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Antileishmanial Potentials of Phytochemicals

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6.1 Introduction

Leishmaniases are a group of neglected tropical diseases (NTD) which affects about 12–15 million people in 88 countries around the world [1]. The protozoan species belonging to Leishmania is the main cause of the disease which is transmitted by sandflies of the genus *Phlebotomus* and *Lutzomyia* [2].

The infection begins when an infected sandfly bites a person, here the promastigote form of the *leishmania* enters the human body. The promastigotes are then up-taken by macrophages where they are transformed into the amastigote which is the intracellular form of *leishmania* [3]. There are three main forms of Leishmaniases, according to its clinical manifestations: Cutaneous leishmaniasis (CL), which affects only localized parts of the skin and is the most common form of the disease; mucocutaneous leishmaniasis (MCL), which has the ability to destroy mucous tissue and is exclusively present in America; and visceral leishmaniasis (VL) which is the less common type of leishmaniasis but causes liver and spleen distention and can be fatal if it does not receive prompt treatment [4].

Pentavalent antimony-based drugs are the main chemotherapeutic treatment against leishmaniases [5]. These drugs not only have serious side effects such as cardiotoxicity, hepatotoxicity, and nephrotoxicity, but also the parasite has acquired resistance to such drugs in many countries, in which leishmaniases is a major public health problem [2]. The WHO has recommended the use of

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medicinal plants and traditional treatments for chronic diseases such as leishmaniasis, where patients are often impaired to seek other medical treatment [6, 7]. In Mexico, approximately 3000 plant species are employed in the practice of traditional medicine and many for the treatment of protozoal infections [8, 9].

6.2 Antileishmanial Activity of Propolis

Propolis is a chemical compound produced by worker bees through combining exudates or parts of the plants, with beeswax and secretions from the salivary glands of the worker bee rich in enzymes. It varies widely in color due to the variation in chemical composition [10]. Due to its antimicrobial power, Propolis is considered the "chemical weapon" that the bees use to maintain an aseptic internal environment in beehives [11, 12]. In addition to its use in dietary supplements and cosmetic formulations, Propolis has many reported biological activities such as anti-inflammatory, antiulcer, antitumor, immunostimulant, hepatoprotective, antibacterial, antifungal, and potential against protozoa [12–14].

The *in vitro* antileishmanial activity of propolis from different regions such as Turkey [15], Brazil [16, 17], Portugal [18], and Cuba [19] have been reported.

6.2.1 Propolis from Turkey

The ethanolic extract of Adana propolis was tested against *Leishmania at* five concentrations (25, 50, 100, 50, 500, and 750 μg/ml). The number of promastigotes in each concentration was calculated using a hemocytometer slide at 24, 48, and 72 hours after being harvested. The Adana propolis samples produced inhibitory effect only at concentrations of 250, 500, and 750 μg/ml through reducing the proliferation of *Leishmania tropica* parasites [20].

Another study by the same authors evaluated the antileishmanial activities of "Bursa" and "Hatay" propolis samples against *Leishmania infantum* and *L. tropica* strains at 50, 100, 250, 500, 750, and 1000 μg/ml concentration of each sample. The growth of leishmania parasites was significantly reduced in the presence of 500, 750, and 1000 μg/ml of Hatay propolis. Bursa propolis was more effective as it showed the inhibitory effects at lower concentrations (250, 500, 750, and 1000 μg/ml). Thus, the *in vitro* results showed that the Hatay and Bursa propolis samples decreased significantly the proliferation of *L. infantum* and *L. tropica* parasites [15].

6.2.2 Propolis from Brazil

The antileishmanial activity and cytotoxicity of brown propolis originating from the semiarid region of Piauí, Brazil were evaluated [16]. The propolis significantly inhibited the growth of *Leishmania amazonensis promastigotes* and reduced the

infection of murine macrophages and the number of internalised amastigotes in these cells. The dichloromethane fraction was the most active and showed the best selectivity index. The study showed that fractionation of the propolis had an improvement in the activity without increasing the cytotoxicity against mammalian cells. Thus the Brazilian brown propolis is a potential source for development of apitherapeutic products for the treatment of leishmaniasis.

6.2.3 Propolis from Portugal

The *in vitro* activity of two different Portuguese propolis extracts; one from the North-eastern Portugal (Bragança county), while the other was collected from the centre of Portugal (Leiria county) together with its major plants *Populus* × *Canadensis* (male and female plants) and *Cistus ladanifer* were evaluated against *Plasmodium falciparum*, *L. infantum*, *Trypanosoma brucei,* and *Trypanosoma cruzi* using the WHO Special Program for Research and Training in Tropical Diseases (TDR). The tested samples showed moderate activity against *T. brucei*. For *L. infantum*, propolis samples and male and female *Populus* showed a similar IC_{50} value of 8.11 μg/ml. *C. ladanifer* showed the lowest activity with an IC_{50} value of 32.46 μg/ml [18].

6.2.4 Propolis from Cuba

In Cuba, 18 *Cuban propolis* extracts of different types such as brown, red, and yellow types collected from various locations were tested for antileishmanial activity using *L. amazonensis* as a model of intracellular protozoa and *Trichomonas vaginalis* as a model of extracellular protozoa. The results showed no correlation between the types of propolis and the obtained antiprotozoal; samples were active against intracellular amastigotes of *L. amazonensis*, but caused toxicity on peritoneal macrophages from BALB/c mice. However, only five samples have inhibitory effect on *T. vaginalis trophozoites* at concentration less than 10 μg/ml [19].

