

Review Article

Plant-derived compounds in treatment of leishmaniasis

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Summary

Leishmaniasis is a neglected public health problem caused by the protozoan species belonging to the genus *Leishmania* affecting mostly the poor populations of developing countries. The causative organism is transmitted by female sandflies. Cutaneous, mucocutaneous, and visceral clinical manifestations are the most frequent forms of leishmaniasis. Chemotherapy still relies on the use of pentavalent antimonials, amphotericin B, paromomycin, miltefosin and liposomal amphotericin B. However, the application of these drugs is limited due to low efficacy, life-threatening side effects, high toxicity, induction of parasite resistance, length of treatment and high cost. Given the fact that antileishmanial vaccines may not become available in the near future, the search for better drugs should be continued. Natural products may offer an unlimited source of chemical diversity to identify new drug modules. New medicines should be less toxic or non-toxic, safe, more efficient, less expensive and readily available antileishmanial agents, especially for low-income populations. In the present review, special focus is on medicinal plants used against leishmaniasis. The bioactive phytochemicals present in the plant derivatives including the crude extracts, essential oils, and other useful compounds can be a good source for discovering and producing new antileishmanial medicines.

Key words: Leishmaniasis, Treatment, Pentavalent antimonials, Plant extracts

Introduction

Some parasitic diseases including toxoplasmosis, Chagas disease, giardiasis, babesiosis and leishmaniasis occur in animals and can transmit to human beings (Rahbari *et al.*, 2008; Heidarpour Bami *et al.*, 2010; Mosallanejad *et al.*, 2011; Esch and Petersen, 2013). Leishmaniasis is located in the ninth place of the global burden of disease among individual infectious diseases. This disease is regarded as a protozoan zoonotic disease affecting approximately 12 million people in 88 (Hosseinienejad *et al.*, 2012; Sarkar *et al.*, 2013; Machado *et al.*, 2014; Mohapatra, 2014) or 98 countries of the world, according to various resources (Calla-Magarinos *et al.*, 2013; Chandrasekaran *et al.*, 2013; Rodrigues *et al.*, 2014) with 2 million new cases annually (1.5 million cutaneous and 0.5 million visceral leishmaniasis) and mortality rate of 60,000 (Mohapatra, 2014; Salehabadi *et al.*, 2014). Leishmaniasis is caused by various species of the protozoa of the genus *Leishmania* (Table 1) (WHO, 2010; Hatam *et al.*, 2013; Salehabadi *et al.*, 2014). Cutaneous leishmaniasis (CL) is the most common form of the disease, among other forms such as visceral (VL) and mucocutaneous (MCL) leishmaniasis. With the advent of the human immunodeficiency virus (HIV), leishmaniasis is rapidly becoming an opportunistic infection in AIDS patients (Diro *et al.*, 2014). On the other hand, an ideal vaccine is not available to control the disease. The standard drugs for leishmaniasis are pentavalent antimonials including sodium stibogluconate (Pentostam[®]) and meglumine antimoniate (Glucantime)

(Diro *et al.*, 2014; Jabini *et al.*, 2014). However, serious side effects including arthralgias, myalgias, leukopenia, pancreatitis, liver problems, cardiotoxicity and cardiac arrhythmia in the patients, prolonged treatment time, and increased parasite resistance (Chakravarty and Sundar, 2010; Lage *et al.*, 2013; Diro *et al.*, 2014). Therefore, alternative drugs to the antimonials such as amphotericin B, pentamidine, paromomycin, and miltefosine have been recommended, but they also come with some side effects such as toxicity, high cost, drug resistance and therapeutic failure (Machado *et al.*, 2012; Wiwanitkit, 2012; Lage *et al.*, 2013). Given the above mentioned reasons, development of new, less toxic and more cost-effective drugs with greater efficacy as well as more accessible alternative therapeutic strategies which could become available for low-income populations to treat the disease has become a necessity (Lage *et al.*, 2013). Since victims of leishmaniasis are generally poor, lengthy treatment using expensive drugs with related costs is far beyond the means of such families (Oryan *et al.*, 2014). Therefore, many patients seek for herbal therapy which is cheaper and readily available. However, most of the herbs have still not been evaluated scientifically. In recent years, there has been growing interest in alternative natural products and plant compounds for the treatment of leishmaniasis (Alviano *et al.*, 2012; Garcia *et al.*, 2012; Ogeto *et al.*, 2013; Chouhan *et al.*, 2014). Anti-leishmanial activity of some plants has been attributed to the presence of the compounds such as alkaloids, chalcones, triterpenoids, naphthoquinones, quinones, terpenes, steroids, lignans,

Table 1: *Leishmania* species causing human disease, reservoir hosts and vectors

<i>Leishmania</i> species	Infection	Geographical location	Reservoir	Vector
<i>L. donovani</i>	VL, PKDL	Old world, except Europe	Humans, rodents	<i>Phlebotomus argentipes</i> , <i>P. orinintalis</i> , <i>P. martini</i>
<i>L. infantum</i>	VL, CL, ML	New world for VL, old world for CL and ML	Rodents, dogs, foxes, jackals	<i>P. perniciosus</i> , <i>P. ariasi</i> , <i>Lutzomyia longipalpis</i>
<i>L. chagasi</i>	VL	New world	Foxes, dogs, opossums	<i>L. longipalpis</i>
<i>L. major</i>	CL, ML	Old world	Rodents, gerbils	<i>P. papatasi</i> , <i>P. duboscqi</i>
<i>L. tropica</i>	CL, ML, VL	Old world	Humans	<i>P. sergenti</i>
<i>L. aethiopica</i>	CL, DCL, MCL	Old world	Hyraxes	<i>P. longipes</i> , <i>P. pedifer</i>
<i>L. mexicana</i>	CL, DCL, MCL	New world	Forest rodents	<i>L. olmeca</i>
<i>L. amazonensis</i>	CL, DCL	New world	Forest rodents	<i>L. flaviscutellata</i>
<i>L. braziliensis</i>	CL, MCL	New world	Forest rodents, peridomestic anteaters	<i>Lutzomyia</i> spp.
<i>L. guyanensis</i>	CL, MCL	New world	Sloths aboreal anteaters	<i>L. umbratilis</i>
<i>L. panamensis</i>	CL, MCL	New world	Sloths	<i>L. trapidoictal</i>
<i>L. peruviana</i>	MCL	New world	Dogs	<i>L. verrucarum</i> , <i>L. pyrenis</i>

VL: Visceral leishmaniasis; PKDL: Post-kala azar dermal leishmaniasis; P: *Phlebotomus*; ML: Mucosal leishmaniasis; CL: Cutaneous leishmaniasis; L: *Lutzomyia*; DCL: Diffuse cutaneous leishmaniasis; MCL: Mucocutaneous leishmaniasis

saponins, and flavonoids (Lage *et al.*, 2013; Sifaoui *et al.*, 2014). Essential oils and extracts of a large number of plants have been shown to be effective against different species of *Leishmania* (Rodrigues *et al.*, 2013; Monzote *et al.*, 2014a). This review attempts to provide information regarding *Leishmania* and the resultant disease, some information about the available drugs, and recent developments in the phytosciences to treat leishmaniasis. Indeed, the current study mostly highlights a range of plant formulations exhibiting leishmanicidal activity.

Main forms of leishmaniasis

Leishmaniasis is the most common neglected tropical disease (NTD) in countries with poor socioeconomic conditions (Neris *et al.*, 2013; Oryan *et al.*, 2014). It is a major public health concern in the tropical and subtropical countries throughout the old (Africa, Asia, Southern Europe) and new world (Latin America) (Daneshbod *et al.*, 2011; de Medeiros *et al.*, 2011; Monzote, 2011). Leishmaniasis is characterized by a spectrum of clinical manifestations ranging from ulcerative skin lesions in the site of the sandfly bite (CL), to the more progressive MCL and VL (Neris *et al.*, 2013; Sachdeva *et al.*, 2014) depending on the parasite species, the host-parasite interaction, the occurrence of co-infections, and the immunological status of the host (de Medeiros *et al.*, 2011). Generally, the proven vectors are species of sandflies of the genus *Phlebotomus* and *Lutzomyia* in the new world and the old world, respectively (Nekouie *et al.*, 2006; Parvizi and Amirkhani, 2008; Alidadi and Oryan, 2014). The most common form of leishmaniasis is CL or oriental sore which is caused by many *Leishmania* species such as *L. major*, *L. tropica*, and *L. aethiopica* in the old world and *L. mexicana*, *L. venezuelensis*, *L. amazonensis*, *L. braziliensis*, *L. panamensis*, *L. guyanensis* and *L. peruviana* in the new world (Table 1) (Oryan *et al.*, 2008; WHO, 2010; Alavi-Naini *et al.*, 2012; Oryan *et al.*, 2013a, b). CL is presented as papules, crusted nodules, plaques, or ulcerative nodular lesions on the face, hands, and/or feet which self-heal within a few months but may leave scars (Asgari *et al.*, 2007; Oryan *et al.*, 2007; Shirian *et al.*, 2014). Diffuse cutaneous leishmaniasis (DCL) is regarded as a chronic, progressive variant of CL that is manifested by disseminated non-ulcerative

skin lesions (Alidadi and Oryan, 2014). The etiologic agent associated with DCL is *L. aethiopica* and *L. amazonensis* in the old and new world, respectively (Alviano *et al.*, 2012; Lindoso *et al.*, 2012). Mucosal leishmaniasis or MCL leads to extensive destruction of naso-oral and pharyngeal or laryngeal regions causing difficulty in eating and increases the risk of the secondary infections (Daneshbod *et al.*, 2011; Alviano *et al.*, 2012; Shirian *et al.*, 2012a; Oryan *et al.*, 2013a). Its main causative organism is *L. aethiopica* in the old world and *L. braziliensis*, *L. Mexicana*, *L. amazonensis* and *L. panamensis* in the new world. In addition, species such as *L. infantum*, *L. major* and *L. tropica* have been isolated from some cases of mucosal leishmaniasis (Shirian *et al.*, 2012a, b). This form is transmitted by the forest sandfly *Lutzomyia* spp. and its reservoir hosts include rodents and dogs (Shirian *et al.*, 2012b; Shirian *et al.*, 2013).

VL, also called kala-azar, is the most severe amongst various forms of leishmaniasis (Wiwanitkit, 2012; Diro *et al.*, 2014). VL is mostly caused by *L. donovani*, *L. infantum*, *L. chagasi* and occasionally *L. tropica* multiplying in the reticuloendothelial system (WHO, 2010; Hosseinijad *et al.*, 2012; Khan *et al.*, 2014). The disease varies from an asymptomatic infection to a fatal condition (Diro *et al.*, 2014). VL is characterized by prolonged fever, weakness, fatigue, severe cachexia and, with advancement of the disease, by the enlargement of spleen and liver, termed hepatomegaly and splenomegaly (hepatosplenomegaly), progressive anemia, and sometimes bleeding and blackening of the skin that gives the disease its common name (Prajapati *et al.*, 2011b; Alviano *et al.*, 2012; Jain and Jain, 2013; Sachdeva *et al.*, 2014a). This disease is usually lethal due to the secondary infections, severe anemia and organ failure if left untreated (Alviano *et al.*, 2012). Dogs are considered as the main reservoir hosts for this form of the disease (Hosseinijad *et al.*, 2012). Post kala-azar dermal leishmaniasis (PKDL) is characterized by the appearance of non-ulcerative cutaneous lesions containing the parasites in the period after successful treatment of VL and recovery from it (Lindoso *et al.*, 2012; Oryan *et al.*, 2014).

Life cycle of *Leishmania*

Leishmania is found in two forms or stages (Figs.

