Plant natural products with leishmanicidal activity

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Covering: mid-1980s to late 2000.

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1 Introduction

This review covers the literature dealing with leishmanicidal activity in natural products from the mid-eighties, the date when more formal and constant research on natural metabolites with leishmanicidal activity was initiated, to late 2000. The review starts with a comprehensive description of the disease and its importance, mentioning both the various forms of the disease and the many methods presently in use for its treatment. The various metabolites are discussed in groups, *i.e.* alkaloids, triterpenes, *etc.*, and a fair amount of discussion on structure–activity relationships is included. Special mention of the activity and the mechanism of action of most of the metabolites is made, and a number of bioassay procedures are listed.

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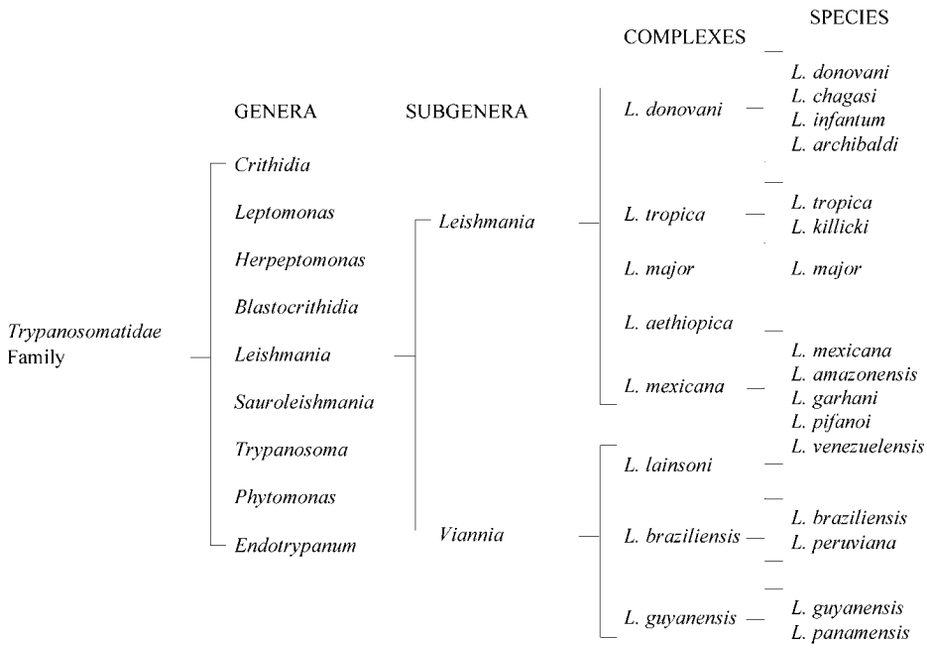
Table 1 Taxonomic classification of pathogenic *Leishmania* spp.[a]



[a] Modified from C. M. Lezama-Dávila and A. P. Isaac-Márquez, *Inmunobiología de las Leishmaniosis*, Universidad Autónoma de Campeche, México, 1995.

2 Generalities on leishmaniasis

Protozoan parasites are among the most common pathogens in the world; they are recognized as the causative agents of some of the most serious tropical diseases in both man and domestic animals. Malaria, amoebiasis, toxoplasmosis, trypanosomiasis, and leishmaniasis, are diseases caused by protozoan parasites that affect approximately 25% of the world's population, most of it in developing countries, causing loss of lives and productivity. Because of this, malaria, together with African trypanosomiasis, Chagas disease, and leishmaniasis, are considered by the World Health Organization among the six most important tropical diseases.[1,2]

Leishmaniasis is a group of tropical diseases caused by a number of species of protozoan parasites belonging to the genus *Leishmania*. This ailment affects around 12 million people in 80 countries and it is estimated that there are about two to three million new cases each year. It is also considered that presently there exists a population of 350 million of people under risk of infection.[3]

In the Old World, leishmaniasis is distributed around the Mediterranean Sea, in East and West Africa, as well as in Afghanistan, India, China and several former Soviet Republics in Asia. In the New World, the disease is found from the southern part of the United States, to the northern parts of Argentina and Paraguay.[4]

In most cases, leishmaniasis is transmitted zoonotically; however, in those cases where an animal reservoir is not known, an anthroponotic transmission is suspected. The main reservoirs for *Leishmania* parasites have been identified as both domestic and wild animals, while the vectors for the disease have been characterized as the female flying insects of the genus *Phlebotomus* (Old World leishmaniasis) and *Lutzomyia* (New World leishmaniasis).[5]

The members of the *Leishmania* genus belong to a biologically diverse group of flagellate parasites of the *Trypanosomatidae* family. Most of the species are pathogenic to both man and lower vertebrates; they have been differentiated, by means of genetic, biochemical and immunological studies, and grouped into different complexes (Table 1).[6]

In humans, *Leishmania* spp. cause a variety of clinical diseases according to the ability of the organism to proliferate in deep tissue (37 °C) or close to the surface of the skin at lower temperatures (25 °C).[7] The various manifestations of the disease have been used by the World Health Organization as the basis to classify leishmaniasis in four clinical forms: a) visceral, b) mucocutaneous, c) cutaneous diffuse or disseminated, and d) cutaneous. Certain species of the parasite have been associated with the different clinical forms of the disease, *e.g.* the *Leishmania donovani* complex causes visceral leishmaniasis, while the *Leishmania tropica* complex is known to induce cutaneous lesions in the Old World and the *Leishmania mexicana* complex causes cutaneous and cutaneous diffuse leishmaniasis in several countries of Latin America.[5,8]

Visceral leishmaniasis is the most severe clinical form of the disease and it can be fatal when not treated; it is characterized by its effect on the internal organs, particularly the liver, the spleen and the bone marrow. Mucocutaneous leishmaniasis often results in facial disfiguration due to erosion in the mucocutaneous sites of the mouth and nose. On the other hand, leishmaniasis cutaneous diffuse or disseminated is characterized by the formation of nodules, plates or multiple lumps, specially around the face and on the external surface of arms and legs. Finally, cutaneous leishmaniasis is the least severe form of the disease and is generally considered as an autolimited infection.[9]

During its biological cycle, parasites of the *Leishmania* genus exist in two forms that develop in a different host: a flagellated extracellular form known as promastigote and an intracellular one designated as amastigote (Fig. 1). The form that infects both man and other vertebrate hosts is the promastigote, which is 15 to 20 μm in length, has a flagellum in its front part, and lives in the digestive tract of the insect transmitter of the disease. The amastigote, the parasitic form, has a diameter of approximately 2.3 μm, a rudimentary flagellate and it is located inside of the host's macrophages.[8]

The vertebrate host is infected with the promastigote form of the parasite as a result of a sting by the vector insect. After this, the promastigotes are quickly phagocytized by the macrophages of the host and inside of them the promastigotes change to the amastigote form. The clinical manifestation

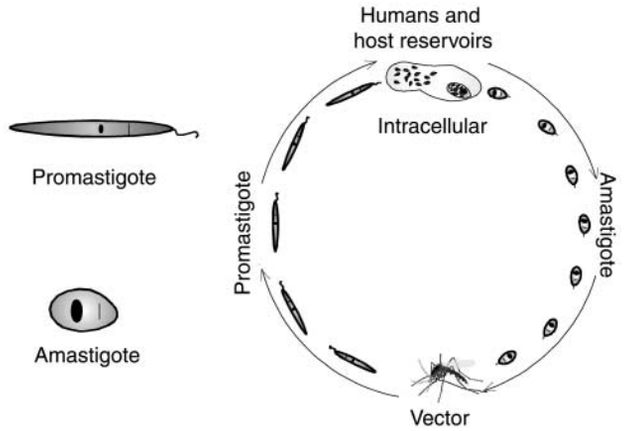


Fig. 1 Forms of *Leishmania* parasite and its biological cycle.

of the disease is a consequence of the multiplication of the amastigotes inside the macrophages.[10]

3 Treatment of leishmaniasis

3.1 Chemotherapy

Historically, the chemotherapy of leishmaniasis has been based on the use of toxic heavy metals, particularly antimony compounds. When this kind of treatment is not effective, other medications used include pentamidine and amphotericin B. All these pharmaceuticals require administration by injection and clinical supervision or hospitalization during treatment due to the severity of the possible secondary effects.[11]

The leishmanicidal agents with the most favourable therapeutic index are the antimony compounds known as antimonials: sodium stibugluconate (Pentosam®) and meglumine antimonate (Glucantime®). However, the clinical formulations of these products contain multiple uncharacterized molecular structures; *e.g.* Pentosam® has been described to contain an unknown number of complexes of antimony with carbohydrates derived from gluconic acid, while in the case of Glucantime®, its constituents have been loosely identified as *N*-methylglucamine derivatives.

