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Natural Products and Synthetic Derivatives as Promising Candidates Against Neglected Tropical Diseases

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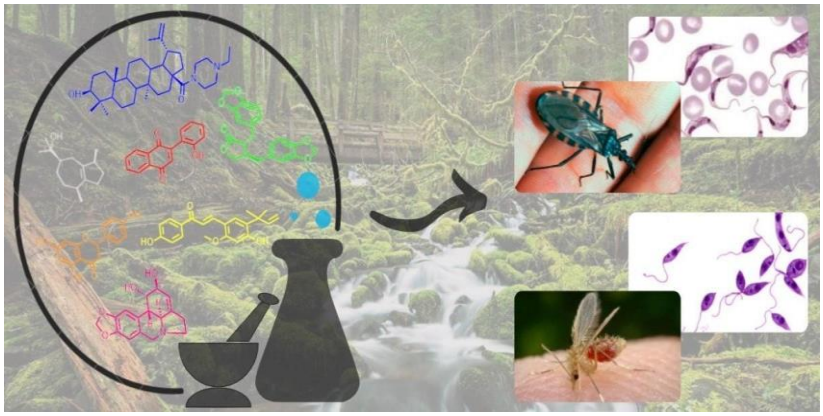
** This manuscript is dedicated to the memory of the recently deceased Yann Le Hyaric Almeida, student of chemistry and son of the co-author, Dr. Mauro Vieira de Almeida.*

Neglected Tropical Diseases (NTD) are a group of endemic diseases that cause up to one million deaths annually. Leishmaniasis and Chagas Disease are classified as NTD whose current treatments are of limited access. There is an urgent need to develop new agents to combat or alleviate the symptoms of these pathologies. The present work highlights the activities of several natural products and their derivatives which have been tested against the causative parasites of both diseases. The activities of natural products illustrate their potential to assist in the combat against NTD. Research in this area could provide an opportunity to advance medical research and to provide treatments to low- and middle-income countries.

Graphical abstract



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1. Introduction

1.1 Leishmaniasis and Trypanosomiasis - Neglected Tropical Diseases

Neglected tropical diseases (NTD), also known as neglected diseases (ND), affect more than one billion people worldwide, predominantly in developing nations [1]. Consequences include impaired child growth and development, reduced intellectual capacity and labour productivities. NTD are not only a consequence of but promotes and propagates poverty [2-4].

The NTD comprise chronic infections caused by parasites, viruses, bacteria and protozoa, corresponding to at least 13 types of disease which are prevalent in approximately 149 countries [5]. The most affected populations live in vulnerable situations due to inadequate sanitary conditions and direct contact with vectors [6]. In Brazil, the population most

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affected by NTD is rural workers and those who carry out extractive activities, as they have more contact with reservoirs, vectors and etiological agents [7].

Among these diseases, parasitic infections caused by protozoa have been the subject of numerous studies, as they affect millions of people and there still do not exist specific treatments or cures. The causative agents of leishmaniasis and trypanosomiasis are flagellated organisms of the genera *Leishmania* and *Trypanosoma* respectively, both containing many sub-genera and species [8,9].

1.2 Leishmaniasis

Leishmaniasis is transmitted mainly by hematophagous mosquitoes of the genera *Phlebotomus* and *Lutzomya* and can exist in three different forms: cutaneous, mucocutaneous and visceral, the latter being the most fatal form [10,11]. The main symptoms are the appearance of skin lesions, nasal wounds, and in cases of the visceral form, haemorrhages complicated by viral and bacterial co-infections [12]. Visceral leishmaniasis is considered a zoonosis, as it is transmissible to both humans and animals. Risk factors range from lack of sanitation to deforestation, with economically challenged regions being most affected [6,13].

In 2021, more than 700 cases of leishmaniasis were recorded in Brazil, 79 of which were fatal with the northern region of the country being most affected [14].