6.2.5 Propolis from Ecuador

In Ecuador, the alcoholic extract of propolis is a common homemade remedy and also 10–20% ethanolic tinctures or powder mixed with honey are sold in the market as antioxidant, anti-inflammatory, and antimicrobial remedies [21]. One study was done by Cuesta-Rubio et al. [21], in which the methanol extract of three propolis samples were collected from three localities of Ecuador (Quito, Guayaquil, and Cotacachi). Based on their chemical composition they were divided into two main samples of propolis: Cotacachi propoli sample (CPS), rich in flavonoids and Quito and Guayaquil samples (QPS and GPS) rich in triterpenic alcohols and acetyl triterpenes.

The three methanol extracts of propolis showed inhibitory activity against *L. amazonensis* growth, where CPS was the most active ($IC_{50} = 17.1 \pm 1.7$ μg/ml) may be due to its high flavonoid content. The samples were also tested *in vitro* against promastigotes of *L. amazonensis* and macrophage from BALB/c mice where all the tested samples were able to inhibit parasite growth, out of which Cotacachi sample was the most active. This propolis sample was also the most promising, displaying a CC_{50} sevenfold higher than the IC_{50}value on parasite. CPS contains the flavonoid sakuranetin, which is reported to be active against *Leishmania* spp. [22]. So it is thought that this flavonoid and its derivatives may be responsible for the observed antiparasitic activity.

6.3 Antileishmanial Activity of Wild Mushrooms

Six mushrooms from Gangetic plane of West Bengal, India which are used as food and for medicinal purposes in traditional folklore by local Santal tribes in India were tested for the anti-proliferating effect against *Leishmania donovani* (MHOM/IN/83/AG83) [23]. These species are *Astraeus hygrometricus* (Pers.) Morgan (Local name – Putko/Kurkure Chatu); *Russula laurocerasi* Melzer (Local name – Jhaal Patra); *Russula albonigra* (Krombh.) Fr. (Local name – Kalo/Kend Patra); *Termitomyces eurhizus* (Berk.) Heim (Local name – Parab Chatu); and *Russula delica* Fr. (Local name – Sada Patra) with them being native to Lateritic zones of five districts of Birbhum, Murshidabad, Burdwan, Bankura, and West Midnapore and *Tricholoma giganteum* Massee (Local name – Dhoodh Chatu). Three extracts were prepared from the milled and freeze dried mature fruitbodies (Basidiocarps) of each species. Eighty percent (80%) ethanol extract, water-soluble polysaccharide fraction and polyphenolic fraction were prepared and tested for their antileishmanial ability against the promastigotes and amastigotes. The 80% Ethanol extract of *A. hygrometricus* and *T. giganteum* significantly inhibited the growth of *L. donovani* promastigotes and interfered in lipid biosynthesis at concentration of 250 μg/ml. These two extracts also induced apoptosis in promastigotes. Water-soluble polysaccharide fraction of *A. hygrometricus, R. laurocerasi, R. albonigra, T. eurhizus, R. delica,* and polyphenolic fraction of *R. laurocerasi* showed dose-dependent inhibition of the intracellular amastigotes replication in macrophages. Significantly, 50% inhibitory concentration of the active extracts against intracellular amastigotes induced release of nitric oxide and interleukin-12 (IL-12) in murine macrophages and dendritic cells assay and also found considerably nontoxic on murine splenocytes.

6.4 Antileishmanial Activity of Medicinal Plants from Various Flora

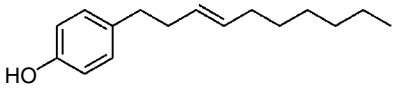
Herbal remedies for leishmaniasis has attracted great interest especially in different countries of Latin America, where it is a public health concern, as in Peru [24], Bolivia [25], Colombia [26], and Brazil [27, 28]. These medicinal plants are native to the tropical Andes located in South America, tracing the Andes Path and run along Venezuela, Colombia, Ecuador, Peru, and Bolivia. They are characterized by complex geography including different altitudes, and wide range of climatic features in the Andes and Amazonian. Therefore, they support an enormous heterogeneity of ecological conditions and rich diversity of flora and fauna, as well [29]. Hence, indigenous peoples of these areas have a long history of treatment of different diseases by medicinal plants and transmitted these practical traditions to following generations [30]. Interestingly, these plants may be native and common in many cases in different flora of these countries.

6.4.1 Peruvian Flora

The flora of Peru is among the principal and most diverse of the world flora. Approximately 25 000 plant species are native and endemic in Peru representing 10% of all plant species in the world. This biodiversity owes to the different geographical conditions found in coastal desert, mountains, jungle, and rainforest providing 28 of the 32 possible climates and 84 of the 103 ecological zones existing in the world [31]. Examples include the best known in South America *Uncaria tomentosa* (cat's claw), which has been traditionally used for 2000 years in in treating inflammation, arthritis, bone pain, asthma, deep wounds, and cancer. In addition, *Phyllantus niruri* (chanca piedra) or *Lepidium meyenii* (maca) is used for fertility improvement and protection of cells against oxidative stress [32].

