Chapter 4

Can the Cure for Chagas' Disease be Found in Nature?

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Additional information is available at the end of the chapter

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Abstract

Nature is a skilled factory that produces a wide variety of secondary metabolites known as natural products. Those compounds synthesized by living organisms are usually related to their vital processes. Many drugs used nowadays, had its origins in medicinal plants and other organisms such as herbs, fungi and sponges. Hence, those sources constitute a viable alternative to conventional medicine in many developing countries. In other hand, protozoan diseases like Chagas, represent a health threat causing mortality to populations around the world. The classic treatment for Chagas' disease is chemotherapic and includes benznidazole and nifurtimox, although, the search for new drugs still remains. Triatomines that may spread Chagas can also be controlled making use of the insecticide property of certain plants. After literature survey it was found, classes of natural products, plant extracts, essential oils, and other natural sources that have shown activity against *T. cruzi*. In this context, many substances were tested *in vitro* and *in vivo* assays to verify trypanocidal efficacy. Promising results were published regarding to compounds arising from plants and sponges that showed high toxicity on different forms of the parasite with low toxicity on mammalian cells, although few were clinically tested on Chagas' disease.

Keywords: medicinal plants, natural products chemistry, Chagas' disease, *Trypanosoma cruzi*

1. Introduction

Plants have been used for many centuries with the purpose of feeding populations worldwide and to establish or bring back health, well-being, and the cure for several illnesses. The use of medicinal plants is very advantageous in terms of resource on chemical and biological research in natural products area. The plant secondary metabolism yields a wide range of chemical compounds, most of them highly bioactive and whose structural diversity is continuously evolving together with plants [1]. In vegetables, these compounds are the main responsible for

chemical defence against fungi, phytopathogens, birds, and other natural predators, being also used by plants to attract pollinators as well, being indispensable to guarantee plant's survival and its spreading through the globe. However, human population takes advantage of these remarkable properties and uses some compounds produced by diverse organisms including plants, fungi, and sponges to develop new medicines. Those metabolites coming from natural sources will promote the desirable healing action, bringing fewer side effects to the users.

In addition to teas, infusions, plasters, and herbal medicines, many traditional "western drugs" that are widely used nowadays had its origins on medicinal plants, such as (1) aspirin (acetylsalicylic acid—*Spiraea* spp.), (2) artemisinin (sweet wormwood—*Artemisia annua*), and more recently, (3) taxol (or paclitaxel from Pacific yew—*Taxus brevifolia*) on **Figure 1** can exemplify [2]. Therefore, nature is an endless source of bioactive substances, as plants can convert just carbon dioxide and water through photosynthesis to produce highly complex organic molecules that could be very useful in human health.

Medicinal plant species constitute a valuable alternative to conventional medicine in many developing countries; especially in poor communities that inhabit rural areas, lacking access to health services. Several of them use plants as the primary health care, as teas, plasters, infusions, and ointments among others. The traditional use of medicinal plants and natural remedies with no established efficacy and safety is a widespread in many countries around the world. Accordingly, all the information about ethnobotany is of utmost importance: this kind of millenary knowledge built during centuries usually combines information from native indigenous culture, together with acquirements brought by the Europeans and the Africans and provides a more rational use for the local biodiversity.

In other hand, protozoan diseases represent an important health threat in countries of tropical and subtropical regions, causing mortality to their populations [3]. Many neglected tropical diseases (NTDs) transmitted by parasites are reported to have life cycles including man as a secondary host, in which they cause disease. About 37 million individuals are presently

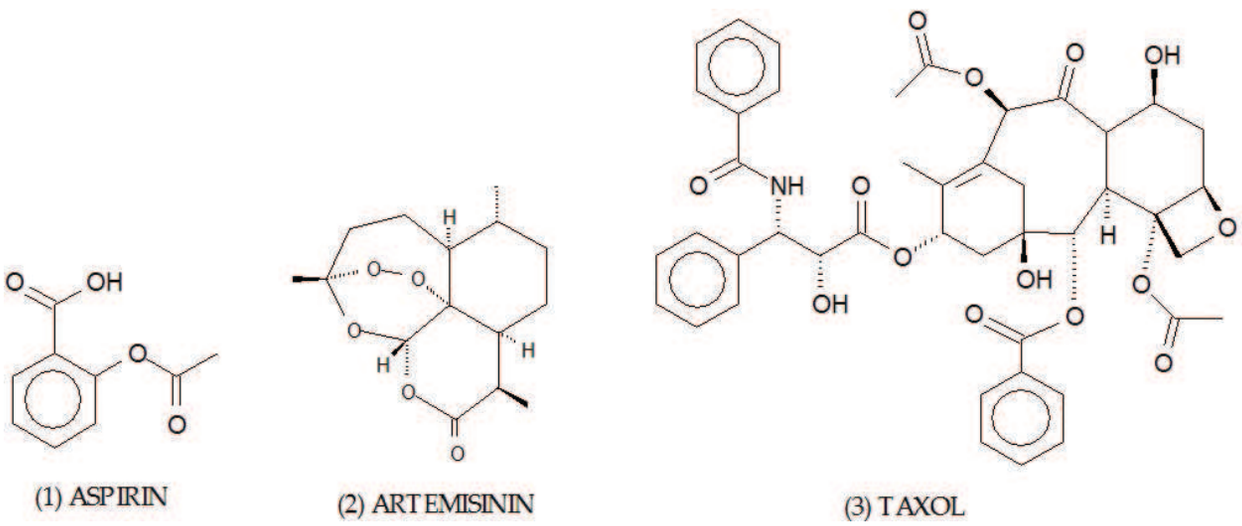

Figure 1. Important drugs from medicinal plants: aspirin (1), artemisinin (2), and taxol (3).

infected by parasites around the world. Together with malaria and amoebiasis, the parasitic illnesses are the main cause of thereabout one million deaths per year. Infections caused by protozoan species such as *Trypanosoma*, *Plasmodium*, and *Leishmania* are a major worldwide health problem causing significant morbidity and mortality in the poorest countries like Africa, Asia, and Latin America for instance.

Leishmaniasis, Chagas' disease, and human African trypanosomiasis (HAT) are among the most important protozoan parasitic illnesses caused by trypanosomatids. Chagas' disease, also known as American trypanosomiasis, is a widespread disease, caused by the kinetoplastid protozoan Tripanosome—*Trypanosoma cruzi*. It is estimated that about 8 million in America are currently infected. However, this disease is expanding worldwide due to migration phenomena. The parasites that have a kinetoplast and a single flagellum are characterized as *Trypanosomatids* [3].

That's why Chagas is recognized as one of the most devastating diseases caused by the parasites of the *Trypanosomatidae* family. The most epidemiologically important form of transmission is through the bite of vector, triatomine hematophagous insects such as *Triatoma infestans* (kissing bug or barbeiro in Brazil). Nevertheless, congenital and transfusion are also relevant for the transmission cycle, since they are responsible for the advancement of this disease in nonendemic areas [4]. These diseases represent significant health problems in endemic countries, and this situation is aggravated by the increasing on treatment failures with available drugs as we will discuss in detail later.

This chapter is therefore aimed to review the great potential of natural products that are available in nature (mainly plants and sponges) regarding to the prevention and treatment of Chagas' disease and the combat of triatomine bugs.

2. Neglected tropical diseases

Neglected tropical diseases (NTDs) are often chronic and debilitating illnesses that currently affect over one billion people worldwide. NTDs are a diverse group of infectious diseases that affect primarily rural and low-income populations residing in tropical and subtropical regions worldwide. The World Health Organization (WHO) officially recognized nowadays 17 NTDs, comprising a highly diverse group of bacterial, protozoan, and helminth infections, transmitted via insects, contaminated food, water, and soil, and/or through human-to-human contact. These diseases cause easily over 200,000 deaths per year affecting many millions more around the globe, although the number of new infections appears to be dwindling. NTDs include the three major protozoan diseases: human African trypanosomiasis (HAT or "sleeping sickness"), Chagas' disease, and leishmaniasis [5]. Dengue, foodborne trematodiases, leprosy, lymphatic filariasis, schistosomiasis, soil transmitted helminthiasis, and trachoma [6] are also classified as NTDs.

