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Leishmaniasis: Plants as a source of antileishmanial agents

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KEYWORDS

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Cutaneous *Leishmaniasis*

Kala-azar

Leishmania

Plant derived antileishmanial compounds

Sandfly

Visceral *Leishmaniasis*

ABSTRACT

Leishmania infection causes a group of tropical diseases and has remained neglected for decades. It spreads by sandfly vector and is one of the most fatal protozoan diseases after malaria. Leishmaniases are a group of diseases caused by the infection of different Leishmania species and display clinically different forms like “Visceral leishmaniasis” (VL), “mucocutaneous leishmaniasis” and “cutaneous leishmaniasis” (CL). Approximately one billion people living in an endemic area are at high risk. Three hundred thousand cases of VL are reported annually and around twenty thousand people die every year, proving it as one of the most lethal forms of leishmaniasis. Until now, no effective vaccine could be made. There is an increase in drug resistance in the case of conventional drugs. New synthetic drugs are either too costly or have side effects. Requirements of new drugs are of utmost importance to control this situation. Plants provide a source of unlimited chemical diversity, which can be screened for antileishmanial activities. Moreover, their low cost and less or no side effects make them idle candidates in the search of new antileishmanial drugs.

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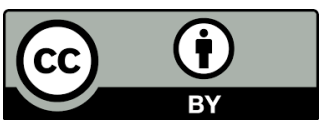


1 Introduction

Leishmaniases, a significant tropical disease is considered the 2nd most significant disease next to malaria (Ghorbani and Farhoudi 2017). The disease has been neglected for decades but now it has attained significant importance because of its geographic spread and growing cases of Leishmania-HIV coinfection (Conceição-Silva and Morgado 2019). Protozoan parasite of the genus Leishmania causes the disease leishmaniasis. Various clinical manifestations of the disease are presented, based on the type of Leishmania parasite infection. CL, VL, and mucocutaneous leishmaniasis (ML) are the major forms of leishmania (Burza et al. 2018). Sandflies transmit *Leishmania* parasites to the mammalian host, as they bite them. The promastigote (flagellated parasitic form) enters into the mammalian host (Figure 1). Inside the host, the parasites become immotile amastigote structures without flagella. Unfortunately, still, we have not been able to find one effective vaccine against leishmaniasis and most of the people depend on synthetic drugs (Ghorbani and Farhoudi 2017).

Pentavalent antimony-derived organic compounds are mostly used drugs. However, they cause stern side effects and also are not fully efficient against this lethal form of leishmaniasis (Guerin et al. 2002). Miltefosine, an available drug also develops resistance as per an *in-vitro* study (Perez et al. 2003). Though lipid formulation of Amphotericin B is an advanced drug, their high cost is a hurdle to make it available for poor people who are most affected (Ouellette et al. 2004; Croft et al. 2006)

Extracts/compounds from natural resources are a huge source of compounds for new drug discoveries of many tropical diseases which have remained neglected. Besides, because of minimum or no side effects and low price, the use of plant extracts as effective anti-leishmanial drugs is gaining importance.

We begin this chapter with the description of the disease-Leishmaniasis, which includes the history of Leishmaniasis, clinical aspects of the disease, and current treatments followed by the rationale behind the need for new plant-derived drugs and discussion on different classes of plant-derived entities which can be a promising antileishmanial agent.

2 Leishmaniasis: a brief history

Evidence suggests that the history of Leishmaniasis dated back to the 1stcentury AD. Many clay curios from Peru & Ecuador and of pre-Inca civilization displayed descriptions of lesions in the skin and facial deformities; these are typically showing mucocutaneous & cutaneous leishmaniasis. Later both Leishman and Donovan discovered the genus, *Leishmania* (Steverding 2017) while studying Kala-azar.

2.1 *Leishmania*/HIV co-infection

The prevalence of HIV/AIDS cases during the last few decades has changed the array of Leishmania infection in both epidemiological and clinical scenarios (Cruz et al. 2006). Since the first report of HIV/Leishmania co-infection was registered during the 1980s (de

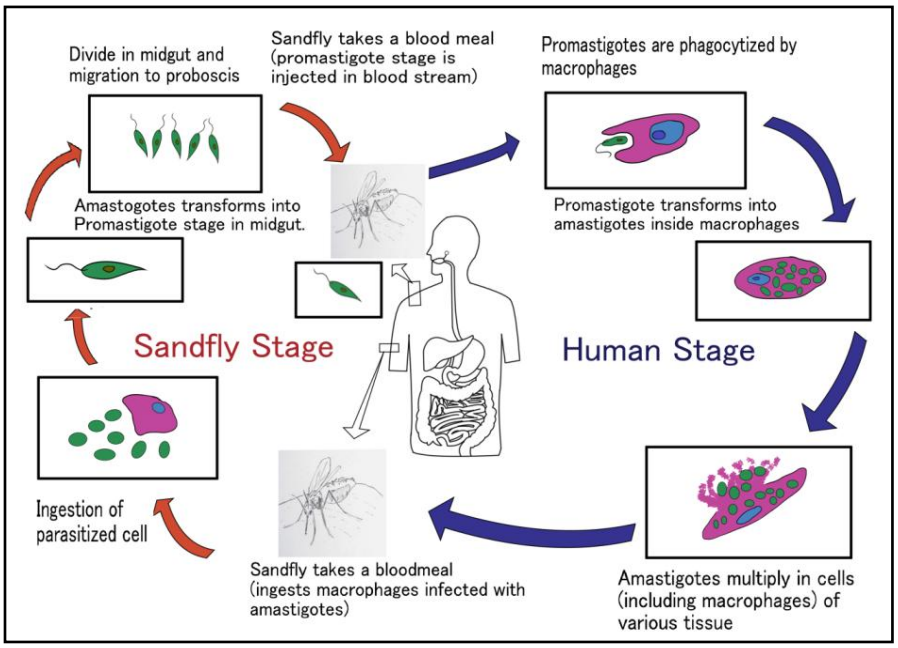


Figure 1 Leishmania life cycle: Sandflies transmit *Leishmania* parasites to the mammalian host, as they bite them. The promastigote (flagellated parasitic form) enters into the mammalian host. Inside the host, the parasites become immotile amastigote structures without flagella and cause the disease.

la Loma et al. 1985), there has seen a continuous surge and now about 35 countries in the world are combating *Leishmania*/HIV coinfection (Desjeux and Alvar 2003; Alvar et al. 2008) the greatest prevalence being in the Mediterranean region. The two diseases are mutually reinforcing. An HIV infection makes a patient more vulnerable to *Leishmania*, and subsequently, *Leishmaniasis* accelerates the replication of HIV and progression to AIDS. Till recently the global impact of HIV/*Leishmania* co-infection has remained underestimated because of the lack of an effective surveillance system. To monitor this, the "World Health Organization", in association with the "United Nations HIV/AIDS" program, has started an active surveillance system LEISHNET since 1998 to find out the actual spread of this problem and the report has been horrifying (Alvar et al. 2008; Cruz et al. 2006).