Currently, the only treatments in Brazil for visceral and cutaneous leishmaniasis are based on amphotericin B and miltefosine, respectively. However, the effectiveness of these treatments for leishmaniasis is limited by their inherent efficacy as well as the increasing frequency of drug resistance. Immunosuppression is an additional collateral effect of these treatments which contributes to outbreaks and further transmission of the disease. [15-17]. For some species such as *L. major* and *L. tropica*, which are responsible for the cutaneous form, the treatment with fluconazole or itraconazole may be effective [18].

For canine leishmaniasis, caused by *L. infantum*, research has been carried out on a safe vaccine and the Leish-tec® vaccine is currently available, however, it is only an auxiliary method because it generates very low immunity. Thus, its use in endemic regions is limited [6,19-21].

1.3 Trypanosomiasis: Chagas disease

Among several protozoa belonging to the genus Trypanosoma, *Trypanosoma cruzi* is the causative agent of American trypanosomiasis, also known as Chagas disease (ChD), which is considered a chronic disease typical of Latin America. This parasite is capable of infecting not only humans but other mammalian species [22].

T. cruzi presents a heteroxenic life cycle, having both a vertebrate host and an invertebrate host, such as insects [23]. There are three well-defined phases of the life cycle of *T. spp.* The first is the trypomastigote form in which there is no potential for parasite replication. This extracellular infecting form is where the parasite infects the host vertebrate and is released into the bloodstream [24]. The amastigote form is characterized by intracellular infection and has the potential for replication in the vertebrate host. These organisms are rounded and have inconspicuous flagella [25]. The third epimastigote form is found in the digestive tract of vectors but is not infective to humans [26].

Currently, the drugs available for the treatment of ChD are benznidazole (BZN - **1**) and nifurtimox (NFX - **2**) (Fig. 1) during the acute and early chronic phases. However, these agents are distant from the criteria of what is considered an ideal drug, such as safety, efficacy in few doses, selectivity, low or no toxicity, and accessibility to low-income patients [27].

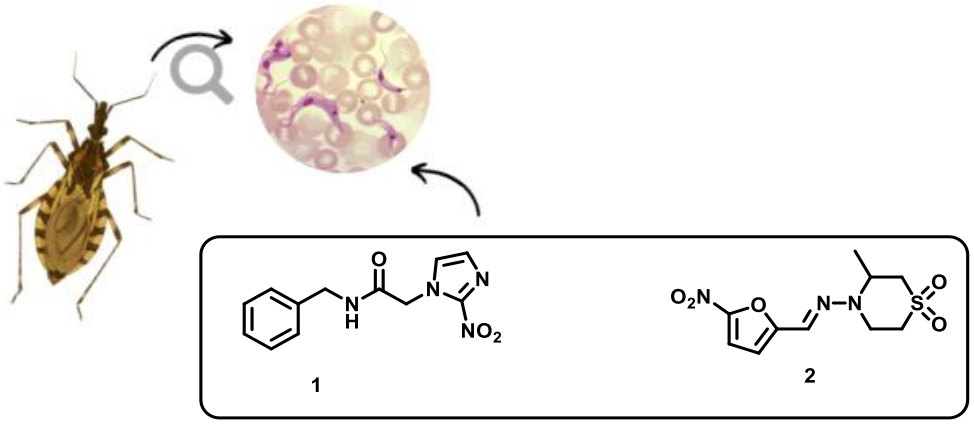

Fig. 1. Transmitting insect, protozoan and available drugs in Leishmaniasis (Adapted from [28, 29]).

As in the case of leishmaniasis, Chagas disease is transmitted by hematophagous insects. In Brazil, the main form of transmission occurs through the faeces of the "kissing bug" (*vinchuca* or *barbeiro*), which are deposited on the skin of the individual. There are other forms of contagion, such as transmission by ticks and fleas as possible vectors of *L. infantum*. Transmission by venereal, transplant or blood transfusion has also been reported [30-32].