The mechanism of action of the antimonials is based on its interference in the bioenergetic processes of the *Leishmania* amastigotes. These products bind to and inhibit different proteins of the parasite, particularly enzymes involved in the glycolysis and oxidation of fatty acids, resulting in a net reduction in the generation of ATP and GTP.[12]

However, the antimonials cause serious side effects that include pain at the site of injection, stiff joints, gastrointestinal problems, cardiotoxicity and, in some cases, hepatic and renal insufficiency. These products also require lengthy treatments and their cost is rather high.[9,13] Finally, the efficacy of the antimonials for the treatment of leishmaniasis has been reported to be around 85%.[14]

Pentamidine is an aromatic derivative of diamidine; it is toxic to a number of protozoa and useful for the treatment of visceral leishmaniasis cases that do not respond to the antimonials. To date, the mechanism of action of pentamidine against protozoa has not been clearly established. At an intracellular level, pentamidine binds to the DNA of the parasite; however, a clear link between the binding of the product to DNA and its efficacy has not yet been established. It has also been suggested that pentamidine interferes with the reception or the function of polyamines. Among the side effects associated with the intravenous administration of pentamidine are hypotension, tachycardia, fainting spells, upset stomach, and severe headaches, in addition to skin eruptions, abnormal function of the liver, hypoglycemia, and renal disfunction.[9]

Amphotericin B is a polyene antifungal agent that represents an adequate and different treatment to the antimonials. Its mechanism of action consists in binding to the ergosterol fraction of the cell membrane of the parasite, thus increasing its permeability. However, this pharmaceutical product is also associated with an important number of side effects, including the alteration of the renal function in approximately 80% of treated individuals.[9,15]

Although there are a number of products being developed, to date none of them has been demonstrated to be fully effective against *Leishmania* parasites. These pharmaceutical products include allopurinol, primaquine, compound WR6062 and the antidepressants imipramine and 3-chloroimipramine.[9,16]

Table 2 Popular methods used in the treatment of cutaneous leishmaniasis[a]

Acids	Heat based treatments
Acetic acid	Hot animal bones
Boric acid	Hot kitchen utensils
Sulfuric acid (car battery acid)	Hot grease (fat, bacon)
	Hot honey
Antibiotics	Hot knives
Penicillin	Hot radio batteries
Unguents for topical use	Hot water/hot water compresses
	Hot wood
Chemical substances	Lit matches
Hydrogen peroxide	
Alcohol	Oil derivatives
Alum	Creosote
Sulfur	Gasoline
Chlorine bleach	Kerosene
Calcium carbonate	
Copper sulfate	Others
Sodium chloride	Ground sea shells
Ether	Nail polish
Formalin	Ashes (tobacco, wood)
Mercury	Liquor
Potassium permanganate	Ground animal bones
Ground lead (from batteries)	Talcum powder
Liquid ferrous sulfate	Soap (bar and powder)
Iodine	Gun powder

[a] Modified and translated from M. M. Weigel, R. X. Armijos, R. J. Racines, C. Zurita, R. Izurieta, E. Herrera and E. Hinojosa, *Bol. Of. Sanit. Panam.*, 1994, **117**, 400.

3.2 Traditional methods

Due to the limited availability of effective pharmaceutical products, most people in areas where leishmaniasis is endemic depend largely on popular treatments and traditional medicine to alleviate the symptoms. Some of the most popular methods for the treatment of leishmaniasis include cauterization procedures using copper sulfate, battery acid or the application of a hot source such as hot water or red hot metal objects (Table 2). These methods, though severe, are of therapeutic value; it has been reported that *Leishmania* amastigotes are thermosensible and that applying heat locally to reach a temperature of 40 °C, or using ultrasound to induce higher temperatures, are efficient ways to accelerate the healing of the ulcers. In some cases, it has been reported that the thermal treatment is as efficient as that with Glucantime®. However, these therapeutic alternatives also have undesirable secondary effects; the use of strong acids, caustic substances and hot metal objects can result in permanent and more serious scars than those caused by the ulcers themselves.[18]

In addition to the various methods already mentioned, the treatment of leishmaniasis following the traditional medical practices of different cultures depends heavily on the use of native plants (Table 3). In traditional medicine, the treatment of leishmaniasis usually consists of the oral administration of crude plant extracts for the systemic form of the disease and as topical preparations of the corresponding extracts for the treatment of skin infections.[3] With this knowledge, and as part

Table 3 Some examples of medicinal plants used for the treatment of leishmaniasis[a]

Common name	Scientific name	Part used
Achiote	*Bixa orellana*	Leaves
Calaguala	*Polypodium calaguala*	Sap
Escobilla	*Sida rhombifolia*	Leaves
Guayaba	*Psidium guajava*	Juice, pulp
Llantén	*Plantago major*	Leaves
Matapalo	*Ficus dendrocida*	Sap
Matico	*Piper angustifolium*	Leaves
Plátano	*Musa paradisiaca*	Peel, sap, fruit
Tauri	*Lupinus tauris*	Leaves
Yerba mora	*Solanum nigra*	Fruit, leaves

[a] Modified and translated from M. M. Weigel, R. X. Armijos, R. J. Racines, C. Zurita, R. Izurieta, E. Herrera and E. Hinojosa, *Bol. Of. Sanit. Panam.*, 1994, **117**, 400.

of its search for new and better pharmaceuticals of high availability and low toxicity, the Tropical Diseases Program of the World Health Organization has considered the investigation of plants used in traditional medicine practices for the treatment of leishmaniasis as essential and of high priority.

3.3 Use of medicinal plants

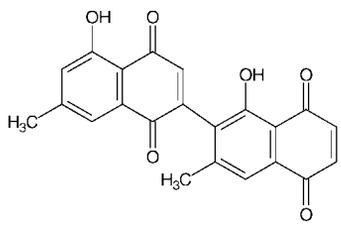
The scientific evaluation of medicinal plants used in the preparation of folk remedies has provided modern medicine with effective pharmaceuticals for the treatment of diseases caused by protozoan parasites. Some of the metabolites obtained from plants used in the treatment of diseases caused by protozoan parasites include quinones, alkaloids and terpenes. As an example, it is important to remember that the first pharmaceutical products developed for the treatment of malaria and amoebiasis were the alkaloids quinine and emetine, obtained from different species of the genus *Cinchona* and *Cephaelis*, respectively. Recently, the clinical use of artemisinin, a sesquiterpene lactone produced by *Artemisia annua*, for the treatment of malaria has prompted interest to discover new pharmaceuticals of plant origin with antiprotozoal activity.[19] As a result of this, most of the studies directed towards obtaining natural products with biological activity against protozoan parasites, have focused mainly on the search for metabolites with antimalarial (*Plasmodium falciparum*) and amoebicidal (*Entamoeba histolytica*) activities and little has been advanced on the search for plant metabolites which are biologically active against species of *Leishmania* and *Trypanosoma*.[20]

Most of the studies directed towards the detection of plant secondary metabolites with leishmanicidal activity, have been done using the promastigote form of the parasite because it is easier to maintain under *in vitro* conditions. However, since the promastigote is not the infective form of the parasite in vertebrate hosts, evaluations done with promastigotes have only an indicative value of the possible leishmanicidal activity of the metabolite tested. As a result of this, a preliminary evaluation using promastigotes must be complemented with an evaluation using intracellular amastigotes in macrophages. At the same time, an evaluation of the possible cytotoxicity of the metabolite must be carried out using non-parasited macrophages, this in order to establish if the *in vitro* activity of the metabolite is due to its general cytotoxic activity or if it possesses a selective activity against the *Leishmania* parasite.[21]

4 Natural products with leishmanicidal activity

4.1 Quinones

Among some of the plant natural products reported to have leishmanicidal activity is diospyrin **1**, a bis-naphthoquinone derivative isolated from the bark of *Diospyros montana* (Ebenaceae). This metabolite is reported to be active against promastigotes of *L. donovani* with an MIC of 1 μg ml^{-1}, although this activity is not selective against the corresponding amastigotes in macrophages.[22] Recently, it has been reported that **1** exerts its leishmanicidal action against *L. donovani* by binding to the parasite's topoisomerase I, thus inhibiting the catalytic activity of the enzyme, or by stabilizing the topoisomerase I–DNA binary complex.[23] On the other hand, the hydroxylated derivative of diospyrin **2**, at a concentration of 3 μM, eliminates 73.8% of amastigotes in infected macrophages. The mechanism of action of this hydroxynaphthoquinone is apparently based on its ability to perturb the electron transport chain in the mitochondria of the parasite or in the generation of free radicals during the interaction between the metabolite and the respiratory chain of the parasite.[24]



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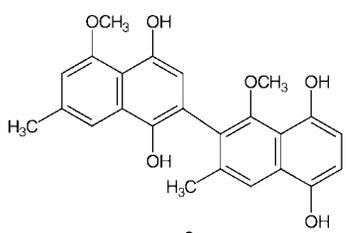

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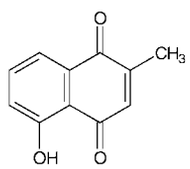
Plumbagin **3**, a naphthoquinone isolated from species of the genus *Plumbago*, is reported to have an activity (IC_{50}) of 0.42 and 1.1 μg ml^{-1} against amastigotes of *L. donovani* and *L. amazonensis*, respectively.[25] Plumbagin **3**, and the dimeric products 3,3′-biplumbagin **4** and 8,8′-biplumbagin **5**, have been isolated from the bark of *Pera benensis* (Euphorbiaceae), a plant used in Bolivia for the treatment of cutaneous leishmaniasis. While **3** and **5** show activity against promastigotes of *L. braziliensis*, *L. amazonensis*, and *L. donovani* at a concentration (IC_{90}) of 5 μg ml^{-1}, **4** shows a lower activity (IC_{90} =



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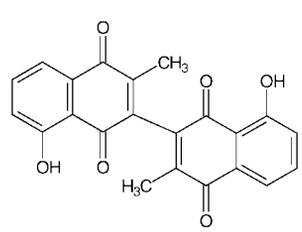

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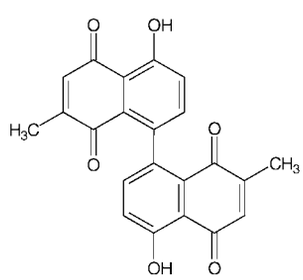

