The aim of this article is to review the current aspects of the pharmacology of leishmaniasis, giving an overview from products have shown a wise way to get a true and potentially rich source of drug candidates against leishmaniasis. The recent researches focused on some synthetic agents like chalcones and natural such as amphotericin B, paromomycin and miltefosine are the other alternatives, but they merely fulfill the requirements of a safe drug. The recent scenario of antileishmanial drugs constitute the results of effort by academics, researchers and sponsorships in order to obtain drugs available, efficient and less toxic to people infected by leishmania parasites.

Keywords: Leishmaniasis, amphotericin B, antiretroviral

RÉSUMÉ [FRANÇAIS/FRENCH]
La leishmaniose, un groupe de maladies tropicales résultant d'une infection des macrophages par des parasites intracellulaires obligatoires du genre Leishmania, est un problème majeur de santé dans le monde entier. L'Organisation mondiale de la Santé a classé la leishmaniose comme une maladie tropicale majeure. L'incidence croissante de la résistance pour le complexe antimoine pentavalent générique pour le traitement dans les régions endémiques et non endémiques a sérieusement entravé leur utilisation. Les médicaments de deuxième intention tels que l'amphotéricine B, la paromomycine et la miltéfosine sont les autres alternatives, mais elles ne font que répondre aux exigences d'un médicament sûr. Les recherches récentes se sont concentrées sur certains agents synthétiques comme chalcones et les produits naturels ont montré une façon sage d'obtenir une véritable source et potentiellement riche de candidats-médicaments contre la leishmaniose. Le but de cet article est d'examiner les aspects actuels de la pharmacologie de la leishmaniose, donnant un aperçu des agents actuels sur le plan clinique utilisés pour de nouveaux composés en cours de développement. Le scénario actuel de médicaments antileishmanienne constituent les résultats de l'effort par des universitaires, des chercheurs et des commandites afin d'obtenir des médicaments disponibles, efficaces et moins toxiques pour les personnes infectées par le parasite Leishmania.

Mots-clés: La leishmaniose, amphoteracin B, antirétroviral

INTRODUCTION
Leishmaniasis is an infection caused by a parasite that is spread to people through the bite of the female phlebotomine sand fly. The parasite exists in many tropical and temperate countries. It has been estimated that there are 2 million new cases of leishmaniasis every year in the world, of which 1.5 million are categorized as cutaneous leishmaniasis, fig,1 and 0.5 million are visceral leishmaniasis. Epidemics occur when people are displaced into affected regions through war or migration or when people in affected regions experience high rates of disease or malnutrition. The leishmaniasis is a complex of diseases caused by at least 17 species of protozoan parasite Leishmania [1]. The disease affects around 12 million people worldwide, with an annual incidence of approximately two million new cases and 350 million are living at risk to be infected. Reported from 88 subtropical and tropical countries has been recorded from Indian subcontinent, Southern Europe and Western Asia to America, including rural and periurban areas [2]. Multiple factors such as the human immune deficient virus (HIV) epidemic, increase of international travel, a lack of effective vaccines, difficulties in controlling vectors, international conflicts and the
development of resistance to chemotherapy could increase the cases of leishmaniasis [3]. The Leishmania are Kinetoplastid protozoans that cause four main clinical syndromes: Cutaneous Leishmaniasis; Mucocutaneous Leishmaniasis (also known as espundia); Visceral Leishmaniasis (VL; also known as kala-azar); and Difuse Leishmaniasis. [4].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 Luztomiya (in the NewWorld) sandfly mosquito and a vertebrate host (human, dog or even a wild vertebrate) [5]. Leishmaniasis is divided into clinical syndromes according to what part of the body is affected most. In visceral leishmaniasis (VL), the parasite affects the organs of the body. Infections from India, Bangladesh, Nepal, Sudan, Ethiopia, and Brazil account for 90% of cases of VL. Cutaneous leishmaniasis (CL) is the most common form of leishmaniasis and, as the name implies, the skin is the predominate site of infection. Mucocutaneous leishmaniasis occurs only in the New World and is most common in Bolivia, Brazil, and Peru. Leishmaniasis is prevalent in tropical and temperate regions of 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 fig 1 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 [6].

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 [7].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 [8]. Today, the greatest prevalence of 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 [9]. 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.

