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Fungal Metabolites and Leishmaniasis: A Review

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Authors' contributions

This work was carried out in collaboration between all authors. Author NF designed the study and wrote the first draft of the manuscript. Authors SAM, IS and AM managed the literature searches. Authors SAM and MD deal with structures. Authors HT and MSS deal with final drafting and editing. All authors read and approved the final manuscript.

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ABSTRACT

Among the most neglected tropical diseases in the world, one is leishmaniasis, which is caused by parasites that belongs to protozoans of the genus *Leishmania*. Leishmaniasis can be controlled profoundly by using chemotherapeutic agents which includes pentavalent antimonials, paromomycin, pentamidine, amphotericin B and miltefosine, as it depends greatly on it. The only oral drug used with high cure rate is miltefosine used for the treatment of visceral leishmaniasis but its observed susceptibility decrease in countries like India where it is extensively used. Hence, the development of novel antileishmanial agents with good potency and better therapeutic profile is very necessary. Here we review diverse classes of secondary metabolites, focusing on anti-parasitic compounds, biosynthesized by fungi.

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Keywords: Leishmaniasis; fungi; secondary metabolites; drug leads.

1. INTRODUCTION

One of the most important parasitic diseases of humans is leishmaniasis, causing significant morbidity and mortality according to the World Health Organization. Species of the genus *Leishmania (Trypanosomatidae)* were first described separately in 1903 by Leishman and Donovan in patients from India. Millions of people are still affected by *Leishmania* and about 2 million new cases appeared annually. Moreover, about 350 million people live at risk of infection with *Leishmania* parasites. The incidence of leishmaniasis is increasing due to projects that are made by man and that have a great impact on environmental, including deforestation, irrigation systems and wells, as well as dams that increase human exposure to the sandfly vector. The risk of *Leishmania*-infected people to develop potentially fatal visceral leishmaniasis increases in people with suppressive immune system such as acquired immunodeficiency syndrome (AIDS) [1]. Natural products from plants have proven to be a valuable source of chemical matter for anti-infective programs. In natural products fungal metabolites play an important role in drug discovery and drug development and they provide lead compounds.

2. ETIOLOGY

There are 17 different species of protozoan parasites belonging to the genus *Leishmania* that cause leishmaniasis. The parasite of *Leishmania* is present in two forms amastigote and promastigote. Amastigote stage is nonflagellated and ovoid shape of *leishmania* [2]. Central round or oval nucleus and adjacent but smaller round or rod shaped kinetoplast can be observed through simple light microscopy. The promastigote form of parasite is found in sandfly host. Its transformation from amastigotes mostly occurs in gut and starts within hours of ingestion of the amastigotes. The complete motile promastigotes are formed within 24-48 h with continue binary division that leads to development of disease [3,4].

3. MODES OF TRANSMISSION

For implementation of an effective intervention strategy, it is important to study complete transmission mechanism of any infectious agent. Pathogens have to be transferred from a source to a susceptible host to cause an infection. The modes of transmission of infection are;

3.1 Vector-borne Transmission

The most common mode of transmission is vector-brone transmission worldwide [5]. *Leishmania* amastigotes are swallowed and circulates freely in the body, after the bite of sandfly to the infected host. Stationary, infective-stage organisms that could be qualified as “metacyclic” promastigotes are formed by the amastigote migration to sand flies proboscis. Upon the bite of infected sandfly to a second host, *e.g.*, a human being, these promastigotes are released on the site of bite or injected and deposited over there, long-lasting erythema is produce by potent vasodilators (*i.e.*, maxadilan) that was deposited along the promastigote [6,7].

3.2 Congenital Transmission

In 1962 the 1st case of congenital leishmaniasis was reported by Low & Cooke [8]. *Leishmania donovani* passed through the placenta of the Syrian hamster and mice but in aborted 5 months fetus, no parasite could be demonstrated in the organs while numerous amastigotes were found in the placenta [9]. This infection might have occurred in most of these cases during the exchange of mother’s blood at the time of passage of fetus through birth canal. The infection of congenital visceral leishmaniasis may change to *Leishmania* acquired through sandfly bite within three months of life [9,10]. The susceptibility to leishmaniasis is high in pregnant women due to shift of cell mediated immunity to humoral immunity [11].