2.2 "Post Kala-azar dermal leishmaniasis (PKDL)"

The cutaneous expression of VL is represented in PKDL. The disease is related to lesions of skin and papules commonly appearing upon the face. The disease may usually appear 2-5 years of successful treatment of VL (Gedda et al. 2020). The disease is confined mainly to two regions – the Indian subcontinent and Sudan and its adjoining area. These two regions are also endemic to visceral leishmaniasis (Ganguly et al. 2010). In Sudanese PKDL, the disease gets cured spontaneously. But in India spontaneous healing does not take place and the disease needs to be treated with SAG (sodium antimony gluconate). However recently the increase in SAG resistance has caused the authorities to use newer drugs like Amphotericin B which are not resistant to *Leishmania*, to combat the disease (Das et al. 2009)

2.3 Zoonotic leishmaniasis

To date,15 well-known Leishmania species infect humans, among these 13 are known to have zoonotic nature. The disease is known to infect a wide range of animals including dogs, rodents, foxes wolves, jackals, and sloths (Gramiccia and Gradoni 2005)

3 Morphology of *Leishmania* parasites

3.1 *Leishmania* parasites have two Stages

3.1.1 Amastigote stage

The amastigote or non-flagellated stage occurs in vertebrate hosts, where the parasite resides in the reticuloendothelial system of man, dog, and hamster. The parasite is oval or round shaped, 2-4 µm long along the longitudinal axis with slight or no motility (figure 2 & 3).

3.1.2 Promastigote stage

It is an extracellular type of Leishmania parasite and is present in vectors (insects) only. However, it can be grown in cultures also. Fully developed promastigotes are 15-20 µm in length and have a long, slender spindle-shaped body. The nucleus lies at the center and the kinetoplastid is present transversely near the anterior end. A flagellum projecting from the front measuring the length of the parasite or even longer is also present (Figure 2 & 3).

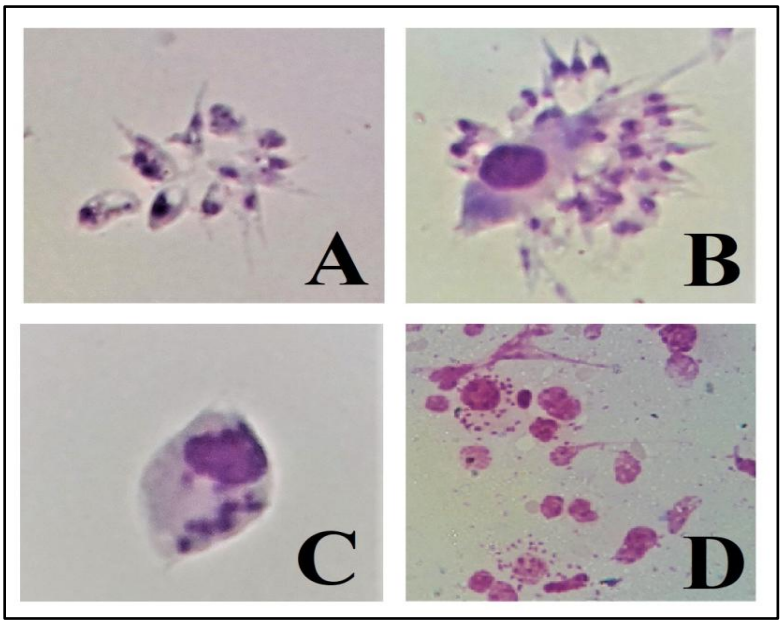


Figure 2 Promastigote stage of *Leishmania donovani* (100X) (A), Promastigotes infecting Mouse cell line (RAW 264.7) at 100X (B), Amastigote stage of *Leishmania donovani* parasites inside RAW 264.7 (100X) (C), *Leishmania donovani* infected liver cells of Balb/c mice model (100X) (D). Cells (A, B, C & D) are stained with Giemsa stain.

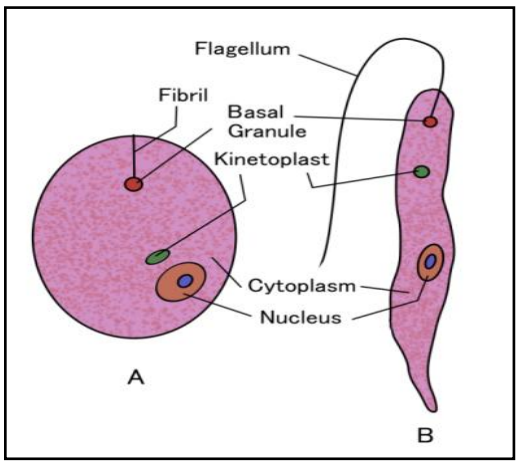


Figure 3 Diagrammatic representation Amastigote (A) and Promastigote (B) form of *Leishmania* parasite

4 Transmission of disease

The prime mode of infection of VL is by the bite of a female phlebotomine Sandfly. The parasite is transmitted by the bites of *phlebotomus* harboring *Leishmania* (Figure 1) in the old world and in the new world by the *Lutzomyia species* (Singh 2006). However other modes of congenital and parental transmission like blood transfusion, needle sharing, and laboratory accidents are also being reported (Herwaldt 1999).

5 Diagnosis

Preliminary diagnosis is dependent on the clinical signs and symptoms of visceral leishmaniasis such as hepatomegaly, splenomegaly, illness with prolonged irregular fever, and weight loss. However, the signs and symptoms may not necessarily represent VL and CL pathogenesis.