**Figure 1:** This figure shows picture of a skin ulcer due to leishmaniasis, hand of Central-American adult. SOURCE: CDC/Dr. D.S. Martin

**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 organell called kinetoplast containing intertwined circular DNA (k DNA) molecules known as maxicircles and minicircles, which make up 5-10% of total DNA [10]. A fully developed promastigote measures about 114.3 to 20 μM in length and 1.5 to 1.8 μM at their widest part [11]. 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, edentuates 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 mid gut 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 figure 2 [12].

**CHEMOTHERAPY OF LEISHMANIASIS**

**Scope of Synthetic Products**

The leishmanicidal agents with the most favorable therapeutic index are the antimony compounds known as antimonials. Pentostam (sodium stibogluconate) and Glucantime, able to interfere with the bioenergetics of the Leishmania amastigotes [13] 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. 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 Sbv [14]. Biophysical analysis, foot-printing studies and the crystal structure has proved that the charged amidinium groups of pentamidine establish hydrogen bonding with O₆ of thymine or N3 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 is a pollen antibiotic that was recommended as first line drug in India by National Expert Committee for Sbv 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 for parasitic cells is the result of its great affinity towards 24-substituted sterols, called ergosterol, the major cell membrane sterols [15]. Miltefosine 2 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 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 in their prescriptions [16]. Paromomycin 3 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 acts by impairing the macromolecular synthesis and alters the membrane properties of Leishmania [17]. Allopurinol The antileishmanial activity of the purine analogue allopurinol was identified over 30 years ago. Because it had oral bioavailability and it was widely used for other clinical Indications, the drug was investigated in clinical trials for CL and VL. However, the results were disappointing. Allopurinol is used as a substrate by various enzymes of the purine salvage pathway of trypanosomatids, and it is selectively incorporated into nucleic acid in the parasite. In recent years, allopurinol was considered as part of a maintenance therapy for canine leishmaniasis [18]. Sitemaqumine 4 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 was found efficacious and well tolerated at various dose levels [19]. As on March 2002, the drug is currently in Phase III trials for the treatment of VL.

**Antiretroviral drugs**

The coinfection Leishmania-HIV is frequent and the most common specie involved is *L. infantum*. In general, the treatment in these cases is similar to that of immunocompetent patients, using primarily antimonials or amphotericine B (standard or lipid or liposomal forms). However, the relapses are very frequent. Therefore, it is important to perform a secondary prophylaxis. Currently, no treatment has been completely effective and the mortality rate is high (approximately 25%) during the first month after diagnosis [20]. Recently, the use of antiretroviral drugs

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has been a considerable impact in coinfected patients. Indinavir and saquinavir, two HIV protease inhibitors, have shown pharmacological activity against *L. major* and *L. infantum*. These results add new insights into the wide-spectrum efficacy of protease inhibitors and suggest studying their action on amastigote forms of Leishmania in order to validate their potential contribution against opportunistic infections in treated seropositive patients [21].

**Figure 2:** This figure shows life cycle of *Leishmania* parasite

![Life cycle of Leishmania parasite](image)

Source: Glew et al. [12]

**IMMUNOMODULATORS**

Cure of leishmaniasis appears to be dependent upon the development of an effective immune response, that activates macrophages to produce toxic nitrogen and oxygen metabolites top kill the intracellular amastigotes. This process is suppressed by the infection itself, which down regulates the requisite signaling between macrophage and T cell such as the interleukin (IL) 12, the interferon (IFN) and the presentation of major histocompatibility complex (MHC). One alternative in leishmaniasis treatment is the association of antileishmanial drugs with products that stimulate the immune system. The purpose is to enhance the immune response by the activation of macrophages and the increase of the nitric oxide production among other mechanisms to eliminate the infection [22]. The first report about the use of immunomodulator was the superiority of human IFN as an adjunct antimony therapy for VL, which was demonstrated in Kenya and India [23]. Amphotericin B in conjunction of IL-12 or IL-10 was more efficient than monotherapy and led to a reduction of the Amphotericin dose. Other studies have been reported, using immunomodulator like BCG [24] and protein A [25].

