



Review

Recent advances in leishmaniasis treatment

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SUMMARY

About 1.5 million new cases of cutaneous leishmaniasis and 500 000 new cases of visceral leishmaniasis occur each year around the world. For over half a century, the clinical forms of the disease have been treated almost exclusively with pentavalent antimonial compounds. In this review, we describe the arsenal available for treating *Leishmania* infections, as well as recent advances from research on plants and synthetic compounds as source drugs for treating the disease. We also review some new drug-delivery systems for the development of novel chemotherapeutics. We observe that the pharmaceutical industry should employ its modern technologies, which could lead to better use of plants and their extracts, as well as to the development of synthetic and semi-synthetic compounds. New studies have highlighted some biopharmaceutical technologies in the design of the delivery strategy, such as nanoparticles, liposomes, cochleates, and non-specific lipid transfer proteins. These observations serve as a basis to indicate novel routes for the development and design of effective anti-*Leishmania* drugs.

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1. Introduction

Leishmaniasis is an infectious disease caused by parasites of the genus *Leishmania* in the family Trypanosomatidae. The disease manifests as three types: cutaneous, mucocutaneous, and visceral leishmaniasis, which is also known as kala-azar.^{1,2} Cutaneous leishmaniasis, the most common form, is a group of diseases with a varied spectrum of clinical manifestations, which range from small cutaneous nodules to gross mucosal tissue destruction.³ Visceral leishmaniasis is the most severe form, in which the parasites have migrated to vital organs. It is a severe, debilitating disease, characterized by prolonged fever, splenomegaly, hypergammaglobulinemia, and pancytopenia. Patients gradually become ill over a period of a few months, and nearly always die if untreated.⁴

Leishmaniasis is transmitted through the bite of female phlebotomine sandflies infected with the protozoan. The parasite is then internalized via macrophages in the liver, spleen, and bone marrow.⁵ *Leishmania* parasites are dimorphic organisms, i.e., with two morphological forms in their life cycle: amastigotes in the mononuclear phagocytic system of the mammalian host, and promastigotes in the digestive organs of the vector.⁶

About 1.5 million new cases of cutaneous leishmaniasis and 500 000 new cases of visceral disease occur each year. Cutaneous

leishmaniasis is endemic in more than 70 countries worldwide, and 90% of cases occur in Afghanistan, Algeria, Brazil, Pakistan, Peru, Saudi Arabia, and Syria.^{3,7,8} Visceral leishmaniasis occurs in 65 countries; the majority (90%) of cases occur in agricultural areas and among the suburban poor of five countries: Bangladesh, India, Nepal, Sudan, and Brazil.^{1,9} The number of cases is increasing globally at an alarming rate.¹⁰ Ecological chaos caused by humans has enabled the leishmaniases to expand beyond their natural ecotopes, and this in turn affects the level of human exposure to the sandfly vectors.¹¹ Cases of *Leishmania* and human immunodeficiency virus (HIV) co-infection have also recently increased.^{12,13}

The classical treatment of leishmaniasis requires the administration of toxic and poorly tolerated drugs. The pentavalent antimonials – meglumine antimoniate (Glucantime) and sodium stibogluconate (Pentostam) – are the first-line compounds used to treat leishmaniasis. Other drugs that may be used include pentamidine and amphotericin B.^{14,15} However, parasite resistance greatly reduces the efficacy of conventional medications.¹⁶ In the last 15 years, clinical misapplication of medications has enabled the development of generalized resistance to these agents in Bihar, India, where half of the global visceral leishmaniasis cases occur.⁷ Moreover, there are no effective vaccines to prevent leishmaniasis.^{17,18}

The purpose of this review is to discuss the current treatment for leishmaniasis and to highlight recent advances in the development of novel chemotherapies.

