Leishmaniasis: Plants as a source of antileishmanial agents

Manoj Kumar Singh\textsuperscript{1,*}, Arpita Das\textsuperscript{1}, Rudra P Saha\textsuperscript{1}, Joydeep Paul\textsuperscript{1}, Debkumar Nandi\textsuperscript{2}

\textsuperscript{1}Department of Biotechnology, Adamas University, Barasat-Barrackpore road, Barabara, P.0 Jagannathpur District- 24 Parganas (North), Kolkata-700126, West Bengal, India
\textsuperscript{2}TCG Life sciences Private Limited, Chembiotek, Salt Lake Electronics Complex, BN Block, Sector V, Kolkata, West Bengal 700091

Received – July 24, 2021; Revision – January 10, 2022; Accepted – January 28, 2022
Available Online – February 28, 2022
DOI: http://dx.doi.org/10.18006/2022.10(1).227.247

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. Leishmaniasis 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.

* Corresponding author
E-mail: manoj.k.singh@adamasuniversity.ac.in (Manoj Kumar Singh)

All the articles published by Journal of Experimental Biology and Agricultural Sciences are licensed under a Creative Commons Attribution-NonCommercial 4.0 International License Based on a work at www.jebas.org.
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 Leishmania, 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 1st century 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 co-infection (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).

3.2 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.](http://www.jebas.org)
Leishmaniasis: Plants as a source of antileishmanial agents

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 Ożinsky 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 (Rodríguez 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 (SbIII) as an anti-Leishmania 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 Amphotericin 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 (SbV) 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 Shβ as compared to Shβ 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; Oloff et al. 1995; Misra et al. 2001; Mohwabali et al. 2004). Many other vaccine candidates like gp46/M2/Parasite Surface Antigen (Montgomery 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 requirement 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 (Flores 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 |
| **Araliaceae**              |            |                  |            |
| *Holarrhena curtisii*       | Leaves extract | LD  | Kam et al. 1998 |
| *Peschiera australis*       | Stem        | 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, LI, LD | Weniger et al. 2001 |
| **Asclepiadaceae**          |            |                  |            |
| *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 | Leishmania sp.  | Panda and Luyten 2018 |
| *Mannizia fournietii*       | Leaves, Stem | LD, LB, LA | Fournet et al. 1994c |
| *Neuraena lobata*           | Leaves      | LM               | Berger et al. 2001 |
| *Ophryosporus piquerioides* | Whole plant | LA, LD, LB  | Fournet et al. 1994c |
| *Perezia multiflora*        | Leaves      | LA, LD, LB       | Fournet et al. 1994c |
| *Pterocaulona lopecearoides*| Whole plant | LD, LB, LA  | Fournet et al. 1994c |
| *Seneio clivicolus*         | Leaves, Stem | LA, LD, LB | Fournet et al. 1994c |
| *Stevia yaconensis*         | Whole plant | LD, LA, LB      | Fournet et al. 1994c |
| Family and Scientific name | Plant part | parasite tested* | References |
|----------------------------|------------|------------------|------------|
| Vernonia squamulosa | Stem | LD, LB | Fournet et al. 1994c |
| Werneria nubigena | Leaves, Stem | LB, LD, LA | Fournet et al. 1994c |
| Xanthium catharticum | Root, Stem | LB, LA, LD | Fournet et al. 1994c |
| Berberidaceae | | | |
| Berberis vulgaris | Root | LM, LT | Mahmaudvand et al. 2014 |
| Berberis buneliaefolia | 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 | | | |
| Mayrenusi 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 |
| Coccinia grandis | Leaves | LD | Lahiry et al. 2018 |
| Dilleniaceae | | | |
| Dillenia philippinensis | Stem | LM | Macahig et al. 2011 |
| Euphorbiaceae | | | |
| Croton cajucaera Benth | Bark | LA | Lima et al. 2015 |
| Fabaceae | | | |
| Acacia nilotica | Bark | LD | Ali et al. 2021 |
| Millettia richardiana | Stem bark | LD | Rajemiariimiraho 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 | Bosquirol 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          | Fournet et al. 1994c |
| *Bocconia pearcei*         | Fruit      | LM              | Fuchino et al. 2010 |
| **Phytolaccaceae**         |            |                  |            |
| *Petiveriaalliiaceae 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 scoparioide*      | Leaves     | LA, LB          | Weniger et al. 2001 |
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* Family and Scientific name | Plant part | parasite tested* | References
--- | --- | --- | ---
*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* | Root | do | Monzote et al. 2016
*Capsicum chinense* | Root | do | Monzote et al. 2016
*Cestrum nocturnum* | Root | do | Monzote et al. 2016
*Nicotiana rustica* | 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. edentula Kuhlm* | 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)

| Plant derived product | Mechanism of action | Reference |
|-----------------------|---------------------|-----------|
| Quercetin | inhibits *Leishmania amazonensis* arginase | Du-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; Cortáz et al. 2007 |
| Luteolin | inhibits *L. donovani* topoisomerase II | Mittra et al. 2000 |
| Licorhclalcone 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 |
| Diphyllell | 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 |
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 “podocarpsflavone 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-dihydroxy-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 IC50 values against...
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). Steroidal 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-Rodriguez 2001).