Nevertheless, the price of immunomodulator is exorbitantly high for poor population [26]. Recently, a new generation of synthetic immunomodulator drugs has shown potential for Leishmania treatment. A Schiff base forming compound, Tucaresol enhance TH1 response and the production of IL-12 and IFN-γ in mice and human in patients with viral infections and cancer. Tucaresol also has activity against infection caused by *L. donovani* in BALB/c mice and C57BL/6 at a dose of 5 mg/Kg [27]. Iminoquimod an imidazoquinoline, is the ingredient of a cream (AldaraTM) used for the treatment of genital warts. This drug has shown to induce nitric oxide production in macrophages and it was effective in vitro against *L. donovani* [28]. This field can be more explored with new products, aiming to validate the use of immunomodulator for treatment of leishmaniasis, particularly in patients infected with strains that can develop ML or other complications.

**COMBINED THERAPY**

After increasing unresponsiveness to most of the monotherapeutic regimens, the combination therapy has found new scope in the treatment of leishmaniasis. The combination of antileishmanial drugs could reduce the potential toxic side effects and prevent drug resistance. Several works have shown that some drugs increase their antileishmanial effect in conjunction [29]. Paromomycin have been used extensively in Sudan in combination with sodium stibogluconate for the treatment of VL in a period of 17 days [30]. The superiority of this combination has been demonstrated in several studies [31, 32]. Combined chemotherapy against VL in Kenya was evaluated using oral allopurinol (21 mg/Kg, three times a day for 30 days) with endogenous pentostam (20mg/Kg once a day). The therapy was efficient, but relapses were found in the first month after treatment [33]. This clinical evidence demonstrated the superiority of the combination therapy and can be a hope to develop new formulations.

**DEVELOPMENT OF NEW DRUGS**

During the past decades have given new impetus to antileishmanial drug discovery; including (i) knowledge of biology, biochemical pathway and genome of parasite, (ii) a revolution in chemical techniques, (iii) several advances in bioinformatics tools and (iii) a higher number of networks, partnerships and consortia to support the development of new antileishmanial agents. Currently, the developments of both synthetic and natural drugs have relevant importance in the search of new therapeutic alternatives.

**ANTILEISHMANIAL SYNTHETIC COMPOUNDS**

The medicinal chemistry is a recent applied science directed to the development of new drugs that evolved significantly due to recent technological advances, mainly in molecular, structural biology and computational
chemistry areas. The generation of structural modifications in an initial molecule (called leading compound) to obtain new derivatives has been one successful approach for the design of new drugs based in known and validated molecular targets in the parasite [34]. The knowledge about the physic-chemical and structural properties of the leading compound and its relation to the pharmacological target or action have provided evidences about the initial pharmacophore group, which is essential to activity [34]. Derivatives with pharmacophore group can be obtained with the aim to increase the activity and modulate toxic and pharmacokinetic characteristics of the compound. This approach together with bioinformatics tools has possibilities the virtual search or in silico of potential drugs. In parallel, the design of specific inhibitors has been explored as a possible means for controlling the parasites growth without damaging the host. A review about potential targets in Leishmania parasite has been written [35]. Some of the most promising targets are: topoisomerases [36], kinetoplast [37], mitochondria [38], trypanothione reductase [39], cisteine protease [40], and fatty acid and sterol pathways [41]. Several synthetic products have demonstrated their antileishmanial potentialities. Per example: azasterols are inhibitors of 24-methyltransferase, which showed activity against promastigotes of L. donovani and axenic amastigotes of L. amazonensis [42]; edelfosine and ilmofosine, new alkyl-lysophospholipid derivatives, demonstrated high in vitro activity against L.donovani promastigotes and amastigotes [43]; nicotinamide is an inhibitor of certain III NAD-dependent deacetylase that caused in vitro inhibition of L. infantum promastigotes and amastigotes [44]; n-acetyl-cysteine, a precursor of glutathione, showed in vivo activity against L. amazonensis in BALB/c mice [45] and 3-substituted quinolines have been demonstrated their potential as activators of macrophages and in vitro activity against L. chagasi promastigotes and amastigotes was observed [46]. On the other hand, the screening of library compounds has been reported. Per example, St. George and col. screened a chemical library of 15000 compounds. Three compounds (NSC#: 13512, 83633 and 351520) were identified to be active against amastigotes of L. major and safe to mammalian host, which represent possible candidates for drug development [47]. The analyzed of library is an advance technology since several compounds can be search and gain information on the chemical class of leaders. The synthetic products have been considered successfully, and some advantages are mentioned such as: cost, time of abstention, novelty and scale-up and low intellectual property complications [48]. However, the synthetic molecules can display a high toxicity and only a low of compounds have been evaluated in clinical studies.

