4. Scope of natural products in fighting against leishmaniasis


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Abstract. Leishmaniasis, a group of tropical diseases resulting from infection of macrophages by obligate intracellular parasites of genus Leishmania, is a major health problem worldwide. Growing incidence of resistance for the generic pentavalent antimony complex for treatment in endemic and non-endemic regions has seriously hampered their use. The second line drugs such as amphotericin B, paromomycin and miltefosine are the other alternatives, but they merely fulfill the requirements of a safe drug. The recent researches focused on natural products have shown a wise way to get a true and potentially rich source of drug candidates against leishmaniasis. The present review initially highlights the current status of leishmaniasis, synergy of the disease with HIV, therapeutic options available and in later sections summarizes natural products that have shown significant antileishmanial activities. In order to highlight any possible mechanism based action, the review has been organized according to chemical structural classes.

1. Introduction

The Leishmania are Kinetoplastid protozoans that cause four main clinical syndromes: Cutaneous Leishmaniasis; Muco-cutaneous Leishmaniasis (also known as espundia); Visceral Leishmaniasis (VL; also known as kala-azar); and Diffuse Leishmaniasis. Leishmaniasis continues to be one of the six entities

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on the World Health Organization tropical disease list [1]. Leishmania species are transmitted by 30 species of sand fly and essentially requires two different hosts: an invertebrate insect vector, Phlebotomus (in the Old World) or Lutzomyia (in the New World) sandfly-mosquito and a vertebrate host (human, dog or even a wild vertebrate) [2].

Leishmaniasis is prevalent in tropical and temperate regions of the world, ranging from rainforests in Central and South America to deserts in West Asia and the Middle East. Current epidemiological reports estimate about 350 million populations at risk with 12 million people affected worldwide, while 1.5-2 million new cases being recorded each year. The visceral leishmaniasis has an estimated incidence of 500,000 new cases and 60,000 deaths each year with more than 90% of cases are centralized to India, Bangladesh, Nepal, Sudan, and Brazil [3].

There are a growing number of reports of Leishmania/human immunodeficiency virus (HIV) co-infections across the world. Leishmania-HIV co-infection has been globally controlled in Southern Europe since 1997 by highly active anti retroviral therapy (HAART), but it appears to be an increasing problem in other countries such as Ethiopia, Sudan, Brazil or India where both infections are becoming more and more prevalent [4]. The situation is particularly alarming in southern Europe, where 50-75% of adult VL cases are HIV positive and among the 45 million people infected by HIV worldwide, an estimated one-third lives in the zones of endemic Leishmania infections [5]. To date, the greatest prevalence of Leishmania/HIV co-infection has been in the Mediterranean basin. Among more than 2,000 cases notified to the WHO, 90% of them belong to Spain, Italy, France and Portugal [6].

The symptoms of leishmaniasis include fever, weight loss, enlarged spleen, swollen glands, skin sores (changing in size and appearance over time), splenomegaly, lymphadenopathy, hepatomegaly, pancytopenia, progressive anemia, and hypergammaglobulinemia with hypoalbuminemia. Leishmaniasis is always fatal when left untreated and some times patients (50% in Sudan and 1-3% in India) develop post kala-azar dermal leishmaniasis (PKDL) [7].

The present review briefly illustrates the current status of Leishmaniasis, occurrence and treatment around the world, and also critically discusses the key points in natural products based drug discovery protocols. Finally, a comprehensive coverage of natural products with significant activity against Leishmania species has been given in detail. In order to highlight any possible structure-activity relationships, the review has been organized according to chemical structural class.
2. Taxonomy of *Leishmania* spp.

The *Leishmania* are protozoa belonging to the order Kinetoplastida and family Trypanosomatidae. Earlier various classifications have been successively applied to the genus *Leishmania*; however the simplest one can be summarized from Figure 1.

![Classification of Leishmania parasite.](image)

3. Morphology and life cycle

*Leishmania* are the obligate intracellular parasites existing in two morphologic forms: promastigotes and amastigotes. Promastigotes are found in digestive tract of sandfly and are long spindle-shaped with a single delicate flagellum (15-28 μM long) attached to cytoplasmic organelle called, kinetoplast containing intertwined circular DNA (kDNA) molecules known as maxicircles and minicircles, which make up 5-10% of total DNA [8]. A fully developed promastigote measures about 114.3 to 20 μM in length and 1.5 to 1.8 μM at their widest part [9]. The small, round to oval bodies called amastigotes (2 to 3 μM in length) are the non-infective *Leishmania* parasites occurring in monocytes, polymorphonuclear leucocytes or endothelial cells of vertebrates (hosts) while promastigotes represent the infective stage in sandfly (vector).
The *Leishmania* promastigotes are transmitted by sandfly to vertebrate hosts e.g. canines, marsupials, edentates and rodents. Once inside the bloodstream of reservoirs for the disease, promastigotes are phagocytosed by the mononuclear phagocytic cells and are transformed to amastigotes that multiply by means of binary fission. On lyse of host cell, the free parasites spread to new cells and tissues of different organs including the spleen, liver and bone marrow. Amastigotes in the blood as well as in the monocytes are ingested during a blood meal by female sandfly. Once ingested, the amastigotes migrate to the midgut of the sand fly and transform into the promastigotes. After a period of four to five days, promastigotes move forward to the oesophagus reach to salivary glands of the sandfly. Infected sandfly during the second blood meal regurgitates the infectious promastigotes from its pharynx into the bloodstream of the host vertebrates and the life cycle is repeated [10].