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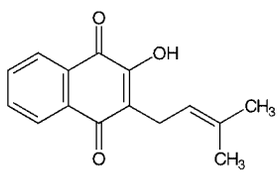
50 μg ml^{-1}) against the same *Leishmania* species. The leishmanicidal activity of plumbagin **3** against the amastigote forms of *L. amazonensis* is observed at 10 μg ml^{-1}, showing an amastigote survival index (SI) of 16.5% and an absence of toxic effects against the macrophages.[26] Finally, the *in vivo* activity of plumbagin **3** is detected at concentrations of 2.5 mg kg^{-1} day^{-1} against *L. amazonensis* and of 5 mg kg^{-1} day^{-1} against *L. venezuelensis*. The mechanism of the action of **3**, just as the one proposed for the hydroxylated derivative of diospyrin **2**, is based on the generation of oxygen free radicals, something that the parasites are not able to defend against.[27]

Another metabolite with a mechanism of action similar to that of **2** and **3** is lapachol **6**, a prenylated hydroxynaphthoquinone obtained from a species of *Tecoma* (*Bignoniaceae*), which is weakly active against amastigotes of *L. donovani* in peritoneal mice macrophages.[28]



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A metabolite isolated from the bark of *Ampelocera edentula* (Ulmaceae), 4-hydroxy-1-tetralone‡ **7**, is known as an intermediate in the biosynthesis of 1,4-naphthoquinones and as an active natural product against promastigotes of *L. braziliensis*, *L. amazonensis* and *L. donovani* with an IC_{90} of 10 μg ml^{-1}. *In vivo* studies carried out with this metabolite on BALB/c mice infected with *L. amazonensis* or *L. venezuelensis*, showed a similar activity to that of Glucantime® (25 mg kg^{-1} day^{-1} *vs.* 56 mg Sb^V kg^{-1} day^{-1}) when administered subcutaneously. However, even though **7** has a stronger activity than Glucantime® (50 mg kg^{-1} day^{-1} *vs.* 112 mg Sb^V kg^{-1} day^{-1}) against *L. amazonensis* when administered close to the infection site, the use of tetralones is limited by the fact that this type of metabolite has been found to be cytotoxic, carcinogenic and mutagenic to laboratory animals.[29]

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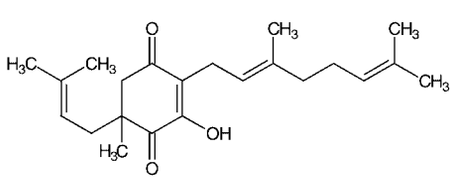

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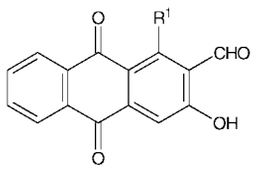
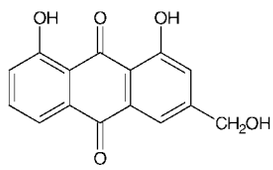
The quinone derivative jacaranone **8**, isolated from the leaves of *Jacaranda copaia* (Bignoniaceae), shows a strong activity against promastigotes of *L. amazonensis* at an ED_{50} of 0.02 mM, although at this concentration the metabolite is toxic to peritoneal mice macrophages. Jacaranone **8** shows a weak activity *in vivo* when administered subcutaneously to mice infected with *L. amazonensis* and a strong cutaneous toxicity when applied inside the lesion.[21]

The prenylated dihydroquinone hydropiperone **9**, isolated from *Peperomia galioides* (Piperaceae), shows toxic activity against promastigote forms of *L. braziliensis*, *L. donovani*, and *L. amazonensis* at a concentration of 25 μg ml^{-1} and causes a total lysis of the parasites at 100 μg ml^{-1}.[30]

Some anthraquinones have been reported to have leishmanicidal activity. The anthraquinone-2-carbaldehydes **10** and **11**, obtained from the roots of *Morinda lucida* (Rubiaceae), have shown selective activity against promastigote forms of *L. major*. The presence of an aldehyde group at C-2 and of a phenolic hydroxy group at C-3 of both structures, suggests that these functional groups are essential for their antiprotozoal activity.[31] Similarly, aloe-emodin **12**, an anthraquinone isolated from the aerial parts of *Stephania dinklagei* (Menispermaceae), presents leishmanicidal activity against promastigotes and amastigotes of *L. donovani* at IC_{50} values of 185.1 μM and 90 μM, respectively.[32]



10 $R^1 = OCH_3$
11 $R^1 = H$



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4.2 Alkaloids

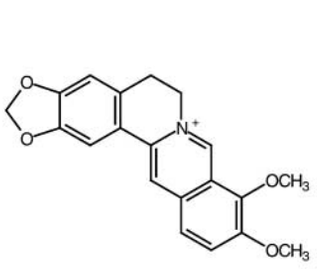
4.2.1 Quinoline and isoquinoline analogues

Berberine **13**, a quaternary isoquinolinic alkaloid found in a number of plant families (*e.g.* Annonaceae, Berberidaceae, Menispermaceae), is one of the alkaloids with the highest leishmanicidal activity. This metabolite is the main constituent in various folk remedies used in the treatment of cutaneous leishmaniasis, malaria and amoebiasis.[33] Berberine **13** has been used clinically for the treatment of leishmaniasis for over 50 years and it has been demonstrated that it possesses significant activity both *in vitro* and *in vivo* against several species of *Leishmania*. At a concentration of 10 μg ml^{-1}, **13** eliminates effectively *L. major* parasites in peritoneal mice macrophages; however, this product shows minimum activity when applied topically on mice cutaneous lesions caused by *L. major*. Similarly, and even though berberine **13** is effective against cutaneous ulcers caused by *L. panamensis* in rats, it has been observed that in these cases viable amastigotes persist on the skin, resulting in the reappearance of the lesion.[3,34]

Another isoquinolinic alkaloid, isoguattouregidine **14**, isolated from the bark of *Guatteria foliosa* (Annonaceae), causes a total lysis of the parasites of *L. donovani* and *L. amazonensis* when evaluated at a concentration of 100 μg ml^{-1}.[35] Anonaine **15** and liriodenine **16**, obtained from the trunk bark and roots of *Annona spinescens* (Annonaceae), have been reported to show activity against promastigotes of *L. braziliensis*, *L. amazonensis* and *L. donovani*.[36] However, while in this report **16** shows leishmanicidal activity with an IC_{100} of 100 μg ml^{-1}, the same metabolite, when reported from the stem bark of *Rollinia emarginata* (Annonaceae), exhibits an IC_{100} of 5 μg ml^{-1} on promastigotes of the same parasites.[37] A possible explanation for the variation in the biological activity of this metabolite against the same species of *Leishmania*, might lie on the use of biphasic or liquid media for the evaluation of leishmanicidal activity in the former and latter reports, respectively.

The benzylisoquinolinic alkaloids are widely distributed in nature and they have been isolated from different plants commonly used in traditional medicine for the treatment of parasitic diseases. In an evaluation of fourteen bisbenzylisoquinolinic alkaloids, strong activity against promastigotes of *L. braziliensis*, *L. amazonensis* and *L. donovani* was found for four of them. Daphnandrine **17**, isolated from *Albertisia papuana* (Menispermaceae), obaberine **18**, obtained from *Pseudoxandra sclerocarpa* (Annonaceae), gyrocarpine **19**, produced by *Gyrocarpus americanus* (Hernandiaceae), and

‡ The IUPAC name for 1-tetralone is 3,4-dihydronaphthalen-1(2*H*)-one.



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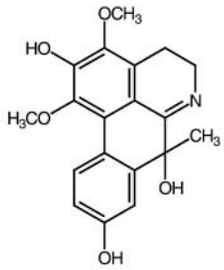

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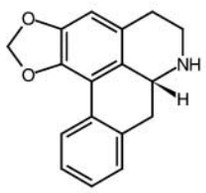

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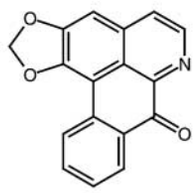
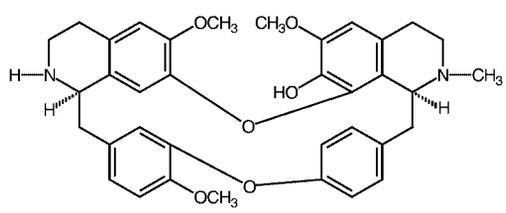


16

limacine **20**, isolated from *Caryomene olivascens* (Menispermaceae); all show leishmanicidal activity at an IC_{100} close to 50 μg ml^{-1}.[38] Gyrocarpine **19** also shows activity *in vitro*, at 10 μg ml^{-1}, against the promastigote forms of *L. braziliensis*, *L. amazonensis* and *L. donovani*. However, in the *in vivo* test against *L. amazonensis*, this metabolite is not as effective as Glucantime® (100 mg kg^{-1} day^{-1} *vs.* 56 mg Sb^{V} kg^{-1} day^{-1}).[39]



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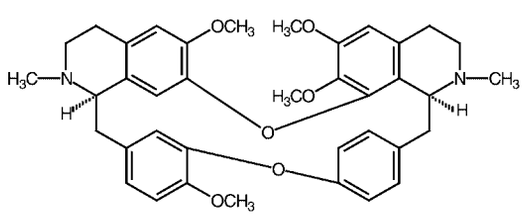

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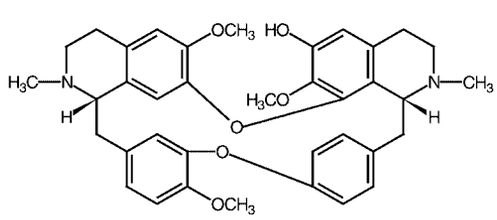

