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REVIEW

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An Update on Natural Antileishmanial Treatment Options from Plants, Fungi and Algae

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Efficient drugs for the treatment of leishmaniasis, which is classified as a neglected tropical disease, are sought for. This review covers potential drug candidates from natural plant, fungus and algae sources, which were described over the last six years. The identification of these natural antileishmanials often based on the knowledge of traditional medicines. Crucial insights into the activities of these natural remedies against *Leishmania* parasites and against infections caused by these parasites in laboratory animals or patients are provided and compared with selected former active examples published more than six years ago. In addition, immuno-modulatory natural antileishmanials and recent developments on combination therapies including natural products and approved antileishmanials are discussed. The described natural products revealed promising data warranting further efforts on the discovery and development of new antileishmanials based on patterns from nature.

Keywords: natural products, leishmaniasis, infectious diseases, neglected tropical diseases.

1. Introduction

Neglected tropical diseases (NTDs) pose a constant threat to world health and comprise a number of infectious diseases, which affect and endanger billions of mainly poor people worldwide. However, the development of accurate treatment options for these poor populations is still not secured.[1] Leishmaniasis is an NTD, which, in its various forms, is endemic to vast areas from South Asia via the Middle East and Africa to South and Central America.[1] It is clinically subdivided into visceral leishmaniasis (VL, caused by *L. infantum* and *L. donovani*), cutaneous leishmaniasis (CL, caused by *L. infantum*, *L. major*, *L. tropica*, *L. mexicana*, *L. amazonensis*, and *L. braziliensis*), and mucocutaneous leishmaniasis (MCL, caused by *L. braziliensis and L. donovani*). VL has a considerable mortality potential while CL is the predominant form of leishmaniasis with up to 1 million new cases annually.[2,3] Current treatments of leishmaniasis patients include antimonials, amphotericin B, miltefosine, paromomycin, and pentamidine, but the systemic toxicity of widely applied pentavalent antimonials and the appearance of drug-resistance necessitate the search for new potent drugs against leishmaniasis.[3] *Leishmania* parasites appear as extracellular motile promastigotes and intracellular/intra-macrophage amastigotes. Both forms are generally applied for antileishmanial research, however, there are differences, which affect the validity of the drug testing experiments. There is general strong support of using the more laborious *in vitro* tests with intracellular amastigotes for antileishmanial drug discovery because they cover host-mediated mechanisms, too. In contrast, cell-free assays with promastigotes are simpler and more proper for automation but can lead to the identification of false positives (i.e., compounds which are active against short-lived promastigotes but inactive against cell-protected amastigotes) and are mainly recommended

for the investigation of test compounds without cellular mechanisms.[4,5]

Natural products and their semi-synthetic derivatives are eminent in terms of activity and percentage

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share of new investigational drugs.[6] In wide parts of the Asian continent, traditional folk medicine such as Traditional Chinese Medicine, Kampo, Ayurveda, Unani, and traditional Greco-Arab and Islamic Medicine, among others, are valuable sources for the discovery of natural drugs.[7,8] But folk medicine is not confined to Asia and is also practiced by native populations in wide parts of Africa and America. For instance, various Bolivian medicinal plants can be considered as suitable treatment options for the management of *Leishmania* infections.[9] The role of natural products in drug discovery is axial, ancient and universal. According to the WHO, over 70% of the world population still relies on herbal remedies in terms of healthcare.[10] The antileishmanial potential of secondary metabolites and extracts of plants was reviewed.[11–13] Natural products from plants such as terpenes, alkaloids and polyphenols have shown distinct activities against leishmaniasis.[14–16] The anti-leishmanial activities of the natural phenol curcumin (**1**) and of naphthoquinones such as plumbagin (**2**, ex. *Plumbago sp.*) and 2-hydroxy-1,4-naphthoquinone (lawsone, ex. *Lawsonia inermis*) including the semi-synthetic derivatives of the latter were recently described.[17–20]

The sources of natural products used against various ailments are not limited to plants. Major groups of organisms, whose extracts and isolates were subjected to antiparasitic studies, include algae, cyanobacteria, and fungi.[21–31] This review aims at a concise description of nature-derived medications and active principles for the treatment of various leishmaniasis forms, which emerged over the last six years. It is focused on plants, algae and fungi samples with considerable antileishmanial activities good enough to be considered as possible treatment options in the future. Further sub-sections include immuno-modulatory natural products, since such immunomodulators experienced a rising interest over the last years, and combination therapies of natural products with currently applied antileishmanials.

2. Natural Anti-Leishmanial Extracts, Oils and Isolates from Plants, Algae and Fungi

Natural plant materials from various parts of the world were investigated. They were extracted with suitable solvents, active natural products were isolated in many cases, and the extracts and/or compounds were tested for their activity against various *Leishmania* strains (*Table 1*, *Figure 1*). Methanolic extracts of whole plants of *Pergularia tomentosa* (Apocynaceae) and *Cleome amblyocarpa* (Capparaceae) from Saudi Arabia were tested for their in vitro activities against *L. major* amastigotes and the *P. tomentosa* extract was slightly more active (IC_{50} = 13.7 μg/mL) than the *C. amblyocarpa* extract (IC_{50} = 21.5 μg/mL, please note that concentrations of extracts and fractions are always and only noted as μg/mL).[32] A methanolic plant extract of Saudi Arabian *Teucrium oliverianum* (Lamiaceae) was more active than the *P. tomentosa* and *C. amblyocarpa* extracts against *L. major* amastigotes (IC_{50} = 7.8 μg/mL) and also showed considerable activity against *L. major* promastigotes (IC_{50} = 26.6 μg/mL).[32]