The socioeconomic impact of NTDs in the developing countries surpasses that of any other infectious disease (with exception of HIV/AIDS) and perhaps may have permanent

socioeconomic effects on many nations. It is such a waste that billions of dollars of productivity are lost to NTDs every year in treatment and prevention costs, besides bearing 149 countries plus the information that the threat of NTDs is no longer confined to nations where these diseases are endemic. Due to globalization and the increasing social, financial, and technological connectedness, the burden to carry NTDs has become global issues.

Massive efforts of community activists, health care workers, scientists, politicians, and economists are required to reduce significantly the significance of public health liability that NTDs oblige. The most effective approach for reducing these diseases is still prevention, due to the absence of affordable or effective curative therapies and the deficiency of preventive vaccines. Between such relevant public health issues and many lives directly or indirectly affected by NTDs, there is education that offers a solution to connect NTD prevention to treatment efforts [7].

2.1. Chagas' disease

Trypanosomiasis is a group of parasitic diseases caused by protozoan from *Trypanosoma* genus. It is caused by trypanosomes of the species *Trypanosoma brucei*. There are two types that infect humans, *Trypanosoma brucei gambiense* (Tbg) and *Trypanosoma brucei rhodesiense* (Tbr). Tbg causes over 98% of reported cases. Both are usually transmitted by the bite of an infected tsetse fly and are most common in rural areas. African trypanosomiasis is a major cause of death in sub-Saharan Africa and poses a major health and economic burden in these regions with an estimated 60 million people at risk of contracting this disease, which is fatal if left untreated [8].

In 1909, the Brazilian physician and researcher Carlos Chagas discovered the etiologic agent of American trypanosomiasis *Trypanosoma cruzi* for which the name was given in honor to his friend Oswaldo Cruz. Since then, this illness received his name and is known worldwide as Chagas' disease [9]. It affects mainly heart and gastrointestinal systems many times being fatal to the bearer. The geographical distribution of reservoirs and vectors of Chagas' infection extends from the Southern USA to Southern Argentina and Chile. Nowadays, it is estimated that 8 million people in Latin America are infected with this pathogen, and 100,000 people are at risk of contracting it each year. Chagas' is also spreading to the USA, Canada, and many parts of Europe and the Western Pacific mainly due migratory flows [10]. There is an estimative that more than 400,000 individuals are currently infected in nonendemic areas like in USA and European countries [11]. These parasites are primarily transmitted by the bite of triatomine bugs from *Triatoma*, *Rhodnius*, and *Panstrongylus* genus [12].

Fever, headache, enlarged lymph glands, and swelling of the eyelid, close to the site of the bite of the insect, are some of the more common mild symptoms of the initial American trypanosomiasis acute phase. This infection is characterized by two distinct clinical stages: the acute phase, with high parasitemia, commonly progresses to a subsequent state of latency, and the chronic phase, with clinical manifestations in various organs. The most common symptoms characteristic of the chronic phase are enlargement of heart ventricles

and enlarged esophagus or colon [13], and these manifestations are occasionally life threatening [14].

2.1.1. Trypanosoma cruzi—life cycle and transmission

Chagas' infection has a wild cycle in nature that exists for millions of years. It is believed that some accidental cases involving humans might have happened at the time, similarly as they occur nowadays: when mankind invades vectors' wild ecotope or when triatomine bugs invade human domiciles. However, *T. cruzi* has been identified infecting human mummies only between 4000 and 9000 years ago. [9, 15].

Triatomines have been known since the sixteenth century but they have only settled down on human households with the beginning of the agricultural cycle. The increasing deforestation through the centuries that marked the livestock cycle leads to the removal of the native animals that once were the main sources of nourishment for the triatomines. Hence, these bugs have adapted progressively to inhabit areas surrounding human residences and the interiors of these dwellings. When humans invaded wild ecotopes and became infected, the transmission of Chagas' disease ceased to be treated as an enzootic disease of wild animals and is so called anthropozoonosis [15].

It is reported for *T. cruzi* to have wild, peridomestic, and domestic life cycles in nature: the wild cycle is merely enzootic and involves triatomine bugs and wild animals, such as rats and common opossum—*Didelphis marsupialis* for example. Meanwhile, the peridomestic cycle is derived from the wild cycle, keeping the infection among domestic animals in areas circumjacent of human residences, through the action of peridomestic triatomines and eventually through interchanges with the wild cycle (like dogs or cats hunting wild animals and wild animals invading areas surrounding human dwellings) [9]. The domestic cycle is characterized by enfold domesticated triatomines that are involved on the transmission of the infection from domestic animals to humans and between humans as well.

In this way, it is possible to perceive that *Trypanosoma cruzi* has a very complex biological cycle, involving several species of triatomines, Trypanosomatids in different stages of growth, wild and domestic mammals, and humans [16]. There are different forms of the *T. cruzi* parasite related to their stage of development: trypomastigote, epimastigote, and amastigote (**Figure 2**).

After triatomines bite an infected mammalian, they ingest the trypomastigotes form of *T. cruzi* from animal bloodstream. Inside the posterior intestine of the triatomine, the trypomastigotes transform into epimastigotes, which are able to proliferate and differentiate into metacyclic forms [17]. These parasitic forms are eliminated by triatomines through the feces, being able to invade new vertebrate cells, where they infect mainly macrophages or cardiac and smooth muscle fibers. Inside the mammalians, they undergo another round of differentiation into the proliferative intracellular amastigote forms. The amastigotes proliferate inside the host cell and give origin to new trypomastigotes when they reach the host's bloodstream. After trypomastigotes arrive at the circulatory system, the infection is disseminated [5].