4. Chemotherapy of leishmaniasis

The leishmanicidal agents with the most favorable therapeutic index are the antimony compounds known as antimonials. Pentostam® (sodium stibogluconate) and Glucantime® (meglumine antimoniate), able to interfere with the bioenergetics of the *Leishmania* amastigotes [11], are the mainstay therapy for VL. They bind to and inhibit enzymes involved in the glycolysis and oxidation of fatty acids. Since ADP phosphorylates to ATP using NADH generated by glycolysis and citric acid cycle, the intracellular ATP levels
essential for the survival of *Leishmania* are depleted. However, due to high cost (approx 200 USD per patients) of branded sodium stibogluconate, a generic sodium antimony gluconate (SAG, Albert David Ltd, India, 13USD per patients) was used to treat patients satisfactorily without any significant difference in final cure. However, due to serious side effects (pain at the site of injection, stiff joints, gastrointestinal problems, cardiotoxicity, hepatic and renal insufficiency) and declining efficacy, the SAG is no longer used in VL hyper endemic regions of India.

**Sodium stibugluconate**

Pentamidine (1) that hampers replication and transcription at the mitochondrial level in pathogen was the first drug used for the treatment of patient refractory to Sb\(^{\text{v}}\) [12]. Biophysical analysis, foot-printing studies and the crystal structure has proved that the charged amidinium groups of pentamidine establish hydrogen bonding with O\(_2\) of thymine or N\(_3\) of adenine and form complexes with the minor groove of DNA. However, the efficacy of 1 has gradually declined over the years and now it cures only 70% of patients producing serious adverse events like shock, hypoglycemia and death in significant proportion.

**Amphotericin B (2)** is a pollen antibiotic that was recommended as first line drug in India by National Expert Committee for Sb\(^{\text{v}}\) refractory regions of VL. At doses of 0.75-1.0 mg/kg for 15 infusions on alternate days its cures more than 97% of patients. The drug can perturb both parasitic and mammalian cells, but the selective lethality of 2 for parasitic cells is the result of its great affinity towards 24-substituted sterols, called ergosterol, the major cell membrane sterols [13].
Miltefosine (3) originally developed as anti tumor agent, was approved in India at 50–100 mg (~2.5 mg/kg) doses for four weeks against VL patients including children. The drug 3 blocks Leishmania proliferation, alters phospholipid and sterol composition and activates cellular immunity. However, due to high cost and serious side effects, medical advisors generally avoid 3 in their prescriptions [14].

Paromomycin (4), an amino glycoside antibiotic originally identified as an antileishmanial drug in the 1960s, acts synergistically with antimonials in vitro, and was demonstrated significant (93% cure rate) at a dose of 16 mg/kg when given intramuscularly for 21 days to VL patients in India. Like other amino glycosides, the drug 4 acts by impairing the macromolecular synthesis and alters the membrane properties of Leishmania [15].
Sitamaquine (5), an orally active analog of 8-aminoquinoline, is in clinical development by the Walter Reed Army Institute in collaboration with GlaxoSmithKline (formerly SmithKline Beecham) to use for the treatment of VL. In a randomized, open label and multicenter Phase II trial in India and Kenya, the drug 5 was found efficacious and well tolerated at various dose levels [16]. As on March 2002, the drug 5 is currently in Phase III trials for the treatment of VL.

5. Natural products as folk medicines for treatment of leishmaniasis

Utility of natural products in drug discovery and development is not surprising as many of medicinal plants i.e. Cinchona calisaya (bark), Strychnos pseudoquina (bark), Deianira erubescens (roots and leaves) and Remijia ferruginea (bark) were historically used against different parasitic diseases. Ancient records as well as recent literature reports have established the effectiveness of natural products as potentially rich sources of new and selective agents for the treatment of important tropical diseases caused by protozoans and other parasites. In 1970s, artemisinin, an important antimalarial drug was identified from traditional Chinese medicine Artemisia annua and since then many artemisinin derivatives were prepared and evaluated in various pre-clinical and clinical trials to use for the treatment of malaria. Likewise, paromomycin 4 (Humatin™, King Pharmaceuticals), obtained from Streptomyces krestomuceticus, is an orphan drug that was approved by Drug-Controller General of India in September 2006 against VL. Paromomycin 4 was originally developed by the Institute for OneWorld Health and is an off-patent antibiotic marketed in the US to treat intestinal parasites also.

Natural products literature provides a growing research on plant derived antileishmanial agents and several natural products so far have been discovered with excellent activity against leishmania parasites, however, none of them have been clinically evaluated in studies or projected to reach the clinical applications in near future. This review is focused to cover the entire formal and constant research on leishmanicidal natural products from the mid-1980 to June 2010 with special attention on structure-activity relationship (SAR) based activity and mechanism of action.