The methanol extract of various Pakistani plants (*Sida cordata*, Malvaceae; *Asparagus gracilis*, Asparagaceae; *Jurinea dolomiaea*, Caryophyllaceae; *Stellaria media*, Caryophyllaceae) was fractionated by extraction with hexane, chloroform, ethyl acetate, butanol and water and finally tested for activity against *L. tropica* promastigotes.[33] The hexane extracts of *S. cordata* (IC_{50} = 9.2 μg/mL) and *J. dolomiaea* (IC_{50} = 7.2 μg/mL) as well as the ethyl acetate (IC_{50} = 5.3 μg/mL) and water extracts (IC_{50} = 6.0 μg/mL) of *J. dolomiaea* showed the highest antileishmanial activities in this study, which were comparable with the activity of glucantime (IC_{50} = 6.0 μg/mL).[33] The extracts were investigated for the presence of alkaloids, saponins, terpenoids, anthraquinones, cardiac glycosides, coumarins, phlobatannins, flavonoids, phenolics, and tannins. The aqueous extract of *J. dolomiaea* contained only saponins, cardiac glycosides and phenols while only alkaloids and tannins were absent in the AcOEt extract of this plant. In contrast, the hexane extract of *J. dolomiaea* showed no phlobatannins, flavonoids, phenolics and alkaloids. These results indicate that various natural compounds of *J. dolomiaea* exert antileishmanial activity. The alcoholic extracts of several plants from Nepal were examined for antileishmanial activity. The ethanol extract of *Paris polyphylla* (Trilliaceae) rhizomes and the methanolic stem extract of *Pedilanthus tithymaloides* (Euphorbiaceae) showed the highest activities against *L. infantum* amastigotes (IC_{50} = 8.8 and 11.8 μg/mL), however, both extracts were also toxic to MRC-5 fibroblasts (CC_{50} = 13.3 and 12.8 μg/mL, selectivity index/SI = 1.4 and 1.1). The methanol extract of Nepali *Ampelocissus tomentosa* (Vitaceae) plants showed distinctly higher selectivity for *L. infantum* amastigotes (IC_{50} = 13.2 μg/mL, SI = 3.5).[34] In addition, chloroform extracts of Arabian *Pulicaria undulata* (Asteraceae) plants showed *in vitro* activity against *L. major* promastigotes and amastigotes (EC_{50} = 3.9 and 3.8 μg/mL, respectively).[35]

Table 1. Plants and their isolates showing antileishmanial activities.

Reference	Extracts/Compounds and Activity	Species	**Plant Family**
[45]	Isolated sesquiterpene coumarins fesolol (**10a**), 8′-*O*-acetyl-asacoumarin A (**10b**) and asacoumarin A (**10c**); *in vitro* activity against *L. infantum*, IC_{50} = 6.8 μM (**10a**) and 12.7 μM (**10b** and **10c**)	*Ferula narthex* Boiss.	Apiaceae
[32]	Methanol plant extract (whole plant); *in vitro* IC_{50} = 13.7 μg/mL (*L. major* amastigotes)	*Pergularia tomentosa* L.	Apocynaceae
[33]	Methanol extraction of aerial plant parts followed by fractionation in hexane, chloroform, ethyl acetate, butanol and water extract fractions; *in vitro* IC_{50} = 33.9 (MeOH), 36.6 (hexane), 28.3 ($CHCl_3$), 13.5 (AcOEt), 18.9 (BuOH) and 12.6 μg/mL (H_2O) (*L. tropica* promastigotes)	*Asparagus gracilis* Royle	Asparagaceae
[35]	Chloroform extract (whole plant); *in vitro* EC_{50} of 3.9 and 3.8 μg/mL (promastigotes and amastigotes of *L. major*)	*Pulicaria undulata* L.	Asteraceae
[48]	Ethanol leaf extract; *in vitro* IC_{50} = 13 μg/mL (*L. amazonensis*), SI = 3	*Jacaranda caroba* DC.	Bignoniaceae
[32]	Methanol extract (whole plant); *in vitro* IC_{50} = 21.5 μg/mL (*L. major* amastigotes)	*Cleome amblyocarpa* Barr.	Capparaceae
[33]	Methanol extract of plant root followed by fractionation in hexane, chloroform, ethyl acetate, butanol and water extracts; *in vitro* IC_{50} = 10.9 (MeOH), 7.2 (hexane), 47.7 ($CHCl_3$), 5.3 (AcOEt), 21.8 (BuOH) and 6 μg/mL (H_2O) (*L. tropica* promastigotes)	*Jurinea dolomiaea* Boiss.	Caryophyllaceae
[35]	Methanol extract of whole plant followed by fractionation in hexane, chloroform, ethyl acetate, butanol and water extracts; *in vitro* IC_{50} = 185.9 (MeOH), 170.4 (hexane), 155.5 ($CHCl_3$), 36.4 (AcOEt), 49.5 (BuOH) and 184.8 μg/mL (H_2O) (*L. tropica* promastigotes)	*Stellaria media* (L.) Vill.	Caryophyllaceae
[39]	Isolated connarin (**4**), activity against *L. amazonensis* (IC_{50} = 2.9 μM, SI = 6.3) and *L. infantum* amastigotes (IC_{50} = 7.7 μM, SI = 2.4).	*Connarus suberosus* Planch.	Connaraceae
[48]	Ethanol leaf extracts, *in vitro* IC_{50} = 120 μg/mL and SI = 0.6 (*L. amazonensis*); hexane stem bark extract, IC_{50} = 161 μg/mL and SI = 2; ethanol root extract, IC_{50} = 93 μg/mL and SI = 7	*Melancium campestre* Naudin	Cucurbitaceae
[34]	Methanol stem extract, *L. infantum* amastigotes IC_{50} = 11.8 μg/mL, MRC-5 fibroblasts CC_{50} = 12.8 μg/mL (SI = 1.1)	*Pedilanthus tithymaloides* (L.) Poit.	Euphorbiaceae
[49]	Isolated (5S,7R,8R,9R,10S)-(−)-7,8-seco-7, 8-oxacassa-13,15-dien-7-ol-17-al (**14**); *in vitro* IC_{50} = 0.88 μM and SI = 33.8 (*L. mexicana*)	*Acacia nilotica* L.	Fabaceae
[48]	Ethanol and hexane leaf extracts; *in vitro* (*L. amazonensis*) IC_{50} = 51 μg/mL and SI = 7 (EtOH), IC_{50} = 0.08 μg/mL and SI = 129 (hexane)	*Dipteryx alata* Vog.	Fabaceae
[48]	Ethanol and hexane leaf extracts; *in vitro* (*L. amazonensis*) IC_{50} = 44 μg/mL and SI = 3 (EtOH), IC_{50} = 35 μg/mL and SI = 1.3 (hexane)	*Hymenaea courbaril* L.	Fabaceae
[48]	Ethanol and hexane leaf extracts; *in vitro* (*L. amazonensis*) IC_{50} = 4.7 μg/mL and SI = 7 (EtOH), IC_{50} = 199 μg/mL and SI = 0.2 (hexane)	*Hymenaea stignocarpa* Mart.	Fabaceae
[46]	Essential plant oil, isolated 6,7-dehydroroyleanone (**11**); *in vitro* IC_{50} = 0.8 and 3 μg/mL/9.5 μM (*L. amazonensis* promastigotes)	*Tetradenia riparia* (Hochst.) Codd	Lamiaceae
[32]	Methanol extract (whole plant): *in vitro* IC_{50} = 7.8 and 26.6 μg/mL (*L. major* amastigotes and promastigotes)	*Teucrium oliverianum* Ging. ex Benth.	Lamiaceae
[33]	Methanol extract (whole plant) followed by fractionation in hexane, chloroform, ethyl acetate, butanol and water extracts; *in vitro* IC_{50} = 41.8 (MeOH), 9.2 (hexane), 125.5 ($CHCl_3$), 56.8 (AcOEt), 228.5 (BuOH) and 259.1 μg/mL (H_2O) (*L. tropica* promastigotes)	*Sida cordata* (Burm. f.) Borss. Waalk.	Malvaceae
[48]	Various extracts; *in vitro* (*L. amazonensis* promastigotes) IC_{50} = 103 μg/mL and SI = 6 (EtOH), IC_{50} = 62 μg/mL and SI = 8 (BuOH), IC_{50} = 96 μg/mL and SI = 6 (AcOEt), IC_{50} = 148 μg/mL and SI = 3 (hexane)	*Campomanesia lineatifolia* Ruiz & Pav.	Myrtaceae
[48]	Hexane leaf extract; *in vitro* IC_{50} = 32 μg/mL and SI = 4 (*L. amazonensis*)	*Syzygium cumini* (L.) Skeels	Myrtaceae
[14, 43]	Leaf essential oil rich in nerolidol (**8**), arginase inhibitor in *L. amazonensis* promastigotes; isolated nerolidol, in vitro IC_{50} = 8 μM (*L. amazonensis* promastigotes), increased lipid dynamics	*Piper clausseniianum* (Miq.) C. DC.	Piperaceae
[40]	Isolated piperine (**5**); *in vitro* IC_{50} = 14.2 μM (*L. amazonensis* promastigotes) and IC_{50} = 28 μM (*L. amazonensis* amastigotes)	*Piper nigrum* L.	Piperaceae
[18]	Isolated plumbagin (**2**), oxidative stress, mitochondria-associated apoptosis induction in *L. donovani*	*Plumbago sp.* L.	Plumbagoceae

