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Therapeutic modalities to combat leishmaniasis, a review

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ABSTRACT

Leishmaniasis is an emerging dermal disorder that causes high morbidity and mortality levels with a wide spectrum of clinical complications. Current situation of chemotherapeutic options with some attempts at immunotherapy has remained a dilemma for the treatment of leishmaniasis. Primary precautionary measure which relies on the managed control of the host and sandfly bite prevention is difficult to establish, as the transmission of the disease is manifested by various *Leishmania* species. Secondary and tertiary prevention is dependent on the medical assistance using clinical guidelines and adequate therapy. However, long course of duration and resistant nature of drugs with pronounced side effects often lead to reduction or cessation of treatment. The aim of this article is to view the current status of chemotherapeutic agents used against leishmaniasis; a review of natural plant extracts exhibiting antileishmanial activities *in vitro* or *in vivo* alone or in combination with recommended drugs seeming to validate their use in folk medicine, topical applications of ointments currently used to develop new compounds under trial, substantial efforts in vaccine development and insights about immunoregulation along with the recommendations and guidelines for future perspectives.

1. Introduction

Leishmania is a tropical neglected disease caused by single cellular, haemoflagellate protozoan parasites of the genus *Leishmania* (family Trypanosomatidae) transmitted by the bite of an infected female sandfly *Lutzomyia* or *Phlebotomus*[1]. Sandflies are the vectors of *Leishmania* parasites that are distributed throughout intertropical and temperate regions of the world. Only 60 of about 600 sandflies species are vectors for *Leishmania* around which 20 *Leishmania* species are described as human pathogens. Leishmaniasis depends on the *Leishmania* spp. involved, site of bite, number of bites, type of sandfly, genus of parasite and genetic potential[2]. Several major risk factors are involved in the emergence and spread of leishmaniasis worldwide: treatment failure and drug resistance, human-made environmental changes and acute predisposition of host immune status[3]. Other factors include: HIV epidemic, inadequate vector or reservoir control, lack of vaccines, international travels and international conflicts, urbanization and deforestation, movement of non-immune persons to endemic regions, massive migration from rural to urban areas, decline in social and economic circumstances[4]. Consolidated data are frequently not available, only estimates have been provided. Worldwide prevalence is about 12 million, endemic in 90 tropical and subtropical countries threatening approximately 350 million people each year and approximately 90% of which occurs in the Sudan and Indian subcontinent[3]. In most countries, the incidence numbers are probably highly underestimated, since many cases are not recognized and there is no obligation to report the disease. Categorically, leishmaniasis is classified into three different clinical forms: cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis and visceral leishmaniasis (VL)[5]. CL is a dermal manifestation representing up to 75% of all the new cases within 1–1.5 million annual incidences commonly caused by *Leishmania major* (*L. major*), *Leishmania tropica, Leishmania aethiopica, Leishmania mexicana* (*L. mexicana*), *Leishmania amazonensis* (*L. amazonensis*), and *Leishmania braziliensis* (*L. braziliensis*) in many parts of the world, particularly in sub-continent and Middle East[6]. CL is characterized by a small red papule which becomes darker and turns into ulcer with raised edges after several weeks. Ulcers can be moist and exude pus with a crusted scab; sores usually appear on exposed body parts of the skin, especially on the face and extremities[2]. Although CL is not lethal, but it can cause significant morbidity and the course of the disease is often accompanied by psychological and social repercussion, stigmatization, painful disfiguration and severe secondary dermal manifestations neoplasms and sarcoidosis. Localized CL is characterized by lesions on face, nose, forehead, and lower limbs that usually heal naturally. Another type of CL is the diffuse cutaneous leishmaniasis that produces symptoms like nodules, plates or lumps on the face, arms and legs never heal spontaneously and relapse after treatment[7]. Mucocutaneous leishmaniasis (espundia) is characterized by nasal obstruction and bleeding, disfiguration and generation of painful mucosal lesions and cartilage of the mouth, ear and pharynx. VL or

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kala-azar is a kind of systemic disease caused by *Leishmania donovani* (*L. donovani*), *Leishmania infantum* (*L. infantum*) and *Leishmania donovani* (*L. chagasi*). Typical symptoms of the disease are irregular fever, weight loss, hepatosplenomegaly, invasion of bone-marrow, skin pigmentation, oral mucosa, and sex cells[2].

2. Chemotherapy

The currently used treatment regimen for leishmaniasis is primarily based on pentavalent antimonial drugs, such as sodium stibogluconate and meglumine antimonate. However, use of these drugs has been limited and linked with perilous side effects, high cost, variable degree of efficacy under same doses and the emergence of drug-resistant. Alternatively, miltefosine, pentamidine, amphotericin B and paromomycin can be used[4].