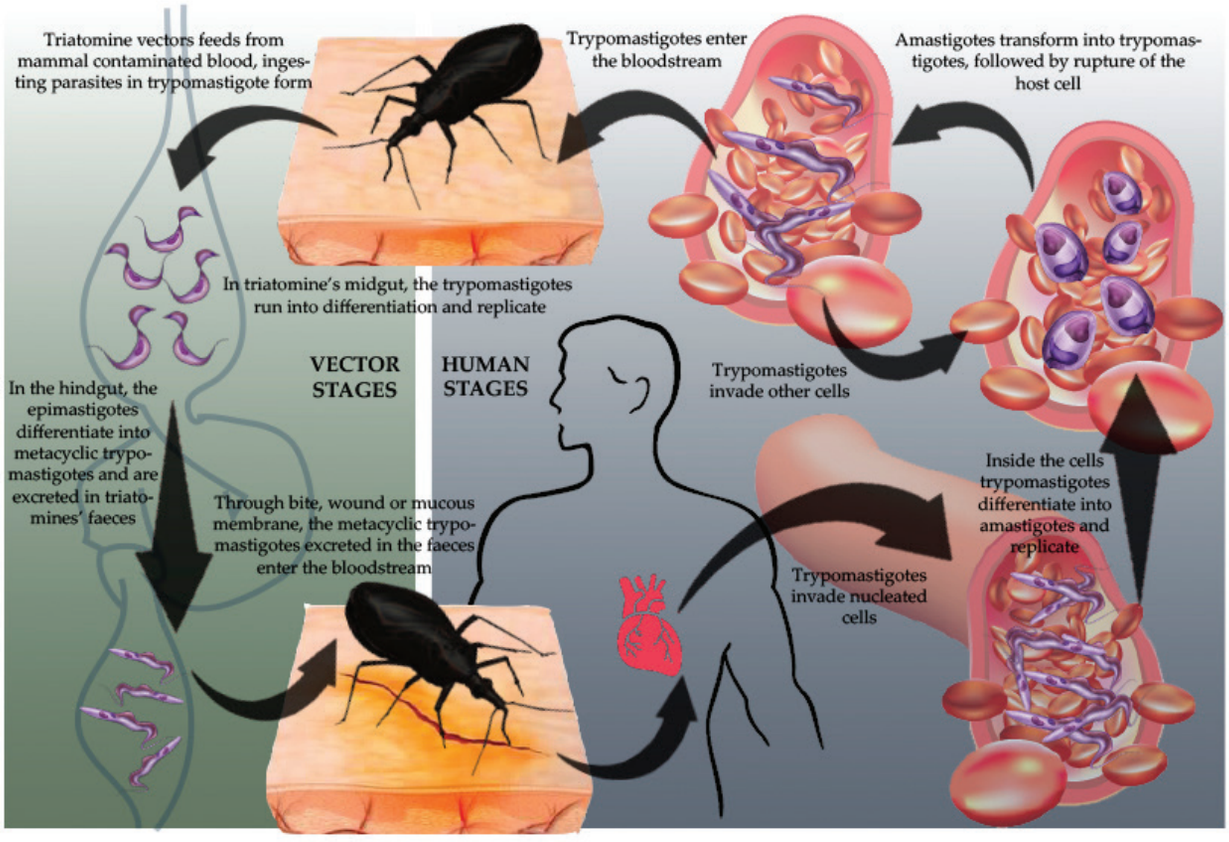

Figure 2. Life cycle of *Trypanosoma cruzi* parasite in triatomine insects and humans.

It is reported that the transmission mechanisms for Chagas' infection can be divided into two distinct groups [9]:

- Principal mechanisms: by means of triatomines (representing around 70% of the cases), blood transfusion (up to 20% of the cases), oral transmission, contaminated food, and placental or birth canal transmission;
- Secondary mechanisms: by means of management of infected animals, organ transplants, laboratory accidents, wounds, sexual transmission, contact with menstrual fluid, or sperm contaminated with parasites, and also, the hypothetic cases of purposeful criminal inoculation and contamination of food with *T. cruzi*.

2.1.2. Traditional Chagas' treatment

The challenge on searching for new Chagas' disease drugs remains for decades. Nowadays, the usual recommended traditional treatment is chemotherapic including either one of the two nitro-aromatic heterocyclic compounds (**Figure 3**) benznidazole (4) and nifurtimox (5).

As cited previously, this infection is clinically characterized by two distinct stages: the acute usually asymptomatic phase, defined by high parasitemia, and a long chronic and progressive phase in which symptoms can manifest after some years. When the patient is in the acute phase of the infection, the treatment with these drugs can cure up to 80% of the cases.

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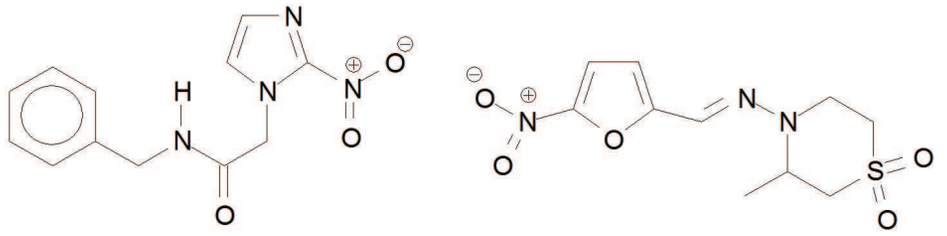

Figure 3. Recommended chemotherapic drugs (4) and (5) used on treatment for Chagas' disease.

Depending on medical orientation, drugs benznidazole (4) and nifurtimox (5) can be administered either separately or simultaneously. However, on the treatment of patients in the chronic phase, drug efficacy decreases dramatically curing only 5–20% of the cases. In addition to this limited therapeutic potential, both compounds feature high toxicity [3].

There are many papers discussing the limitations of the conventional therapeutic approach [10, 12]:

- the high dosages of drugs used and the long duration of the treatment, both necessary to produce the desired medicinal effect;
- the ineffectiveness of such drugs against all the stages of the disease and all strains of the parasite;
- problems related to the lack of efficiency in drugs' production and distribution;
- several toxic effects carried out by these drugs on the patients;
- their limited effectiveness during the chronic stage;
- regional degrees of effectiveness due to drug resistance and;
- the presence of severe side effects leading to the immediate interruption of treatment in a high percentage of the patients.

All those reasons highlight the urgent need for research on new Chagas' drugs and/or safer alternative treatments.

3. Natural sources for Chagas' treatment

Through the last decades, many efforts have been made, aiming for an effective treatment for Chagas' disease without major prejudice to patients' health. There were meritorious advances regarding to molecular biology field and pathophysiology of Chagas' disease. However, according to Coura and Viñas [14], those efforts were yet unsuccessful due to:

- the usual lack of symptoms in the illness' acute phase;
- the occurrence of various parasites strains (with different drug resistance profile);

- the hardness to find a selective and more suitable drug for the parasites and;
- the inefficient fund distribution for research while most of investments are aimed to prevention and to develop diagnostic tests.

Most of the current knowledge about parasites' biology, the identification of potential molecular targets, together with the potential natural molecules from the plant kingdom, has encouraged researchers to keep searching sorely for new drugs against *T. cruzi* in nature [14].

3.1. Plant extracts

Nature is a skilled factory that produces a wide variety of chemical substances with broad structural patterns that researchers call as natural products. Most of them are secondary metabolites synthesized by plants that are directly or indirectly related to their vital processes from metabolism to chemical defense and every single way that vegetables relate to the environment.

Searching in the literature, it is possible to find many works about broad classes of secondary metabolites that have proven to be active against *T. cruzi* [16]. Usually, as part of preliminary investigation, medicinal plant extracts, fractions, isolated natural products, or pure compounds are subjected to chemical characterization tests and *in vitro* assays for screening their biological activity. Based on the evaluated biological response, it is possible to infer which chemical classes may be present in each case [1] and decide if it is suitable for advise them in a treatment or not. Historically, plant produces many active classes of natural compounds, such as alkaloids, terpenoids, flavonoids, and quinones and many of them widely reported as promising sources of antiparasitic agents.

Bioactive natural compounds despite being very attractive sources for new drugs in their original form can also be subjected to derivatization reactions or via synthetic steps, aiming to change chemically functional groups to magnify their bioactivity [14]. In this way, many classes of secondary metabolites, pure compounds, and its derivatives have been specifically tested *in vitro* and *in vivo* assays to verify their trypanocidal efficacy. More recently, promising results were published regarding to terpenes and sesquiterpene lactones arising from plant's leaves that presented high toxicity on different evolutional stages of parasites with low toxicity on mammalian cells. Some other substances even have showed strong activity *in vitro*, but only few of them were clinically tested on Chagas' disease yet.

Abdel-Sattar and co-workers [8] investigated the *in vitro* activity of the methanol extracts from 51 plants collected in Saudi Arabia. Among these, 15 exhibited pronounced activity against *T. cruzi* (IC_{50} < 2 μg ml^{-1}: *Hypoestes forskaolii* (white ribbon bush), *Capparis spinosa* (caper bush), *Kleinia odora, Psiadia punctulata, Cucumis prophetarum* (*concombre du prophète*), *Ricinus communis* (castor oil plant), the latex of *Euphorbia ammak* (candelabra spurge), *Euphorbia schimperiana* (dafeuina), *Marrubium vulgare* (horehound), *Commicarpus grandiflorus, Argemone ochroleuca* (chicalote), *Solanum villosum* (hairy nightshade), *Withania somnifera* (winter cherry), *Peganum harmala* (African hue), and *Tribulus macropterus* (Shershir).