1A-G): amastigotes which are intracellular, spherical, without flagellum or with an internalized flagellum, non-motile and reside and multiply within the phagolysosomes of phagocytic cells such as macrophages of the vertebrate host. The other form, termed promastigotes that present inside the sandflies are extracellular, elongated, spindle-shaped, flagellated and

motile (Mishra *et al.*, 2009; Monzote, 2011; Alviano *et al.*, 2012; Esch and Petersen, 2013; Mohapatra, 2014). The life cycle of *Leishmania* takes place in two hosts, the mammals and sandflies (Fig. 2) (Motazedian *et al.*, 2006). The adult female blood-sucking sandfly bites an individual infected with *Leishmania* and thereby the parasite is ingested within the host's blood (Motazedian

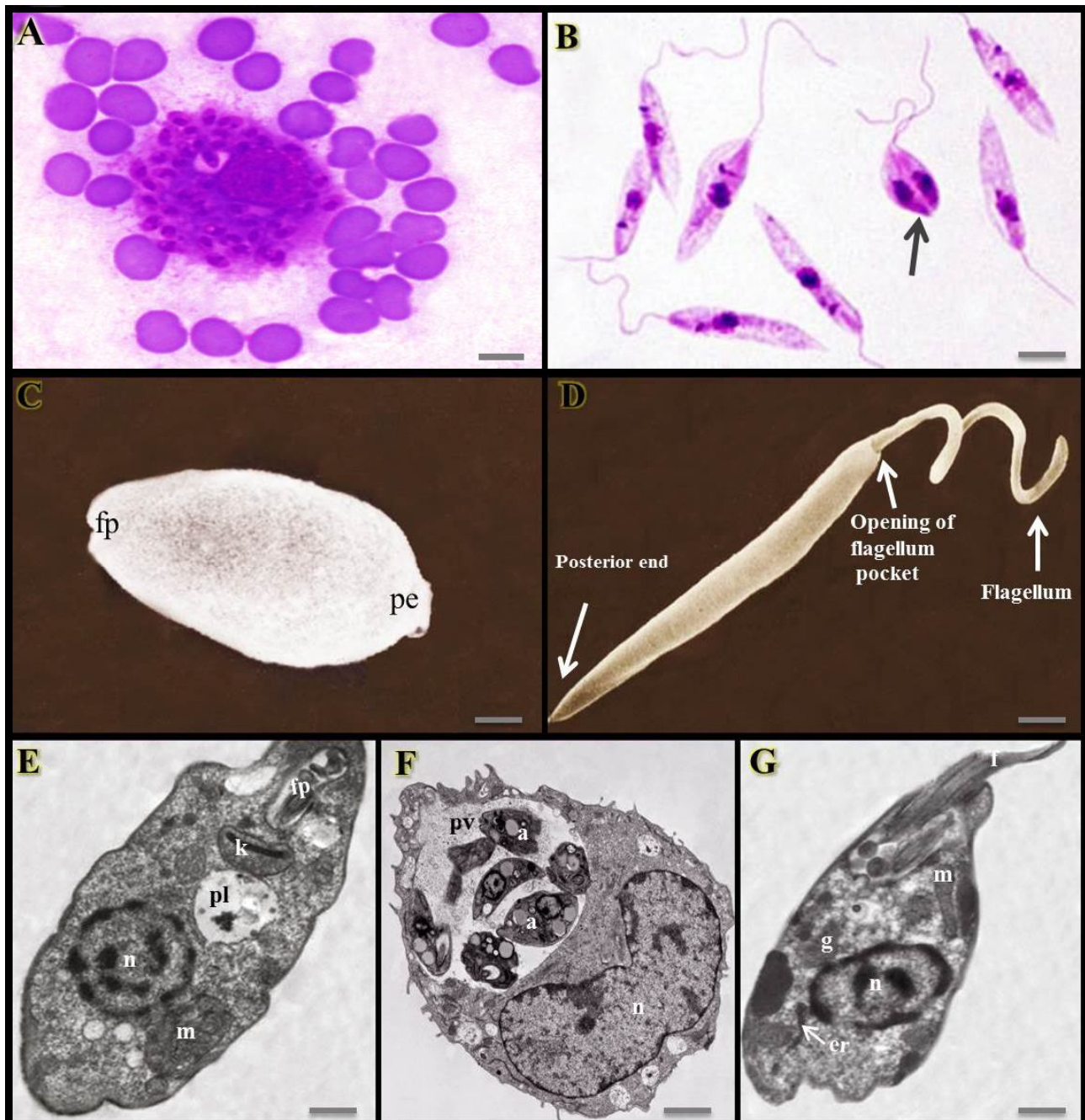


Fig. 1: Two stages of *Leishmania* parasite. (A) Intracellular and non-motile stage called amastigote within the macrophages, (B) Extracellular and motile form called promastigote possessing a flagellum. The promastigotes multiply by binary division (arrow), giemsa stain ($\times 1,000$). Scanning electron micrograph of (C) leishmanial amastigote (fp: Flagellum pocket, and pe: Posterior end) and (D) promastigote, (scale bars = 1 μm). (E) Transmission electron micrograph of a mastigote. The flagellum is completely enclosed within the flagellar pocket. n: Nucleus, m: Mitochondrion, pl: Phagolysosome, k: Kinetoplast, and fp: Flagellar pocket. (F) A macrophage containing large parasitophorous vacuole filled with leishmanial amastigotes. n: Nucleus of macrophage, a: Amastigotes, and pv: Parasitophorous or parasite-containing vacuole. (G) TEM of a promastigote showing different organelles. er: Endoplasmic reticulum, n: Nucleus, g: Golgy apparatus, m: Mitochondrion, and f: Flagellum, (scale bars = 15 μm) (Asaln *et al.*, 2013; Hatam *et al.*, 2013)

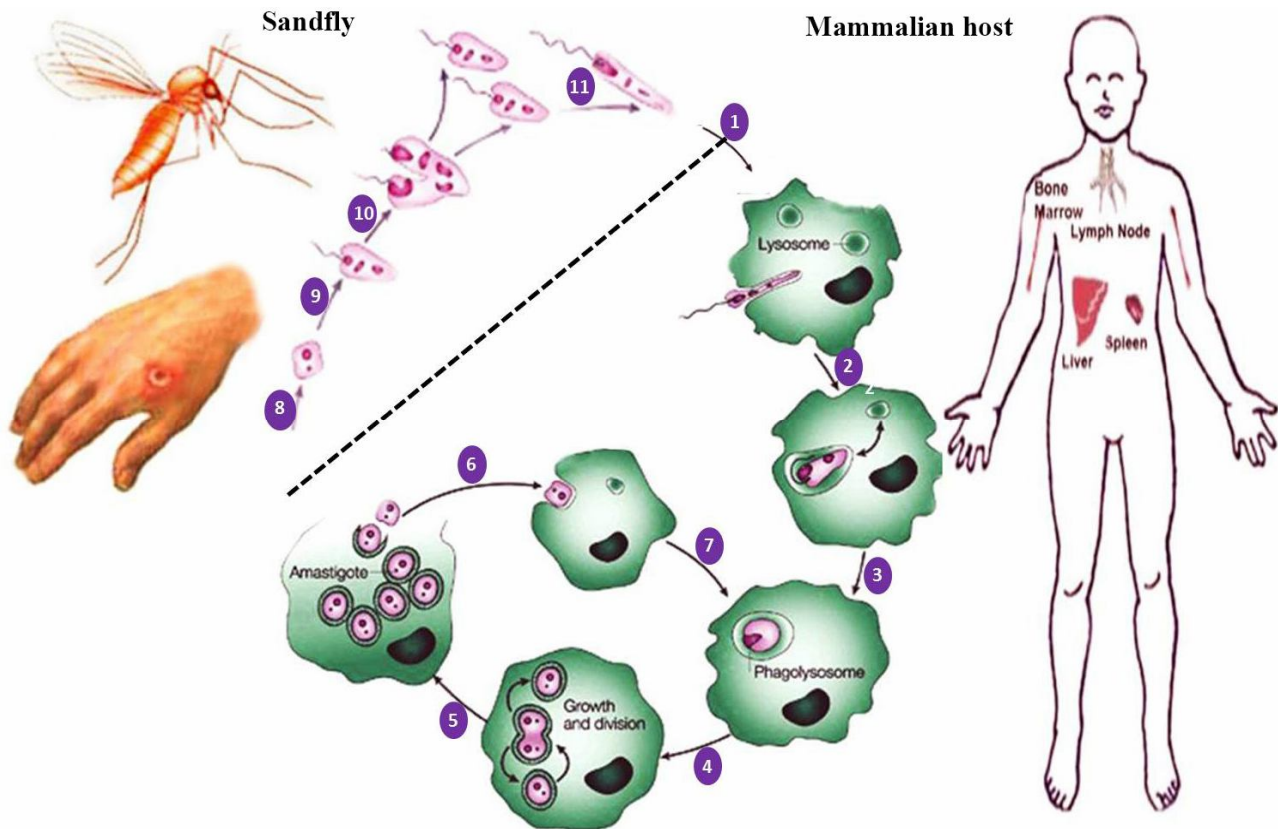


Fig. 2: Leishmanial infection in sandfly and mammalian hosts. (1) Delivery of promastigotes into human skin by the biting sandfly and attachment of promastigotes to a macrophage by phagocytosis; (2) entry of promastigote into the phagosome of macrophage; (3) fusion of the phagosome containing a promastigote with lysosomes in an infected macrophage and production of phagolysosome; (4) differentiation of promastigote into amastigote within the phagolysosome of the macrophage and then multiplication of amastigotes by binary division; (5) continuing proliferation of amastigotes and rupture of heavily infected macrophages and release of amastigotes; (6) either phagocytosis of the released amastigotes by new macrophages and (7) phagocytosis of amastigotes and formation of phagolysosome and the cycle continues; (8) ingestion of macrophages containing amastigotes by sandfly following a blood meal taken from the infected and/or reservoir host; (9-11) development of promastigotes in the sandfly vector (transformation of amastigotes into promastigotes in sandfly's midgut; division of promastigotes in midgut and migration to proboscis of sandfly; taking a blood meal from the host by sandfly; and then repetition of the cycle (1))

et al., 2006; Parvizi and Amirkhani, 2008). Inside the gut of the insect, the amastigotes are transformed into promastigote form. They live extracellularly in the alimentary tract and then migrate to the saliva of the sandfly, until it releases promastigotes from its proboscis into the bite site via biting the next person. Inside the human host, the promastigotes invade macrophages which transform back into the amastigote form (Motazedian *et al.*, 2006; Alviano *et al.*, 2012; Aslan *et al.*, 2013; Esch and Petersen, 2013). The amastigotes multiply inside the macrophages by binary fission and may lyse the cell and spread within the host. The parasite mainly affects the reticuloendothelial system including spleen, liver, bone marrow and lymphoid tissues (Mishra *et al.*, 2009; Alviano *et al.*, 2012; Esch and Petersen, 2013; Mohapatra, 2014).