2.1. Miltefosine

Miltefosine is an alkylphosphocholine analogue (hexadecylphosphocholine) that was originally developed as an oral antineoplastic agent but later proved to be a novel oral drug for treatment of VL with successful results in immunocompetent and immunocompromised patients. The antileishmanial mechanism of action of this compound can be extrapolated from its effect on mammalian cells, where it causes modulation of cell surface receptors, inositol metabolism, phospholipase activation, protein kinase C and other mitogenic pathways, eventually culmination in apoptosis[8]. Adverse effects of miltefosine include reversible gastrointestinal disturbances, renal toxicity and teratogenic in nature[9].

2.2. Pentamidine

Pentamidine is an aromatic derivative of diamidine which was chemically defined as 4-[5-(4-carbamimidoyl-phenoxy) pentoxy] benzenecarboximidamide, originally synthesized as hypoglycemic drugs and later its activity was demonstrated against *Leishmania* infections particularly VL. Pentamidine acts on the genome of parasite by hampering replication and transcription at the mitochondrial level[10]. In addition, pentamidine interferes with the reception or the function of polyamines. Commonly, the treatment with pentamidine causes pain at the site of injection, nausea, headache, burning sensation and hypotension in addition to skin eruptions, abnormal liver, renal dysfunctions and rarely diabetes mellitus[9].

2.3. Amphotericin B

Amphotericin B is a macrolide polyene antifungal agent, which was discovered from a bacterium *Streptomyces nodosus* and is recommended as a second line drug therapy against all forms of leishmaniasis[11]. The antileishmanial activity of amphotericin B is attributed to binding to its selectivity for 24 substituted sterols, namely, ergosterol fraction of the cell membrane of the parasite thus increasing its permeability and so its selectivity towards the microorganism[12]. However, this pharmaceutical product is also associated with serious side effects including fever accompanied by rigor and chills, thrombophlebitis, toxicities like myocarditis, severe hypokalaemia, renal dysfunction and even death[10]. Currently, toxic effects of amphotericin B have been largely improved with the advancement of lipid formulations fungizone (micellar AmBisome and sodium deoxycholate) resulting in increased efficacy and reduced toxicity as compared to the parent drug formulation. Advantages of this route of administration include targeting higher drug concentrations to the site of infection, lowering systemic toxicity, decreasing cost and fastening healing time[13].

2.4. Paromomycin

Paromomycin (aminosidine) is an aminoglycoside aminocyclitol produced by *Streptomyces rimosus* var. It is an effective remedy against several bacterial, protozoal and leishmanial spp.[14]. The mechanism of action of paromomycin in *Leishmania* is believed to be associated with the inhibition of protein synthesis by binding to the 30S ribosomal subunit at the start codon of mRNA, leading of accumulation of abnormal initiation complex[15]. In parallel, paromomycin promoted ribosomal subunit association of both cytoplasmatic and mitochondrial forms following low Mg^{2+} concentration, induced dissociation and also caused dysfunction in respiratory systems experimental models. Side effects associated with the ointment formulation are the ototoxicity, liver dysfunction, skin rashes and local pruritus[16].

3. Plant products with antileishmanial activity

The fascination and use of potential natural plants for the treatment of various ailments based on traditional practice with known medicinal properties have been used for centuries[17]. However, rapid advances in synthetic organic chemistry and advent of high screening techniques have made the process of finding new drugs and plant metabolites focus on bulk screening faster and easy. The discovery of pure compounds in plants as active principles and the art of exploring natural products has become a major part of the molecular sciences. Early studies on plant products were followed by an era of organic chemistry that led to the development of antimonial and arsenical (sodium stibogluconate, melarsoprol) and diamidine (pentamidine) antiprotozoal drugs. Plants are an important source for drug candidates, particularly against leishmaniasis because of their long association with parasites. More than hundred plants have been known to be active against various forms of leishmanial parasites[18,19]. With the objective of contributing to these studies, a literature search on the use of natural products (crude plant extracts, semi-purified fractions and chemically defined molecules) which have already been evaluated against leishmaniasis has been carried out. Several studies have been reported and carried out as described below on antileishmanial activity of crude extracts and/or chemically defined compounds derived from plants *in vitro* against promastigotes and amastigotes or *in vivo* against *Leishmania* infected animals.