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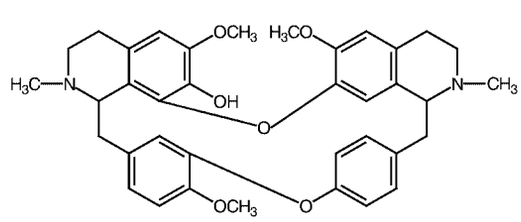
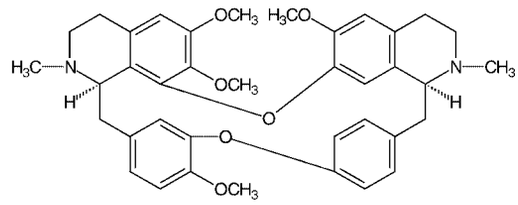


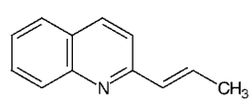
20

Another bisbenzylisoquinolinic alkaloid, that shows leishmanicidal activity at 10 μg ml^{-1} against the promastigote forms of *L. braziliensis*, *L. amazonensis*, and *L. donovani*, is isotetradrin **21**, a metabolite isolated from *Limaciopsis loangensis* (Menispermaceae). The *in vivo* activity of this product, at 100 mg kg^{-1} day^{-1} in BALB/c mice, is comparable to that of Glucantime® (56 mg Sb^{V} kg^{-1}) when tested against *L. amazonensis* and is slightly less effective against *L. venezuelensis*.[39] Finally, bisbenzylisoquinolinic alkaloids isolated from the stem bark of *Guatteria boliviana* (Annonaceae) have also been reported to show moderate activity when tested against promastigotes of *L. donovani*, *L. amazonensis* and *L. braziliensis*.[40]



21

The alkaloids derived from 2-alkylquinoline, chimanine B **22** and chimanine D **23**, isolated from the leaves of *Galipea longiflora* (Rutaceae), show activity at an IC_{90} of 25 μg ml^{-1} against promastigotes of *L. braziliensis*, while the 2-*n*-propylquinoline **24** shows activity at an IC_{90} of 50 μg ml^{-1}. When tested *in vivo* in cutaneous lesions caused by *L. amazonensis* and *L. venezuelensis*, **24** proved active at a concentration of 100 mg kg^{-1} day^{-1}. This metabolite, when administered orally (0.54 mmol kg^{-1}), suppresses by 99.9% the presence of *L. donovani* parasites in the liver of BALB/c mice after ten days of treatment. Finally, **23** also has activity *in vivo* against parasites of *L. amazonensis* and *L. donovani*.[41–43]



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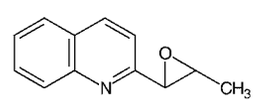

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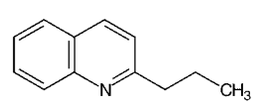
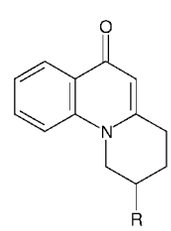


24

Dictylomides A **25** and B **26**, two alkaloids derived from quinolin-4-one isolated from the bark of *Dictyoloma peruviana* (Rutaceae), cause a total lysis of promastigotes of *L. amazonensis* at 100 μg ml^{-1} and show a minor activity on promastigotes of *L. braziliensis* at the same concentration.[44]



25 R = $CH_2CH_2CH{=}CHCH_2CH_3$

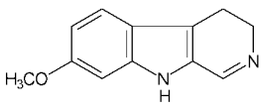
cis

26 R = $CHOHCH_2CH_2CH_2CH_2CH_3$

4.2.2 Indole analogues

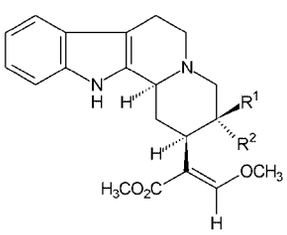
Among some of the indole alkaloids reported to possess antiprotozoal activity is harmaline **27**, the main constituent of a number of plants utilized in traditional medicine to cure leishmaniasis, including *Peganum harmala* and *Passiflora incarnata*. The possible mechanism of the antiprotozoal action of **27** has been postulated as its being able to intercalate DNA or by interfering with the metabolism of aromatic amino acids

in the parasite. However, because of its activity as an inhibitor of monoamino oxidase A, harmaline **27** produces psychopathic effects that prevent its use as a therapeutic agent.[34,45]

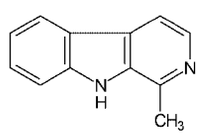


27

Five indole alkaloids obtained from the bark of *Corynanthe pachyceras* (Rubiaceae) present marked activity against promastigotes of *Leishmania major*. Among these alkaloids are dihydrocorynantheine **28**, corynantheine **29** and corynantheidine **30**, which present activity values of IC_{50} below 3 μM. These metabolites do not show significant cytotoxic activity against the drug sensitive KB-3-1 and multidrug-resistant KB-V1 cell lines, indicating an important selectivity in their antiprotozoal activity. It is proposed that the alkaloids of *C. pachyceras* have a relatively planar tetracyclic structure and that their mechanism of action is based on the inhibition of the respiratory chain of the parasite.[46] Several additional indole alkaloids such as harmane **31**, pleiocarpine **32** and buchtienine **33**, isolated from the stem bark and leaves of *Kopsia griffithii* (Apocynaceae), have been reported to possess toxic activity against promastigotes of *L. donovani*.[47]



28 $R^1 = H, R^2 = C_2H_5$
29 $R^1 = H, R^2 = C_2H_3$
30 $R^1 = C_2H_5, R^2 = H$



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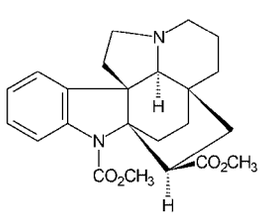
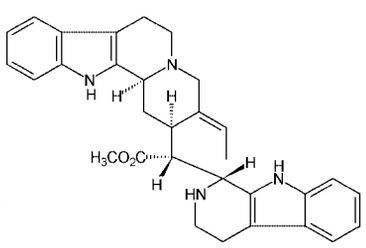
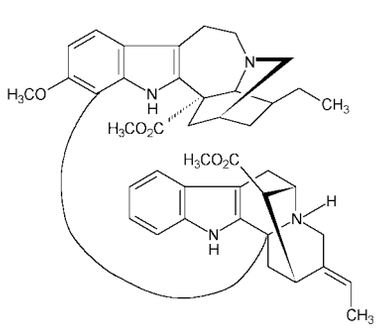


32



33

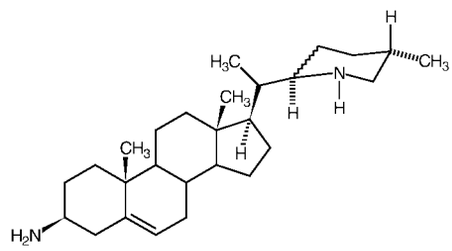
The bis-indole alkaloid, gabunine **34**, isolated from the stem bark of *Peschiera van heurkii* (Syn. *Tabernaemontana van heurkii*; Apocynaceae), shows *in vitro* activity against promastigotes of *L. amazonensis* and *L. braziliensis*. Nevertheless, even though **34** exhibits strong *in vitro* activity against amastigotes of *L. amazonensis* with a survival index (SI) of amastigotes of 3% at 25 μg ml^{-1}, this metabolite has no activity in the *in vivo* assay, possibly due to its inactivation in the host.[48]



34

4.2.3 Steroidal alkaloids

Sarachine **35**, a steroidal alkaloid isolated from leaves of the Bolivian plant *Saracha punctata* (Solanaceae), completely inhibits the growth of the promastigote forms of *L. brazilienis*, *L. donovani* and *L. amazonensis* at a concentration of 10 μg ml^{-1}; however, at the same concentration it presents a strong toxic activity against mice peritoneal macrophages.[49] Similarly, eight steroidal alkaloids obtained from the leaves of *Holarrhena curtisii* (Apocynaceae) exhibit leishmanicidal activity against promastigotes of *L. donovani* and significant cytotoxic activity against the HL-60 cell line. These include holamine **36**, 15-α-hydroxyholamine **37**, holacurtine **38**, and *N*-desmethylholacurtine **39** as the most active.[50]



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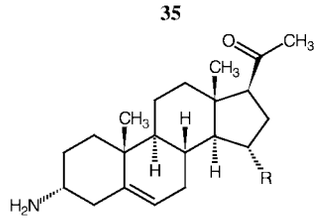
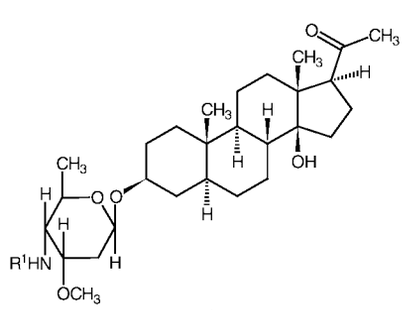


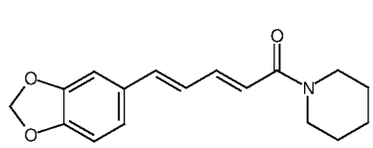
36 R = H
37 R = OH



38 $R^1 = CH_3$
39 $R^1 = H$

4.2.4 Other alkaloids

The well known main component of *Piper* species, the alkaloid piperine **40**, and benzoxazol-2(3*H*)-one **41**, obtained from the leaves of *Acanthus illicifolius* (Acanthaceae), show activity against promastigotes of *L. donovani* and their activities are comparatively similar to that of pentamidine.[51,52]