6 Parasite survival strategy: immune evasion mechanisms

Several factors are present that cause immunosuppression in host cells by *Leishmania* parasites as revealed during studies in animal models (hamster/mouse). Studies on mice models have revealed the role of T cells (Blackwell and Ulczak 1984), T-Helper II cells, and adherent cells as responsible for immunosuppression (Conceição-Silva and Morgado 2019). Reports say that *Leishmania* infection can cause the Suppression of superoxide production (Underhill and Ozinsky 2002; Pham et al. 2005; Lodge and Descoteaux 2006), decrease in NO (nitric oxide), production (Nandan and Reiner 1955; Blanchette et al. 1999; Wanderley et al. 2006), and inhibition of Interleukin-12 production (Rodriguez et al. 2001). *Leishmania* infection Delays apoptosis which is an adaptive mechanism and a defense strategy (Moore and Matlashewski 1994; Akarid et al. 2004; Donovan et al. 2009).

7 Control of leishmaniasis

There has been a lot of research for an effective drug or vaccine against leishmaniasis, but the search for a non-toxic, cost-effective and highly potent drug or vaccine is still going on. The first chemotherapeutic treatment of *Leishmaniasis* was developed in Germany by Ehrlich and his group in the late nineteenth century. Their novel approach of differential toxicity of the drugs towards pathogen and host is still the fundamental approach to all the drug development in modern-day also. It has been seen that in visceral leishmaniasis in India, the *Leishmania tropica* is the co-endemic mediator, this explains the reason behind the increase in the frequency of unresponsiveness towards therapy using sodium antimony gluconate. This further complicates the treatment in the Indian sub-continent. Thus, these compounds are slowly being replaced by newer formulations like the liposomal delivery of amphotericin B and other drugs like miltefosine and paromomycin (Chappuis et al. 2007).

7.1 Currently used therapeutic drugs

7.1.1 Antimonials

Macado and Vianna were the first to publish the use of trivalent antimonials (Sb^{III}) as an anti-*Leishmanial* chemotherapeutic agent in 1913 for CL. Later, in 1915 antimonials were introduced in India for VL treatment (Berman 1997).

7.1.2 Pentamidine isethionate

Pentamidine is used for 2nd line of treatment against VL. However, its exact working principle is not clear so far. Pentamidine is generally used as a competitive inhibitor of arginine-transport & noncompetitively inhibits spermidine and putrescine, it is speculated that its antileishmanial efficacy is probably facilitated via its ability to disrupt mitochondrial membrane potential and by effecting polyamine biosynthesis.

7.1.3 Amhotericin B

Amphotericin B, isolated from *Streptomyces nodosus* was initially an antifungal macrolide antibiotic and its antileishmanial activity was first shown in the early 1960s. Amphotericin B exhibits highly potent leishmanicidal activity. Because of growing Antimony ($Sb^{V)}$ unresponsiveness towards VL in the Indian subcontinent during the last couple of decades, amphotericin B has emerged as an alternate with high efficacy. 15 to 20 infusions, each having a dosage amount of 0.750 to 1 mg/kg bodyweight for either every other day or daily has reliably produced about 97 percent cure rates (Vertut et al. 1994).

7.1.4 Paromomycin

Paromomycin (identical to aminosidine), belonging to the class of aminocyclitol aminoglycosides is isolated from *Streptomyces rimosus* cultures and contains both antibacterial & antiprotozoal activity. The drug was made in the 1960s as an anti-leishmanial drug. But the compound remains neglected, until 1980 (Chunge et al. 1990). It provides a synergistic effect in combination with Sb^{v} as compared to Sb^{V} alone in several studies from India (Thakur et al. 1992, 1995)

7.1.5 Miltefosine

In 1987, Croft and Hogg (1988) described the activity of alkylphosphocholines, including the drug "miltefosine" to kill amastigotes of *L. donovani* under *in-vivo* (mice model) and *in-vitro* conditions (Croft et al. 1988). The introduction of miltefosine is a landmark in antileishmanial therapy as it is the first oral drug having satisfactory efficacy and toxicity. It has undergone phase I, II, and III trials and was found to be 94% successful (Sundar et al. 2002; Bryceson 2001).

7.1.6 Vaccine

Since the drug resistance developed due to chemotherapeutic treatment is a big health issue, exhaustive efforts have been taken towards the development of an effective vaccine. Increasing knowledge of immunological response and host-pathogen interactions involved in *Leishmania* infection has led to significant advancement in the search for vaccine candidates in *Leishmania* control.

Earlier studies in the search of suitable vaccine candidates included live as well as attenuated *Leishmania* parasites. Though live vaccines were effective these were discontinued because of problems associated with the virulence of effective vaccine (Gradoni 2001). DNA vaccines are more appealing newer generation vaccines but chances of DNA integration into host cell chromosomes and adverse immune response against DNA are some big hurdles (Watts and Kennedy 1999). Some vaccines, which have been tried exhaustively against *Leishmania*, are Leishmanisation (Tabbara et al. 2005) and Killed Vaccines (Russel and Alexander 1988; Handmanet et al. 1990; Olobo et al. 1995; Misra et al. 2001; Mohebali et al. 2004). Many other vaccine candidates like gp46/M2/Parasite Surface Antigen (Montgomary et al. 2000), LACK/p36 (Julia et al. 1996) dp72 and P0 (Rachamim and Jaffe 1993) PapLe22 (Fragaki et al. 2001), Amastigote cysteine proteases (Rafati et al. 2002) and Amastigote P4 and P8 (Soong et al. 1995) have been tried for their efficacy in different forms of *Leishmania*.

8 Natural products: source of antileishmanial Agent

Till now there is no effective vaccine against *Leishmania*. Most of the pentavalent antimony compounds had been developed as drugs to treat *Leishmania* infection before 1959. But increasing toxicity of these drugs, rising resistance, and various persistent side effects are still a matter of great concern. There has been an exhaustive exploration for antileishmanial agents and alternate therapy, like amphotericin B and pentamidine has been discovered but they also show unpleasant side effects (Brajtburg and Bolard, 1996; Sundar and Chakravarty 2015a; Hefnawy et al. 2018). Therefore, development for alternative drugs is urgently needed in this sector. Plants are a source of enormous chemical entities and the insistent require for alternative drugs has encouraged the researchers to find natural plant products for possible application in the treatment of leishmaniasis. Even, WHO has approved the usage of conventional medication in distant rural areas where primary health facilities are inaccessible (Chan-Bacab and Peña-Rodríguez 2001). Many people in rural areas, where appropriate health services are unavailable and people are unable to avail good medical treatment because of poverty and the absence of other agencies, people mostly rely on folk medicines which are generally extracted from natural resources like plants. The plant products occurring in nature are credible resources with an amazing variety and accessibility in their chemical composition. Many of the folk medicines which are being used for centuries have been scientifically proved to be potent. Data presented in Table 1 revealed the natural products which are a credible source of antileishmanial agents that have been explored extensively and many compounds are potent against different species of *Leishmania* (Vermelho et al. 2014). Improved drug designing and advancement against leishmaniasis is necessary at the current juncture if we want to continue the battle against the emerging drug-resistant variants of the deadly pathogen. New drug targets and the designing of novel compounds against the newly identified drug targets are necessary for clinical trials and toxicity studies (Table 2) (Hefnawy et al. 2017). Unfortunately, there has been little progress in developing alternative methods for managing *leishmaniasis* (Flórez et al. 2010; Pawar et al. 2014; Mol et al. 2015). Bioinformatic analysis can significantly reduce costs associated with the expensive clinical trials by identifying and analyzing drug candidates to the existing drug targets and also help us to identify new targets *in silico*. Bioinformatic analysis can facilitate characterization of the identified drug candidates and also predict their side effects and resistance. The analysis of the high-throughput genomics, proteomics, and metabolomics data through Bioinformatics may significantly contribute towards the discovery of new drugs against leishmaniasis (Xia 2017; Dos et al. 2018). Therefore, in this era of the constant onset of drug-resistant pathogens, bioinformatics can be used to accelerate the development of novel drugs against leishmaniasis.