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2. Current treatment and recent advances

Pentavalent antimonials were developed in 1945, and remain the first-choice treatment for both visceral and cutaneous leishmaniasis in most parts of the world. Amphotericin B and pentamidine are the second-line antileishmanial drugs, although they require long courses of parenteral administration.¹⁹ The choice of treatment also depends on the causative *Leishmania* species.²⁰ The most common syndrome is localized cutaneous leishmaniasis (CL), which is most frequently caused by *Leishmania major* and *Leishmania tropica* in the Old World (Mediterranean basin, Middle East, and Africa), and by *Leishmania braziliensis*, *Leishmania mexicana*, and related species in the New World (Mexico, Central America, and South America).²¹ A study of 103 patients with CL in Peru showed that among patients infected with *Leishmania (Viannia) peruviana* (47.6%), *Leishmania (Viannia) guyanensis* (23.3%), and *Leishmania (Viannia) braziliensis* (22.3%), 21 of them (21.9%) did not respond to pentavalent antimonial chemotherapy. Therefore, accurate identification of the parasite is of great clinical importance, because it will guide the choice of an appropriate treatment.²⁰ Although spontaneous cure is the rule, the rate of recovery varies depending on the species of *Leishmania*, and may require months or years to complete healing.³

Most of the commonly used drugs are toxic and do not cure, i.e., eliminate the parasite, from infected individuals.¹⁹ Failure to treat leishmaniasis successfully is often due to increased chemoresistance of the parasite.^{15,22}

Because treatment is a growing problem, the development of new medicines that can replace or complement the presently available therapeutic alternatives is necessary. Encouraging advances in chemotherapy have been made in recent years. Antileishmanial chemotherapy has improved since the development of lipid formulations of amphotericin B, which is a much less toxic treatment for fungal infections, and has been exploited for the treatment of leishmaniasis.²³ The unilamellar liposomal formulation (AmBisome®), lipid complex (Abelcet®), and colloidal dispersion (Amphocil™) have all been evaluated in clinical trials for visceral leishmaniasis and/or mucocutaneous leishmaniasis. Yardley and Croft (2000) found that AmBisome and Amphocil are more effective (50% effective dose (ED₅₀) values 0.3 and 0.7 mg/kg, respectively) than Abelcet (ED₅₀ 2.7 mg/kg) against *Leishmania donovani* in a mouse model. AmBisome (25 mg/kg) was the most successful in reducing the size of lesions caused by *L. major*, and Amphocil (12.5 mg/kg) also showed activity, whereas Abelcet was inactive against this species.²⁴

However, the high cost of these amphotericin B preparations precludes their widespread use in developing countries. New formulations involving microcapsules made of albumin, which is a cheap and effective carrier system and provides effective protection against phagocytic cells, have been tested. Microspheres of hydrophilic albumin with three amphotericin B aggregation states (monomeric, dimeric, and multiaggregate) and a multiaggregate form encapsulated with two commercial polymers were tested against *Leishmania infantum* (both extracellular promastigote and intracellular amastigote forms). The albumin-encapsulated forms showed no toxicity to murine cells and had lower 50% effective concentration (EC₅₀) values (0.003 µg/ml) for amastigotes than did the free formulations (0.03 µg/ml). These promising results have increased interest in amphotericin B encapsulated in microspheres, and in exploring new chemotherapeutic approaches.²⁵

Miltefosine, an alkylphospholipid, was developed as an oral antineoplastic agent (for cutaneous cancers) and has subsequently been applied to treat leishmaniasis.²⁶ The discovery that miltefosine is effective against *Leishmania* led to the identification of a modern group of antiprotozoal medicines. Following clinical studies, miltefosine was approved as Impavido™ and has become

the first oral treatment for leishmaniasis in some countries.²⁷ It is an effective treatment for visceral and cutaneous leishmaniasis, including for antimony-resistant infections. However, this drug may not necessarily be superior to parenteral therapies for all forms of leishmaniasis.²⁸ The requirement for a long treatment period (28 days) will necessitate the formulation of strategies for more rational use, in order to prevent patients from developing resistance to the drug. Studies of the resistance mechanisms have shown that possible mechanisms include a decrease in drug uptake, differential plasma membrane permeability, more rapid drug metabolism, and efflux of the drug.²⁹ Miltefosine was registered in India for the treatment of visceral leishmaniasis in 2002.³⁰ It has exhibited teratogenic potential, and therefore should not be administered to pregnant women.³¹

Other alkylphospholipids such as edelfosine and ilmofosine,³² as well as perifosine,³³ have proved to possess potent in vitro antiparasitic activity. In 2008, Cabrera-Serra et al. tested edelfosine and perifosine orally in Balb/c mice infected with *Leishmania amazonensis*. This pre-clinical study showed that perifosine had higher activity in the *in vivo* assay and may be a possible alternative treatment against cutaneous leishmaniasis.³⁴