**SCOPE OF NATURAL PRODUCTS**

**Alkaloids**

The alkaloids constitute an important class of natural products exhibiting significant anti-leishmanial activities. The quinoline alkaloids, 2-n-propyquinoline 5, chimanine-D 6 and chimarine-B 7, isolated from Galipea longiflora (Rutaceae), exhibit antileishmanial activity against L.braziliensis promastigotes with an IC90 values of 50, 25 and 25μg/mL, respectively. Oral in vivo studies was performed on 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 given for 5 days results in 72.9% parasite suppression only. Likewise, dictyolomide-A 8 and B 9 isolated from the bark of Dictyoloma peruviana (Rutaceae), causes total lyses of L. amazonensis promastigotes at 100 μg/mL concentrations [49].

![Chemical structures of alkaloids](image)

**Indole alkaloids**

Dihydrocorynantheine 10, corynantheine 11 and corynantheidine 12 isolated from the bark of Corynanthe pachyceras (Rubiaceae) are the respiratory chain inhibitors exhibiting IC50 of 3μM against L.major. Pleiocarpine isolated from stem bark of Kopsia griffithii (Apocynaceae), shows in vitro antileishmanial activity with an IC50<25μg/mL against L.donovani promastigotes. Gabunine a bis-indole alkaloid obtained from stem bark of Peschiera van heurkii (Apocynaceae), exhibits in vitro activity with an IC50 25μg/mL against L. amazonensis amastigotes [50].
Isoquinoline alkaloids liriodenine 13 and O-methylmoschatoline 14, isolated from Annona foetida (Annonaceae), display in vitro activity against promastigote forms of *L. braziliensis* with an IC50 < 60μM [51]. The SAR study among these oxoaporphine alkaloids reveals that methylenedioxy moiety is eight times more active against *L. braziliensis* and *L. guyanensis* than the O-methylmoschatoline. Berberine, occurring in many plant species of Annonaceae, Menispermaceae and Berberiferae, exhibits in vivo leishmanicidal activity with an IC50 value of 10 μg/mL against *L. major*. Isoguattouregidine isolated from Guatteria foliosa (Annonaceae), shows activity at 100 μg/mL concentrations against *L. donovani* and *L. amazonensis*. Anonaine isolated from Annona spinescens (Annonaceae), exhibits activity against promastigotes of *L. braziliensis* and *L. donovani* [52]. The alkaloids, (+)-neolitsine and cryptodorine, isolated from Guatteria dumetorum (Annonaceae), display significant activity against promastigotes of *L. maxicana* at 15 and 3 μM concentrations, respectively. Xylopine, an aporphine alkaloid isolated from Guatteria amplifolia (Annonaceae), show activity against promastigotes of *L. mexicana* (IC50 value 3 μM) and *L. panamensis* (IC50 value 6 μM) [53]. Unonopsine, a dimeric aporphine alkaloid isolated from the Unonopsis buchtienii (Annonaceae), displays antileishmanial activity (IC100 value 25μg/mL) against *L. donovani* promastigotes [54].

Ancistrocladine A 16 and B 17 isolated from yet undescribed Congolese Ancistrocladaceae species, require 2.61 and 1.52 μg/mL concentrations, respectively to reach the IC50 towards *L.major* promastigotes. An apoptosis-like death pathway is the possible mode of action for above compounds. Ancistrocladidine, isolated from Ancistrocladus tanzaniensis (Ancistrocladaceae) shows relatively weak activity by a factor of 2 against *L. donovani* when compared to ancistrotanzanine-B (IC50 = 1.6 μg/mL), while by a factor of 10 in comparison to miltefosin (positive control). Likewise, ancistrotanazanine-A 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.

**Bisbenzyl Isoquinolinic Alkaloids**

Daphanandrine 18 isolated from Albertisia papuana obaberie obtained from *Pseudoxandra sclerocarpa* (Annonaceae), gyrocarpine 19 produced by *Gyrocarpus americanus* (Hernandiaceae) and limacine 21 isolated from *Caryomene olivasans* (Menispermaceae), display activity against *L. donovani, L. braziliensis* and *L. amazonensis* with an IC100 of ~50 μg/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 [55].