3.3 Sexual Transmission

Promastigotes were found in cultures of urine and prostatic fluid from patients with visceral leishmaniasis. Transmission via a sexual route is common; include transmission from a man to his wife and in homosexual man suffering from AIDS [12].

3.4 Occupational (Needle Stick) Exposures

Number of cases of AIDS and about 200 cases of HIV-associated leishmaniasis were detected earlier in Spain, in which more than 85 per cent

occurred among intravenous drug users (IVDUs). The infection with amastigote is so common in 17% of 111 bone marrow aspirates with HIV positive subject. Alvar et al. [1] described *L. infantum* zymodemes, in blood of the persons who are drug user and share syringes and, therefore, they act as a carrier. In another study, Molina et al. [13] tested the indirect xenodiagnosis of visceral leishmaniasis in 10 HIV-infected patients, of whom nine were IVDUs thereby indicating the possibility of needle-mediated transmission [13,14].

4. CLINICALLY DIFFERENT FORMS OF LEISHMANIASIS

There are three clinically distinguishable forms of leishmaniasis: cutaneous, mucocutaneous, and visceral.

4.1 Cutaneous Leishmaniasis

Skin is involved in it and exposed parts of the body such as the face, arms and legs may get skin ulcer due to cutaneous leishmaniasis. A large number of lesions ranging from one to dozens are produced. According to the infecting *Leishmania* species, the lesions may appear as smooth nodules, ulcers, flat plaques or hyperkeratotic wart-like lesions. Immuno-suppressive conditions like infection with the human immunodeficiency virus (HIV) or the steroid treatment can result in unusually severe cutaneous leishmaniasis [15].

4.2 Mucocutaneous Leishmaniasis

Mucocutaneous leishmaniasis is a rare form of the disease usually occurs in Latin America. It tends to occur months or years after the healing of a cutaneous leishmaniasis ulcer, but it can also appear during the skin ulcers. Ulcerations and erythema at the nose are the first signs, which may develop into a destructive inflammation to involve the nasal septum, the pharynx or larynx. Mucocutaneous leishmaniasis does not heal spontaneously, and the destructive inflammation may cause severe disfigurement of face due to the nasal septum perforate, or pharynx or larynx blockade [16].

4.3 Visceral Leishmaniasis

The most common symptoms of visceral leishmaniasis, are an intermittent fever, decreased appetite, abnormal weight loss, anemia, and abdominal distension with enlargement of the liver and spleen. The sensitivity to infections with other microorganisms increases with thrombo-cytopenia, a low platelet count, result in abnormal bleeding, including hemorrhages or mucous membranes petechiae, and leukopenia. This form of the disease is always fatal, due to other infections and complications, as no treatment was provided. In patients co-infected with HIV, this disease is very fatal [17].

5. ANTILEISHMANIAL DRUGS AND PRESENT STATUS

***Amphotericin B* (1)** is a polyene antibiotic with selective and high affinity for ergosterol a major sterol in fungi and *Leishmania* parasites. Therefore this antibiotic is considered selective drug against these organisms [18-20]. The sensitivity of different species (having differences in the type and quantity of sterols in the membranes) of *Leishmania* to amphotericin B is not compared. Amphotericin B leads to accumulation of 14-α-methyl sterols by inhibiting 14-α-demethylation of lanosterol which is mediated by cytochrome 450 enzymes. The accumulation of 14-α-methyl sterols cause blockage of synthesis of ergosterol in *Leishmania* parasites [21-23].

5.1 Pentavalent Antimonials (Pentostam and Glucantime)

More than half of a century, pentavalent antimonials (antimony compounds) including sodium stibogluconate **(Pentostam) (2)** and meglumine antimoniate **(Glucantime) (3),** have been used for treatment of leishmaniasis. Despite of several side effects such as need for daily parenteral administration and drug resistance, they are still considered as 1st line therapy. The antimonial's mechanism of action, molecular structure and metabolism are still under investigation. The final active form whether is sodium stibogluconate Sb(V) or Sb(III), is still unclear. Studies showed that the mechanism of action of pentavalent antimonials can be proposed by three different models [24]. Prodrug model; According to this model, pentavalent antimony Sb(V) behaves as a prodrug, which undergoes biological reduction to much more active/toxic trivalent form of antimony Sb(III) that exhibits antileishmanial activity. Intrinsic

antileishmanial activity model; According to this model, Sb(V) has intrinsic antileishmanial activity. Early studies show that macromolecular biosynthesis in amastigotes [25] is inhibited by Sb(V) by energy metabolism perturbation due to inhibition of glycolysis and fatty acid beta oxidation [26]. However, there is still no identification of the specific targets in these pathways.