Sesquiterpene lactone isolated from the leaves of *Munnozia maronii* (Asteraceae) inhibited the growth of eleven species of *Leishmania* promastigotes at concentrations between 2.5 and 10.0 μg/mL. Diterpenoids isolated from Euphorbiaceae species possessed toxic activity against the promastigote forms of *L. braziliensis* and *L. amazonensis* at concentrations IC_{100} (0.75 and 5 μg/mL respectively) [20]. Fournet *et al*. also demonstrated berberine, a quaternary isoquinoline alkaloid with highest leishmanicidal activity as it effectively eliminated *L. major* parasites at a concentration of 10 μg/mL in peritoneal mice macrophages[21]. Jonathan *et al.* evaluated activity of berberine and several of its derivatives for efficacy against *L. donovani* and *Leishmania brazliensis panamensis* in golden hamsters. Tetrahydroberberine was less toxic and more potent than berberine against *L. donovani* but was less potent to meglumine antimonate. Only berberine and 8-cyanodihydroberberine showed significant activity (increase in 50% suppression of lesion size) against *Leishmania brazliensis panamensis*[22].

Luize *et al.* studied antileishmanial effect of several plants including *Baccharis trimera, Cymbopogen citratus, Matricaria chamomela, Mikania glomerata, Ocimum gratissimum, Piper regnelli, Prunus demestica, Psium guajava, Sambucus canadensis, Stryphnodendron adstringens, Tanacetum parthenium* and *Tanacetum vulgare* and showed significant inhibitory growth effects (49.5%–99.0%) on promastigote and axenic amastigote forms of *L. amazonensis in vitro* at 100 μg/mL with no haemolytic effect observed[23]. Antileishmanial

activity of xantholides isolated from *Xanthium macrocarpum* (Asteraceae) was evaluated on *L. infantum* and *L. mexicana* promastigotes *in vitro*. The compounds had growth inhibitory activities ranging from 3.6% ± 0.7% to 40% ± 8%[24]. A study carried out by Khalid *et al.* on *Allium sativum* and *Azadirachta indica* revealed antileishmanial effect with IC_{50} of 4.94 μg/mL and 10.2 μg/mL respectively[25]. Antileishmanial activity of methanolic extract of *Vernonia amygdalina* (Asteraceae) was studied *in vitro* with a 50% effective dose of 13.3–18.5 μg/mL against promastigote and 45.8–74.4 μg/mL against amastigote forms of *Leishmania aethopica*[26]. Similarly, ethanolic extracts of medicinal plants *Vernonia polyanthes* and *Ocimum gratissimum* used in treatment of infectious and inflammatory disorders in Brazil were evaluated on *L. amazonensis* and *L. chagasi* promastigotes. Extracts were most active against *L. amazonensis* at IC_{50} (4 μg/mL) and *L. chagasi* at IC_{50} (71 μg/mL)[27]. Methanolic extracts of *Gongronema latifolia, Dorstenia multiradiata, Picralima nitida, Cola attiensis* and *Desmodium gangeticum* against VL isolate were tested and were found active at concentration of 50 mg/mL[28]. Other plants with marked activity against *L. donovani* were *Khaya senegalensis* and *Anthostema senegalense* with IC_{50} values of 9.8 and 9.1 mg/mL respectively[29]. The ethanolic extract of *Yucca filamentosa* showed potent activity against *L. amazonensis* at a concentration of 5 mg/mL[30].

The dichloromethane extracts of *Eryngium ternatum, Origanum dictamnus* and *Origanum microphyllum* and the methanol extracts of *Eryngium amorginum* had IC_{50} < 10 μg/mL with no cytotoxicity[31]. A study carried out on methanolic extracts of the leaves of *Polyalthia suaveolens, Dioscorea preussii, Augouardia letestui* and stem bark of *Cola lizae* plants on *L. infantum* showed the highest effect (IC_{50} < 5 μg/mL)[32]. The antileishmanial activity of plants extracts from *Sarcocephalus latifolius, Zanthoxylum zanthoxyloides, Entada africana, Bobgunnia madagascarensis, Pseudocedrela kotschyi* and *Psorospermum guineense* used in traditional management of parasitic infections in Mali had significant effects against promastigote and amastigote forms of *L. major*[33]. Mori *et al*. screened the effect of crude methanol extract isolated from *Cordia fragrantissima* wood against *Leishmania* species. The crude extract had minimal inhibitory concentration of 12.5 μg/mL against *L. major* promastigotes[34].

Le *et al*. revealed the search of new products in microorganism or marine sources, such as a glycoprotein isolated from the sponge *Pachymatisma johnstonii*, which showed a high activity *in vitro* against *L. donovani, L. braziliensis* and *L. mexicana* and aphidicolin, a fungal metabolite isolated from nigrospora sphaerica, which inhibited the growth of promastigotes and amastigotes of *L. donovani*[35].

