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Role of natural plant extracts for potential antileishmanial targets–In-depth review of the molecular mechanism

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ABSTRACT

A group of protozoan parasites known as *Leishmania* species can cause a variety of chronic illnesses, ranging from self-healing lesions to fatal outcomes. Drug-resistant pathogens have become common due to the lack of safe and effective medications, which has sparked the development of new therapeutic interventions, particularly plant-based natural extracts. As a way to avoid chemotherapy's side effects, natural herbal remedies have drawn more attention. In addition to having anti-inflammatory, anticancer, and cosmetic properties, the secondary metabolites of plants, such as phenolic compounds, flavonoids, alkaloids, and terpenes, have many positive effects on our health. Natural metabolites such as naphthoquinone, alkaloids, benzophenones, etc. that have antileishmanial and antiprotozoal activity have been the subject of extensive research. In this review paper, it can be concluded that these natural extracts can be developed into excellent therapeutic agents against Leishmaniasis.

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Introduction

Leishmaniasis is an infectious parasitic disease caused by the genus *Leishmania* protozoa, which belongs to a member of the *Trypanosomatidae* family (1). The parasite infects humans when an insect vector female sand fly of the *Lutzomyia* or *Phlebotomus* genera feeds on human blood (2). Both a mammalian host and an insect vector are required for *Leishmania's* life cycle, which has two morphological forms: promastigote and amastigote (3). The promastigote form is injected into the skin when it bites the host (4). The promastigote was transformed into an amastigote form after being phagocytosed by macrophages. Depending on the species of *Leishmania*, they can spread to different tissues and grow infected cells. When sandflies feed on the blood of an infected host, cells with amastigotes or free amastigotes are consumed. Procyclic promastigotes develop from amastigotes in the insect's gut, multiply, and migrate to the anterior midgut (stomodeal valve), where they undergo metacyclogenesis and differentiate into metacyclic infective forms. Regurgitation is then used to transmit these forms to a new mammalian host during a blood meal (5,6).

There are three clinical manifestations of leishmaniasis: Cutaneous Leishmaniasis (CL), Mucocutaneous Leishmaniasis (MCL), and Visceral Leishmaniasis (VL) (7). Since 350 million people worldwide are at risk of developing one of the disease's clinical forms in 102 different countries, the World Health Organization (WHO) classifies it as a neglected tropical disease (WHO, 2018) (8). *Leishmania major, Leishmania tarentolae, Leishmania donovani, Leishmania tropica, Leishmania infantum,Leishmania panamensis*, and *Leishmania braziliensis* are just a few of the more than 20 *Leishmania* species that can cause disease (9). CL is the most common form of leishmaniasis, and it causes skin sores, usually ulcers, on exposed body parts and leaves behind scars that last a lifetime. VL is the most severe form of leishmaniasis and is potentially fatal if untreated. It is exclusively brought on by species of the *Leishmania donovani* complex (10).

Over the past 60 years, pentavalent antimonials have been the first-choice treatment for this illness, but because of patient toxicity and parasite resistance (11), as an alternative to pentavalent antimonial, second-line medications like Miltefosine, amphotericin B, and paromomycin have been used to treat various forms of leishmaniasis (11). All forms of leishmaniasis are currently treated with drugs, which can have serious side effects on people, such as renal failure, hepatotoxicity, leukopenia, neurotoxicity, cardiotoxicity, etc. However, the search for new medicines becomes more crucial due to their toxicity and inconsistent efficacies in humans (12-14).

The need for new medicines, however, is growing due to their toxicity and inconsistent efficacies in humans.

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Numerous investigations using natural products have revealed encouraging anti-Leishmanial activity (15). Natural herbal remedies have received more attention as a means of avoiding the drawbacks of chemotherapy (16). In search of fresh and effective leishmaniasis treatments, numerous research teams have examined natural products. These nutrients are present in fruits and vegetables and are essential components of a diet that is balanced. They are frequently present in medicinal plants as well, and many of the therapeutic effects of conventional medicines have been attributed to these phytochemicals (17-19). The secondary metabolites of plants, such as phenolic compounds, flavonoids, alkaloids, and terpenes, have a variety of beneficial effects on our health, including anti-inflammatory, anticancer, and cosmetic properties. Additionally, they support the treatment of cancer and tropical parasites like *Leishmania* species (10). The objective of this review is to update and summarise knowledge on the early drug discovery process based on crude extracts, fractions, and isolated compounds obtained from natural products specifically compounds derived from herbs, to treat leishmaniasis (1).