A few other methanolic extracts showed moderate activity while 20 were considered to be inactive against *T. cruzi* (IC_{50} > 15 μg ml^{-1}). The methanolic extract of the Solanaceae

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W. somnifera that showed potent activity for *T. cruzi*, parasites (IC_{50} of 1.93 μg ml^{-1}), after submitted to solvent-solvent partition, the chloroform fraction showed to be more potent with IC_{50} = 0.6 μg ml^{-1}, comparable to that of the standard Chagas' drug benznidazole (1) (IC_{50} of 0.52 μg ml^{-1}). The authors justify this as the chloroformic fraction concentrates the more active compounds, and this fact leads to the increasing on the biological activity of the considered fraction [8]. The chemical composition of this fraction still requires further investigation.

Another investigated Solanaceae is *Physalis angulata* L. (gooseberry), a widespread vegetable occurring mainly in tropical regions and used in folk medicine due to its active compounds and antiparasitic properties. The great medicinal potential of this species is often associated to the presence of physallins: *seco*-steroids (**Figure 4**) that have showed strong trypanocidal activity against different evolutive forms of *T. cruzi*, *Plasmodium falciparum*, and different *Leishmania* species as well.

Some results are very promising though one of the major problems faced by many research groups on natural products chemistry worldwide is related to the difficulty to obtain pure active secondary metabolites from natural sources. This fact could not be different for physalins: to isolate these compounds and obtain them in the pure form, it is quite difficult and time consuming, usually affording low yields at high costs. So economically, it can become very unattractive to treat any NTDs using pure isolated plant compounds like physalins for example.

On the other hand, the use of potential compounds from natural sources usually presents good alternative. Activity assays were performed on crude ethanolic extract of *P. angulata* that concentrates the active constituents and showed to be effective against different studied parasite species [11]. The extract was evaluated against epimastigotes and trypomastigotes forms of *T. cruzi*,

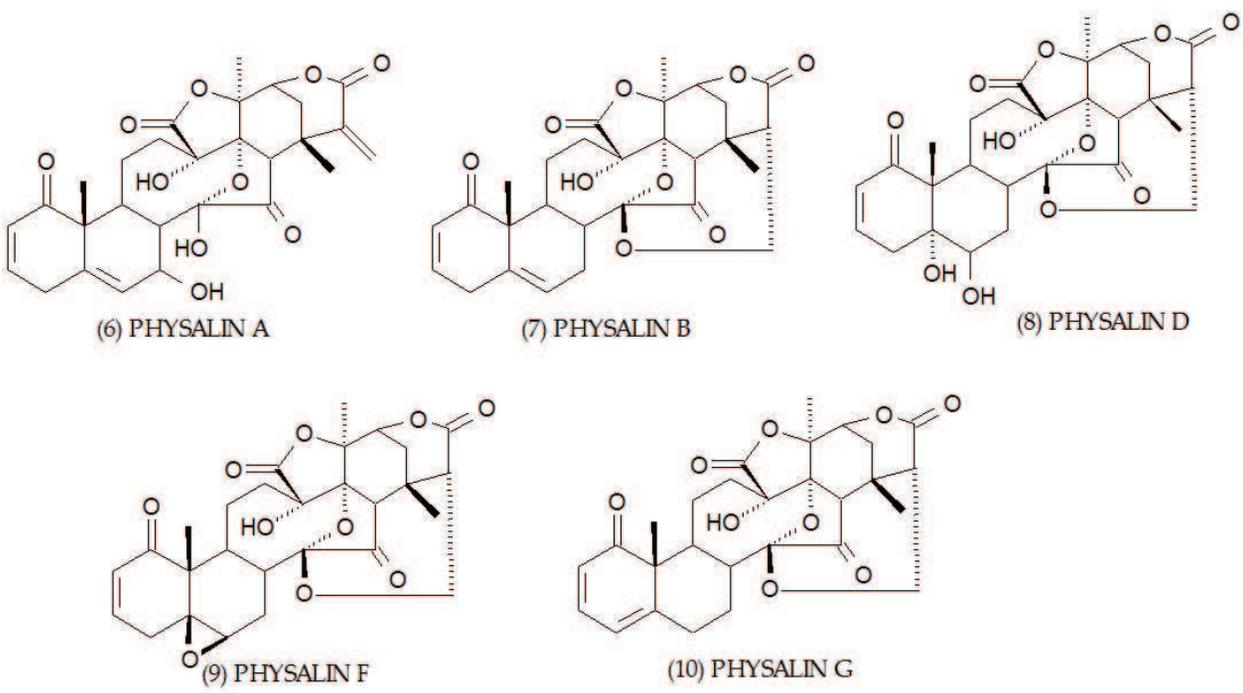

Figure 4. Physalins A (6), B (7), D (8), F (9), and G (10) isolated from *Physalis angulata*.

showing potent anti–*T. cruzi* activity, being able to inhibit the proliferation of the epimastigote forms and lyse of trypomastigotes. Beyond being active, the use of the crude plant extract has its advantages is easily obtained, nonmutagenic, and presented low toxicity in mice and high stability, which many times help to avoid degradation of the compounds of interest. Herein, it is evident that in an extract, a rich mixture of natural compounds, their chemical interactions can combine synergistically and thus alter the effect that each would have by itself.

Furthermore, the presence of phenolic compounds (**Figure 5**) like chlorogenic acid (11), rosmarinic acid (12), and coumarin (13) and flavonoids (**Figure 6**) luteolin (14), kaempferol (15), and vitexin (16) in low concentrations may have been responsible for the weak bioactivity of *L. paniculata* and *P. crucis* ethanolic extracts against *T. cruzi* [16].

In vivo studies were also performed by Meira and collaborators [11] to evaluate the effects of the same extracts against *T. cruzi* infection in mice on acute phase. The treatment reduced significantly blood parasitemia in mice when compared to those treated only with vehicle. The authors suggest that the potent activity of concentrated ethanolic extract from *P. angulata* on different strains of *T. cruzi* and *in vivo* on an acute model of infection is due to its richness in physalins (**Figure 4**).

3.1.1. Steroidal alkaloids from Solanum genus

In *Solanaceae* family, distributed in tropical and subtropical regions of Americas, Africa, and Australia, the genus *Solanum* is the most representative comprising about 1400 species [18]. The glycoalkaloids (**Figure 7**) solamargine (18) and solasonine (19) are the typical metabolites of *Solanum* genus; however, several other classes of compounds, such as flavonoids, phenolic acids, steroids, tannins, and triterpenes, were also recognized.

Several *Solanum* species have their biological activities intensively investigated, being proved the antiviral, diuretic, antifungi, antispasmodic, anti-inflammatory, and other pharmacodynamic properties. Recent studies evidenced that extracts of wolf apple, *Solanum lycocarpum* and its glycoalkaloids α-solamargine (18) and α-solasonine (19), were active against parasites, flagellated protozoa, *Trypanosoma cruzi*, *Leishmania infantum*, and *Leishmania amazonensis*, as well as against helminthes *Strongyloides stercoral* and *Schistosoma mansoni* [19]. In the light of chemical ecology, the antiparasitic effect of *S. lycocarpum* in the wild is evident: the largest canid of South America, the maned-wolf (*Chrysocyon brachyurus*), eats the ripen fruits

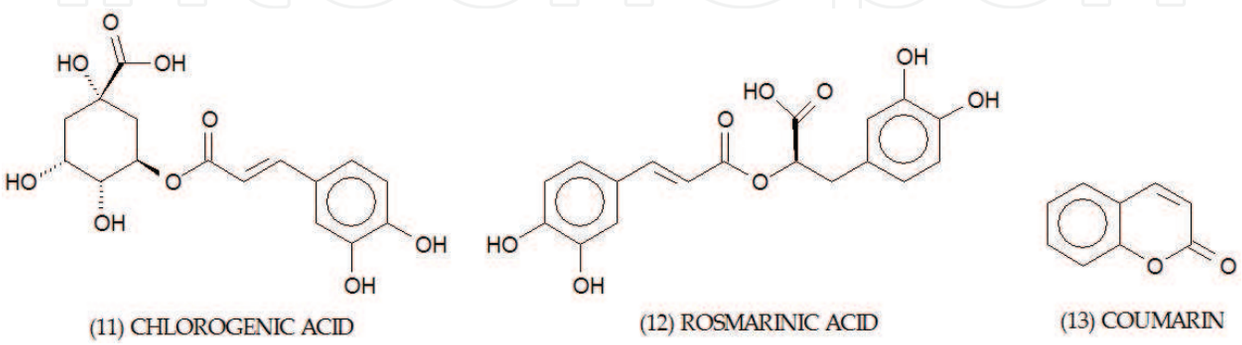

Figure 5. Active phenolic compounds (11), (12) and (13) from ethanol extracts of *L. paniculata* and *P. crucis*.