Table 1. (cont.)

Reference	Extracts/Compounds and Activity	Species	**Plant Family**
[34]	Ethanol extract of rhizomes; *in vitro* activity against *L. infantum* amastigotes IC_{50} = 8.8 μg/mL, MRC-5 fibroblasts CC_{50} = 13.3 μg/mL (SI = 1.4)	*Paris polyphylla* Sm.	Trilliaceae
[34]	Methanol extract; *in vitro* activity against *L. infantum* amastigotes IC_{50} = 13.2 μg/mL, MRC-5 fibroblasts CC_{50} = 47.1 μg/mL (SI = 3.5)	*Ampelocissus tomentosa* (Heyne ex Roth) Planch.	Vitaceae
[17]	Isolated curcumin (**1**), apoptosis induction and S phase arrest in *L. major* promastigotes	*Curcuma longa* L.	Zingiberaceae

Curcumin (**1**), the major aromatic constituent of the Indian spice turmeric (*Curcuma longa*, Zingiberaceae), showed only moderate *in vitro* activity against *L. major* promastigotes (LC_{50} = 35 μM) but was able to induce apoptosis and to arrest cell cycle in the S phase of *L. major* promastigotes.[17] Previous reports about the antileishmanial activities of the naphthoquinone plumbagin (**2**) from Plumbagoceae plants (ex. *Plumbago sp.*, e.g., *Plumbago indica* and *Plumbago zeylanica*) showed that plumbagin interfered with oxidative phosphorylation and inhibited trypanothione reductase in *Leishmania* parasites. A closer look at the cell death inducing properties of plumbagin revealed that it induced mitochondria mediated apoptosis-like cell death accompanied by ATP depletion, caspase 3/7-like protease activation, cytosolic calcium level increase, and lipid peroxidation in *L. donovani* based on plumbagin-associated ROS formation and oxidative stress.[18] As soon as in the 1990's, natural plumbagin was investigated for its antileishmanial activities, and it was found moderately active against *L. braziliensis*, *L. amazonensis* and *L. donovani in vitro* (IC_{50} = 5 μg/mL, 26.6 μM) while it inhibited lesion formation at doses of 2.5-5 mg/kg/d in mice infected with *L. amazonensis* and *L. venezuelensis*.[36,37] The considerable antileishmanial activity of plumbagin-type naphthoquinones was corroborated by the bis-naphthoquinone burmanin A (**3**) (ex. *Diospyros burmanica*, Ebenaceae, Burma), which showed high activity against and selectivity for *L. major* promastigotes (IC_{50} = 0.053 μM, SI = 453) comparable with the approved antileishmanial drug amphotericin B (IC_{50} = 0.035 μM).[38] The isoprenylated bisphenol connarin (**4**), which was isolated from *Connarus suberosus* (Connaraceae) plants in Brazil, showed distinct activity against *L. amazonensis* (IC_{50} = 2.9 μM, SI = 6.3) and *L. infantum* amastigotes (IC_{50} = 7.7 μM, SI = 2.4). Compound **4** was 2–4 times more active against the amastigotes than the approved drug miltefosine, while it showed reduced activity against *L. amazonensis* and *L. infantum* promastigotes (IC_{50} = 11.4 and 13.3 μM). Impaired mitochondrial function and changes in parasite lipid profile were described as possible modes of action of **4**.[39]

Several antileishmanial aromatic natural products were discovered in plants, which belong to the widespread Piperaceae plant family. The alkaloid piperine (**5**), the major component of *Piper nigrum* (black pepper), was found to be only moderately active against *L. amazonensis* promastigotes (IC_{50} = 14.2 μM) and amastigotes (IC_{50} = 28 μM).[40] Its immune-modulatory properties in *Leishmania* parasites are mentioned below. In contrast, chalcone **6** (2,6-dihydroxy-4-methoxychalcone) from *Piper aduncum* (IC_{50} = 0.5 μg/mL, *L. amazonensis* promastigotes) and the alkaloid piplartine (piperlongumine, **7**) from *Piper retrofractum* (IC_{50} = 7.5 μM, *L. donovani* promastigotes) showed considerably higher *in vitro* activities than piperidine, and piplartine even exhibited distinct *in vivo* activity (30 mg/kg i.p., reduction of spleen parasitic burden in *L. donovani* infecte d hamsters by 36%).[41,42] In addition to these aromatic compounds, the essential oil of the leaves of *Piper claussenianum* rich in the sesquiterpene nerolidol (**8**) showed arginase inhibition in *L. amazonensis* promastigotes.[43] Purified nerolidol itself exhibited *in vitro* activity against *L. amazonensis* promastigotes (IC_{50} = 8 μM) and increased lipid dynamics in treated promastigotes.[14] In this context it has to be mentioned that linalool (**9**), a structurally simple natural monoterpene, and the linalool-enriched fraction from the essential oil of the leaves of *Croton cajucara* (Euphorbiaceae) plants from the Amazonas region were found to be highly effective against *L. amazonensis* promastigotes (IC_{50} = 8.3 ng/mL) and amastigotes (IC_{50} = 8.7 ng/mL).[44] The sesquiterpene coumarin fesolol (**10a**) was isolated from the exudate of *Ferula narthex* (Apiaceae) plants of North Pakistan and was active against *L. infantum* (IC_{50} = 6.8 μM), however, with low selectivity (IC_{50} = 8 μM for peritoneal murine macrophages and human fetal lung fibroblasts).[45] The analogs 8'-O-acetyl-asacoumarin A