Crude extract – Plant sources

Since ancient times, people have recognized that certain plants have healing properties. Plant-based medications have been used to treat a wide range of pathological conditions. These medications don't isolate their active ingredients; instead, they are used as concentrated plant extracts or a mixture (20). The preferred drug source, particularly for anticancer, antiparasitic, and antimicrobial agents, has been natural products from plants (21,22). The following are a few plants' crude extracts and fractions that are used as medicinal plants to treat leishmaniasis.

A. Embilica officinalis L. [EO] (Figure 1-a) is commonly known as Indian gooseberry or Amla and is widely distributed in tropical and subtropical regions of the world. Besides, it has been used in the Indian traditional system of medicine. The Methanolic crude extract from the fruit of EO have IC_{50} values of 21.67 µg/ml and 34.82 µg/ml in promastigote and amastigote, respectively. EO fruit extract Induced ROS and apoptotic-like cell death, disruption of mitochondrial membrane potential, and generation of superoxide in mitochondria in *Leishmania donovani* (23).

B. Ziziphus spina-christi, also known as jujube and found in various Saudi Arabian regions, is a member of the *Rhamnaceae* family (Figure 1-b). The plant has a variety of medicinal uses and has demonstrated numerous beneficial traits, such as antioxidant, antimicrobial, anti-inflammatory, etc. The plant's antileishmanial properties result in a methanolic extract with an IC_{50} value of 54.6 µg/ml in *Leishmania major* amastigotes. The extract demonstrated antileishmanial mechanisms like increased plasma membrane permeability, decreased parasite load, and stimulation of NO production (24).

C. One of the mangrove species, known as grey or white mangrove, is the *Avicennia marina*, and it can be seen in Iran along the Persian Gulf coast (Figure 1-c). The hydroalcoholic extract of plant leaves may have an inhibitory effect on the promastigote and amastigote forms of *Leishmania tropica*, which have IC_{50} values of 125 µg/ml and 73.19 µg/ml, respectively. The inhibitory effect results in increased ROS production, decreased arginase level, induced apoptosis in promastigote, and decreased parasite growth(25).

D. The Brazilian Atlantic Forest is home to *Piper cabralanum* C. DC. (Figure 1-d), whose leaves extract has activity against *Leishmania amazonensis*. The IC_{50} values for The methanol extract, hexane extract, dichloromethane extract, and ethyl acetate extract having IC_{50} values144.54 µg/ml and 0.51 µg/ml,59.92 µg/ml and 21.08 µg/ml, 64.87 µg/ml and 35.63 µg/ml, >800 µg/ml and 27.19 µg/ml in promastigote and amastigote respectively. The effect of crude extract on parasites is demonstrated to be the induction of a Th-2 type immune response, inhibition of promastigote form growth, inhibition of macrophage internalized amastigote form, and stimulation of NO production (26).

E. Commonly known as stinging nettle or common nettle, *Urtica dioica* is found in Iran, India, Malaysia, and the United States (Figure 1-e). This plant has a variety of beneficial properties, including anticancer, antioxidant, and anti-inflammatory effects. *Leishmania major*'s growth was inhibited by an aqueous crude extract from *U. dioica* leaves with an IC_{50} value of 4500 µg/ml (27).

F. Acacia nilotica, also known as Babul, is an Indian gum arabic tree that belongs to the *Fabaceae* family. The methanolic extract from its bark has an IC_{50} value of 19.6 µg/ml for *Leishmania donovani* promastigote and 77.52 µg/ml for amastigote, respectively. *A. nilotica* bark extract decreased the parasite's intramacrophage activity. This plant is primarily found in the Middle East, Africa, and the Indian subcontinent, and its various parts are used to treat a variety of diseases (28).

G. The *Cassia fistula* plant, also known as the Golden Shower Tree and Indian Laburnum (Figure 1-g), is frequently used in various forms of traditional medicine for the treatment and prevention of disease. The IC_{50} values for promastigote and amastigote of *Leishmania donovani* for the methanolic extract from *C. fistula* leaf are 43.31 µg/ml and 80.76 µg/ml, respectively. Promastigote growth was inhibited, as was the growth of intra-macrophagic amastigote, and methanolic extract caused apoptosis in the parasite (29).

