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# Mexican Medicinal Plants as an Alternative for the Development of New Compounds Against Protozoan Parasites

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Additional information is available at the end of the chapter

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## Abstract

The protozoan parasites *Plasmodium*, *Leishmania*, *Trypanosoma*, *Entamoeba histolytica*, *Giardia lamblia*, and *Trichomonas vaginalis*, cause high morbidity and mortality in developed and developing countries. *P. falciparum* is responsible for malaria, one of the most severe infectious diseases in Africa. Hundreds of million people are affected by *Trypanosoma* and *Leishmania* that cause African and South American trypanosomiasis, and leishmaniasis. *E. histolytica* and *G. lamblia* contribute to the enormous burden of diarrheal diseases worldwide; trichomoniasis is the most common nonviral sexually transmitted disease in the world. Because of the important side effects of current treatments and the decrease in drug susceptibility, there is a renewed interest for the search of therapeutic alternatives against these pathogens. Natural products obtained from medicinal plants and their derivatives have been recognized for many years as a source of therapeutic agents. There are numerous reports about medicinal plants that are used by indigenous communities to treat gastrointestinal complaints. Importantly, phytochemical studies have allowed the identification of several secondary metabolites with anti-parasite activity. Our review revealed that Mexican medicinal plants have a great potential for the identification of new molecules with activity against protozoan parasites of medical importance worldwide and their potential use as new therapeutic compounds.

**Keywords:** *Plasmodium*, *Leishmania*, *Trypanosoma*, *Entamoeba*, *Giardia*, *Trichomonas*, Mexican medicinal plant

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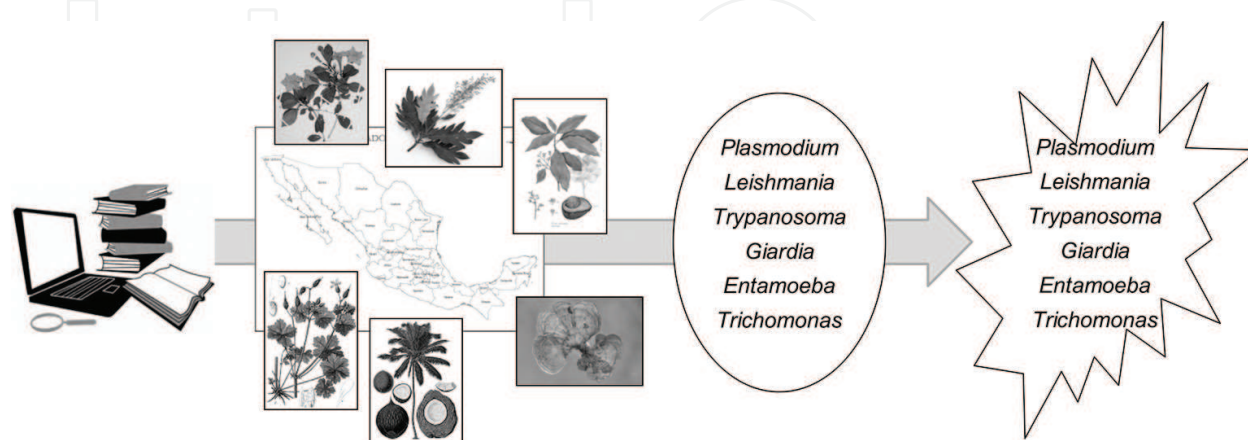
## 1. Introduction

Protozoan parasites represent a large public health problem worldwide, from tropical and developing regions to developed countries. Among them, *Plasmodium* spp. that produces malaria is considered as the first parasitic cause of death both in people living in endemic areas and travelers returning from these regions, affecting 240 million people in 2009 and producing more than 1 million deaths in children each year in Africa alone [1]. The hemoflagellates of the *Trypanosomatidae* family, *Leishmania* spp. and *Trypanosoma* spp. are responsible for three major human diseases, leishmaniasis (cutaneous, mucocutaneous, and visceral leishmaniasis), sleeping sickness (African trypanosomiasis), and Chagas disease (American trypanosomiasis), respectively [2]. Other highly prevalent infective parasites include the intestinal anaerobic protozoa, *Entamoeba histolytica* and *Giardia intestinalis* (commonly referred to as *G. lamblia* or *G. duodenalis*) that contribute to the enormous burden of diarrheal diseases worldwide, as well as *Trichomonas vaginalis*, which is the most common nonviral sexually transmitted disease in the world [3–5]. The control of these protozoan parasites is usually based on the improvement of sanitary conditions to avoid infection, and the treatment of infected individuals. Several drugs, such as metronidazole (MTZ), pentamidine, amphotericin B and derivatives, among others, are available for the treatment of these parasitic infections. However, significant side effects have been reported, and there is a decrease in drug susceptibility [6]. In the case of *Trypanosoma*, *Leishmania*, and *Plasmodium*, an alternative approach is the interruption of disease transmission by either preventing contacts between human beings and vectors, killing or altering the vector life cycle. However, the effectiveness of vector control is limited by the development of insecticide resistance [7–9]. Therefore, it is necessary to improve the current chemotherapy arsenal against these protozoan parasites and their vectors. Natural treatments based on probiotics [10, 11], propolis [12, 13], or lactoferrin [14] may represent potential therapeutic agents against protozoan parasites. The so-called “eco-friendly control tool of mosquito vectors” based on natural molecules derived from plants is another growing line of investigation [15–21]. The search for new, safe, and efficient agents usually involves the identification of a biochemical target in parasites and the development of specific inhibitors from *in silico* (computational), *in vitro*, and *in vivo* experiments. Another strategy relies on the screening of known and unknown molecules to identify active compounds. The identification of new drugs can result from chemical modifications of existing molecules, evaluation of drugs that are currently used to treat other diseases, screening of chemical libraries, and assessment of natural compounds derived from plants that are commonly employed in traditional medicine [22, 23].

Plants synthesize a large number of organic compounds also called primary metabolites that contribute to the production of carbohydrates, lipids, and proteins, among others, that are necessary for their growth. They also generate a small amount of a variety of secondary metabolites known as phytochemicals that are represented by alkaloids, carotenoids, flavonoids, saponins, hydroxycinnamic acids, and triterpenoids, among others. To date, more than 4000 of these compounds have been discovered; some of them are responsible for color and organoleptic properties of plants, such as the red color of grapes or the characteristic smell of lavender; others act as a natural protection system against pathogens or grazing animals [24]. Traditional medicines all around the world have identified the benefit of plants for human

health and have taken advantage of the biological properties of phytochemicals for the empiric treatment of common human diseases. More recently, a number of scientific experiments have been performed to determine how a specific phytochemical can act at the molecular and cellular levels to protect human cells against oxidative damage, to stimulate enzymes, to interfere with the DNA replication, or to affect infection processes. These works confirmed that natural molecules obtained from medicinal plants and their derivatives are a valuable source of new therapeutic agents for the treatment of common human diseases and the control of protozoan parasites and their vectors. Importantly, the key importance of natural product research was recently highlighted by the awarding of the 2015 Nobel prize to Youyou Tu for the discovery of the antimalarial drug artemisinin [25].

In this context, Mexico has more than 3000 species of medicinal plants that have been empirically used by indigenous communities for years [26]. Some of the herbal expertise of pre-Columbian Olmec, Toltec, Aztec, Maya, Zapotec, Mixteca and Perupacha civilizations has been used by European doctors and scientists from the time of the conquest, which contributed to increase the therapeutic arsenal and enrich the universal pharmacology through centuries. Although a number of Mexican plants are currently cultivated in most countries of Europa and other continents, there is still a large number of endemic species in Mexico that remain uncharacterized. As part of the efforts to explore their potential, several groups of investigation have initiated chemical, toxicological, pharmacological, or clinical investigations in order to provide rational elements for their therapeutic effects against diseases that affect the Mexican population, mainly central nervous system disorders, diabetes, metabolic syndrome, inflammatory processes, and gastrointestinal disorders [27]. Notably, extensive review of ethnobotanical data identified medicinal plants that are used by indigenous communities in Mexico to treat complaints that fit with symptoms of parasitic infections. In addition to terrestrial plants, marine algae represent a potential source of distinct secondary metabolites related to their specific metabolism. In most cases, a general *in vitro* evaluation of the selected plants was performed to confirm the traditional use. But in some cases, phytochemical studies have allowed the isolation and identification of secondary metabolites with antiparasitic activity (Figure 1). In this chapter, we describe the current knowledge about the effects of several



**Figure 1.** Strategy to search and review works about the evaluation of Mexican medicinal plants as an alternative for the development of new compounds against protozoan parasites.