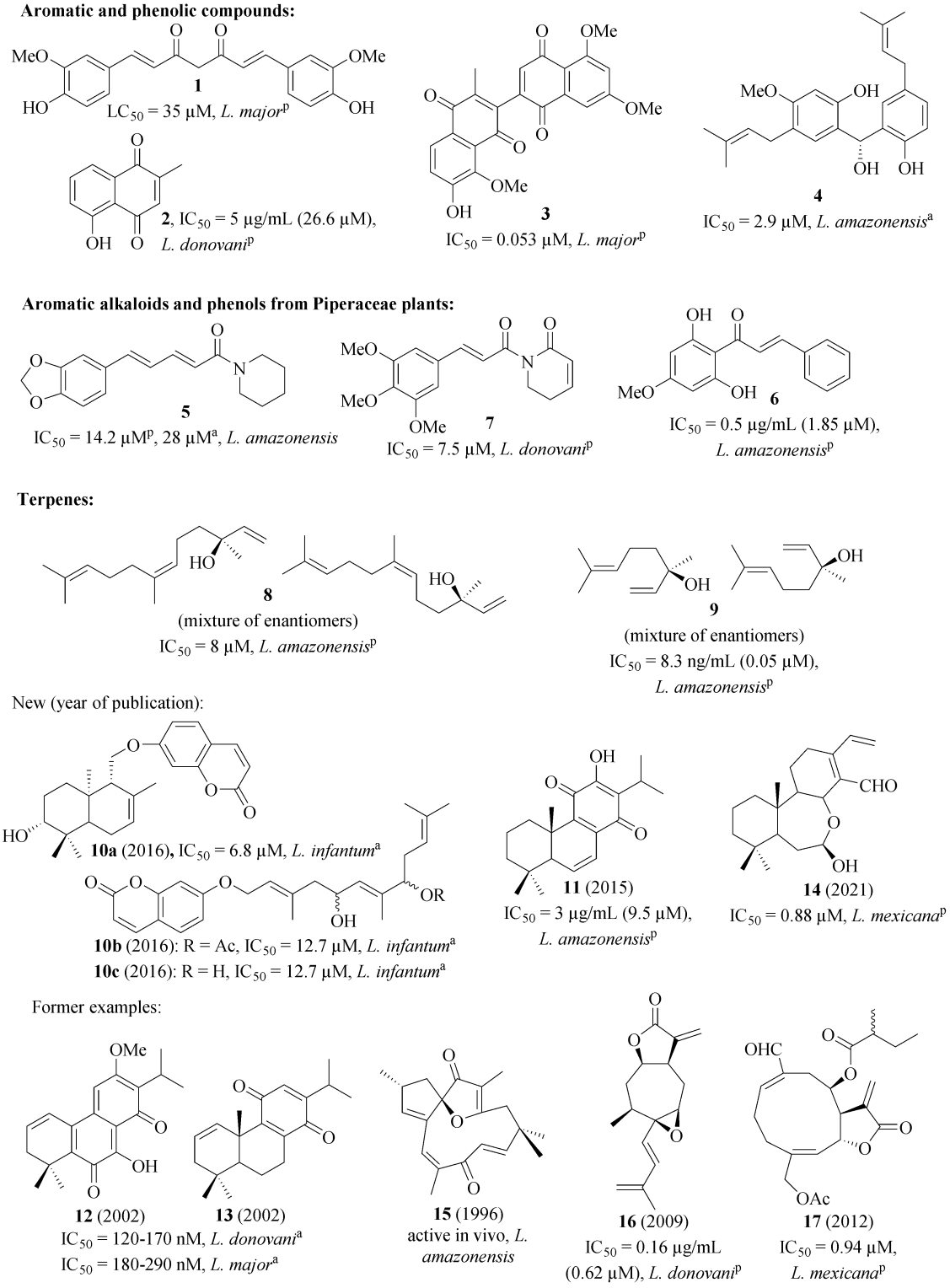

Figure 1. Structures of isolated plant constituents with promising antileishmanial activities against promastigotes ([P]) and/or amastigotes ([a]).

(**10b**) and asacoumarin A (**10c**) were less antileishmanial (IC_{50} = 12.7 µM for both compounds) but displayed a higher selectivity (IC_{50} = 32 µM for peritoneal murine macrophages). Interestingly, the acetyl group of **10b**

led to reduced toxicity to fetal lung fibroblasts (IC_{50} = 31.7 μM) when compared with deacetylated analog **10c** (IC_{50} = 11.7 μM).

The essential oil and the isolated benzoquinone diterpene 6,7-dehydroroyleanone (**11**) of *Tetradenia riparia* (Lamiaceae), a medicinal plant of Africa and South America, were active against *L. amazonensis* promastigotes (IC_{50} = 0.8 μg/mL for the oil and 3 μg/mL for the quinone).[46] Structurally related natural quinoid abietanones **12** [7-hydroxy-12-methoxy-20-nor-abieta-1,5(10),7,9,12-pentaen-6,14-dione] and **13** (abieta-8,12-dien-11,14-dione) isolated from the Turkish Lamiaceae plant *Salvia cilicica* with excellent *in vitro* activity against *Leishmania donovani* (IC_{50} = 120–170 nM) and *Leishmania major* amastigotes (IC_{50} values = 180-290 nM) were published two decades ago, which underline the potential of such natural quinones as antileishmanial drugs.[47]