H. White jute, also known as *Corchorus capsularis* L. (Figure 1-h), has been shown to have antiparasitic properties against *Leishmania donovani* and β-sitosterol$_{CCL}$ extract from plant leaves. The 17.7 µg/ml IC_{50} of the extract of β-sitosterol$_{CCL}$ demonstrated that it inhibits the growth of the parasite promastigote form, disturbs the redox balance via intracellular ROS production, increases the storage of lipid bodies, and inhibits trypanothione reductase (30).

I. Sterculia villosa (SVE) is also known as the Sardol in northeast India and the Elephant rope tree (Figure 1-i). Whole plant extracts are also used to treat skin conditions. The *Leishmania donovani* promastigote is treated with the SVE bark methanol extract, which has an IC_{50} value of 17.5 µg/ml. During oxidative stress, the SVE bark extract lowers cell viability and the induction of lipid peroxidation(31).

J. The *Caryocar coriaceum* Wittm species (Figure 1-j) is a member of the *Caryocaraceae* family that is found throughout South and Central America as a Cerrado vegetation type. The pulp and peel extract from the *Caryocar* fruit have a variety of properties, including anti-inflammatory, antimicrobial, and antineoplastic effects. Fruit pulp

and peel extract had an impact on *Leishmania amazonesis* promastigote and amastigote, which displayed decreased NO and ROS production, decreased mitochondrial membrane potential, induced phospholipid exposure, damaged plasma membrane, and increased expression of the proteins Nrf2, HO-1, and ferritin. (8).

Diospyros gracilescens, a member of the *Ebenaceae* family, is primarily found in the West and Center regions of Cameroon (Figure 1-k). *Diospyros* species are frequently used in traditional African medicine, primarily in the treatment of leprosy. Trunk crude extract has an IC_{50} value of 5.84 µg/ml and 35.69 µg/ml, while hexane fraction has an IC_{50} value of 0.79 µg/ml and 8.06 µg/ml, dichloromethane extract has an IC_{50} value of 1.63 µg/ml and 10.97 µg/ml, ethyl acetate has an IC_{50} value of 2.36 µg/ml and 16.05 µg/ml and n-butanol fraction has an IC_{50} value of 1.11µg/ml and 22.08µg/ml in promastigote and amastigote respectively. The promastigote and amastigote stages of *Leishmania donovani* exhibit the effects of moderate inhibition. (32).

Important plant natural/crude extract- treatment in Leishmaniasis

The early stages of drug discovery for leishmaniasis were based on isolated, crude extracts, fractions, and natural product-derived compounds, particularly those derived from herbs and a plentiful source of substances with anti-leishmanial activity found in natural products. Some of the secondary metabolites of plant extract are investigated in the creation of novel leishmaniasis medications using natural products as inhibitors.

Shakeri and co-workers, (33) studied that a class of secondary metabolites known as 7-prenyloxycoumarins is primarily found in plants from the *Rutaceae* and *Apiaceae* families. Some examples of prenylated coumarins include auraptene, umbelliprenin (UM), and 7-isopentenyloxy-coumarin. UM can be found in many edible plant species, especially in *Ferula* species, as well as celery, coriander, angelica, lemon, and other citrus fruits. Despite being isolated for more than 50 years, researchers have only recently begun to examine UM's biological activities. In addition to its biological effects on cancer, this natural compound has been found to have anti-inflammatory, antioxidant, and antileishmanial properties.

Caryocar coriaceum Wittm fruit extract was used as a *Leishmania* inhibitor by the Tomiotto-Pellissierresearch group (8). *Caryocar* is a genus of plants rich in phenolic compounds, and leaf extracts from this plant have been used to treat leishmaniasis. As a result, the fruit's pulp and peel extract affected promastigote and amastigote forms of *Leishmania amazonensis,* and both extracts had an anti-promastigote effect. This effect was caused by the induction of an apoptosis-like process, which produced reactive oxygen species (ROS), damaged the mitochondria and plasma membrane, and exposed phosphatidylserine. The treatment decreased the infection of these cells even though the fruit extracts did not affect the viability of macrophages. The extracts then demonstrated antioxidant properties by lowering NO, ROS, and MDA levels in the in-vitro infection context. Additionally, both extracts increased the expression of Nrf2/HO-1/Ferritin and increased the total iron-bound in infected macrophages, which led to a reduction in the amount of iron that was available for

Figure 1. Different plants part used as a natural extract in the treatment of leishmaniasis.