Mexican plants against selected protozoan parasites of medical importance worldwide, including Mexico, namely *Plasmodium* spp., *Leishmania* spp., *Trypanosoma* spp., *G. lamblia*, *E. histolytica*, and *T. vaginalis*. We also report the identification of some phytochemical compounds with antiparasitic activity.

## 2. Mexican medicinal plants against *Plasmodium falciparum*

### 2.1. *Plasmodium* and malaria

For decades, Malaria has been considered as the most important parasitic infectious disease worldwide, with high morbidity and mortality rates, as well as a huge socioeconomic impact in tropical and subtropical regions. In 2015, the World Malaria Report of the World Health Organization (WHO) estimated 214 million infected people and 438,000 deaths worldwide. Most cases and deaths occurred in Africa (88%), followed by the South-East Asia Region. However, for the first time, the incidence of malaria, which takes into account population growth, has been reduced by about 37% between 2000 and 2014, and the death rate has also been decreased by 60% worldwide. These encouraging numbers are the result of the efficient prophylactic and therapeutic management of malaria. Notably, the case number was reduced by 75% in several endemic countries from Asia region and South Africa and by 67.5% in Latin America. In this region, seven countries, namely Argentina, Belize, Costa Rica, Ecuador, El Salvador, Mexico, and Paraguay, are now in the elimination phase. In contrast, other countries including Panama, Nicaragua, Honduras, and Guatemala still maintain a significant transmission. Despite significant advances in the control of malaria worldwide, approximately 3.2 billion people in Asia, Latin America, and to a lesser extent, Middle East, i.e., nearly half of the world's population, were still at risk for malaria in 2015 [9, 28–30].

Malaria is caused by protozoan parasites of the genus *Plasmodium*. Among the five parasites known to infect human (*P. falciparum*, *P. malariae*, *P. ovale*, *P. vivax*, and *P. knowlesi*), *P. falciparum* is the most virulent, causing approximately 200 million clinical cases each year, while *P. vivax* is estimated to affect 13.8 million people [31]. *P. falciparum* is an intracellular parasite whose life cycle requires two hosts, *Anopheles* mosquito (sexual stages) and human (asexual stages). More than 70 different *Anopheles* species can transmit malaria, which contributes to the high spread of the disease [32]. Infection begins with the bite of an infected female mosquito; infective sporozoites rapidly move to the liver and proliferate (schizogony) in hepatocytes to form 30,000–40,000 merozoites that further escape into blood. In red blood cells, merozoites transform into trophozoites that invade new erythrocytes; some trophozoites differentiate themselves into microgametocytes (male) and macrogametocytes (female) that can be ingested by another *Anopheles* mosquito. These sexual parasite forms develop into a zygote, which progresses into an ookinete and an oocyst that releases sporozoites to infect a new host [33, 34].

The first symptoms of malaria also called the “primary attack” correspond to the hepatic phase and may resemble any febrile illness. In the erythrocytic phase, fever is accompanied by shivering, vomiting, joint pain, anemia, and retinal damage. Then, the typical symptoms of malaria, consisting in fever with sudden coldness and sweating, occur in periodic intervals of 2–3 days known as “short-term relapses.” In some patients, “long-term relapses” of 20–60 days



may occur due to reactivation of infection in the liver (*P. ovale* and *P. vivax*) or persistent infection in blood (*P. falciparum* and *P. malariae*) [35]. *P. falciparum* covers the surface of the infected blood cells with PfEMP1 proteins (*P. falciparum* erythrocyte membrane protein 1) to be stucked to blood vessels and escape destruction in the spleen. With time, this creates hemorrhagic events and obstruction of circulatory vessels, which leads to cerebral malaria [33].

WHO recommends artemisinin-based combination therapy (ACT) as the first-line treatment for *P. falciparum* malaria in all endemic regions. ACT combines a fast acting but rapidly cleared artemisinin derivative with a longer-lasting partner drug. The main combinations are lumefantrine (LMF) with artemether (ATM), which constitutes the most widely used ACT, mefloquine (MFQ) with artesunate (AS), amodiaquine (ADQ) paired with AS, and piperazine (PPQ) combined with dihydroartemisinin (DHA). However, the increasing prevalence of artemisinin resistant *P. falciparum* across Southeast Asia and Africa threatens to destabilize malaria control worldwide. Artemisinin resistance is caused by over 20 different mutations in the *kelch13* gene [36]. The multidrug resistance 1 gene (*Pfmdr1*) and chloroquine resistance transporter gene (*Pfcr1*) may also confer resistance to a great number of antimalarial drugs, including ATC [37, 38]. The recently developed vaccine, RTS,S/AS01 (RTS,S) (Mosquirix™) should help to protect young children against *P. falciparum* [39, 40]. However, malaria management in adult populations is still an extreme challenge and new antimalarials with distinct mechanisms of action are needed to circumvent existing or emerging drug resistance [41].

## 2.2. Relevant studies about Mexican plant with activity against *Plasmodium*

Plants are recognized as important sources of antimalarial compounds, such as artemisinin obtained from *Artemisia annua*, quinine present in western Amazonian *Cinchona* spp., as well as quassinoids and limonoids in plants of the *Simaroubaceae* and *Meliaceae* families, respectively [42, 43]. In Mexico, about 113 species are traditionally used to treat malaria symptoms, from which only several have been pharmacologically characterized (Table 1).

In 1990, Noster and Kraus performed the first investigations about the relevance of Mexican medicinal plants for the development of new antimalarial compounds. These authors examined two plants of the *Rubiaceae* family, *Coutarea latiflora* Sesse & Moc. ex. DC. (*Hintonia latiflora* Bullock) and *Exostema caribaeum* (Jacq.) Roem. et Schult. that were collected in Puebla, Mexico. Notably, *C. latiflora* also known as *Copalchi* is recommended to treat diabetes, stomachaches, gastric ulcers, diarrhea, skin problems, kidney problems, fever, typhus, and malaria. *E. caribaeum* is used for the treatment of gastritis, ulcers, diarrhea, stomachaches, to increase appetite and blood pressure, and to eliminate tapeworms; bark extracts are also efficient against fever, especially fever related to malaria. The hydrolyzed ethyl acetate extracts of the stem bark were shown to have the most potent antimalarial activity *in vitro*. Notably, one phenylcoumarin derivative isolated from the ether extract of *E. caribaeum* showed a moderate activity against chloroquine and pyrimethamine-sensitive FCH-5/Tanzania strain of *P. falciparum* [44]. Later, fractionation of lipophilic and hydrophilic extracts from the stem bark and branches of a related species, *E. mexicanum*, revealed the presence of two new 4-phenylcoumarins: 4',8-dihydroxy-5,7-dimethoxy-4-phenylcoumarin (exomexin A) and 3',4'-dihydroxy-5,7,8-trimethoxy-4-phenylcoumarin (exomexin B). Exomexin

Scientific names	Common names in Mexico	Portions	Extracts	Properties	Metabolites	References
<b>Mexican medicinal plants against <i>Plasmodium</i></b>						
<i>Exostema caribaeum</i>	<i>Quina, melena de león</i>	Stem bark	Ethyl acetate	IC <sub>50</sub> = 3.2 µg/ml	Phenylcoumarin derivative	[32]
<i>Hintonia latiflora</i> (= <i>Cuntarea latiflora</i> )	<i>Copalchi, palo amargo</i>	Stem bark	Ethyl acetate	IC <sub>50</sub> = 7.3 µg/ml <i>In vivo</i> activity against schizonts at 40 mg/kg. IC <sub>50</sub> = 24.7 and 25.9 µM IC <sub>50</sub> = 25.9 µM	Phenylcoumarin derivative 5-O-β-D-glucopyranosyl-7,4'-dimethoxy-3'-hydroxy-4-phenylcoumarin 5-O-β-D-glucopyranosyl-7-methoxy-3',4'-dihydroxy-4-phenylcoumarin	[32] [34]
<b>Mexican medicinal plants against <i>T. cruzi</i></b>						
<i>Aristolochia taliscana</i>	<i>Guaco</i>	Roots	Methanol	<i>In vitro</i> activity on epimastigotes at 0.5 mg/ml	Neolignans (aupomatenoid-7 licarin A, aupomatenoid-1 and licarin B) and lignans (austrobailignan-7, and fragransin E1).	[42]
<i>Persea americana</i>	<i>Aguacate</i>	Seeds	Methanol	IC <sub>50</sub> (epimastigotes) = 82 µM IC <sub>50</sub> (trypomastigotes) = 49 µM	1,2,4-Tri-hydroxyheptadec-16-ene, 1,2,4-tri-hydroxyheptadec-16-yne and 1,2,4-trihydroxinonadecane derivatives	[43]
<i>Senna villosa</i>	<i>Booxsaal che, saal che, black bean</i>	Leaves	Chloroform	<i>In vitro</i> activity on epimastigotes at 1.6 mg/ml	8-Hydroxymethylen)-tricosanyl	[44]
<i>Haematoxylum brasiletto</i>	<i>Palo de brasil</i>	Leaves and aerial parts	Methanol	<i>In vitro</i> activity on epimastigotes at 7.92 mg/ml	Hematoxylin, Brazilin, Caffeic acid, Gallic acid, Methyl gallate, phloroglucinol, 4-hydroxycinnamic acid and 5-methoxypsoralen	[46]
<b>Mexican medicinal plants against <i>Leishmania</i></b>						
<i>Pentalinon andrieuxii</i>	<i>Bejuco guaco, cantibtec, contrayerba</i>	Leaves and roots	Water	<i>In vitro</i> activity on promastigotes at 10 µg/ml	6,7-Dihydroneridienone, Cholest-4-en-3-one	[53]
<i>Laennecia confusa</i>	ND	Aerial parts	Water Chloroform	IC <sub>50</sub> ~20 µg/ml	Flavonoids, Cyanogenic Glycosides and Cardiotonic Saponins, Sesquiterpene Lactones and Triterpenes	[55]