Anigorufone, an antifungal phenyl–phenalenone phytoalexin, isolated from the banana plant (*Musa acuminata*) and synthesis is triggered by infection caused by a saprophytic pathogenic fungus, *Fusarium oxysporum*, which causes Panama's disease in the banana plant, shows antibiotic activity against the fungus and leishmanicidal activity on *L. donovani* promastigotes and *L. infantum* amastigotes with 50% lethal concentration at 12 μg/mL and supports the use of plant extract enriched in antifungal phytoalexins as a more rationale for a new antileishmanial drug or leismanicidal drug[36]. Kayser *et al.* identified that monomeric and dimeric naphthoquinones against extracellular promastigotes of *L. donovani*, *L. infantum, Leishmania enriettii* and *L. major* using a direct cytotoxicity assay with potent *in vitro* leishmanicidal activity in the range of EC_{50} 0.9–17.0 μg/mL[37]. *In vitro* leishmanicidal activity of aqueous extract of *Syagrus coronata* on *L. amazonensis* was evaluated with minimal inhibitory concentration of 8.3 μg/mL and observed by light microscopy with no cytotoxic effect as well as no allergic reactions in mammalian cells, suggesting good prospects for the development of new antileishmanias drugs[38]. Juliana *et al*. also evaluated *in vitro* activity of the crude hydroalcoholic extract of the aerial parts of *Miconia langsdorffii* against the promastigote forms of *L. amazonensis* in humans and displayed moderate activity with an IC_{50} value of 175.4 μg/mL[39].

Moheb *et al*. evaluated the efficacy of 25% and 40% hydroalcoholic extract of *Cassia fistula* against CL ulcers in small white mice and caused significant reduction of CL ulcers as compared with the control group. Combination of 75% *Cassia fistula* plus 2% dimethyl-sulfamide also significantly reduced diameter of ulcers as compared with glucantime for the treatment of CL in mice[40]. The combination of traditional drugs *Euphorbia milli*, *Aloe vera* with animal fat and turmeric exhibited considerably good antilieshmanial activity representing more efficient therapy leading to tissue softening and lesion and wound healing ulcer in comparison to chemical drug glucantime in mice model[41]. Efficacy of herbal extracts of *Thymus vulgaris* (thyme) and *Achillea millefolium* (yarrow), propolis hydroalcoholic extract were found more effective than systemic glucantime or alcohol against CL in BALB/c mice under trial for six weeks causing the reduction of the mean of ulcer size. The highest efficacy was observed for propolis, followed by *Achillea millefolium* and then *Thymus vulgaris*[42].

Maesabalide III (MB-III), an oleate triterpene saponin isolated from the Vietnamese plant *Maesa balansae* was studied against *L. donovani* in golden hamsters after administration of a single subcutaneous dose prophylactically against liposomal amphotericin B, administered intravenously at 5 mg/kg of body weight. Prophylactic administration of MB-III at 0.2 mg/kg reduced liver amastigote burdens by 99.8% within 7 days. Both MB-III at 0.8 mg/kg and liposomal amphotericin B were 100% effective against liver stages. It is concluded that administration of a single dose of MB-III at 0.8 mg/kg has efficacy potentially comparable to that of a single dose of liposomal amphotericin B at 5 mg/kg and is, therefore, considered a promising new antileishmanial lead compound[43]. The antileishmanial activity of several plants *Alstonia scholaris, Swertia chirata, Tibouchina semidecandra, Tinospora cordifolia* and *Nyctanthes arbortristis* from India were carried out against *L. donavani* infected hamsters and showed more than 75% inhibition of parasites on day 7 and/or 28 post treatment with an increased survival period[44].

The crude extract *Teucrium stocksianum* and its subsequent organic fractions showed that ethyl acetate fraction was found slightly more effective than the parent extract at lower concentrations which further gave a clue that leishmanicidal constituents of *Teucrium stocksianum* were concentrated in ethyl acetate fraction[45]. Antileishmanial dose dependant antipromatigote activity of *Aloe vera* leaf and *Tamarix aphylla* bark against *Leishmania tropica* has been investigated with statistically highly significant effect on motility of the parasites[46].

A preliminary examination of the crude methanol extracts of plant species collected from the Sudan, *viz. Azadirachta indica, Maytenus senegalensis* and *Eucalyptus globulus* showed IC_{50} values of 11.5, 55 and 78 mg/mL, respectively exhibited considerable *in vitro* antileishmanial activity on *L. major* promastigotes at a concentration of 0.5 mg/mL[47].

Arabino-galactins isolated from *Echinacea purpurea* showed preventative effect against the promastigotes in mice infections. Later it was shown that the LD_{50} antileishmanial activity was based on the immunostimulatory function and macrophage activation of the *Echinacea purpurea* used both orally (for immunostimulation) and topically (for wound healing) in leishmaniasis[48].