Diagnosis of leishmaniasis

Laboratory diagnosis of the disease is achieved by the demonstration of the parasites in amastigote form, in materials obtained from the patient including skin lesions in the CL cases, and in the spleen (as the most reliable

sample), bone marrow and lymph node aspirates in the cases with VL (Khan *et al.*, 2014). The smears are stained with giemsa/leishman stains for the presence of amastigotes inside the macrophages. The aspirates can be inoculated into BALB/c mice which are genetically susceptible to *Leishmania*, and, as a result, they will become infected over time (Khan *et al.*, 2014; Shirian *et al.*, 2014; Yamamoto *et al.*, 2014). For this purpose, the use of methods such as splenic puncture, aspiration of bone marrow, fine-needle biopsy of lymph nodes and culture is helpful in the cases of VL (Alidadi and Oryan, 2014; Khan *et al.*, 2014). However, these procedures are risky and invasive. There are several serological tests including direct agglutination test, complement fixation tests, enzyme-linked immunosorbent assay (ELISA), and indirect immunofluorescent antibody test (Daneshbod *et al.*, 2011; Khan *et al.*, 2014; Shirian *et al.*, 2012b). However, they are unable to distinguish past and current infections. Moreover, cross-reaction of antibodies against *Leishmania* may occur with those of other diseases including trypanosomiasis and toxoplasmosis (Mehrabani *et al.*, 2011; Shirian *et al.*, 2014). Other methodologies include cytology and culture of the aspirates,

histopathologic examination and immunohistochemistry (Mehrabani *et al.*, 2007, 2011; Daneshbod *et al.*, 2011; Khan *et al.*, 2014; Shirian *et al.*, 2014). Molecular assays such as polymerase chain reaction (PCR) assay could be applied for diagnosis of the parasite in the tissues and organs of the patients and also in the blood of asymptomatic healthy persons living in the endemic regions who may serve as parasite reservoirs, sometimes with 100% sensitivity (Abbasi *et al.*, 2013; Shirian *et al.*, 2014; Sudarshan *et al.*, 2014).

Control strategies for leishmaniasis

To date, there is no suitable vaccine against *Leishmania*. However, several vaccine preparations are in more or less advanced stages of testing (Bacon *et al.*, 2013; Mutiso *et al.*, 2013). The vaccines evaluated so far include live, live-attenuated (containing the parasites which are infectious but not pathogenic) and killed (with both prophylactic and therapeutic purposes) (Bacon *et al.*, 2013; Mutiso *et al.*, 2013; Alidadi and Oryan, 2014). In addition to these, there are further vaccines such as fractionated/subunit, naked DNA (cloning of the gene encoding the vaccine candidate in a vector with the injection of DNA), recombinant and synthetic (containing the recombinant DNA-derived antigens and peptides), non-protein antigens (such as *Leishmania* lipophosphoglycin), anti-sandfly saliva components (indirect enhancement anti-parasite immunity) and adjuvants for augmenting the effect of vaccination (Bacon *et al.*, 2013; Mutiso *et al.*, 2013). Efficient management of the disease is based on early diagnosis and treatment to lower morbidity and prevent mortality with an efficient vaccination, if that is possible (Alidadi and Oryan, 2014). On the other hand, disease control and prevention is achieved by identification of risk factors of the disease. For this purpose, control of vector and reservoir hosts through the use of insecticides and impregnated bednets can be useful (Votykpa *et al.*, 2012; Coura-Vital *et al.*, 2013; Diro *et al.*, 2014). An emerging and extremely serious problem in the field of VL control is its co-infection with HIV. In these conditions, infection with HIV increases the risk of progression of asymptomatic leishmanial infection to clinical VL and vice versa (Alidadi and Oryan, 2014; Diro *et al.*, 2014). Immunosuppression, malnutrition, poverty, and generally any factor exposing people to infected sandflies and reservoir hosts may increase the probability of the infection and disease (Votykpa *et al.*, 2012; Coura-Vital *et al.*, 2013; Alidadi and Oryan, 2014).

Current chemotherapy for leishmaniasis

The drug used for treatment of leishmaniasis should be selected based on several factors such as patient characteristics, pharmacogenetical characteristics of the drug, causative *Leishmania* species, risk factors and geographical area (Lindoso *et al.*, 2012; Sarkar *et al.*, 2013; Fernandez *et al.*, 2014). An ideal drug for treatment of leishmaniasis should provide several

requirements for patients including being effective in one or few doses, low cost, not be teratogenic, no side effects, no need for hospitalization, and no induction of resistance (Alviano *et al.*, 2012). Chemotherapy based on available drugs that are not highly effective and cause serious effects, remains the only treatment available for this disease (Seifert, 2011; Alviano *et al.*, 2012). Since the 1950's ago, pentavalent antimony compounds have been proposed as the first-line drugs for treatment of leishmaniasis at dose of 20-28 mg/kg/day for 28-30 days, intravenously (IV) or intramuscularly (IM) (Jain and Jain, 2013). The drugs currently used for treatment of leishmaniasis are still based on pentavalent antimonials which are available in two formulations, sodium stibogluconate (SSG, Pentostam[®], C₁₂H₃₈O₂₆Sb) and meglumine antimoniate (Glucantime[®], C₁₄H₂₉O₁₀N₂Sb), that target the amastigotes of both CL and VL (Alviano *et al.*, 2012; Dastgheib *et al.*, 2012; Lindoso *et al.*, 2012; Jabini *et al.*, 2014; Mohapatra, 2014; Ribeiro *et al.*, 2014). The antimonials mechanism of action is not still clear. However, these drugs inhibit the activity of the glycolytic and oxidative pathways of the fatty acids for the reduction of ATP in the amastigotes (Lindoso *et al.*, 2012). In addition, it has been stated that the pentavalent form utilizes thiols from the parasite and the cell surface of the host and is reduced into the trivalent which is a more active and toxic form inside the macrophages (Alviano *et al.*, 2012; Lindoso *et al.*, 2012; Mohapatra, 2014). Thiol metabolism and its high intracellular levels play prominent roles in developing antimonial resistance. Thiol molecule increases the oxidative stress within the macrophages preventing antioxidant formation and reduction of pentavalent antimonials to trivalent form (Jain and Jain, 2013; Mohapatra, 2014). Inhibition of fatty acid oxidation and induction of apoptosis and also induction of the DNA topoisomerase enzyme are other mentioned mechanisms (Alviano *et al.*, 2012). Nevertheless, pentavalent antimonials have drawbacks and side effects including anorexia, vomiting, dizziness, arthralgia, myalgia, fever, parenteral administration, high cost and long course of treatment (Fernandez *et al.*, 2014; Gamboa-Leon *et al.*, 2014). These drugs have limitations for pregnant women, the elderly, and individuals with cardiac, renal and hepatic diseases due to toxicity for these organs and pancreas (Neves *et al.*, 2011; Sen and Chatterjee, 2011; Lindoso *et al.*, 2012). Moreover, increased parasite resistance and treatment failures associated with the use of these compounds in leishmaniasis patients have been reported (Chakravarty and Sundar, 2010; Valadares *et al.*, 2012; Jain and Jain, 2013). Despite the side effects of pentavalent antimonials and the prolonged need for daily parenteral injection (daily IM or IV injection), they are used for a long time (Salehabadi *et al.*, 2014). When the presence of restrictions or treatment failure with these drugs occur, alternative drugs named the second-choice drugs should be used. The alternative drugs to pentavalent antimony compounds include amphotericin B, pentamidine and paromomycin (Prajapati *et al.*, 2011a, b; Valadares *et al.*, 2012; Wiwanitkit, 2012). The two-dimensional chemical

structure related to the available chemotherapeutic agents against leishmaniasis is presented in Fig. 3.

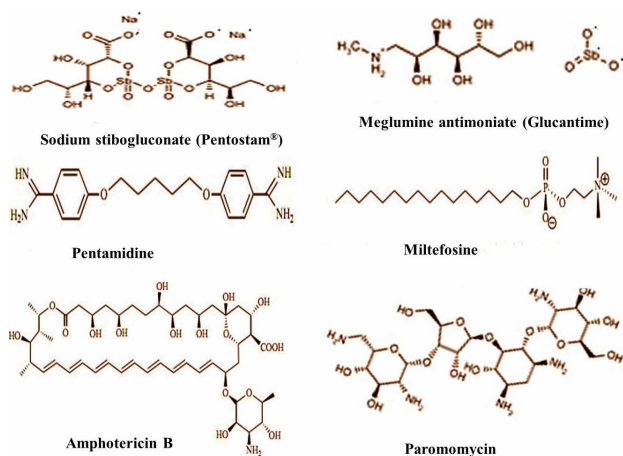


Fig. 3: Two-dimensional structure of drugs most commonly used against leishmaniasis

Amphotericin B (fungizone, $C_{47}H_{73}NO_{17}$, AmbB), a polyene antifungal agent, is extracted from the filamentous bacteria called *Streptomyces nodusus*. This compound is administered as the second line drug for the treatment of patients with leishmaniasis and acts on amastigotes and also promastigotes, at dose of 0.75-1 mg/kg for 15 days, IM (Lindoso *et al.*, 2012; Jain and Jain, 2013). It exists in four forms namely, deoxycholate amphotericin B, amphotericin colloidal dispersion (Amphocil), amphotericin B lipid complex (Abeclet) and liposomal amphotericin B (AmBisome) (Monzote, 2011; Mohapatra, 2014). AmpB tends to and targets sterols such as ergosterol and cholesterol which are important for parasite membrane constitution. It binds to cholesterol and forms pores in the cell membrane of the parasite leading to cell death due to increased permeability and the leakage of cellular content. Moreover, it may lead to generation of oxygen free radicals causing damage to cell, followed by cell death (Jain and Jain, 2013; Mohapatra, 2014). Despite the therapeutic property of AmpB, its resistance may occur due to high frequency of its use, but by the unknown mechanism. It is believed that a molecular mechanism may be involved in the development of the drug resistance (Mohapatra, 2014). Deoxycholate amphotericin B is associated with more severe effects including nausea, vomiting, fever, anemia, hapokalemia, cardiotoxicity and nephrotoxicity (Lindoso *et al.*, 2012; Neris *et al.*, 2013; Gamboa-Leon *et al.*, 2014). Liposomal amphotericin B is less toxic and better tolerated by the patients in comparison with conventional AmpB, but it is very expensive, exhibits short circulating half-life and reaches higher concentrations in the liver and spleen (Lindoso *et al.*, 2012; Lage *et al.*, 2013). Moreover, leishmaniasis has emerged as an opportunistic infection in human immunodeficiency virus-infected patients, making the treatment even more challenging (Bhattacharya *et al.*, 2013). Given the reports regarding high rates of toxicity of antimonials, AmpB has been

introduced as the preferential first line treatment for VL patients co-infected with HIV (Lage *et al.*, 2013; Diro *et al.*, 2014). Paromomycin (PM, Humatin, paromomycin sulfate, $C_{23}H_{47}O_{18}S$) is a broad spectrum antibiotic belonging to the aminoglycoside group extracted from *Streptomyces rimosus* variety paromomycinus (Lindoso *et al.*, 2012; Wiwanitkit, 2012; Mohapatra, 2014). PM and imiquimod can be applied topically for the treatment of localized CL (Tiuman *et al.*, 2012). The drug is administered intramuscularly at dose of 15 mg/kg for 21 days (Jain and Jain, 2013). This drug exerts these effects by interfering with ribosomes and mitochondrial membrane and inhibiting protein synthesis and respiration (Lindoso *et al.*, 2012; Wiwanitkit, 2012). To treat CL, three ointments of PM have been developed as follows: PM 15% methylbenzethonium chloride, 12%, PM 15% plus urea 10% and PM with gentamycin 0.5% (Monzote, 2011). The main side effects of this drug include hepatotoxicity, ototoxicity and local pain at the injection site (Lindoso *et al.*, 2012; Sachdeva *et al.*, 2013). Nonetheless, low cost, negligible side effects, good efficacy and short duration of administration all have made this drug a candidate for the first line therapy for leishmaniasis patients (Mohapatra, 2014). Because of the possibility of resistance to monotherapy with PM, newer formulations such as PM loaded with albumin microsphere and PM with liposome have been provided for achieving better outcomes (Wiwanitkit, 2012). Pentamidine (pentamidine isethionate, $C_{19}H_{24}N_4O_2$), a type of dibenzimidazole, interferes with the synthesis of *Leishmania* DNA and by affecting on the mitochondrial membrane leads to the parasite death. This drug is recommended at dose of 4 mg/kg, three times per week (Jain and Jain, 2013). Although pentamidine is administered for all forms of leishmaniasis, low efficacy and toxicity have limited its application (Monzote, 2011). Its other side effects are hypotension, myalgia, hypoglycemia, diabetes mellitus and abscess formation at the injection site (Lindoso *et al.*, 2012). Miltefosine (hexadecylphosphocholine, Impavido®), an anticancer drug, is orally administered as the second line treatment in VL and PKDL patients who fail to respond to antimonial drugs, at dose of 2.5 mg/kg/day for a total of 28 days (Shakya *et al.*, 2011; Jain and Jain, 2013; Fernandez *et al.*, 2014; Mohapatra, 2014). The mechanism of action of this drug is not well understood. But, it can bind to the *Leishmania* cell membrane, become internalized and finally cause cell death (Mohapatra, 2014). Nevertheless, because of misuse of the drug, its long half-life and probably inactivation of genes responsible for drug uptake are reasons for resistance to this drug. Additionally, failures in treatment and relapse have been observed in some cases treated with this compound (Alviano *et al.*, 2012; Sachdeva *et al.*, 2013; Fernandez *et al.*, 2014). Also, adverse effects including toxicity to the gastrointestinal, hepatic, and renal systems have limited administration of miltefosine. In addition, it is teratogenic and so it should not be used in pregnant women (Lindoso *et al.*, 2012). In addition, sitamaquine, an 8-amino-quinoline, is the second oral