Fabaceae plants from Arabia, Africa and South America were investigated for antileishmanial activity. Leaves of Brazilian Fabaceae plants such as *Dipteryx alata*, *Hymenaea courbaril* and *Hymenaea stignocarpa* were extracted with ethanol or hexane, which showed good to moderate activities against *L. amazonensis*. For instance, the hexane extract of *Dipteryx alata* was particularly active (IC_{50} = 0.08 μg/mL) with a high selectivity (SI = 129).[48] The root extract of *Acacia nilotica* plant material from Nigeria led to the isolation of the diterpene **14** [(5S,7R,8R,9R,10S)-(−)-7,8-seco-7,8-oxacassa-13,15-dien-7-ol-17-al], which showed an excellent IC_{50} value of 0.88 μM against *L. mexicana* promastigotes as well as considerable selectivity (SI = 33.8).[49] The high activity of **14** is in line with previous reports about similar plant-derived sesqui- and diterpenes. For instance, the diterpene jatrophone (**15**, isolated from *Jatropha isabellii*, Euphorbiaceae, plants in Paraguay) was subcutaneously administered (25 mg/kg ×13 days) in mice infected with *L. amazonensis* and it was more active than the approved drug glucantime (112 mg/kg×13 days).[50] Further sesquiterpene lactones **16** (8-epixanthatin-1β,5β-epoxide, ex. *Xanthium brasilicum*, Asteraceae, Sudan) and **17** (15-acetoxy-8-[(2-methylbutyryloxy)]-14-oxo-4,5-*cis*-acanthospermolide, ex. *Acanthospermum hispidum*, Asteraceae, Benin) showed strong activity against *Leishmania donovani* (IC_{50} = 0.16 μg/mL) and *L. mexicana* (IC_{50} = 0.94 μM) promastigotes.[51,52]

Further Brazilian plants were investigated such as *Jacaranda caroba* (Bignoniaceae), whose ethanol leaf extract showed *in vitro* activity (IC_{50} = 13 μg/mL, *L. amazonensis*) and selectivity (SI = 3).[48] The ethanol root extract (IC_{50} = 93 μg/mL and SI = 7) of *Melancium campestre* (Cucurbitaceae) was more active against and selective for *L. amazonensis* than the ethanol leaf extract (IC_{50} = 120 μg/mL and SI = 0.6) and the hexane stem bark extract (IC_{50} = 161 μg/mL and SI = 2) of the same plant.[48] Various solvent extracts of *Campomanesia lineatifolia* (Myrtaceae) were tested for activity against *L. amazonensis* promastigotes and the butanol extract showed some activity and selectivity (IC_{50} = 62 μg/mL and SI = 8).[48] Finally, the hexane leaf extract of Brazilian *Syzygium cumini* (Myrtaceae) showed moderate activity against *L. amazonensis* (IC_{50} = 32 μg/mL and SI = 4) and adds to the number of promising Brazilian plants with antileishmanial activities.[48] The structures of the antileishmanial natural compounds of the described plants are shown and summarized in *Figure 1*.

Summing up, the described antileishmanial natural compound classes comprise terpenes, quinones, alkaloids, and various aromatic compounds such as phenols and flavones. Natural products such as linalool, nerolidol, curcumin, and plumbagin are structurally simple and in parts commercially available, which should enable the future semi-synthesis of derivatives of these compounds with improved antileishmanial activities. Flavones such as luteolin are also easily and commercially available and might serve as starting points for the semi-syntheses of new antileishmanial flavone derivatives. *Table 1* summarizes the plants and their isolates, which were used for the treatment of various types of leishmaniasis. More plant derived products with immune-modulatory properties are mentioned separately for a better clarity.

In addition to plants, other natural sources such as algae and fungi provide compounds with antileishmanial activities. Various hexane/ethyl acetate eluent fractions were obtained from the Antarctic *Iridaea cordata* red alga by chromatographic purification and two polar fractions (i.e., fractions obtained with an eluent with a high content of ethyl acetate) showed activity against *L. amazonensis* promastigotes (IC_{50} = 17.4 μg/mL for hexane: AcOEt = 1:4 fraction, 24.3 μg/mL for hexane: AcOEt = 2:3 fraction) and amastigotes (IC_{50} = 12.4 μg/mL for hexane: AcOEt = 1:4 fraction, 4.0 μg/mL for hexane: AcOEt = 2:3 fraction).[23] The highest activity was observed against amastigotes for the hexane: AcOEt = 2:3 fraction and further efforts to purify the active principle(s) of this fraction appear to be promising. Iberian *Cystosteira* macroalgae were investigated for antileishmanial activities and the hexane extracts of *C. baccata* and *C. barbata* displayed reasonable activities against *L. infantum* amastigotes (IC_{50} = 5.1 μg/mL and 6.8 μg/mL.[53] Carotenoids, ste-

roids, meroterpenoids, fatty acids and triacylglycerols were identified as components of the *Cystosteira* extracts, which warrant further exploration as possible antileishmanial drugs.

Various fungi were tested against *Leishmania* parasites. Water soluble polysaccharides were isolated from the Indian wild mushrooms *Termitomyces eurhizus* (IC_{50} = 100 μg/mL) and *Russula laurocerasi* (IC_{50} = 86.9 μg/mL), which were moderately active against *L. donovani* amastigotes.[25] Ethanol and ethyl acetate extracts of the mushroom *Grifola frondosa* were also moderately active against *L. donovani* promastigotes (IC_{50} = 93.9 μg/mL for EtOH extract and 412.5 μg/mL for AcOEt extract).[28] A fungal extract library was also established for new antileishmanial compounds. In this way, the new bisabolane sesquiterpene lactone derivative HD871-1 (**18**) was discovered with considerable activity against *L. mexicana* amastigotes (IC_{50} = 3.73 μM).[54] The activity of **16** matches with the activities of other fungal sesquiterpenes such as hypnophilin (**19**, isolated from the mushroom *Lentinus strigosus*), which exhibited distinct activity against amastigotes of *L. amazonensis* (IC_{50} = 2.16 μg/mL, 8.7 μM).[29] A direct comparison is difficult since different *Leishmania* strains were applied for testing. A higher activity than those of **18** and **19** against amastigotes of *L. donovani* was observed for fungal quinones of the endophytic fungus *Edenia sp.* such as **20** (palmarumycin CP18, IC_{50} = 0.62 μM, SI = 245).[26] Nevertheless, compound **18** is clearly of interest as a potential new antileishmanial drug candidate, which might be successfully optimized to new analogs with improved antileishmanial activities by (semi-)synthetic methods in the future. Harzialactone a (**21**), which was recently isolated from the marine-derived fungus *Paecilomyces* sp 7 A22, also shows a lactone scaffold. But its antileishmanial activities were only moderate against *L. amazonensis* promastigotes (IC_{50} = 5.25 μg/mL, 27.3 μM) or weak against *L. amazonensis* amastigotes (IC_{50} = 18.18 μg/mL, 94.6 μM).[55]

Table 2 and *Figure 2* summarize the outcome of the studies with algae and fungal sources for the identification of antileishmanial constituents.