Keeping in view the significance of this knowledge and the art of exploring natural products will lead to the discovery of the pure compounds as active principles in plants with antileshmanial activity and will provide new impetus to obtain rational drug design at low cost and plans of new drugs.

4. Topical formulations

Topical treatments are an attractive alternative recommended choice for localized, self-limiting form of the disease and offer significant

advantages, such as ease of administration, more patient-compliance and fewer adverse effects with perceived efficacy at low cost over systemic therapy. A combination of 15% paromomycin sulfate plus 12% methyl benzethonium chloride in paraffin ointment results in reduced ulcer size of *L. major* and *L. mexicana* in BALB/c mice. In a parallel study, paromomycin in combination with quaternary ammonium disinfectants and/or dimethyl sulfoxide in paraffin showed significant level of antileishmanial activity[49]. A topical formulation of miltefosine comprised of 3-propyloxypropylene glycol, 3-hexylpropylene glycol and 3-nonylpropylene glycol has been under trial in Syria and Colombia for the treatment of nodular cutaneous malignancies. The response of CL in patients with miltex has been reported in experimental studies that using *L. mexicana*- and *L. major*-infected BALB/c mice has reduced and healed lesion size. A study was carried out on the formulation of 15% aminosidine and 10% urea in white soft paraffin in experimental models. Experimental studies with *L. amazonensis*-infected BALB/c mice proved the phenothiazine chlorpromazine and the tricyclic antidepressant amitriptyline, as 10% ointments in petroleum, to be ineffective[50]. However, the antimycobacterial drug clofazimine and the naphthoquinone plumbagin had a suppressive effect on lesion growth. Elsewhere, the dinitroaniline herbicide trifluralin, a compound with proven *in vitro* antileishmanial activity in a 15% topical formulation, had suppressive effects on *L. major* and *L. mexicana* lesions in BALB/c mice[51]. Other topical formulations have been in clinical trials, but many results have been equivocal and no major breakthroughs have been achieved. Studies are being designed to investigate other topical treatments of leishmaniasis including quinacrine, miconazole, clotrimazole, chlorpromazine, amphotericin and garlic cream.

5. Vaccine development

Studies are being carried out that to improve the understanding of the immune response to *leishmania* in particular and to rationalize vaccine development. The rationale for vaccine development is provided by the evidence that most individuals who had leishmaniasis are resistant to subsequent clinical infections[52]. Various types of vaccines (*e.g.*, killed or attenuated whole parasites, synthetic or recombinant peptides, or recombinant live vaccine vectors), with or without cytokines or other adjuvants, are being investigated. Substantial effort has been spent in developing a *Leishmania* vaccine; so far, inspite of extensive knowledge, progress in developing a protective vaccine has been limited. New approaches are now being investigated in the experimental models with several *Leishmania* species and sandfly saliva proteins[53]. Coler *et al*. give a useful update of work on killed or live attenuated vaccines, recombinant vaccines, synthetic peptides, nonprotein antigens, and 'naked' DNA vaccine. Simple nature of the parasite lifecycle and no reoccurrence of infection after healing in experimental models have shown that protection and possibility to develop a vaccine against leishmniasis could be achieved by using parasite-specific proteins, DNA or genetically attenuated parasites. In early trials, the Mayrink's vaccine was a mixture of killed, sonicated promastigotes of *L. amazonensis*, *L. braziliensis*, and unknown *Leishmania* species. Although tested vaccines were safe and immunogenic significant, yet long-lasting protection could not be shown[54]. The immune response and protective efficacy of volunteers vaccinated with BCG plus killed promastigotes of *L. mexicana* of commercially available vaccine against CL have been claimed maximum in Eucador[55]. However, for several basic and logistic reasons, like difficulties in maintaining parasite virulence, risk of unacceptable lesions in some recipients, vaccination is not currently recommended by World Health Organization[56].