Specifically, several Peruvian medicinal plants have been reported to have potential *in vitro* leishmanicidal activity mainly against *L. amazonensis* promastigote and amastigote stages. Most of these medicinal plants are traditionally used by the Chayahuita, an ethnic group from the Peruvian Amazon, that treats symptoms of leishmaniasis [33, 34]. However, compounds responsible for this activity have not been identified and isolated yet completely. Examples include;

- ***Piper hispidum* var. hispidum Sw. and *Piper strigosum* (Fam. Piperaceae)**
Leaves are crushed and applied on skin wounds and swellings as a dressing for treating symptoms of CL including inflammation. Chalcones were shown to be the bioactive compounds, but in other related *Piper* species (e.g., *Piper elongatum*) against extracellular promastigotes of *Leishmania braziliensis in vitro* [35]. These



Gibbilimbol B

Figure 6.1 The alkenylphenol chemical structure (Gibbilimbol B) isolated from *Piper malacophyllum*. *Source:* de Oliveira et al. [38].

observations were confirmed by synthesis of chalcone analogues, which demonstrated improved and selective activity against the parasites comparing with the natural chalcones [36, 37]. However, in others (e.g., *Piper malacophyllum*), an alkenylphenol (Gibbilimbol B, Figure 6.1) was found to have a plasma membrane disruption activity [38]. These findings have supported the traditional use of *P. hispidum* and *P. strigosum* in treatment of leishmaniasis.

- ***Caladium steudneriifolium*** **Engl. (Fam. Araceae)**

Caladium steudneriifolium is commonly known as the elephant ear. Its root could be finely grated and applied locally on cutaneous lesions, until it is healed.

- ***Capirona decorticans*** **Spruce (Fam. Rubiaceae)**

For small lesions and at the very beginning of the disease, the bark aqueous extract could be taken twice daily until cicatrisation.

- ***Siparuna*** **sp. (Fam. Monimiaceae)**

The woody stem is grated and boiled. The preparation is drunk three times daily for eight days.

- ***Smilax papyracea*** **Duhamel (Fam. Smilacaceae)**

Roots extract is drunk many times daily, until relieved of cutaneous symptoms.

- ***Tabernaemontana sananho*** **Ruiz & Pav. (Fam. Apocynaceae)**

The root is rasped and applied locally on the affected area. Several monomeric anddimeric indole alkaloids have been isolated from *Tabernaemontana* species. The dimeric alkaloids were shown in the Bolivian species *Tabernaemontana van heurckii* Mull. Arg. to be responsible for activity against *L. amazonensis in vitro* and *In vivo* investigations [39].

- ***Euterpe oleracea*** **Maritus (açai palm) (Fam. Arecaceae)**

Euterpe oleracea Maritus is a palm tree and historically known as açai. It is native to the Varzea area of the Amazon, especially in Brazil, Peru, Suriname, and Trinidad and Tobago. Its global importance came from the fruit juice, which is marketed as an energy drink. It is rich in polyphenolic compounds with high antioxidant and anti-inflammatory properties. These compounds include orientin, isoorientin, and vanillic acid, as well as anthocyanins cyanidin-3-glucoside and cyanidin-3-rutinoside [40]. Recently, Da Silva, et al., evaluated the *in vitro* selective effects of clarified açai palm juice on the promastigotes and amastigotes of *L. amazonensis* (MHOM/BR/26361) and *L. infantum* (=*L. chagasi*) (MHOM/BR/27840).

The results showed that the açai juice induced cellular morphological changes and reduced the number of *L. amazonensis* promastigotes (39.68–96.05%) and *L. infantum* promastigotes (59.12–93.88%) in 72 and 48 hours, respectively and without causing harmful effects to the host macrophages cells [41].

6.4.2 Ecuadorean Flora

Ecuador's natural wealth provides the richest source of plants in South America, in proportion to its area (283 561 km^2). It contains approx. 270 families of vascular plants and 20 000 species [42].

Based on interviews with healers using traditional medicine, recognized there as Agents of Traditional Medicine (ATMs) and that found in the literature, Gachet et al. [43] assessed the *in vitro* antiprotozoal potential of plants traditionally usedin Ecuador in the treatment of leishmaniasis. The authors of this study found that, out of 431 use-records, 145 plants are used for the treatment of leishmaniasis and the 10 most frequently reported taxa accounted for 37.7% of all records. From a representative selection of 39 species, a total of 140 extracts were screened *in vitro* against *L. donovani*. The leishmanicidal activity was observed in 14 plant species, among them were *Elephantopus mollis*, *Minquartia guianensis*, *Bocconia integrifolia*, *Gouania lupuloides*, *Scoparia dulcis*, an as-yet-unidentified species of *Piper* and *Brugmansia* [43].

- ***Elephantopus mollis*** **(Fam. Astraceae)**

Elephantopus mollis is reported to be used in folk medicine for the treatment of various diseases, including nephritis, edema, dampness, chest pain, fever, scabies, joint pain due to wound and cough [44]. In addition, an *in vitro* leishmanicidal activity has proven against *Leishmania major*. For this activity, seven sesquiterpenoid lactones were isolated, identified and investigated from the whole plant of the related Peruvian and Brazilian collections. They were named as 2,5-epoxy-2β-hydr oxy-8α-(2-methylpropenoyloxy)-4(15),10(14),11(13)-germacratrien-12,6α-olide, (4βH)-8α-(2-methylpropenoyloxy)-2-oxo-1(5),10(14),11(13)-guaiatrien-12,6α-olide and (4βH)-5α-hydroxy-8α-(2-methylpropenoyloxy)-1(10),11(13)-guaiadiene-12,6α-olide, together with molephantin, elephantopin, isoelephantopin, and 2-deethoxy-2β-methoxyphantomolin. It was found that the α-methylene-γ-butyrolactone moiety was essential for the leishmanicidal activity [45].