Table 1 List of natural compounds showing antileishmanial activity

Family and Scientific name	Plant part	parasite tested*	References
Annonaceae			
Annona crassifora	Stem, root, bark	*LB, LD*	Brígido et al. 2000
Annona foetida	bark	*LB*	Brígido et al. 2000
Annona mucosa	Leaves, seed	*LB*	Brígido et al. 2000
Annona glabra	Leaves	*LD, LA*	Brígido et al. 2000
Annona purpurea	bark	*LD*	Brígido et al. 2000
Apocynaceae			
Aspidosperma spruceanum	Stem, bark, leaves	*LI*	Reina et al. 2014
Aspidosperma desmanthum	Stem, bark, leaves	*LI*	Reina et al. 2014
Peschiera australis	Stem	*LA*	Delorenzi et al. 2001
Pentalinon andrieuxii	Root	*LM*	Lezama-Dávila et al. 2014
Plumeria bicolor	Bark	*LD*	Sharma et al.2011
Himatanthus sucuuba	Bark	*LA*	Castillo et al. 2007
Altsonia scholaris	leaves	*LD*	Sharifi-Rad et al. 2018
Holarrhena curtisii	Leafe extract	*LD*	Kam et al. 1998
Peschiera australis	Stem, bark	*LA*	Delorenzi et al. 2001
Peschiera var. heurkii	Leaves, stem bark	*LA*	Muñoz et al. 1994
Picralima nitida	Seed	*LC*	Iwu et al. 1992
Tabernaemontana obliqua	Leaves	*LM, LA, LB,LB, LI, LD*	Weniger et al. 2001
Araliaceae			
Hedera helix	Leaves	*LM*	Hooshyar et al. 2014
Oreopanax floribundus	Leaves	*LP*	Cardona et al. 2020
Asclepiadaceae			
Gongronema latifolia	Leaves	*LC*	Iwu et al. 1992
Asteraceae			
Acanthospermum hispidum	Whole plant	*LA, LB*	Fournet et al. 1994b
Baccharis salicifolia	Leaves	*LB*	Fournet et al. 1994b
Chersodoma jodopappa	Leaves, Stem	*LA, LB, LD*	Fournet et al. 1994b
Cnicothamnus lorentzii	Leaves, Stem	*LA, LD, LB*	Fournet et al. 1994b
Echinacea purpurea	Whole plant	*Leishmaniasp.*	Panda and Luyten 2018
Munnozia fournetii	Leaves, Stem	*LD, LB, LA*	Fournet et al. 1994c
Neurolaena lobata	Leaves	*LM*	Berger et al. 2001
Ophryosporus piquerioides	Whole plant	*LA, LD, LB*	Fournet at al. 1994c
Perezia multiflora	Leaves	*LA, LD, LB*	Fournet at al. 1994c
Pterocaulona lopecuroideum	Whole plant	*LD, LB, LA*	Fournet at al. 1994c
Senecio clivicolus	Leaves, Stem	*LA, LD, LB*	Fournet at al. 1994c
Stevia yaconensis	Whole plant	*LD, LA, LB*	Fournet at al. 1994c

Family and Scientific name	Plant part	parasite tested*	References
Vernonia squamulosa	Stem	*LD, LB, LA*	Fournet at al. 1994c
Werneria nubigena	Leaves, Stem	*LB, LD, LA*	Fournet at al. 1994c
Xanthium catharticum	Root, Stem	*LB, LA, LD*	Fournet at al. 1994c
Berberidaceae			
Berberis vulgaris	Root	*LM, LT*	Mahmaudvand et al. 2014
Berberis bumeliaefolia	Bark	*LD, LB, LA*	Fournet et al. 1994b
Berberis cf. laurina	Stem	*LD, LB, LA*	Fournet et al. 1994b
Bombacaceae			
Hubero dendronpatino	Bark	*LP*	Weniger et al. 2001
Burseraceae			
Protium altsonii	leaves	*LA*	Santana et al. 2020
Protium hebetatum	leaves	*LA*	Santana et al. 2020
Celastraceae			
Maytenusi licifolia	Root bark	*LA*	Dos et al. 2013a
Clusiaceae			
Calophyllum brasiliense	Stem bark	*LI*	Da Silva et al. 2021
Crassulaceae			
Bryophyllum pinnatum (Lam.) Kurz	Leaves	*LA*	Rossi et al. 2000
Cucurbitaceae			
Coccinia grandis	Leaves	*LD*	Lahiry et al. 2018
Dilleniaceae			
Dillenia philippinensis	Stem	*LM*	Macahig et al. 2011
Euphorbiaceae			
Croton cajucara Benth	Bark	*LA*	Lima et al. 2015
Fabaceae			
Acacia nilotica	Bark	*LD*	Ali et al. 2021
Millettia richardiana	Stem bark	*LD*	Rajemiarimiraho et al.2014
Desmodium gangeticum L.	Whole plant	*LD*	Mishra et al. 2005
Gentianaceae			
Swertia chirata	Whole plant	*LD*	Medda et al. 1999
Lauraceae			
Endlicheria bracteolata	Leaves	*LA*	Rottini et al. 2019
Nectandra hihua	Stem bark, leaves	*LI*	Bosquiroli et al. 2017
Nectandra oppositifolia	Twig	*L. (L.) infantum chagasi*	Da Costa-Silva et al. 2019
Liliaceae			
Allium sativum L.	Bub	*LT*	Mahmoudvand et al. 2016
Melastomaceae			
Miconia langsdorffii	Ariel part	*LA*	Peixoto et al. 2011
Tibouchina paratropica	Ariel part	*LD*	Tracanna et al. 2015