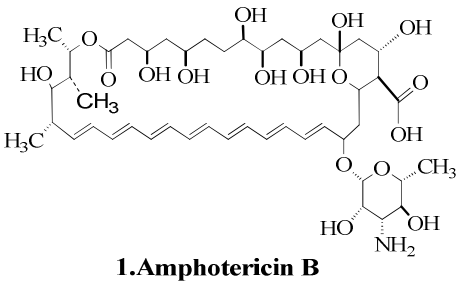
Host immune activation model; According to this model, antimonials cause the activation of host immune system that kills the intracellular *Leishmania* parasites. Sodium antimony gluconate can induce effective antileishmanial immune response by activating both innate and adaptive immune system which would help to eradicate the existing infection as well as prevent the chances of relapse [24].

5.2 Pentamidine

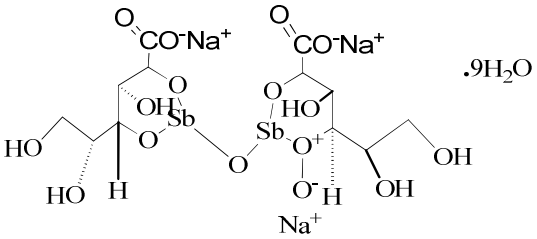
Pentamidine (pentamidine mesylate) **(4)** is an antiparasitic and apoptotic drug used for treatment of leishmaniasis [23,27].

5.3 Miltefosine

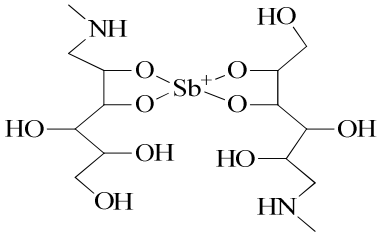
Miltefosine **(5)** is an alkylphosphocholine which acts by interfering cell signal-transduction pathways and inhibits phospholipids and sterol biosynthesis and has a major role in cancer treatment. It inhibits ornithine decarboxylase and polyamine biosynthesis irreversibly. It has also been developed for treatment of leishmaniasis. It is effective against both the stages of leishmanial parasites i.e. promastigotes and amastigotes and is used orally [28,29]. Natural products are considered an important domain for antileishmanial drug discovery.



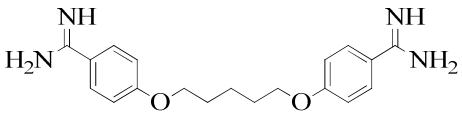
1.Amphotericin B



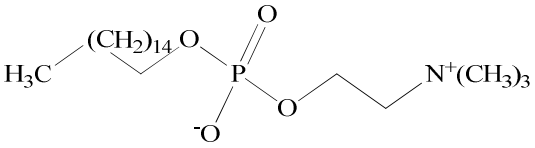
2.Sodium stibogluconate



3.Meglumine antimoniate



4.Pentamidine



5.Miltefosine

Fig. 1. Existing antileishmanial drugs (1-5)