- ***Minquartia guianensis*** **(Fam. Olacaceae)**

Ecuador natives use the bark infusion for intestinal infections treatment caused by parasites, against muscular pain and cutaneous inflammation including leishmaniasis [46, 47]. Triterpenes (e.g., lupeol, taraxerol, lupenona, and squalene), xanthone and minquartynoic acid were isolated in previous phytochemical studies on *M. guianensis*. Specifically, the minquartynoic acid isolated from bark showed activity against *P. falciparum* and *L. major* [48].

- ***Bocconia integrifolia*** **(Fam. Paperveraceae)**

Rocha et al. [49] have reviewed medicinal plants and bioactive natural products with antileishmanial activity [49]. It was shown that the alkaloidal fraction of the leaves and stem bark of *B. integrifolia* were responsible for the activity against *L. amazonensis* and *L. braziliensis* [25].

6.4.3 Mexican Flora

Mexico is a unique biogeographic area reflecting a diverse endemic flora. It comprises 24 360 vascular plant species, among them 10 235 (42%) are endemic. The biodiversity in Mexico and Central America is odd, owing to its transition zone between the Nearctic and Neotropical biotas [50].

The aerial parts of *Cleoserrata serrata* (Jacq.) Iltis are commonly applied in South-Central Mexico to treat wounds and bacterial skin infections and in Panama by Kuna, Ngöbe-Buglé, and Teribe Indians for tropical warm baths and by Kunas in the form of "Ina kuamakalet" for snakebites. Recently, *C. serrata* CH_2Cl_2/MeOH extract was incubated at different concentration *with L. Mexicana* amastigotes and promastigotes *in vitro*, as well as with bacteria that usually coinfect skin ulcers. The results showed a significant inhibition of the parasite survival (which was more effective in infective amastigotes) and additionally inhibited growth of the coinfective bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa* [51].

Delgado-Altamirano et al. [52] has investigated recently a few Mexican plants extracts against promastigotes and intracellular amastigotes of *L. amazonensis*. This study found that CH_2Cl_2 and CH_2Cl_2/MeOH *Schinus molle*, CH_2Cl_2 of *Lantana camara* and aqueous *Prosopis laevigata* extracts were active and selective against *L. amazonensis* promastigotes. Moreover, the lower IC_{50} and higher selectivity index of these extracts indicated presence of valuable candidates with leishmanicidal activity [52].

Furthermore, in other related studies, 20 Mexican medicinal plants have been evaluated against *Leishmania Mexicana*. Among them, *Tridax procumbens*, *Lonchocarpus xuul*, and *Pentalinon andrieuxii* showed potential activity and from these plants, active compounds have been isolated, such as the 3(*S*)-16,17-didehydrofalcarinol or Oxylipin, cholestra-4,20,24-trien-3-one or pentalinosterol, 24-methylcholest-4-24(28)-dien-3-one, cholest-4-en-3-one, 6,7-dihydroneridie-none, neridienone, cholest-5,20,24-trien-3β-ol, and isocordoin. One of these compounds; pentalinonsterol, has been synthesized and assayed experimentally in a VL caused by *L. donovani* using BALB/c mice as a model. In addition, the I.V administration of liposomal formulation of pentalinonsterol demonstrated a significant reduction of parasite load in mouse liver and spleen [53].

6.4.4 Bolivian Flora

Like other ethnic groups around the world, Tacana people living in the northern area of the Department of La Paz have their traditional medicinal plants. They used these plants to treat skin problems, including leishmania ulcers, skin infections, inflammation, and wound healing. Evaluation of 38 Tacana medicinal plants extracts was performed *in vitro* against promastigotes of *L. amazonensis* and *L. braziliensis*. The results revealed that about 60% demonstrated leishmanicidal activity, showing relatively higher species activity against the *L. braziliensis* strain than *L. amazonensis*. Among these plants, a total of five crude extracts can be considered as promising leishmanicidal species: *Jacaranda glabra* (Fam. Bignoniaceae), *S. dulcis* (Fam. Plantaginaceae), *Tessaria integrifolia* (Fam. Astraceae), *Thalia geniculata* (Fam. Marantaceae), and Ejije Bid'u leaves [54].

6.4.5 Iranian Flora

Since leishmaniasis is endemic in Iran and the CL is the most common type, a total of 68 articles including 188 experiments (140 *in vitro* and 48 *in vivo*) in nine databases between 1999 and 2015 have discussed herbal treatment of leishmaniasis and around 98 types of plants were examined against three genera of *Leishmania* sp. Systematic and meta-analysis review of this study showed that the most Iranian plants with antileishmanial activity were *Artemisia* sp., *Allium sativum*, *Achilleamille folium*, *Peganum harmala,* and *Thymus vulgaris* [55].

Experimentally, 17 ethanolic extract of different Iranian plants were investigated *in vitro* against *L. major* promastigotes and the mouse macrophage cell line J774 by Manjili, H. K., et al. in 2012 based on the Iranian ethnobotanical history. Only four plants, *Caesalpinia gilliesii* (Fam. Fabaceae), *Satureia hortensis* (Fam. Labiatae), *Carum copticum heirm* (Fam. Umbellifererae), and *Thymus migricus* (Fam. Lamiaceae), exhibited higher selectivity against the leishmania promastigotes relative to the macrophage cell line, based on the measured IC_{50} values. These data indicated that these plants contain not yet known active ingredient which require further investigations for isolation and identification [56].