Family and Scientific name	Plant part	parasite tested*	References
Meliaceae			
Azadirachta indica	Bark, Leaves, Seed	*LD*	Chouhan et al. 2015
Carapa guianensis	Seed oil	*LA*	Oliveira et al. 2018
Menispermaceae			
Cissampelos sympodialis Eichl.	Leaves	*LC*	Cavalcanti da Silva et al. 2012
Chondodendron tomentosum	Bark	*LI*	González-Coloma et al. 2012
Chasmanthera dependens	Stem, Bark	*LA*	Githinji et al. 2010
Tinospora sinensis	Stem	*LD*	Singh et al. 2008
Cissampelos sympodialis	Leaves	*LC*	Cavalcanti da Silva et al. 2015
Moraceae			
Pourouma guianensis	Leaves	*LA*	Torres-Santos et al. 2004
Moringaceae			
Moringa Oleifera	Flowers	*LD*	Singh et al. 2015
Myristicaceae			
Otoba novogranatensis	Leaves	*LA, LB, LI, LA, LB, LI*	Weniger et al. 2001
Otoba parvifolia	Bark	*LA, LB*	Weniger et al. 2001
Virola surinamensis	Leaves	*LD*	Barata et al. 2000
Myrsinaceae			
Maesabalansae	Leaves	*LD*	Maes et al. 2004
Papaveraceae			
Bocconia integrifolia	Leaves, Stem bark	*LA, LB, LD*	Fournet et al. 1994c
Bocconia pearcei	Leaves	*LA, LB, LD*	Fournet et al. 1994c
Bocconia pearcei	Fruit	*LM*	Fuchino et al. 2010
Phytolaccaceae			
Petiveriaalliaceae L	Leaves	*LA*	Garcia et al. 2017
Piperaceae			
Piper longum L	Ariel part	*LD*	Singh et al. 2011
Piper auritum	Ariel part	*LD, LM, LD, LB*	Monzote et al. 2010
Rubiaceae			
Corynanthe mayumbensis	Stem, Bark	*LI*	Lamidi et al. 2005
Rutaceae			
Citrus sinensis	Leaves	*LA*	Garcia et al. 2017
Galipea longiflora	Leaves, Root bark	*LD, LB, LA*	Fournet et al. 1994a
Swinglea glutinosa	Bark	*LA, LB, LI*	Weniger et al. 2001
Sapindaceae			
Dodonaea viscosa	Leaves	*LA*	Al-Sokari et al. 2015
Cupania dentate	Bark	*LM*	Peraza-Sánchez et al. 2007
Scrophulariaceae			
Conobea scoparioides	Leaves	*LA, LB*	Weniger et al. 2001

Family and Scientific name	Plant part	parasite tested*	References
	Solanaceae		
Brugmansia suaveolens	Flowers, Leaves	*LA*	Monzote et al. 2016
Brunfelsia cestroides	Leaves, Stem	*do*	Monzote et al. 2016
Capsicum annum.	Root	*do*	Monzote et al. 2016
Capsicum frutescens L.	Root	*do*	Monzote et al. 2016
Capsicum chinense	Root	*do*	Monzote et al. 2016
Cestrum nocturnum L.	Root	*do*	Monzote et al. 2016
Nicotiana rustica L.	Root	*do*	Monzote et al. 2016
Solanum americanum	Leaves	*do*	Monzote et al. 2016
Solanum lycopersicon	Fruits	*do*	Monzote et al. 2016
	Sterculiaceae		
Corchorus capsularis L.	Leaves	*do*	Pramanik et al. 2019
	Ulmaceae		
A. edentulaKuhlm	Stem bark	*LB*	Fournet et al. 1994b
	Verbenaceae		
Vitex heterophylla	Leaves	*LD*	Bhakuni et al. 1988

** LA (L. amazonensis), LC (L. chagasi), LB (L. braziliensis), LI (L. infantum), LD (L. donovani), LM (L. maxicana), LT (L.tropica), LM (L. panamensis)*

Table 2 Antileishmanial compounds and mechanism of action

Plant derived product	Mechanism of action	Reference
Quercetin	Inhibits *Leishmania amazonensis*arginase	Da-Silva et al. 2012
	Generation of reactive oxygen species and mitochondrial disruption of *L. amazonensis* promastigotes	Fonseca-Silva et al. 2011
	Inhibits topoisomerase II in leishmania sp.	Mittra et al.2000; Cortázar et al. 2007
Leteolin	Inhibits *L. donovani*topoisomerase II	Mittra et al. 2000
Licochalcone A	Disrupts the function and ultrastructure of leishmanial mitochondria	Zhai et al. 1995
	Inhibits leishmania Fumarate Reductase	Chen et al. 2001
leiocarpin	Disrupts mitochondrial membrane potential	Morais et al. 2020
Amarogentin	Inhibits L. donovani DNA-topoisomerase I	Ray et al. 1996
Plumbagin	Inhibits trypanothione reductase in leishmania	Sharma et al. 2012
Diphyllin	Inhibits parasite phagocytosis by macrophages	Di Giorgio et al. 2005
Artemisinin	Heme-triggered activation of Artemisinin	Geroldinger et al. 2020
Epigallocatechin 3-gallate	Inhibits *leishmania* arginase	Dos et al. 2013b; Khademvatan et al. 2019

Other natural compounds which have been explored are marine sources or microorganisms. For example, a protein containing carbohydrate moiety extracted from the sponge *Pachymatisma johnstonii*, demonstrated strong efficacy against *Leishmania braziliensis*, *Leishmania mexicana*, and *Leishmania donovani*, under *in-vitro* conditions. Another fungal metabolite, aphidicolin extracted from *Nigrospora sphaerica*, has also been found to suppress *L.donovani* amastigotes and promastigotes growth (Chan-Bacab and Peña-Rodríguez 2001). Nonetheless, plants have been the most explored natural source. The different classes of natural compounds explored have been discussed subsequently.