6. Alkaloids

The alkaloids constitute an important class of natural products exhibiting significant anti-leishmanial activities. The quinoline alkaloids,
2-n-propylquinoline (6), chimanine-D (7) and chimanine-B (8), isolated from *Galipea longiflora* (Rutaceae), exhibit antileishmanial activity against *L. braziliensis* promastigotes with an IC$_{90}$ values of 50, 25 and 25 $\mu$g/mL, respectively. Oral *in vivo* studies using 6 in BALB/c mice demonstrates 99.9% suppression of liver parasites while subcutaneous treatment with 7 causes 86.6% parasite suppression when given for 10 days at 0.54 mmol/kg [17]. However, oral treatment with 7 for 5 days results in 72.9% parasite suppression only. Likewise, dictylomide-A (9) and B (10), isolated from the bark of *Dictyoloma peruviana* (Rutaceae), causes total lyses of *L. amazonensis* promastigotes at 100 $\mu$g/mL concentration [18].

6.1. Indole alkaloids

Dihydrocorynantheine (11), corynantheine (12) and corynantheidine (13), isolated from the bark of *Corynanthe pachyceras* (Rubiaceae), are the respiratory chain inhibitors exhibiting IC$_{50}$ of 3 $\mu$M against *L. major*. Pleiocarpine (14) isolated from stem bark of *Kopsia griffithii* (Apocynaceae), shows *in vitro* antileishmanial activity with an IC$_{50}$ < 25 $\mu$g/mL against *L. donovani* promastigotes. Gabunine (15), a bis-indole alkaloid obtained from stem bark of *Peschiera van heurkii* (Apocynaceae), exhibits *in vitro* activity with an IC$_{50}$ 25 $\mu$g/mL against *L. amazonensis* amastigotes [19].

6.2. Isoquinoline alkaloids

*O*-methylmoschatoline (16) and liriodenine (17), isolated from *Annona foetida* (Annonaceae), display *in vitro* activity against promastigote forms of
*L. braziliensis* with an IC<sub>50</sub> < 60 μM [20]. The SAR study among these oxoaporphine alkaloids reveals that 17 bearing methylenedioxy moiety is eight times more active against *L. braziliensis* and *L. guyanensis* than the 16. Berberine (18), occurring in many plant species of Annonaceae, Menispermaceae and Berberifaceae, exhibits *in vivo* leishmanicidal activity with an IC<sub>50</sub> value of 10 μg/mL against *L. major*. Isoguattouregidine (19) isolated from *Guatteria foliosa* (Annonaceae), shows activity at 100 μg/mL
concentrations against *L. donovani* and *L. amazonensis*. Anonaine (20) isolated from *Annona spinescens* (Annonaceae), exhibits activity against promastigotes of *L. braziliensis* and *L. donovani* [21].

The alkaloids, (+)-neolitsine (21) and cryptodorine (22), isolated from *Guatteria dumetorum* (Annonaceae), display significant activity against promastigotes of *L. maxicana* at 15 and 3 μM concentrations, respectively. Xylopine (23), an aporphine alkaloid isolated from *Guatteria amplifolia* (Annonaceae) shows activity against promastigotes of *L. mexicana* (IC<sub>50</sub> value 3 μM) and *L. panamensis* (IC<sub>50</sub> value 6 μM) [22]. Unonopsine (24), a dimeric aporphine alkaloid isolated from the *Unonopsis buchtienii* (Annonaceae), displays antileishmanial activity (IC<sub>100</sub> value 25 μg/mL) against *L. donovani* promastigotes [23].
6.3. Naphthylisoquinoline alkaloids

Among the naphthylisoquinoline alkaloids, ancistroealaine-A (25) isolated from *Ancistrocladus ealaensis* (Ancistrocladaceae), exhibits activity against *L. donovani* promastigotes with an IC$_{50}$ value 4.10 μg/mL. Ancistrocladinium A (26) and B (27) isolated from yet un-described Congolese Ancistrocladaceae species, require 2.61 and 1.52 μg/mL concentrations, respectively to reach the IC$_{50}$ towards *L. major* promastigotes. An apoptosis-like death pathway is the possible mode of action for compounds 26 & 27. Ancistrocladidine (28), isolated from *Ancistrocladus tanzaniensis* (Ancistrocladaceae) shows relatively weak activity by a factor of 2 against *L. donovani* when compared to ancistrotanzanine-B (29) (IC$_{50} = 1.6$ μg/mL), while by a factor of 10 in comparison to miltefosin (positive control). Likewise, ancistrotanzanine-A (30), exhibits activity against promastigotes of *L. donovani*. SAR based studies among the alkaloids suggest that the compound bearing C,C-biaryl axis connecting the naphthyl and isoquinoline moiety shows weak or no leishmanicidal activity [24].

6.4. Bisbenzylisoquinolinic alkaloids

Daphanandrine (31) isolated from *Albertisia papuana* (Menispermaceae), obaberine (32) obtained from *Pseudoxandra sclerocarpa* (Annonaceae), gyrocarpine (33) produced by *Gyrocarpus americanus* (Hernandiaceae) and limacine (34) isolated from *Caryomene olivasans* (Menispermaceae), display
activity against *L. donovani*, *L. braziliensis* and *L. amazonensis* with an IC\textsubscript{100} of \(~50\ \mu g/mL). SAR studies among these alkaloids demonstrate that alkaloids with methylated nitrogen are more active than those with non-substituted or aromatic nitrogens while quaternization of one or more nitrogen atoms results in the loss of antileishmanial activity [25].