6. Conclusions and recommendations

This study emphasized on the extensive review of identified commercial and herbal products which were used for the treatment of leishmaniasis to synthesize modified compounds by using rational drug design. The potential efficacy of antileishmanial plant extracts needs to be evaluated and analyzed in all forms of leishmaniasis, which is irrespective of the species. The potent leishmanicidal activities of certain chemically defined molecules isolated from natural origins represent an exciting advance in the search for novel antileishmanial agents. Future development of topical drugs must employ rational drug design to take account of physicochemical properties and their effect on percutaneous absorption. This leads to the identification of several scaffolds for the future synthesis of second-generation compounds with optimized pharmacological profile for the treatment of leishmaniasis. Further enhancement in the control of leishmaniasis can be conducted through knowledge about the genome of parasite, information of drug used for other infection or pathologies and compounds isolated from new natural sources.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Handman E, Bullen DV. Interaction of *Leishmania* with the host macrophage. *Trends Parasitol* 2002; **18**(8): 332-4.
[2] Markle WH, Makhoul K. Cutaneous leishmaniasis: recognition and treatment. *Am Fam Physician* 2004; **69**(6): 1455-60.
[3] Desjeux P. The increase in risk factors for leishmaniasis worldwide. *Trans R Soc Trop Med Hyg* 2001; **95**(3): 239-43.
[4] Sereno D, Holzmuller P, Lemesre JL. Efficacy of second line drugs on antimonyl-resistant amastigotes of *Leishmania infantum*. *Acta Trop* 2000; **74**(1): 25-31.
[5] Monzote L. Current treatment of leishmaniasis: a review. *Open Antimicrob Agents J* 2009; **1**: 9-19.
[6] Donald JA. *Burger's medicinal chemistry and drug discovery*. New York: John Wiley and Sons; 2003, p. 1347-52.
[7] Davies CR, Kaye P, Croft SL, Sundar S. Leishmaniasis: new approaches to disease control. *BMJ* 2003; **326**(7385): 377-82.
[8] Verma NK, Dey CS. Possible mechanism of miltefosine-mediated death of *Leishmania donovani*. *Antimicrob Agents Chemother* 2004; **48**(8): 3010-5.
[9] Sundar S, Chatterjee M. Visceral leishmaniasis-current therapeutic modalities. *Indian J Med Res* 2006; **123**(3): 345-52.
[10] Mishra J, Saxena A, Singh S. Chemotherapy of leishmaniasis: past, present and future. *Curr Med Chem* 2007; **14**(10): 1153-69.
[11] Singh S, Sivakumar R. Challenges and new discoveries in the treatment of leishmaniasis. *J Infect Chemother* 2004; **10**(6): 307-15.
[12] Ramos H, Valdivieso E, Gamargo M, Dagger F, Cohen BE. Amphotericin B kills unicellular leishmanias by forming aqueous pores permeable to small cations and anions. *J Membr Biol* 1996; **152**(1): 65-75.
[13] Yardley V, Croft SL. Activity of liposomal amphotericin B against experimental cutaneous leishmaniasis. *Antimicrob Agents Chemother* 1997; **41**(4): 752-6.
[14] Tracy JW, Webster LT Jr. Chemotherapy of parasitic infections: drugs used in the chemotherapy of protozoal infections-malaria. In: Hardman JG, Limbird LE, editors. *Goodman & Gilman's: the pharmacological basis of therapeutics*.10th ed. New York: McGraw-Hill; 2001, p. 1097-120.
[15] Sundar S, Chakravarty J. Paromomycin in the treatment of leishmaniasis. *Expert Opin Investtiq Drugs* 2008; **17**(5): 787-94.
[16] Sundar S, Jha TK, Thakur CP, Sinha P, Bhattacharya SK. Injectable paromomycin for visceral leishmaniasis in India. *N Engl J Med* 2007; **356**: 2571-81.
[17] Qayum M, Nisar M, Shah MR, Zia-Ul-Haq M, Kaleem WA, Marwat IK.

Biological screening of oils from *Impatiens bicolor* Royle. *Pak J Bot* 2010; **44**(3): 355-9.

[18] Kvist LP, Christensen SB, Rasmussen HB, Mejia K, Gonzalez A. Identification and evaluation of Peruvian plants used to treat malaria and leishmaniasis. *J Ethnopharmacol* 2006; **106**(3): 390-402.

[19] Rocha LG, Almeida JR, Macêdo RO, Barbosa-Filho JM. A review of natural products with antileishmanial activity. *Phytomedicine* 2005; **12**(6-7): 514-35.

[20] Fournet A, Muñoz V, Roblot F, Hocquemiller R, Cavé A, Gantier JC. Antiprotozoal activity of dehydrozaluzanin C, a sesquiterpene lactone isolated from *Murinozia maronii* (Asteraceae). *Phytother Res* 1993; **7**: 111-5.

[21] Fournet A, Barrios AA, Muñoz V. Leishmanicidal and trypanocidal activities of Bolivian medicinal plants. *J Ethnopharmacol* 1994; **41**: 19-37.

[22] Vennerstrom JL, Lovelace JK, Waits VB, Hanson WL, Klayman DL. Berberine derivatives as antileishmanial drugs. *Antimicrob Agents Chemother* 1990; **34**(5): 918-21.