### 8.4 Terpenes

Linalool (Figure 7), a monoterpen 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...
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 lyse 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-hydroxyrosenolactone 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 Dolichocarpus 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 Centella asiatica 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-napthoquinone, 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).
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-Deacetylbbaccatin III, a toxoid isolated from the Taxaceae family (T. baccata) shows potent leishmanicidal efficacy against L. donovani amastigotes. It has been reported that 10-Deacetylbbaccatin III is non-toxic to the host macrophages as much as a concentration of 5 μM. 10-deacetylbbaccatin 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).
Leishmaniasis: Plants as a source of antileishmanial agents

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 Leishmania, 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.

Acknowledgments

We thank Mr. Titas Ghosh (Research scholar, Department of Biotechnology, Adamas University) for his help in preparing the diagrams.

References

Akarid, K., Arnoult, D., Micic-Polianski, J., Sif, J., et al. (2004). Leishmania major-mediated prevention of programmed cell death induction in infected macrophages is associated with the repression of mitochondrial release of cytochrome c. Journal of Leukocyte Biology, 76(1), 95–103.

Ali, R., Tabrez, S., Rahman, F., et al. (2021). Antileishmanial Evaluation of Bark Methanolic Extract of Acacia nilotica: In Vitro and In Silico Studies. ACS Omega, 6(12), 8548–8560.

Al-Sokari, S. S., Ali, N. A., Monzote, L., & Al-Fatimi, M. A. (2015). Evaluation of Antileishmanial Activity of Albaha Medicinal Plants against Leishmania amazonensis. BioMed Research International, 2015, 938747.

Alvar, J., Aparicio, P., Aseffa, A., et al. (2008). The relationship between leishmaniasis and AIDS: the second 10 years. Clinical Microbiology Reviews, 21(2), 334–359.

Barata, L. E., Santos, L. S., Ferri, H.P., et al. (2000). Anti-leishmanial activity of neolignans from Virola species and synthetic analogues. Phytochemistry, 55(6), 589–595.

Berger, I., Passreiter, C. M., Cáceres, A., & Kubelka, W. (2001). Antiprotozoal activity of Neurolaena lobata. Phytotherapy Research : PTR, 15(4), 327–330.

Berman J. D. (1997). Human leishmaniasis: clinical, diagnostic, and chemotherapeutic developments in the last 10 years. Clinical Infectious Diseases, 24(4), 684–703.

Bhakuni D.S., Goel A.K., Jain S., et al. (1988) Screening of Indian plants for biological activity. Part XIII. Indian Journal of Experimental Biology, 26, 883–904.

Bhaumik, S. K., Paul, J., Naskar, K., Karmakar, S., & De, T. (2012). Asiaticoside induces tumour-necrosis-factor-α-mediated nitric oxide production to cure experimental visceral leishmaniasis.

Figure 12 Artemisinin, Dehydrozaluzanin C
caused by antimony-susceptible and -resistant Leishmania donovani strains. *The Journal of Antimicrobial Chemotherapy*, 67(4), 910–920.

Blackwell, J. M., & Ulczak, O. M. (1984). Immunoregulation of genetically controlled acquired responses to Leishmania donovani infection in mice: demonstration and characterization of suppressor T cells in noncure mice. *Infection and immunity*, 44(1), 97–102.

Blanchette, J., Racette, N., Faure, R., Siminovich, K. A., & Olivier, M. (1999). Leishmania-induced increases in activation of macrophage SHP-1 tyrosine phosphatase are associated with impaired IFN-gamma-triggered JAK2 activation. *European Journal of Immunology*, 29(11), 3737–3744.

Bosquiroli, L., Dos Santos Ferreira, A. C., Farias, K. S., et al. (2017). In Vitro antileishmanial activity of sesquiterpene-rich essential oils from Nectandra species. *Pharmaceutical Biology*, 55(1), 2285–2291

Brajtburg, J., & Bolard, J. (1996). Carrier effects on biological activity of amphotericin B. *Clinical Microbiology Reviews*, 9(4), 512–531.

Brígido H.P.C., Barbosa J.C., da Silva J.V., et al. (2020). Antileishmanial activity of Annona species (Annonaceae). *SN Applied Sciences*. 2. 10.1007/s42452-020-03340-7.

Bryceson A. (2001). A policy for leishmaniasis with respect to the prevention and control of drug resistance. *Tropical Medicine & International Health*, 6(11), 928–934.

Burza, S., Croft, S. L., & Boelaert, M. (2018). *Leishmaniasis. Lancet (London, England)*, 392(10151), 951–970.

Cardona, G.W., Robledo, S., Alzate, F., et al. (2020). Antileishmanial and cytotoxic activities of four Andean plant extracts from Colombia. *Veterinary World*, 13(10), 2178–2182

Castillo, D., Arevalo, J., Herrera, F., et al. (2007). Spirolactone iridoids might be responsible for the antileishmanial activity of a Peruvian traditional remedy made with *Himatanthus sucauba* (Apocynaceae). *Journal of Ethnopharmacology*, 112(2), 410–414

Cavalcanti da Silva, E., Dias Rayol, C., Medeiros, et al. (2012). Antileishmanial activity of warifteine: a bisbenzylisoquinoline alkaloid isolated from *Cissampelos sympodialis* Eichl.(Menispermaceae). *The Scientific World Journal*, 2012, 516408

Chan-Bacab, M. J., & Peña-Rodríguez, L. M. (2001). Plant natural products with leishmanicidal activity. *Natural Product Reports*, 18(6), 674–688.