Scientific names	Common names in Mexico	Portions	Extracts	Properties	Metabolites	References
<b>Mexican medicinal plants against <i>G. lamblia</i> and <i>E. histolytica</i></b>						
<i>Zanthoxylum liebmannianum</i>	Colopahtle	Leaves	Ethanol	IC50 (Eh) = 503.48 µg/ml IC50 (Gl) = 58 µg/ml	Asarinin	[64]
<i>Teloxys graveolens</i>	Epazote de zorrillo	Aerial parts	Methanol	IC50 (Eh) = 12.5 µg/ml IC50 (Gl) = 16.8 µg/ml IC50 (Eh) = 17.2 µg/ml	Melilotoside Narcissin	[65]
<i>Rubus coriifolius</i>	Zarzamora	Aerial parts	Methanol:dichloro methane	IC50 (Eh) = 11.6 µg/ml IC50 (Gl) = 55.6 µg/ml	(-)-Epicatechin	[66]
<i>Geranium mexicanum</i>	Pata de León	Roots	Dichloromethane–MeOH	IC50 (Eh) = 1.9 µg/ml IC50 (Gl) = 1.6 µg/ml	(-)-Epicatechin	[68]
<i>Decachaeta incompta</i>	ND	Leaves	Dichloromethane	IC50 (Eh) = 2.6 µg/ml IC50 (Gl) = 18.1 µg/ml	Incomptine A	[71]
<i>Salvia polystachya</i>	Chia	Aerial parts	Acetone	IC50 (Eh) = 22.9 µM IC50 (Gl) = 28.2 µM IC50(Eh) from 117.0 to 160.6 µM IC50 (Gl) from 107.5 to 134.7 µM	Linearolactone Polystachynes A, B and D	[73]
<i>Lepidium virginicum</i>	pich' tuluk'	Roots	Methanol	IC50 (Eh) = 100.1 µg/ml	Benzyl glucosinolate	[76]
<i>Lippia graveolens</i> <i>Ruta chalepensis</i>		Aerial parts	Methanol	IC50 (Eh) = 44.3 µg/ml IC50 (Eh) = 45.95 µg/ml	Carvacrol Chalepensisin	[77]
<i>Adenophyllum aurantium</i>	<i>Arnica silvestre</i>	Roots	Ethyl acetate	IC50 (Eh) = 230 µg/ml	Thiophenes	[78]
<i>Hippocratea excelsa</i>	Cancerina	Roots	Hexane/ethanol	IC50 (Gl) = 0.11 µM IC50 (Gl) = 0.74 µM	Pristimerine tingenone	[83]
<i>Geranium mexicanum</i> <i>Rubus coriifolius</i> <i>Cuphea pinetorum</i> <i>Helianthemum glomeratum</i>	Pata de león Zarzamora Cenicilla o hierba de la gallina	Roots Aerial parts Aerial parts Leaves	Dichloromethane/ methanol	ED50 (Gl) = 0.072 µmol/kg ED50 (Gl) = 2.057 µmol/kg ED50 (Gl) = 1.429 µmol/kg	(-)-Epicatechin Kaempferol Tiliroside	[84]



Scientific names	Common names in Mexico	Portions	Extracts	Properties	Metabolites	References
Mexican medicinal plants against <i>T. vaginalis</i>						
<i>Carica papaya</i>	<i>Papaya</i>	Seeds	<i>Methanol</i>	IC50 = 5.6 µg/ml	Sanguinarine alkaloid	[99]
<i>Cocos nucifera</i>	<i>Cocotero, coyolli</i>	husk fiber		IC50 = 5.8 µg/ml		
<i>Bocconia frutescens</i>	<i>ts'ixte'</i>	Aerial parts		IC50 from 30.9 to 60.9 µg/ml		
<i>Geranium mexicanum</i>	<i>Geranio de olor, Pata de león</i>	Roots				
<i>Lygodium venustum</i>	<i>Bejuco chino, crispillo</i>	Aerial parts				
<i>Lobophora variegata</i>	ND	Whole	Dichloromethane/ methanol	IC50 = 1.3 ± 0.7 µg/ml	Sulfoquinovosyl-diacylglycerols 1-3	[100, 101, 103]
<i>Udotea conglutinata</i>	ND			IC50 = 1.6 ± 0.1 µg/ml		
ND = not determined. ED50, effective dose 50; IC50, half-maximal inhibitory concentration; MC100, concentration inducing 100% of the maximum response. Gl, <i>G. lamblia</i> ; Eh, <i>E. histolytica</i> .						

**Table 1.** Names and metabolites of the most relevant Mexican medicinal plants with activity against selected protozoan parasites.

A, the most lipophilic molecule, had the strongest *in vitro* activity against the chloroquine-sensitive strain (poW) and the chloroquine-resistant strain (Dd2) of *P. falciparum*, with half-maximal inhibitory concentration (IC<sub>50</sub>) values of 3.6 and 1.6 µg/ml, respectively [45]. In another study, Argotte-Ramos et al. [46] confirmed that ethyl acetate extract of the stem bark of *H. latiflora* was also able to suppress parasitemia in mice infected with *P. berghei*. Bioassay-directed fractionation of the extract showed that this activity was due to two 4-phenylcoumarins, the new 5-O-β-D-glucopyranosyl-7,4'-dimethoxy-3'-hydroxy-4-phenylcoumarin and the previously reported 5-O-β-D-glucopyranosyl-7-methoxy-3',4'-dihydroxy-4-phenylcoumarin. This latter molecule suppressed the development of schizonts by 70.8% at the dose of 40 mg/kg in the *in vivo* model. Both compounds were also effective against *P. berghei* schizonts in *in vitro* experiments with IC<sub>50</sub> values of 24.7 and 25.9 µM, respectively. More recently, Rivera et al. [47] reported that methanolic extract of *H. latiflora* stem bark (HlMeOHe) also has an antimalarial efficacy. Toxicity assays showed that median lethal dose (LD<sub>50</sub>) was 2783.71 mg/kg. *P. yoelii yoelii*-infected mice treated with 600 and 300 mg/kg died after 6 and 7 days, respectively, with parasitemia around 45% versus 70% in untreated mice. Interestingly, treatment with 1200 mg/kg led to a 23 days survival time with a residual parasitemia of 23.6%. However, HlMeOHe seemed to be mutagenic since the average number of micronuclei significantly increased from 0.9 in untreated to 4.8 in treated mice. The authors concluded that the identification of the chemical composition of HlMeOHe should help to reduce its genotoxic potential.

*Artemisia ludoviciana ssp. mexicana* of the *Compositae* family has been empirically used for the treatment of intermittent fever and other symptoms. Malagon et al. [48] prepared ethanolic extracts from stems, leaves, and flowers to evaluate their activity in mice infected with *P. yoelii yoelii*. Results showed that parasite reproduction was inhibited up to 98.6% at the 5th day; the effective dose 50 (ED<sub>50</sub>) was of 29.2 mg/kg with a security margin 50 (SM<sub>50</sub>) of 28.7. Surprisingly, this extract did not seem to contain the artemisinin molecule discovered in the leaves of *A. annua* and that is the basis of ACT, which suggests the anti-*Plasmodium* effect may be due to another active molecule.