antiparasitic drug used in the treatment of leishmaniasis. It seems that this compound targets succinate dehydrogenase causing oxidative stress in *Leishmania*. Its application is limited due to hematological toxicity such as methemoglobinemia and hemolysis (Carvalho *et al.*, 2011). Additionally, it has been demonstrated that cisplatin (cis-diaminedichloro-platinum (II), CDDP), an anti-neoplastic DNA-binding drug, exhibits anti-leishmanial activity in the *in vitro* tests (Sachdeva *et al.*, 2013). However, this compound causes nephrotoxicity and hepatotoxicity through the generation of reactive oxygen species (ROS) and induction of lipid peroxidation due to decreased antioxidant defense system (Neris *et al.*, 2013; Sachdeva *et al.*, 2013; Sachdeva *et al.*, 2014a, b). Further, two distinct classes of the antifungal drugs including imidazoles (ketoconazole) and triazoles (fluconazole and itraconazole) are used in leishmaniasis especially CL due to the less toxicity compared to pentavalent antimonials and their oral administration (Alviano *et al.*, 2012; Lindoso *et al.*, 2012). The latter is of slower metabolism rate and less toxic than the former. A randomized clinical trial evaluated and compared the efficacy and tolerability of meglumine antimoniate, pentamidine and amphotericin B in treatment of CL due to *L. (Viannia) guyanensis* in 186 patients (Neves *et al.*, 2011). No difference was observed among the groups in relation to sex, age and number or site of lesions. Several adverse effects were found in 40% of patients including 20.3% arthralgia for meglumine group, 35.1% pain and 10.8% induration at the injection site in the pentamidine group and 75.7% from the amphotericin B group refused to continue participating in the study. As none of the drugs mentioned above are free of side effects, the search for alternative therapeutic agents is essential in control and prevention of leishmaniasis (de Medeiros *et al.*, 2011).

Combination therapy for leishmaniasis

Drug resistance related to monotherapy regimes were found in about 10 to 15% and 50 to 60% of CL and VL patients, respectively (Salehabadi *et al.*, 2014). Combination therapy of anti-leishmanial drugs is currently considered as one of the most rational and promising approaches that has advantages such as reduced toxicity, synergic effects, limited drug resistance development, low treatment failure rate, and shorter treatment regimens (Gazanion *et al.*, 2011; Monzote *et al.*, 2011). For example, combined therapy of botulin derivative BT06 and the betulinic acid derivative AB13 with miltefosine via synergistic interactions has been considered as a promising compound in the field of new alternative therapy for leishmaniasis (Sousa *et al.*, 2014). In addition, Jabini *et al.* (2014) suggested that silymarin in conjunction with glucantime, but not alone, can have promising effects on CL caused by *L. major*. Combination of the treatment with cryosurgery and an antileishmanial drug such as glucantime can be much more effective than each of the two modalities when used alone (Alavi-Naini *et al.*, 2012). Nonetheless, two

treatment protocol including combined oral azithromycin capsule 10 mg/kg/day and allopurinol, a drug with anti-leishmanial activity, tablet 10 mg/kg/day for two months and IM injection of glucantime 20 mg/kg for 20 days showed low efficacy in the treatment of old world CL. However, combination therapy had a slightly better outcome after a two-month period of follow-up in 86 patients. The authors reported no severe adverse effect in their study (Dastgheib *et al.*, 2012). Nicotinamide (NAm), known as vitamin B₃, is able to exert *in vitro* anti-leishmanial activity. It has been found that NAm significantly improves the anti-leishmanial activity of trivalent antimony in a synergistic manner while it shows additive activity with amphotericin B and slightly antagonizes pentamidine activity (Gazanion *et al.*, 2011). In addition, it has been indicated that NAm significantly increases the toxicity of pentavalent antimony against the amastigotes of *L. infantum*, *L. amazonensis* and *L. braziliensis* (Gazanion *et al.*, 2011).

Other treatment approaches

One method for improving a drug's anti-leishmanial efficacy is nanonization. This approach is identified as foreign bodies/nanoparticles phagocytosed by the macrophages allowing targeted drug delivery. Application of nanoparticles of amphotericin B (amphotericin B attached to functionalized carbon nanotubes) is another therapeutic modality and the *in vivo* and *in vitro* experiments have shown promising effects on promastigotes as well as amastigotes of *L. Donovanii* (Prajapati *et al.*, 2011a). Another simple and cost-effective modality for improving the treatment with antimony compounds is thermotherapy. The potential mechanism of action of this method is probably direct destruction of parasites by heat (Alavi-Naini *et al.*, 2012). In a randomized controlled trial conducted on 382 patients, the effectiveness of a single localized treatment by thermotherapy was compared with intralesional administration of glucantime for 5 days, for the treatment of CL by a six-month period of follow up (Safi *et al.*, 2012). The treatment rate for the thermotherapy group was 82.5%, while it was 74% in the glutamine group. Consequently, thermotherapy was more efficient and cost-effective, with fewer side effects, shorter therapy duration and better patient compliance than intralesional glutamine. In a randomized controlled trial (RCT), patients with leishmaniasis due to *L. tropica* were treated with an initial removal of skin lesions through electrocoagulation using a bipolar high-frequency electrosurgery instrument followed by being kept moist with or without pharmaceutical sodium chlorite (DAC N-055) daily (Jebran *et al.*, 2014). Electrocoagulation reduced the parasite load and also created a fresh wound bed for producing subsequent granulation tissue and epithelialization. Additionally, sodium chlorite initiated tissue-regeneration and was also cytotoxic against promastigote and amastigote forms of *L. tropica*. This modality was stated as a highly effective, inexpensive and well-tolerated treatment option for patients with CL

due to other *Leishmania* such as *L. major*, *L. mexicana* and *L. braziliensis*, either the old world or the new world (Jebran *et al.*, 2014). Also, topical administration of honey can be effective in the treatment of leishmaniasis. It influences the collagen formation, wound contraction, and epithelialization with minimal scar formation and has antimicrobial effects (Oryan and Zaker, 1998; Alavi-Naini *et al.*, 2012). It can enhance the speed of wound healing and therefore it can be effectively used in patients with CL (Alavi-Naini *et al.*, 2012).

Herbal or plant therapy

As previously mentioned, lack of an efficient vaccine and resistance to drugs administered for the treatment of leishmaniasis, coupled with their high cost, parenteral route of administration and toxicity, have been regarded as a great concern especially in endemic areas of developing countries. Hence, there could be no doubt that the search for novel agents having anti-leishmanial or leishmanicidal potency is one of the critical challenges in the field of the current drug discovery program and is of worldwide concern (Guimaraes *et al.*, 2010; Sen and Chatterjee, 2011; Iqbal *et al.*, 2012; Wink, 2012; Ogeto *et al.*, 2013; Mahmoudvand *et al.*, 2014). For this purpose, some researchers have been looking for new alternatives in nature as an important source of drugs used in medicine (de Medeiros *et al.*, 2011; Rondon *et al.*, 2012; Wink, 2012; Manjolin *et al.*, 2013; Gamboa-Leon *et al.*, 2014; Sifaoui *et al.*, 2014). It has been estimated that there are about 250,000 medical plant species in the world. However, the biological activities of only about 6% of them have been screened. Furthermore, only approximately 0.75% medical herbal compounds have been studied in clinical trials (Sen and Chatterjee, 2011; Jameel *et al.*, 2014). The major merits of herbal medicine include their low cost, low incidence of serious adverse effects and good efficacy (Garcia *et al.*, 2010; Wink, 2012; Sarkar *et al.*, 2013). Unfortunately, the efficacy of most of these compounds has been shown only in *in vitro* studies and hence they are still in their initial stages of preclinical trials to be applicable in clinical practice and translation of their results into clinical practice is a neglected field. It is estimated that herbal products are involved directly or indirectly in production of about 25% of medicines (Colares *et al.*, 2013; Vila-Nova *et al.*, 2013). Several studies have shown that various plant species possess inhibitory activity against certain types of parasites such as *L. major* (Ogeto *et al.*, 2013), *L. amazonensis* (Guimaraes *et al.*, 2010; de Medeiros *et al.*, 2011), *L. tropica* (Iqbal *et al.*, 2012), *L. aethiopicum* (Bekele *et al.*, 2013), *L. braziliensis* (Yamamoto *et al.*, 2014), *L. mexicana* (Gamboa-Leon *et al.*, 2014), *L. infantum* (Mansour *et al.*, 2013; Machado *et al.*, 2014), *L. chagasi* (Rondon *et al.*, 2012) and *L. donovani* (Sachdeva *et al.*, 2014a, b).

The number of recent *in vitro* and *in vivo* animal studies regarding the effectiveness of medicinal plants and plant-derived compounds such as the crude extracts

and essential oils against CL and VL caused by different *Leishmania* species has been summarized in Table 2. In order to evaluate the anti-leishmanial or leishmanicidal activity of a compound, several criteria are considered including MIC, IC50 as well as selectivity index (Mahmoudvand *et al.*, 2014). MIC is defined as the minimum inhibitory concentration of a compound or drug against the parasite. IC50 is described as the concentration of drug that causes 50% growth inhibition of amastigote or promastigote forms of *Leishmania* (Monzote *et al.*, 2014a). IC50 is used for macrophages of the host, too. CC50 is the cytotoxicity concentration of drug that results in 50% of mortality of macrophage from usually BALB/c mice (Islamuddin *et al.*, 2012). An anti-leishmanial drug or natural compound is safe when selectivity index is greater than 10 (Mahmoudvand *et al.*, 2014). Rondon *et al.* (2011) evaluated the leishmanicidal potential of the fractions obtained from the leaves of *Aloe vera* (aloe), and *Ricinus communis* (castor) and the seeds of *Coriandrum sativum* (coriander) against amastigotes and promastigotes of *L. infantum*. These compounds were compared at different concentrations of 6.25, 12.5, 25, 50, and 100 µg/ml with 40 µg/ml pentamidine and amphotericin B as the positive controls for tests related to promastigotes and amastigotes, respectively. They used three extracts obtained from these plants including ethyl acetate, chloroform, and methanol. All fractions were efficient against *L. infantum* promastigotes but did not show significant difference with pentamidine. On the other hand, ethyl acetate and chloroform fractions of *R. communis* and *C. sativum* methanol extract were the most efficacious against amastigotes with no significant difference with amphotericin B. Amongst these, ethyl acetate fraction of *R. communis* showed the least toxicity and the best results. This action was attributed to iron-dependent enzymes and membrane lysis of the parasite due to the presence of flavonoids, alkaloids, phytoestrogens and saponins. The authors proposed the necessity of undertaking *in vivo* studies with *R. communis* ethyl acetate fraction due to its low cytotoxicity as well as high effectiveness against the parasite. As well, 2-substituted quinolone, a medicinal plant derivative, is efficacious against promastigotes and amastigotes of *L. infantum*, and *L. amazonensis* (Seifert, 2011), and more effective than miltefosin against *L. donovani* (Loiseau *et al.*, 2011). Recently, Ogungbe *et al.* (2014) used 352 phenolic phytochemicals exhibiting antiparasitic activity, including 10 aurones, 6 cannabinoids, 34 chalcones, 20 chromenes, 52 coumarins, 92 flavonoids, 41 isoflavonoids, 52 lignans, 25 quinones, 8 stilbenoids, 9 xanthenes, and 3 miscellaneous phenolic compounds along with 24 *Leishmania* enzymes. It was demonstrated that two aurones (bracterin triacetate and 4,6-dibenzoyl-2-[phenylhydroxymethyl]-3(2H)-benzofuranone), one chalcone (crotaorixin), five coumarins (mammeaA/AA, mammeaB/BA, mammeaB/BA cyclo F, scoparone, and umckalin), six flavonoids (diploacene, quercetin-3',4',7'-trimethyl ether, cannflavin A, 3'-O-methyldiploacene, 3'-O-methyldiploacol, and 4'-O-methyldiploacene), one