Table 1. Current drugs for leishmaniasis

Medication	Leishmania species	Clinical efficacy	References
Topical			
15% paromomycin/12% methylbenzethonium chloride	New World cutaneous leishmaniasis	76%	[30]
15% paromomycin/12% methylbenzethonium chloride combined with parenteral meglumine antimoniate	New World cutaneous leishmaniasis	90%	[30,31]
Paromomycin/gentamicin	Ulcerative *L major* in Tunisia	81%	[32]
Paromomycin	Ulcerative *L major* in Tunisia	82%	[32]
Imiquimod	*L infantum*	1 of 1 (case report)	[33]
Ethanolic amphotericin B (5%)	*L major* in Israel	50%	[34]
Intralesional			
Sodium stibogluconate	Old World cutaneous leishmaniasis	95%	[35]
Intramuscular			
Pentamidine	*L v guyanensis* Colombia	58.1%	[36]
Meglumine antimoniate	*L v guyanensis*	55.5%	[36]
Miltefosine	*Bolivia*	88%	[37]
	L braziliensis in Bolivia	75%	[38]
	L (V) braziliensis in Guatemala	90%	[39]
	L Mexicana in Guatemala	60%	[39]
	L (V) guyanensis	53.6%	[40]
Sitamaquine	*L donovani*	87%	[41]
Intravenous			
Sodium stibogluconate or meglumine antimoniate	Old and New World cutaneous, mucocutaneous, visceral	>90%	[39]
Sodium stibogluconate	*L (V) braziliensis* in Bolivia	70%	[42]
Liposomal amphotericin B or amphotericin B deoxycholate	Old World visceral	100%	[43]
Liposomal amphotericin B	*L (V) braziliensis*	1 of 1 (case report)	[44]
Meglumine antimoniate	*L (V) braziliensis* in Peru	75%	[45]
Pentamidine	*L (V) braziliensis* in Peru	35	[45]

6. NATURAL PRODUCTS AND LEISHMANIASIS

Despite of the continuous efforts in drug discovery and drug development against leishmaniasis current therapies are not much effective and there is a need for discovery of more effective antileishmanial agents [46].

In view of the present scenario, development and introduction of new antileishmanial compounds would be an urgent need due to the toxicity of the clinically used drugs and the continuous side-effects even after adjustment of dose and duration of treatment.

In the ongoing search for new drugs, natural products are gaining ground being easily available and relatively cheap. Furthermore, valuable synthetic compounds can be obtained from the leads with antileishmanial activity obtained from natural sources (plants and microorganisms). Thus, in the present study we have reviewed antileishmanial compounds from fungi which is less explored site.

6.1 Fungal Metabolites with Antileishmanial Activity

Fungal secondary metabolites possess broad bioactive applications such as immunosuppressants, agrochemicals, anti-parasitics, antimicrobial and antitumor agents [47-49]. Different classes of fungal metabolites have been reported for their antileishmanial potential.

The fungal metabolite aphidicolin **(6),** a tetracyclic diterpene antibiotic and DNA synthesis inhibitor isolated from *Nigrospora sphaerica* have antiparasitic potential against *Leishmania* and *Trypanosoma* species with EC_{50} values ranging between 0.02 -1.83 µg/ml [50-51].

In another study, it is reported that perylene quinonoids, hypocrellin A and B **(7, 8)** isolated from fungus *Hypocrella bambusae*. Hypocrellin A showed significant antileishmanial activity against *L. donovani* (IC_{50} 0.27±0.03 µg/ml), while B was moderately active with IC_{50} 12.7± 3.1 µg/ml) [52]. In 2005 Marinho and his collegues studied antileishmanial potential of methanolic extract of an endophytic fungus *Penicillium janthinellum* isolated from the fruit of Brazilian plant *Melia azedarach* (*Meliaceae*). They isolated a known polyketide metabolite citrinin **(9)** which showed 100% inhibition of *L. mexicana* at 40 µg/ml [53]. A biphenyl derivative, altenusin **(10)** isolated from *Alternaria* sp., showed inhibitory activity against *L. amazonensis* [54]. Crude ethyl acetate extract from *phaseolorum* sp., *Phyllosticta* sp., *Phomopsis* sp. and *Cercospora kikuchii* showed inhibition of growth of *L. tarentolae* [55]. Another fungus *Cochliobolus* sp., isolated from *Piptadenia adiantoides* was also studied for antileishmanial effects. Alcoholic extract showed significant results and two compounds cochlioquinone A and isocochlioquinone A were purified by its further fractionation **(11,12)**. Both compounds showed antileishmanial activity against *L. amazonensis*, with EC_{50} values of 1.7 µM and 4.1 µM, respectively [56]. Phytochemical investigation of the fungus *Chaetomium* sp., leads to isolation of three new xanthone compounds. These chaetoxanthones A−C **(13-15)** exhibited significant activities against *L. donovani* with IC_{50} values of 5.3, 3.4, and 3.1 µg/ml, respectively [57].