Chappuis, F., Sundar, S., Hailu, A., et al. (2007). Visceral leishmaniasis: what are the needs for diagnosis, treatment and control?. *Nature Reviews. Microbiology*, 5(11), 873–882.

Chen, M., Christensen, S. B., Blom, J., et al. (1993). Licochalcone A, a novel antiparasitic agent with potent activity against human pathogenic protozoan species of Leishmania. *Antimicrobial Agents and Chemotherapy*, 37(12), 2550–2556.

Chen, M., Christensen, S. B., Theander, T. G., & Kharazmi, A. (1994). Antileishmanial activity of licochalcone A in mice infected with Leishmania major and in hamsters infected with *Leishmania donovani*. *Antimicrobial Agents and Chemotherapy*, 38(6), 1339–1344.

Chen, M., Zhai, L., Christensen, S. B., Theander, T. G., & Kharazmi, A. (2001). Inhibition of fumarate reductase in Leishmania major and *L. donovani* by chalcones. *Antimicrobial Agents and Chemotherapy*, 45(7), 2023–2029

Chouhan, G., Islamuddin, M., Want, M. Y., et al. (2015). Apoptosis mediated leishmanicidal activity of *Azadirachta indica* bioactive fractions is accompanied by Th1 immunostimulatory potential and therapeutic cure in vivo. *Parasites and Vectors*, 8, 183

Chunge, C. N., Owate, J., Pamba, H. O., & Donno, L. (1990). Treatment of visceral leishmaniasis in Kenya by aminosidine alone or combined with sodium stibogluconate. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 84(2), 221–225.

Coimbra, E. S., Antinarelli, L. M., Silva, et al. (2016). Quinoline derivatives: Synthesis, leishmanicidal activity and involvement of mitochondrial oxidative stress as mechanism of action. *Chemico-Biological Interactions*, 260, 50–57.

Conceição-Silva, F., & Morgado, F. N. (2019). Leishmania Spp-Host Interaction: There Is Always an Onset, but Is There an End?. *Frontiers in Cellular and Infection Microbiology*, 9, 330

Cortázar, T. M., Coombs, G. H., & Walker, J. (2007). Leishmania panamensis: comparative inhibition of nuclear DNA topoisomerase II enzymes from promastigotes and human macrophages reveals anti-parasite selectivity of fluoroquinolones, flavonoids and pentamidine. *Experimental Parasitology*, 116(4), 475–482

Croft, S. L., & Hogg, J. (1988). Limited activity of bacterial DNA topoisomerase II inhibitors against *Leishmania donovani* and *Trypanosoma cruzi* amastigotes in vitro. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 82(6), 856.

Croft, S. L., Steifert, K., & Yardley, V. (2006). Current scenario of drug development for leishmaniasis. *The Indian Journal of Medical Research*, 123(3), 399–410.
Cruz, L., Nieto, J., Moreno, J., et al. (2006). Leishmaniasis/HIV co-infections in the second decade. The Indian Journal of Medical Research, 123(3), 357–388.

da Costa-Silva, T. A., Conserva, G., Galisteo, A. J., Jr, Tempone, A. G., & Lago, J. (2019). Antileishmanial activity and immunomodulatory effect of secophilic subamolide, a butanolide isolated from Nectandra oppositifolia (Lauraceae). The journal of Venomous Animals and Toxins Including Tropical Diseases, 25, e20190008

da Silva, E. R., Maquiaveli, C., & Magalhães, P. P. (2012). The leishmanicidal flavonols quercetin and quercitin target Leishmania (Leishmania) amazonensis arginase. Experimental Parasitology, 130(3), 183–188

Da Silva, L. G., Gomes, K. S., Costa-Silva, T. A., et al. (2021). Calanolides E1 and E2, two related coumarins from Calophyllum brasiliense Cambess. (Clusiaceae), displayed in vitro activity against amastigote forms of Trypanosoma cruzi and Leishmania infantum. Natural Product Research, 35(23), 5373–5377

Das VN, Pandey K, Verma N, et al. (2019). Short report: Development of post-kala-azar dermal leishmaniasis (PKDL) in miltefosine-treated visceral leishmaniasis. American Journal of Tropical Medicine and Hygiene, 80(3):336-8.

de la Loma, A., Alvar, J., Martinez Galiano, E., et al. (1985). Leishmaniasis or AIDS?. Transactions of the Royal Society of Tropical Medicine and Hygiene, 79(3), 421–422.

Delorenzi, J. C., Attias, M.,Gattass, C. R., et al. (2001). Antileishmanial activity of an indole alkaloid from Peschieraaustralis. Antimicrobial Agents and Chemotherapy, 45(5), 1349–1354

Desjeux P, & Alvar J. (2003). Leishmaniasis/HIV co-infections: epidemiology in Europe. Annals of Tropical Medicine & Parasitology, 97 (Suppl 1), 3-15.

Di Giorgio, C., Delmas, F., Akhmedjanova, V., et al. (2005). In vitro antileishmanial activity of diphyllyn isolated from Haplophyllumbucharicum. Planta Medica, 71(4), 366–369

do Socorro S Rosa, Mendonça-Filho, M., R. R., Bizzo, et al. (2003). Antileishmanial activity of a linalool-rich essential oil from Croton caju-cara. Antimicrobial Agents and Chemotherapy, 47(6), 1895–1901.