Sitamaquine is a promising oral treatment for visceral leishmaniasis in Africa. A 28-day course of treatment was efficacious and well tolerated in 61 Kenyan patients infected by *L. donovani*, with the tested dose of 2.0 mg/kg/day; however, further studies are required to define the optimal dose. Some adverse effects included abdominal pain, headache, and a severe renal event. The effects of sitamaquine on the kidney need further investigation.³⁵

Paromomycin is the only aminoglycoside with clinically important antileishmanial activity. Both visceral and cutaneous forms can be treated with this antibiotic, but poor oral absorption has led to the development of parenteral and topical formulations for the visceral and cutaneous forms, respectively.³⁶ In a study of patients with a visceral form of leishmaniasis in India, Sundar et al. (2007) found that paromomycin administered by deep gluteal intramuscular injection (11 mg/kg/day) for 21 days was equally effective as infusion of amphotericin B (1 mg/kg/day) for 30 days.³⁷ In 1992 in central Tunisia, patients with cutaneous leishmaniasis caused by *L. major* were treated with paromomycin ointment, but there was no difference between the treated and control groups.³⁸ However, new topical formulations of paromomycin have given good results. A randomized, controlled study was undertaken to compare the therapeutic efficacy of two paromomycin topical preparations with meglumine antimoniate. The results showed that topical paromomycin can be a therapeutic alternative for cutaneous leishmaniasis, although a longer period is required for clinical healing.³⁹ A hydrophilic gel containing 10% paromomycin was evaluated in Balb/c mice infected with *L. amazonensis* and hamsters infected with *L. braziliensis*. Compared to the antimony treatment, the activity of the paromomycin gel was significantly higher against *L. amazonensis*, whereas these two medications were equally effective against *L. braziliensis*. The gel formulation may represent an alternative topical treatment for cutaneous leishmaniasis.⁴⁰

3. Plants as medicine for leishmaniasis

Plants are clearly a potential source of new antiprotozoal drugs. The biological activity of plant extracts has been attributed to compounds belonging to diverse chemical groups including alkaloids, flavonoids, phenylpropanoids, steroids, and terpenoids.^{41–43} To obtain a herbal medicine or an isolated active compound, different research strategies can be employed, among them, investigation of the traditional use, the chemical composi-

tion, the toxicity of the plants, or the combination of several criteria.⁴⁴

In the extraction processes, different plant parts and different solvents have generally been used. In screening for biological activity, there is clearly substantial room for improvement in the extraction methodologies, since a variety of techniques can be used to prepare extracts.^{45,46} Usually, solvents of different polarity are employed for the extractions. For purification and isolation, the active extracts of the plant are sequentially fractionated, and each fraction and/or pure compound can be evaluated for biological activity and toxicity. This strategy is called bioactivity-guided fractionation, which allows tests that are simple, reproducible, rapid, and low-cost.^{46,47}

Promastigote, axenic amastigote, and intracellular amastigote forms of *Leishmania* can be used to screen for biologically active plant substances. Tests with standard drugs have shown that *in vitro* tests on axenic amastigotes not only yield significant results when compared to tests on promastigotes, but they are also easy to manipulate and quantify.^{47–49} This can be achieved by using a cell counter, a colorimetric method with Alamar blue or acid phosphatase activity, evaluating the viability of the cell population with an MTT-based method, determining ornithine decarboxylase activity, or using a fluorescent dye such as propidium iodide and a fluorescence-activated cell sorter (FACS).^{47,50–52} Interestingly, the ability to derive transgenic *Leishmania*-expressing reporter genes, such as the green fluorescent protein (GFP) or luciferase, has opened up new alternatives for the development of drug-screening tests.^{53,54} For screening the intracellular forms of the parasite, a colorimetric β -lactamase assay has been used to assess the potential of new antileishmanial drugs in the clinical isolates.⁵⁵ Table 1 compares some antileishmanial activities that have been reported in the last 5 years.^{56–87}

In vitro screenings are only the first steps to prove the efficacy and safety of medicinal plants for application in the treatment of leishmaniasis. In addition, variation in the efficacy of drugs in treating leishmaniasis may often result from differences in the drug sensitivity of *Leishmania* species, the immune status of the patient, or the pharmacokinetic properties of the drug.¹⁶

In reviewing the literature on the use of natural products (plant crude extracts, fractions, isolated compounds, and essential oils), we have become aware of the great effort by researchers around the world to locate compounds with antileishmanial activity. These efforts are now bearing fruit, obtaining good results and validating natural products as genuine sources for drug discovery.