3. Antileishmanial Natural Products with Immuno-Modulatory Activities

Immuno-modulatory strategies are of growing relevance for the treatment of leishmaniasis infections. Several natural products of plants, which showed significant *in vitro* activity against *Leishmania* parasites, were reported to modulate immune response in infected cells and organisms (*Table 3*, *Figure 3*).[15] Natural tannins such as ellagitannin **22** from *Pelargonium* species (Geraniaceae) displayed IFN-like properties and released nitric oxide/NO as well as tumor necrosis factor-α/TNF-α in macrophages.[16,56] The iridoid-enriched fraction picroliv isolated from *Picrorhiza kurroa* (Plantagenaceae) showed immune-stimulant

Table 2. Natural products with antileishmanial activities from algae and fungi.

Organism	Species	Fractions/Compounds and Activity	Reference
Algae	*Iridaea cordata* (Turner) Bory de Saint-Vincent	Various hexane: ethyl acetate eluent fractions with activity against *L. amazonensis* promastigotes, IC_{50} = 138.6 μg/mL (hexane: AcOEt = 4:1), IC_{50} = 17.4 μg/mL (hexane: AcOEt = 1:4), IC_{50} = 24.3 μg/mL (hexane: AcOEt = 2:3); activity against amastigotes, IC_{50} = 23.6 μg/mL (hexane: AcOEt = 4:1), 12.4 μg/mL (hexane: AcOEt = 1:4) and 4.0 μg/mL (hexane: AcOEt = 2:3)	[23]
	Cystosteira baccata (S. G. Gmelin) P.C. Silva *and Cystosteira barbata* (Stackh.) C. Agardh	Hexane extracts with activity against *L. infantum* amastigotes, IC_{50} = 5.1 μg/mL for *C. baccata*, IC_{50} = 6.8 μg/mL for *C. barbata*	[53]
Fungus	*Fungal Extract Library*	Isolated HD871-1 (18) with activity against *L. mexicana* amastigotes, IC_{50} = 3.73 μM	[54]
	Grifola frondosa (Dicks.) Gray	Ethanol and ethyl acetate extracts active against *L. donovani* promastigotes IC_{50} = 93.9 μg/mL (EtOH) and 412.5 μg/mL (AcOEt)	[28]
	Paecilomyces sp 7 A22 Bainier	Isolated harzialactone a (21) with activity against *L. amazonensis* promastigotes (IC_{50} = 5.25 μg/mL, 27.3 μM) and amastigotes (IC_{50} = 18.18 μg/mL, 94.6 μM)	[55]
	Russula laurocerasi Britzelm.	Water soluble polysaccharides, IC_{50} = 86.9 μg/mL (*L. donovani* amastigotes)	[25]
	Termitomyces eurhizus (Berk.) R. Heim	Water soluble polysaccharides, IC_{50} = 100 μg/mL (*L. donovani* amastigotes)	[25]

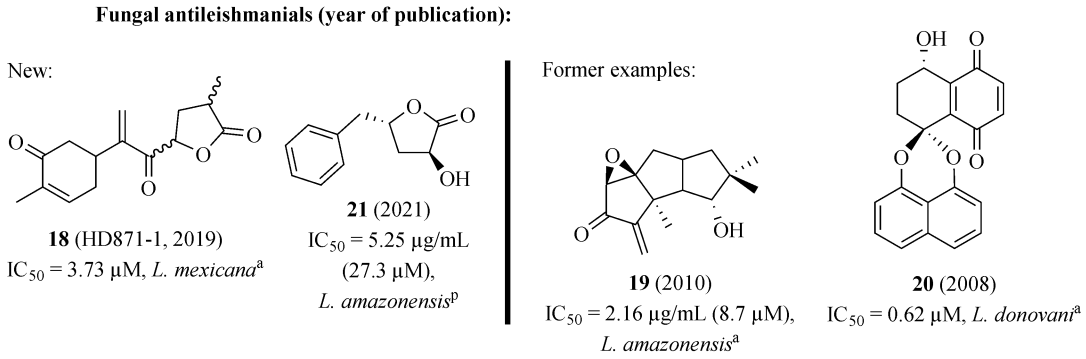

Figure 2. Structures of fungal antileishmanials with activity against promastigotes ([p]) or amastigotes ([a]).

Table 3. Plant-derived natural antileishmanials with immuno-modulatory activities.

Family	Species	Fractions/Compounds and Activity	Reference
Asteraceae	*Tanacetum parthenium* (L.) Sch.Bip.	Isolated parthenolide (**26**), inhibition of IκB kinase β	[64,65]
Euphorbiaceae	*Croton caudatus* Geisel.	Semi-purified hexane extract (JDHex), increased formation of NO, TNF-α and IL-12 *in vitro*, IFN-γ induction and IL-10 suppression *in vivo*	[68]
Fabaceae	*Glycyrrhiza glabra* L.	Isolated 18β-glycyrrhetinic acid (**25**), induction of IL-12, IFN-γ, TNF-α and inducible NO synthase, downregulation of IL-4 and IL-10	[63]
Geraniaceae	*Pelargonium sp.* L'Hér. ex Aiton	Isolated ellagitannin **22**, IFN-like properties, NO release, TNF-α release in macrophages	[16,56]
Malpighiaceae	*Lopanthera lactescens* Ducke	Isolated 6α,7α,15β,16β,24-pentacetoxy-22α-carbomethoxy-21β,22β-epoxy-18β-hydroxy-27,30-bisnor-3,4-secofriedela-1,20(29)-dien-3,4R-olide (**28**, LLD-3), effects on B and T cell proliferation and B cell immunoglobulin production	[67]
Piperaceae	*Piper claussenianum* (Miq.) C. DC.	Leaf essential oil rich in nerolidol, increased NO formation by 20.5 %	[43]
Piperaceae	*Piper nigrum* L.	Isolated piperine (**5**), suppression of NF-κB and activation of IκB kinase, suppression of MCP-1, TNF-α, and NO production	[40,58]
Plantagenaceae	*Picrorhiza kurroa* Royle ex. Benth.	Picroliv (iridoid-enriched fraction), immune stimulant: increased macrophage migration index/MMI, [^{14}C]-glucosamine uptake, phagocytosis of *E. coli*, chemiluminescence of peritoneal macrophages, [^{3}H]- thymidine uptake	[57]
Salicaceae	*Salix sp.* L.	Salicylic acid (**23**) and acetyl salicylic acid (**24**), NO formation	[59,60]
Rutaceae	*Raputia heptaphylla* Pittier	Isolated 11α,19β-dihydroxy-7-acetoxy-7-deoxoichangin (**27**), formation of IL-12p70, TNF-α and NO	[66]

activities in mouse lymphocytes (increased macrophage migration index/MMI, [^{14}C]-glucosamine uptake, phagocytosis of *E. coli*, chemiluminescence of peritoneal macrophages, [^{3}H]- thymidine uptake), which increased the resistance of treated hamsters to *L. donovani* infection.[57] Piperaceae derived compounds were also investigated, and piperine (**4**, see *Figure 1*) showed immune-modulatory effects by suppression of NF-κB and activation of IκB kinase as well as by suppression of MCP-1, TNF-α, and NO production.[40,58] In contrast to that, nerolidol (**8**, ex. *Piper claussenianum*, Piperaceae) led to an increase of NO formation by 20.5 %.[43]