isoflavonoid (sophoronol E), three lignans (aristolignin, 3,3',4,5-tetramethoxy-4',5'-methylenedioxy-7,7'-epoxy-lignan and 4,5,4',5'-dimethylenedioxy-3,3'-dimethoxy-7,7'-epoxylignan), and one stilbenoid (machaeriol B) can be considered as promising drugs and are worthy of further investigation for the treatment of leishmaniasis (Ogungbe *et al.*, 2014). Essential oils or volatile extracts as natural compounds obtained from different parts of plants such as the flowers, leaves, bark, stems, wood, roots, fruits or seeds are commonly used in folk medicine to treat leishmaniasis (Colares *et al.*, 2013). Essential oils prepared by hydrodistillation are composed of different small hydrophobic molecules such as sesquiterpenes and terpenes, especially monoterpenes diffusing easily across cell membranes and consequently gaining access to intracellular targets (Machado *et al.*, 2012; Colares *et al.*, 2013; Machado *et al.*, 2014). Terpenes are hydrocarbons formed from units of isoprene, can easily penetrate the lipid bilayer of cell membrane and produce changes in the integrity of cell structures and mitochondrial membrane (Colares *et al.*, 2013). There are several reports on the activity of essential oils on endemic *Leishmania* species responsible for CL and VL (Machado *et al.*, 2012; Radulovic *et al.*, 2013; Machado

et al., 2014). Pharmacological application of the essential oils against human parasitic infections is unclear due to the potential risk for toxicity (Machado *et al.*, 2012). However, several studies have demonstrated that the crude essential oils and their major compounds present low or no toxicity to host cells at the effective concentrations (Rodrigues *et al.*, 2013). It has recently been reported that essential oils from *Artemisia annua* (Radulovic *et al.*, 2013), *Croton argyropyloides* (de Franca-Neto *et al.*, 2012), *Ligustim chuanxiong* (Zhang *et al.*, 2012) and *Menta villosa* (Da Silva *et al.*, 2012) are effective and non-toxic in animal models with leishmaniasis. Machado *et al.* (2014) have also demonstrated that essential oils derived from *T. capitellatus* have anti-leishmanial activity without significant cytotoxicity against macrophages. In addition to the anti-leishmanial activity of these essential oils, they have also shown insecticidal activity, which is extremely important in the control of sandflies and leishmaniasis (Machado *et al.*, 2014). Moreover, some essential oils derived from plants such as *Croton cajucara* (red sacaca) have immunomodulatory effects and this makes them useful in the treatment of leishmaniasis (Rodrigues *et al.*, 2013).

Table 2: Antileishmanial activity of plant-derived compounds against CL and VL as tested *in vitro* and *in vivo*

Scientific name	Plant part used	Preparation	Concentration/dose	Organism tested	Way of use	Main outcomes	Reference
Against CL (<i>in vitro</i> and <i>in vivo</i> studies)							
<i>Croton pullei</i> var. <i>glabrior</i>	Stem bark	julocrotine, alkaloid isolated from <i>C. pullei</i>	Concentration of 1 mg/ml, IC50: 19.8 µM	Amastigote and promastigotes of <i>L. amazonensis</i>	<i>In vitro</i>	80% inhibition, significant reduction in the number of amastigotes and morphological changes in promastigotes without any toxicity	Guimaraes <i>et al.</i> , 2010
<i>Lippia sidoides</i> Cham. (<i>Alecrim pimenta</i>)	Aerial parts	Essential oil	Concentration of 250-15.6 µg/ml, IC50: 44.38 µg/ml	Amastigotes and promastigotes of <i>L. amazonensis</i>	<i>In vitro</i>	Reduced both amastigote and promastigote survival without cytotoxic effects	de Medeiros <i>et al.</i> , 2011
<i>Valeriana wallichii</i> DC	Root	Water, methanol and chloroform extracts	(Promastigotes): 1-500 µg/ml for the extracts and 1-20 µg/ml for the fractions, amphotericin B: 0.01-10 µM; (amastigote): 0.001 to 100 µg/ml for the extracts and fractions	Promastigotes of <i>L. donovani</i> and promastigotes and amastigotes of <i>L. major</i>	<i>In vitro</i>	Methanol and chloroform (F3: the most active with IC50 of 3-7 µg/ml for both promastigotes and 0.3 µg/ml against amastigotes of <i>L. major</i>) extracts showed activity against all three options via apoptotic cell death	Ghosh <i>et al.</i> , 2011
<i>Aloe vera</i> vs. <i>Tamarix aphylla</i>	Leaf vs. bark	Methanolic extract	25-100 µg/ml	Promastigotes of <i>L. tropica</i>	<i>In vitro</i>	Especially <i>T. aphylla</i> except for the lowest concentration had high significant effect on parasite motility and with anti-promastigote activity	Iqbal <i>et al.</i> , 2012
<i>Cymbopogon citratus</i> and citral	Aerial parts	Essential oil	Concentration: 10-400 µg/ml, and IC50: ranging from 25 to 52 µg/ml and 34-42 µg/ml, respectively	Promastigotes of <i>L. infantum</i> , <i>L. tropica</i> and <i>L. major</i>	<i>In vitro</i>	both were effective in inhibiting the parasites growth by apoptosis without cytotoxicity induction	Machado <i>et al.</i> , 2012
<i>Galipea longiflora</i> Krause	Bark	Quinolinic alkaloid extracts vs. glucantime	10 µg/ml extract, 14 µg/ml glucantime; in mice, IP: 6.25, 12.5 mg the extract or 25 mg glucantime and 12.5 mg extract with 25 mg the drug	Promastigotes of <i>L. amazonensis</i>	<i>In vitro</i> and <i>in vivo</i> in mouse	The combined therapy was more efficient than individual use, reduced the parasite load and the footpath thickness	Calla-Magarinos <i>et al.</i> , 2012
<i>Calophyllum brasiliense</i>	Leaf	Mammea A/BB, coumarin obtained from <i>C. brasiliense</i>	18 mg/kg/d IM or 0.2% topically vs. 27 mg/kg/d of Glucantime for 30 days	Promastigotes of <i>L. amazonensis</i>	In mouse with CL	Reduced size of skin lesions, similar anti-leishmanial activity to that of 27 mg/kg/d, IM, Glucantime®	Tiuman <i>et al.</i> , 2012
<i>Moringa stenopetala</i>	Root	1,3-dilinoleoyl-2-olein and 1,3-dioleoyl-2-linolein (triglycerides)	Concentrations: 1.00, 0.33, 0.11, 0.037, 0.012, 0.004, and 0.0013 mg; IC50: 0.079 and 242.5 µg/ml vs. 0.004 and 0.136 µg/kg for amphotericin B and miltefosine	Promastigotes of <i>L. aethiopia</i>	<i>In vitro</i>	Were active against promastigote and amastigote stages of <i>L. aethiopia</i> , but relatively lower than the reference compounds	Bekele <i>et al.</i> , 2013
<i>Vanillosmopsis arborea</i> and <i>α-bisabolol</i>	Stem	Essential oil	IC50: 7.35 and 4.95 µg/ml for promastigotes and IC50: 12.58 and 10.70 µg/ml for amastigotes	Promastigotes and amastigotes of <i>L. amazonensis</i>	<i>In vitro</i>	Showed leishmanicidal activity in the absence of obvious cytotoxicity on macrophages	Colares <i>et al.</i> , 2013
<i>Curcuma longa</i> Linn.	Spice turmeric	Curcumin, gallium curcumin, indium	5 µg/ml for all, IC50: 38, 32, 26, 52 and 20 µg/ml	Promastigotes of <i>L. major</i>	<i>In vitro</i>	Indium curcumin was the most effective than others, followed by	Fouladvan <i>et al.</i> ,