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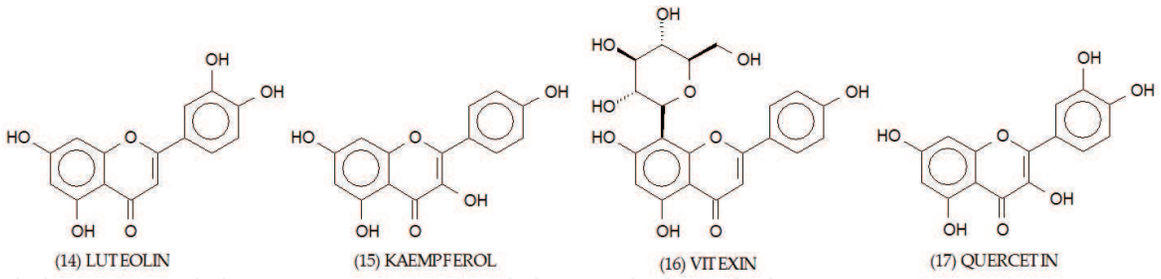

Figure 6. Active flavonoids (14), (15), (16) and (17) from ethanol extracts of *L. paniculata* and *P. crucis*.

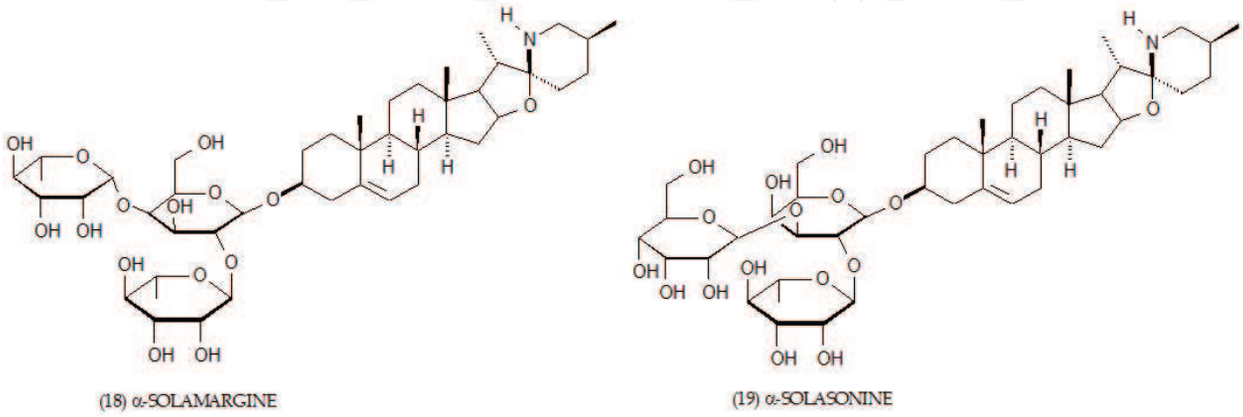

Figure 7. *Solanum* glycoalkaloids α-solamargine (18) and α-solasonine (19).

containing glycoalkaloids; this helps to control some parasitic diseases that affect it. At least that is believed by some authors [19].

The steroidal glycoalkaloid α-solamargine (18) was found on the ripe fruits of *Solanum palinacanthum* (*joá-bagudo*) as well and showed an IC_{50} of 15.3 μg ml^{-1} against *T. cruzi*, closely similar to benznidazole (1) with IC_{50} of 9.0 μg ml^{-1} [20]. Although the mechanism of action is not rightly understood, the authors speculated that the positioning of the terminal sugars in α-solamargine (18) binds more favorably with the parasites' mucin-rich cell surface when compared to α-solasonine (19). The glycoalkaloid α-solamargine (18) demonstrated to be active in the trypanocidal effect could be suitable as a candidate to prepare new therapeutic substance.

Solanum nudum Dunal (or zapata) has been used ethnopharmacologically to treat fevers. Extracts from leaves were reported to have antimalarial activity *in vitro* against asexual blood forms of protozoan *Plasmodium falciparum*. Based on this, Londoño and collaborators [12] evaluated the leishmanicidal, tripanocidal, antiplasmodial, and cytotoxic activity of eight extracts from *Solanum ovalifolium* (*cucubo*) and *Solanum arboreum* (*hoja hedionda*) obtained in different polarities, aiming to contribute to new therapeutic alternatives against protozoan diseases.

An early phytochemical analysis showed a very similar profile of secondary metabolites for both species extracts, revealing the presence of triterpenes, phenols, saponins, flavonoids, coumarins, and anthocyanosids on polar extracts. The authors found that biological activity of *S. ovalifolium* dichloromethane and hexane extracts was selective for *T. cruzi*, while the

ethanol extract was selective for *T. cruzi* and *Leishmania panamensis*. Meanwhile, the ethanol and dichloromethane extracts from *S. arboreum* showed activity against all tested parasites: *L. panamensis*, *T. cruzi,* and *P. falciparum.* The ethanol extract activity was comparable to benznidazole (4), probably due to the identification of polar compounds, known to exhibit antiprotozoal activity such as saponins, flavonoids, and coumarins. In the dichloromethane extract was found the presence of steroids such as diosgenone, which can explain its activity [12]. The cytotoxicity is related to the cell type, although steroids of *Solanum* species are also important for their cytotoxicity.

Based on activity observed for dichloromethane and ethanol extracts of *S. arboreum* on intracellular amastigotes of *L. panamensis* and *T. cruzi* and total forms of *P. falciparum*, it suggests that these extracts could be considered as promising in the search for new antiprotozoal compounds. However, additional studies on toxicity using other cell lines are required in order to discriminate whether the toxicity shown by these extracts is against tumoral or nontumoral cells [12].

3.1.2. Terpenoids

More than 20,000 known compounds are triterpenoids produced by plants through squalene cyclization. The terpenes are considered to be the most representative group of phytochemicals [21] being the structural base for several classes of derivatives. Hence, compounds from these classes are very abundant in nature being an attractive group to be screened for biological activities of interest. Hundreds of new terpene-derived molecules exhibiting trypanocidal activity have been described on the past 10 years; some of them have already been assayed *in vitro* and *in vivo* against *T. cruzi* [14].

The diterpenoids with an abietane-type skeleton (**Figure 8**) present in many plants are known to possess a wide range of biological activities, including anti-inflammatory, antibacterial, antifungal, and antimalarial among others. For example, the phenolic abietane ferruginol (20), isolated from the roots of the herb *Craniolaria annua* (Martyniaceae) known locally as *escorzonera*, showed activity against trypomastigote and epimastigote forms of *T. cruzi*. Though, it also showed cytotoxic effects against fibroblastic Vero cells. *C. annua* is a perennial herb that grows in American tropical areas and is broadly used in traditional medicine. Previous examination of this plant has led to the isolation of montbretol derivative (22) which showed trypanocidal activity against trypomastigote (IC_{50} = 25 μM) and epimastigote (IC_{50} = 69 μM) forms of *T. cruzi* [18]. Some semi-synthetic abietane-type diterpenoids isolated from *Plectranthus barbatus* Andrews (*boldo de jardim*), *Dracocephalum komarovii* Lipsky, *Salvia cilicica* Boiss, and *Juniperus procera* Hochst. ex Endl. (African juniper) berries have shown promising trypanocidal activity together with a quinone derivative of dehydroabietic acid, 12-methoxycarnosic acid, and a few others [3]. A complete survey of abietane type terpenois and their biological activities is reviewed by Gonzalez [22], covering literature from 1980 up to 2014.

