. odorata* was surveyed by Kvist et al., 2006 *in vitro* against *L. major*, for which it showed very low activity.

A thorough discussion of the presence of secondary metabolites for each mentioned plant species with activity would go beyond the scope of the study, however, it should be mentioned that *Minquartia guaiensis* contains minquartyonic acid, a toxic polyacetylenic acid, which is known for anti-protozoal activity (Rasmussen et al., 2000). For *E. mollis*, the presence of toxic and anti-leishmanial sesquiterpene lactones has been demonstrated earlier (Fuchino et al., 2001).

## 5. Conclusions and Outlook

The screening for *in vitro* activity is a helpful tool to discover plants that may have a potential in drug development. In the field of ethnopharmacology, however, a more substantial approach should include the testing of preparations as made by ATMs. Therefore, our results may be considered in part preliminary.

Leishmaniasis as a disease may be misinterpreted by ATMs, because of their frequent inability to diagnose the protozoa as the underlying cause of the apparent symptoms (e.g. wounds, sores, ulcers). Therefore, the great number of species reported could be in part due to the lack of knowledge about the real reason for the efficacy of a plant or group of plants, and demonstrates the trial and error aspect of finding an appropriate remedy.

This study focused on the traditional treatment of cutaneous leishmaniasis and its different variations, which are the only forms present in Ecuador. The biological testing against local strains of *Leishmania* was not possible as part of our work, but would be a valuable future research direction.

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