Brief Report

Exploration of Antileishmanial Compounds Derived from Natural Sources

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Abstract:Leishmaniasis is considered one of the different neglected tropical diseases by the World Health Organization. Over the past few decades to tackle leishmaniasis, effective and novel drugs have progressed. But few are expensive some other medication shows poor effects and few drugs with long treatment lead to cause resistance. But it is very important to start a better medication against *leishmania* so researchers came in front of the utilization of natural products which are considered a better option. Finding active compounds in medicinal plants is another alternative to currently accessible medications. **Materials and methods**:This study examined and reported the far more potential natural products used to treat disease caused by Leishmania spp. Leishmaniasis, plant metabolites, in vivo, in vitro, and treatment against leishmaniasis have been used as search terms in the Google Scholar, PubMed, and Science Direct databases, and only papers published between 2015 and 2021 have been chosen for further analysis.**Results:** The use of novel natural compounds with leishmanicidal action as well as the leishmanicidal activity of natural compounds against promastigote, axenic, and intracellular amastigote forms were included in roughly 20 research papers that were reviewed. **Conclusion:** Due to their capacity to selectively target parasites without harming host cell viability, herbal plants are a possible source of new anti-leishmanial medication. Future leishmaniasis treatments will draw on the isolated compounds as a source, completing those already offered in clinics.

Keywords: Leishmaniasis; drugs; parasites; herbal plants; treatments

INTRODUCTION

Leishmania is a Eukaryotic protozoan and dimorphic intracellular parasite from the family Trypanosomatidae[1]. *Leishmania* is responsible to cause an infectious parasitic disease called leishmaniasis. From the bite of infected female phlebotomine sand flies, *Leishmania* is transmitted and over 20 *Leishmania* species are infective to humans. *Leishmania's* life cycle consists of two distinct morphological forms in the mammalian host and the sand fly vector: intracellular amastigotes and motile(spindle shaped flagellated) promastigotes[2] The female phlebotomine sand fly vector consumes amastigotes together with the blood meal that is taken from the individual infected vertebrate host during the blood meal. Amastigotes are then transformed into promastigotes, which begin reproducing in the sand fly's gut and are considered as procyclic. Promastigotes that have undergone metacyclogenesis from their procyclic state are now extremely infectious, motile, and moving toward the sandfly's mouth and buccal cavity. These are known as metacyclic promastigotes. The mouth parts release these infectious promastigotes, which are then deposited into the wound created by the subsequent blood feeding. These promastigotes are phagocytosed by neutrophils and macrophages, which are referred to as the macro-

 doi:10.20944/preprints202211.0185.v1

phages, as part of the individual's host immunological response. However, these promastigotes continue to exist inside the macrophages and quickly transform into amastigotes, which are immobile and capable of reproducing in the acidic environment of the phagolysosome. At this point, the macrophages are lysed, and the amastigotes are then prepared to infect additional macrophages[3]. Refer figure 1 for diagrammatical understanding.

Table 1: Different *Leishmania* species that are infective to humans, along with their main clinical infection, reservoir, vector and the geographical distribution[4][5]

***Leishmania* species**	**Main clinical Infection**	**Reservoir**	**Vector**	**Main geographical distribution**
L. infantum	VL, CL, ML	Rodents, dogs, foxes, jackals	*P. pcrniciosufi*, *P. arias*, *Lutzomyia longipalpis*	Europe, north Africa, Central America, South America
L. donovani	VL, PKDL	Humans, rodents	*Phlebotomus argentipes*, *P. orinntalis*, *P. martini*	Africa, central Asia, southeast Asia
L. amazonensis	CL, DCL	Forest rodents	*L. flaviscutellata*	South America
L. Mexicana	CL, DCL, MCL	Forest rodents	*L. olmeca*	Central America, Mexico, USA
L. aethiopica	CL, DCL, MCL	Hyraxes	*P. longipes*, *P. pedifer*	Ethiopia, Kenya
L. tropica	CL, ML, VL	Humans	*P. sergenti*	Central Asia, middle east, parts of north Africa, southeast Asia
L. major	CL, ML	Rodents, gerbils	*P. papatasi*, *P. duboscqi*	Central Asia, north Africa, middle east, East Africa
L. guyanemis	CL, MCL	Sloths arboreal anteaters	*L. umbratilis*	South America
L. peruviana	MCL	Dogs	*L. verrucarurn*, *L. pvmenis*	Peru
L. panamensis	CL, MCL	Sloths	*L. trapidoictal*	Northern South America and southern Central America

VL: Visceral leishmaniasis; CL: Cutaneous leishmaniasis; MCL: Mucocutaneous leishmaniasis; PKDL: Post-kala azar dermal leishmaniasis; P: *Phlebotomus*; ML: Mucosal leishmaniasis; L: *Lutzomyia*; DCL: Diffuse cutaneous leishmaniasis.

Visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), and mucocutaneous leishmaniasis (MCL) are the 3 different types of leishmaniasis[6]. Among these three the most dangerous form of the disease is visceral leishmaniasis (kala-azar) as it prefers to live in the liver, spleen, and bone marrow. *L. donovani*, *L. infantum*, *L. chagasi*, and rarely *L. tropica* proliferating in the reticuloendothelial system is the primary cause of VL[7][8][9]. If untreated, visceral leishmaniasis typically results in death[10]. The most prevalent one is cutaneous leishmaniasis, a chronic skin ulcer that develops at the site of a sandfly bite and might take months to heal. Leishmania species including L. major, L. tropica, and L. aethiopica in the old world and *L. mexicana, L. venezuelensis, L. amazonensis, L. braziliensis, L. panamensis, L. guyanensis*, and *L. peruviana* are responsible for the most common form of Cutaneous leishmaniasis[11][9][12][13]. Cutaneous leishmaniasis (CL), a term used to describe skin clinical symptoms brought on by several species of obligate intramonocular phagocytic cells. Skin ulcers caused by protozoan parasites typically heal spontaneously but leave an scar[14]. Diffuse cutaneous (DCL), recidivans (RL), and post kala-azar dermal (PKDL) leishmaniasis are examples of cutaneous manifestations[10]. Mucocutaneous leishmaniasis first results in lesions that resemble comparable skin ulcers that heal but later return, typically in the mucous tissue of the nose and mouth[10]. Each year, there are almost 2 million instances of cutaneous leishmaniasis, 50000 cases of visceral leishmaniasis, and 60000 leishmaniasis-related deaths[15]. According to WHO In September 2021, 55 VL-endemic countries which are 70%, and 56 CL-endemic countries which are 63% have submitted data to the WHO Global Leishmaniasis Programme for the year 2020..When it comes to VL 87% of global cases are reported from India, Eritrea, Brazil, Ethiopia, Sudan, Somalia, Kenya and 80% of CL are from Pakistan, Algeria, Colombia, Iraq, Brazil, Afghanistan, and the Syrian Arab Republic and reported more than 5000 CL cases in 2020[6].

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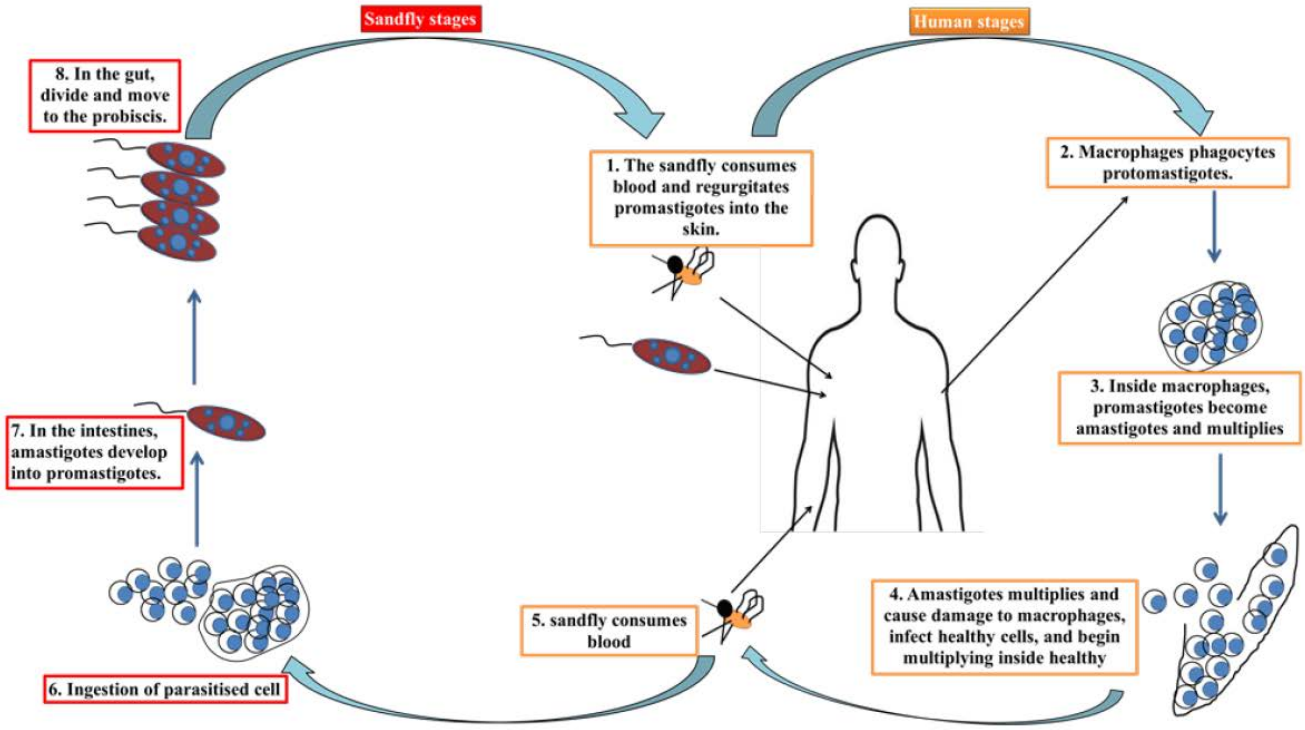

Figure 1: life cycle of Leishmania

For the treatment of leishmaniasis as the drugs of choice Traditionally pentavalent antimonials (sodium stibogluconate and meglumine antimoniate) have been used[16][17]. Due to drug resistance, it is no longer recommended[18]. Shorter regimens and preventing resistance have been the focus of recent clinical studies. The Asian elimination initiative approved miltefosine (first oral drug) as the first-line treatment in 2005 and liposomal amphotericin B emerged as an alternative treatment, but both these drugs are expensive, but there is evidence of decreasing efficacy in both visceral leishmaniasis and post kala-azar cutaneous leishmaniasis after a decade of therapy[19].