Salicylic acid (**23**), the oxidation product of natural salicin from *Salix* sp. plants (Salicaceae), was described as a component of a possible topical therapy for CL, which induced the formation of NO as a mode of action, and cured 12 % of *L. tropica* infected patients in a clinical study, with a further 28 % of the patients experiencing an improvement.[59] Mice, infected with *L. major*, were fed with the widely applied drug acetyl salicylic acid (**24**, 400 mg/kg/day for 13 weeks), which inhibited *L. major* visceralization and reduced lesion size and hepato-/splenomegaly by increased NO formation.[60] Copper complexes with ligands based on the salicyl fragment also showed distinct activities against *L. tropica* and *L. donovani*.[61,62]

The triterpene **25** (18β-glycyrrhetinic acid) from *Glycyrrhiza glabra* (Fabaceae) displayed *in vitro* and *in vivo* activity against *L. donovani* by induction of IL-

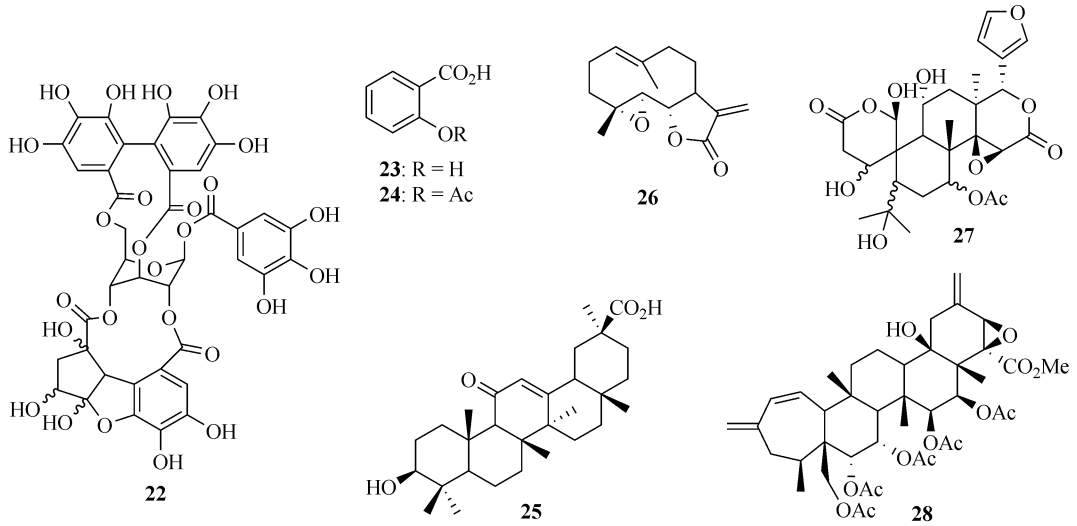

Figure 3. Structures of antileishmanial natural products from plants with immuno-modulatory activities (piperine **5** is shown in *Figure 1*).

12, IFN-γ, TNF-α and inducible NO synthase, as well as downregulation of IL-4 and IL-10.[63] Similar effects were reported of the triterpenes oleanolic acid and ursolic acid.[11] Parthenolide (**26**), a sesquiterpene lactone isolated from *Tanacetum parthenium* (Asteraceae), was described as a strong inhibitor of *L. amazonensis* promastigotes (IC_{50} = 0.37 μg/mL, 1.49 μM) and of IκB kinase β.[64,65] The *seco*-limonoid **27** (11α,19β-dihydroxy-7-acetoxy-7-deoxoichangin) from *Raputia heptaphylla* showed distinct activity against *L. panamensis* amastigotes (EC_{50} = 7.9 μM) accompanied by increased formation of IL-12p70, TNF-α and NO.[66] Nor-triterpene **28** (6α,7α,15β,16β,24-pentacetoxy-22α-carbomethoxy-21β,22β-epoxy-18β-hydroxy-27,30-bisnor-3,4-secofriedela-1,20(29)-dien-3,4R-olide, LLD-3) isolated from *Lopanthera lactescens* (Malpighiaceae) was also strongly active against *L. amazonensis* amastigotes (IC_{50} = 0.41 μg/mL, 0.16 μM) and had effects on the proliferation of B and T cells as well as on B cell immunoglobulin production.[67] A semi-purified hexane extract (JDHex) of *Croton caudatus* leaves (Euphorbiaceae) exhibited a higher activity against *L. donovani* amastigotes (IC_{50} = 2.5 μg/mL) than against promastigotes (IC_{50} = 10 μg/mL), associated with an increased formation of NO, TNF-α and IL-12 and distinct *in vivo* activity of the extract, which reduced the spleen and liver parasite burden, accompanied by IFN-γ induction and IL-10 suppression.[68]

The mentioned natural products can either suppress or induce immune responses in infected cells. Piperine (**5**) led to a suppression of TNF-α, and NO production and, thus, it can contribute to a control of an excessive immune reaction upon *Leishmania* infection. In addition, a combination of piperine, which exhibited antileishmanial activities (see above), with immune response inducing compounds might enhance the activity of piperine against *Leishmania*. Hence, the induction of immune response based on increased levels of NO, TNF-α, and IFN-γ, for example, is of great importance for the treatment of *Leishmania* infections. A few more plant products including compounds with immuno-modulatory activities will be described in the synergy effects section below.

4. Synergy Effects of Natural Extracts, Oils and Isolates in Combination with Approved Drugs

The promising effects of natural products in combination with currently approved antileishmanial drugs are briefly described in this section. For instance, the combination of natural products with antimonials can lead to synergy effects and improved treatment outcomes. Meglumine antimoniate in combination with the well-known plant spices capsaicin (**29**, ex. *Capsicum* sp., Solanaceae) and piperine (**5**, Piperaceae, see *Figure 1*) led to enhanced antileishmanial *in vitro* activities of both natural compounds against *L. infantum* promastigotes and amastigotes.[69] In addition, a clinical study with patients suffering from CL using glucantime plus ozonated olive oil exhibited synergy effects leading to distinct lesion size

reductions.[70] Such combination strategies can also be suitable options in order to reduce the systemic toxicity of antimonials in case that lower effective antimonial doses can be administered.