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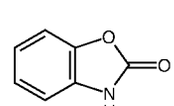


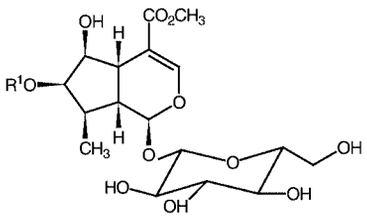
41

4.3 Terpenes

4.3.1 Iridoids

Another group of plant metabolites that have shown leishmanicidal activity are the iridoids, monoterpenoid glycosides

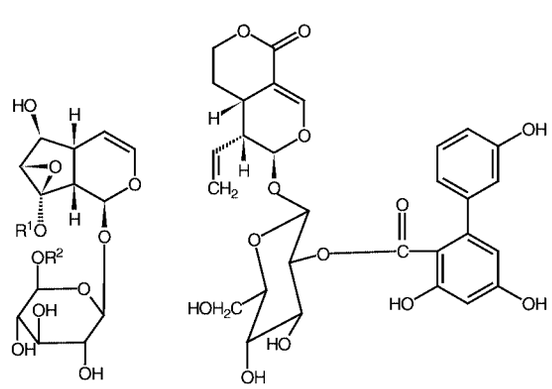
with the cyclopenta[*c*]pyran skeleton recognized as biosynthetic precursors of indole alkaloids. Among these metabolites, arbortristosides A **42**, B **43**, C **44**, and 6-β-hydroxyloganin **45**, isolated from the seeds of *Nyctanthes arbortristis* (Oleaceae), are reported to possess *in vitro* activity against amastigotes of *L. donovani*. In the *in vivo* test using infected hamsters with *L. donovani*, **42** presented leishmanicidal activity at a concentration of 10 mg kg^{-1} for 5 days when administered intraperitoneally and at 100 mg kg^{-1} for 5 days when administered orally.[53] Extracts of *N. arbortristis* have been reported to exhibit immunostimulant activity in BALB/c albino mice and it has been argued that this activity plays an important role in the expression of leishmanicidal activity.[54]



42 R^1 = *p*-methoxycinnamoyl
43 R^1 = caffeoyl
44 R^1 = coumaroyl
45 R^1 = H

Similarly, Picroliv, a standardized fraction of iridoid glycosides picroside I **46** and kutkoside **47**, obtained from the roots and rhizomes of *Picrorhiza kurroa*, is reported to increase the nonspecific immune response and to induce a high degree of protection against the infection of promastigotes of *L. donovani* in hamsters.[55] Picroliv has also demonstrated an ability to prevent the liver damage experienced by laboratory animals as a side effect commonly associated with the use of sodium stibogluconate (Pentostam®).[56] On the basis of these results, Picroliv has been proposed as an adjuvant to increase the efficacy of leishmanicidal drugs.

Amarogentin **48**, a secoiridoid glycoside isolated from *Swertia chirata* (Gentiaceae), when evaluated at a concentration higher than 60 μM, can inhibit the catalytic activity of the topoisomerase I from *L. donovani*. Topoisomerase I is reported to catalyse the relaxation of DNA and, therefore, it plays an important role in maintaining the cellular functions of the parasite. Apparently, **48** exerts its inhibitory effect by binding to the enzyme and preventing the formation of a binary complex with DNA. A similar mechanism of action has been reported for Pentostam®.[57] Recently, the evaluation of amarogentin **48** in the form of liposomes and niosomes, has been reported to show a greater leishmanicidal activity (and with no toxic effects) than those observed for free **48** when tested in hamsters. On the basis of these results this metabolite has been proposed for clinical application in the treatment of leishmaniasis.[58]



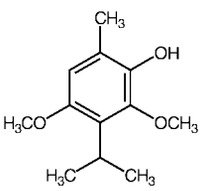
46 R^1 = vanilloyl, R^2 = H
47 R^1 = H, R^2 = cinnamoyl

48

4.3.2 Monoterpenes

Espintanol **49**, a monoterpenoid isolated from the bark of *Oxandra espintana* (Annonaceae), is reported to exhibit significant activity against promastigotes of twelve species of *Leishmania*, including *L. mexicana*. However, even though this metabolite showed a high toxicity toward macrophages, it showed only a weak activity when tested *in vivo* in mice infected with *L. amazonensis*.[59]

Two monoterpenoid derivatives from *Peperomia galoides*, grifolin **50** and piperogalin **51**, cause the total lysis of the promastigote forms of *L. braziliensis*, *L. donovani* and *L. amazonensis* when tested at a concentration of 100 μg ml^{-1}; piperogalin **51**, at 10 μg ml^{-1}, is reported to cause the lysis of more than 90% of the promastigotes.[60]



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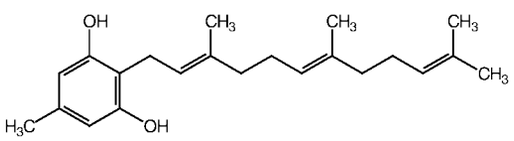

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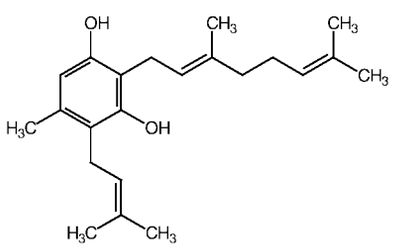
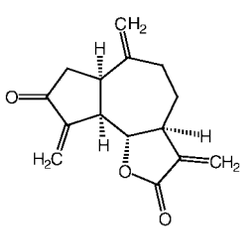


51

4.3.3 Sesquiterpenes

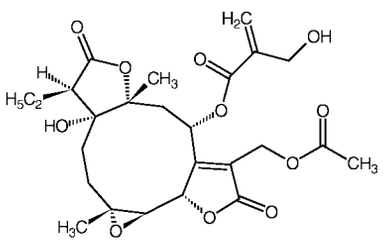
Dehydrozaluzanin C **52**, a sesquiterpene lactone isolated from the leaves of *Munnozia maronii* (Asteraceae), inhibits the growth of eleven species of *Leishmania* promastigotes at concentrations between 2.5 and 10 μg ml^{-1}. The activity against parasites of *L. mexicana* and *L. amazonensis* is observed at an IC_{90} of 25 μg ml^{-1}. In the *in vivo* test, this metabolite reduces the severity of the lesions caused by *L. amazonensis* in BALB/c mice.[61]



52

Two species belonging to the Asteraceae family that contain terpenes with leishmanicidal acitivity are *Vernonia brachycalyx* and *Jasonia glutinosa*. The major antiprotozoal metabolite of *V. brachycalyx* has been identified as the sesquiterpene dilactone 16,17-dihydrobrachycalyoxide **53**, which shows activity (IC_{50} 17 μg ml^{-1}) against the promastigote forms of *L. major*. However, at this same concentration, **53** inhibits the proliferation of human lymphocytes, suggesting that its antiprotozoal

activity is due to its general toxicity and that its administration could suppress the immune mechanism in humans.[62] On the other hand, kudtriol **54**, a sesquiterpene alcohol obtained from the aerial parts of *J. glutinosa*, has shown toxic activity against promastigotes of *L. donovani* at a concentration of 250 μg ml^{-1}, and it is proposed that the presence of a C-5 hydroxy group in the α orientation is essential for the expression of its leishmanicidal activity.[63]



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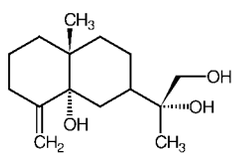
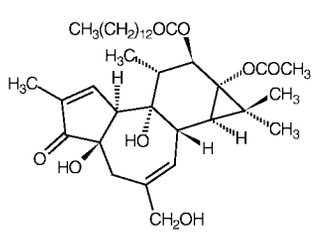


54

4.3.4 Diterpenes

Plant species of the family Euphorbiaceae are characterized for their content of diterpenoid phorbol esters, metabolites well known as tumour promoters and highly cytotoxic. One of these phorbol esters, 12-*O*-tetradecanoyl phorbol-13-acetate (TPA) **55**, is able to cause a variety of structural changes in the parasites of *L. amazonensis* at a concentration of 20 ng ml^{-1}. The proposed mechanism of action for **55** involves the activation of protein kinase C, an important enzyme in the development of several cellular functions.[34,64] Other diterpenoids with leishmanicidal activity isolated from Euphorbiaceae species include jatrogrossidione **56** and jatrophone **57**. These metabolites possess toxic activity against the promastigote forms of *L. braziliensis*, *L. amazonensis* and *L. chagasi*; even though the activity of **56** is greater than that of **57** (IC_{100} = 0.75 and 5 μg ml^{-1}, respectively), jatrogrossidione **56** shows toxicity against macrophages and it does not have activity *in vivo*.[65]



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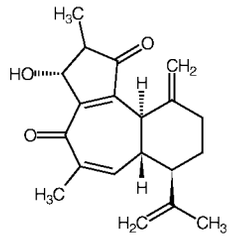

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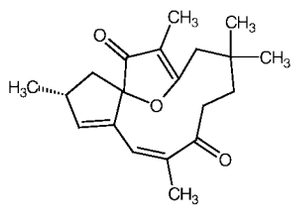
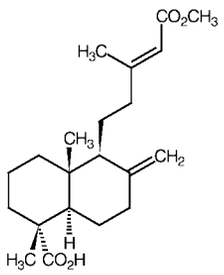