The most successful source of possible therapeutic leads has been natural compounds (secondary metabolites)[20]. Natural products continue to provide exceptional structural variety compared to typical combinatorial chemistry, allowing for the discovery of mostly new low molecular weight lead compounds[21]. In several cases, natural products with their secondary metabolites have opened new avenues for combating neglected tropical diseases[22]. Numerous studies have suggested that natural herbal antiparasitic herbs, particularly in the case of CL, are unlikely to cause side effects. Traditional medicine has documented the treatment of cutaneous leishmaniasis in a variety of modalities, including oral medicines, ointment, and poultice. Herbs have also been employed in this treatment for their topical and systemic effects[23].

MATERIALS AND METHODS

This paper is focused on the most recent updates on the novel drug development process for treating leishmaniasis using different natural products which are crude extracts, fractions, and isolated compounds, particularly herbal-derived compounds. Through the help of Google Scholar, PubMed, and Science direct databases the information has been gathered where the keywords; leishmaniasis, natural products, *in vivo*, *in vitro*, and treatment against leishmaniasis, have been used and only those papers have been selected which were published from 2015 to 2021. Few research papers have been considered which show a prominent effect over *leishmania* with different IC50 and IC100 (50% and 100% inhibitory concentration). Articles and research papers examining the

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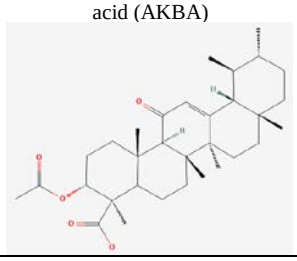
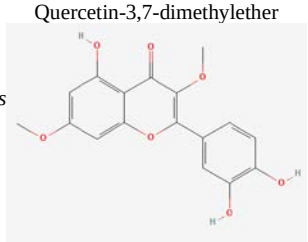
leishmanicidal activity of natural compounds against promastigote, axenic, and intracellular amastigote forms were the selection criteria. The utilisation of innovative natural compounds with leishmanicidal activity against promastigotes of Leishmania spp. was discussed in a few articles.

Anti-leishmanial compounds

Herbal plants are a viable option for new anti-leishmanial drugs by showing selective activity on parasites without compromising host cell viability[24]. Under this category, *Urtica dioica* is one among them, a member of the Urticaceae family and it can be employed as a novel compound found naturally against *Leishmania*, which can be found in Europe and Asia. To determine the anti-leishmanial (**L. major**) activity of any compound inhibitory (IC50), cytotoxic (CC50), and effective concentrations (EC50) are measured, similarly the anti-leishmanial activity of *U. dioica* extract from leaves was determined to suppressing promastigotes and amastigotes growth and the best concentrations were 3500 and 6000 µg/ml[25].

Table 2: Leishmanicidal activities of different extractions of plant parts

s.no	Class	Plant	Part	Secondary metabolite	*Leishmania spp*	Values	References
1	Crude extracts and fractions	*Urtica dioica L.*	Leaves	-	**L. major**	macrophages CC_{50}: 20,000 µg/ml promastigotes IC_{50} 3500µg/ml amastigotes IC_{50} 6000 µg/ml	[25]
2	Powdered *Boswellia serrata* resin extract	*Boswellia serrata*	Resin	3-O-acetyl-11-keto-β-boswellic acid (AKBA) 	*L. donovani*	axenic amastigotes IC_{50}: 0.88 µM	[26]
3	Crude extracts and fractions	*Croton gratissimus* and *Cuscuta hyalina*	Fruits of *Croton gratissimus* Stems of *Cuscuta hyalina*	Quercetin-3,7-dimethylether 	*L. donovani*	IC_{50}: 4.5 ± 0.3 µM	[27]
4	Essential oil extraction	*Levandula officinalis*	Flowering branches and Fresh lavender	-	*L. major*	leishmaniocidal impact was likewise seen in concentrations of 10%	[28]
5	Essential oil extraction	*Medicago lupulina*	leaves	-	*L. major*	Promastigotes IC_{50}: 250 µg/ml	[29]
6	Crude extracts and fractions	*Portulaca oleracea*	Stems and leaves	-	*L. major*	herb's leaves Promastigotes IC_{50}: 360 µg/ml stems essence Promastigotes IC_{50}: 680 µg/ml	[30]
7	Crude extracts and fractions	*Crataegus microphylla*	Leaves	-	*L. major*	Promastigotes IC_{50}: 1094 µg /ml	[31]
8	Crude extracts and fractions	*Phoenix dactylifera L*	Fruit and Pit Extracts	-	*L. major*	Pit exract promastigotes IC_{50}: 23 mg/mL Fruit promastigotes IC_{50}: 500 mg/mL	[32]
9	Fruit extract	*Crataegus aronia*	Fruit	-	*L. major*	Amastigote IC_{50}: 49.13 µg/ml	[33]
10	Essential oil extraction	*Tetradenia riparia*	Leaves	6,7-dehydroroyleanone	*L. amazonensis*	TrEO LD_{50}: 0.8 mg/mL TrROY LD_{50}: 3 mg/mL	[34]