Moreover, methanolic extracts of *Calendula officinalis* (Fam. Astraceae) flowers, *Datura stramonium* (Fam. Solanaceae) seeds, and *Salvia officinalis* leaves were evaluated *in vitro* against *L. major* promastigotes (strain MROH/IR/75/IR). The antipromastigote and anti-amastigote activities of *D. stramonium* seeds and *S. officinalis* leaves were less potent than that of the *C. officinalis* flower extract. However, all extracts reduced the infectivity of *L. major* amastigotes in macrophages [57].

6.5 Antileishmanial Activity of Chemical Constituents

The current available synthetic drugs for treatment of leishmaniasis, including miltefosine, pentavalent antimonials, and amphotericin B, are highly toxic. Additionally, these drugs are highly expensive and parasite resistance is also a major concern. Natural products constitute a sustainable, cost-effective, and effective source for promising candidates with leishmanicidal potential. In this context, several classes of secondary metabolites have been reported as antileishmanial chemical constituents. The following section introduces different classes and examples of phytochemicals that have antileishmanial activities (Figure 6.2).

6.5.1 Alkaloids

The crude extract as well as subfractions of *Guatteria latifolia* (family Annonaceae) were reported to have an *in vitro* antileishmania amazonensis activity [58]. This activity was connected to reduction of nitric oxide (NO) production in gamma interferon (IFN-γ) and lipopolysaccharide (LPS)-stimulated macrophages [58]. The activity was attributed to the presence of puterine, oxoputerine, and lysicamine in from *G. latifolia* [58] (Figure 6.2).

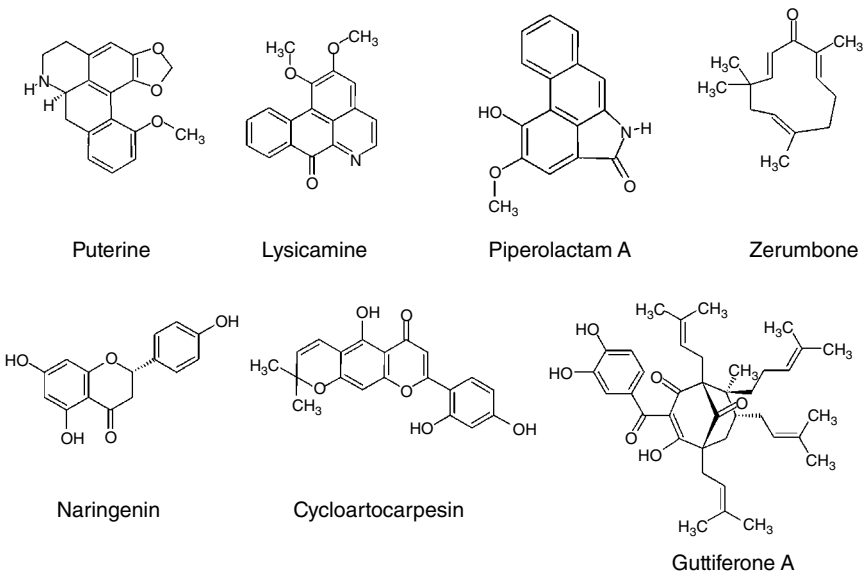


Figure 6.2 Examples of phytoconstituents with antileishmania activity. *Source:* Based on Ferreira et al. [58].

Piperolactam A (Figure 6.2), an aristolactam alkaloid isolated from *Piper betle* root extract, showed promising antileishmanial activity against *L. donovani* wild-type, and drug-resistant strains (sodium stibogluconate and paromomycin) and field isolated (GE1) resistant strains [59]. Inclusion of Piperolactam A in hydroxypropyl-β-cyclodextrin (HPBCD) nanoparticles resulted in significant increase in selectivity leishmanicidal potential [59].

6.5.2 Flavonoids

The extract of *Codonopsis clematidea* and its bioactive flavonoid naringenin have been evaluated for their antileishmanial potential [60]. This study showed that naringenin can be considered a potential candidate against VL, owing to its ability to activate $CD4^+$ and $CD8^+$ T cells as well as trigger NFκB and iNOS production [60].

Among the prenylated flavonoids, cycloartocarpesin has been isolated from the rhizomes of *Dorstenia contrajerva*. This compound showed a promising *in vitro* antileishmanial activity [61]. Cycloartocarpesin has been also found in many fruits.

6.5.3 Terpenes

Macrocyclic lathyrane diterpenoids, isolated from *Jatropha multifidi*, owed a promising *in vitro* antileishmanial activity [62]. Among diterpenoids, 14-deoxy-1β -hydroxy-4(4*E*)-jatrogrossidentadione and 15-deoxy-1β-hydroxy-4(4*E*)-jatrogrossi dentadione showed promising activity [62].

Zerumbone, a monocyclic sesquiterpene isolated from *Zingiber zerumbet*, showed a promising *in vitro* antileishmanial activity [63]. This activity was attributed to its ability to induce ROS mediated apoptosis in *L. donovani* promastigotes [63].

6.5.4 Glycoglycerolipids

Glycoglycerolipids are known as ubiquitous structural and signaling molecules in living organisms [64]. Among glycoglycerolipids, 1-*O*-linolenoyl-2-*O*-stearoy l-3-*O*-β-D-galactopyranosyl glycerol has been isolated from the rhizomes of *D. contrajerva*. This compound showed a promising *in vitro* antileishmanial activity [61].