4. Synthetic compounds as an alternative

Recent years have seen growing interest in new therapies and the use of natural products, especially those derived from plants, as sources of new chemotherapeutic compounds with greater activity and fewer side effects. In view of the present unsatisfactory situation, it is highly desirable to study new molecules obtained from medicinal plants for leishmaniasis treatment.

Researchers worldwide continue to search for new molecules for the treatment of leishmaniasis, and several screenings with synthetic compounds and their derivatives have been carried out. Brenzan et al. (2008) studied the structure–activity relationship of coumarin (–) mammea A/BB isolated from the CH₂Cl₂ extract of *Calophyllum brasiliense* leaves against promastigote and intracellular amastigote forms of *L. amazonensis*. The derivative compounds displayed significant activity. This study demonstrated that several aspects of the structure were important for antileishmanial activity.⁸⁸

Delfin et al. (2009) studied the antileishmanial activity of BTB 06237 (2-[(2,4-dichloro-5-methylphenyl)sulfanyl]-1,3-dinitro-5-

(trifluoromethyl) benzene), a compound previously identified through quantitative structure–activity relationship (QSAR) studies, which possesses potent and selective activity against *Leishmania* parasites. In this study, several analogues of BTB 06237 were synthesized and analyzed for activity against axenic amastigotes, their ability to reduce the level of parasitemia in peritoneal macrophages, and their ability to generate reactive oxygen species (ROS) in *L. donovani* promastigotes.⁸⁹ Al-Qahtani et al. (2009) tested the *in vitro* antileishmanial activity of 44 derivatives of 1,3,4-thiadiazole and related compounds against promastigote forms of *L. donovani*. Micromolar concentrations of these agents were used to study the inhibition of multiplication of promastigotes. Seven compounds were identified as potential antigrowth agents against the parasite.⁹⁰

In addition, a series of 2,4,6-trisubstituted pyrimidines and 1,3,5-triazines were synthesized and screened for antileishmanial activity against *L. donovani*. The compounds that showed the highest *in vitro* activity against the parasite were screened for *in vivo* activity in golden hamsters (*Mesocricetus auratus*) infected with the MHOM/IN/80/Dd8 strain of *L. donovani*, and showed moderate *in vivo* inhibition of 48–56% at a dose of 50 mg/kg $\times$ 5, intraperitoneal route for 5 days.⁹¹ Barbosa et al. (2009) evaluated the biological activity of seven aromatic compounds prepared through the Baylis–Hillman reaction (BHR) against the promastigote form of *Leishmania chagasi*, and all the compounds showed high bioactivity.⁹² Musonda et al. (2009) demonstrated the synthesis and evaluation of 2-pyridyl pyrimidines with *in vitro* antileishmanial activity. Moreover, they analyzed the relation between the physicochemical properties, to demonstrate a link between lipophilicity and antiparasitic activity. Cross-screening of the library against cultured *L. donovani* parasites revealed that compounds of this class are potent inhibitors of parasite development *in vitro*.⁹³

Poorrajab et al. (2009) carried out the synthesis and *in vitro* biological evaluation of nitroimidazolyl-1,3,4-thiadiazole-based antileishmanial agents against *L. major*. Most of the compounds exhibited antileishmanial activity against the promastigote form of *L. major* at non-cytotoxic concentrations. Interestingly, these compounds were effective against intracellular *L. major*, and significantly decreased the infectivity index.⁹⁴ Srinivas et al. (2009) demonstrated the simple synthesis and potent activity of aryloxy cyclohexyl imidazoles, a new structural class of azoles with antileishmanial activity, both *in vitro* and *in vivo*.⁹⁵ A series of 1-phenylsubstituted β -carboline containing an *N*-butylcarboxamide group at C-3 of the β -carboline nucleus were synthesized and evaluated *in vitro* against promastigotes of *L. amazonensis*. Among all compounds tested, two derivatives showed potent activity against the parasite. The results demonstrated that the synthesized β -carboline-3-carboxamide derivatives have potential for use as new drugs for the treatment of leishmaniasis.⁹⁶ Aponte et al. (2010) reported the leishmanicidal and cytotoxic activities of tetrahydrobenzothieno-pyrimidines.⁹⁷ The synthesis and *in vitro* activity of *R*(+)-limonene derivatives against *Leishmania* were described by Graebin et al. (2010). Seven compounds showed better *in vitro* activity against *L. braziliensis* than the standard drug pentamidine.⁹⁸ Nava-Zuazo et al. (2010) synthesized a new series of quinoline tripartite hybrids from chloroquine, ethambutol, and isoxyl drugs, using a short synthetic route. The compounds were tested *in vitro* against *L. mexicana*, and (4-butoxyphenyl)-*N'*-{2-[(7-chloroquinolin-4-yl)amino]ethyl}urea proved to be the most active compound against the parasites.⁹⁹