		curcumin, and diacetylcurcumin vs. amphotericin B	respectively			gallium curcumin	2013
<i>Piper regnellii</i> var. <i>pallascens</i>	Leaf	Eupomatenoid (a neolignan)	Concentrations of 30.0, 85.0 and 170.0 µM; IC50:	Promastigotes of <i>L. amazonensis</i>	<i>In vitro</i>	Induced cell death in the parasite, increased mitochondrial ROS and O ₂ -production and G0/G1 phase cell cycle arrest	Garcia <i>et al.</i> , 2013
<i>Strychnos pseudoquina</i>	Stem bark	Flavonoids of quercetin 3-O-methyl ether and strychnobilavonethyl purified from acetate extract	0.78-100 µg/ml, and fractions (each one, 50 µg/ml), IC50: 24.9 ± 5.2 µg/ml	Promastigotes of <i>L. amazonensis</i>	<i>In vitro</i>	The ethyl acetate extract and two purified flavonoids showed anti-leishmanial potential with no significant toxicity	Lage <i>et al.</i> , 2013
<i>Vitis vinifera</i> L.	Leaf	Ethanollic and aqueous extracts	50, 25, and 6.25 µg/ml vs. 50, 25, and 6.25 mg/ml ethanollic vs. aqueous extracts, IC50: 0.108 mg/ml	Promastigotes of <i>L. infantum</i>	<i>In vitro</i>	Ethanollic extract was more effective and destructed <i>L. infantum</i> promastigotes due to higher anthocyanin contents	Mansour <i>et al.</i> , 2013
<i>Physalis angulata</i>	Stem	Ethanollic extract	1.2-100 µg/ml; IC50: 5.35 µg/ml and 4.50 µg/ml against <i>L. amazonensis</i> and <i>L. braziliensis</i> promastigotes	Promastigotes of <i>L. amazonensis</i> and <i>L. braziliensis</i>	<i>In vitro</i>	Significantly reduced the parasites, was non-mutagenic and presented a promising anti-leishmanial effect without significant cytotoxicity	Nogueira <i>et al.</i> , 2013
<i>Aloe secundiflora</i>	Leaf	Methanollic and water extract	1-1000 µg/ml, IC50: 279.488 and 42.824 µg/ml for water and methanollic extracts	Premastigotes of <i>L. major</i>	<i>In vitro</i>	Significantly inhibited the growth of <i>L. major</i> vs. amphotericin B	Ogeto <i>et al.</i> , 2013
<i>Annona muricata</i> acetogenins, annonacinone and corosolone, and <i>Platymiscium floribundum</i> coumarin scoparone	Stem of <i>P. floribundum</i> and leaves and seeds of <i>A. muricata</i>	One coumarin, scoparone, from <i>P. floribundum</i> and two acetogenins, annonacinone and corosolone, from <i>A. muricata</i>	100, 50, 25, 12.5 and 6.25 µg/ml	Promastigotes of <i>L. donovani</i> , <i>L. mexicana</i> and <i>L. major</i>	<i>In vitro</i>	<i>L. donovani</i> and <i>L. major</i> were more susceptible to annonacinone and <i>L. mexicana</i> to annonacinone and scoparone, three species had similar susceptibility to corosolone, and corosolone was the most toxic	Vila-Nova <i>et al.</i> , 2013
<i>Senna Spectabilis</i>	Flower	Crude ethanollic extract, fractions (dichloromethane and n-butanol) and two major alkaloidal metabolites of (-)-cassine/(-)spectaline	Extracts and fractions: serial concentrations of 100, 30, 10, 3, 1 and 0.3 µg/ml; Pentamidine and alkaloids: of 100, 30, 10, 3, 1 and 0.3 M	Promastigotes of <i>L. major</i>	<i>In vitro</i>	Fractions and flavonoids exhibited significant leishmanicidal activity than others without toxic effects on murine macrophages	de Albuquerque Melo <i>et al.</i> , 2014
<i>Thymus capitellatus</i> Hoffmanns. and Link	Aerial parts	Volatile extract (essential oil) and its compounds (1,8-cineole and borneol)	Concentration from 10 to 400 µg/ml. IC50: ranging from 35 to 62 µg/ml	Promastigotes of <i>L. infantum</i> , <i>L. tropica</i> and <i>L. major</i>	<i>In vitro</i>	Promoted leishmanicidal activity via induction of apoptosis, without significant cytotoxic effects	Machado <i>et al.</i> , 2014
<i>Berberis vulgaris</i> and <i>B. barbering</i> (its major principle, an isoquinoline alkaloid)	Root (rich in berberine)	Aqueous, methanollic and chloroform extracts vs. glucantime	IC50: 2.1-26.6 µg/ml; berberine had the least IC50% about 2.1 and 2.9 µg/ml for <i>L. major</i> and <i>L. troica</i> , respectively; CC50: 27.3-362.6 µg/ml	Promastigotes of <i>L. major</i> and <i>L. tropica</i>	<i>In vitro</i>	Were effective in inhibiting promastigotes of both <i>Leishmania</i> with more cytotoxicity by berberine; showed no significant cytotoxicity	Mahmoud vand <i>et al.</i> , 2014
<i>Chenopodium ambrosioides</i>	Not available	Essential oil and its components: ascaridole, carvacrol and caryophyllene oxide	10 µg/kg; ascaridole with the least IC50 for promastigotes, and macrophages	Amastigotes and promastigotes of <i>L. amazonensis</i>	<i>In vitro</i>	Were active against both forms the parasite and ascaridole had the better activity; the oil had the greatest selectivity index	Monzote <i>et al.</i> , 2014a
<i>Arrabidaea chica</i>	Leaf	Five fractions from crude hexanic extract of <i>A. chica</i>	1-500 µg/ml, IC50: 37.2 and 18.6 µg/ml for <i>L. amazonensis</i> and <i>L. infantum</i>	Promastigotes of <i>L. amazonensis</i> and <i>L. infantum</i>	<i>In vitro</i>	Especially B2 fraction inhibited completely the peptidase activity of promastigotes	Rodrigues <i>et al.</i> , 2014
<i>Olea europea</i> , 5 olive tree varieties (Limouni, Zarrazi, Dhokkar, Toffehi, Chemlali, Tataouine)	Leaf	Ethanol, methanol and mixture extracts	IC50: 2.130-71.570 µg/ml for <i>L. donovani</i> , 17.622-48.94 and 14.661-58.95 µg/ml for <i>L. tropica</i> and <i>L. major</i> , 2.94-16.95 µg/ml for <i>L. amazonensis</i>	Promastigotes of <i>L. donovani</i> , <i>L. amazonensis</i> , <i>L. tropica</i> and <i>L. major</i>	<i>In vitro</i>	All had interesting leishmanicidal activity, the Limouni variety had the highest phenolic content and the strongest activity. <i>L. amazonensis</i> was the most sensitive species	Sifaoui <i>et al.</i> , 2014
<i>Bixa orellana</i>	Seed	Essential oil (ishwarane and geranylgeraniol) and 71 other compounds in the oil)	30 mg/kg, IP, 14 days; amphotericin B 1 mg/kg; IC50: 8.5 vs. 0.03 µg/ml for amphotericin B	Amastigote and promastigote forms of <i>L. amazonensis</i>	<i>In vitro</i> and <i>in vivo</i> in BALB/c mouse	Showed activity against amastigote form and ability to control disease progression in mice	Monzote <i>et al.</i> , 2014b
<i>Tanacetum parthenium</i> (L.) Schultz-Bip	Aerial part	Sesquiterpene and lactone-rich dichloromethane fraction	IC50: 2.4±0.76 and 1.76±0.25 µg/ml for promastigotes and amastigotes, respectively, IM	Amastigotes and promastigotes of <i>L. amazonensis</i>	<i>In vitro</i> and <i>in vivo</i> in mouse	Decreased the parasite population with low toxicity, decreased the growth and size of lesions in mice	Rabito <i>et al.</i> , 2014
<i>Tridax procumbens</i> alone and in combination with <i>Allium</i>	Whole plant and bulbs, respectively	Methanol and aqueous, respectively	20 mg/kg separately, and 40 mg/kg of the mixture for 2 weeks, IP	Promastigotes of <i>L. mexicana</i> , CL	In mouse with CL	Increase in IgG2/IgG1 ratio and Th1-type immune response with the mixture. The mixture was better at controlling the infection without cytotoxicity	Gamboa-Leon <i>et al.</i> , 2014

<i>sativum</i> (garlic)							
<i>Berberis vulgaris</i> L.	Root bark	Ethanol extract	10% and 20% root bark extract	Promastigotes of <i>L. major</i>	In mouse with CL	Caused 90% and 55% recovery in mice treated with 20% and 10% root extract, respectively	Salehabadi <i>et al.</i> , 2014
<i>Baccharis uncinella</i>	Leaf	Oleanolic- and ursolic-containing terpenic fraction from <i>B. uncinella</i> vs. amphotericin B	1.0 and 5.0 mg/kg of the fraction (IP) vs. 10.0 mg/kg of amphotericin B; for 5 days	Promastigotes and amastigotes of <i>L. amazonensis</i> and <i>L. braziliensis</i>	In mouse with CL	Decreased lesion size, did not cause alteration in the tissues, a curative effect similar to the drug	Yamamoto <i>et al.</i> , 2014
Against VL (in vitro and in vivo studies)							
<i>Lippia sidoides</i> Cham.	Leaf	Thymol- and carvacrol-rich essential oil	IC50: 74.1 and 54.8 µg/ml for thymol- and carvacrol-rich oil	<i>L. chagasi</i> promastigotes	<i>In vitro</i>	Both essential oil showed significant activity, but carvacrol-rich oil was more effective	Farias-Junior <i>et al.</i> , 2012
<i>Mangifera indica</i> L.	Leaf	Petroleum ether, chloroform, and methanol extracts	Concentrations of 3, 5, 10, 15 and 30 µg/ml; IC50: 2.99, 2.91 and 2.74 µg/ml, respectively	Promastigotes of <i>L. donovani</i>	<i>In vitro</i>	All significantly inhibit the growth of the parasite, the methanol extract was the most active followed by chloroform and petroleum extracts	Haldar <i>et al.</i> , 2012
<i>Artemisia annua</i> (artemisinin)	Leaf and seed with floral part	n-hexane fractions and seeds vs. pentamidine	All in concentration of 100 µg/ml; IC50: 6.6 and 5.05 µg/ml vs. 1 µg/ml of pentamidine	Promastigotes of <i>L. donovani</i>	<i>In vitro</i>	Showed anti-leishmanial activity via programmed cell death (apoptosis) without cytotoxic effect	Islamuddin <i>et al.</i> , 2012
<i>Trichosanthes dioica</i> Roxb. (pointed gourd)	Root	Triterpenoid enriched extract (fraction)	Graded concentrations of 2.5, 5, 10, 20, 40 and 80 µg/ml; IC50: 18.75 µg/ml	Promastigotes of <i>L. donovani</i>	<i>In vitro</i>	Inhibited significantly the growth of <i>L. donovani</i> promastigotes	Bhattacharya <i>et al.</i> , 2013
Withanolides (<i>Withania somnifera</i> , Ashwagandha)	Leaf	Two fractions (F5 and F6) from ethanol extract,	60 and 15 µg/ml, respectively	Promastigotes of <i>L. donovani</i>	<i>In vitro</i>	Induced apoptotic death by the production of ROS from mitochondria and destruction of mitochondrial membrane	Chandrasekaran <i>et al.</i> , 2013
<i>Croton Cajucara</i> (red <i>sacaca</i>)	Leaf	Essential oil and 7-hydroxycalamenene	Concentration of 1-1000 µg/ml; IC50: 66.7, 1.37 and 0.01 µg/ml for the oil, the fraction and amphotericin B, respectively	Promastigotes of <i>L. chagasi</i>	<i>In vitro</i>	Caused mitochondrial changes in promastigotes, reduced parasite/macrophage interaction by 52.8%, increased NO production by 80% without any cytotoxicity	Rodrigues <i>et al.</i> , 2013
<i>Eryngium foetidum</i> L.	Aerial part	Methanol, n-hexane, ethyl acetate, and 50% methanol, a daucane sesquiterpene	20 µg/ml; IC50: 7.84 µM	Promastigotes of <i>L. donovani</i>	<i>In vitro</i>	Showed promising growth inhibition without any significant cytotoxicity	Rojas-Silva <i>et al.</i> , 2013
<i>Pleumeria pudica</i>	Leaf	Petroleum ether, chloroform and methanol extracts	3, 5, 10, 15 and 30 µg/ml and IC50: 3.04, 2.98 and 2.93 µg/ml for ether, chloroform and methanol extracts	<i>L. donovani</i> promastigotes	<i>In vitro</i>	All extracts inhibited growth of <i>L. donovani</i> promastigotes; methanol extract was the most active followed by chloroform and petroleum ether extracts	Sarkar <i>et al.</i> , 2013
<i>Fumaria parviflora</i> Lam.	Whole plant	Methanolic extract vs. pentamidine	Ranged from 1000 to 1.038 µg/ml; CI50: 5.35 µg/ml	Promastigotes of <i>L. donovani</i>	<i>In vitro</i>	had significant anti-leishmanial activity	Jameel <i>et al.</i> , 2014
<i>Milletia</i> species	Stem bark	Water and methanol crude extracts and six fractions of methanol extract	Concentration: 10 mg/ml; IC50: 11.8 µg/ml	Promastigotes of <i>L. donovani</i>	<i>In vitro</i>	dichloromethane fraction showed the best <i>in vitro</i> anti-leishmanial activity, with low cytotoxicity due to high terpenoids and alkaloids levels	Rajemiari miraho <i>et al.</i> , 2014
<i>Withania somnifera</i> (winter cherry)	Whole plant	Aqueous extract	350 mg/kg, for 15 days, orally, 5 mg/kg cisplatin, for 5 days, IP	Promastigotes of <i>L. donovani</i>	In mouse with VL	Increased anti-leishmanial efficacy of cisplatin, augmented levels of IgG2 over IgG1 and enhanced Th1 type immunity	Sachdeva <i>et al.</i> , 2013
<i>Tinospora cordifolia</i> in combination with cisplatin	Whole plant	Pure herb extract (tablet form)	<i>T. cordifolia</i> : 100 mg/kg, 15 days; Cisplatin: 5 mg/kg for 5 days, IP	Promastigotes of <i>L. donovani</i>	In mouse with VL	Enhanced proliferation and differentiation of lymphocytes, induced Th1 immune response and IFN-γ and IL-2, but declined IL-4 and IL-2 levels	Sachdeva <i>et al.</i> , 2014a
Combination of <i>Asparagus racemosus</i> (shatavari) with cisplatin	Whole plant	Tablet form	<i>A. racemosus</i> : 650 mg/kg for 15 days, oral, and cisplatin: 5 mg/kg for 5 days, IP	<i>L. donovani</i> promastigotes	In mouse with VL	Enhanced protective immune response (IgG) and boosted both cellular and humoral immunity	Sachdeva <i>et al.</i> , 2014b