![Chemical Structures](image)

**6.5. Steroidal alkaloids**

Among the alkaloids, holamine (35), 15-\(\alpha\)-hydroxyholamine (36), holacurtine (37) and *N*-desmethylholacurtine (38), obtained from *Holarrhena curtisii* (Apocynaceae), the metabolite 35 exhibits strongest activity against *L. donovani* (1.56>IC\textsubscript{50}>0.39 \(\mu g/mL)) in compared to 36, 37 and 38 (6.25>IC\textsubscript{50}>1.56 \(\mu g/mL)) [26].

![Chemical Structures](image)


6.6. Benzoquinolizidine alkaloids

Klugine (39), cephaeline (40), isocephaeline (41) and emetine (42), demonstrating significant leishmanicidal activity against *L. donovani* have been isolated from *Psychotria klugii* (Rubiaceae). Among these metabolites, the compound 39 (IC\(_{50}\) of 0.40 μg/mL) and 41 (IC\(_{50}\) 0.45 μg/mL) exhibit <13- and <15-fold less potent activity in compared to 40, while compound 40 with IC\(_{50}\) of 0.03 μg/mL demonstrates >20- and >5-fold more *in vitro* activity against *L. donovani* when compared to pentamidine and amphotericin-B, respectively. The alkaloid 42, exhibits activity against *L. donovani* with an IC\(_{50}\) value 0.03 μg/mL, however produces toxicity in treatment of cutaneous leishmaniasis caused by *L. major* [27].

\[
\begin{align*}
39 & \quad R_1 = \text{OH}; \quad R_2 = \text{OH} \\
40 & \quad R_1 = \text{OCH}_3; \quad R_2 = \text{H} \\
41 & \quad R = \text{OCH}_3 \\
42 & \quad R = \text{OH}
\end{align*}
\]

6.7. Diterpene alkaloids

The alkaloids, 15,22-\(O\)-Diacetyl-19-oxo-dihydroatisine (43), azitine (44) and isoazitine (45), isolated from *Aconitum*, *Delphinium* and *Consolida* species, show significant leishmanicidal activities. The metabolite 45 exhibits strongest activity against promastigotes of *L. infantum* with IC\(_{50}\) values 44.6, 32.3 and 24.6 μM at 24, 48 and 72 h of culture, respectively. The compound 44 and 43 with IC\(_{50}\) values of 33.7 and 27.9 μM at 72 h of culture, respectively, exhibit activity against promastigotes of *L. infantum* [28].

\[
\begin{align*}
43 & \quad \text{OAc} \\
44 & \quad \text{OAc} \\
45 & \quad \text{OAc}
\end{align*}
\]
6.8. Pyrrolidinium alkaloids

The pyrrolidinium alkaloid (2S,4R)-2-carboxy-4-(E)-p-coumaroyloxy-1,1-dimethylpyrrolidinium inner salt (46), isolated from Phlomis brunneogaleata (Lamiaceae), display activity with an IC\textsubscript{50} of 9.1 μg/mL against axenic amastigotes of L. donovani [29].

6.9. Acridone alkaloids

The rhodesiacridone (47) and gravacridonediol (48) isolated from Thamnosma rhodesica (Rutaceae), exhibit 69% and 46% inhibition at 10 μM concentration, respectively against promastigotes of L. major. The compounds also display activity against L. major amastigotes and cause over 90% and 50% inhibition at 10 and 1 μM concentration, respectively [26].

6.10. β-Carboline alkaloids

The harmaline (49), isolated from Peganum harmala (Nitrariaceae), exhibits amastigote-specific activity (IC\textsubscript{50} of 1.16 μM). Harmine (50) isolated from same plant species reduces spleen parasite load by approximately 40, 60, 70 and 80% in free, liposomal, niosomal and nanoparticulate forms, respectively in mice model. Canthin-6-one (51) and 5-methoxycanthin-6-one (52) occurring in plant species of Rutaceae and Simaroubaceae, demonstrate \textit{in vivo} activity against L. amazonensis in BALB/c mice model. \textit{N}-hydroxyannomontine (53) and annomontine (54) isolated from Annona foetida (Annonaceae), show efficient leishmanicidal potentials. The SAR studies suggest that the metabolite 54 (IC\textsubscript{50} = 34.8 μM) displays 6 times more activity compared to 53 against L. braziliensis promastigotes. The compound 53 also exhibits activity against promastigotes of L. guyanensis while 54 remain inactive [25].
6.11. Alkaloids from marine sources

Many marine sponges e.g. *Amphimedon viridis*, *Acanthostrongylophora* species, *Neopetrosia* species, *Plakortis angulospiculatus* and *Pachymatistema johnstonii* serve as rich sources of alkaloids with significant antileishmanial potentials. Renieramycin A (55) isolated from *Neopetrosia* species, is a La/egfp (expressing enhanced green fluorescent protein) inhibitor that shows efficient antileishmanial activity against *L. amazonensis* with IC$_{50}$ 0.2 μg/mL. Araguspongin C (56), isolated from a marine sponge *Haliclona exigua*, displays leishmanicidal activity against promastigotes as well as amastigotes at 100 μg/mL concentrations [30].