6.5.5 Guttiferone A as a Lead Compound for Semisynthesis of Antileishmanial Molecules

Guttiferone A, a catechol pharmacomodulation, has been isolated from Clusiaceae family including *Symphonia globulifera*, *Garcinia livingstonei,* and *Garcinia macrophylla* [65]. *In vitro* evaluation of Guttiferone A toward *L. donovani* on the

promastigote form showed a promising antileishmanial activity for Guttiferone A ($IC_{50} = 5.83\,\mu M$) [65].

Guttiferone A belongs to the PolyPrenylated AcylPhloroglucinols (PPAPs) family. The synthesis of ether and ester derivatives of the catechol moiety revealed that the monoacetyl derivative produced similar antileishmanial activity [65].

6.6 Conclusion

As a conclusion, nearly all the experiments were performed *in vitro*, based on traditional folklore medicine and did not reveal the exact mechanism by which these extracts and/or compounds act. Additionally, in comparison with the chemical standard treatment (e.g., Miltefosine), the natural or herbal treatment showed less activity and potency. In other cases, the leishmanicidal activity of several herbal treatments are not completely clarified, due to unknown or unidentified composition. Therefore, further *in vivo* investigations, isolation and identification of responsible compounds involved in the leishmancidal activity should be carried out and focused on. These investigations may lead to hypotheses upon which theories and structure activity relationships could be constructed and consequently much potent plants-derived antileishmanial medicaments.

References

1 WHO (2017). *Leishmaniasis*. WHO Regional Office for Africa. http://wwwafrowhoint/health-topics/Leishmaniasis.
2 Tiuman, T.S., Santos, A.O., Ueda-Nakamura, T. et al. (2011). Recent advances in leishmaniasis treatment. *Int. J. Infect. Dis.* 15 (8): e525–e532.
3 Murray, W.H., Berman, D.J., Davies, R.C., and Saravia, G.N. (2005). Advances in leishmaniasis. *Lancet* 366: 1561–1577.
4 Akhoundi, M., Kuhls, K., Cannet, A. et al. (2016). A historical overview of the classification, evolution, and dispersion of leishmania parasites and sandflies. *PLoS Negl. Trop. Dis.* 10 (3): e0004349.
5 Bifeld, E. and Clos, J. (2015). The genetics of *Leishmania virulence*. *Med. Microbiol. Immunol.* 204 (6): 619–634.
6 WHO (2010). Control of the leishmaniases. *World Health Organ. Tech. Rep. Ser.*: 22–26.
7 WHO (2013). Traditional medicine strategy 2014–2023. *Altern. Integr. Med.*: 1–76.
8 Alonso-Castro, A., Dominguez, F., José Maldonado-Miranda, J. et al. (2016). Use of medicinal plants by health professionals in Mexico. *J. Ethnopharmacol.* 198: 81–86.

9 Atlas INI (1994). *Atlas INI de las Plantas de la Medicina Tradicional Mexicana*. Insitiuto Nacional Indigenista. Tomo I,II, III.

10 Cuesta-Rubio, O., Piccinelli, A.L., Campo Fernandez, M. et al. (2007). Chemical characterization of *Cuban propolis* by HPLC − PDA, HPLC − MS, and NMR: the brown, red, and yellow *Cuban* varieties of propolis. *J. Agricult. Food Chem.* 55 (18): 7502–7509.

11 Sforcin, J.M. and Bankova, V. (2011). Propolis: is there a potential for the development of new drugs? *J. Ethnopharmacol.* 133 (2): 253–260.

12 Bankova, V., Popova, M., and Trusheva, B. (2014). Propolis volatile compounds: chemical diversity and biological activity: a review. *Chem. Central J.* 8 (1): 28.

13 Farooqui, T. and Farooqui, A.A. (2012). Beneficial effects of propolis on human health and neurological diseases. *Front. Biosci. (Elite Ed.)* 4: 779–793.

14 Catchpole, O., Mitchell, K., Bloor, S. et al. (2015). Antiproliferative activity of New Zealand propolis and phenolic compounds vs human colorectal adenocarcinoma cells. *Fitoterapia* 106: 167–174.

15 Duran, N., Muz, M., Culha, G. et al. (2011). GC-MS analysis and antileishmanial activities of two Turkish propolis types. *Parasitol. Res.* 108 (1): 95–105.

16 Santana, L.C., Carneiro, S.M.P., Caland-Neto, L.B. et al. (2014). Brazilian brown propolis elicits antileishmanial effect against promastigote and amastigote forms of *Leishmania amazonensis*. *Nat. Prod. Res.* 28 (5): 340–343.

17 Ayres, D.C., Marcucci, M.C., and Giorgio, S. (2007). Effects of Brazilian propolis on *Leishmania amazonensis*. *Mem. Inst. Oswaldo Cruz* 102: 215–220.

18 Falcao, S.I., Vale, N., Cos, P. et al. (2014). *In vitro* evaluation of Portuguese propolis and floral sources for antiprotozoal, antibacterial and antifungal research. *Phytother. Res. PTR* 28 (3): 437–443.

19 Monzote Fidalgo, L., Sariego Ramos, I., Garcia Parra, M. et al. (2011). Activity of *Cuban propolis* extracts on *Leishmania amazonensis* and *Trichomonas vaginalis*. *Nat. Prod. Commun.* 6 (7): 973–976.

20 Duran, G., Duran, N., Culha, G. et al. (2008). *In vitro* antileishmanial activity of *Adana propolis* samples on *Leishmania tropica*: a preliminary study. *Parasitol. Res.* 102 (6): 1217–1225.