As part of an ethnobotanical study in Yucatan, Mexico (February 1994–June 1995; September 1996–October 1996), medicinal plants used by Mayan communities were collected from Chikindzonot, Ekpedz, and Xcocomil villages and surroundings to confirm their pharmacological relevance [49]. Notably, several species that are commonly recommended against fever or pain were screened *in vitro* for antimalarial activity, such as *Cestrum nocturnum*, also known as night-blooming jasmine, an evergreen woody shrub of the *Solanaceae* family, *Casearia corymbosa*, a 15-m high tree belonging to the *Salicaceae* family, and *Caesalpinia gaumeri*, a tree with deeply fluted and perforated trunk that belongs to the *Fabaceae* family. They also evaluated *Ehretia tinifolia*, a 25-m tree of the *Boraginaceae* family, whose pinguicas are traditionally used for nervous disorders and kidney problems, while the bark is used for wound healing, as well as *Manilkara zapota*, commonly known as the *sapodilla*, a long-lived, evergreen tree native from southern Mexico, Central America, and the Caribbean, which has curative properties against dysentery and diarrhea, fever, diuretics, high blood pressure, and pain caused by picket scorpion. Interestingly, nonpolar extracts of leaves from *C. nocturnum*, *C. corymbosa*, *C. gaumeri* and *E. tinifolia* showed different levels of antimalarial activity against both chloroquine-sensitive HB3 and chloroquine-resistant K1 strains of *P. falciparum*, with IC<sub>50</sub> ranging from

172.49 to >500 µg/ml. In the case of *M. zapota*, nonpolar extract of stem bark was the most effective with an IC<sub>50</sub> value higher than 500 µg/ml.

### 3. Mexican medicinal plants against *Trypanosoma cruzi*

#### 3.1. *Trypanosoma cruzi* and American trypanosomiasis

*T. cruzi* is the causative agent of American trypanosomiasis or Chagas disease, which is the third cause of death in Latin America after malaria and schistosomiasis. Between 16 and 18 million people are affected by this disease that kills annually about 50 thousand people; importantly, 100 million people (25% of the population of Latin America) are at risk of contracting this infection [50].

*T. cruzi* is mainly transmitted by a triatomine bug (*Triatoma infestans*). In the vector, trypomastigote goes into the epimastigote stage that reproduces through binary fission in midgut to form metacyclic trypomastigotes. This infectious stage enters the human host through the bite wound or by crossing mucous membranes, and transforms into amastigotes in infected cells. Intracellular amastigotes can evolve into trypomastigotes that burst out of the cell and enter the blood stream to be transmitted to another triatomine bug. Nonvector transmission has also been described, mainly through oral infection, blood transfusions, congenital transmission, organ transplantation, and laboratory accidents [51].

Chagas disease has an acute and a chronic phase. The acute phase lasts for the first few weeks or months of infection; it can be asymptomatic or include fever, fatigue, and local swelling (called chagoma). In the chronic phase, patients usually have cardiac abnormalities, as well as digestive, neurological, or mixed alterations; recently, it has been shown that they also have behavioral changes, such as psychomotor alterations, attention and memory deficits, as well as depression [52]. Chemotherapy involves the use of two drugs: nifurtimox and benznidazole. However, both agents have variable efficacy in the acute phase and are ineffective in the chronic stage; moreover, they produce severe adverse effects [53].

#### 3.2. Relevant studies about Mexican plant with activity against *Trypanosoma cruzi*

Because of the epidemiologic relevance of Chagas disease in Mexico, the traditional medicine has identified several Mexican plants that can help to control this infection (Table 1). Based on this knowledge, Abe et al. [54] performed a screening of crude methanolic extracts of several medicinal plants (20 families and 37 species) against epimastigotes of *T. cruzi*. Results showed that 18 extracts had a trypanocidal effect at a concentration of 2 mg/ml, and 13 extracts showed a trypanocidal activity at 1 mg/ml. The methanolic extract of root from *Aristolochia taliscana*, a medicinal species known as *guaco* that is used to treat bites of snakes, cough, diarrhea, and dermatological conditions, had the highest biological activity immobilizing all epimastigotes at a concentration of 0.5 mg/ml. Phytochemical study allowed the identification of six secondary metabolites: four neolignans (aupomatenoid-7 licarin A, aupomatenoid-1, and licarin B) and two lignans (austrobailignan-7 and fragransin E1). The best trypanocidal activity was

found for aupomatenoid-7 and fragrasin E1, with a minimum concentration (MC100) value of 25 and 50 µg/ml, respectively. The structure-activity relationship (SAR) analysis determined that loss of the hydroxyl group reduces the trypanocidal activity. In addition, the authors suggested that steric effects might be affecting the biological behavior.

Following with the search of new options for the treatment of Chagas disease, Abe et al. [55] studied another set of Mexican medicinal plants belonging to 41 families and 65 species. Only one extract had a strong trypanocidal activity against epimastigotes of *T. cruzi*, while 10 extracts presented a weak activity. However, 39 extracts showed a good activity against trypomastigotes since concentrations inducing 100% of the maximum response (MC100) were between 125 and 500 µg/ml. The methanolic extract of seed from *Persea americana* (Lauraceae family), a tree native from Central Mexico that produces avocado, showed the best activity on epimastigotes. The phytochemical analysis of the extract identified three 1,2,4-tri-hydroxyheptadec-16-ene derivatives, three 1,2,4-tri-hydroxyheptadec-16-yne derivatives, and two 1,2,4-trihydroxinonadecane derivatives. The most active compound was a 1,2,4-tri-hydroxyheptadec-16-ene (IC<sub>50</sub> = 82 and 49 µM against epimastigotes and trypomastigotes, respectively). The SAR analysis determined that the transformation of the group 16-ene terminal by a group 16-yne reduces the activity.

*Senna villosa* is a leguminous plant of southeastern Mexico, with antifungal and antimicrobial activities, usually used to treat stomach disorders (laxative), dysmenorrhea, or fungal infection. Phytochemicals analysis has identified alkaloids, sterols, flavonoids, and anthraquinones as secondary metabolites. Particularly, Jimenez-Coello et al. [56] showed that crude chloroformic extracts had trypanocidal activity *in vitro* against epimastigotes of *T. cruzi* at a concentration of 1.6 mg/ml. The main metabolite responsible for the activity in *in vitro* experiments and *in vivo* models (33.6 mg/g) was (8-hydroxymethylen)-trieicosanyl acetate. Therefore, the same group of investigations tested chloroformic extracts of *S. villosa* leaves against amastigotes of *T. cruzi* during the acute phase of infection. Results showed a reduction in the number of amastigotes in cardiac tissue at a dose of 3.3 mg/g compared with untreated mice [57].

Molina-Garza et al. [58] evaluated the trypanocidal activity of 10 plants used in traditional Mexican medicine for the treatment of parasitic infections: *Artemisia Mexicana*, *Castela texana*, *Cymbopogon citratus*, *Eryngium heterophyllum*, *Haematoxylum brasiletto*, *Lippia graveolens*, *Marrubium vulgare*, *Persea americana*, *Ruta chalepensis*, and *Schinus molle*. Methanolic extracts (150 mg/ml) of *E. heterophyllum*, *H. brasiletto*, *M. vulgare*, and *S. molle* produced growth inhibition (88–100%) of *T. cruzi* epimastigotes. *C. citratus* and *A. mexicana* led to 83% inhibition, *P. americana* and *R. chalepensis* to 70%, and *C. texana* and *L. graveolens* to 33% inhibition. The highest values of trypanocidal activity (7.92 and 11.24 mg/ml) were for *H. brasiletto* and *E. heterophyllum*, respectively. The phytochemical characterization of *H. brasiletto* indicated the presence of hematoxylin, brazilin, caffeic acid, gallic acid, methyl gallate, phloroglucinol, 4-hydroxycinnamic acid, and 5-methoxypsoralen. Constituents of *E. heterophyllum* extracts have not been described yet, although the presence of (*E*)-2-dodecanal, a metabolite with trypanocidal activity, has been found in the related specie *E. foetidum*. *H. brasiletto* extracts also have unsaturated compounds, including carbonyl groups, carboxyl groups, triterpenes, sesquiterpene lactone, quinones, flavonoids, and tannins [59].