These studies result from the urgent need to develop cost-effective new drugs and to discover novel molecules with potent antiparasitic activity and improved pharmacological characteristics. Although many advances have been made in the treatment of leishmaniasis, much still remains to be understood.

Table 1Plant crude extracts, fractions, isolated compounds, and essential oils evaluated against the *Leishmania* genus

Family/plant species	Extracts or compounds	<i>Leishmania</i> species	IC ₅₀ (μg/ml)		Ref.
			PRO	AMA	
Aloeaceae					
<i>Aloe nyriensis</i>	Methanolic extract	<i>L. major</i>	68.4	ND	56
	Aqueous extract	<i>L. major</i>	53.3	ND	56
Annonaceae					
<i>Annona coriacea</i>	Total alkaloids extract	<i>L. chagasi</i>	41.6	ND	57
<i>Annona crassiflora</i>	Total alkaloids extract	<i>L. chagasi</i>	24.9	ND	57
<i>Annona muricata</i>	Ethyl acetate extract	<i>L. amazonensis</i>	25.0	NT	58
<i>Guatteria australis</i>	Total alkaloids extract	<i>L. chagasi</i>	37.9	ND	57
<i>Polyalthia suaveolens</i>	Methanolic extract	<i>L. infantum</i>	1.8	8.6	59
<i>Pseudomalmea boyacana</i>	Ethyl acetate extract	<i>L. amazonensis</i>	48.9	NT	58
<i>Rollinia exsucca</i>	Hexane extract	<i>L. amazonensis</i>	20.8	NT	58
<i>Rollinia pittieri</i>	Hexane extract	<i>L. amazonensis</i>	12.6	NT	58
<i>Xylopia aromatica</i>	Methanolic extract	<i>L. amazonensis</i>	20.8	NT	58
Apocynaceae					
<i>Himatanthus sucuuba</i>	Ethanol extract	<i>L. amazonensis</i>	20.0	5.0	60
<i>Pagianta cerifera</i>	Dichloromethane extract	<i>L. amazonensis</i>	25.0	12.5	61
Asteraceae					
<i>Achillea millefolium</i>	Essential oil	<i>L. amazonensis</i>	7.8	6.5	62
<i>Anthemis auriculata</i>	Anthecotulide	<i>L. donovani</i>	NT	8.18	63
	4-Hydroxyanthecotulide	<i>L. donovani</i>	NT	3.27	63
	4-Acetoxyanthecotulide	<i>L. donovani</i>	NT	12.5	63
<i>Baccharis dracunculifolia</i>	Crude extract	<i>L. donovani</i>	45.0	NT	64
	Hautriwaic acid lactone	<i>L. donovani</i>	7.0	NT	64
	Ursolic acid	<i>L. donovani</i>	3.7	NT	64
	Uvaol	<i>L. donovani</i>	15.0	NT	64
	2α-Hydroxy-ursolic acid	<i>L. donovani</i>	19.9	NT	64
<i>Calea montana</i>	Ethanol extract	<i>L. amazonensis</i>	NT	10.0	65
<i>Elephantopus mollis</i>	Dichloromethane extract	<i>L. donovani</i>	NT	0.6	66