Despite the predominance in South America, vector and host migration and blood transfusion rates have increased the spread of the disease to developing countries, where there were no previous reports of the disease [33,34]. Therefore, there is an urgent need to develop safe and effective new drugs for the treatment of chronic Chagas disease.

Thus, it is evident the need for novel therapies to combat the disadvantages associated with available treatments including adverse side effects, high costs, drug resistance and limited efficacy [35]. The ideal form of prevention would be the discovery of a safe and effective vaccine. However, thus far, the only disease mitigation feasible is through monitoring of symptoms for early identification of symptoms of the disease and by combating transmission vectors.

Unfortunately, the pharmaceutical industry has shown little interest in developing new drugs for neglected diseases, due to low prospects of financial returns. As such, most research on NDTs has been conducted within an academic setting. To further complicate matters, many of the most affected countries have major socio-economic problems.

Hence, natural products emerge as a promising alternative in the form of traditional medicinal plant preparations but also as lead compounds for drug prototypes. This strategy makes it possible to take advantage of the biodiversity present in these emerging countries to provide novel and practical treatments.

In the search for novel, promising molecules, research groups are investigating and trying to identify the active sites of biomolecules for future *in vitro* and *in vivo* assays. Buschiazzo and colleagues have isolated *T. cruzi* trans-sialidase (TcTS), a non-homologous enzyme, which does not show significant sequence similarity at the primary level, in neither humans nor hosts. This is expressed mainly by trypomastigotes and is being considered a promising pharmacological target of ChD [36]. This enzyme catalyzes the transfer of sialic acid from the glycoconjugate surface of the host to the surface of the mucin-like glycoprotein of *T. cruzi* while assisting to evade the host's immune system [37].

As such, researchers are testing substances of natural origin and derivatives thereof against parasites. For example, Azeredo and co-workers [38], evaluated the bioactivity of an oxazolone derived from alanine (**3**), illustrated in Fig. 2, against the epi- and amastigote forms of *T. cruzi*. The structure of this compound contains a Michael acceptor subunit, which is known for its reactivity with soft nucleophiles, such as cysteine [39].

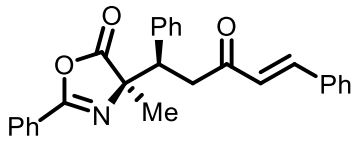

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Fig. 2. Chemical structures of oxazolone derived from alanine.

In 2020, the World Day for Neglected Tropical Diseases was established by the World Health Assembly, to be celebrated on January 30, to increase awareness of these diseases and plan strategies for the well-being of the affected populations. The WHO reinforces the need for countries to take steps to prevent these diseases and to promote the development of better, safer, more accessible and easier-to-use methods and medicines [6,40].

Natural products have long been used to develop new drug prototypes. They have long been employed as a promising strategy for the discovery of new drug candidates. Perhaps equally important, natural product-based treatments have been employed to meet the needs of endemic regions with little or no access to allopathic medicine.

1.4 Natural Products

Natural Products (NPs), by definition, are produced by plants and other diverse living organisms. Their production is believed to be involved in the evolution of defense mechanisms for millions of years. One example is the production of secondary metabolites. Among these metabolites are alkaloids, phenols, terpenoids, flavonoids, saponins, steroids, tannins, glycosides and others, which have application in human, veterinary and agricultural medicine [41]. Throughout human history, many plants and other natural organisms have been used as therapeutics and, even today, natural substances are widely used, mainly by developing countries, as a form of primary medicinal treatment. Although they have been replaced or supplemented by the use of standardized "modern" medicines, the use of these crude drug preparations continues to be an important source of research for drug discovery. Work on the isolation and purification of an active molecule, and the subsequent optimization of its pharmacological profile and activity by methods of synthesis continues to be an extremely active area of interest to medicinal chemists worldwide [42,43].