Although very efficient, amphotericin B is an expensive antileishmanial drug having side-effects and so synergy effects of natural products with amphotericin B were studied in order to find ways to reduce the necessary amphotericin B doses for leishmaniasis treatments. A study of the ethanolic leaf extract of the African medicinal plant *Moringa oleifera* (Moringaceae) showed its activity against *L. major* promastigotes (IC_{50} = 6.87 μg/mL) and amastigotes (IC_{50} = 9.31 μg/mL) and revealed a considerable antileishmanial synergy effect in combination with amphotericin B (fractional inhibitory concentration/FIC = 0.375). The active principles of *M. oleifera* leaf extract were identified as resorcinol (**30**, IC_{50} = 3.79 μg/mL, 34.4 μM), luteolin 7-*O*-glucoside (**31**, IC_{50} = 10.7 μg/mL, 23.9 μM) and syringic acid (**32**, IC_{50} = 13.4 μg/mL, 67.6 μM).[71] Already two decades ago, the aglycone luteolin (isolated from *Vitex negundo*, Lamiaceae) and its structurally related flavonoid quercetin (isolated from *Fagopyrum esculentum*, Polygonaceae) were shown to reduce splenic parasite load in hamsters infected with *L. donovani* by 80% (luteolin, 3.5 mg/kg) and 90% (quercetin, 14 mg/kg) while luteolin remained non-toxic to human cells.[72]

Various propolis extracts, oils and compounds showed distinct *in vitro* and *in vivo* activities against *Leishmania* strains and infections.[73] The essential oil of Tunisian propolis, which was made by bees (*Apis mellifera*) in a region where various *Citrus* plants (Rutaceae) grow, exhibited strong activities against *L. major* and *L. infantum* promastigotes (IC_{50} = 5.3 μg/mL and 3.7 μg/mL) and amastigotes (IC_{50} = 7.4 μg/mL and 5.0 μg/mL), it activated macrophages by NO formation, and it displayed synergy effects when combined with amphotericin B (FIC = 0.37).[74] Cadambine acid (**33**) isolated from the *Naucleas diderichii* (Rubiaceae) medicinal plant growing in West and Central Africa was active against and selective for *L. infantum* amastigotes (IC_{50} = 1.2 μM, SI > 209) by induction of NO formation, and it acted synergistically in concert with amphotericin B.[75] Flavolignans were isolated from milk thistle (*Silybum marianum*, Asteraceae), and although dehydroisosilybin A (**34**) showed only low activity against *L. donovani* and *L. infantum* promastigotes (IC_{50} = 90.2 μM), it exhibited a moderate synergy effect together with amphotericin B, which made it possible to reduce the applied dose of dehydroisosilybin A by a factor of more than 4 and the dose of amphotericin B by a factor of 2.[76]

Taken together, there is a well-equipped arsenal of natural products, which were successfully combined with approved antileishmanial drugs such as antimonials and amphotericin B. Quite a few of them such as piperidine, capsaicin, resorcinol, syringic acid, luteolin, olive oil, and propolis are easily available and, thus, can be suitable additives for currently applied treatments in order to reduce costs and side-effects/toxicities. Structures of natural products with synergy effects when combined with clinically approved drugs are shown in *Figure 4*.

5. Conclusions

This review underlines the great potential of natural products as valuable drug candidates for the treat-

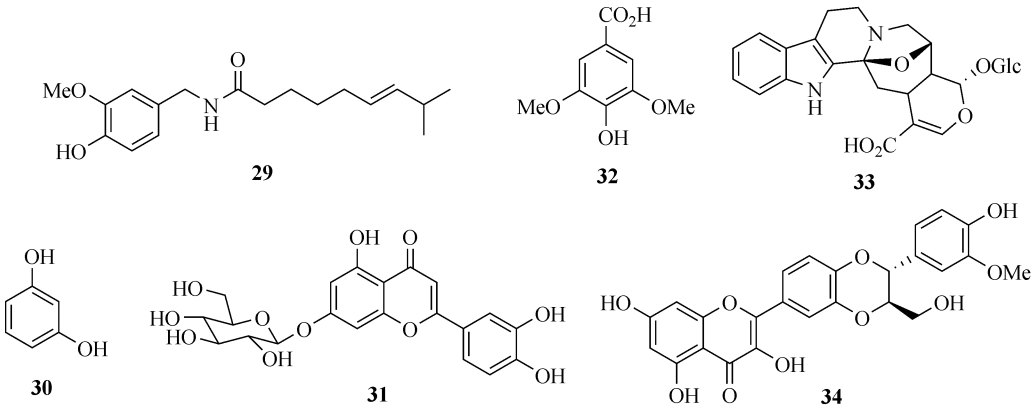

Figure 4. Structures of antileishmanial natural products with synergy effects in combination with approved antileishmanial drugs (piperine **5** is shown in *Figure 1*).

ment of leishmaniasis. Nature can provide many possible treatment options for various forms of leishmaniasis. This review does not include bacterial sources, yet, a considerable number of antileishmanial plant, fungal and algae products. These natural sources are already being applied by traditional healers in tropical and sub-tropical regions as treatments for various infectious diseases. A good deal of the described extracts and natural products was derived from plants. Medicinal plant products can enhance the effects of conventional antileishmanials such as antimonials and amphotericin B, which can decrease treatment costs and improve the quality of life of patients due to reduced side-effects. Various products of algae and fungi also revealed promising antileishmanial activities not inferior to nor less promising than those effects of the plant-based products. Several extracts and natural products showed excellent *in vitro* and/or *in vivo* antileishmanial activities, which warrant further in-depth studies. The immuno-modulating properties of certain compounds and fractions are particularly interesting since they have the potential to lead to an immunization against *Leishmania* infection and, thus, they can also be of relevance for other human diseases including infectious diseases such as viral infections.

This review is intended to show the potential of nature-derived antileishmanials and to encourage readers either to start or to continue research in the field of nature-inspired drugs against NTDs such as leishmaniasis. These efforts can comprise the exploitation of currently known plants, fungi, and algae products with known antileishmanial effects, as well as the identification of new natural sources with antileishmanial activities in order to develop cost-effective and easily accessible therapies, which can add to or even replace current standard therapies for leishmaniasis diseases. In addition, chemists can provide synthetic approaches to the described highly active natural products in order to achieve an easier access to these molecules and to generate new (semi-)synthetic derivatives with improved biological properties.

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Author Contribution Statement

Waleed S. Koko carried out the literature search on plants, Ibrahim S. Al Nasr wrote the manuscript and revised the manuscript, Tariq A. Khan carried out the literature search on fungal and animal sources. Bernhard Biersack carried out the literature search on natural products and wrote the manuscript. Rainer Schobert revised and proofread the manuscript.

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