**Steroidal Alkaloids:** Among the alkaloids, holamine 22, 15-α hydroxyholamine, holacurtine 23 and N-desmethylholacurtine obtained from *Holarrhena curtisii* (Apocynaceae), the metabolite holamine exhibits strongest activity against *L. donovani* (1.56>IC50>0.39μg/mL) in compared to holacurtine and N-desmethyl holacurtine (6.25>IC50>1.56μg/mL) [56].
Benzoquinolizidine Alkaloids: Klugine 24, cephaeline 25, isocephaeline 26 and emetine 27 demonstrating significant leishmanicidal activities against L. donovani have been isolated from Psychotria klugii (Rubiaceae). Among these metabolites, klugine (IC50 of 0.40 μg/mL) and isocephaeline (IC50 0.45 μg/mL) exhibit <13- and <15-fold less potent activity in compared to cephaeline with IC50 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. Emetine exhibits activity against L. donovani with an IC50 value of 0.03 μg/mL, however produces toxicity in treatment of cutaneous leishmaniasis caused by L. major [57].

Diterpene Alkaloids: The alkaloids, 15, 22-O-Diacetyl-19-oxo-dihydroatisine, azitine 28 and isoazitine 29, isolated from Aconitum, Delphinium and Consolida species, show significant leishmanicidal activities. The metabolite isoazitine exhibits strongest activity against promastigotes of L. infantum with IC50 values 44.6, 32.3 and 24.6 μM at 24, 48 and 72 h of culture, respectively. azitine with IC50 values of 33.7 and 27.9 μM at 72 h of culture, respectively, exhibit activity against promastigotes of L. infantum [58].

Pyrrolidinium Alkaloid: (2S,4R)-2-carboxy-4-(E)-coumaroyloxy-1,1-dimethylpyrrolidin salt, isolated from Phlomis brunneogaleata (Lamiaceae), display activity with an IC50 of 9.1 μg/mL against axenic amastigotes of L.donovani.

Acridone Alkaloids: The rhodesiacridone 30 and gravacidonediol 31 isolated from Thamnosma rhodesica (Rutaceae), exhibit 69% and 46% inhibition at10μM concentration, respectively against promastigote 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.

β-Carboline Alkaloids: The harmaline 32, isolated from Peganum harmala (Nitrariaceae), exhibits amastigotesspecific activity (IC50 of 1.16 μM). Harmine 33 isolated from same plant species reduces spleen parasite load by approximately 40, 60, 70 and 80% in free, liposomal, niosomal and nanoparticlar forms, respectively in mice model. Canthin-6-one and 5-methoxycanthin-6-one occurring in plant species of Rutaceae and Simaroubaceae, demonstrate in vivo activity against L. amazonensis in BALB/c mice model. N-hydroxyannomontine and annomontine isolated from Annona foetida (Annonaceae), show efficient leishmanicidal potentials.

Alkaloids from Marine Source: Marine sponges e.g. Amphimedonviridis, Acanthostrongylophora species, Neopetrosia species, Plakortis angulospiculatus and Pachymatisma johnstonii serve as rich sources of alkaloids with significant antileishmanial potentials. Renieramycin A isolated from Neopetrosia species, is a La/cgfp (expressing enhanced green fluorescent protein) inhibitor that shows efficient antileishmanial activity against L.amazonensis with IC50 0.2 μg/mL. Araguspongin C,
isolated from a marine sponge Haliclona exigua, displays
leishmanicidal activity against promastigotes as well as
amastigotes at 100 μg/mL concentrations [59]. Among the
ciliatamides A-C [34, 35, 36] isolated from Aaptos ciliata, the
peptide ciliatamides at 10.0 μg/mL concentrations inhibit
50% growth L. major promastigotes [60]. The lipopeptides,
almiramides A-C [37, 38, 39] isolated from cyanobacterium
Lyngbya majuscula, exhibit significant in vitro antileishmanial activity against L. donovani. Dragonamide
A, E and herbamide B isolated from same cyanobacterium
strain, exhibit in vitro activity against L. donovani with
EC50 values of 6.5, 5.1 and 5.9 μM, respectively.