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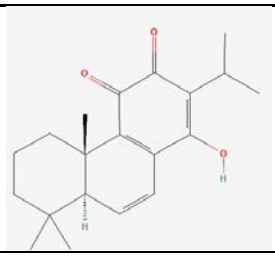
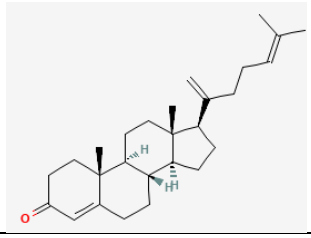
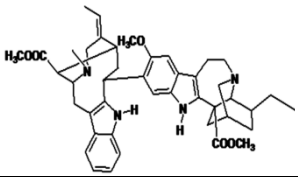
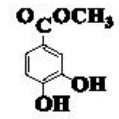
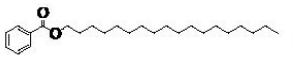
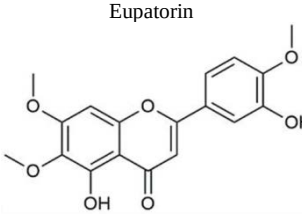
							
				Pentalinonsterol			
11	A novel sterol Synthesis	*Pentalinon andrieuxii*	Root		*L. donovani*	2.5 mg/kg liposome-encapsulated showed significant reduction in parasite in mice	[35]
				Voacamine			
12	Isolation of dimeric indole alkaloid	*Tabernaemontana coronaria*	Stem bark		*L. donovani* *L. amazonensis*	IC_{50}: 14.702± 0.101 µM	[36]
13	Crude extracts and fractions	*T. oliverianum, A. javanica and C. amblyocarpa*	Dry plant powder	-	*L. major*	IC_{50} of *T. oliverianum*: 7.8 µg/mL, IC_{50} of *A. javanica* 13.7 µg/mL and IC_{50} of *C. amblyocarpa* 21.5 µg/mL	[37]
14	Benzoic acid derivatives	*Ifloga spicata*	Dried whlole plant	methyl 3,4-dihydroxybenzoate  octadecyl benzoate 	*L. tropica*	LD_{50} of 3,4-dihydroxybenzoate 10.40±0.09 µg/Ml and LD_{50} of octadecyl benzoate 14.11±0.11µg/mL	[38]
15	Essential Oil extraction	*Achillea santolina*	Aerial parts of **A. santolina** plant	-	*L. infantum*	After 72 h efficiency concentration was 1000 mL/mg	[39]
16	Extraction of the Essential Oil	*Endlicheria bracteolata*	leaves	-	*L. amazonensis*	IC_{50} for Promastigote 7.945±1.285µg/mL(24hrs) IC_{50} for Promastigote 6.186±1.226µg/mL (48 h) IC_{50} for intracellular amastigote 3.546±1.184µg/mL (24 h)	[40]
17	Leaf extract	*Corchorus capsularis L*	Leaf	-	*L. donovani*	IC_{50} 79.00 ± 0.3 µg/ml for promastigotes	[41]
18	Crude extracts and fractions	*Aerva sanguinolenta*	Whole plant	-	*L. donovani*	IC_{50} values was between 0.1 ug/ml to 1 ug/ml	[42]
				Eupatorin			
19	Flavonoid extraction and fractions	*Baccharis trimera*	Aerial parts		*L. amazonensis*	IC_{50} value was 1.6 ± 0.1 µM	[43]
20	Crude extracts and fractions	*Euphorbia cactus, Euphorbia ammak*	Stems and leaves	-	*Leishmania spp*	Euphorbia cactus Ehrenb, Euphorbia ammak Forssk has IC_{50} value <15.6 µg/ml	[14]

In other research studies[26] 3-O-acetyl-11-keto-β-boswellic acid (AKBA) is extracted from the *Boswellia serrata* resin which showed antileishmanial activity. The activity was

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seen against *L. donovani.* Boswellia serrata Roxb is a deciduous tree with a greyish papery bark that produces a fragrant yellowish oleo-gum-resin known as Indian frankincense or Olibanum, which has anti-inflammatory properties[44]. 3-O-acetyl-11-keto-boswellic acid (AKBA) had an IC50 of 0.88 μM against axenic amastigotes but was ineffective against intracellular amastigotes in murine macrophages[26].