<i>Tanacetum parthenium</i>	Plant powder	<i>L. amazonensis</i>	490	74.8	67
	Dichloromethane extract	<i>L. amazonensis</i>	3.6	2.7	67
	Parthenolide	<i>L. amazonensis</i>	0.37	0.81	68
	Guaianolide	<i>L. amazonensis</i>	2.6	ND	69
<i>Vernonia polyanthes</i>	Methanolic extract	<i>L. amazonensis</i>	4.0	NT	70
Caricaceae					
<i>Carica papaya</i>	Ethanol extract	<i>L. amazonensis</i>	NT	11.0	65
Celastraceae					
<i>Maytenus putterlickoides</i>	Methanolic extract	<i>L. major</i>	60.0	ND	56
Clusiaceae					
<i>Calophyllum brasiliense</i>	(–) Mammee A/BB	<i>L. amazonensis</i>	3.0	0.88	71
Crassulaceae					
<i>Kalanchoe pinnata</i>	Quercetin diglycoside	<i>L. amazonensis</i>	NT	45.0	72
Fabaceae					
<i>Acacia tortilis</i>	Aqueous extract	<i>L. major</i>	52.9	ND	56
<i>Albizia coriaria</i>	Aqueous extract	<i>L. major</i>	66.7	ND	56
<i>Copaifera reticulata</i>	Oleoresin	<i>L. amazonensis</i>	5.0	15.0	73
Flacourtiaceae					
<i>Laetia procera</i>	Casearilucine A	<i>L. amazonensis</i>	11.1	5.98	74
	Caseamembrol A	<i>L. amazonensis</i>	11.0	10.5	74
	Laetiaprocerine A	<i>L. amazonensis</i>	10.9	47.4	74
	Laetiaprocerine D	<i>L. amazonensis</i>	50.9	30.3	74
	Butanolide	<i>L. amazonensis</i>	111.0	129.0	74
Ginkgoaceae					
<i>Ginkgo biloba</i>	Isoginkgetin	<i>L. amazonensis</i>	NT	1.9	75
Goodeniaceae					
<i>Scaevola balansae</i>	Dichloromethane extract	<i>L. amazonensis</i>	8.7	NT	76
Lamiaceae					
<i>Hyptis lacustris</i>	Ethanol extract	<i>L. amazonensis</i>	NT	10.0	65
<i>Ocimum gratissimum</i>	Essential oil	<i>L. amazonensis</i>	135.0	100.0	77
	Eugenol	<i>L. amazonensis</i>	80.0	NT	77
	Methanolic extract	<i>L. chagasi</i>	71.0	NT	70
<i>Premna serratifolia</i>	Dichloromethane extract	<i>L. amazonensis</i>	4.4	NT	76
Lecythidaceae					
<i>Careya arborea</i>	Arborenin	<i>L. donovani</i>	15.0	12.5	78
Liliaceae					
<i>Asparagus racemosus</i>	Methanolic extract	<i>L. major</i>	58.8	ND	56
	Aqueous extract	<i>L. major</i>	56.8	ND	56
Malpighiaceae					
<i>Lophanthera lactescens</i>	LLD3	<i>L. amazonensis</i>	NT	0.41	79
Meliaceae					
<i>Dysoxylum binectariferum</i>	Chloroform fraction	<i>L. donovani</i>	50.0	ND	80
	Rohitukine	<i>L. donovani</i>	100.0	ND	80
Menispermaceae					
<i>Cissampelos ovalifolia</i>	Total alkaloids extract	<i>L. chagasi</i>	63.9	ND	57
Olaceae					