57

The 15-monomethyl ester of dehydropinifolic acid **58**, a labdane diterpene obtained from the stem bark of *Polyalthia macropoda* (Annonaceae), and ribenol **59**, an *ent*-manoyl oxide derivative isolated from *Sideritis varoi* (Lamiaceae), have been reported to show *in vitro* activity against promastigotes of *L. donovani*.[66,67] It is worth pointing out that whereas **59** also has activity against the amastigotes of *L. donovani*, different derivatives of this metabolite, obtained through chemical or biological transformations, exhibit a stronger leishmanicidal activity. Additionally, 6-β-hydroxyrosenonolactone **60**, a diterpene isolated from the bark of *Holarrhena floribunda* (Apocynaceae), has a moderate and weak activity against promastigotes and amastigotes of *L. donovani*, respectively, and a low toxicity against macrophage cells.[68]



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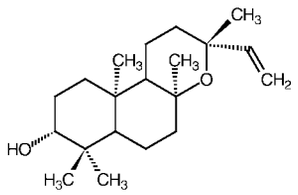

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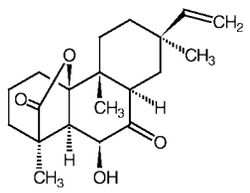


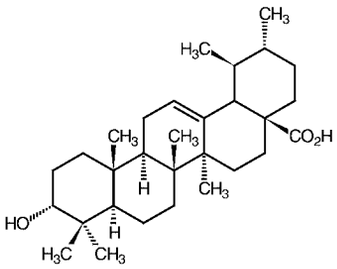
60

4.3.5 Triterpenes

Another group of metabolites having antileishmanial activity are the triterpenes. These include ursolic acid **61** and betulinaldehyde **62**, obtained from the bark of *Jacaranda copaia* and the stem of *Doliocarpus dentatus* (Dilleniaceae), respectively. Both metabolites are active against the amastigotes of *L. amazonensis*, but **62** is toxic to the peritoneal macrophages in mice and **61** exhibits limited activity in the *in vivo* test.[21,69] Two triterpenes obtained from the leaves of *Celaenodendron mexicanum* (Euphorbiaceae), (24*Z*)-3-oxotirucalla-7,24-dien-26-oic acid **63** and *epi*-oleanolic acid **64**, also exhibit leishmanicidal activity on promastigotes of *L. donovani* (IC_{50} = 13.7 and 18.8 μM, respectively). Apparently, the presence of the carboxylic acid group in their structure confers the antiprotozoal activity.[70] The quassinoids, a certain kind of degraded triterpenes occurring in different species of the family Simaroubaceae, possess leishmanicidal activity. Among these metabolites, simalikalactone D **65** and 15-β-heptylchaparrinone **66**, show activity against promastigotes of *L. donovani*. However, as is the case for other triterpenes, these metabolites are also toxic to macrophages.[34]

4.3.6 Saponins

Among the saponins that possess toxic activity against promastigotes of *L. infantum* and *L. tropica* are α-hederin **67**, β-hederin **68** and hederagenin **69**, all of them obtained from the leaves of *Hedera helix* (Araliaceae). Of these three metabolites, only **69** shows significant activity against the amastigote forms of the same *Leishmania* species.[71] In a different study both **67** and **68** showed the expected activity against promastigotes and amastigotes of *L. infantum*, but they also showed a strong antiproliferative activity on human monocytes. Apparently, these saponins inhibit the growth of the promastigote forms by acting on the membrane of the parasite where they induce a drop in membrane potential.[72] Similarly, hederecolchiside A_1 **70**, obtained from *Hedera colchica*, exhibits strong activity against the promastigote and amastigote forms of *L. infantum*, but it also shows a notable activity on human monocytes. Again, this metabolite inhibits the growth of promastigotes by significantly altering the external membrane of the parasite.[72] Another saponin that also shows activity against promastigotes of *L. infantum* is mimengoside A **71**, isolated from the leaves of *Buddleja madagascariensis* (Loganiaceae).[73,74] Finally, muzanzagenin **72**, a sapogenin obtained from the roots of *Asparagus*



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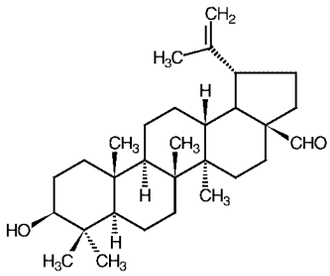

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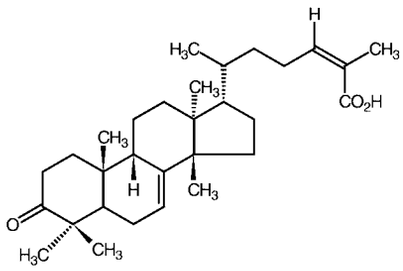
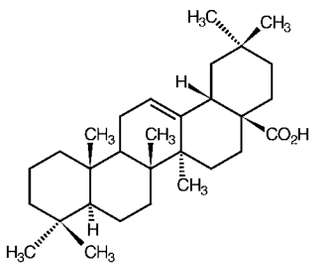


63



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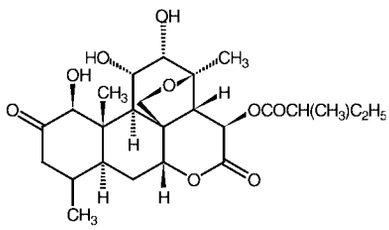

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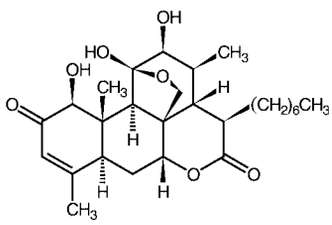


66

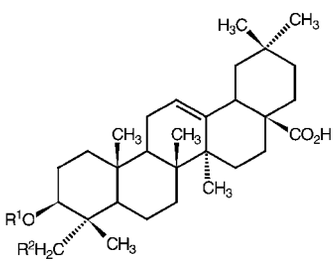
africanus (Liliaceae), shows activity against promastigotes of *L. major* (IC_{50} 31 μg ml^{-1}), but it also inhibits the proliferation of human lymphocytes, thus indicating that it does not possess a selective toxicity against *Leishmania* parasites.[75]

4.4 Phenolic derivatives

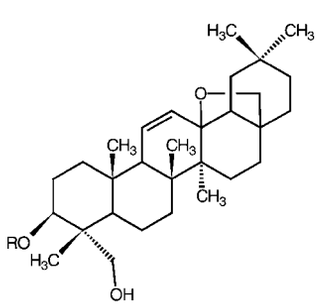
4.4.1 Chalcones

Some of the phenolic products with antileishmanial activity include chalcones and flavonoids that occur in a variety of plant species. The chalcone (*E*)-1-[2,4-hydroxy-3-(3-methylbut-2-enyl)phenyl]-3-[4-hydroxy-3-(3-methylbut-2-enyl)phenyl]-prop-2-en-1-one **73** is reported to be toxic to promastigotes of *L. donovani*,[76] while 2′,6′-dihydroxy-4′-methoxychalcone (DMC) **74**, isolated from inflorescences of *Piper aduncum* (Piperaceae), is reported to show a significant activity *in vitro* against promastigotes and amastigotes of *L. amazonensis*. This last metabolite affects the ultrastructure of the parasite mitochondria without causing damage or inducing NO production in the macrophages.[77] Recently, the leishmanicidal activity of **74**, when tested *in vitro* against amastigotes and *in vivo* in BALB/c mice infected with *L. amazonensis*, was improved by its encapsulation in poly(D,L-lactide) nanoparticles.[78] The oxygenated chalcone, licochalcone A **75**, obtained from roots of the Chinese licorice plant (*Glycyrrhiza* spp., Fabaceae), inhibits the *in vitro* growth of promastigotes of *L. major* and *L. donovani*. This metabolite also exhibits a remarkable capacity to eliminate amastigotes of *L. major* in human peripheral blood monocyte-derived macrophages and in U937 cells; its intraperitoneal administration prevents the development of lesions in BALB/c mice infected with *L. major* and both its intraperitoneal and oral administration reduce the parasite load in the spleen and liver of hamsters infected with *L. donovani*.[79,80] The proposed mechanism of action for **75** involves the alteration of the ultrastructure and the function of mitochondria, thus exerting its effect on the parasite respiratory chain without damaging the organelles of macrophages or their phagocytic function.[81] However, *in vitro* tests have revealed that at lower concentrations licochalcone A **75** inhibits the proliferation of human lymphocytes. Thus, the use of chalcones for the treatment of leishmaniasis may have the suppression of the immune system as an undesirable side effect.[82]

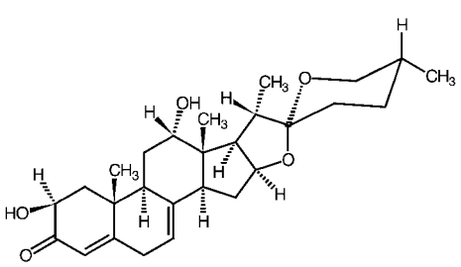
The aurones, a group of metabolites related biosynthetically to the chalcones, have demonstrated antileishmanial activity against the promastigote forms of *L. major*, *L. donovani*, *L. infantum* and *L. enrietti*. These metabolites have also been



67 R^1 = Ara 2-1 Rha, R^2 = OH
68 R^1 = Ara 2-1 Rha, R^2 = H
69 R^1 = H, R_2 = OH
70 R^1 = Ara [Glc 4-1] 2-1 Rha, R^2 = H
Ara: α-L-arabinopyranose
Glc: β-D-glucopyranose
Rha: α-L-rhamnopyranose
Fuc: β-D-fucopyranose