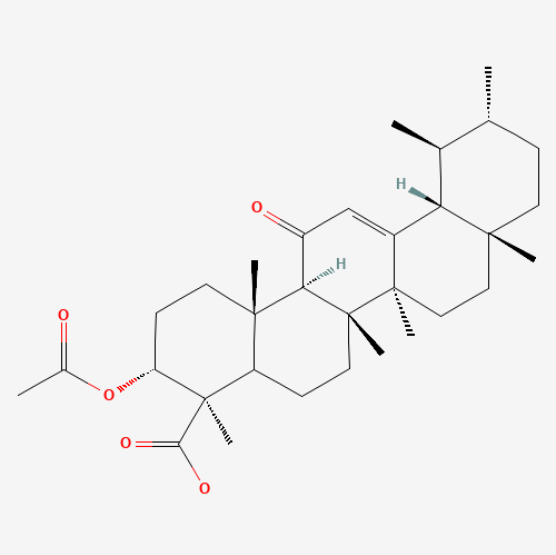

Figure 2: 3-O-acetyl-11-keto-β-boswellic acid (AKBA).

In 2020 Mahmoud et. al[27] used Sudanese medicinal plants for antiprotozoal activity, to this Powdered materials of *C. gratissimus* fruits and *C. hyalina* stems were used. Flavonoids, terpenoids, and essential oil were the main secondary metabolites for these plants[45][46]. Different Compounds were Isolated and their Structure was Elucidated where The chloroform extracts were found to be effective against *Leishmania donovani.* The most active form of flavonoid found is Quercetin3,7-dimethyl ether against *Leishmania donovani* with IC50 of 4.5 ± 0.3 μM.

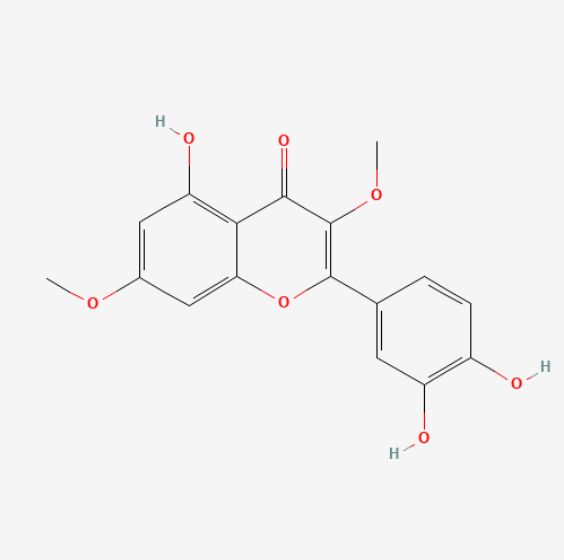

Figure 3: Quercetin3,7-dimethyl ether.

Levandula Officinalis essence was tested against *Leishmania major* where essential oil was extracted from the fresh dried. Different concentration of essential oil (0.5, 1.5, 5, 10, 15, and 20 %) was added into a *Leishmania major in vitro* culture against promastigotes and

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found that the leishmanicidal impact was likewise seen in concentrations of 10% or greater in that no viable parasite was seen in the major groups at hour 72. Additionally, in comparison to the chemical drug group, a significant difference was seen in the number of live parasites at concentrations of 0.5, 1.5, and 5 %[28].

Medicago lupulina, often known as black alfalfa, is an effective treatment for cutaneous leishmaniasis. Phytochemical preparations of this plant were estimated to contain flavonoids, alkaloids, phenols, tannins, and diterpenes[47]. Leaves of the plant were collected and dried and then the essential oils were extracted and tested on promastigotes of *Leishmania major* by MTT assay[29]. The essential oil's IC50 against typical parasite promastigotes was 250 μg/ml, respectively.

Portulaca oleracea is mostly known as Purslane plant a traditional medicine that could be used to develop new pharmaceutical drugs or lead against *Leishmania* species, with different active agents and alkaloids as their most significant chemicals.[48]. Eskandari et.al [30] tested the Antileishmanial effect of *Portulaca oleracea* extract by collecting the stem and leaves of the plant, drying them, and extracting the essence through them. Then they tested this essence with a different concentration on *L. major* promastigotes i*n vitro* by MTT assay. The IC50 values for the purslane herb's leaves and stems essence were 360 and 680 μg/ml, respectively, after 48 hours.

S. Shirazi and M. Dodi [31] used Crataegus microphylla (Red herb hawthorn) leaf extract on Leishmania major promastigotes. The stems and leaves of the plant were collected and dried. On L. major promastigotes, the biological activity of herb extract (DMSO dissolved) and drug susceptibility was assessed. Crataegus microphylla alcohol extracts have an IC50 of 1094 μg /ml.

Albakhit et.al has tested *Phoenix dactylifera L* date Fruit and Pit Extracts against Leishmania major promastigotes[32]. Date pits and fruit have been taken as herbal remedies throughout history by people of various cultures. Anti-inflammation, anti-tumor, antioxidant, antibacterial, antidiabetic, nephroprotective, hepatoprotective, and sex hormone modulator are the medicinal qualities of *Phoenix dactylifera* that have been mentioned[49]. Date fruit and pit methanolic extracts were produced, and their IC50 value was determined using the MTT assay on log-phase promastigotes by Albakhit et. al[32]. For methanolic extracts of pit and fruit, IC50s were estimated to be 23 mg/mL and 500 mg/mL.