Donovan, M. J., Maciuba, B. Z., Mahan, C. E., & McDowell, M. A. (2009). Leishmaniasis infection inhibits cycloheximide-induced macrophage apoptosis in a strain-dependent manner. Experimental Parasitology, 125(1), 58–64.

Dos Reis, M. B., Manjolin, L. C., Maquiaveli, C., Santos-Filho, O. A., & da Silva, E. R. (2013a). Inhibition of Leishmania (Leishmania) amazonensis and rat arginases by green tea EGCG, (+)-catechin and (-)-epicatechin: a comparative structural analysis of enzyme-inhibitor interactions. PloS one, 8(11), e78387

Dos Santos Vasconcelos, C. R., de Lima Campos, T., & Rezende, A. M. (2018). Building protein-protein interaction networks for Leishmania species through protein structural information. BMC bioinformatics, 19(1), 85.

Dos Santos, V. A., Leite, K. M., da Costa Siqueira, M., et al. (2013b). Antiprotozoal activity of quinomethide triterpenes from Maytenusilicifolia (Celastraceae). Molecules (Basel, Switzerland), 18(1), 1053–1062

Dutta, A., Ghoshal, A., Mandal, D., et al. (2007). Racemoside A, an anti-leishmanial, water-soluble, natural steroidal saponin, induces programmed cell death in Leishmania donovani. Journal of medical microbiology, 56(Pt 9), 1196–1204.

Flórez, A. F., Park, D., Bhak, J., et al. (2010). Protein network prediction and topological analysis in Leishmania major as a tool for drug target selection. BMC Bioinformatics, 11, 484.

Fonseca-Silva, F., Inacio, J. D., Canto-Cavalheiro, M. M., & Almeida-Amaral, E. E. (2011). Reactive oxygen species production and mitochondrial dysfunction contribute to quercetin induced death in Leishmania amazonensis. PLoS one, 6(2), e14666

Fonseca-Silva, F., Inacio, J. D., Canto-Cavalheiro, M. M., et al. (2016). Oral Efficacy of Apigenin against Cutaneous Leishmaniasis: Involvement of Reactive Oxygen Species and Autophagy as a Mechanism of Action. PLoS Neglected Tropical Diseases, 10(2), e0004442.

Fournet, A., Angelo, A., Muñoz, V., Roblot, F., Hocquemiller, R., et al. (1992). Biological and chemical studies of Perabenensis, a Bolivian plant used in folk medicine as a treatment for cutaneous leishmaniasis. Journal of Ethnopharmacology, 37(2), 159–164.

Fournet, A., Barrios, A. A., & Muñoz, V. (1994a). Leishmanicidal and trypanocidal activities of Bolivian medicinal plants. Journal of Ethnopharmacology, 41(1-2), 19–37.

Fournet, A., Barrios, A. A., Muñoz, V., Hocquemiller, R., Roblot, F., et al. (1994b). Antileishmanial activity of a tetralone isolated from Ampelocereadentula, a Bolivian plant used as a treatment for cutaneous leishmaniasis. Planta Medica, 60(1), 8–12

Fournet, A., Gantier, J. C., Gautheret, A., et al. (1994c). The activity of 2-substituted quinoline alkaloids in BALB/c mice.
infected with Leishmania donovani. The Journal of Antimicrobial Chemotherapy, 33(3), 537–544.

Fragaki, K., Sufia, I., Ferrua, B., et al. (2001). Immunisation with DNA encoding Leishmania infantum protein papLe22 decreases the frequency of parasiticemic episodes in infected hamsters. Vaccine, 19(13-14), 1701–1709.

Fuchino, H., Kawano, M., Mori-Yasumoto, K., et al. (2010). In vitro leishmanicidal activity of benzophenanthridine alkaloids from Bocconia pearcei and related compounds. Chemical & Pharmaceutical Bulletin, 58(8), 1047–1050

Ganguly, S., Das, N. K., Barbhuiya, J. N., & Chatterjee, M. (2010). Post-kala-azar dermal leishmaniasis—an overview. International Journal of Dermatology, 49(8), 921–931.

Garcia, A. R., Amaral, A., Azevedo, M., et al. (2017). Cytotoxicity and anti-Leishmania amazonensis activity of Citrus sinensis leaf extracts. Pharmaceutical Biology, 55(1), 1780–1786

Gedda, M. R., Singh, B., Kumar, D., et al. (2020). Post kala-azar dermal leishmaniasis: A threat to elimination program. PLoS Neglected Tropical Diseases, 14(7), e0008221

Germónprez, N., Maes, L., Van Puyvelde, L., et al. (2005). In vitro and in vivo anti-leishmanial activity of triterpenoid saponins isolated from Maesabalansae and some chemical derivatives. Journal of Medicinal Chemistry, 48(1), 32–37.

Geroldinger, G., Tonner, M., Quirgst, J., et al. (2020). Activation of artesiminin and heme degradation in Leishmania tarentolae promastigotes: A possible link. Biochemical Pharmacology, 173, 113737

Ghorbani, M., & Farhoudi, R. (2017). Leishmaniasis in humans: drug or vaccine therapy? Drug Design, Development and Therapy, 12, 25–40

Githinji, E. K., Irungu, L. W., Tonui, W. K., et al. (2010). In vitro effects of Warburgia ugandensis, Psidium puncultata and Chasmanthera dependens on Leishmania major promastigotes. African Journal of Traditional, Complementary, and Alternative Medicines, 7(3), 264–275

González-Coloma, A., Reina, M., Sáenz, C., et al. (2012). Antileishmanial, antitrypanosomal, and cytotoxic screening of ethnopharmacologically selected Peruvian plants. Parasitology research, 110(4), 1381–1392

Gradoni L. (2001). An update on antileishmanial vaccine candidates and prospects for a canine Leishmania vaccine. Veterinary Parasitology, 100(1-2), 87–103.