*Carica papaya*, a giant herbaceous plant in the *Caricaceae* family, is originated in Central America and widely distributed in southern Mexico. It is traditionally used for diabetes treatment and birth control, as antiseptic, antimicrobial, or diuretic, to control parasites, lower blood pressure and cholesterol, and reduce inflammation, among others. Some data also indicated that it has antiprotozoal activity. Therefore, Jimenez-Coello et al. [60] evaluated the effects of extracts and a mixture of the main components of *C. papaya* against *T. cruzi* amastigotes during subacute phase and chronic disease. Results showed that chloroformic extract was able to reduce the number of amastigotes (55.5 and 69.7%) in cardiac tissue of infected mice during the subacute phase at a concentration of 50 and 75 mg/kg, respectively. The fatty acids mixture also exhibited a similar trypanocidal activity (56.45%); however, the total elimination of the parasite was not achieved. In the chronic phase of infection, the number of amastigotes was only reduced to 46.8 and 5.13% using the same concentrations. Therefore, the authors suggested the use of this extract in combination with other reference drug for a more efficient pharmacological treatment of Chagas disease.

*T. brucei* is the other pathogen genus that is responsible for the African trypanosomiasis also known as sleeping sickness. Due the medical relevance of this parasitic infection and problems with conventional treatments, medicinal plants have been investigated to develop alternative drugs. Unfortunately, the potential of Mexican medicinal plants against this parasite does not seem to have been investigated yet; therefore, we did not include this topic in the present review.

## 4. Mexican medicinal plants against *Leishmania*

### 4.1. *Leishmania* and leishmaniasis

Cutaneous, mucocutaneous, and visceral (kala-azar) leishmaniasis are caused by more than 20 species of *Leishmania*, mainly *L. donovani*, *L. infantum*, or *L. chagasi*, in the case of visceral disease, while cutaneous forms can be due to more than 15 different species. All species are morphologically identical, but specific biochemical and molecular characteristics allow their identification through isoenzyme analysis, molecular methods, or monoclonal antibodies. This set of parasitic infections affects 88 countries worldwide, 67 in the old world, and 21 in America. The large majority (90%) of visceral leishmaniasis cases is reported in only five countries: Bangladesh, India, Nepal, Sudan, and Brazil, while cutaneous leishmaniasis mainly affects seven countries: Afghanistan, Algeria, Brazil, Iran, Peru, Saudi Arabia, and Syria. The annual incidence is estimated at 1.5 million cases of cutaneous, mucocutaneous, and diffuse cutaneous leishmaniasis and, 500,000 cases of visceral leishmaniasis. There are 350 million people at risk of contracting the disease, which is associated with about 2.4 million people with disabilities and about 70,000 deaths per year [61, 62].

*Leishmania* infection begins with the inoculation of promastigotes by a sand fly from *Phlebotomus* or *Lutzomyia* genus during blood meals. After being phagocytized by macrophages or other mononuclear phagocytic cells, parasite evolves to amastigotes that multiply and infect other mononuclear cells. The life cycle is continued when a sand fly feeds on an



infected person and ingests amastigotes within macrophages. In the insect gut, amastigotes transform into promastigotes that migrate to the proboscis to be transmitted to another human host [63].

Cutaneous leishmaniasis is characterized by skin ulcers that are cured by themselves, while mucocutaneous leishmaniasis is associated with progressive infection with invasion and destruction of the nasopharyngeal mucosa. Symptoms of visceral leishmaniasis mainly include fever, weight loss, enlargement of spleen and liver, as well as low blood counts. Treatment depends on the *Leishmania* species, the clinical signs, the geographic region, and the immunologic status of the patient. Visceral leishmaniasis is usually treated with liposomal amphotericin B, and recently by miltefosine (MilteX), although the pentavalent antimony (SbV) and paromomycin (Humatin) are also used in developing countries. The same treatments can also be used for severe cases of cutaneous or mucocutaneous disease. Most of these drugs cause serious problems, including renal insufficiency. In addition, their high cost makes treatment unaffordable for most infected people. Therefore, a large number of patients discontinue the treatment, which promotes the emergence of resistant strains [64].

#### 4.2. Relevant studies about Mexican plant with activity against *Leishmania*

Besides the relevance of Leishmaniasis in Mexico, the study of Mexican plants as an option for the treatment of this infectious disease has been limited (Table 1). *Pentalinon andrieuxii*, a flowering plant in the *Apocynaceae* family, is a native plant of the state of Yucatan, Mexico that has been commonly used for the treatment of cutaneous leishmaniasis in southeastern Mexico. Lezama-Davila et al. [65] reported that aqueous and organic extracts of *P. andrieuxii* root (10 µg/ml) have activity on promastigotes of *L. mexicana* *in vitro*. The extracts of leaves and roots contain various secondary metabolites, mainly cardenolides, flavonoids, pregnane sterols, trinosesquiterpenoids, and triterpenoids. Phytochemical analyses of root hexane extract revealed 16 sterol derivatives, three coumarins, and one triterpenoid. The evaluation of their effect on promastigotes and amastigotes of *L. mexicana* showed that five sterols have a greater inhibitory effect than the reference drug, pentostam, after 48 h exposure on promastigotes; notably, 6,7-dihydroneridienone was the most active metabolite with an IC<sub>50</sub> value of 9.2 µM. These compounds were as effective as pentostam against amastigotes, with IC<sub>50</sub> values from 1.4 to 3.5 µM versus 2.7 µM, respectively. Cholest-4-en-3-one was the most active metabolite with an IC<sub>50</sub> value of 0.03 µM against the amastigote form. The SAR analysis showed the importance of fragment 4-ene-3-oxo in the steroidal system for the leishmanicidal effect, while the 3-ol-5-ene system reduced the antiparasitic activity. Variations in chain size on D ring of five members also influenced the activity. Interestingly, none of these compounds showed cytotoxic effects (IC<sub>50</sub> >100 µg/ml) on noninfected bone marrow-derived macrophages from C57BL16 mouse. Authors suggested that these compounds may act as antagonists of endogenous sterols, interfering or inhibiting sterol biosynthesis, causing alterations in membrane of *L. mexicana*, and leading to morphological abnormalities and destruction of amastigotes [66].

Infusion of *Laennecia confusa* (*Asteraceae* family), native of the states of Chihuahua and Chiapas, Mexico, is used as sedative and treatment for alcohol addiction. The genus *Laennecia* contains several secondary metabolites, such as terpenoids, terpenes, saponoides, flavonoids, sterols,

lactones, and tannins, among others. When Martínez-Ruiz et al. [67] evaluated the trypanocidal potential of *L. confusa*, they confirmed the presence of flavonoids, cyanogenic glycosides and cardiotonic, saponins, sesquiterpene lactones, and triterpenes, in 71 fractions obtained from aqueous, hexanic, methanolic, and chloroformic extracts. Aqueous and chloroformic extracts caused a significant growth reduction of *L. donovani* with IC<sub>50</sub> values around 20 µg/ml; interestingly, the IC<sub>50</sub> value decreased to 200 µg/ml for a specific fraction of the chloroformic extract. Unfortunately, all compounds exhibited toxicity on macrophages.

*Lopezia racemosa* (also known as *L. mexicana*, *L. hirsute* Jacq.), widely distributed in Mexico, is traditionally used for skin infections, stomach cancer, and urinary retention, among others. It has been reported that plants of the *Onagraceae* family contain tannins, flavonoids, and sterols as metabolic constituents; however, *L. racemosa* has not been submitted to phytochemical studies yet. Cruz-Paredes et al. [68] evaluated the effect of hexane, chloroform, and methanol extracts (HE, CE, and ME) of *L. racemosa* and their fractions on *L. donovani* promastigotes. Interestingly, HE 11-14b and ME 28-36 fractions and CE produced a high reduction (88%) in parasites number when compared with untreated controls. However, most extracts and fractions had a toxic effect on human-derived macrophages (THP-1); only fraction 28–36 ME showed no significant cytotoxicity (below 25%) (IC<sub>50</sub> = 770 µg/ml). The authors hypothesized that the high amount of polyphenols (tannins and flavonoids) present in this plant may be responsible for the biological activity.