Fruit from hawthorn (*Crataegus Aronia*) possesses antibacterial and antioxidant properties. It is believed that the plant is generally safe and well-tolerated and that it is mostly utilized for treating cardiovascular disorders[50][51]. Leishmania major amastigotes were supplemented with hawthorn fruit extract and after 24 hours IC50 was 49.13 μg/ml[33].

I.G. Demarchi et. al[34] isolated essential oil (TrEO) and 6,7-dehydroroyleanone (TrROY) from *Tetradenia Riparia* commonly known as Iboza Riparia and Moschosma riparium and the plant is a herbaceous shrub, Infectious and inflammatory disorders are treated with it as a traditional medicine in Africa[52][53][54]. The terpenoids and other compounds are some sources of essential oils of *Tetradenia Riparia* [55]. There may be some biological effects of the diterpene 6,7-dehydroroyleanone isolated from *T. Riparia* (TrROY), such as antioxidant and anti-termite efficacy[56]. TrEO and TrROY had LD50 of 0.8 mg/mL and 3 mg/mL, respectively, which caused 50% Leishmania mortality against *L. (L.) amazonensis* promastigote and amastigote [34].

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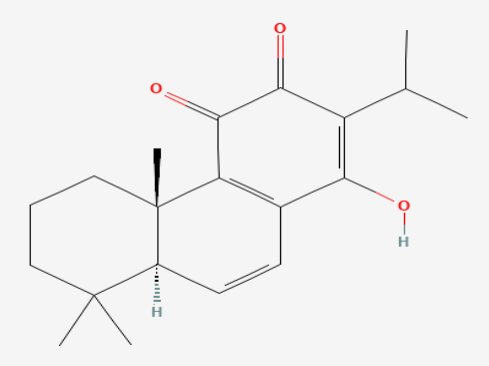

Figure 4: 6,7-dehydroroyleanone.

G. Gupta et al.,[35] have effectively proven the antileishmanial activity by synthesis of the new sterol pentalinonsterol (PEN), which naturally occurs in the root of a Mexican medicinal plant called Pentalinon andrieuxii. The Yucatan Peninsula of Mexico is home to the plant Pentalinon andrieuxii , whose roots have long been employed by Mayan traditional healers and have been demonstrated to possess a variety of biological activities, including antileishmanial characteristics[57][58]. Pentalinonsterol was encapsulated in a liposomes and proceeded with *in vivo* studies. By administering liposome-encapsulated PEN (2.5 mg/kg) intravenously to experimental BALB/c mice with *L. donovani* infection, the parasite burden in the liver and spleen was significantly reduced. Mice that were infected and given liposomal PEN demonstrated a significant TH1 immune response that was characterised by the generation of IFN-γ and the development of mature hepatic granulomas

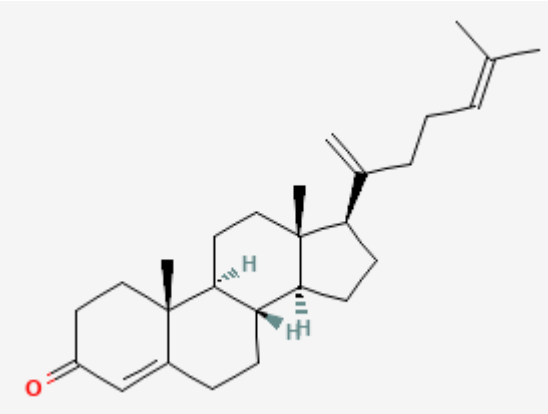

Figure 5: Pentalinonsterol (PEN).

A wide range of biological functions, such as anti-protozoal activity, are present in indole alkaloids. It has already been effective to use indole alkaloids derived from several plant sources for antibacterial, anticancer, antidiabetic, antiulcerative, and anti-protozoal applications. Voacamine is an indole alkaloid and an antiprotozoal substance that has been identified from the plant Tabernaemontana coronaria and is effective against a variety of trypanosomatid parasites, including the Brazilian and Indian strains of Leishmania amazonensis and Trypanosoma cruzi[59]. In a *in vitro* assay Voacamine inhibited L. donovani topoisomerase IB's catalytic activity and acted in an uncompetitive manner at IC_{50} value 14.702 ± 0.101 μM . In further studies *in vivo* and *in vitro*, vacamine stabilises the DNA-topoisomerase IB cleavage complex[36].

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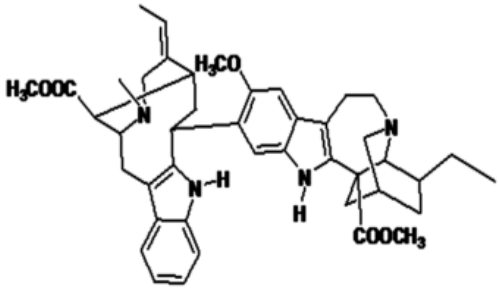

Figure 6: Voacamine.