[23] Luize PS, Tiuman TS, Morello LG, Maza PK, Ueda-Nakamura T, Dias-Filho BP, et al. Effects of medicinal plant extracts on growth of *Leishmania (L.) amazonensis* and *Trypanosoma cruzi*. *Braz J Pharm Sci* 2005; **41**: 85-94.

[24] Lavaurelt M, Landreau A, Larcher G, Bouchara JP, Pagniez F, Pape PL. Antileishmanial and antifungal activity of xanthenolides isolated from *Xanthium macrocarpum*. *Fitoterapia* 2005; **76**(3-4): 363-6.

[25] Khalid FA, Abdalla N, Mohomed HEO, Toum AM, Magzoub MMA, Ali MS, et al. Treatment of cutaneous leishmaniasis with some local Sudanese plants (Neem, Garad & Garlic). *Turkiye Parazitoloji Dergisi* 2004; **28** (3): 129-32.

[26] Tadesse A, Gebre-Hiwot A, Asres K, Djote M, Frammol D. The *in vitro* activity of *Vernonia amygdalina* on *Leishmania aethopica*. *Ethiop Med J* 1993; **31**(3): 183-9.

[27] Braga FG, Bouzada ML, Fabri RL, de O Matos M, Moreira FO, Scio E, et al. Antileishmanial and antifungal activity of plants used in traditional medicine in Brazil. *J Ethnopharmacol* 2007; **111**(2): 396-402.

[28] Iwu MM, Jackson JE, Tally JD, Klayman DL. Evaluation of plant extracts for antileishmanial activity using a mechanism-based radiorespirometric microtechnique (RAM). *Planta Med* 1992; **58**(5): 436-41.

[29] Abreu PM, Martins ES, Kayser O, Bindseil KU, Siems K, Seemann A, et al. Antimicrobial, antitumor and antileishmania screening of medicinal plants from Guinea-Bissau. *Phytomedicine* 1999; **6**(3): 187-95.

[30] Plock A, Sokolowska-Köhler W, Presber W. Application of flow cytometry and microscopical methods to characterize the effect of herbal drugs on *Leishmania* spp. *Exp Parasitol* 2001; **97**(3): 141-53.

[31] Fokialakis N, Kalpoutzakis E, Tekwani BL, Khan SI, Kobaisy M, Skaltsounis AL, et al. Evaluation of the antimalarial and antileishmanial activity of plants from the Greek island of Crete. *J Nat Med* 2007; **61**(1): 38-45.

[32] Lamidi M, Digiorgio C, Delmas F, Favel A, Mve-Mba CE, Rondi ML, et al. *In vitro* cytotoxic, antileishmanial and antifungal activities of ethnopharmacologically selected Gabonese plants. *J Ethnopharmacol* 2005; **102**(2): 185-90.

[33] Ahua KM, Ioset JR, Ioset KN, Diallo D, Mauël J, Hostettmann K. Antileishmanial activities associated with plants used in the Malian traditional medicine. *J Ethnopharmacol* 2007; **110**(1): 99-104.

[34] Mori K, Kawano M, Fuchino H, Ooi T, Satake M, Agatsuma Y, et al. Antileishmanial compounds from *Cordia fragrantissima* collected in Burma (Myanmar). *J Nat Prod* 2008; **71**(1): 18-21.

[35] Le Pape P, Zidane M, Abdala H, Moré MT. A glycoprotein isolated from the sponge, *Pachymatisma johnstonii*, has anti-*leishmania*l activity. *Cell Biol Int* 2000; **24**(1): 51-6.

[36] Luque-Ortega JR, Martínez S, Saugar JM, Izquierdo LR, Abad T, Luis JG, et al. Fungus-elicited metabolites from plants as an enriched source for new leishmanicidal agents: antifungal phenyl-phenalenone phytoalexins from the banana plant (*Musa acuminata*) target mitochondria of *Leishmania donovani* promastigotes. *Antimicrob Agents Chemother* 2004; **48**(5): 1534-40.

[37] Kayser O, Kiderlen AF, Bertels S, Siems K. Antileishmanial activities of aphidicolin and its semisynthetic derivatives. *Antimicrob Agents Chemother* 2001; **45**(1): 288-92.

[38] Rodrigues LA, Alviano DS, Gomes MT, Silva DO, Antoniassi R, Silva AJR, et al. *In vitro* anti-*Leishmania amazonensis* activity of the polymeric procyanidin-rich aqueous extract from *Syagrus coronate*. *J Med Plants Res* 2011; **5**(16): 3781-90.