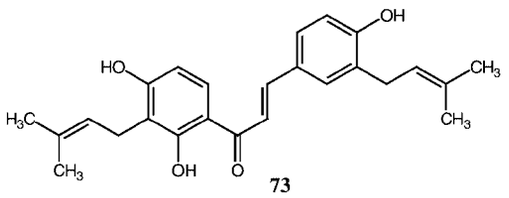


71 3-*O*-α-L-rhamnopyranosyl-(1-4)-β-D-glucopyranosyl-(1-3)-[β-D-glucopyranosyl-(1-2)]-β-D-fucopyranoside of 16-dehydroxysaikogenin G



72

reported to be active against the amastigote forms of *L. donovani*, but some of them are toxic to bone marrow-derived macrophages. Sulfuretin, 2-[(3,4-dihydroxyphenyl)methylene]-



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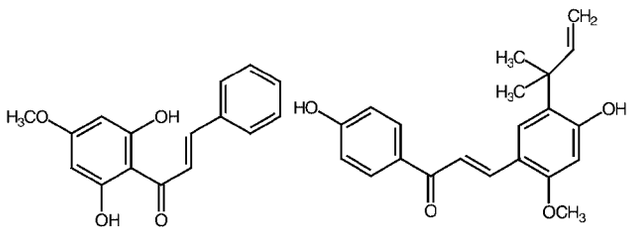
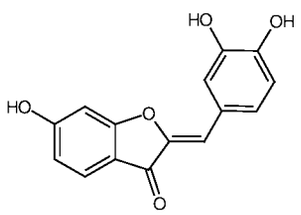


74 **75**

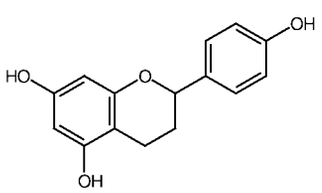
6-hydroxybenzofuran-3(*2H*)-one **76**,§ is an aurone with activity against promastigotes of *Leishmania* spp. (EC_{50} = 0.09–0.11 μg ml^{-1}) and against amastigotes of *L. donovani* (EC_{50} = 1.24 μg ml^{-1}), but non-toxic to bone marrow-derived macrophages.[83]



76

4.4.2 Flavonoids

The phenolic compound 5,7,4′-trihydroxyflavan **77** exhibits toxic activity on amastigotes of *L. amazonensis*,[84] while the biflavonoids amentoflavone **78**, podocarpusflavone A **79** and B **80**, isolated from the leaves of *Celanodendron mexicanum*, only show a weak activity against promastigotes of *L. donovani*.[70]



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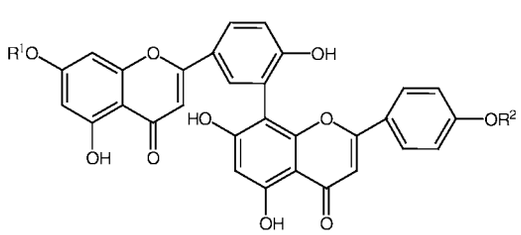
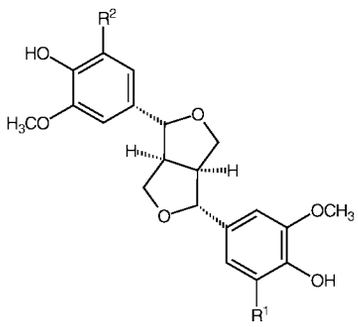


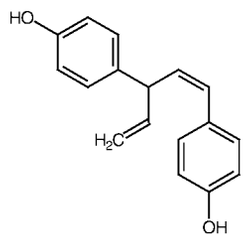
78 R^1 = H, R^2 = H
79 R^1 = H, R^2 = OCH_3
80 R^1 = OCH_3, R^2 = OCH_3

4.4.3 Other phenolic derivatives

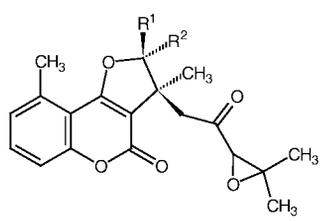
The lignans (+)-medioresinol **81** and (−)-lirioresinol B **82** are active against the amastigote forms of *L. amazonensis*;[69] whereas (+)-nyasol **83** shows high selectivity in its activity against the promastigotes of *L. major*.[75] The coumarin isomers of the epoxide of 2-epicycloisobrachycoumarinone **84** and the epoxide of cycloisobrachycoumarinone **85**, isolated from *Vernonia brachycalyx* (Asteraceae), also have selective activity against promastigotes of *L. major*.[85] Finally, the curcumins, a group of phenolic diketones that include curcumin **86**, desmethoxycurcumin **87** and bis-desmethoxycurcumin **88**, isolated from the rhizomes of *Curcuma longa* also exhibit leishmanicidal activity when tested against promastigotes of *L. major*. However, these metabolites also inhibit the proliferation of human lymphocytes and thus they, as other groups, do not possess selective antiprotozoal activity.[86]



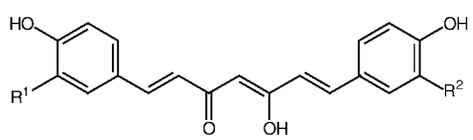
81 R^1 = R^2 = OCH_3
82 R^1 = R^2 = CH_3



83



84 R^1 = CH_3, R^2 = H
85 R^1 = H, R^2 = CH_3



86 R^1 = R^2 = OCH_3
87 R^1 = H, R^2 = OCH_3
88 R^1 = H, R^2 = H

4.5 Other metabolites

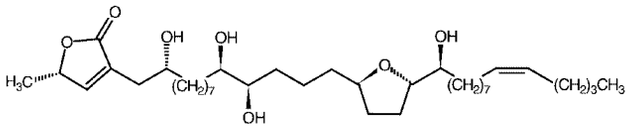
4.5.1 Acetogenins

Some acetogenins such as senegalene **89**, squamocine **90**, asimicine **91** and molvizarine **92**, isolated from the seeds of *Annona senegalensis* (Annonaceae), show activity against promastigotes of *L. major* and *L. donovani* at concentrations that vary between 25 and 100 μg ml^{-1}. However, these metabolites also show a cytotoxicity greater than that of vinblastine against KB and VERO cell lines.[87] Other acetogenins such as rolliniastatin-1 **93**, obtained from the stem bark of *Rollinia emarginata* (Annonaceae), and annonacin A **94** and goniothalamicin **95**, obtained from the seeds of *Annona glauca* (Annonaceae), have been reported to show activity against the promastigote forms of *L. braziliensis*, *L. donovani*, *L. amazonensis*.[37,88] Although a clear structure–activity relationship has not been established for the acetogenins, their leishmanicidal activity has been attributed to the number of hydroxy groups or to the presence of a single tetrahydrofuran ring in their structure.

4.5.2 Various

Argentilactone **96**, an α,β-unsaturated δ-lactone obtained from the roots of *Annona haemantantha* (Annonaceae), has a significant activity against promastigotes of *L. donovani*, *L. major*

§ The name given for compound **76** in ref. 83 is 4,6-dihydroxy-2-(2,3-dihydroxyphenylmethylene)benzofuran-3(2*H*)-one, but the structure given in this reference, and in other literature sources, has only 3 hydroxy groups.



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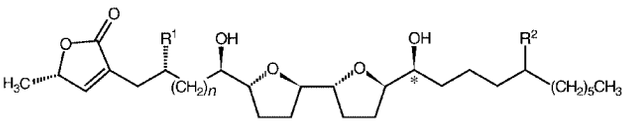
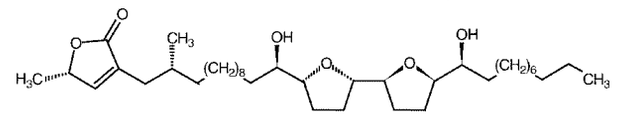
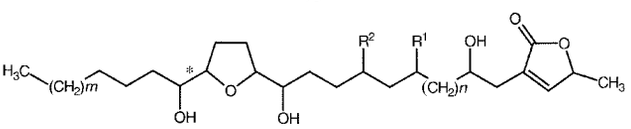


*threo-trans-threo-trans-**

90 R^1 = H, R^2 = OH, n = 10, *= *erythro*
91 R^1 = OH, R^2 = H, n = 10, *= *threo*
92 R^1 = OH, R^2 = H, n = 8, *= *erythro*

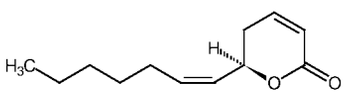


93

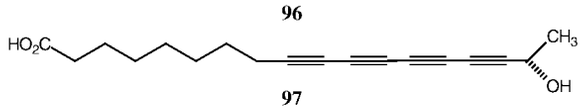


94 R^1 = OH, R^2 = H, n = 5, m = 8, *= *erythro*
95 R^1 = H, R^2 = OH, n = 3, m = 10, *= *threo*

and *L. amazonensis*. It has been reported that the subcutaneous administration of this metabolite reduces the size of the lesion caused by *L. amazonensis* and decreases the number of parasites in the spleen of BALB/c mice.[89]



96



97

Minquartynoic acid **97**, a fatty acid derivative isolated from the cortex of *Minquartia guianensis*, shows a moderated *in vitro* activity against *Leishmania major* attributed to its general cytotoxicity.[90]