Among the ciliatamides A-C (57-59) isolated from *Aaptos ciliate*, the peptide 57 and 58 at 10.0 μg/mL concentrations inhibit 50% growth *L. major* promastigotes [31]. The lipopeptides, almiramides A-C (60-62) isolated from cyanobacterium *Lyngbya majuscula*, exhibit significant *in vitro* antileishmanial activity against *L. donovani*. The SAR studies among these peptides suggest that 61 and 62 exhibit strong activity against *L. donovani*. 
with EC\textsubscript{50} values of 2.4 and 1.9 \(\mu\)M, respectively. The metabolites 61 and 62 also display weak cytotoxicity to mammalian Vero cells at 52.3 and 33.1 \(\mu\)M concentrations, respectively [32]. Dragonamide A (63), E (64) and herbamide B (65), isolated from same cyanobacterium strain, exhibit \textit{in vitro} activity against \textit{L. donovani} with EC\textsubscript{50} values of 6.5, 5.1 and 5.9 \(\mu\)M, respectively [33].

Viridamide A (66) isolated from \textit{Oscillatoria nigro-viridis}, shows activity against \textit{L. mexicana} with EC\textsubscript{50} of 1.5 \(\mu\)M [34]. Venturamides A (67) and B (68) obtained from cyanobacterium \textit{Oscillatoria} species, exhibit activity against \textit{L. donovani} with EC\textsubscript{50} >19.0 \(\mu\)M. Valinomycin (69), a dodecadepsipeptide isolated from \textit{Streptomyces} strains, exhibits activity against promastigotes of \textit{L. major} with EC\textsubscript{50} < 0.11 \(\mu\)M, but at the same time shows cytotoxicity to 293T kidney epithelial cells and J774.1 macrophages [35].

7. Quinones

Primin (2-methoxy-6-pentylcyclohexa-2,5-diene-1,4-dione), occurring in \textit{Primula obconica} and other species (Primulaceae), shows significant leishmanicidal activity against \textit{L. donovani} with an IC\textsubscript{50} of 0.711 \(\mu\)M. Diospyrin (70), a bis-naphthoquinone inhibiting topoisomerase I, isolated from the bark of \textit{Diospyros Montana} (Ebenaceae), demonstrates antileishmanial activity against \textit{L. donovani} promastigotes with an MIC of 1.0 \(\mu\)g/mL [36]. The hydroxylated derivative of 70 at 3 \(\mu\)M concentration eliminates 73.8\% of amastigotes in infected macrophages [37]. Plumbagin (72), originally isolated from \textit{Plumbago zylenica}, shows leishmanicidal activity against amastigotes of \textit{L. donovani} (IC\textsubscript{50} = 0.42 \(\mu\)g/mL) and \textit{L. amazonensis} (IC\textsubscript{50} = 1.1 \(\mu\)g/mL). At a concentration of 10 \(\mu\)g/mL, the
compound 72 presents an amastigote survival index (SI) of 16.5% against L. amazonensis with the absence of toxic effects against the macrophages. The metabolite 72 also shows in vivo activity against L. amazonensis and L. venezuelensis at concentrations 2.5 and 5 mg/kg/day, respectively. The mechanism of the action of compounds 72 and 71 involves generation of oxygen free radicals from which the parasites remain unable to defend.

The dimeric products 3,3-biplumbagin (73) and 8,8′-biplumbagin (74), isolated from the bark of Pera benensis (Euphorbiaceae), display significant
antileishmanial activity. Among these, the metabolite 73 shows lower activity (IC$_{90}$ = 50 μg/mL) compared to 72 and 75 (IC$_{90}$ = 50 μg/mL) against L. braziliensis, L. amazonensis, and L. donovani promastigotes [38,39]. Lapachol (75), a prenylated hydroxynaphthoquinone isolated from Tecoma species (Bignoniaceae), displays activity with mechanism of action similar to 71 and 72 against L. donovani amastigotes in peritoneal mice macrophages. The metabolite 3,4-dihydronaphthalen-1(2H)-one (76), isolated from the bark of Ampelocera edentula (Ulmaceae), exhibits leishmanicidal activity (IC$_{90}$ of 10 μg/mL) against L. braziliensis, L. amazonensis and L. donovani promastigotes. The metabolite 76 demonstrates strong in vivo activity on subcutaneous treatment in BALB/c mice infected with L. amazonensis or L. venezuelensis when compared to Glucantime® (25 mg/kg/day vs 56 mg Sb$/^\text{V}$/kg/day). However, the use of tetralones is limited due to cytotoxic, carcinogenic and mutagenic properties in animals [40].

Jacaranone (77), a quinone isolated from the leaves of Jacaranda copaia (Bignoniaceae), exhibits a strong activity with an ED$_{50}$ of 0.02 mM against L. amazonensis promastigotes but at the same concentration shows toxicity to peritoneal mice macrophages. The prenylated dihydroquinone hydropiperone (78), isolated from Peperomia galioides (Piperaceae), shows activity at a concentration of 25 μg/mL against promastigote forms of L. braziliensis, L. donovani and L. amazonensis. At 100 μg/mL concentration the metabolite 78 causes total lysis of the parasites [41].
The anthraquinone-2-carbaldehydes, 79 and 80, isolated from the roots of *Morinda lucida* (Rubiaceae), shows leishmanicidal potential selective to *L. major* promastigotes. SAR studies suggest that presence of an aldehyde group at C-2 and a phenolic hydroxy group at C-3 in both structures, are essential for their antiprotozoal activity [42].