8.1 Flavonoids

The flavonoids, quercetin, and Luteolin isolated from a Polygonaceae *(Fagopyrum esculentum*) and a Verbenaceae (*Vitex*

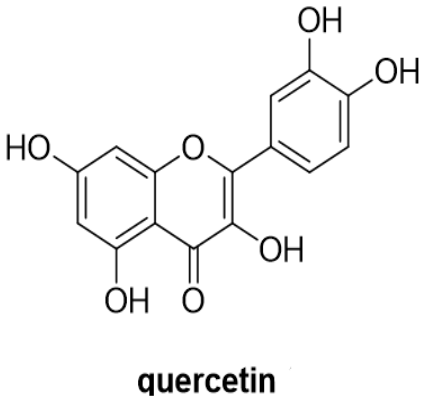
negundo) are effective antileishmanial compounds having IC50 as 12.5 micromoles and 14.5 micromoles respectively, against amastigote form of *Leishmania donovani*. Both these drugs are capable of stimulating topoisomerase II-dependent cleavage of kinetoplastid DNA in *Leishmania* donovani. Studies in animal models indicate that Luteolin gives protection of up to 80 percent in the *Leishmania* infected spleen when treated with 3.5mg/kg of body weight. Similarly, quercetin reduces parasite load up to ninety percent at a dosage of 14 mg/Kg weight of the body. Luteolin is nontoxic to human T-cells (Mittra et al. 2000). It is reported that quercetin and luteolin (figure 4) specifically inhibit a typical bi-subunit topoisomerase (topoisomerase I) present in *Leishmania* parasites (da Silva et al. 2012). The phenolic compound"5,7,4'-trihydroxyflavan" shows anti-amastigotes activity against *L. amazonensis* (Fonesca-Silva et al. 2016). The bioflavonoids "podocarpusflavone A", amentoflavone, and podocarpusflavone B, extracted from *C. maxicanum* leaves, show a mild action when used against *L. donovani* (Chang Bacab and Peña-Rodríguez 2001).

8.2 Chalcones

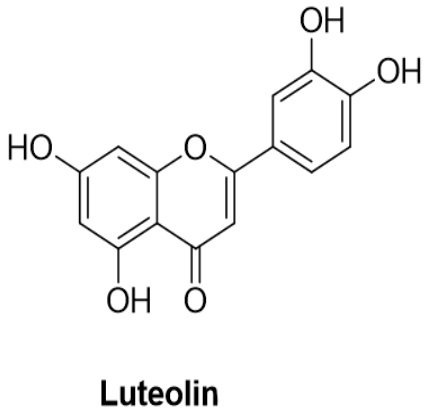
Licochalcone A (Figure 5) is an oxygenated chalcone that has been obtained from Chinese liquorice *Glycyrrhiza spp*. (Fabaceae), shows robust activity against leishmania, by preventing the proliferation of amastigote and promastigotes of *Leishmania donovani* and *Leishmania major* (Chen et al. 1993; Croft et al. 2006). A 96% reduction in the number of the parasite in the spleen and liver was seen under *in vivo* studies in hamsters (Chen et al. 1994; Croft et al. 2006). Licochalcone A and associated chalcones can destroy the mitochondrial ultrastructure of the *Leishmania* parasite. Moreover, the compounds can strongly inhibit Fumarate Reductase in *L. major* and trypanothione reductase in *L. donovani* (Chen et al. 2001; Ortalli et al. 2018). 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" is toxic towards *Leishmania donovani* promastigotes, while "2',6'-dihydroxy-4'-methoxychalcone", obtained from flowers of "*Piper aduncum"*, is shown to exhibit a considerable *in vitro* action towards amastigotes and promastigotes of *L. amazonensis* (Chang Bacab and Peña-Rodríguez 2001)

8.3 Saponin

Mesabalides I-VI, all the six oleane tri-terpenoid saponins extracted from *Maesabalansae* (Myrsinaceae) has been reported to have potent *antileishmanial* action. Maesabalide III and IV (Figure 6) were most effective having IC(50) values against

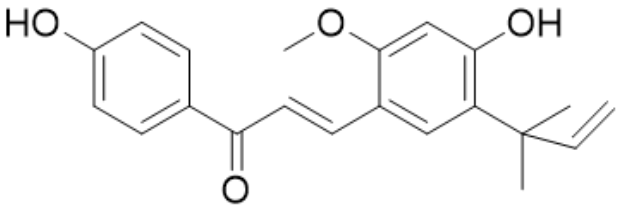


quercetin



Luteolin

Figure 4 Quercetin and Luteolin



Licochalcone A

Figure 5 Licochalcone A

amastigotes residing inside the macrophages at a dosage of 7ng/ml and 14 ng/ml respectively far less as compared to standard drug sodium stibogluconate (IC50, 5.6 µg/ml) for treating *Leishmania* infection (Germonprez et al. 2005). Similarly, a comparable efficiency of mesabalide III (0.8mg/kg for 1 day) to Amphotericin-B (5 mg/kg for one day) has been demonstrated in *L. donovani* infected hamsters (Maes et al. 2004). Racemoside A (Figure 6), a steroidal saponin derived from *Asparagus racemosus* (Liliaceae), was capable to cause apoptosis in *Leishmania donovani* amastigotes (Dutta et al. 2007). Asteroidal saponin extracted from bulbs of *Allium paradoxum* exhibited an anti-leishmanial effect by directly killing *L.major* promastigotes (Rezaee et al. 2018). These saponins decrease the parasitic membrane potential and inhibit the growth of promastigotes. However, the cytotoxic activity of some antileishmanial saponins on the host cell is quite concerning (Chan-Bacab and Peña-Rodríguez 2001).

8.4 Terpenes

Linalool (Figure 7), a monoterpene derived from a Euphorbiaceae, *Croton cajucara*, shows potent action against *L. amazonensis* (both promastigotes & amastigotes). Linalool was able to decrease the intracellular parasite burden by 50% in infected macrophages. It also increases NO production. *In vitro* mitochondrial swelling and destruction of chromatin and kinetoplastid was observed, ultimately undergoing cell lysis (do Sorocco et al. 2003). Another