Table 1 (Continued)

Family/plant species	Extracts or compounds	Leishmania species	IC ₅₀ (μg/ml)		Ref.
			PRO	AMA	
<i>Minquartia guianensis</i>	Dichloromethane extract	<i>L. donovani</i>	NT	2.8	66
Papaveraceae					
<i>Bocconia integrifolia</i>	<i>n</i> -Hexane extract	<i>L. donovani</i>	NT	1.8	66
	Dichloromethane extract	<i>L. donovani</i>	NT	0.5	66
	Methanol extract	<i>L. donovani</i>	NT	0.7	66
Piperaceae					
<i>Piper auritum</i>	Essential oil	<i>L. donovani</i>	12.8	22.3	81
<i>Piper dennisii</i>	Ethanol extract	<i>L. amazonensis</i>	NT	10.0	65
<i>Piper hispidum</i>	Ethanol extract	<i>L. amazonensis</i>	69.0	5.0	82
<i>Piper regnellii</i>	Eupomatenoid-5	<i>L. amazonensis</i>	9.0	5.0	83
<i>Piper strigosum</i>	Ethanol extract	<i>L. amazonensis</i>	>100	7.8	82
<i>Piper sp</i>	Dichloromethane extract	<i>L. donovani</i>	NT	2.2	66
Poaceae					
<i>Cymbopogon citratus</i>	Essential oil	<i>L. amazonensis</i>	1.7	3.2	84
	Citral	<i>L. amazonensis</i>	8.0	25	84
Rhamnaceae					
<i>Gouania lupuloides</i>	Dichloromethane extract	<i>L. donovani</i>	NT	1.9	66
	Methanol extract	<i>L. donovani</i>	NT	2.9	66
Rutaceae					
<i>Galipea panamensis</i>	Coumarin compound 1	<i>L. panamensis</i>	NT	9.9	85
	Coumarin compound 2	<i>L. panamensis</i>	NT	10.5	85
	Phebalosin	<i>L. panamensis</i>	NT	14.1	85
	Artifact murralongin	<i>L. panamensis</i>	NT	>100	85
	Murrangatin acetone	<i>L. panamensis</i>	NT	NT	85
Scrophulariaceae					
<i>Scoparia dulcis</i>	Dichloromethane extract	<i>L. donovani</i>	NT	1.8	66
<i>Scrophularia cryptophila</i>	Cryptophilic acid A	<i>L. donovani</i>	NT	12.8	86
	Cryptophilic acid C	<i>L. donovani</i>	NT	5.8	86
	Harpagide	<i>L. donovani</i>	NT	2.0	86
	Acetylharpagide	<i>L. donovani</i>	NT	6.9	86
	Buddlejasaponin III	<i>L. donovani</i>	NT	6.2	86
Solanaceae					
<i>Brugmansia sp</i>	Dichloromethane extract	<i>L. donovani</i>	NT	3.0	66
Umbelliferae					
<i>Ferula szowitsiana</i>	Auraptene	<i>L. major</i>	5.1	NT	87
	Umbelliprenin	<i>L. major</i>	4.9	NT	87
Verbenaceae					
<i>Lantana sp</i>	Ethanol extract	<i>L. amazonensis</i>	NT	10.0	65
Zingiberaceae					
<i>Hedychium coronarium</i>	Ethanol extract	<i>L. amazonensis</i>	NT	10.0	65

PRO, promastigote; AMA, amastigote; IC₅₀, concentration in μg/ml that inhibits growth of 50% of the cells; NT, not tested; ND, not determined.

5. What is the future of antileishmanial agents?

Leishmaniasis represents a significant global burden and a great challenge to drug discovery and delivery, because of the intracellular nature and disseminated locations of the parasite. The potential of some colloidal drug carriers such as emulsions, liposomes, and nanoparticles is of great interest, mainly due to their versatile nature and attractive advantages in the context of parasitic diseases.

The potential of medicine delivery systems based on the controlled release of drugs, especially nanoparticles (NPs), is attracting increased attention. NPs are submicron moieties (between 1 nm and 100 nm) and are considered 'intelligent' particles with a magnetic core, a recognition layer, and a therapeutic load. They are made of inorganic or organic materials, which may or may not be biodegradable.¹⁰⁰

Biodegradable nanoparticles (such as PLGA, PLA, chitosan, gelatin, polycaprolactone, and polyalkylcyanoacrylates) are frequently used as drug delivery vehicles because of their therapeutic value in reducing the risks of toxicity of some medicinal drugs. They can increase the bioavailability, solubility, and retention time of many potent drugs that are difficult to deliver orally.¹⁰¹

Scientists who work with modern technologies for oral formulations must overcome the hurdle of poor aqueous solubility of many drugs. Many reports have demonstrated the advantages of nanoformulations for improving oral bioavailability *in vivo*. Nanosizing will attract increased attention as an option for

formulations affording immediate release for oral administration.¹⁰²

Nanoparticles can be useful in treating some macrophage-mediated diseases. Mononuclear phagocyte cells engulf *Leishmania* parasites, but also remove drug particles from the body circulation. The characteristic of macrophages to recognize cell-surface ligands is exploited in nanoparticulate systems, by anchoring specific entities to ensure their internalization into the cells.¹⁰³

Liposomes and nanoparticles have shown great potential for improving the efficacy and tolerability of antileishmanial drugs such as liposomal amphotericin B. Solid lipid nanoparticles and nanostructured lipid carriers represent a second generation of colloidal carriers, mainly because of their better stability and ease of commercialization.¹⁰⁴