CL: Cutaneous leishmaniasis; VL: Visceral leishmaniasis; ROS: Reactive oxygen species; NO: Nitric oxide; IP: Intraperitoneal; IM: Intramuscular; IC50: Concentration of drug that caused 50% of growth inhibition of amastigote or promastigote form of *Leishmania*

The antileishmanial activity of some plant extracts has been attributed to flavonoids (Manjolin *et al.*, 2013; Wong *et al.*, 2014). Flavonoids are a group of polyphenolic compounds that naturally present in fruits and vegetables and are known as antioxidants and anticancers with a significant protective effect against membrane damage (Manjolin *et al.*, 2013; Sifaoui *et al.*, 2014). Flavonoids such as catechins are able to form complexes with the parasite cell wall to influence processes requiring cell linking, and hence inhibit the parasite growth (Ogeto *et al.*, 2013). In a research work carried out by Manjolin *et al.* (2013), it has been shown that dietary flavonoids such as fisetin (the most potent

alkaloid), quercetin, luteolin and 7,8-hydroxyflavone with low cytotoxicity characteristics are able to inhibit arginase enzyme from *L. amazonensis*. Arginase plays a central role in the biosynthesis of polyamine which is very important and essential for protecting the parasite against oxidative stress and ROS produced by the host's defense system.

Mushrooms and their metabolites have been tested for their probable anti-leishmanial potential (Mallick *et al.*, 2014). Mallick *et al.* (2014) evaluated the anti-leishmanial effect of mushrooms against promastigote and amastigote forms of *L. donovani*. They used 18 extracts derived from six wild mushrooms (ethanol

extracts and water-soluble polysaccharide and polyphenolic fractions). Among these, *Astraeus hygrometricus* and *Tricholoma giganteum* induced apoptosis and inhibited the growth of *L. donovani* promastigotes. On the other hand, water-soluble fractions of *A. hygrometricus*, *Russula laurocerasi*, *Russula albonigra*, *Termitomyces eurhizus*, *Russula delica* and polyphenolic fractions of *R. laurocerasi* could inhibit the proliferation of intracellular amastigotes in macrophages. Regarding intracellular amastigotes, the compounds were non-toxic and induced release of NO and IL-12 in murine cells. They showed that such mushrooms can open the way for further phytochemical and pharmacological investigations towards the search of novel anti-leishmanial compounds (Mallick *et al.*, 2014). A review conducted by Mishra *et al.* (2009) has reported the leishmanicidal activity of various alkaloids including indole, quinoline, isoquinoline, pyrimidine-carboline, steroidal and diterpene derived from various plants as well as those obtained from marine sources. These alkaloids were from the following origins: indole alkaloids including those obtained from stem bark and leaf of *Kopsia griffithii* (Apocynaceae), *Peschiera australis* (Apocynaceae), the bark of *Corynanthe pachyceras*, (Rubiaceae), stem bark of *Aspidosperma ramiflorum* (Apocynaceae), and the stem bark of *Peschiera van heurkii* (Apocynaceae); isoguinoline alkaloids like those isolated from *Annona foetida* (Annonaceae), the bark of *Guatteria foliosa* (Annonaceae), the young leaves of plant *Guatteria dumetorum* (Annonaceae), the stem bark of *Rollinia emarginata* (Annonaceae), *Guatteria* sp., and the *Unonopsis buchtienii* R. E. Fries (Annonaceae); naphthylisoquinoline alkaloids including those derived from leaves and twigs of *Ancistrocladus griffithii* (Ancistrocladaceae), the roots of *Ancistrocladus likoko* J. Leonard (Ancistrocladaceae), *Ancistrocladaceae* sp., and *Ancistrocladus tanzaniensis* (Ancistrocladaceae); bisbenzylisoquinoline alkaloids such as those obtained from *Albertisia papuana* (Menispermaceae), *Pseudoxandra sclerocarpa* (Annonaceae), *Gyrocarpus americanus* (Hernandiaceae), *Caryomene olivasans* (Menispermaceae), *Limaciopsis loangensis* (Menispermaceae), and the stem bark of *Guatteria boliviana* (Annonaceae); and quinolone alkaloids including those isolated from the plants including *Galipea longiflora* Krause (Rutaceae), and the bark of *Dictyoloma peruviana* (Rutaceae). Others included steroidal alkaloids isolated from the leaves of the Bolivian plant *Saracha punctata* (Solanaceae), and the leaves of *Holarrhena curtisii* (Apocynaceae), benzoquinolizidine alkaloids derived from *Psychotria klugii* (Rubiaceae), diterpene alkaloids isolated from the species of genera *Aconitum*, *Delphinium* and *Consolida* (Ranunculaceae), pyrimidine- β -carboline alkaloids obtained from the bark extract of *Annona foetida* (Annonaceae), leaves of *Acanthus illicifolius* (Acanthaceae), the marine alkaloids isolated from marine sponges possessing antileishmanial activity like *Amphimedon viridis*, *Acanthostrongylophora* sp.,

Neopetrosia sp., *Plakortis angulospiculatus* and *Pachymatisma johnstonii*, and finally the miscellaneous alkaloids from leaves of *Acanthus illicifolius* (Acanthaceae), and from stem bark of *Zanthoxylum chiloperone* var. *angustifolium* (Rutaceae). Saponins are high molecular weight glycosylated plant metabolites with anti-inflammatory, anti-tumor and anti-HIV activity (Makwali *et al.*, 2012). The anti-leishmanial activity of acridine and dinitroaniline herbicides in combination with triterpenoid saponin extract and extracts prepared from roots of *Plumbago capensis* was investigated against *L. major* in BALB/c mice (Makwali *et al.*, 2012). The combined therapy eliminated the parasites from the lesions and reduced parasite load in the internal organs so that complete clearance of parasitemia from both sites occurs. The authors demonstrated that the combination therapy with saponin, acriflavine, trifluralin and plumbagin is a promising treatment for the infection with *L. major*. Sen and Chatterjee (2011) established the mechanism of action of several plant-derived compounds, as follows: plant-derived compounds including chalcones (alter the ultrastructure of parasite mitochondria resulting in inhibition of the parasite respiratory chain), flavonoids (inhibit the parasite DNA synthesis, and induce the production of ROS triggering apoptosis), saponins (able to react with *Leishmania* membrane, induce a decrease in membrane potential and cause ultimately loss of membrane integrity), quinones (increase the free radicals generation by the parasite and induce apoptotic-like cell death), alkaloids (interfer with the macromolecular biosynthesis and inhibit respiration of amastigotes, modulation of mitogen activated protein kinase, a regulatory enzymes for apoptosis and inflammation, induction apoptotic-like death cell mediated by free radicals and NO production), lignans (interact with macromolecules resulting in cell cycle arrest and prevent the parasite attachment to macrophages and their entry), tannins (increase the NO production in infected macrophages and enhance the expression of cytokines including IL-10, IL-12, TNF- α and IFN- γ), terpenoids (increase the production of NO, induce apoptosis via inhibition of parasite DNA topoisomerase, and inhibit the parasitic growth), and finally oxylipins (inhibit super-oxidase dismutase leading to increased ROS generation and the parasite killing, and enhance generation of NO and TNF- α within macrophages). da Costa *et al.* (2014) evaluated the activity of hexane and ether acetate extracts of stem wood, root wood and bark from *Connarus suberosus* Planch (Connaraceae), *Neea theifera* Oerst. (Nyctaginaceae) and *Myrcia linearifolia* Cambess. (Myrtaceae) against promastigotes of *L. amazonensis* at a concentration of 100 $\mu\text{g/ml}$. Six out of 10 *C. suberosus* extracts were effective against the parasite and among these the root bark hexane extract had the most activity. The inhibitory concentration index for 50% of the parasites (IC₅₀) ranged from 27.57 to 94.00 $\mu\text{g/ml}$. *N. theifera* and *M. linearifolia* showed no anti-leishmanial activity against *L. amazonensis*. The root bark ether acetate extract was selected for chemical fractionation

resulting in a mixture of rapanone and a new compound called suberonone, a mixture of β -sitosterol and stigmasterol, oleic acid, geranylgeraniol, and two derivatives obtained from the first two mixtures. Several researchers have evaluated anti-leishmanial properties of the most common plants available in their countries. For example, Ribeiro *et al.* (2014) investigated the *in vitro* antileishmanial activity of 44 extracts and fractions derived from 16 Brazilian plant species against *L. amazonensis*. They used plants with minimum inhibitory concentrations (IC₅₀) in *Leishmania* and their cytotoxic effects on murine macrophages (CC₅₀) for determining the selectivity index (SI) of each extract or fraction. The products that presented higher SI values were used in the treatment of macrophages infected with *L. amazonensis*. These plants included *Bowdichia virgiloides* Kunth, *Campomanesia lineatifolia* Ruiz, *Cecropia pachystachya* Trecul, *Chrysobalanus icaco* L., *Diospyros hispida*, D.C., *Dipteryx alata* Vog., *Syzygium cumini* L., *Eugenia uniflora* L., *Hymenaea courbaril* L., MS *Hymenaea stignocarpa* Mart. ex. Hayne, *Jacaranda caroba* Vell., *Jacaranda cuspidifolia* Mart., *Jacaranda ulei* Bureau and K. Schum, and *Licania tomentosa* Benth. The most potent extracts against *L. amazonensis* were the hexanic extract of *D. alata* (IC₅₀ of 59.08 μ g/ml), the hexanic extract of *S. cumini* (IC₅₀ of 31.64 μ g/ml), the ethanolic and hexanic extracts of leaves of *H. courbaril* (IC₅₀ of 44.10 μ g/ml and 35.84 μ g/ml, respectively), the ethanolic extract of *H. stignocarpa* (IC₅₀ of 4.69 μ g/ml), the ethanolic extract of *J. caroba* (IC₅₀ of 13.22 μ g/ml), and the ethanolic extract of *J. cuspidifolia* leaves (IC₅₀ of 10.96 μ g/ml). Extracts obtained from *D. alata* and *J. cuspidifolia* presented higher selectivity index, with high leishmanicidal activity and low cytotoxicity in the mammalian cells. Generally, the ethanolic extracts were less effective and more toxic than the hexanoic extracts and buthanolic, dichloromethane ethyl acetate and hexanic fractions in the mammalian cells. Because of the lack of NO production in the treated macrophages, they suggested that this activity of *D. alata* and *J. cuspidifolia* is due to mechanisms other than macrophage activation mediated by NO production. Garcia *et al.* (2012) examined the anti-leishmanial activity of 48 extracts obtained from 46 Cuban plants mainly from leaves but from seeds and roots in concentration between 6.25 and 100 μ g/ml against promastigotes and mastigotes of *L. amazonensis*. Only 4 extracts including *Bambusa vulgaris*, *Hura crepitans*, *Mangifera indica*, and *Simarouba glauca* exhibited selective activity against the parasite and then were evaluated against intracellular amastigotes of *L. amazonensis*. Among these, three showed inhibition of growth with IC₅₀ value \leq 50 μ g/ml. However, *M. indica* extract showed an IC₅₀ value of 60.1 μ g/ml. The IC₅₀ of *B. vulgaris*, *S. glauca* and *H. crepitans* was 41.5 μ g/ml, 45.5 μ g/ml, and 27.7 μ g/ml, respectively. Collectively, among the 46 evaluated plant species, *H. crepitans* caused the highest inhibition of promastigotes growth (IC₅₀ = 16.4 μ g/ml) and showed lower toxicity against host cells (IC₅₀ = 390.5 μ g/ml), with an excellent selectivity index equal to 24. In

general, the extracts prepared from *H. crepitans*, *B. vulgaris*, and *S. glauca* showed more promising antileishmanial activity against both stages of the parasite. Therefore, the authors suggested identifying and purifying the active compounds of these plants. A study conducted in Saudi Arabia evaluated the *in vitro* antiprotozoal potential of the methanol extracts of 51 plants and some of their petroleum ether, chloroform, ethyl acetate and aqueous fractions against *L. infantum* amastigotes and also *Plasmodium falciparum*, *Trypanosoma brucei* and *T. cruzi* (Abdel-Sattar *et al.*, 2010). Among these, only nine crude extracts (18%) showed pronounced anti-leishmanial activity with IC₅₀ from 0.25 to 2.03 μ g/ml. Three plants including *Euphorbia schimperiana*, *Tribulus macropterus* and *Hypoestes forsskalii* showed IC₅₀ less than 0.25 μ g/ml. The highest activity was associated with the extracts of *Solanum villosum*, *Verbesina encelioides* and *T. macropterus* with IC₅₀ of 0.51, 0.35 and less than 0.25 μ g/ml and SI of greater than 125, 99.3 and 87.5, respectively.