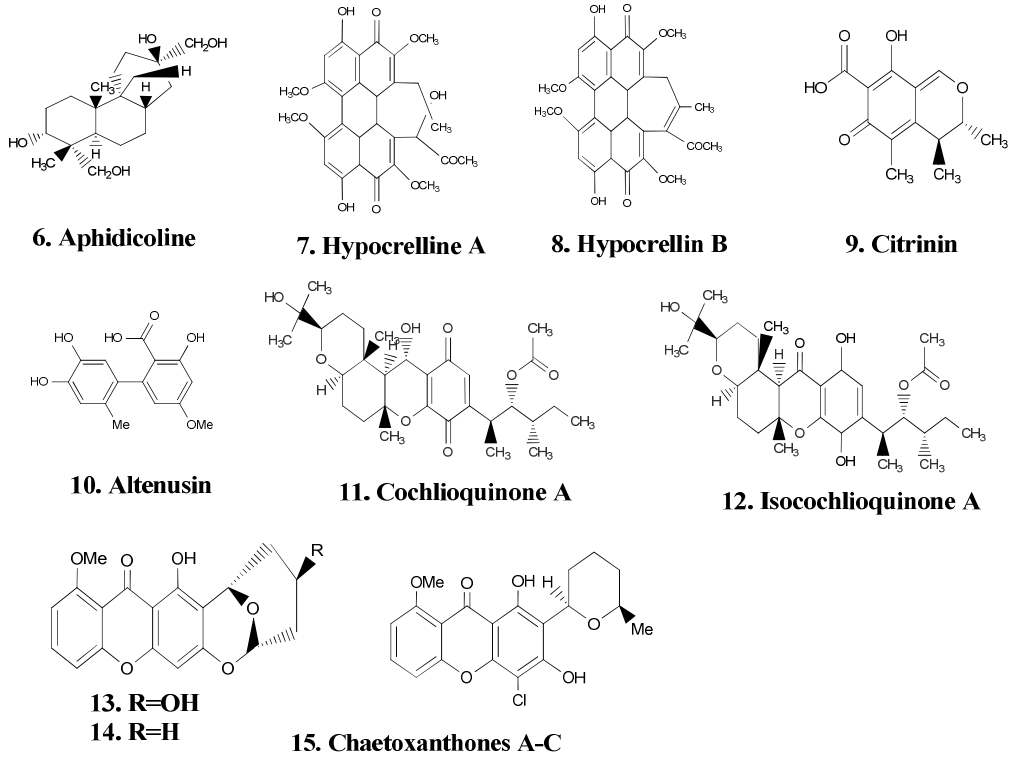


Fig. 2. Antileishmanial compounds from fungi (6-15)

Rosa et al. [58] studied 34 extracts from different fungi of *Basidiomycota* and found positive results against *L. amazonensis*. From endophytic *Edenia* sp., isolated from *Petrea volubilis*, five potent antileishmanial compounds were separated. These compounds **16-20** were preussomerin EG_1 **(16)**, palmarumycin CP_2 **(17)**, palmarumycin CP_{17} **(18)**, palmarumycin CP_{18} **(19)** and CJ-12, 37 **(20)**. Preussomerin EG1 showed potent antileishmanial activity against *L. donovani* with low IC_{50} value like amphotericin B [59].

In another study, 11 extracts of *Alternaria*, *Arthrinium*, *Cochliobolus*, *Colletotrichum*, *Penicillium*, *Fusarium* and *Gibberella* showed inhibition against *L. amazonensis* with IC_{50} values ranging from 4.60 to 24.40 µg/ml [57]. In 2012 it is also reported that extracts of 12 fungi belonging to the genera *Alternaria*, *Antarctomyces*, *Cadophora*, *Davidiella*, *Helgardia*, *Herpotrichia*, *Microdochium*, *Oculimacula* and *Phaeosphaeria* inhibited the proliferation of *L. amazonesis* [60].

Gao and his colleagues in 2012 studied secondary metabolites of *Eurotium repens* which were examined for in vitro antibacterial, antifungal, antimalarial, and antileishmanial activities [61]. They reported 8 compounds **(21-28)** from this fungus. Compounds **21–26** showed antileishmanial activity against *L. donovani* promastigotes with IC_{50} values ranging from 6.2 to 23 µg/ml. Compounds **25** and **26** showed higher antileishmanial activities (IC_{50} values of 7.5 and 6.2 µg/ml, respectively) than **21–24** (IC_{50} values ranging from 19–23µg/ml). No antiprotozoal effect showed by compounds **27** and **28**.