21 Cuesta-Rubio, O., Fernández, M.C., Hernández, I.M. et al. (2017). Chemical profile and anti-leishmanial activity of three *Ecuadorian propolis* samples from Quito, Guayaquil and Cotacachi regions. *Fitoterapia* 120: 177–183.

22 Grecco, S.S., Reimão, J.Q., Tempone, A.G. et al. (2012). *In vitro* antileishmanial and antitrypanosomal activities of flavanones from *Baccharis retusa* DC. (Asteraceae). *Exp. Parasitol.* 130 (2): 141–145.

23 Mallick, S., Dutta, A., Dey, S. et al. (2014). Selective inhibition of *Leishmania donovani* by active extracts of wild mushrooms used by the tribal population of India: an *in vitro* exploration for new leads against parasitic protozoans. *Exp. Parasitol.* 138: 9–17.

24 Kvist, L.P., Christensen, S.B., Rasmussen, H.B. et al. (2006). Identification and evaluation of Peruvian plants used to treat malaria and leishmaniasis. *J. Ethnopharmacol.* 106 (3): 390–402.

25 Fournet, A., Barrios, A.A., and Muñoz, V. (1994). Leishmanicidal and trypanocidal activities of Bolivian medicinal plants. *J. Ethnopharmacol.* 41 (1): 19–37.

26 Weniger, B., Robledo, S., Arango, G.J. et al. (2001). Antiprotozoal activities of Colombian plants. *J. Ethnopharmacol.* 78 (2): 193–200.

27 Muzitano, M.F., Cruz, E.A., de Almeida, A.P. et al. (2006). Quercitrin: an antileishmanial flavonoid glycoside from *Kalanchoe pinnata*. *Planta Med.* 72 (1): 81–83.

28 Muzitano, M.F., Tinoco, L.W., Guette, C. et al. (2006). The antileishmanial activity assessment of unusual flavonoids from *Kalanchoe pinnata*. *Phytochemistry* 67 (18): 2071–2077.

29 Maldonado Fonkén MS. An introduction to the bofedales of the Peruvian High Andes. *Mires Peat*. 2014;15: Art. 5, 1–13.

30 Gonzales, G. and Valerio, L. (2006). Medicinal plants from Peru: a review of plants as potential agents against cancer. *Anti Cancer Agents Med. Chem.* 6 (5): 429–444.

31 Cermal-Editions (2014). Typical Peruvian plants. https://www.peruinformation.org/typical-plants (accessed 18 March 2019).

32 Berłowski, A., Zawada, K., Wawer, I., and Paradowska, K. (2013). Antioxidant properties of medicinal plants from Peru. *Food Nutr. Sci.* 04 (08): 71–77.

33 Odonne, G., Valadeau, C., Alban-Castillo, J. et al. (2013). Medical ethnobotany of the Chayahuita of the Paranapura basin (Peruvian Amazon). *J. Ethnopharmacol.* 146 (1): 127–153.

34 Estevez, Y., Castillo, D., Pisango, M.T. et al. (2007). Evaluation of the leishmanicidal activity of plants used by Peruvian Chayahuita ethnic group. *J. Ethnopharmacol.* 114 (2): 254–259.

35 Hermoso, A., Jiménez, I.A., Mamani, Z.A. et al. (2003). Antileishmanial activities of dihydrochalcones from *Piper elongatum* and synthetic related compounds. Structural requirements for activity. *Bioorg. Med. Chem.* 11 (18): 3975–3980.

36 Boeck, P., Bandeira Falcao, C.A., Leal, P.C. et al. (2006). Synthesis of chalcone analogues with increased antileishmanial activity. *Bioorg. Med. Chem.* 14 (5): 1538–1545.

37 de Mello, T.F., Bitencourt, H.R., Pedroso, R.B. et al. (2014). Leishmanicidal activity of synthetic chalcones in *Leishmania* (Viannia) *braziliensis*. *Exp. Parasitol.* 136: 27–34.

38 de Oliveira, A., Mesquita, J.T., Tempone, A.G. et al. (2012). Leishmanicidal activity of an alkenylphenol from *Piper malacophyllum* is related to plasma membrane disruption. *Exp. Parasitol.* 132 (3): 383–387.

39 Munoz, V., Moretti, C., Sauvain, M. et al. (1994). Isolation of bis-indole alkaloids with antileishmanial and antibacterial activities from *Peschiera van heurkii* (syn. *Tabernaemontana van heurkii*). *Planta Med.* 60: 455–459.

40 Yamaguchi, K.K.L., Pereira, L.F.R., Lamarão, C.V. et al. (2015). Amazon acai: chemistry and biological activities: a review. *Food Chem.* 179: 137–151.

41 Da Silva, B.J.M., Souza-Monteiro, J.R., Rogez, H. et al. (2018). Selective effects of *Euterpe oleracea* (acai) on *Leishmania* (*Leishmania*) *amazonensis* and *Leishmania infantum*. *Biomed. Pharmacother.* 97: 1613–1621.

42 Flora-of-Ecuador (2018). Dept of Biological and Environmental Sciences, University of Gothenburg, in co-operation with Pontificial Catholic University of Ecuador, Q. Flora of Ecuador. https://bioenv.gu.se/forskning/huvudforskningsomraden/systematik (accessed 20 March 2019).

43 Gachet, M.S., Lecaro, J.S., Kaiser, M. et al. (2010). Assessment of anti-protozoal activity of plants traditionally used in Ecuador in the treatment of leishmaniasis. *J. Ethnopharmacol.* 128 (1): 184–197.