[39] Peixoto JA, Andrade E Silva ML, Crotti AEM, Cassio Sola Veneziani R, Gimenez VM, Januário AH, et al. Antileishmanial activity of the hydroalcoholic extract of *Miconia langsdorffii*, isolated compounds, and semi-synthetic derivatives. *Molecules* 2011; **16**(2): 1825-33.

[40] Mohebali M, Chenari A, Nazari M. The effect of *Cassia fistula* extracts on *Leishmania major* ulcers in laboratory mice. *Pajouhandeh* 1999; **4**: 9-14.

[41] Mohammad BA. Antileishmanial effects of traditional herbal extracts against cutaneous leishmaniosis *in vivo*. *Adv Environ Biol* 2011; **5**(10): 3188-95.

[42] Nilforoushzadeh MA, Shirani-Bidabadi L, Zolfaghari-Baghbaderani A, Saberi S, Siadat AH, Mahmoudi M. Comparison of *Thymus vulgaris* (Thyme), *Achillea millefolium* (Yarrow) and propolis hydroalcoholic extracts versus systemic glucantime in the treatment of cutaneous leishmaniasis in balb/c mice. *J Vector Borne Dis* 2008; **45**(4): 301-6.

[43] Maes L, Germonprez N, Quirijnen L, Van Puyvelde L, Cos P, Vanden Berghe D. Comparative activities of the triterpene saponin maesabalide III and liposomal amphotericin B(AmBisome) against *Leishmania donovani* in hamsters. *Antimicrob Agents Chemother* 2004; **48**(6): 2056-60.

[44] Singha UK, Guru PY, Sen AB, Tandon JS. Antileishmanial activity of traditional plants against *Leishmania donovani* in golden hamsters. *Int J Pharmacogn* 1992; **30**(4): 289-95.

[45] Shah SM, Ayaz M, Khan A, Ullah F, Farhan, Shah AA, et al. 1,1-Diphenyl,2-picrylhydrazyl free radical scavenging, bactericidal, fungicidal and leishmanicidal properties of *Teucrium stocksianum*. *Toxicol Ind Health* 2013; doi: 10.1177/0748233713487250.

[46] Iqbal H, Khattak B, Ayaz S, Rehman A, Ishfaq M, Abbas MN, et al. Comparative efficacy of *Aloe vera* and *Tamarix aphylla* against cutaneous leishmaniasis. *Int J Basic Med Sci Pharm* 2012; **2**(2): 42-5.

[47] Tahir AE, Ibrahim AM, Satti GMH, Theander TG, Kharazmi A, Khslid SA. The potential antileishmanial activity of some Sudanese medicinal plants. *Phytother Res* 1998; **12**(8): 576-9.

[48] Soudi S, Hashemi SM, Hosseini AZ, Ghaemi A. Antileishmanial Effect of *Echinacea purpurea* root extract cultivated in Iran. *Iran J Pharm Res* 2007; **6**: 147-9.

[49] Chen M, Christensen SB, Theander TG, Kharazmi A. Antileishmanial activity of licochalcone A in mice infected with *Leishmania major* and in hamsters infected with *Leishmania donovani*. *Antimicrob Agents Chemother* 1994; **38**(6): 1339-44.

[50] Evans AT, Croft SL, Peters W. Failure of chlorpromazine or amitriptyline ointments to influence the course of experimental cutaneous leishmaniasis. *Trans R Soc Trop Med Hyg* 1988; **82**(2): 226.

[51] Chan MM, Grogl M, Chen CC, Bienen EJ, Fong D. Herbicides to curb human parasitic infections: *in vitro* and *in vivo* effects of trifluralin on the trypanosomatid protozoans. *Proc Natl Acad Sci U S A* 1993; **90**(12): 5657-61.

[52] Requena JM, Iborra S, Carrión J, Alonso C, Soto M. Recent advances in vaccines for leishmaniasis. *Expert Opin Biol Ther* 2004; **4**(5): 1505-17.

[53] Kubar J, Fragaki K. Recombinant DNA-derived *leishmania* proteins: from the laboratory to the field. *Lancet Infect Dis* 2005; **5**(2): 107-14.

[54] Coler RN, Reed SG. Second-generation vaccines against leishmaniasis. *Trends Parasitol* 2005; **21**(5): 244-9.

[55] Ben Salah A, Louzir H, Chlif S, Mokni M, Zaatour A, Raouene M, et al. The predictive validity of naturally acquired delayed-type hypersensitivity to leishmanin in the resistance to *Leishmania major*-associated cutaneous leishmaniasis. *J Infect Dis* 2005; **192**(11): 1981-7.

[56] Croft SL, Coombs GH. Leishmaniasis-current chemotherapy and recent advances in the search for novel drugs. *Trends Parasitol* 2003; **19**(11): 502-8.