Leishmania amazonensis replication. The three flavonoids present in the *Caryocar coriaceum* extracts can act as synergistic inhibitors of *Leishmania* proteins and compete for the active site, according to in silico molecular modeling experiments. Due to its stronger interaction binding strength, rutin is also preferred at the active site.

Bioactivity-guided fractionation was applied to the *Saururus cernuus* leaf extract in MeOH by Brito JR *et al.* (34). Purified compounds were tested *in vitro* for anti-*Leishmania amazonensis* activity against promastigote and amastigote forms.MeOH extract from *Saururus cernuus* leaves was fractionated according to bioactivity, and yielded two related tetrahydrofuran dineolignans: threo, threo-manassantin A (1), and threo, erythro-manassantin A. (2). The EC_{50} values for compounds 1 and 2 were higher than those found for the positive control miltefosine (EC_{50} of 28.7 ± 3.5 µM) against promastigotes (EC_{50} of 35.4 ± 7.7 and 17.6 ± 4.2 µM, respectively) and amastigotes (EC_{50} of 20.4 ± 1.9 and 16.0 ± 1.1 µM, respectively). For both substances, less cytotoxicity toward host cells was seen. Additionally, electron microscopy revealed ultrastructural modifications in promastigotes that changed their structural morphology. These substances also altered the morphology and physiology of the plasmatic membrane of *Leishmania amazonensis*. The dineolignans 1 and 2 could be used as a scaffold to create new, effective leishmaniasis drug candidates.

Maamri *et al.* studied (35) that terpenoids and polyphenols are effective antiparasitic substances found in nature. The binding affinity of two key phytochemicals, Masticadienonic acid, and 3-Methoxycarpachromene, towards the trypanothione reductase as target drugs, responsible for

the defense mechanism against oxidative stress and virulence of these parasites, a new in silico analysis was carried out using molecular docking and the Autodock vina program. The molecular docking results show an elective binding profile for ligands inside the active site of this important enzyme, which is an exciting and novel positive finding. In the ADMET study, 3-Methoxycarpachromene has the highest likelihood of being absorbed by the human intestine. This research demonstrates the potential value of 3-Methoxycarpachromene and Masticadienonic Acid in the search for new drugs, particularly for the treatment of leishmaniasis.

Leishmaniasis is difficult to control and eradicate due to the high toxicity of current *anti-Leishmania* parasite medications and their numerous side effects. Natural products provide an intriguing and diverse chemical environment for the discovery of novel antileishmanial medications. An internal database of 360 kauranes (tetracyclic diterpenes) was created to help find new treatment options. Herrera-Acevedo *et al.,*(36) used a combined ligand- and structure-based virtual screening (VS) approach to find potential inhibitors of *Leishmania major* (Lm) pteridine reductase I. (PTR1). The LmPTR1 enzyme inhibition assay was used to test the most effective kauranesto confirm the viability of the VS approach. When the random forest (RF) model was used to analyze the half-maximal inhibitory concentration (IC_{50}) values of a few different bioactive compounds, the results showed that 2β-hydroxy-menth-6-en-5β-ylent-kaurenoate (135) and 3α-cinnamoyloxy-ent-kaur-16-en-19-oic acid (302) were both below the 10 μM threshold. Similar to substance 302, 3α-*p*-coumaroyloxy-ent-kaur-16-en-19-oic acid (302a) was also created and demonstrated the greatest anti-LmPTR1 activity. Finally, to investigate the targeting potential of these kauranes to other species-dependent variants of this enzyme, molecular docking calculations, and molecular dynamics simulations were carried out for the VS-selected, most-active kauranes within the active sites of PTR1 hybrid models created from three *Leishmania* species known to cause cutaneous leishmaniasis in the new world i.e*., Leishmania braziliensis, Leishmania panamensis, and Leishmania amazonensis*.