![Chemical structures](image)

The aloe-emodin (81) isolated from *Stephania dinklagei* (Menispermacae), shows leishmanicidal activity at IC$_{50}$ values of 185.1 and 90 $\mu$M against *L. donovani* promastigotes and amastigotes, respectively [43]. Vismione D isolated from *Vismia orientalis* (Clusiaceae) exhibits activity against axenic amastigotes of *L. donovani* with an IC$_{50}$ value of 0.37 $\mu$g/mL but shows cytotoxicity when tested on human L6 cells (IC$_{50}$ of 4.1 $\mu$g/mL) [29].

8. Terpenes

8.1. Iridoids

Iridoids, a class of monoterpenoid glycosides often serve as intermediates in the biosynthesis of indole alkaloids are well known for significant leishmanicidal activity. The arbortristosides-A (82), B (83), C (84) and 6-$\beta$-hydroxyloganin (85), isolated from *Nyctanthes arbortristis* (Oleaceae) exhibit *in vitro* activity against *L. donovani* amastigotes. The *in vivo* studies using intraperitoneal and oral treatment (10 and 100 mg/kg concentrations for 5 days) of hamsters infected with *L. donovani*, the metabolite 82 displays significant leishmanicidal activities [44]. Picroside I (86) and kutkoside (87),
obtained from *Picrorhiza kurroa*, exhibits a high degree of protection against the infection of promastigotes of *L. donovani* in hamsters [45]. Picroliv, a standardized fraction of iridoid glycosides 86 and 87, increases the nonspecific immune response and induces a high degree of protection against the infection of promastigotes of *L. donovani* in hamsters. Picroliv is an adjuvant proposed to increase the efficacy of leishmanicidal drugs and has demonstrated excellent therapeutic index in Phase I and II clinical trials [46].

Amarogentin (88), a secoiridoid glycoside isolated from *Swertia chirata* (Gentiaceae), produces leishmaincidal effect at a concentration > 60 μM against *L. donovani* through inhibition of catalytic activity of topoisomerase I [47]. The metabolite 88 exerts inhibitory effect with a mechanism of action similar to Pentostam® i.e. by binding to the enzyme and preventing the formation of a binary complex with DNA. The evaluation of 88 in the form of liposomes and niosomes shows an enhanced leishmanicidal activity (without toxic effects) than those observed for free 88 when tested in hamsters [48].
8.2. Monoterpenes

Espintanol (89), isolated from the bark of Oxandra espintana (Annonaceae), shows antileishmanial activity against promastigotes of twelve Leishmania species. However, the metabolite 89 exhibits only a weak activity in vivo in mice infected with L. amazonensis. Grifolin (90) and piperogalin (91) obtained from Peperomia galoides, causes total lysis of L. braziliensis, L. donovani and L. amazonensis promastigotes at 100 μg/mL concentrations. At 10 μg/mL concentration, metabolite 91 causes more than 90% lysis of the promastigotes [49].

8.3. Sesquiterpenes

A sesquiterpene lactone, dehydrozaluzanin C (92), isolated from the leaves of Munnozia maronii (Asteraceae), shows activity at concentrations between 2.5-10 μg/mL against promastigotes of eleven Leishmania species. The in vivo test using the metabolite 92 in BALB/c mice results in reduction of the lesions caused by L. amazonensis [50].

Sesquiterpene dilactone, 16,17-dihydrobrachycalyoxide (93), isolated from Vernonia brachycalyx (Asteraceae), exhibits activity (IC$_{50}$ = 17 μg/mL) against L. major promastigote but also inhibits the proliferation of human lymphocytes [51]. Kudtriol (94), a sesquiterpene alcohol isolated from the aerial parts of Jasonia glutinosa (Asteraceae), shows toxic activity against promastigotes of L. donovani at 250 μg/mL concentration. SAR study with metabolite 94 indicates that the presence of a C-5 hydroxy group in the α-orientation is essential for the expression of the leishmanicidal activity [52]. The (+)-curcuphenol (95), isolated from sponge Myrmekioderma styx, exhibits in vitro anti-leishmanial activities against L. donovani with an EC$_{50}$ of 11.0 μM [53].
8.4. Diterpenes

A phorbol diester, 12-\(O\)-tetradecanoyl phorbol-13-acetate (TPA) \(96\), also known as phorbol 12-myristate 13-acetate (PMA), was originally identified from the croton plant, which at a concentration of 20 ng/mL displays ability to cause a variety of structural changes in the parasites of \(L.\ amazone{n}sis\) by activation of protein kinase C, an important enzyme in the development of several cellular functions \([54]\). Among the other diterpenoids isolated from Euphorbiaceae species with leishmanicidal potentials are \(jatrogrossidione (97)\) and \(jatrophone (98)\). These metabolites possess toxic activity against the promastigote forms of \(L.\ braziliensis\), \(L.\ amazone{n}sis\) and \(L.\ chagasi\). SAR studies with these metabolites revealed that \(97\) with IC\(_{100}\) value of 0.75 \(\mu\)g/mL displays activity higher than \(98\) (IC\(_{100}\) = 5 \(\mu\)g/mL), but remains inactive \textit{in vivo} \([55]\).