## 5. Mexican medicinal plants against gastrointestinal protozoan parasites

### 5.1. *Entamoeba histolytica* and *Giardia lamblia*

Gastrointestinal diseases occur worldwide and are associated with poor sanitary conditions, overcrowding, poor water quality control, and low socioeconomic level. Different microorganisms can produce these symptoms; two of them are the protozoan parasites *E. histolytica* and *G. lamblia* (or *G. intestinalis* or *G. duodenalis*). *E. histolytica* is responsible for human amoebiasis. Trophozoites live and proliferate in the intestinal tract by eating bacteria and cellular debris. In some cases, parasites cross the epithelial wall to reach the bloodstream and spread throughout the body to invade other organs, mainly liver, as well as lungs, brain, or spleen. Trophozoites can also form cysts that are eliminated with feces. Most infected patients are asymptomatic; others present a wide range of symptoms including diarrhea, stomachache, and hemorrhagic colitis. The extraintestinal localization of trophozoites can produce fatal abscesses. Amoebiasis remains a major health problem, affecting more than 10% of the world's population, mainly in developing countries. Globally, it accounts for 50 million clinical cases and is responsible for approximately 110,000 deaths annually, which makes it the second-leading cause of death from a protozoan parasite after malaria [69–71]. Giardiasis, also called Beaver fever, is the other common intestinal infection associated with diarrhea, producing over 250 million symptomatic human infections per year worldwide, with a high prevalence in children in developing countries. The flagellated protozoan *G. lamblia* is the causal agent of giardiasis. Colonization of the small intestine produces acute or chronic diarrhea, mal absorption, excess gas, stomach or abdominal cramps, nausea, and failure to thrive. *Giardia* infection also alters

child linear growth and psychomotor development, due to iron-deficiency anemia, micronutrient deficiencies and growth retardation associated with diarrhea and malabsorption syndrome [72]. Both *E. histolytica* and *G. lamblia* are transmitted by the fecal-oral route, through ingestion of food and water that have been contaminated by feces of an infected host.

Metronidazole and other 5-nitroimidazoles are the drugs of choice against *E. histolytica* and *G. lamblia*; however, there are some reports about their mutagenicity in bacteria and their carcinogenic effects in rodents. Additionally, metronidazole provokes several side effects, including headache, dry mouth, metallic taste, glossitis, and urticaria [73–75].

## 5.2. Relevant studies about Mexican plants with activity against *Entamoeba histolytica* and *Giardia lamblia*

Mexican native communities use a large number of plants to treat intestinal ailments. However, only few species have been scientifically evaluated to confirm their potential such as anti-*Giardia* or anti-*Entamoeba* treatments (**Table 1**). *Zanthoxylum liebmannianum*, commonly known as *Colopahtle*, is recommended for the treatment of stomachaches, amoebiasis, intestinal parasites, and as a local anesthetic agent. The crude ethanol extract from leaves of *Z. liebmannianum* exhibited an inhibitory effect on the proliferation of *E. histolytica* and *G. lamblia* trophozoites with IC<sub>50</sub> values of 3.48 and 58.00 µg/ml, respectively. Asarinin, hyperin, β-sitosterol, and β-sitosterol glucoside were isolated from this extract. Among them, asarinin was the most active compound with IC<sub>50</sub> values of 19.86 µg/ml for *E. histolytica* and 35.45 µg/ml for *G. lamblia* [76].

In 2003, Calzada et al. [77] reported the isolation and antiprotozoal activity of one coumaric acid derivative, named melilotoside, and the flavonoids pinocembrine, pinostrobin, chrysin, narcissin, and rutin from *Teloxys graveolens*, a medicinal plant traditionally used to control some gastrointestinal diseases. Melilotoside exhibited the most potent activity toward *E. histolytica* and *G. lamblia* with IC<sub>50</sub> values of 12.5 and 16.8 µg/ml, respectively. Interestingly, narcissin showed selectivity against *E. histolytica* (IC<sub>50</sub> = 17.2 µg/ml).

The same year, Alanís et al. [78] isolated (-)-epicatechin, (+)-catechin, hyperin, nigaichigoside F1, B-sitosterol 3-O-β-D-glucopyranoside, gallic acid, and ellagic acid from *Rubus coriifolius*, a medicinal plant used by the Maya communities in southern Mexico to treat bloody diarrhea. These compounds had activity against *E. histolytica* and *G. lamblia* trophozoites, being (-)-epicatechin the most potent molecule with the IC<sub>50</sub> values of 1.9 and 1.6 µg/ml, respectively. (-)-Epicatechin is also obtained from *Geranium mexicanum*, with the vernacular name *pata de leon*, an endemic Mexican species used as purgative, and as a remedy against tonsillitis, cough, whooping cough, urticaria, dysentery, and diarrhea. This flavonoid was active against *E. histolytica* and *G. lamblia* with IC<sub>50</sub> values ranging from 1.9 to 79.2 µg/ml for *E. histolytica* and from 1.6 to 100.4 µg/ml for *G. lamblia*. In addition, *G. mexicanum* contains (+)-catechin, tyramine, and β-sitosterol 3-O-β-D-glucopyranoside, but they only had a moderate activity against these protozoan parasites [79].

In northeast Mexico, indigenous populations use infusion of leaves from *Artemisia ludoviciana* as an antidiarrheal treatment. Aqueous, methanolic, acetonic, and hexanic leaf extracts from plants collected in Monterrey City, Mexico, were found to be active *in vitro* against both

*E. histolytica* and *G. lamblia* trophozoites. Particularly, the acetonic (IC<sub>50</sub> = 117.2 µg/ml) and hexanic (122.7 µg/ml) extracts showed an interesting activity against *E. histolytica*, while the hexanic extract had the highest effect upon *G. lamblia* (IC<sub>50</sub> = 137.4 µg/ml) [80]. *A. ludoviciana* was also studied by Ramos-Guerra et al. [81], together with *M. vulgare*, *Mentha spicata*, and *Chenopodium ambrosioides* that are also popularly used against intestinal disorders. Surprisingly, *A. ludoviciana* was inactive against both protozoan species (IC<sub>50</sub> >100 µg/mL) in this work. Acetonic and methanolic extracts from *M. vulgare* were very active against *G. lamblia* with an IC<sub>50</sub> = 7 and 12 µg/ml, respectively, and slightly to moderately toxic to *E. histolytica* (IC<sub>50</sub> = 90 and 34 µg/ml, respectively). Hexanic, acetonic, and methanolic extracts from *M. spicata* were also very potent against *G. lamblia* (IC<sub>50</sub> = 17, 13, and 8 µg/ml, respectively) while only the acetonic extract was slightly active against *E. histolytica* (IC<sub>50</sub> = 98 µg/ml). Hexanic and acetic *C. ambrosioides* extracts were moderately active against amoeba (IC<sub>50</sub> = 57 and 58 µg/ml). The highest activity against both protozoan species was obtained with organic extract from *M. vulgare* and *M. spicata*, which require further studies to identify the active compounds.

*Decachaeta incompta* is a Mesoamerican flowering plant that has been traditionally used in Oaxaca, as well as in Chiapas, Colima, Guerrero, Michoacán, Mexico State, Jalisco, and Puebla, Mexico. Its antiprotozoal properties have been confirmed since the dichloromethane extract of leaves was effective against *E. histolytica* and *G. lamblia* trophozoites (IC<sub>50</sub> values of 132.5 and 141.4 µg/ml, respectively). Bioassay-guided fractionation of crude extract resulted in the isolation of four sesquiterpene lactones named incomptines. Incomptine A, a sesquiterpene lactone of the heliangolide type, appeared to be the most potent antiamoebic and anti giardial compound with IC<sub>50</sub> values of 2.6 µg/ml for *E. histolytica* and 18.1 µg/ml for *G. lamblia*. Its potency against *E. histolytica* was close to that of emetine (IC<sub>50</sub> 1.05 µg/ml) [82]. Recently, we used a proteomic approach based on two-dimensional gel electrophoresis and electrospray ionization tandem mass spectrometry (ESI-MS/MS) analysis to get insights into the molecular mechanisms involved in the antiamoebic activity of incomptine A. Our results evidenced the differential expression of 21 *E. histolytica* proteins in response to incomptine A treatment. Notably, three glycolytic enzymes, namely enolase, pyruvate:ferredoxin oxidoreductase and fructose-1,6-biphosphate aldolase, were downregulated. In addition, we observed an increased number of glycogen granules through ultrastructural analysis of trophozoites by electronic microscopy. Based on these data, we proposed that incomptine A could affect *E. histolytica* growth through alteration of energy metabolism [83].

*Salvia polystachya* Ort. (*Lamiaceae*), popularly known as *chia* is used in Mexican traditional medicine as a purgative, antigastralgalic, antipyretic, and to treat dysentery. In 2010, Calzada et al. [84] evaluated the possible antiprotozoal *in vitro* activity of the crude extract and four neo-clerodane diterpenoids from *S. polystachya*. They found that linearolactone was the most potent antiamoebic and anti giardial compound with IC<sub>50</sub> values of 22.9 and 28.2 µM, respectively. Polystachynes A, B, and D showed moderate antiprotozoal activity with IC<sub>50</sub> values ranging from 117.0 to 160.6 µM for *E. histolytica* and from 107.5 to 134.7 µM for *G. lamblia*.