Njanpa CA and the group (32)studied the anti-leishmanial natural products from *Diospyros gracilescen*s L. (*Ebenaceae*). The plant extracts were made by macerating them in a solution of H_2O: EtOH (30:70, v/v) and then fractionating them with the aid of biological cues. In vitro tests using triplicate samples of plant extracts, fractions, and isolated compounds against *Leishmania donovani* promastigotes and amastigotes were conducted. Using the resazurin colorimetric assay, the antileishmanial potency and cytotoxicity on RAW 264.7 cells were assessed. Based on in-depth spectroscopic analyses, including 1D and 2D NMR, HR-ESI-MS, and a comparison of their results with those reported in the literature, the structures of all compounds were clarified. The most effective antileishmanial activity was demonstrated by the hydroethanolic crude extract of plant trunk (IC_{50} = 5.84 μg/mL) and further fractionation of this extract produced seven compounds with moderate potency (IC_{50} = 13.69-241.71 μM) against *Leishmania donovani* and four fractions, of which the hexane fraction had the highest activity (IC_{50} = 0.79 g/mL). With IC_{50} values of 241.71 μM and 120 μM, respectively, compound 1-deoxyinositol (7) inhibited the promastigote and amastigote forms of *Leishmania donovani*. It also demonstrated the highest selectivity against *Leishmania donovani* promastigotes (SI > 5.04). The promising hexane fraction significantly slowed parasite growth at various concentrations, but over a 120-hour exposure period, there was no sign of a tidal effect and the hydroethanolic extract from the trunk of *Diospyros gracilescens* and the resulting hexane fraction has a very strong inhibitory effect on parasite promastigotes and amastigotes of *Leishmania donovani* that are grown in culture.

The study of trypanothione reductase of *Leishmania mexicana was* done by Matadamas-Martínez *et al.* (37). The survival of the parasite depends on *Leishmania mexicana* trypanothione reductase (LmTR), an NADPH-dependent flavoprotein oxidoreductase crucial to thiol metabolism. This enzyme is a desirable target for the development of novel anti-*Leishmania* drugs due to its absence in the mammalian host. In this study, a tridimensional model of LmTR was built, and 20 molecules from the ZINC database were molecularly docked. Five substances (ZINC04684558, ZINC09642432, ZINC12151998, ZINC14970552, and ZINC11841871) were chosen and tested against recombinant LmTR (rLmTR) and *Leishmania mexicana* promastigote. The results ranged from docking scores of -10.27 kcal/mol to -5.29 kcal/mol. The complexes of compounds chosen by the LmTR were successfully simulated using molecular dynamics. The rLmTR activity was inhibited by the five chosen substances in the 32.9 to 40.1% range. It is described how specific compounds bind to LmTR by forming various hydrogen bonds with various residues of the molecule monomers A and B. Out of all the compounds chosen, compound ZINC12151998 had the highest leishmanicidal activity (IC_{50} = 58 μM), inhibited 32.9% of the enzyme activity (100 μM), and had a docking score of -10.27 kcal/mol. Although it had a higher half-maximal cytotoxicity concentration (CC_{50} = 53 μM) than the other four compounds, it was less cytotoxic than glucantime and more active than amphotericin B. To find new anti-*Leishmania* drugs that are more effective and less cytotoxic, the compound ZINC12151998 offers a promising starting point for a hit-to-lead process.

The cathepsins from *Leishmania* spp. are promising molecular targets for treating leishmaniasis. Inhibit the recombinant cathepsin L of *Leishmania mexicanam* was studied by de Sousa LR and group (38). *Leishmania mexicana* cathepsin L is crucial to the parasite life cycle and a key component of its virulence in mammals. To create inhibitors against the *Leishmania mexicana* cathepsin L-like rCPB2.8, natural products that have been demonstrated to exhibit antileishmanial activity were screened. Among them, are agathisflavone (1), tetrahydro-robusta flavone (2), 3-oxo-urs-12-en-28-oic acid (3), and quercetin (4) demonstrated significant inhibitory activity on rCPB2.8 with IC_{50} values ranging from 0.43 to 18.03 μM. Compounds 1-3 had Ki values in the low micromolar range (Ki = 0.14-1.26 μM), and the mechanisms of inhibition for these compounds were identified. While biflavanone 2 is an uncompetitive inhibitor, biflavone 1 and triterpene 3 are partially noncompetitive inhibitors. The leishmanicidal natural products' established mechanisms of action offer a fresh perspective on the search for *Leishmania*-fighting medications.