Gramiccia, M., & Gradoni, L. (2005). The current status of zoonotic leishmaniases and approaches to disease control. International Journal for Parasitology, 35(11-12), 1169–1180.

Guerin, P. J., Olliaro, P., Sundar, S., et al. (2002). Visceral leishmaniasis: current status of control, diagnosis, and treatment, and a proposed research and development agenda. The Lancet Infectious diseases, 2(8), 494–501.

Handman, E., Button, L. L., & McMaster, R. W. (1990). Leishmania major: production of recombinant gp63, its antigenicity and immunogenicity in mice. Experimental Parasitology, 70(4), 427–435.

Hazers, S., Ghosh, S., Das Sarma, et al. (2013). Evaluation of a diospyrin derivative as antileishmanial agent and potential modulator of ornithine decarboxylase of Leishmania donovani. Experimental Parasitology, 135(2), 407–413.

Hefnawy, A., Berg, M., Dujardin, J. C., & De Muylder, G. (2017). Exploiting Knowledge on Leishmania Drug Resistance to Support the Quest for New Drugs. Trends in Parasitology, 33(3), 162–174.

Hefnawy, A., Cantizani, J., Peña, I., et al. (2018). Importance of secondary screening with clinical isolates for anti-leishmania drug discovery. Scientific Reports, 8(1), 11765.

Herwaldt B. L. (1999). Leishmaniasis. Lancet (London, England), 354(9185), 1191–1199.

Hooshayr, H., Talari, S., & Feyzi, F. (2014). Therapeutic Effect of Hedera helix Alcoholic Extract Against Cutaneous Leishmaniasis Caused by Leishmania major in Balb/c Mice. Jundishapur Journal of Microbiology, 7(4), e9432

Iwu, M. M., Jackson, J. E., Tally, J. D., & Klayman, D. L. (1992). Antileishmanial, antitrypanosomal, and cytotoxic screening of ethnopharmacologically selected Peruvian plants. Parasitology research, 110(4), 1381–1392.

Julia, V., Rassoulzadegan, M., & Glächenhaus, N. (1996). Resistance to Leishmania major induced by tolerance to a single antigen. Science (New York, N.Y.), 274(5286), 421–423.

Kam, T. S., Sim, K. M., Koyano, T., et al. (1998). Cytotoxic and leishmanicidal aminoglyco steroids and aminosteroid derivatives. Biochemical Pharmacology, 57(4), 491–500.