The triterpenes ursolic acid (23) and oleanolic acid (24) obtained in their pure form from *Miconia* species (Melastomataceae) were tested and shown to be active against the blood form of *T. cruzi*. Animals treated with both substances presented low parasitemia when compared to animals treated with benznidazole (4) [5]. It was also demonstrated that ursolic acid (23)

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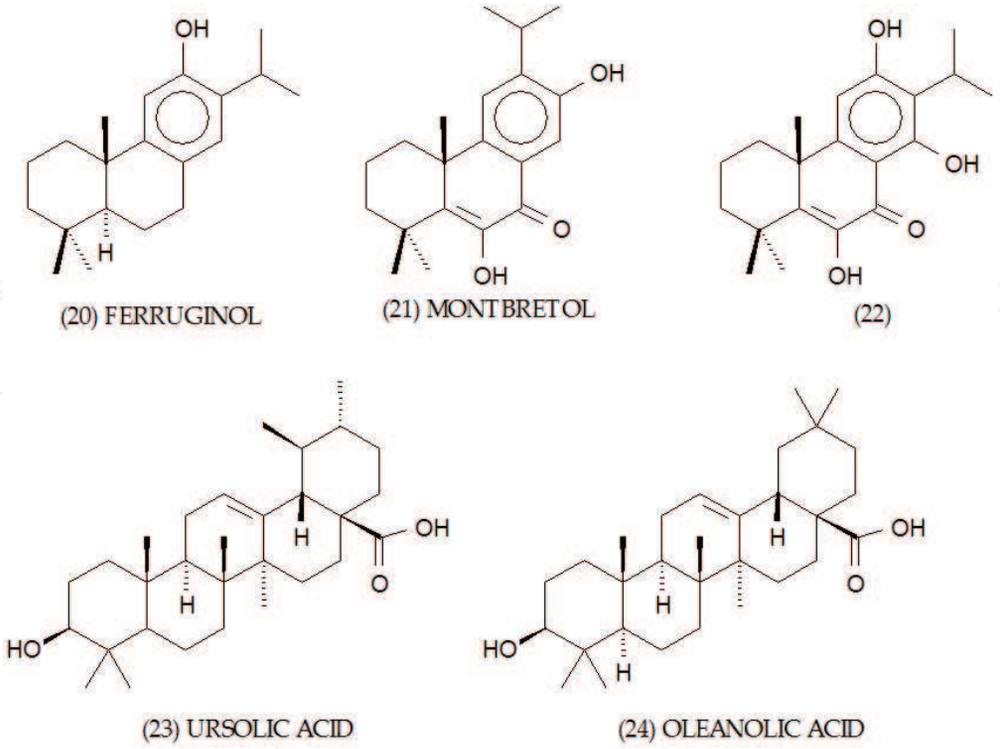

Figure 8. Active terpenoids (20, 21, and 22) and triterpenes (23 and 24) isolated from plants.

and oleanolic acid (24) were capable of controlling the peak of parasitemia in infected mice and, interestingly, treated mice did not show any alterations in their biochemical parameters, reinforcing the idea that these triterpenes are not toxic for animals. Considering the low or absent level of toxicity of triterpenes for mice, as well as their high trypanocidal activity, these results suggest that both compounds can be used for the development of new drugs against *T. cruzi* [21].

The sesquiterpene caryophyllene (25) and the phenylpropanoid eugenol (26) can be found in nature on many essential oils (**Figure 9**). Both were tested *in vitro* in their pure form against antiepimastigote and antipromastigote forms of parasites *L. brasiliensis* and *T. cruzi* [23]. The authors also tested the substances caryophyllene (25) and eugenol (26) regarding their cytotoxicity.

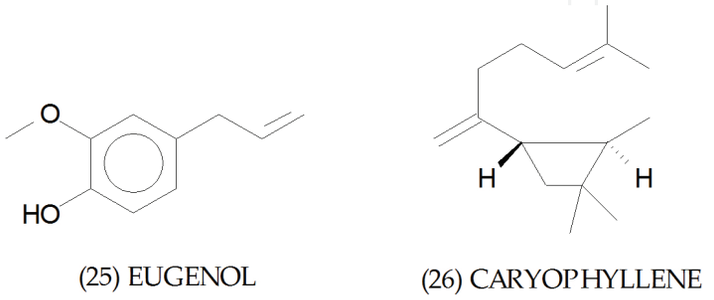

Figure 9. Structures of active compounds: caryophyllene (25) and eugenol (26).

Caryophyllene (25) showed higher percentage of parasite inhibition, being capable of eliminate 100% of *L. brasiliensis* in concentrations of 100 and 50 μg mL^{-1}. About *T. cruzi*, caryophyllene (25) inhibits 67% of the sample in concentration of 100 μg mL^{-1}, with an additional advantage: caryophyllene (25) did not exhibit cytotoxicity in concentration of 12.5 μg mL^{-1}. Similarly, eugenol (26) in concentration of 100 μg mL^{-1} showed percentage of inhibition of 17.34% and 40% for *T. cruzi* e *L. brasiliensis*, respectively. Eugenol (26) did not exhibit cytotoxicity in concentration of 50 μg mL^{-1}.

Sesquiterpene lactones (**Figure 10**) are terpenoid derivatives and usually have α,β-unsaturated carbonyl groups that are primarily responsible for mediate their wide spectrum of biological activities. Many compounds from this chemical class often show high activity against *T. cruzi* and have been isolated from the aerial parts of plants while their mechanism of action is currently under clarification. Some scholars in the area suspect that these compounds have the power to generate free radicals within trypanosomes [14]. Complementary ultra structural studies demonstrated that many compounds from this chemical class may affect mitochondrial function. It is known that the α-methylene-γ-lactone of sesquiterpene lactones is responsible for most of the biological properties of these compounds. Some authors have suggested that the interaction of the α-methylene portion from those lactones, with sulphydryl groups present in some parasites enzymes that are crucial for its survival, accounts the cytotoxicity of these compounds [14]. It is also practicable that these sequiterpene lactones may affect calcium metabolism, once they are similar to thapsigargin (28), a potent inhibitor of this ion. However, this hypothesis has not been tested yet.

Interestingly, some of these terpenic molecules are feasible to chemical modification in order to comprehend their mechanisms of action in such organisms or intended to optimize their effectiveness on elimination of parasites. It is possible to strategically perform chemical reactions on specific functional groups on some known natural products. This approach proved to be very effective, once with the increasing on lipophilicity of isolated diterpenes lead to a substantial improvement on their trypanocidal activity, for example. It is also reported that

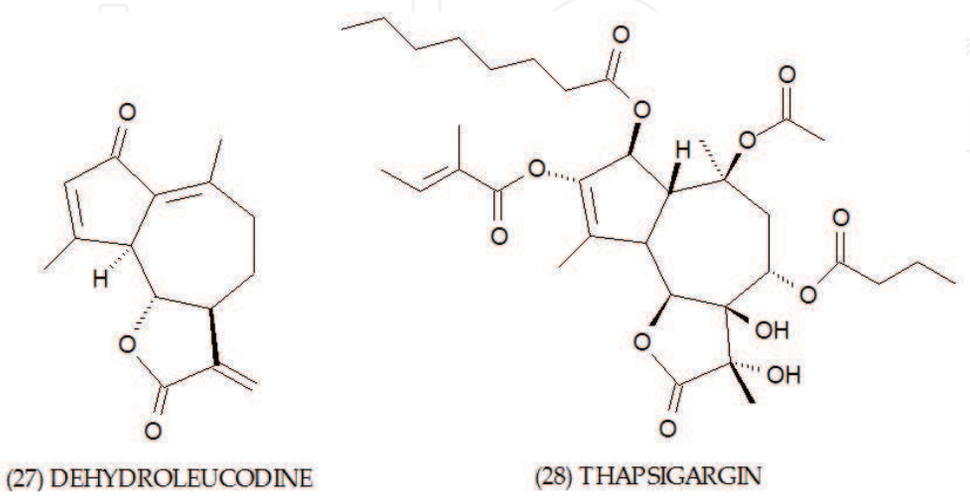

Figure 10. Sesquiterpene lactones: dehydroleucodine (27) and thapsigargin (28).

parasites have a rudimentary defence system highly sensitive to oxidative stress, being their main vulnerability [14].