Protozoan infections of the genus *Leishmania* are a significant global health issue, with high endemicity in

developing nations. The pentavalent antimonials, which are toxic to the heart and kidneys, are the preferred medications for the treatment of leishmaniasis. Pereira IO*et al.,*(39) studied that *Garcinia braziliensis* extract was used as a *Leishmania* protease inhibitor. To check the ability of different extracts like hexanoic, ethyl-acetate, ethanolic, and fukugetin, a bioflavonoid purified from the ethyl-acetate extract of the pericarp of the fruit of *Garcinia brasiliensis*, a tree native to Brazilian forests, to inhibit the *Leishmania* protease in vitro. Nuclear magnetic resonance (NMR), mass spectrometry, ultraviolet, and infrared spectral analyses were used to characterize the isolated compound. The ethyl-acetate extract and the substance fukugetin demonstrated significant activity as inhibitors of *Leishmania*'s proteases, with a mean (± SD) IC_{50} values of 15.0 ± 1.3 µg/mL and 3.2 ± 0.5 µM/mL and respectively, defining a bio guided assay. Furthermore, neither the promastigote nor amastigote forms of *Leishmania amazonensis* nor mammalian cells were responsive to this isolated compound. These findings imply that fukugetin is an effective protease inhibitor of *Leishmania amazonensis* and that it is not toxic to mammalian or *Leishmania* cells in vitro. This research creates novelleishmanicidal drugs derived from natural sources that specifically target the parasite's proteases.

Patel B and her co-workers (40) studied that Ile209 of *Leishmania donovani* xanthine phosphoribosyl transferase plays a key role in determining its purine base specificity. The purine salvaging enzymes of *Leishmania donovani*, Xanthine phosphoribosyltransferase (XPRT), and Hypoxanthine-Guanine phospho-ribosyltransferase (HGPRT), have different 6-oxopurine specificities. In contrast to LdHGPRT, which only phosphoribosylates hypoxanthine and guanine, LdXPRT preferentially phosphoribosylates xanthine, hypoxanthine, and guanine. LdXPRT was employed as a model to comprehend these purine base specificities. The conserved residue Ile209 in HGPRTs, when changed to V, decreased the affinity of LdXPRTfor xanthine and caused it to resemble HGXPRT. The Y208F mutation in the active site demonstrated that purine base specificity is not imparted by aromatic residue interactions with the purine ring, which are restricted to pi-pi binding forces. Enzyme activity was impacted by removing LdXPRT's distinctive motif (L55-Y82). The research identified I209 as a crucial residue influencing LdXPRT's 6-oxopurine specificity.

Diphenyl propanoids, or lignans, have a wide range of biological activities. Saha S and his group (41) studied the anti-leishmanial properties of lyoniside and saracoside, two lignan glycosides. These substances stabilize the LdTopIB-mediated cleavage complex formation both in vitro and in *Leishmania* promastigotes, inhibit the religation of the cleaved strand, and inhibit the catalytic activities of *Leishmania donovani* topoisomerase IB (LdTopIB) in a non-competitive manner. These two substances can interact with the free enzyme LdTopIB in addition to poisoning it. Additionally, we have demonstrated that promastigotes and intracellular amastigotes are cytotoxic to lyoniside and saracoside. The parasite experiences apoptosis-like cell death as a result of the protein-DNA complex formation, which causes double-strand breaks in the parasite's DNA. These two compounds are potential antileishmanial candidates due to their cytotoxicity toward the AG83 strain, which is sensitive to sodium antimony gluconate (SAG), as well as their capacity to eradicate the GE1 strain, which is SAG resistant. In addition to killing *Leishmania donovani* amastigotes inside macrophages in a lab setting, lyoniside and saracoside also showed potent antileishmanial efficacies in the leishmaniasis model in BALB/c mice. Nitric oxide and reactive oxygen species are produced during treatment with these lignan/ glycosides, which lead to nearly complete clearance of the liver and splenic parasite burden.These substances had a poor cytotoxic effect on cultured murine peritoneal macrophages that were not infected up to 100 mM concentrations and did not inhibit human topoisomerase IB up to 200 mM concentrations. When considered collectively, it can be said that these compounds have the potential to become excellent therapeutic agents against the deadly disease leishmaniasis.