The *in vitro* anti-leishmanial efficacy of nine medicinal plants from eight distinct families against the promastigote and amastigote stages of Leishmania major was examined [37]. All the plants met the criteria for anti-leishmanial activity against the L. major, includes Sonchus oleraceus, Echinops spinosissimus, Trichodesma africana, Pergularia tomentosa, Teucrium oliverianum, Blepharis ciliaris, Citrllus colocynthis, Cleome amblyocarpa, and Aerva javanica. From these plants the crude extracts of Only three of the studied plant extracts—T. oliverianum, A. javanica, and C. amblyocarpa—showed action against *L. major* promastigote stage over 60% inhibition at the higher concentration 50 g/mL. For the same concentration of 50 g/mL, all the other species displayed inhibition % ranging from 25 to 59 %. The IC_{50} values differ in these three plants *T. oliverianum*: 7.8 µg/mL, *A. javanica* 13.7 µg/mL and IC_{50} of *C. amblyocarpa* was 21.5 µg/mL

The anti-leishmanial properties of benzoic acid derivatives, such as methyl 3,4-dihydroxybenzoate and octadecyl benzoate , obtained from the significant *Ifloga spicata* [38] plant an annual herb from the family Asteraceae, is used whole for the treatment of skin conditions like dermatosis and allergies as well as in decoction form for the treatment of heart conditions[60][61][62]. FT-IR, mass spectrometry, and multinuclear (1H and 13C) NMR spectroscopy were used to identify the chemical structures of methyl 3,4-dihydroxybenzoate and octadecyl benzoate. The potency of both compounds was tested in axenic cultures of *L. tropica* promastigotes. Both substances demonstrated strong anti-leishmanial activity, with LD50 values of 110.40 ± 0.09 and 14.11 ± 0.11 µg/mL for methyl 3,4-dihydroxybenzoate and octadecyl benzoate , respectively.

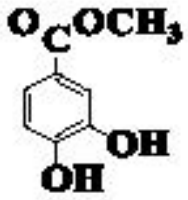

Figure 7: Methyl 3,4-dihydroxybenzoate.

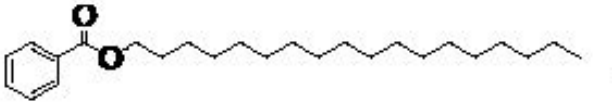

Figure 8: Octadecyl benzoate.

In a other research Achillea L has been test against L. infantum[39]. For thousands of years, yarrow (Achillea) species have been used in ethnopharmacology all across the world. Most of the species in the Achillea L. (Asteraceae) genus are found in Europe and Asia. It has long been used in Iran as an anti-inflammatory and to relieve anxiety[63]. Since yarrow has been studied for the longest time, its active principle, which is a natural blend of chemical components, looks to be essential oil[64]. Ayrom et al has prepared

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Achillea santolina essential oil by Using the Clevenger apparatus, the aerial components of the A. santolina plant were harvested, dried, and hydro-distilled. Using the MTT and trypan blue test techniques, the effects of various concentrations of saline, Achillea santolina essential oil, and glucantime were evaluated at 24-, 48-, and 72-hour intervals against Leishmania infantum promastigote. In comparison to the control group, the use of Achillea santolina essential oil decreased viability in all concentrations. After 72 h efficiency concentration was 1000 mL/mg for Achillea santolina essential oil.

In other studies, *Leishmania amazonensis* is treated with essential oil from *Endlicheria bracteolata*. The plant Endlicheria bracteolata (Meisn.) C.K. Allen, belonging to the Lauraceae family, is known as laurel in the Amazonian states of Amapá, Pará, Amazonas, and Acre. Several members of the *Lauraceae* family have relevant antiprotozoal activity. Promastigotes and intracellular amastigotes were used to test the antileishmanial activity against, while J774.G8 was used to test the cytotoxicity of, *E. bracteolata* essential oil at varying concentration. With E. bracteolata essential oil IC50, flow cytometry and transmission electron microscopy were used. The IC50 for E. bracteolata essential oil in promastigote forms was 7.945 ± 1.285 μg/mL after 24 h and 6.186 ± 1.226 μg/mL after 48 h, while it was 3.546 ± 1.184 μg/mL for intracellular amastigote forms for 24 h. Indicating that E. bracteolata essential oil is less toxic to macrophages than to parasites, the CC50 was 15.14 ± 0.090 μg/mL. Transmission electron microscopy shown that the treatment of promastigote and intracellular amastigote forms with E. bracteolata essential oil can result in mitochondrial damage[40].

In 2019 P.K. Pramanik, et al conducted a research on *Corchorus capsularis L.* leaf extract (CCEx) as a replacement leishmanicide for *Leishmania donovani*. The widespread plant *Corchorus capsularis L.*, also known as white jute, has a variety of therapeutic benefits[41]. The leaves of C. capsularis L. have strong antimicrobial, anti-inflammatory and antinociceptive effects[65][66]. Leaf extract was made using the *C. capsularis L.* leaves that had been shade dried. With an IC50 value of 79.00 ± 0.3 μg/ml, the leaf extract exhibits strong antileishmanial activity against L. donovani promastigotes. Leaf extract dramatically increases the quantity of non-protein thiols in virulent parasites while also significantly increasing intracellular reactive oxygen species (ROS).