3.1.3. Flavonoids

In addition to terpenoids, other group of natural products with very interesting bioactivity is the flavonoids (**Figures 6** and **11**). They are very abundant in nature being responsible for many interesting properties like antioxidant, anti-inflammatory, and free-radical scavengers. The ethanol leaf extract from the bay cedar, *Guazuma ulmifolia* Lam. (Malvaceae), was active *in vitro* against the tested parasite strains of *T. cruzi*, *L. brasiliensis,* and *L. infantum*, possibly due the presence of quercetin (17), a potent known leishmanicidal flavonoid from flavones group [16]. The cytotoxicity presented by the aforementioned extract reinforces the need for further tests, including *in vivo* trials, like antineoplastic activity in tumor cells, before considerate *G. ulmifolia* ethanol extracts as a potential alternative source of natural compounds against Chagas' disease.

Flavanones (**Figure 11**) naringenin (29), sakuranetin (30), and its methylated derivative sakuranetin-4′-methyl ether (31) have their antiparasital activity tested *in vitro* against four parasites from *Leishmania* spp. species and *T. cruzi* trypomastigotes and amastigotes [24].

In this study, the authors reported that sakuranetin (30) presented good activity against all tested *Leishmania* species and against *T. cruzi* trypomastigotes. Hence, sakuranetin (30) was chemically transformed thru methylation procedure furnishing sakuranetin-4′-methyl ether (31). This chemical modification yielded an inactive compound against the tested parasite species. However, this result is interestingly important once evidenced that the presence of hydroxyl group at C-4′ and of methoxyl group at C-7 in related flavanone are directly associated to the aforementioned activity. In conclusion, Grecco and collaborators [24] provided flavanone important structural information required for comprehension about anti-protozoan activity of these flavonoids. This kind of information could be very useful for the design of novel and more effective agents against Leishmaniasis and Chagas' disease for example.

3.1.4. Lectins

Lectin is the name given to a group containing all sugar-specific agglutinins of nonimmune origin. Those substances were found to be valuable because they could recognize and bind

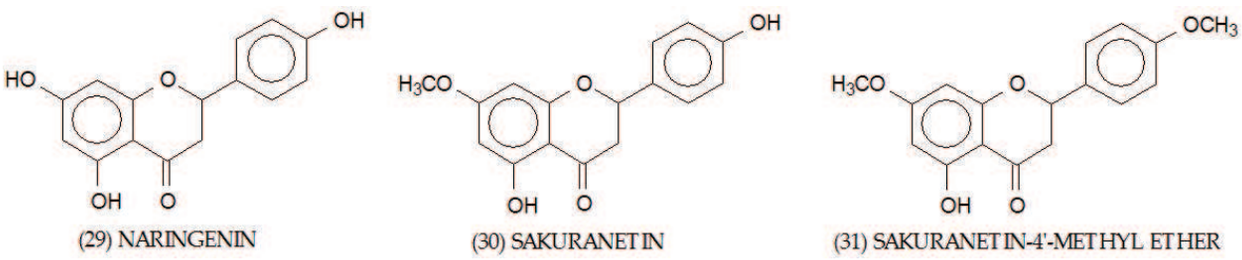

Figure 11. Flavanones: naringenin (29), sakuranetin (30), and sakuranetin-4′-methyl ether (31).

carbohydrates specifically and reversibly. Hence, the lectins have great potential and value in the study of glycoproteins, helping to comprehend the mechanisms of many physiological and pathological processes [25]. The bonding between lectins and some protozoans' sugars is believed to cause interference in chemical or biological processes that eventually lead to the death of these parasites. Therefore, lectin isolated from triatomine insect *Rhodnius prolixus* (Reduviidae) showed to interfere on the life cycle of *Trypanosoma rangelii* effectively. Apparently, carbohydrates on the surface of *T. rangelii* and *T. cruzi* cells interact with lectins extracted from soy beans, *Glycine max* (Fabaceae), and castor-oil beans, *Ricinus communis* (Euphorbiaceae), suggesting that they could be helpful to determinate the presence of *T. cruzi* from the feces of *R. prolixus,* one of vectors of Chagas' parasite [26].

3.2. Essential oils

It is evident that many medicinal plants from *Artemisia* genus (Asteraceae) have ethnopharmacological importance. The classic example refers to *Artemisia annua* that furnished artemisinin (2) as aforementioned. Likewise, the species *Artemisia absinthium* L. (absinthe) had composition and biological effects of the essential oil and the extracts widely studied. Different researchers have demonstrated antimicrobial and antiprotozoal effects against *T. cruzi*, *Leishmania aethiopica*, *Leishmania donovani*, and *Leishmania infantum*.

Among the major constituents identified on *A. absinthium* essential oils, are (**Figure 12**) *α*-thujone (32), *β*-thujone (33), sabinene (34), *β*-pinene (35), myrcene (36), *trans*-sabinyl acetate (37), 1,8-cineole (38), linalool (39), *cis*-epoxyocimene (40), artemisiaketone (41), camphor (42), bornyl acetate (43), myrtenol (44), chrysanthenyl acetate (45) hydrocarbon monoterpenes, and sesquiterpene lactones, depending on the plant origin, mixtures of these components could be found in different ratio concentrations.

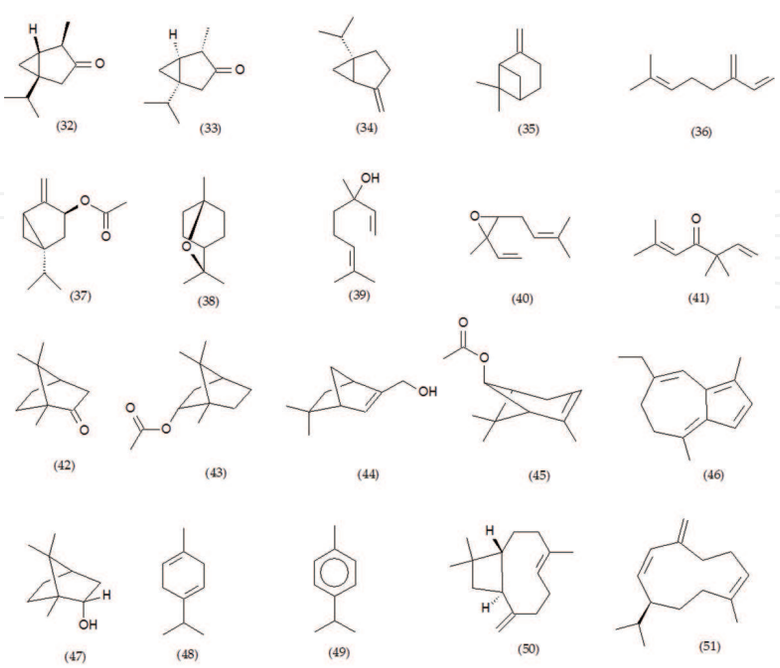

Figure 12. Some chemical constituents (32 – 51) of active plants essential oils.

Usually the collection of wild herbal populations can result in extracts and essential oils with variable compositions [10]. So, after *A. absinthium* essential oils chromatographic fractionation, the antiparasitic effects of some fractions revealed that compounds dihydrochamazulene (46) and *trans*-caryophyllene (50) (main compounds on their respective fractions) could be related to the observed activity.