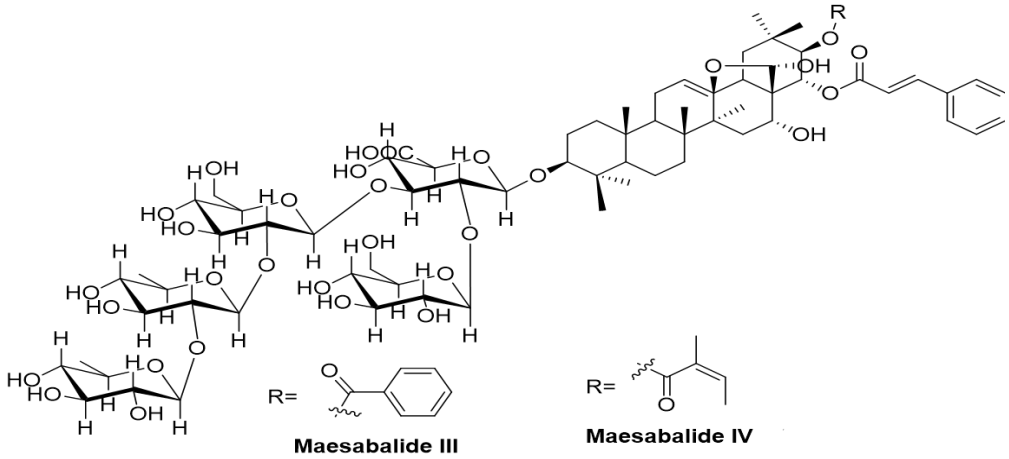


Figure 6 Maesabalide III, Maesabalide IV, Recemoside A

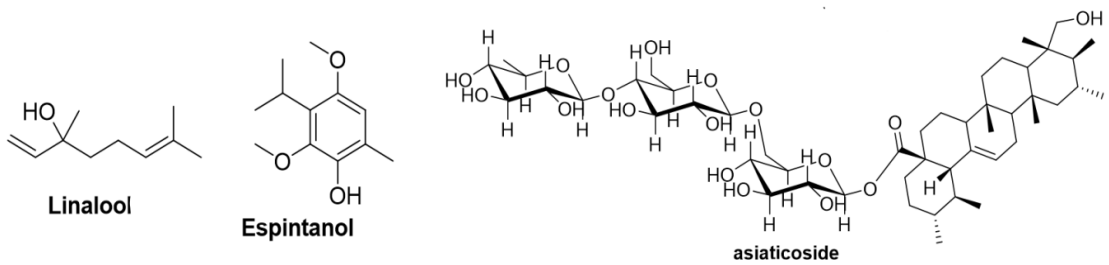


Figure 7 Linalool, Espintanol, Asiaticoside

monoterpenoid, Espintanol (Figure 7) obtained from *Oxandraespintana* (Annonaceae) bark, show strong efficacy against promastigotes of 12 different species of Leishmania. Nevertheless, *in-vivo* efficacy was very weak. Two monoterpenoid derivatives grifolin and piperogalin extracted from *Peperomia* completely lyses *L. donovani* and *L. braziliensis* promastigotes at an amount of 100 microgram/ml. Diterpenoids also show leishmanicidal activity. Jatrogrossidione and jatrophone, extracted from Euphorbiaceae species exhibit toxicity against different species of Leishmania parasite (*L. braziliensis, L. amazonensis, L. chagasi.)* promastigotes. 6-β-hydroxyrosenonolactone is another diterpene derived from *Holarrhena floribunda* (Apocynaceae) bark, showing moderate activity against *L. donovani*, and is less cytotoxic to host macrophage. Triterpenes are also another group of metabolites showing antileishmanial action, which consists of betulinaldehyde and ursolic acid, isolated from *Doliocarpus dentatus* (Dilleniaceae) stem and *Jacaranda copaia* bark, respectively. Both the compounds showed efficacy against *L. amazonensis* amastigotes. However, *in-vivo* results were not satisfactory and cytotoxicity was also a concern. Two new terpenoids named sugikurojin A and asiaticoside (figure 7) synthesized from *Cryptomeria japonica* and *Centellaasiatica* show antileishmanial activity against *L. infantum* (IC50 value-14.0 µM) and *Leishmania donovani* (IC50-11.2µM) (Bhaumik et al. 2012).

8.5 Iridoids

Amarogentin, a secoiridoid glycoside extracted from *Swertia chirata* (Gentianaceae) and the iridoid Molucidin (Figure 8) extracted from *M. lucida*, show strong *in-vivo* antileishmanial activity. Amarogentin potentially inhibits *Leishmania* topoisomerase-I (Ray et al. 1996). The non-ionic surfactant vesicle system i.e. niosomal formulation decreased the parasite numbers by ninety percent in the spleen after treating for six days with 2.5 mg per of the drug. Pathological studies, staining of histological slides, and the amount of certain liver enzymes show minimum cytotoxic activity.

8.6 Napthoquinone

Plumbagin, a naphthoquinone extracted from the Euphorbiaceae *"Perabenensis"*, inhibits the growth of intracellular amastigotes and promastigotes of *L. donovani*. Another bis-naphthoquinone, Diospyrin (Figure 9) (a semi-synthetic derivative) isolated from *Diospyros Montana* Roxb also shows potent activity against leishmania parasite under *in-vitro* and *in-vivo* studies (Hazra et al. 2013).