Since amoebiasis and giardiasis share intestinal symptoms, several groups of investigation used a screening approach to simultaneously evaluate the antiamoebic and anti giardial effects



of a large number of Mexican medicinal plants that are recommended for gastrointestinal diseases. In 2006, Calzada et al. [85] studied 26 plants and found that methanolic extract obtained from *Chiranthodendron pentadactylon*, *Annona cherimola*, and *Punica granatum* was the most effective on *E. histolytica* with  $IC_{50} < 30 \mu\text{g/ml}$ . Interestingly, *C. pentadactylon* had an  $IC_{50}$  value of  $2.5 \mu\text{g/ml}$ , which is close to the  $IC_{50}$  value of emetine, but far less than metronidazole used as control drugs. On the other hand, extracts of *Dorstenia contrajerva*, *Senna villosa*, and *R. chalepensis* were the most active toward *G. lamblia* with  $IC_{50} < 38 \mu\text{g/ml}$ . Recently, Camacho-Corona et al. [86] showed that the dichloromethane/methanol extract of *Larrea tridentata*, also known as *gobernadora* (governess) and *hediondilla* (little smelly one) in Mexico, exhibits a moderate inhibitory activity against *E. histolytica* ( $IC_{50} = 100 \mu\text{g/ml}$ ). The extract of *Hyptis albida* was the most active against *G. lamblia* with an  $IC_{50}$  value of  $16.11 \mu\text{g/ml}$ . Extracts of *Crataegus mexicana*, *Ocimum basilicum*, and *L. tridentata* exhibited a moderate activity against *G. lamblia* with  $IC_{50}$  values of 153 and  $116 \mu\text{g/ml}$ , respectively.

### 5.3. Relevant studies about Mexican plant with activity against *Entamoeba histolytica*

Although amoebiasis and giardiasis share several symptoms, the protozoan parasites that are responsible for these infectious diseases are quite different. Therefore, several investigators focused their research on *Entamoeba* or *Giardia* to confirm the ethnobotanical properties of Mexican medicinal plants used to treat intestinal diseases and identify the phytochemicals that are responsible for their activity against these endemic pathogens (Table 1). Thus, Calzada et al. [87] reported scientific findings that support the ethnomedical use of roots of *Lepidium virginicum*, a herb of the highlands of Chiapas, Mexico, which is recommended for the treatment of diarrhea and dysentery. The crude extract of *L. virginicum* roots exhibited *in vitro* activity against *E. histolytica* trophozoites ( $IC_{50} = 100.1 \mu\text{g/ml}$ ). Extract fractionation revealed that benzyl glucosinolate is responsible for this activity with an  $IC_{50}$  of  $20.4 \mu\text{g/ml}$ . Later, Quintanilla-Licea et al. [88] performed an anti-amoebic screening among methanolic extracts of 32 plants used in northeast Mexican traditional medicine. Six extracts induced more than 80% growth inhibition at a concentration of  $150 \mu\text{g/ml}$ . *L. graveolens* Kunth and *R. chalepensis* Pers. showed the most significant antiprotozoal activity (91.54 and 90.50% growth inhibition at a concentration of  $150 \mu\text{g/ml}$  with  $IC_{50}$  values of 59.14 and  $60.07 \mu\text{g/ml}$ , respectively). Bioassay-guided fractionation of the methanolic extracts afforded carvacrol ( $IC_{50} = 44.3 \mu\text{g/ml}$ ) and chalepentin ( $IC_{50} = 45.95 \mu\text{g/ml}$ ), respectively, as bioactive compounds. Recently, Herrera-Martínez et al. [89] reported that ethyl acetate extract of *Adenophyllum aurantium* root exhibits anti-amoebic activity *in vitro* with an  $IC_{50}$  of  $230 \mu\text{g/ml}$ . This extract was also able to inhibit the encystation process of *E. invadens*, the protozoan parasite of reptiles. Interestingly, this extract affected virulence properties of amoeba, since the intraperitoneal administration (2.5 or 5 mg) to *E. histolytica*-infected hamsters prevented the development of amoebic liver abscesses in 48.5 or 89.0% of the animals, respectively. Moreover, adhesion and erythrophagocytosis were 28.7 and 37.5% inhibited, respectively. These effects were associated with alterations in trophozoite organization, namely a reduced number of vacuoles and alterations in the actin cytoskeleton. Thiophenes were identified as the major components by carbon-13 nuclear magnetic resonance ( $^{13}\text{C}$ -NMR) analysis; however, their relevance for anti-amoebic activity remains to be confirmed.



#### 5.4. Relevant studies about Mexican plant with activity against *Giardia lamblia*

In an attempt to characterize the *in vitro* activity against *Giardia*, Ponce-Macotella et al. [90] evaluated 14 medicinal plants commonly used as antidiarrheic and antiparasitic treatment in Mexico. Nine species presented a clear anti-*giardial* effect when they were used at the concentrations traditionally recommended. Notably, *Justicia spicigera*, *Lipia berlandieri*, and *Psidium guajava* produced a higher mortality ( $91 \pm 0.5\%$ ,  $90 \pm 0.6\%$ , and  $87 \pm 1.0\%$ , respectively) than tinidazole used as reference drug ( $79 \pm 1.9\%$ ). Later, the same group of investigation reported that trophozoites exposed to *J. spicigera* extract have significant changes in ultrastructure, mainly modification in size and shape, as well as damage in nucleus structure, which may be due to alterations in the pattern of nucleoskeleton proteins as a result of the effects of plant phytochemicals [91, 92].

The state of Yucatan, Mexico, is a rich source of Mayan medicinal plants for treatment of dysentery, gastritis, gastric ulcers, and other intestinal problems. In 2002, Ankli et al. [49] confirmed that six species have activity against *G. lamblia* with IC<sub>50</sub> values less than 100 µg/ml, three of them with minimum inhibitory concentration (MIC) values less than 100 µg/ml. The most active extract was the nonpolar extract A of *Crossopetalum gaumeri* (MIC = 6.3 µg/ml), whereas the polar extract B showed a very weak antiprotozoal activity. The nonpolar and polar extracts of *Psidium sartorianum*, *Piscidia piscipula*, *Bidens squarrosa* and *Casimiroa tetrameria* and the nonpolar fraction of *Bauhinia divaricata* showed weak activity with IC<sub>50</sub> values between 20 and 90 µg/ml. Later, Peraza-Sánchez et al. [93] demonstrated the *in vitro* anti-*giardial* activity of another set of 10 native plants from Yucatan, Mexico: *Byrsonima crassifolia* (L.) Kunth, *Cupania dentata* DC., *Diphysa carthagenensis* Jacq., *Dorstenia contrajerva* L., *Gliricidia sepium* (Jacq.) Kunth ex Walp., *Justicia spicigera* Schldl., *Pluchea odorata* (L.) Cass., *Spigelia anthelmia* L., *Tridax procumbens* L., and *Triumfetta semitriloba* Jacq. The extract obtained from *T. procumbens* was the most active (IC<sub>50</sub> = 6.34 µg/ml), followed by *C. dentata* (IC<sub>50</sub> = 7.59 µg/ml), *D. carthagenensis* (IC<sub>50</sub> = 11.53 µg/ml), and *B. crassifolia* (IC<sub>50</sub> = 15.55 µg/ml). *G. sepium*, *J. spicigera*, *P. odorata*, *S. anthelmia*, and *T. semitriloba* were active in the range from 46.41 to 117.41 µg/ml. *Hippocratea excelsa* is another Mayan medicinal plant with a confirmed anti-*Giardia* activity. From the different triterpenoids that have been isolated from the root bark of *H. excelsa*, pristimerine and tingenone were the most active compounds with IC<sub>50</sub> values of 0.11 and 0.74 µM, respectively [94].

Barbosa et al. [95] isolated the flavonoids kaempferol, tiliroside and (–)-epicatechin from *G. mexicanum*, *Cuphea pinetorum*, *Helianthemum glomeratum*, and *Rubus coriifolius*, which are medicinal plants used for the treatment of gastrointestinal disorders in Mexico, and evaluated their antiprotozoal activity in suckling females CD-1 mice infected with *G. lamblia*. The most active flavonoid was (–)-epicatechin (ED<sub>50</sub> = 0.072 µmol/kg); its activity was even stronger than that of metronidazole and emetine used as reference drugs. In the case of kaempferol and tiliroside, their potency was close to that of metronidazole, but far less than emetine (ED<sub>50</sub>=2.057 and 1.429 µmol/kg, respectively).

*C. dentata* (*Sapindaceae* family) is traditionally used against inflammation in Veracruz, Mexico and pain in Quintana Roo, Mexico. Hernández-Chávez et al. [96] showed that methanolic, hexanic, dichloromethane, ethyl acetate and butanolic extracts of *C. dentata* are able to inhibit

the proliferation of *Giardia* trophozoites (IC<sub>50</sub> = 8.17, 4.42, 2.12, 9.52 and 6.5 µg/ml, respectively). The phytochemical study of fractions resulted in the isolation of taraxerone, taraxerol, scopoletin, and two mixtures of steroidal compounds. Among them, taraxerone was the metabolite with the highest giardicidal activity (IC<sub>50</sub> = 11.33 µg/ml).