Amphotericin B has poor solubility, and because it is not absorbed in the gastrointestinal tract, it must be administered by slow intravenous injection. An oral delivery system is desirable, and modern studies aimed at increasing oral absorption have evaluated nanosuspensions.¹⁰⁵ Amphotericin B formulated in a nanosuspension for oral delivery was tested against *L. donovani* infection in a Balb/c mouse model. The amphotericin B nanosuspensions significantly reduced parasite numbers in the liver.¹⁰⁶

Cochleates, a novel lipid particle-based delivery system, also have potential for oral administration of hydrophobic drugs such as amphotericin B. The cochleate system promotes the uptake of amphotericin B from the gastrointestinal tract, and has been shown to be effective in a mouse model of systemic candidiasis.

This route of administration may have potential for therapeutic application.¹⁰⁷

Mendoza et al. (2008) proposed a novel formulation for edelfosine by developing a lipid nanoparticulate system, with the purpose of decreasing systemic toxicity and improving the therapeutic potential of the drug. The lipid employed Compritol[®], which presents advantages *in vitro* as a matrix material for nanoparticle development and the controlled release of edelfosine.¹⁰⁸

Non-specific lipid transfer proteins (nsLTPs) from plants are small basic proteins, and can be subdivided into nsLTP1 (10 kDa) and nsLTP2 (7 kDa) according to their molecular weight.¹⁰⁹ These proteins can bind a broad range of lipid molecules, and have attracted increasing interest as potential drug carriers. nsLTPs have been purified from a variety of plants including barley seeds and hops,¹¹⁰ rice,¹¹¹ tomato,¹¹² the pandan *Pandanus amaryllifolius*,¹¹³ and cumin *Cuminum cyminum*.¹¹⁴

These carriers have a transfer activity that should make them useful when drugs must cross through the lipid membranes. The study of complex formation between nsLTP1 and several drugs is likely to allow additional pharmaceutical applications. A study by Pato et al. (2001) demonstrated that wheat nsLTP1 can bind ether phospholipid analogues (edelfosine, ilmofosine) and an antifungal conazole derivative (BD56) with similar affinity. Pato and co-workers also showed an interaction between amphotericin B and nsLTP1, although they could demonstrate no affinity. Their study suggests a potential application of nsLTPs as antileishmanial drug carrier systems.¹¹⁵

Combination chemotherapy has improved prospects for curbing the emergence of drug resistance, and has proven to increase activity through the use of compounds with synergistic or additive activity, reducing required doses and toxic side effects.¹⁶ In a recent study, WR279,396, a topical formulation containing 15% paromomycin and 0.5% gentamicin was evaluated in a phase II trial in Tunisia and France. The treatment for 20 days was safe and effective against CL caused by *L. major*.¹¹⁶

The effectiveness of drug combinations in chemotherapy is measured by the interaction index. This methodology uses combination and individual drug dose–effect data and employs isobolar analysis, and fractional inhibitory concentrations (FIC) can be calculated.¹¹⁷ Monzote et al. (2007) showed that the essential oil from *Chenopodium ambrosioides* had a synergic effect with pentamidine against promastigotes of *L. amazonensis*, but found an indifferent effect for combinations with meglumine antimoniate or amphotericin B.¹¹⁸ The current trend is to investigate the possibility of increasing the effects of antibiotics or synthetic drugs by combining them with natural products such as plants used in traditional medicine. Some new projects aim to develop a new generation of phytochemicals, which can be used alone or in combination with antibiotics or synthetic drugs. These new formulations of combinations of medicine could be used to cure diseases that are presently treated using synthetic drugs alone.¹¹⁹

6. Conclusions

Pharmaceutical research on natural products represents a major strategy for discovering and developing new drugs. There are many possibilities for research, but priority should be given to tropical infectious and chronic diseases for which current medications have severe drawbacks, and to the scientific appraisal of plant-based remedies that might be safer, cheaper, and less toxic than existing prescription medicines. This is an area rich in possibilities, and the world's flora represents an enormous source of material for testing. What is needed now is funding to support this type of research.

Industry should play a role, because of its greater knowledge of modern technologies, which may lead to better utilization of plants and their extracts, and their funding and marketing. Development of new molecules to treat leishmaniasis is necessary. The development of drug combinations and new pharmaceutical technologies is still an open area for research. The biological and biopharmaceutical issues should be considered in the design of delivery strategies for treating parasitic infections such as leishmaniasis.

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