The 15-monomethyl ester of dehydropinifolic acid \((99)\), obtained from the stem bark of \textit{Polyalthia macropoda} (Annonaceae), and ribenol \((100)\), an \textit{ent}-manoyl oxide derivative isolated from \textit{Sideritis varoi} (Lamiaceae), show \textit{in vitro} activity against promastigotes of \(L.\ donovani\) \([56]\). Also the different derivatives of this metabolite, obtained through chemical or biological transformations, exhibit strong leishmanicidal activity. Additionally, 6-\(\beta\)-hydroxyrosenonolactone \((101)\), a diterpene isolated from the bark of \textit{Holarrhena floribunda} (Apocynaceae), has a moderate and weak activity against promastigotes and amastigotes of \(L.\ donovani\), respectively \([57]\).
8.5. Triterpenes

The ursolic acid (102) and betulinaldehyde (103), obtained from the bark of *Jacaranda copaia* and the stem of *Doliocarpus dentatus* (Dilleniaceae), respectively show activity against the amastigotes of *L. amazonensis*. However, the metabolite 103 exhibits toxicity to peritoneal macrophages in mice while 102 displays limited activity *in vivo*.

The triterpenes, (24Z)-3-oxotirucalla-7,24-dien-26-oic acid (104) and *epi*-oleanolic acid (105), isolated from the leaves of *Celaenodendron mexicanum* (Euphorbiaceae), display leishmanicidal activity against *L. donovani* with IC_{50} values of 13.7 and 18.8 μM, respectively. The quassinoids, simalikalactone D (106) and 15-β-heptylchaparrinone (107), obtained from species of Simaroubaceae family show activity against promastigotes of *L. donovani* but at the same time exhibit toxicity to macrophages [58]. Triterpene glycosides obtained from marine sources e.g. holothurins A (108), isolated from the sea cucumber *Actinopyga lecanora*, causes 73.2 ± 6.8% and 65.8 ± 6% inhibition of *L. donovani* promastigotes and amastigotes, respectively at 100 μg/mL concentration. The other isomer B (109) obtained from same source shows 82.5 ± 11.6% and 47.3 ± 6.5% inhibition against promastigotes of *L. donovani* at 100 and 50 μg/mL concentrations, respectively [59].
9. Saponins

The $\alpha$-hederin (110), $\beta$-hederin (111) and hederagenin (112), obtained from the leaves of *Hedera helix* (Araliaceae), show lishmanicidal activity against *L. infantum* and *L. tropica*. Among these, the metabolite 112 also shows significant activity against the amastigote forms while both 110 and 111 exhibit strong anti-proliferative activity on human monocytes [60]. The saponins 110-112 appear to inhibit the growth of *Leishmania* promastigotes by acting on the membrane of the parasite with induction of a drop in membrane potential [61]. The hederecolchiside-A1 (113), isolated from *Hedera colchica*, shows strong activity against the promastigotes and amastigotes of *L. infantum*, but also displays a notable activity on human monocytes.

The saponin, mimengoside-A (114), isolated from the leaves of *Buddleja madagascariensis* (Loganiaceae) [62], exhibits activity against promastigotes of *L. infantum*. Muzanzagenin (115), obtained from the roots of *Asparagus africanus* (Liliaceae), displays activity with an IC$_{50}$ value 31 $\mu$g/mL against the *L. major* promastigotes. However, the metabolite 115 also inhibits the proliferation of human lymphocytes [63].

10. Phenolic derivatives

10.1. Chalcones

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 (116) shows toxicity to promastigotes of *L. donovani*, while 2',6'-dihydroxy-4'-methoxylchalcone (117), isolated from inflorescences of *Piper aduncum* (Piperaceae), exhibits significant *in vitro* activity against promastigotes and amastigotes of *L. amazonensis* by affecting the ultrastructure of the parasite mitochondria without causing damage or inducing NO production in the macrophages [64,65].

The metabolite 117 with an IC$_{50}$ value of 0.5 $\mu$g/mL shows strong antileishmanial activity against the promastigotes of *L. amazonensis*, while exhibit lower activity (IC$_{50}$ = 24 $\mu$g/mL) against amastigote forms. Encapsulated formulation of 117 when administered at 1.0 $\mu$g/mL causes the reduction in the level of *L. amazonensis* infected macrophages by 53% [66]. Ultrastructural studies suggest that 117 produces selective toxicity to the intracellular amastigotes without affecting macrophage organelles even when exposed to 80 $\mu$g/mL concentration. The licochalcone-A (118), isolated from roots of the Chinese licorice plant *Glycyrrhiza* species (Fabaceae), shows *in vitro*
110 \( R_1 = \text{Ara 2-1 Rha, } R_2 = \text{OH} \)
111 \( R_1 = \text{Ara 2-1 Rha, } R_2 = \text{H} \)
112 \( R_1 = \text{H, } R_2 = \text{OH} \)
113 \( R_1 = \text{Ara [Glc 4-1] 2 Rha, } R_2 = \text{H} \)

\( \text{Ara: } \alpha \text{-L-arabinopyranose} \)
\( \text{Glc: } \beta \text{-D-glucopyranose} \)
\( \text{Rha: } \alpha \text{-L-rhamnopyranose} \)
\( \text{Fuc: } \beta \text{-D-fucopyranose} \)