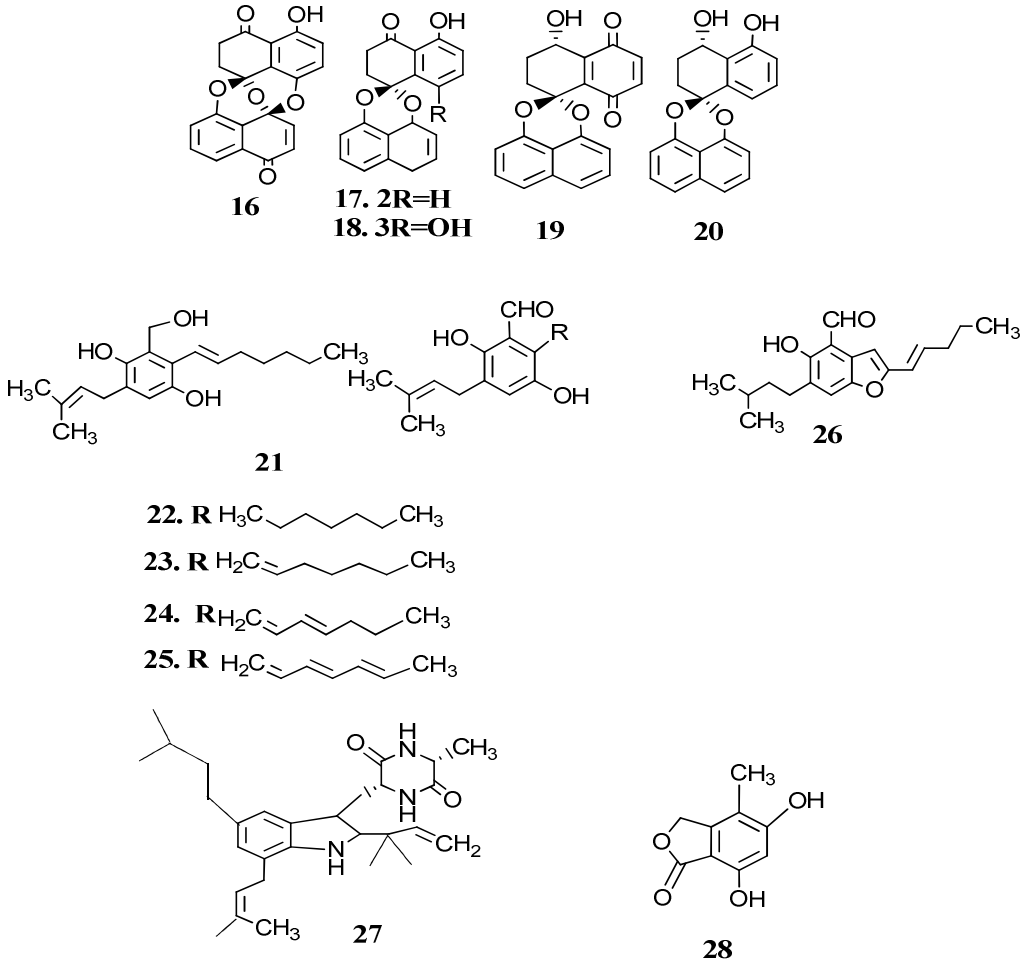


Fig. 3. Antileishmanial compounds from fungi (16-28)

In 2013, 82 fungal extracts were selected and screened against promastigotes of *L. mexicana*. Only four fungal extracts of *Fusarium* sp. TA50, *Fusarium* sp. TA54, *Verticillium* sp. TH28, and the unidentified 2TA2 showed significant antileishmanial activity against *L. mexicana* promastigotes with IC_{50} values ranging between 14.23–100 µg/ml [62]. More than 2700 fungal endophytes isolated form angiosperms and ferns of different regions of Panama were studied for their antileishmanial potential. Results showed that 15.4% of extracts samples were active against causative agents of leishmaniasis [63].