44 Kabiru, A. (2013). Elephantopus species: traditional uses, pharmacological actions and chemical composition. *Adv. Life Sci. Technol.* 15: 6–14.

45 Fuchino, H., Koide, T., Takahashi, M. et al. (2001). New sesquiterpene lactones from *Elephantopus mollis* and their leishmanicidal activities. *Planta Med.* 67 (7): 647–653.

46 Cursino, L.M.C., Nunez, C.V., RCd, P. et al. (2012). Triterpenes from *Minquartia guianensis* (Olacaceae) and *in vitro* antimalarial activity. *Química Nova* 35: 2165–2168.

47 Atta-ur-Rahman (2012). *Studies in Natural Products Chemistry (Bioactive Natural Products (Part P))*, 1e. Elsevier Academic Press.

48 Cristina, R., Paula, D., Fernanda, M. et al. (2012). Triterpenes from *Minquartia guianensis* (Olacaceae) and *in vitro* antimalarial activity. *Química Nova* 35 (11): 2165–2168.

49 Rocha, L.G., Almeida, J.R.G.S., Macêdo, R.O., and Barbosa-Filho, J.M. (2005). A review of natural products with antileishmanial activity. *Phytomedicine* 12 (6): 514–535.

50 Sosa, V., De-Nova, J.A., and Vásquez-Cruz, M. (2018). Evolutionary history of the flora of Mexico: dry forests cradles and museums of endemism. *J. Syst. Evol.* 56 (5): 523–536.

51 Alamilla-Fonseca, L.N., Delgado-Domínguez, J., Zamora-Chimal, J. et al. (2018). *Leishmania mexicana* cell death achieved by *Cleoserrata serrata* (Jacq.) Iltis: learning from Maya healers. *J. Ethnopharmacol.* 211: 180–187.

52 Delgado-Altamirano, R., Monzote, L., Pinon-Tapanes, A. et al. (2017). *In vitro* antileishmanial activity of Mexican medicinal plants. *Heliyon* 3 (9): e00394.

53 Gutierrez-Rebolledo, G.A., Drier-Jonas, S., and Jimenez-Arellanes, M.A. (2017). Natural compounds and extracts from Mexican medicinal plants with anti-leishmaniasis activity: an update. *Asian Pac. J. Trop. Med.* 10 (12): 1105–1110.

54 Arevalo-Lopez, D., Nina, N., Ticona, J.C. et al. (2018). Leishmanicidal and cytotoxic activity from plants used in Tacana traditional medicine (Bolivia). *J. Ethnopharmacol.* 216: 120–133.

55 Soosaraei, M., Fakhar, M., Hosseini Teshnizi, S. et al. (2017). Medicinal plants with promising antileishmanial activity in Iran: a systematic review and meta-analysis. *Ann. Med. Surg. (Lond.)* 21: 63–80.

56 Kheiri Manjili, H., Jafari, H., Ramazani, A., and Davoudi, N. (2012). Anti-leishmanial and toxicity activities of some selected Iranian medicinal plants. *Parasitol. Res.* 111 (5): 2115–2121.

57 Nikmehr, B., Ghaznavi, H., Rahbar, A. et al. (2014). *In vitro* anti-leishmanial activity of methanolic extracts of *Calendula officinalis* flowers, *Datura stramonium* seeds, and *Salvia officinalis* leaves. *Chin. J. Nat. Med.* 12 (6): 423–427.

58 Ferreira, C., Passos, C.L., Soares, D.C. et al. (2017). Leishmanicidal activity of the alkaloid-rich fraction from *Guatteria latifolia*. *Exp. Parasitol.* 172: 51–60.

59 Bhattacharya, P., Mondal, S., Basak, S. et al. (2016). *In vitro* susceptibilities of wild and drug resistant *Leishmania donovani* amastigotes to piperolactam A loaded hydroxypropyl-beta-cyclodextrin nanoparticles. *Acta Trop.* 158: 97–106.

60 Kaur, G., Chauhan, K., and Kaur, S. (2018). Immunotherapeutic potential of *Codonopsis clematidea* and naringenin against visceral leishmaniasis. *Biomed. Pharmacother.* 108: 1048–1061.

61 Peniche-Pavía, H.A., Medrano-Nahuat, D., Torres-Tapia, L.W. et al. (2016). Metabolites isolated from the rhizomes of *Dorstenia contrajerva* with anti-leishmanial activity. *Phytochem. Lett.* 18: 140–143.

62 Falodun, A., Imieje, V., Erharuyi, O. et al. (2014). Isolation of antileishmanial, antimalarial and antimicrobial metabolites from *Jatropha multifida*. *Asian Pac. J. Trop. Biomed.* 4 (5): 374–378.

63 Mukherjee, D., Singh, C.B., Dey, S. et al. (2016). Induction of apoptosis by zerumbone isolated from *Zingiber zerumbet* (L.) Smith in protozoan parasite *Leishmania donovani* due to oxidative stress. *Braz. J. Infect. Dis.* 20 (1): 48–55.

64 Manzo, E., Ciavatta, M.L., Pagano, D., and Fontana, A. (2012). An efficient and versatile chemical synthesis of bioactive glyco-glycerolipids. *Tetrahedron Lett.* 53 (7): 879–881.

65 Fromentin, Y., Gaboriaud-Kolar, N., Lenta, B.N. et al. (2013). Synthesis of novel guttiferone A derivatives: in-vitro evaluation toward *Plasmodium falciparum*, *Trypanosoma brucei* and *Leishmania donovani*. *Eur. J. Med. Chem.* 65: 284–294.