The study of *Ferula szowitsiana* for the antileishmanial activity of 7-prenyloxycoumarins against promastigotes was done by Iranshahi and team (42).From the roots of *Ferula szowitsiana*, two new sesquiterpene coumarins, designated szowitsiacoumarin A and B (1) and szowitsiacoumarin B (2), as well as a phenylpropanoid derivative called 2-epihelmanticine (3) and nine previously identified compounds, including auraptene (4), umbelliprenin (5), galbanic acid (6), methyl galbanate, etc. Numerous spectroscopic techniques, such as HR-MALDI-MS analysis and 1D-(1H and 13C) and 2D-NMR experiments (DQF-COSY, HSQC, HMBC, and ROESY), were used to elucidate the structures of these compounds. Due to incomplete prior knowledge of the configuration of 2-epihelmanticine, a relative configurational analysis of its four stereocenters was conducted using the recently published J-based method. Galbanic acid (6), as well as auraptene (4) and umbelliprenin (5), two prenylated coumarins, and the Me(2) CO extract of *Ferula szowitsiana* (*Apiaceae*) roots have been tested for their inhibitory effects on promastigotes of *Leishmania major* and after a 48-hour incubation period, umbelliprenin and auraptene both exhibited significant activities, with IC_{50} values of 4.9 µg/ml (13.3 µM) and 5.1 µg/ml (17.1 µM), respectively.

Four Ethiopian plants were chosen, and their antileishmanial efficacy against two Leishmanial parasites was tested by Nigatu *et al*., (43). The plant materials were macerated in methanol (80%). Using the Alamar Blue assay, the crude extracts were then tested for in-vitro antipromastigote activity against promastigotes and axenically cultured amastigotes of clinical isolates of *Leishmania aethiopica* and *Leishmania donovani*. Cell viability was then determined fluorometrically. Amphotericin B was used as a positive control, and 1% DMSO and the media were used as a negative control. The extracts also underwent a preliminary phytochemical analysis. *Ferula communis* and *Otostegia integrifolia* demonstrated the best activity among the four plant extracts, with respective IC50 values of 11.38±0.55 and 13.03±0.87 µg/mL against *L. aethiopica*. The IC50 values for the same plant extracts against *L. donovani*, however, were lower at 23.41±2.32 and 17.24±1.29 µg/mL, respectively. *O. integrifolia* had the strongest effects on *L. aethiopica* and *L. donovani* amastigotes (IC50: 16.84±0.65 and 14.55±0.38 µg/mL, respectively). The second-highest growth inhibitor of *L. aethiopica* and *L. donovani* amastigotes was *F. communis*, with IC50 values of 14.32±0.54 and 31.12±0.19 µg/mL, respectively. The extracts contained phenol, flavonoids,

tannins, saponins, terpenoids, and alkaloids, according to the phytochemical analysis.The results of this study show that *F. communis* and *O. integrifolia* crude extracts demonstrated promising antileishmanial activity against *L. aethiopica* and *L. donovani*, which may be attributed to the presence of various secondary metabolites.

Conclusion

Even though leishmaniases are a group of diseases with drug-based treatments, they continue to pose a significant challenge to research fields because the arsenal of drugs currently in use is limited in comparison to the number of species that cause these diseases. For creative drug development strategies, a paradigm shift is necessary due to the low success rate of drug discovery. Innovative drug development begins with natural remedies as a source of inspiration for the efficient treatment of disease conditions. Innovative natural product drug discovery has the potential to boost the success rate of new therapeutic moieties in this era of developing scientific technology. Discovering new drugs from natural products will play a significant role in addressing global health issues and advancing the goals of sustainable development for health.

In this review, the extraction from different plant parts and act on different *Leishmania* spp. The inhibitory effect of plant extract was shown on the promastigote and amastigote forms of the *Leishmania* parasite. However, no information has yet to be discovered regarding the potential modes of action of these extracts on the extracellular and intracellular parasites. Regarding the mode of action of these natural products, several substances have the potential to change the mitochondrial membrane potential, elevating intracellular ROS levels, lowering ATP concentration, and inducing programmed cell death. Some natural substances were seen to lower the parasite load and function as immunomodulators in vivo.This study reports comprehensive and detailed information aiming antileishmanial activity of natural plant extract for the treatment of leishmania disease. Natural extracts reported in this paper are promising drug candidates for the development of phyto drugs against the deadly disease leishmaniasis.

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Conflicts of Interest

There is no conflict of interest.

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