**Quinones:** Primin (2-methoxy-6-pentylcyclohexa-2,5-
diene-1,4-dione), present in Primula obconica and Primulaceae, shows significant leishmanicidal activity against L. donovani with an IC50 of 0.711 μM. Diospyrin 40, a bis-naphthoquinone inhibiting topoisomerase I, isolated from the bark of Diospyros Montana (Ebenaceae), demonstrates antileishmanial activity against L. donovani promastigotes with an MIC of 1.0 μg/mL [63]. The hydroxylated derivative of 70 at 3 μM concentration eliminates 73.8% of amastigotes in infected macrophages [64]. Plumbagin 41, originally isolated from Plumbago zeylanica, shows leishmanicidal activity against amastigotes of L. donovani (IC50=0.42μg/mL) and L. amazonensis(IC50=1.1μg/mL). At a concentration of 10 μg/mL, the compound 41 presents an amastigote survival index (SI) of 16.5% against L. amazonensis with the absence of toxic effects against the macrophages. The metabolite 41 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 41 and 40 involves generation of oxygen free radicals from which the parasites remain unable to defend. The dimeric products 3,3-biplumbagin 42 and 8,8'-biplumbagin 43, isolated from the bark of Pera benensis (Euphorbiaceae), display significant antileishmanial activity. Among these, the metabolite 42 shows lower activity (IC90 = 50 μg/mL) compared to 41 and 44 (IC90 = 50 μg/mL) against L. braziliensis, L. amazonensis, and L. donovani promastigotes [65, 66]. Lapachol 44, a prenylated hydroxynaphthoquinone isolated from Tecomia species (Bignoniaceae), displays activity with mechanism of action similar to 41 against L. donovani amastigotes in peritoneal mice macrophages. The metabolite 3, 4-dihydroxynaphthalen-1(2H)-one, isolated from the bark of Ampelocera eldentula (Ulmaceae), exhibits leishmanicidal activity (IC90 of 10 μg/mL) against L. braziliensis, L. amazonensis and L. donovani promastigotes. It 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/kg/day). However, the use of tetralones is limited due to cytotoxic, carcinogenic and mutagenic properties in animals [67]. Jacaranone, a quinone isolated from the leaves of Jacaranda copia (Bignoniaceae), exhibits a strong activity with an ED50 of 0.02 mM against L. amazonensis promastigotes but at the same concentration shows toxicity to peritoneal mice macrophages. The prenylated dihydroxy naphthoquinone hydropiperone, isolated from Peperomia galiioides (Piperaceae), shows activity at a concentration of 25 μg/mL against promastigote forms of L. braziliensis, L. donovani and L. amazonensis. At100 μg/mL concentration causes total lyses of the parasites. The anthraquinone-2-carbaldehydes, 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 [68]. The aloe-emodin 45 isolated from Stephania dinklagei (Menispermaceae), shows leishmanicidal activity at IC50 values of 185.1 and 90 μM against L. donovani promastigotes and amastigotes, respectively [69]. Vismione D isolated from Vismia orientalis (Clusiaceae) exhibits activity against axenic amastigotes of L. donovani with an IC50 value of 0.37 μg/mL but shows cytotoxicity when tested on human L6 cells (IC50 of 4.1 μg/mL).
Terpenes

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 46, B 47, C 48 and 6-β-hydroxyloganin 49, 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 46 displays significant leishmanicidal activities [70]. Picroside I 50 and kutkoside 51 obtained from Picrorhiza kurroa, exhibits a high degree of protection against the infection of promastigotes of L. donovani in hamsters. Picroliv, a standardized fraction of iridoid glycosides 50 and 51, increases the nonspecific immune response and induces a high degree of protection against the infection of promastigotes of L. donovani in hamsters. Picrolive is an adjuvant proposed to increase the efficacy of leishmanicidal drugs and has demonstrated excellent therapeutic index in Phase I and II clinical trials. Amarogentin, a secoiridoid glycoside isolated from Swertia chirata (Gentianaceae), produces leishmanicidal effect at a concentration > 60 μM against L. donovani through inhibition of catalytic activity of topoisomerase I. The metabolite amarogentin exerts inhibitory effect with a mechanism of action similar to Pentostam® by binding to the enzyme and preventing the formation of a binary complex with DNA. The evaluation of amarogentin in the form of liposomes and niosomes shows an enhanced leishmanicidal activity (without toxic effects) than those observed for free amarogentin when tested in hamsters.