A number of toxic proteins such as PAP-S and PAP-R, obtained from the seeds and roots of *Phytolacca americana*, and ricin, produced by *Ricinus communis*, have the capacity to inhibit the function of ribosomes isolated from *L. infantum*. However, these proteins have been shown to be inactive against the intact parasites due to their inability to penetrate the cell membrane.[34]

5 Recent developments

In recent years, several screenings of medicinal plants used for the treatment of leishmaniasis have been carried out in regions such as Spain, Sudan and Guinea-Bissau.[91–93] These studies have confirmed the importance of many plant species as potential sources for the isolation of novel metabolites with leishmanicidal or immunostimulant activities. At the same time, a study carried out with a herbal extract denominated Z-HE, which contains a mixture of Iranian medicinal plants, has demonstrated that this extract, when applied topically, is more efficient in alleviating the symptoms of cutaneous leishmaniasis (74.4%) than meglumine antimoniate (27.1%).[94]

Even though there exists an important number of natural products from plant sources that have demonstrated potential as possible leishmanicidal agents (Table 4), most of them do not meet all the requirements considered to be essential for their potential commercialization: to be administered topically or orally, to be effective at moderate doses, and not to cause severe side-effects.[95] To date only berberine **13**, applied parenterally, is used clinically for the treatment of cutaneous leishmaniasis,[3,28] whereas chimanine D **23** and 2-*n*-propylquinoline **24** have reached the clinical evaluation phase for the treatment of cutaneous leishmaniasis.[96] Both **23** and **24** have also proved to be active against visceral leishmaniasis in mice when administered orally.[43]

One of the main reasons for which a number of plant metabolites with leishmanicidal activity have not made it to clinical evaluation is their high cytotoxicity. This lack of selectivity is evident in products such as the phorbol esters, the quassinoids and the acetogenins. On the other hand, there are metabolites such as gabunine **34** and ursolic acid **61** that lack cytotoxicity, but have only a weak *in vivo* leishmanicidal activity. Perhaps chemical transformation of the latter metabolites could improve their antiprotozoal activity while, at the same time, maintaining their lack of cytotoxicity.

The lack of an appropriate correlation between the results obtained in the *in vitro* bioassays and those corresponding to the *in vivo* evaluations, as well as the need to have simple and rapid evaluation procedures in which all species and strains of *Leishmania* can be included, have motivated the search for new bioassay methodologies for the detection of leishmanicidal activity. One of the tests developed recently known as radiorespirometric microtechnique (RAM) has demonstrated, using promastigotes, a good correlation between its results and the response of patients to the therapy with pentavalent antimony. This technique is based on the capacity of the product being evaluated to inhibit, in the parasite, the catabolism of different ^{14}C-labelled substrates. RAM has been used to evaluate the leishmanicidal activity in medicinal plant extracts from Nigeria; the results obtained show that of the eleven extracts evaluated, five inhibited the catabolism of two or more substrates (*i.e.* amino acids, sugars and fatty acids) in the parasite.[97]

Another technique used recently to detect leishmanicidal activity in plant extracts, is the bioassay based on the inhibition of [^{3}H]timidine uptake by *Leishmania* parasites. This method has been used successfully in the detection of antileishmanial activity of (+)-nyasol **83** and the epoxide of cycloisobrachycoumarinone **85**, both metabolites reported as having selective activity on parasites of *L. major*.[75,76,85]

On the other hand, as a result of the search for new therapeutic agents against diseases caused by protozoan parasites, and taking into account that, in most cases, the mechanism of action of plant metabolites with leishmanicidal activity is not known, an approach based on the detection of the biochemical targets where these are effective has been proposed.[34,98] Presently, the number of investigations directed towards the detection of natural products with the ability to inhibit key enzymes of *Leishmania* parasites is limited, both amarogentine **48** and diospyrin **1** have been reported as inhibitors of topoisomerase I, the enzyme recognized as the ideal target for the development of drugs with trypanocidal activity.[99]

It is important to take into account that activities such as the immunostimulant activity of the iridoids contained in Picroliv or the antiinflammatory activity of ursolic acid **61**, can contribute to the treatment of leishmaniasis by increasing the activity of other antileishmanial drugs.

6 New strategies

Finally, Multidrug Resistance (MDR) phenotype, due to P-glycoprotein (Pgp), has been established as an efficient mechanism to reduce the intercellular drug accumulation in tumour cells and protozoan parasites including *Plasmodium*, *Entamoeba* and *Leishmania*. At present, in the search for new modulators of Pgp, sesquiterpenes isolated from *Crossopetalum*

Table 4 Leishmanicidal activity from groups of natural products

Quinones
Diospyrin **1** [a,b,c]
Hydroxydiospyrin **2** [b]
Plumbagin **3** [a,b,d]
3,3′-Biplumbagin **4** [a]
8,8′-Biplumbagin **5** [a]
Lapachol **6** [b]
4-Hydroxy-1-tetralone **7** [a,d]
Jacaranone **8** [a,d]
Hydropiperone **9** [a]
Anthraquinone-2-carbaldehydes **10**, **11** [a]
Aloe-emodin **12** [a,b]

Alkaloids
Berberine **13** [b,d]
Isoguattouregidine **14** [a]
Anonaine **15** [a]
Liriodenine **16** [a,b]
Daphnandrine **17** [a]
Obaberine **18** [a]
Gyrocarpine **19** [a,d]
Limacine **20** [a]
Isotetrandrine **21** [d]
Chimanine B **22** [a]
Chimanine D **23** [a,d]
2-*n*-Propylquinoline **24** [a,d]
Dictyolomide A **25** [a]
Dictyolomide B **26** [a]
Harmaline **27** [b]
Dihydrocorynantheine **28** [a]
Corynantheine **29** [a]
Corynantheidine **30** [a]
Harmane **31** [a]
Pleiocarpine **32** [a]
Buchtienine **33** [a]
Gabunine **34** [a,b]
Sarachine **35** [a]
Holamine **36** [a]
15-α-Hydroxyholamine **37** [a]
Holacurtine **38** [a]
N-Demethylholacutine **39** [a]
Piperine **40** [a]
Benzoxazol-2(3*H*)-one **41** [a]

Phenolic compounds
Podocarpusflavone A **79** [a]
Podocarpusflavone B **80** [a]
(+)-Medioresinol **81** [b]
(−)-Lirioresinol B **82** [b]
(+)-Nyasol **83** [a]
Epoxide of 2-epicyclobrachycoumarinone **84** [a]
Epoxide of 2-cyclobrachycoumarinone **85** [a]
Curcumin **86** [a]
Demethoxycurcumin **87** [a]
Bis-dimethoxycurcumin **88** [a]

Terpenes
Arbortristoside A **42** [b,d]
Arbortristoside B **43** [b]
Arbortristoside C **44** [b]
6-β-Hydroxyloganin **45** [b]
Picroside I **46** [e]
Kutkoside **47** [e]
Amarogentine **48** [c]
Espintanol **49** [a,d]
Grifolin **50** [a]
Piperogalin **51** [a]
Dehydrozaluzanin C **52** [a,d]
16,17-Dihydrobrachycalyoxide **53** [a]
Kudtriol **54** [a]
12-*O*-Tetradecanoyl phorbol-13-acetate **55** [a,b]
Jatrogrossidione **56** [a]
Jatrophone **57** [a]
Dehydropinifolic acid 15-monomethyl ester **58** [a]
Ribenol **59** [a,b]
6-β-Hydroxyrosenonolactone **60** [a,b]
Ursolic acid **61** [b,d]
Betulinaldehyde **62** [b]
(24*Z*)-3-Oxotirucalla-7,24-dien-26-oic acid **63** [a,b]
epi-Oleanolic acid **64** [a,b]
Simalikalactone D **65** [a]
15-β-Heptylchaparrinone **66** [a]
α-Hederin **67** [a,b]
β-Hederin **68** [a,b]
Hederagenin **69** [a,b]
Hederacolchiside A_1 **70** [a,b]
Mimengoside A **71** [a]
Muzanzagenin **72** [a]

Phenolic compounds
(*E*)-1-[2,4-Dihydroxy-3-(3-methylbut-2-enyl)-phenyl]-3-[4-hydroxy-3-(3-methyl-but-2-enyl)phenyl]prop-2-en-1-one **73** [a]
2′,6′-Dihydroxy-4′-methoxychalcone **74** [a,b]
Licochalcone A **75** [a,b,d]
Sulfuretin **76** [a,b]
5,7,4′-trihydroxyflavan **77** [b]
Amentoflavone **78** [a]

Other metabolites
Senegalene **89** [a]
Squamocine **90** [a]
Asimicine **91** [a]
Molvizarine **92** [a]
Rolliniastatin-1 **93** [a]
Annonacin A **94** [a]
Goniothalamicin **95** [a]
Argentilactone **96** [a,d]
Minquartynoic acid **97** [a]

[a] *In vitro* activity against promastigotes. [b] *In vitro* activity, against amastigotes. [c] Inhibitory activity of the topoisomerase I. [d] *In vivo* activity. [e] Immunostimulant activity.

tonduzzi and *Maytenus macrocarpa* (Celastraceae) and different flavonoids have demonstrated their ability to reverse the multi-drug resistance in an MDR *Leishmania tropica* line.[100,101] On the other hand, the leishmanicidal effect of the leaf extract from *Kalanchoe pinnata* on amastigotes of *L. amazonesis* has been identified as an induction of NO production in macrophages, and not to a direct effect of the extract on the parasite.[102] These two last examples show the potential of new strategies in the search for an effective drug in the treatment of leishmaniasis.

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