Khademvatan, S., Eskandari, K., Hazrati, N., et al. (2019). In silico and in vitro comparative activity of green tea components against Leishmania infantum. Journal of Global Antimicrobial Resistance, 18, 187–194.
| Lahiry, S., Das, A. K., Das, S. N., & Manna, M. (2018). Ethanolic leaf extract of Coccinia grandis is effective against both drug resistant and drug sensitive clinical isolates of Indian Kala-azar. *Journal of Parasitic Diseases, 42*(3), 433–441 |
| Lamidi, M., DiGiorgio, C., Delmas, et al. (2005). In vitro cytotoxic, antileishmanial and antifungal activities of ethnopharmacologically selected Gabonese plants. *Journal of Ethnopharmacology, 102*(2), 185–190 |
| Lezama-Dávila, C. M., Pan, L., Isaac-Márquez, et al. (2014). Pentalinonandrieuxi root extract is effective in the topical treatment of cutaneous leishmaniasis caused by *Leishmania mexicana*. *Phytotherapy research, 28*(6), 909–916 |
| Lima, G. S., Castro-Pinto, D. B., Machado, G. C., Maciel, M. A., & Echevarria, A. (2015). Antileishmanial activity and trypanothione reductase effects of terpenes from the Amazonian species *Croton cajucara* Benth (Euphorbiaceae). *Phytomedicine, 22*(12), 1133–1137 |
| Lodge, R., & Descoteaux, A. (2006). Phagocytosis of *Leishmania donovani* amastigotes is Rac1 dependent and occurs in the absence of NADPH oxidase activation. *European Journal of Immunology, 36*(10), 2735–2744. |
| Macahig, R. A., Matsunami, K., & Otsuka, H. (2011). Chemical studies on an endemic Philippine plant: sulfated glucoside and seco-A-ring triterpenoids from *Dillenia philippinensis*. *Chemical & Pharmaceutical Bulletin, 59*(3), 397–401 |
| Maes, L., Germonprez, N., Quirijnen, L., et al. (2004). Comparative activities of the triterpene saponin maesabalide III and liposomal amphotericin B (AmBisome) against *Leishmania donovani* in hamsters. *Antimicrobial Agents and Chemotherapy, 48*(6), 2056–2060 |
| Mahmoudvand, H., Sepahvand, P., Jahanbakhsh, S., & Azadpour, M. (2016). Evaluation of the antileishmanial and cytotoxic effects of various extracts of garlic (*Allium sativum*) on *Leishmania tropica*. *Journal of Parasitic Diseases, 40*(2), 423–426 |
| Medda, S., Mukhopadhyay, S., & Basu, M. K. (1999). Evaluation of the in-vivo activity and toxicity of amarogentin, an antileishmanial agent, in both liposomal and niosomal forms. *The Journal of Antimicrobial Chemotherapy, 44*(6), 791–794 |
| Mishra, P. K., Singh, N., Ahmad, G., Dube, A., & Maurya, R. (2005). Glycolipids and other constituents from *Desmodium gangeticum* with antileishmanial and immunomodulatory activities. *Bioorganic & Medicinal Chemistry Letters, 15*(20), 4543–4546 |
| Misra, A., Dube, A., Srivastava, B., Sharma, P., Srivastava, et al. (2001). Successful vaccination against *Leishmania donovani* infection in Indian langur using alun-precipitated autoclaved *Leishmania* major with BCG. *Vaccine, 19*(25-26), 3485–3492. |
| Mittra, B., Saha, A., & Chowdhury, A. R. et al. (2000). Luteolin, an abundant dietary component is a potent anti-leishmanial agent that acts by inducing topoisomerase II-mediated kinetoplast DNA cleavage leading to apoptosis. *Molecular medicine, 6*(6), 527–541 |
| Mohebali, M., Khamesipour, A., Mobedi, I., Zarei, Z., & Hashemi-Fesharki, R. (2004). Double-blind randomized efficacy field trial of alun precipitated autoclaved *Leishmania* major vaccine mixed with BCG against canine visceral leishmaniasis in Meshkin-Shahr district, I.R. Iran. *Vaccine, 22*(29-30), 4097–4100. |
| Mol, M., Kosey, D., & Singh, S. (2015). Nano-Synthetic Devices in Leishmaniasis: A Bioinformatics Approach. *Frontiers in Immunology, 6*, 323. |
| Montgomery, J., Ilg, T., Thompson, J. K., Kobe, B., & Handman, E. (2000). Identification and predicted structure of a leucine-rich repeat motif shared by *Leishmania* major proteophosphogly can and Parasite Surface Antigen 2. *Molecular and Biochemical Parasitology, 107*(2), 289–295 |
| Monzote, L., García, M., Montalvo, A. M., Scull, R., & Miranda, M. (2010). Chemistry, cytotoxicity and antileishmanial activity of the essential oil from *Piper auritum*. *Memorias do Instituto Oswaldo Cruz, 105*(2), 168–173 |
| Monzote, L., Jiménez, J., Cuesta-Rubío, O., et al. (2016). In Vitro Assessment of Plants Growing in Cuba Belonging to Solanaceae Family Against *Leishmania amazonensis*. *Phytotherapy research, 30*(11), 1785–1793 |
| Moore, K. J., & Matlashewski, G. (1994). Intracellular infection by *Leishmania donovani* inhibits macrophage apoptosis. *Journal of Immunology, 152*(6), 2930–2937. |
| Morais, L. S., Dusi, R. G., Demarque, D. P., et al. (2020). Antileishmanial compounds from *Connarussubamazilis* - Metabolomics, isolation and mechanism of action. *PloS one, 15*(11), e0241855 |
| Muñoz, V., Moretti, C., Sauvain, M., et al. (1994). Isolation of bis-indole alkaloids with antileishmanial and antibacterial activities from *Peschiera van heurkii* (syn. *Tabernaemontana van heurkii*). *Planta Medica, 60*(5), 455–459 |
| Nandar, D., & Reiner, N. E. (1995). Attenuation of gamma interferon-induced tyrosine phosphorylation in mononuclear phagocytes infected with *Leishmania donovani*: selective"
inhibition of signaling through Janus kinases and Stat1. *Infection and Immunity, 63*(11), 4495–4500.

Oliveira, I., MoragasTellis, C. J., Chagas, M., et al. (2018). *Carapa guianensis* Aublet (Andiroba) Seed Oil: Chemical Composition and Antileishmanial Activity of Limonoid-Rich Fractions. *BioMed Research International*, 2018, 5032816

Olobo, J. O., Anjili, C. O., Gicheru, M. M., et al. (1995). Vaccination of vervet monkeys against cutaneous leishmaniosis using recombinant *Leishmania* ‘major surface glycoprotein’ (gp63). *Veterinary Parasitology*, 60(3-4), 199–212.

Ortall, M., Ilari, A., Colotti, G., et al. (2018). Identification of chalcone-based antileishmanial agents targeting trypanothione reductase. *European Journal of Medicinal Chemistry, 152*, 527–541.

Ouellette, M., Drummelsmith, J., & Papadopoulou, B. (2004). *Leishmaniasis*: drugs in the clinic, resistance and new developments. *Drug Resistance Updates, 7*(4-5), 257–266.

Panda, S. K., & Luyten, W. (2018). Antiparasitic activity in Asteraceae with special attention to ethnomedical use by the tribes of Odisha, India. *Activitéantiparasitaire chez les Asteraceae avec une attention particulière pour l’utilisationethnotanotique par les tribus’Odisha*Inde. *Parasite (Paris, France), 25*, 1-10

Pawar, H., Kulkarni, A., Dixit, T., Chaphekar, D., & Patole, M. S. (2014). A bioinformatics approach to reanalyze the genome annotation of kinetoplastid protozoan parasite *Leishmania donovani*. *Genomics, 104*(6 Pt B), 554–561.