Given the high prevalence of CL by *L. major* in Iran, Kheiri Manjili *et al.* (2012) assessed the anti-leishmanial potential of the ethanolic extracts of 17 medicinal plants against *L. major* promastigotes. Four plants including *Caesalpinia gilliesii*, *Satureia hortensis*, *Carum copticum* heirm, and *Thymus migricus* showed high anti-leishmanial activity with IC₅₀ of 9.76 ± 1.27 , 15.625 ± 3.76 , 15.625 ± 5.46 , and 31.25 ± 15.44 μ M, respectively. These plants were toxic against macrophages at concentrations higher than those needed to inhibit the parasite cell growth (IC₅₀, 45.13 ± 3.17 , 100.44 ± 17.48 , 43.76 ± 0.78 , and 39.67 ± 3.29 μ M, respectively). Moreover, glucantime inhibited the growth of *L. major* promastigotes with IC₅₀ = 254 μ g/ml without affecting the growth of macrophages. The results obtained from this study revealed the effectiveness of *C. gilliesii*, *S. hortensis*, *C. copticum* heirm, and *T. migricus* extracts due to the presence of the active compounds which could serve as alternative agents in the control of CL. Although herbal remedies have been used for a long period of time for the treatment of leishmaniasis, a systematic scientific evaluation of the effectiveness of herbal extracts has rarely been performed. A double-blind, randomized, clinical trial carried out by Sattar *et al.* (2012) investigated the antileishmanial potency of *Morinda citrifolia* (Noni) stem extract against CL. Among 40 patients, 30% indicated good improvement and 50% exhibited excellent response. In addition, morindicone and morinthone, two major constituents derived from the extract, displayed good antileishmanial activity. Another double-blind randomized clinical trial by Zerehsaz *et al.* (1999) compared the efficacy of intramuscular administration of meglumine antimonial (glucantime) (85 patients, for 20 days, 15-20 mg/kg/day) with topical herbal extract consisting of a mixture of the pure extracts of *Althaea rosa*, *Althaea officinalis* and members of the families Leguminosae, Faliaceae, Malvaceae, and Lythraceae (Z-HE) (86 patients, as a crude paste covered by a dressing, for 5 days) on patients with CL. Complete

cure was obtained in 74.4% and 24.1%, partial cure in 11.6% and 14.1% as well as failure in 14.0% and 58.8% in the glucantime group and the Z-HE group, respectively. Moreover, adverse effects due to treatment including urticarial and generalized pruritus only were observed in the glucantime group (Zerehsaz *et al.*, 1999). It was concluded that the mechanism of action could be the enhancement of hosts' cellular immunity. A thorough investigation on anti-leishmanial activity of the plants present here along with their toxicity, mechanism of action and physiochemical properties for development of an optimum formulation is urgently needed to confirm their efficacy in the treatment of leishmaniasis (Jain and Jain, 2013).

Future directions

Some studies have focused on a way to prevent side effects of the drugs effective on leishmaniasis such as cisplatin through the simultaneous supplementation of preventive agents. One of these methods includes induction of antioxidant enzymes with free radicals scavenging activities. The extracts of several plants have been shown to exhibit effective protection against drug (cisplatin)-induced toxicities and ameliorative effect (Sachdeva *et al.*, 2013; Sachdeva *et al.*, 2014a, b). Since infective form, amastigote, is located inside macrophages, the treatment of leishmaniasis is difficult (de Medeiros *et al.*, 2011). It has been demonstrated that synthetic flavonoid dimers can increase intercellular drug accumulation and thereby avert drug resistance via impairment of the ATPase pump (Wong *et al.*, 2014). For example, Wong *et al.* (2014) examined the *in vitro* and *in vivo* anti-amastigote and anti-promastigote activities of flavonoid dimer 39 (39.HCL) compared with well-known anti-leishmanial drugs. For *in vivo* study, mice acquired CL through subcutaneous inoculation of *L. amazonensis* promastigotes into the footpad. Then, they received 0.5 or 2.5 mg/kg of 39.HCL dissolved in 50% polyethylene glycol, 28 mg/kg SSG dissolved in 0.9% NaCl from day 16 to 48 post-infection. Compound 39 displayed significant anti-promastigote (IC₅₀ from 0.19 to 0.69 μ M) and anti-amastigote (IC₅₀, 0.17 to 2.2 μ M) activities towards promastigotes of all species including *L. major*, *L. amazonensis*, *L. braziliensis*, and *L. tropica*. In mice, 39.HCL reduced thickness of the footpad by 36% and the amastigote load present in the lesions was reduced 20-fold. Importantly, compound 39 was not toxic to peritoneal macrophages. Therefore, the authors introduced flavonoid dimer 39 as a novel safe and efficient leishmanicidal agent against leishmaniasis. Apart from a combination of two or more antileishmanial drugs, researchers are also investigating the effect of immunotherapy due to immunomodulators obtained from plants in conjunction with anti-leishmanial drugs (Sachdeva *et al.*, 2013). Indeed, the use of products and formulations that stimulate the immune system can be regarded as a promising alternative for the treatment of leishmaniasis (Sachdeva *et al.*, 2013; Sachdeva *et al.*, 2014b). It is necessary to consider the immune response

elicited by the treatment as well as effect of therapy on the immune response since the Th1 and Th2 responses play a prominent role in recovery from leishmaniasis and shift from Th2 to Th1 response helps in providing resistance to infection (Chowdhury *et al.*, 2012; Yamamoto *et al.*, 2014). Some studies have demonstrated the benefits of combining conventional treatment with other compounds that can control immune response or they have immunomodulatory property (Calla-Magarinos *et al.*, 2012; Chandrasekaran *et al.*, 2013; Gamboa-Leon *et al.*, 2014). An *in vivo* study on anti-leishmanial and immunomodulatory activity of *Withania somnifera* against *L. donovani* has shown that it is more effective with the combination of cisplatin and *W. somnifera* compared to the drugs alone (Sachdeva *et al.*, 2013). This inhibitory effect of *W. somnifera* has been attributed to the presence of withaferin A (steroidal lactone) and withanolide A. Withaferin A can induce *Leishmania*-affected cells apoptosis via inhibition of protein kinase C affecting apoptosis (Sachdeva *et al.*, 2013). In addition, *W. somnifera* due to its hepatoprotective and antioxidant properties can eliminate hepatotoxicity and the liver dysfunction either induced by cisplatin used for the treatment of VL or caused by the parasite itself (Sachdeva *et al.*, 2013). Plant extracts, secondary metabolites or biomolecules can exert immunostimulatory properties (Chouhan *et al.*, 2014). It is believed that the most common mechanism for exerting immunomodulatory activity is the stimulation of NO or ROS production that is principle effector molecule for killing of *Leishmania* amastigotes (Niranthin, lignan isolated from aerial parts of *Phyllanthus amarus*) (Chowdhury *et al.*, 2012). The triterpene fraction containing oleanolic and ursolic acids can modulate immune cells to produce and increase levels of IL-12 and IFN- γ (Yamamoto *et al.*, 2014). Tannins and related compounds kill *Leishmania* via a NO-mediated mechanism (Chouhan *et al.*, 2014). Increase in cytokines related to Th1 lymphocytes (*Berberine chloride*) (Saha *et al.*, 2011), decrease in those associated with Th2 cells (*Asparagus racemosus*) (Sachdeva *et al.*, 2014b) or high IgG2/IgG1 ratio levels (mixture of *Tridax procumbens* and *Allium sativum*) (Gamboa-Leon *et al.*, 2014) are regarded as other potential mechanisms for anti-leishmanial, leishmanicidal or immunomodulatory activities of plant-derived formulations (Chouhan *et al.*, 2014). Leishmanicidal activity of *Allium sativum* (garlic) has been demonstrated against infection with *L. major* and *L. maxicana*, so that it induces a Th1-type response and stimulates INF- γ and NO production in macrophage, and thus prevents the progression of the infection (Gharavi *et al.*, 2011; Gamboa-Leon *et al.*, 2014). To improve the therapeutic efficacy and reduce toxicity, the above mentioned natural molecules can be applied as either scaffold for producing and exploring new immunodrugs or natural immunomodulators in synergy and in combination with existing drugs (Prajapati *et al.*, 2011a; Jain and Jain, 2013). Targeting of anti-leishmanial drugs to macrophages via drug delivery systems reflects a promising strategy

overcoming the drawbacks associated with the current treatment protocols. Various novel drug carriers including liposomes, nanoparticles, carbon nanotubes and microspheres among others may target macrophages in both ways, actively and passively (Jain and Jain, 2013). Liposomes and nanoparticles among others are naturally taken up by macrophages via phagocytosis and hence can target the drugs passively to target site. It is attempted to alleviate high toxicity of the available drugs by the site-specific delivery to macrophage through development of an ideal drug delivery system (Prajapati *et al.*, 2011a; Jain and Jain, 2013). Gold nanoparticles conjugated with quercetin (a widely available herbal flavonoid) have been successfully used against leishmanial infections by Das *et al.* (2013). They utilized synergistic effect of quercetin and the gold nanoparticles in impairing the oxygen metabolism of the parasite in macrophages. This compound was effective and presented promising results compared to sodium stibogluconate and paramomycin resistant strains. The value of the plants mentioned above and their derivations as drug candidates cannot be fully assessed, since data achieved from *in vivo* animal model studies and clinical trials have not been greatly generated.

Conclusion

Despite good knowledge on the epidemiology of leishmaniasis, it still remains uncontrollable. Researchers from every corner of the world should devote more attention and time for leishmaniasis, as one neglected protozoan disease with public health importance. In general, identification of the most efficient surveillance system and control strategies to reduce the mortality and morbidity rate, a suitable approach to control vectors, identifying and combatting risk factors, a safe, non-invasive, short and cheap treatment, all should be regarded as the most important approaches for satisfactory control towards complete eradication of leishmaniasis from particular developing countries. The present study shows a wide range of plant extracts exhibiting interesting antileishmanial properties *in vitro* and *in vivo* animal experimental studies. But the necessity of generalizing the results obtained from the *in vitro* and also the *in vivo* studies on the effectiveness of plant extracts, metabolites or formulations against different *Leishmania* species to clinical practice should be stressed to validate their activities.

Conflict of interest

Nothing to declare.

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