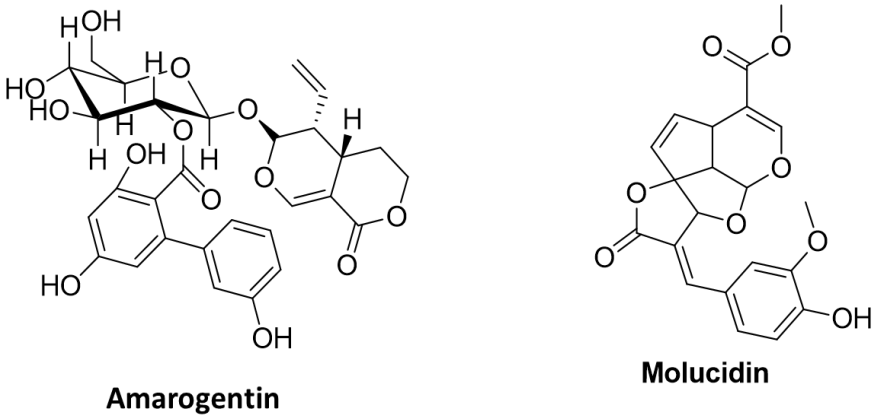


Figure 8 Amarogentin, Molucidin

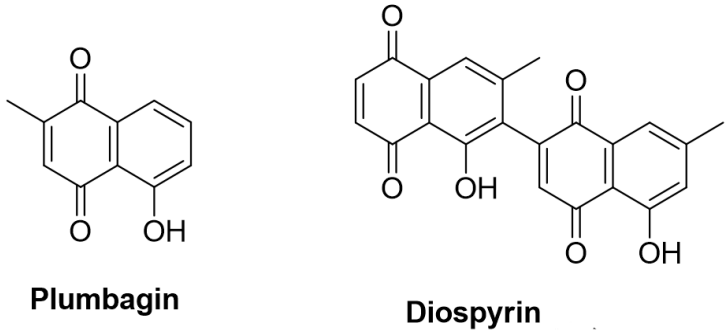


Figure 9 Plumbagin, Diosyrin

8.7 Quinoline Alkaloids

2- Substitute quinolines extracted from "*G. longiflora"* have been shown to exert potent activity against CL and VL. It is reported that China mine B (Figure 10) treatment at 50 mg/kg body reduced the lesion size by seventy-four percent and the leishmania burden by ninety percent in the cutaneous leishmaniasis mice model with a regime of 5 injections, each given at 4 days of interval (Fournet et al. 1994a). Treating with Chinamine D (Figure 10) resulted in eighty-seven percent hepatic parasite reduction (at a dose of 100 mg/kg of body weight for 5 days). "2-n-propylquinoline" lowers the parasite burden in the liver by 99.9 percent in an animal model of VL at a dose of 94 mg/kg body weight for ten days 10 days (Fournet et al. 1992). Recent studies with several derivatives of synthetic quinoline presented a notable decrease in the parasite number (80-90% in the lesions of *L. donovani* and *L.amazonensis* infected mice when treated orally with 25 mg/kg body weight for 10 days, twice daily (Coimbra et al. 2016).

8.8 Lignans

Diphyllin (Figure 11), a lignan derived from *H.bucharicum* (Rutaceae) showed leishmanicidal efficacy against *L. infantum* with IC50 values of 14.4 microgram/kg4.4 µg/ml and 0.2 µg/ml correspondingly. The derivative "s-ketosulfide (3,4-dimethoxy)-8-(40-methylthiophenoxy)- propiophenone of the neolignan 3,4,5-trimethoxy-8-[20,60-dimethoxy-40-(E)-propenylphenoxy]-phenylpropane" extracted from Myristicaceae (*Virolapavonis)* has shown moderate activity against the promastigote and amastigote form of *L. donovani* (Polonio and Efferth 2008). Another two important lignan glycosides (lyoniside and saracoside) and lignin derivative- niranthin kill *L. donovani* both under *in-vivo* and *in-vitro* conditions by inhibiting parasite-specific topoisomerases (Saha et al. 2013).

8.9 Toxoid

10-Deacetylbaccatin III, a toxoid isolated from the Taxaceae family (*T. baccata*) shows potent leishmanicidal efficacy against *L. donovani* amastigotes. It has been reported that 10-Deacetylbaccatin III is non-toxic to the host macrophages as much as a concentration of 5 µM. 10-deacetylbaccatin produces Nitric oxide to show antileishmanial activity (Polonio and Efferth 2008).

8.10 Sesquiterpenes

Artemisinin (Figure 12), an effective anti-malarial drug, extracted from *Artemisia annua* (Asteraceae) shows antileishmanial activity. The IC50 values are 22 µM against intracellular amastigotes and 160 µM against promastigotes. Dehydrozaluzanin C (Figure 12), a sesquiterpene lactone extracted from *Munnozia maronii* (Asteraceae) leaves, blocks the promastigote survival in 11 different *Leishmania* (at concentration raging 2.5 to 10 µg /ml). This has also been seen under *in-vivo* conditions through the reduction of lesion severity due to *L. amazonensis* infection in mice (Chan-Bacab and Peña-Rodríguez 2001; Polonio and Efferth 2008). In addition, Sesquiterpenes-rich compounds extracted from *Copaifera* spp. Showed leishmanicidal efficacy against *L. amazonensis* intracellular amastigotes (Soares et al. 2013).

Figure 10 ChimanineB, Chimanine D

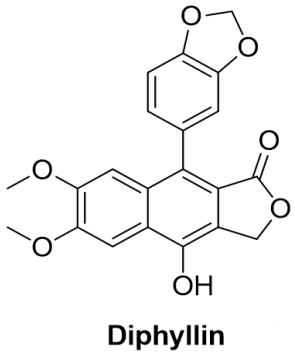


Figure 11 Diphyllin

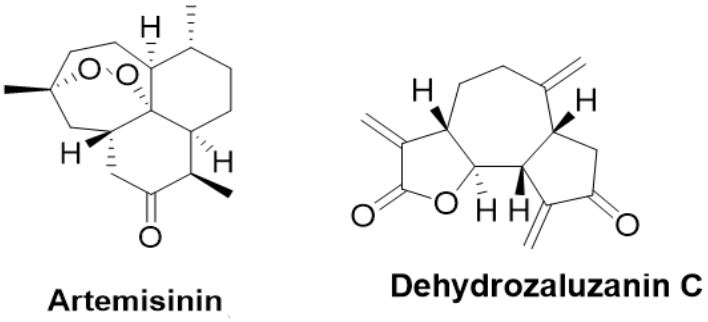


Figure 12 Artemisinin, Dehydrozaluzanin C

9 Investigational Drugs against Leishmaniasis

Among several natural products, which have shown potent anti-leishmanial activity, few have reached phase II trials. Two nitroimidazole compounds, PA-824, and fexinidazole have shown potent anti-leishmanial responses in pharmacokinetic studies in humans. Also, derivatives of quinoline scaffold, like Indolyl quinoline analogs and the Naphthoquinones derivatives like Buparvaquone currently have been listed for phase II trials (Sundar and Chakravarty 2015b).

Conclusion

There has been a lot of research for an effective drug or vaccine against *Leishmaniasis*, but the search for a non-toxic, cost-effective and highly potent drug or vaccine is still going on. Still, an effective vaccine against leishmania is lacking. Drugs based on Pentavalent antimony compounds are still the main course drugs. Nevertheless, the persistence of side effects and the growing drug resistance are still a matter of great concern. This advocates the urgent need of developing alternative drugs. Plant-based or plant-extracted materials may probably provide an important resource of new medicinal drugs, which could be used as alternative therapeutic strategies. So, research must be undertaken to screen natural products, especially, plant-derived products for probable use in *Leishmania* therapy. They have the advantage of having fewer side effects and low cost. Though initial studies are abundant, there is more need to further do research, isolate lead compounds and study the mechanism of action. Despite so many encouraging findings, these compounds have not been able to make it in the market or even in clinical trials. We advocate that the authorities should encourage more product-oriented initiatives along with R&D for this poor man's disease.

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