## 6. Mexican medicinal plants against *Trichomonas vaginalis*

### 6.1. *Trichomonas vaginalis* and trichomoniasis

*T. vaginalis* is an anaerobic flagellated protozoan that lives and replicates by binary fission in the urogenital tract of humans, namely vulva, vagina, or urethra, in women, and urethra, prostate and epididymis in men. The “pear” shaped trophozoite (10–20 µm in length) is the unique morphological stage for this monoxen parasite for which human is the only host. Trichomoniasis represents the most prevalent nonviral sexually transmitted infection in the world, affecting around 250 million people annually [97]. In Mexico, a recent report revealed that trichomoniasis is at the 12th place among the 20 principal causes of infectious diseases with a rate of 104.23 cases per 100,000 individuals. Women are more affected than men at a ratio of 36:1 and women aged 25–44 years represent the mayor number of cases (almost 60,000 infected women in 2011) [98]. At least 50% of infected individuals are asymptomatic; they are neither detected nor treated, which makes trichomoniasis a neglected parasitic infection that can silently spread worldwide [99]. Symptomatic women develop vaginitis, cervicitis, urethritis, a malodourous seropurulent vaginal discharge and infertility. Moreover, *Trichomonas* infection has been linked to bad pregnancy outcomes (preterm birth, low birth weight, and respiratory infections in the newborn). Importantly, trichomoniasis is an enhanced risk factor of getting or spreading other sexually transmitted infections, such as human immunodeficiency virus (HIV), papilloma virus (HPV) and herpes simplex virus II (HSV-2) [100–102]. Men usually represent the short-term reservoir of *T. vaginalis*, but they may also suffer from urethritis [103]. In addition, an association with worse prostate cancer prognosis has been reported [104].

Since the early 60s, the drug of choice for treating trichomoniasis is metronidazole and its derivatives (tinidazole and secnidazole) [105]. As in the case of other anaerobic protozoan pathogens, important side effects have been reported, including headache, nausea, gastrointestinal disturbance, and anorexia, as well as cytotoxic effects, which limit the efficacy of the treatment [106, 107]. However, the main cause of treatment failure is the resistance of parasite to 5-nitroimidazole derivatives. MTZ resistance has been observed in 5–20% of patients [108] and around 10% of clinical isolates are 5-nitroimidazoles resistant *in vitro* [109]. In this context, new treatments for trichomoniasis are necessary.

### 6.2. Relevant studies about Mexican plant with activity against *Trichomonas vaginalis*

With the purpose of searching for new drugs for the control of trichomoniasis, several groups performed *in vitro* susceptibility assays to identify the anti-*Trichomonas* activity of Mexican plants that were selected on the basis of chemotaxonomical criteria, as well as ethnobotanical

and ethnopharmacological uses for the treatment of clinical signs associated with trichomoniasis, such as abdominal pain, colic, and vaginal discharge (**Table 1**).

In 2007, Calzada et al. [110] reported the antitrichomonal effect of methanol extracts of *Carica papaya* and *Cocos nucifera* (IC<sub>50</sub> values of 5.6 and 5.8 µg/ml, respectively), as well as *Bocconia frutescens*, *G. mexicanum*, and *Lygodium venustum* (IC<sub>50</sub> values ranging from 30.9 to 60.9 µg/ml) collected in six states of the country, namely Mexico City, State of Mexico, Hidalgo, Guanajuato, Sinaloa, and Yucatan, Mexico. The genotoxicity of the sanguinarine alkaloid present in *B. frutescens* could explain the antiprotozoal activity of the extract.

In another study, Moo-Puc et al. [111] evaluated dichloromethane:methanol extracts of 25 tropical seaweeds (12 *Rhodophyta*, 5 *Phaeophyta*, and 8 *Chlorophyta*) from the coast of Gulf of Mexico and Caribbean in Yucatan, Mexico. The most active algal extracts were from *Lobophora variegata* (*Phaeophyta*) and *Udotea conglutinata* (*Chlorophyta*), with IC<sub>50</sub> values of  $1.3 \pm 0.7$  and  $1.6 \pm 0.1$  µg/ml, respectively. Although their investigation did not involve structure elucidation, the authors suggested that this effect could be due to the presence of terpenes and polyphenols that are known antiprotozoal compounds [112]. Interestingly, extracts were not toxic for Madin-Darby canine kidney (MDCK) cells. The further characterization of the brown alga *L. variegata* revealed its antioxidant activity [113]. Fractionation using different solvents and isolation of antiprotozoal constituents indicated that the chloroformic fraction was the most effective against *T. vaginalis* due to the presence of sulfoquinovosyl-diacylglycerols 1–3 (SQDGs 1–3) according to chromatographic fractionation on Sephadex LH-20, chemical and enzymatic hydrolysis, as well as analysis of fast atom-mass spectrometry (FAB-MS) and NMR spectroscopic data. The mixture of SQDGs 1–3 only had a moderate activity against *T. vaginalis* trophozoites (IC<sub>50</sub> = 8 µg/ml), being less effective than the whole extract. The authors concluded that crude extract and nonpolar fractions from *L. variegata*, mainly the ethyl acetate fraction, should contain the major inhibitory compounds [114].

In addition to their hypolipemic [115] and hypoglycemic [116] effects in animal models, extract obtained from *P. americana* seeds has activity against several fungi [117], bacteria [118], and protozoan parasites [90]. Notably, Jiménez-Arellanes et al. [119] showed that chloroformic and ethanolic extracts of *P. americana* seeds obtained from the town of Ario de Rosales in the state of Michoacan, Mexico, displayed significant activity against *T. vaginalis* (IC<sub>50</sub> = 0.524 and 0.533 µg/ml, respectively). According to a preliminary analysis, these extracts contain β-sitosterol, phytol and palmitic acid, and catechin and epicatechin, respectively, which could be responsible for the antiprotozoal activity.

## 7. Conclusion

It is clear that antiparasitic drugs currently available have been essential to control, at least partially, the spread and illnesses related to malaria, trypanosomiasis, leishmaniasis, amoebiasis, giardiasis, and trichomoniasis. However, besides the existence of this chemotherapeutic arsenal, these infections still represent a huge threat for human health worldwide, particularly in developing countries. Failure in parasite elimination is mainly due to drug toxicity and

emergence of drug resistance in both parasites and vectors. Thus, one of the main contemporary challenges in global health is to find new, efficient and safe alternatives to prevent the establishment of drug resistance strains. Mexican medicinal plants are recognized as important sources of therapeutic compounds. Particularly, the present review supports the popular uses of plants from different regions of Mexico for the treatment of some of the most prevalent parasitic infections (**Figure 1**). In addition, it clearly highlights their potential for the isolation and identification of new antiparasitic molecules. Unfortunately, it is worth noting that most extracts, fractions, or isolated molecules tested were less or as efficient as the drug of choice for each pathogen. This could be resolved by chemical modifications of the initial structure to improve the stability of the molecule and its antiparasitic activity. The identification of the biochemical targets could also allow the design of more active molecules through bioinformatics screening and docking studies. On the other hand, prospective studies aimed to improve delivery systems *in vivo* should help to circumvent the drawbacks related to stability, bioavailability, and integrity of natural compounds. Some of these techniques currently used with phytochemicals include nano- and microencapsulation in polymers of natural or synthetic origin, or lipids. Another important point is the necessity of toxicity and mutagenicity tests to confirm the safety of the most promising molecules.

## Abbreviations

<sup>13</sup> C-NMR	Carbon-13 nuclear magnetic resonance
ACT	Artemisinin-based combination therapy
ADQ	Amodiaquine
AS	Artesunate
ATM	Artemether
CE	Chloroform extract
DHA	Dihydroartemisinin
ED50	Effective dose 50
ESI-MS/MS	Electrospray ionization tandem mass spectrometry
FAB-MS	Fast atom bombardment-mass spectrometry
HE	Hexane extract
IC50	Half maximal inhibitory concentration
LD50	Median lethal dose
LMF	Lumefantrine
MC100	Concentration inducing 100% of the maximum response
ME	Methanol extract
MeOHe	Methanolic extract
MFQ	Mefloquine
MIC	Minimum inhibitory concentration
MTZ	Metronidazole
PPQ	Piperaquine
SM50	Security margin 50
WHO	World Health Organization



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