In a other study *Aerva sanguinolenta* is used against *L. donovani* [42], Perennial herb *Aerva sanguinolenta*, is a member of the *Amaranthaceae* family. The herb has traditionally been utilised extensively as a traditional medicine. It contains a lot of phytochemicals, including betacyanins, flavonoids, terpenoids, sphingolipids, and tannins[67]. Extract from *A. sanguinolenta* shown positive anti-leishmanial activity. The range of IC50 values was between 0.1 ug/ml and 1 ug/ml against *L. donovani*.

In 2020 L. D. C. CLEMENTINO et. al[43], the compounds from *Baccharis trimera* aerial parts were extracted and tested for their effectiveness against *Leishmania amazonensis* and macrophage cells. Its anti-inflammatory, antioxidant, and hepatoprotective properties have been proven by pharmacological research[68][69]. Eupatorin (3',5-dihydroxy-4',6,7-trimethoxyflavone) was shown to be a flavonoid. For a new antiparasitic medications, flavonoid, eupatorin, was identified and described against *L. amazonensis* with cytotoxicity assessments in murine macrophages. In comparison to intracellular amastigotes, eupatorin was 3.7 times more effective with IC50 value of 1.6 ± 0.1μg/ml.

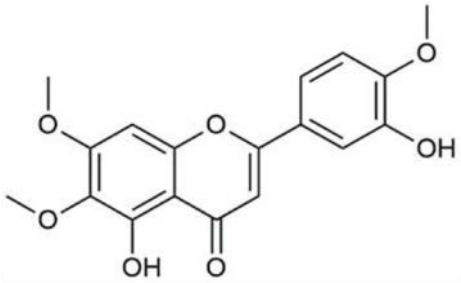

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Figure 9: Eupatorin.

Al-Hajj et al. [14] in 2018 evaluated *Euphorbia cactus Ehrenb, Euphorbia ammak Forssk, Euphorbia inarticulate Schweinf, and Pergularia tomentosa L* for their anti-leishmanial efficacy by using Stems and leaves extract. In these *Euphorbia cactus, Euphorbia ammak* showed anti-leishmanial activity. To check the activity the patient who had cutaneous leishmaniasis, Leishmania spp. were isolated. After a 24-hour incubation period of *Leishmania spp* cells with serial concentrations of plant extracts, the IC50 values for Euphorbia cactus Ehrenb and Euphorbia ammak Forssk against *Leishmania spp* promastigotes were less than < 15.6µgml-1.

DISCUSSION

Various herbal plants are available in nature. These are considered the alternatives for developing new anti-leishmanial drugs. A growing number of novel herbal components are pharmacologically active, particularly anti-inflammatory chemicals. These naturally occurring substances appear to have a selective effect against parasites without affecting the survival of the host cell. In addition, they are more accessible to isolated populations than conventional medication[70][71][72][73][74]. Certain findings lend support to the theory of developing a successful vaccination against *Leishmania*. However, the development of a potent vaccine to prevent the disease is highly desired due to the rise in first-line drug resistance and the toxicity of second-line drugs. The need for a potent cell-mediated immune response to mediate protection from Leishmania and numerous other intracellular infections presents a significant obstacle to vaccination[75]. The differences between human diseases and animal models, as well as the translation of laboratory-based research to the field, represent another obstacles to the creation of an effective vaccine[76].

The development of inhibitors that can be natual secondary metabolites and the strengthening of the pipeline for the eradication of disease can both benefit from the discovery of new therapeutic targets. For example Topoisomerase inhibitors, which have the potential to be used as antileishmanial medications, have been discovered to be fatal targets for DNA topoisomerases that regulate the over- or underwinding of DNA[77]. As a natural source to cure leishmania, secondary metabolites from microorganisms, including fungus and marine creatures, have also been identified[78]. Leishmania parasites have been discovered to be eliminated by plant defensins by disruption of the plasma membrane, rupture of the mitochondrial membrane, and generation of reactive oxygen species[79].

Antileishmanial medications that are safe to use and derived from medicinal plants are abundant in endemic nations. Studies of many kinds have been done on effective medications to decrease parasite infection throughout the world. The effectiveness of herbal therapy in treating various *Leishmania spp* parasites has been studied. Pharmaceutical companies and various researchers have looked at various drug delivery techniques and nano-particle therapy to assess the efficacy of herbal remedies and disease therapies. The *in vivo* and *in vitro* activities of various plant metabolites and crude extract have been covered in this review along with research papers of various kinds where these compounds have shown some promising outcomes against various *Leishmania* species. As plant-based compounds may be suitable substitutes for pharmaceutical drugs for a number of disorders, it is vital to discover standardised and secure preparation before suggesting them for more clinical applications.

Therefore this review concludes that the isolated compounds mentioned will be used as a source for future leishmaniasis therapies, complementing those presently provided in clinics.

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