Peixoto, J. A., Andrade E Silva, M. L., Crotti, A. E., et al. (2011). Antileishmanial activity of the hydroalcoholic extract of *Miconia langsdorffii*, isolated compounds, and semi-synthetic derivatives. *Molecules, 16*(2), 1825–1833.

Peraza-Sánchez, S. R., Cen-Pacheco, F., Noh-Chimal, A., et al. (2007). Leishmanicidal evaluation of extracts from native plants of the Yucatan peninsula. *Fitoterapia, 78*(4), 315–318

Pérez-Victoria, F. J., Castanyes, S., & Gamarro, F. (2003). *Leishmania donovani* resistance to miltefosine involves a defective inward translocation of the drug. *Antimicrobial Agents and Chemotherapy, 47*(8), 2397–2403.

Pham, N. K., Mouriz, J., & Kima, P. E. (2005). Leishmania piñanoi amastigotes avoid macrophage production of superoxide by inducing heme degradation. *Infection and Immunity, 73*(12), 8322–8333.

Polonio, T., & Efferth, T. (2008). *Leishmaniasis*: drug resistance and natural products (review). *International Journal of Molecular Medicine, 22*(3), 277–286.

Pramanik, P. K., Paik, D., Pramanik, A., & Chakraborti, T. (2019). White jute (*Corchorus capsularis* L.) leaf extract has potent leishmanicidal activity against *Leishmania donovani*. *Parasitology International, 71*, 41–45

Rachamim, N., & Jaffe, C. L. (1993). Pure protein from *Leishmania* donovani protects mice against both cutaneous and visceral leishmaniasis. *Journal of Immunology, 150*(6), 2322–2331.

Rafati, S., Kariminia, A., Seyde-Eslami, S., Narimani, M., Taheri, T., & Lebbatard, M. (2002). Recombinant cysteine proteinase-based vaccines against *Leishmania major* in BALB/c mice: the partial protection relies on interferon gamma producing CD8(+) T lymphocyte activation. *Vaccine, 20*(19-20), 2439–2447.

Rajemiariamiraho, M., Banzouzi, J. T., Nicolau-Travers, M. L., et al. (2014). Antiprotozoal activities of *Millettia richardiana* (Fabaceae) from Madagascar. *Molecules, 19*(4), 4200–4211

Ray, S., Majumder, H. K., Chakravarty, A. K., Mukhopadhyay, S., Gil, R. R., et al. (1996). Amarogentin, a naturally occurring secoiridoid glycoside and a newly recognized inhibitor of topoisomerase I from *Leishmania* donovani. *Journal of Natural Products, 59*(1), 27–29

Reina, M., Ruiz-Mesia, L., Ruiz-Mesia, W., et al. (2014). Antiparasitic indole alkaloids from *Aspidosperma desmanthum* and *A. spruceanum* from the Peruvian Amazonia. *Natural Product Communications, 9*(8), 1075–1080.

Rezaee, F., Zolfaghari, B., & Dinani, M. S. (2018). Isolation of dioscin-related steroideal saponin from the bulbs of *Allium paradoxum* L. with leishmanicidal activity. *Research in Pharmaceutical Sciences, 13*(5), 469–475.

Rodriguez-Sosa, M., Monteforte, G. M., & Satoskar, A. R. (2001). Susceptibility to *Leishmania* mexicana infection is due to the inability to produce IL-12 rather than lack of IL-12 responsiveness. *Immunology and Cell Biology, 79*(4), 320–322.

Rossi B.R., Torres-Santos E.C., Santos A.P.P.T., et al. (2000) Treatment of cutaneous leishmaniasis with *Kalanchoe pinnata*: experimental and clinical data. *Phytomedicine 7*, 115.

Rottini, M. M., Amaral, A., Ferreira, J., et al. (2019). *Endlicheria bracteolata* (Meisn.) Essential Oil as a Weapon against *Leishmania amazonensis*: In Vitro Assay. *Molecules, 24*(14), 2525
Russell, D. G., & Alexander, J. (1988). Effective immunization against cutaneous leishmaniasis with defined membrane antigens reconstituted into liposomes. *Journal of Immunology*, 140(4), 1274–1279.

Saha, S., Mukherjee, T., Chowdhury, S., et al. (2013). The lignan glycosides lyoniside and saracoxide poison the unusual type IB topoisomerase of *Leishmania donovani* and kill the parasite both in vitro and in vivo. *Biochemical Pharmacology*, 86(12), 1673–1687.

Santana, R. C., Rosa, A., Mateus, M., et al. (2020). In vitro leishmanicidal activity of monoterpenes present in two species of *Protium* (Burseraceae) on *Leishmania amazonensis*. *Journal of Ethnopharmacology*, 259, 112981.

Sharifi-Rad, M., Salehi, B., Sharifi-Rad, J., Setzer, W. N., & Irriti, M. (2018). *Pulicaria vulgaris* Gaertn. essential oil: an alternative or complementary treatment for *Leishmania*. *Cellular and Molecular Biology*, 64(8), 18–21.

Sharma, N., Shukla, A. K., Das, M., & Dubey, V. K. (2012). Evaluation of plumbagin and its derivative as potential modulators of redox thiol metabolism of *Leishmania* parasite. *Parasitology Research*, 110(1), 341–348.