Monoterpenes: Espinanol 52, isolated from the bark of Oudnstra espinitana (Annonae), shows antileishmanial activity against promastigotes of twelve Leishmania species. However, the metabolite 52 exhibits only a weak activity in vivo in mice infected with L. amazonensis. Grifolin 53 and piperogalin 54 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 54 causes more than 90% lysis of the promastigotes.

Sesquiterpenes: A sesquiterpene lactone, dehydrozaluzanin C 55, isolated from the leaves of Munnoria 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 55 in BALB/c mice results in reduction of the lesions caused by L. amazonensis. Sesquiterpene dilactone, 16, 17-dihydrobrachycalyxoxide, isolated from Vernonia brachycalyx (Asteraceae), exhibits activity (IC50 = 17 μg/mL) against L. major promastigote but also inhibits the proliferation of human lymphocytes. Kudtriol 56, a sesquiterpene alcohol isolated from the arial
parts of *Jasonia glutinosa* (Asteraceae), shows toxic activity against promastigotes of *L. donovani* at 250 μg/mL concentration. SAR study with metabolite 56 indicates that the presence of a C-5 hydroxy group in the α-orientation is essential for the expression of the leishmanicidal activity. The (+)-curcuphenol 57, isolated from sponge *Myrmekioderma styx*, exhibits in vitro anti-leishmanial activities against *L. donovani* with an EC50 of 11.0 μM.

**Diterpenes** A phorbol diester, 12-O-tetradecanoyl phorbol-13-acetate (TPA), 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. amazonensis* by activation of protein kinase C, an important enzyme in the development of several cellular functions. Among the other diterpenoids isolated from Euphorbiaceae species with leishmanicidal activities are jatrogossidione 58 and jatrophone 59. These metabolites possess toxic activity against the promastigote forms of *L. braziliensis*, *L. amazonensis* and *L. chagasi*. SAR studies with these metabolites revealed that 58 with IC100 value of 0.75 μg/mL displays activity higher than 59 (IC100 = 5 μg/mL), but remains inactive in vivo. The 15-monomethyl ester of dehydropinifolic acid, obtained from the stem bark of *Polyalthia macropoda* (Annonaceae), and ribenol, an enol-manoyl oxide derivative isolated from *Sideritis canariensis* (Lamiaceae), show in vitro activity against promastigotes of *L. donovani*. Also the different derivatives of this metabolite, obtained through chemical or biological transformations, exhibit strong leishmanicidal activity. Additionally, 6-β-hydroxyrosenono lactone, a diterpene isolated from the bark of *Holarrhena floribunda* (Apocynaceae), has a moderate and weak activity against promastigotes and amastigotes of *L. donovani*, respectively [71].

**Triterpenes**: The ursolic acid 60 and betulinaldehyde 61, obtained from the bark of *Jacaranda copia* and the stem of *Doliocarpus dentatus* (Dilleniaceae), respectively show activity against the amastigotes of *L. amazonensis*. However, the metabolite 61 exhibits toxicity to peritoneal macrophages in mice while 60 displays limited activity in vivo. The triterpenes, (24Z)-3-oxotirucall-7,24-dien-26-oic acid 62 and epi-oleanolic acid 63, isolated from the leaves of *Celaenodendrum mexicanum* (Euphorbiaceae), display leishmanicidal activity against *L. donovani* with IC50 values of 13.7 and 18.8 μM, respectively. The quassinoids, simalikalactone D 64 and 15-β-heptylchaparrinone, obtained from species of Simaroubaceae family show activity against promastigotes of *L. donovani* but at the same time exhibit toxicity to macrophages [72]. Triterpene glycosides obtained from marine sources e.g. holothurins A, 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 obtained from same source shows 82.5 ± 11.6% and 47.3 ± 6.5% inhibitions against promastigotes of *L. donovani* at 100 and 50 μg/mL concentrations, respectively.