Rodrigues and his coworkers studied *in vitro* and *in vivo* antileishmanial effect of KA (Kojic acid) **(29)** on *L. amazonensis*. It was observed that Kojic acid at concentration of 50 µg/ml reduce the growth of amastigotes with values of 79% (IC_{50} 27.84 µg /ml) while in case of promastigotes 62% growth reduction was found with IC_{50} 34 µg /ml. Secondly it was observed that after 4 weeks of treatment with Kojic acid collagen fiber production was increased and parasitic burden was reduced drastically which indicate potential of this compound against leishmaniasis [64]. Malik and his coworkers in 2014 studied ethyl acetate extract of filamentous fungus *Geosmithia langdonii* and found significant results against *L. donovani.* Further fractionation led to isolation of two new compounds **(30, 31)** from extract and 10 known compounds **(32-41).** These compounds were identified as 4-[2,4-dihydroxy-6-(hydroxymethyl)benzyl]benzene-1,2-diol **(30)**, (4R,5R,6R)-4,5-dihydroxy-6-(6′-methylsalicyloxy)-2-methyl-2-cyclohexen-1-one **(31)** were found to be new, (+)-epiepoformin **(32)**, (−)-dihydroepiepoformin **(33)**, (4S,5S)-4,5-dihydroxy-2-methylcyclohex-2-enone **(34)**, 6-methylsalicylic acid **(35)**, gentisylquinone **(36)**, 3,4-dihydroxytoluene **(37)**, 2,5-dihydroxybenzaldehyde **(38)**, 3-hydroxybenzyl alcohol **(39)**, 2,5-dihydroxybenzyl alcohol **(40)**, and 3-hydroxytoluene **(41)**. Compounds **32**, **38**, **40**, and **41** were found to be active against *L. donovani* with IC_{50} values of 6.9, 3.3, 8.5, and 9.2 µM, respectively. Three compounds **30**, **34** and **39** showed moderate activities against *L. donovani* with IC_{50} values of 13.0, 47.3, and 34.0 µM respectively [65].

In another study, three new perylenequinones **(42-44)** were isolated from *Alternaria* sp. (DC401) an endophytic fungus isolated from *Pinus ponderosa*. These compounds were tested for their antileishmanial potential. Compound **43** and **44** showed antileishmanial activity against *L. donovani* with IC_{50} values of 3.14 and 1.4 µg/ml respectively [66].

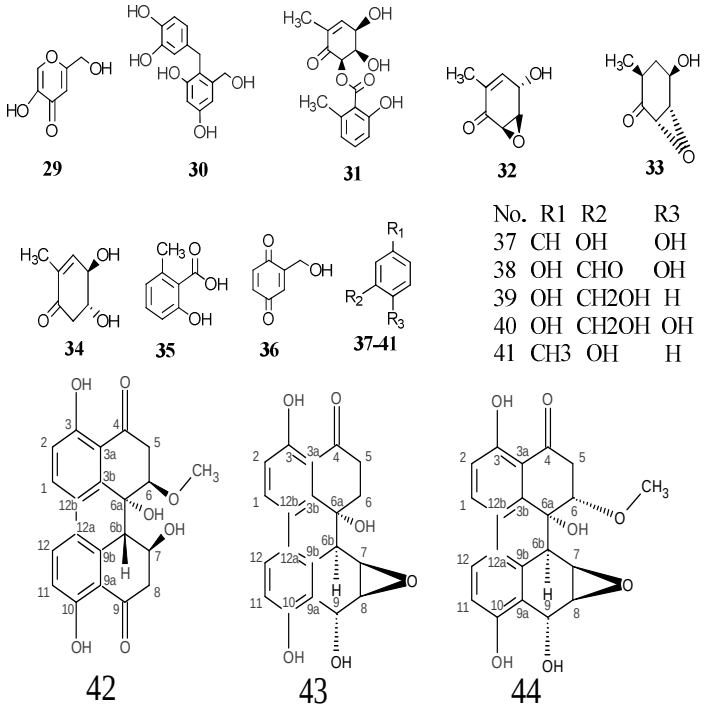


Fig. 4. Antileishmanial compounds from fungi (29-44)

7. CONCLUSION

Leishmaniasis mostly affects people of developing countries living below poverty line and is a life threatening disease. The main cause of leishmaniasis pathogenesis and manifestations is poor sensitizations, malnutrition and unhealthy living environment factors. Increased resistance to the available drug regimen urges the need for the development of new cost effective drugs. This review signified the antileishmanial potential of fungal metabolites for further drug development.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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