Essential oils extracted from fresh leaves of velame, *Croton pedicellatus,* and sangre de drago, *Croton leptostachyus* (Euphorbiaceae), showed to be active against the extracellular forms of *T. cruzi in vitro*. The main compounds identified on crotons' oils were borneol (47), γ-terpinene (48), *p*-cymene (49), *trans*-caryophyllene (50), and germacrene D (51). The difference observed for the oils' activity could be related to the presence of these components in variable proportions or due to the existence of other minor components in volatile content. Unfortunately, despite of being active, Neira and co-workers [27] found out that these oils were toxic for Vero cells.

3.3. Marine organisms

3.3.1. Sponges

The crescent need for bioactive molecules that can be used as potential natural drugs, being able to cure diseases and reducing undesirable side effects at the same time, leads the researches all around the world to look to the sea. Many papers available in the literature report the search for new active compounds, and they have found that marine biodiversity is a promising source of natural products with remarkable biological activities. To the best of our knowledge, studies involving marine sponges yield close to 200 new pharmacologically active metabolites every year [28].

Being ancient organisms, some sponges contain diverse groups of metabolically active compounds. Hence, the investigation of biological activity is an important source to obtain extracts or compounds with potential biomedical action. So much that the effect of acetone extract from lyophilized Brazilian and Spanish marine sponges, *Chondrosia reniformis* (esponja de vidro—glass sponge), *Tethya rubra* (the red golfball sponge), *Tethya ignis* (esponja de fogo—fire sponge), *Mycale angulosa* (common sponge), and *Dysidea avara* (soft sponge), was evaluated on growth of *T. cruzi* forms. All the tested extracts showed activity against epimastigote forms of the parasite. The extracts of *D. avara* (IC_{50} = 23.4 μg ml^{-1}), *M. angulosa* (IC_{50} = 67.3 μg ml^{-1}), and *C. reniformes* (IC_{50} = 28.6 μg ml^{-1}) were the most active. Moreover, the extracts showed no toxic effects in normal cells ($LLCMK_2$) at concentrations that inhibited 50% of the parasites [28]. In this study, the marine sponges have some compounds identified by GC–MS (**Figure 13**): the steroids, stigmasterol (52), *β*-sitosterol (53), and brassicasterol (54) were found in larger quantities in sponges' organic extracts and show activity against *T. cruzi*.

The trypomastigotes were sensitive to the presence of different concentrations of marine sponge extracts as well. Although the action mechanism of steroids is unknown, it is accepted that these compounds may be initiated at the cell membrane but also via intracellular receptor binding. In addition, steroids may participate in growth regulation, proliferation and

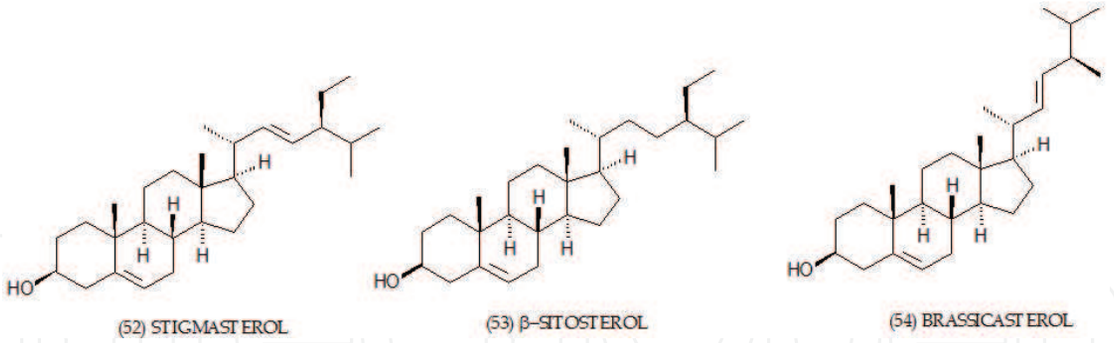

Figure 13. Steroids stigmasterol (52), *β*-sitosterol (53), and brassicasterol (54) found in marine sponges.

cell death, and redox mechanisms [12]. These compounds could participate in a conjugated addition of nucleophilic amino acid residues present in target enzymes on *Leishmania*. This reaction occurs usually via Michael type mechanism that was also reported for other α,β-unsaturated compounds such as lactones and chromones [13].

3.4. Combating triatomine bed

As discussed through this chapter, triatomine bugs can affect human health acting as vectors transmitting Chagas' disease to many populations worldwide. The inappropriate use of synthetic insecticides, usually been used to control these insects, is closely linked to the development of resistance in pests, human diseases, and contamination of food and the environment. Resistance to the pyrethroid deltamethrin and other nonnatural insecticides, for example, has been reported in different areas of the Gran Chaco region of Argentina and Bolivia for *Triatoma infestans*, the major Chagas' disease vector in southern South America [29]. Nevertheless, the biological action of natural products and essential oils with insecticidal activity represents a very important alternative, which allows an environmental friendly management of pest insects without affecting people's health. Plants can produce a wide diversity of compounds that are involved in their chemical defense [30]. Those compounds are usually volatile and can be found concentrated on their essential oils [31]. Among these natural products, terpene compounds have been shown to have a significant potential for insect control [31] killing or at least repealing the insects away. However, little is known about the molecular properties related to their insecticidal activity.

For example, Nieto-Sanchez *et al.*, [32] have recently prospected in southern Ecuador for traditional Chagas' disease control strategies employed by general population. Among those actions they have found:

- the active search and elimination of triatomines;
- insecticide-based fumigation on infected places;
- educational activities managing population.

Those prevention methods are effective in short term for reducing triatomine infestation, although do not prevent reinfestation in the long run. Interestingly, they have also reported practices such as sweeping with brooms made from plants believed to have natural insecticide

properties by local residents: herbs such as porotillo (*Fallopia convolvulus*), moshquera (*Croton* spp.), florblanca (*Buddleja utilis* also known as monteramirez), and chamana (*Dodonaea viscosa*) are considered to be highly acidic plants by local populations; so they become a natural insecticide when turned into brooms. The natives described that sweeping more than once a day in and outside the domiciles using water to create less dust and to prevent the dirt to stick on the floor is the most common way.

Those findings suggest that multiple tasks are required to control bug recolonization, especially in poorly constructed houses. The usual use of synthetic chemical insecticides constitutes a fragile short-term solution for controlling Chagas' disease. There is a need to develop more sustainable long-lasting solutions for Chagas' disease transmission in areas that have high occurrence of triatomine infestation.

4. Final considerations

The studies reviewed briefly in this chapter along with many others that have been carried out since the 50s have brought valuable information aiming to contribute to the understanding about the parasite's life cycle and to highlight the crescent need to clarifying some biomolecular targets and enzymatic mechanisms that could be useful to the development of new natural drugs against *T. cruzi* and other parasites.

It is personally believed that the cure for Chagas' disease is hidden somewhere in nature; the scientists are currently working as explorers, prospecting this greatness in molecular levels, searching in every single bush for a viable solution that helps populations suffering for Chagas worldwide. Here in few lines, it was showed the great potential of natural products for the treatment of this parasitic disease. The plentiful Mother Nature furnishes material to the obtention of useful substances emerging from crude extracts, essential oils, and many fractions possessing very complex, variable, and rich composition. In this chapter, was portrayed, several groups of secondary metabolites, such as diterpenes, terpenes, triterpenes, sesquiterpenes, sesquiterpene lactones, steroids, flavonoids, polyketides, lectins, and many others.

Massive efforts of community activists, health care workers, politicians, and economists are also required to reduce significantly the significance of public health liability that NTDs oblige. The most effective approach for reducing these diseases is still prevention, due to the absence of affordable or effective curative therapies and the deficiency of preventive vaccines. Between such relevant public health issues and many lives directly or indirectly affected by NTDs, there is education that offers a solution to connect NTD prevention to treatment efforts.

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