**Saponins**: The α-hederin 65, β-hederin 66 and hederagenin 67, obtained from the leaves of *Hedera helix* (Araliaceae), show leishmanicidal activity against *L. infantum* and *L. tropica*. Among these, the metabolite 67 also shows significant activity against the amastigote forms while both 65 and 66 exhibit strong anti-proliferative activity on human monocytes. The saponins 65-67 appear to inhibit the growth of Leishmania promastigotes by acting on the membrane of the parasite with induction of a drop in membrane potential. The hederecolchiside-A1 68, 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 69, isolated from the leaves of *Buddleja madagascariensis* (Loganiaceae), exhibits activity against promastigotes of *L. infantum*. Muzanazegenin 70, obtained from the roots of *Asparagus africanus* (Liliaceae), displays activity with an IC50 value 31 μg/mL against the *L. major* promastigotes. However, the metabolite 70 also inhibits the proliferation of human lymphocytes.
Phenolic Derivatives

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\(^71\) shows toxicity to promastigotes of *L. amazonensis*, while exhibiting lower activity (IC\(_{50}=24\mu\text{g/mL}\)) against amastigote forms. Encapsulated formulation of 72 when administered at 1.0 μg/mL causes the reduction in the level of *L. amazonensis* infected macrophages by 53% [73]. Ultrastructural studies suggest that 72 produces selective toxicity to the intracellular amastigotes without affecting macrophage organelles even when exposed to 80 μg/mL concentration.

The licochalcone-A 73, isolated from roots of the Chinese licorice plant *Glycyrrhiza* species (Fabaceae), shows *in vitro* activity against *L. major* and *L. donovani* promastigotes. The intraperitoneal administration of 73 prevents the development of lesions in BALB/c mice infected with *L. major*. The intraperitoneal and oral administration of 73 significantly reduces the parasite load in the spleen and liver of hamsters infected with *L. donovani*. The compound 73 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 73 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-hydroxyl...
benzofuran-3(2H)-one) 74, is an aurone, a group of metabolites related biosynthetically to the chalcones, exhibit activity with EC50 values of 0.09-0.11μg/mL against promastigotes of Leishmania species. The metabolite 74 with an EC50 value of 1.24μg/mL displays activity against L. donovani amastigotes, but remains nontoxic to bone marrow-derived macrophages.

**Flavonoids:** The compound 5, 7, 4′-trihydroxyflavan 75 shows activity against the amastigotes of L. amazonensis, while the biflavonoids amentoflavone, podocarpusflavone A 76 and B 77, isolated from the leaves of Celandodendron mexicanum, shows weak activity against L. donovani promastigotes. The flavones fisetin 78 (isolated from Acacia greggi and A. berlandieri), 3-hydroxyflavone, luteolin (isolated from Salvia tomentosa), and quercetin (isolated from plants of family Alliaceae) exhibit potent antileishmanial activity against the intracellular forms of the L. donovani with IC50 values 0.6, 0.7, 0.8 and 1.0 μg/mL, respectively. Biochanin A, an O-methylated isoflavone occurring in legumes, shows activity against L. donovani with an IC50 value of 2.5 μg/mL. Coumarins: The coumarin isomers 2-epicycloisobrachy coumarinone 79 and cycloisobrachy coumarinone 80, isolated from Vernonia achyranthis (Asteraceae), display selective activity against promastigotes of L. major.

**Curcumine:** The curcumin, curcumin81, desmethoxycurcumin 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 [75].

**Other Metabolites**

Acetogenins like senegalen 82, squamocine 83, asimicine 84 and molvizarine 84, isolated from the seeds of Annonasenegalensis (Annonaceae), show activity against promastigotes of L. major and L. donovani at concentrations that vary between 25 and 100μg/mL. However, these metabolites also show cytotoxicity greater than that of vinblastine against KB and VERO cell lines. Other acetogenins such as rolliniastatin-1, isolated from Rollinia emarginata (Annonaceae), annonacin A and goniolaftaminic, obtained from Annona glauca (Annonaceae), display promising activity against the promastigote of L. braziliensis, L. donovani, Lamazonensis, however a clear SAR has not been established.

**FUTURE SCOPE**

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 increase 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 leishmani 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.

![Chemical structure](image1)

![Chemical structure](image2)

![Chemical structure](image3)

![Chemical structure](image4)

![Chemical structure](image5)

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ACKNOWLEDGEMENT / SOURCE OF SUPPORT
Authors are many thankful to University of Pune, National Chemical Laboratory, Pune, for providing Library facilities Also thankful to Prof. T.J. Sawant, Founder Secretary, JSPM, Pune for providing necessary facilities.

CONFLICT OF INTEREST
No conflict of interest was declared by authors.

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