114 3-\( \alpha \text{-L-rhamnopyranosyl-(1-4)-} \beta \text{-D-glucopyranosyl-} \)
\( (1-3)-[\beta \text{-D-glucopyranosyl-(1-2)}]-\beta \text{-D-fucopyranoside} \)
\text{of 16-dehydroxysaikogenin G}
activity against *L. major* and *L. donovani* promastigotes. The intraperitoneal administration of 118 prevents the development of lesions in BALB/c mice infected with *L. major* [67,68]. The intraperitoneal and oral administration of 118 significantly reduces the parasite load in the spleen and liver of hamsters infected with *L. donovani*. The compound 118 appears to affect the parasite respiratory chain without damaging the organelles of macrophages or phagocytic function by altering the ultrastructure and function of mitochondria only. However, at lower concentrations 118 inhibits the proliferation of human lymphocytes. Substituents that hinder free rotation in chalcones have been demonstrated to be inactive. The introduction of polar chemical moieties (like hydroxyl and glycosyl groups) led to a reduction of the antileishmanial activity. The modification at the α,β-double bond in chalcones results in marginal reduction of the leishmanicidal activity compared to parent compounds, thus this part is just a chemical spacer.
necessary only. The sulfuretin (2-[(3,4-dihydroxyphenyl)methylene]-6-
hydroxybenzofuran-3(2H)-one) (119), is an aurone, a group of metabolites
related biosynthetically to the chalcones, exhibit activity with EC_{50} values of
0.09-0.11 μg/mL against promastigotes of Leishmania species. The metabolite
119 with an EC_{50} value of 1.24 μg/mL displays activity against L. donovani
amastigotes, but remains non-toxic to bone marrow-derived macrophages [69].

10.2. Flavonoids

The compound 5,7,4′-trihydroxyflavan (120) shows activity against the
amastigotes of L. amazonensis [70], while the biflavonoids amentoflavone
(121), podocarpusflavone A (122) and B (123), isolated from the leaves of
Celanodendron mexicanum, exhibit weak activity against L. donovani
promastigotes. The flavones fisetin (124) (isolated from Acacia greggii and
A. berlandieri), 3-hydroxyflavone (125), luteolin (126) (isolated from Salvia
tomentosa), and quercetin (127) (isolated from plants of family Alliaceae)
exhibit potent antileishmanial activity against the intracellular forms of the
L. donovani with IC_{50} values 0.6, 0.7, 0.8 and 1.0 μg/mL, respectively.
Biochanin A (128), an O-methylated isoflavone occurring in legumes, shows
activity against L. donovani with an IC_{50} value of 2.5 μg/mL [3].

10.3. Lignans

The lignans (+)-medioresinol (129), (-)-lirioresinol B (130) and (+)-
nyasol (131), show activity against the amastigotes of L. amazonensis,
whereas 131 also exhibits high selectivity in its activity against the
promastigotes of L. major. Dyphillin, isolated from Haplophyllum
bucharicum (Rutaceae), modulates phagocytosis of macrophages and
selectively inhibits the amastigotes of L. infantum with an IC_{50} value
0.2 μg/mL [71].
10.4. Coumarins

The coumarin isomers 2-epicycloisobrachycoumarinone (132) and cycloisobrachycoumarinone (133), isolated from Vernonia brachycalyx (Asteraceae), display selective activity against promastigotes of L. major.

10.5. Curcumins

The curcumins, curcumin (134), desmethoxycurcumin (135) and bis-desmethoxycurcumin (136), isolated from the rhizomes of Curcuma longa, show significant anti-leishmanial activity against promastigotes of L. major. However, these metabolites also inhibit the proliferation of human lymphocytes [72].

11. Other metabolites

Acetogenins like senegalene (137), squamocine (138), asimicine (139) and molvizarine (140), 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. However, these metabolites also show cytotoxicity greater than that of
vinblastine against KB and VERO cell lines [73]. Other acetogenins such as rolliniastatin-1 (141), isolated from Rollinia emarginata (Annonaceae), annonacin A (142) and goniothalamicin (143), obtained from Annona glauca (Annonaceae), display promising activity against the promastigote of L. braziliensis, L. donovani, L. amazonensis, however a clear SAR has not been established [74].

Future perspectives

Despite the advances in the parasitological and biochemical researches using various species of Leishmania, the treatment options available against leishmaniasis are far from satisfactory. In current situation, development of new drugs to combat leishmaniasis require increased input from the disciplines of chemistry, pharmacology, toxicology and pharmaceutics to complement the advances in molecular biology that have been made in past 21 years.

Natural products are potential sources of new and selective agents for the treatment of important tropical diseases caused by protozoans and other parasites. The tremendous chemical diversity present in natural products and the promising leads that have already been demonstrated significant against parasitic diseases are needed to be addressed also against leishmania
parasites. The development of antileishmanial natural products or their analogs in accordance to the considerations outlined above would have a dramatic positive impact on the treatment of leishmaniasis. A safe, non-toxic and cost-effective drug is urgently required to eliminate this problem from every corner of world. A safer, shorter & cheaper treatment, identification of the most cost effective surveillance system and control strategies, suitable vector control approach are among some important aspect for the control and complete eradication of this deadly disease.

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References