Sharma, U., Singh, D., Kumar, P., Dobhal, M. P., & Singh, S. (2011). Antiparasitic activity of plumericin & isoplumericin isolated from *Plumeria bicolor* against *Leishmania donovani*. *The Indian Journal of Medical Research*, 134(5), 709–716.

Singh S. (2006). New developments in diagnosis of leishmaniasis. *The Indian Journal of Medical Research*, 123(3), 311–330.

Singh, M. K., Paul, J., De, T., & Chakraborti, T. (2015). Bioactivity guided fractionation of *Moringa oleifera* Lam. flower targeting *Leishmania donovani*. *Indian Journal of Experimental Biology*, 53(11), 747–752.

Singh, N., Kumar, A., Gupta, P., et al. (2008). Evaluation of antileishmanial potential of *Tinospora sinensis* against experimental visceral leishmaniasis. *Parasitology Research*, 102(3), 561–565.

Singh, S. K., Bimal, S., Narayan, S., et al. (2011). *Leishmania donovani*: assessment of leishmanicidal effects of herbal extracts obtained from plants in the visceral leishmaniasis endemic area of Bihar, India. *Experimental Parasitology*, 127(2), 552–558.

Soares, D. C., Portella, N. A., Ramos, M. F., Siani, A. C., & Saraiva, E. M. (2013). Trans- β-Caryophyllene: An Effective Antileishmanial Compound Found in Commercial Copaiba Oil (Copaifera spp.). *Evidence-Based Complementary and Alternative Medicine*, 2013, 761323.

Soong, L., Duboise, S. M., Kima, P., & McMahon-Pratt, D. (1995). *Leishmania pifanoi* amastigote antigens protect mice against cutaneous leishmaniasis. *Infection and Immunity*, 63(9), 3559–3566.

Steverding D. (2017). The history of leishmaniasis. *Parasites & Vectors*, 10(1), 82.

Sundar, S., & Chakravarty, J. (2015a). An update on pharmacotherapy for leishmaniasis. *Expert Opinion on Pharmacotherapy*, 16(2), 237–252.

Sundar, S., & Chakravarty, J. (2015b). Investigational drugs for visceral leishmaniasis. *Expert Opinion on Investigational Drugs*, 24(1), 43–59.

Sundar, S., Jha, T. K., Thakur, C. P., et al. (2002). Oral miltefosine for Indian visceral leishmaniasis. *The New England journal of Medicine*, 347(22), 1739–1746.

Tabbara, K. S., Peters, N. C., Afrin, F., et al. (2005). Conditions influencing the efficacy of vaccination with live organisms against *Leishmania* major infection. *Infection and Immunity*, 73(8), 4714–4722.

Thakur, C. P., Bhowmick, S., Dolfi, L., & Olliaro, P. (1995). Aminosidine plus sodium stibogluconate for the treatment of Indian kala-azar: a randomized dose-finding clinical trial. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 89(2), 219–223.

Thakur, C. P., Olliaro, P., Gothoskar, S., et al. (1992). Treatment of visceral leishmaniasis (kala-azar) with aminosidine (= paromomycin) -antimonial combinations, a pilot study in Bihar, India. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 86(6), 615–616.

Torres-Santos, E. C., Lopes, D., Oliveira, R. R., et al. (2004). Antileishmanial activity of isolated triterpenoids from *Pourouma guianensis*. *Phytomedicine*, 11(2-3), 114–120.

Tracanna, M. I., Fortuna, A. M., Cárdenas, A. V., et al. (2015). Anti-leishmanial, anti-inflammatory and antimicrobial activities of phenolic derivatives from *Tibouchina paratropica*. *Phytotherapy Research*, 29(3), 393–397.

Underhill, D. M., & Ozinsky, A. (2002). Phagocytosis of microbes: complexity in action. *Annual Review of Immunology*, 20, 825–852.
Vermelho, A.B., Supuran, T., Cardoso, V., et al. (2014) Leishmaniasis: possible new strategies for treatment. In: Claborn D. (ed) *Leishmaniasis-Trends in Epidemiology, Diagnosis and Treatment*, In Tech, Rijeka,-Croatia.

Vertut-Doi, A., Ohnishi, S. I., & Bolard, J. (1994). The endocytic process in CHO cells, a toxic pathway of the polyene antibiotic amphotericin B. *Antimicrobial Agents and Chemotherapy, 38*(10), 2373–2379.

Wanderley, J. L., Moreira, M. E., Benjamin, A., Bonomo, A. C., & Barcinski, M. A. (2006). Mimicry of apoptotic cells by exposing phosphatidylserine participates in the establishment of amastigotes of *Leishmania* (L) amazomensis in mammalian hosts. *Journal of Immunology, 176*(3), 1834–1839.

Watts, A. M., & Kennedy, R. C. (1999). DNA vaccination strategies against infectious diseases. *International Journal for Parasitology, 29*(8), 1149–1163.

Weniger, B., Robledo, S., Arango, G. J., et al. (2001). Antiprotozoal activities of Colombian plants. *Journal of Ethnopharmacology, 78*(2-3), 193–200

Xia, X. (2017). Bioinformatics and Drug Discovery. *Current Topics in Medicinal chemistry, 17*(15), 1709–1726

Zhai, L., Blom, J., Chen, M., Christensen, S. B., & Kharazmi, A. (1995). The antileishmanial agent licochalcone A interferes with the function of parasite mitochondria. *Antimicrobial Agents and Chemotherapy, 39*(12), 2742–2748