Natural products are relevant in the discovery of new therapies for neglected tropical diseases. This, unfortunately, is because many countries afflicted by these infirmities are populated by people with a low-income profile [44]. This being as it may, NPs present an excellent research opportunity. In addition to being an important source of bioactive compounds in and of themselves, natural products provide a wealth of chemical structural diversity and poorly studied or unknown biological activities. However, it should be emphasized the difficulty in isolating substances from NPs is a result of low yields, high manufacturing costs, questions of intellectual property, environmental impact, and difficult work in this area of chemistry [45].

The scope of the present review seeks to select some specific classes of NPs, their semi-synthetic derivatives and synthetic analogues that show promising potential to act against pathogens of the *Leishmania* and *Trypanosoma* genera.

In the present review, we cite and emphasize publications concerning natural products as well as their derivatives and synthetic analogues and derivatives as well. We have focused on journal articles published in the previous the last 20 years and exclude patent literature. The relevant compounds have been divided into the following classes: essential oils, triterpenoids, alkaloids, chalcones, flavonoids, quinones and naphthoquinones, saponins and lignans.

1.5 Essential oils

Essential oils are constituents of aromatic plants located in different morphological structures, such as leaves, flowers, fruits, seeds, buds, roots, rhizomes and bark [46]. These compounds may be obtained by various techniques, such as hydrodistillation, solvent extraction, cold pressing or supercritical fluid extraction. They possess many substances that have biological properties [47].

The essential oil extracted from the leaves of *Cryptocarya aschersoniana*Mez. (LauraceaeJuss.), popularly known in Brasil as "*canela-nhutinga*", is composed predominantly of monoterpene hydrocarbons (Fig 3), the racemic mixture (*S*) and (*R*)-limonene (**4** and **5**), linalool (**6**) and nerolidol (**7**) and were studied by Andrade and co-workers [48], against the promastigote forms of *L. amazonensis* in 2018. Until this moment, there were no reports on the chemical composition and the antileishmanial and cytotoxic activities of the essential oil of *C.aschersoniana*. The authors carried out hydrodistillation in a Clevenger-type apparatus and analyzed the chemical composition by gas chromatography coupled to mass spectrometry (GC-MS) and Gas Chromatography with Flame Ionization Detector (GC-FID). The essential oil under study exhibited high leishmanicidal activity when tested against promastigote forms of *L. amazonensis*. There was an

increase in parasite lysis dependent on the concentration of essential oil, which demonstrated a 50% inhibitory concentration (IC_{50}) of *L. amazonensis* at a concentration of 4.46 μg mL^{-1}. The high leishmanicidal activity of the essential oil from *C. aschersoniana* leaves was related to the presence of its main chemical components:(**4**) and (**5**), and the oxidized monoterpenes (**6**) and (**7**) whose structures are illustrated in Fig. 3. This hypothesis is supported by results obtained previously, which showed that these compounds were, separately, active against different forms of the disease.

Although the activities were good, the essential oil showed inferior inhibitory activity (IC_{50} = 4.46 μgmL^{-1}) when compared to the Amphotericin B reference standard. In addition, it showed a higher cytotoxic concentration (CC_{50} = 7.71 μg.mL^{-1}) against peritoneal macrophage cells of mice, almost seven times more toxic than Amphotericin B (CC_{50} = 51.86 μg.mL^{-1}). The authors postulated that the strong anti-*L. amazonensis* activity, concomitant with the high cytotoxicity could be explained by the absence of specific cellular targets of the principal constituents of the oil. In other words, these molecules can interact with enzymes present in both healthy cells and protozoan cells. This was attributed to the physicochemical properties of these compounds, which are nonpolar and lipophilic species. As such, they are highly permeable to cytoplasmic membranes. Once they cross the membrane, they can cause cytoplasmic coagulation, denature proteins, and disrupt metabolic pathways such as lipid biosynthesis, leading to cell death by necrosis and apoptosis [48].

In another study, **4** and **5**, present in the essential oils of *Citrus limonia* and *Citrus latifolia*, were responsible for the leishmanicidal activity [47], while the activity of **6** was reported against the promastigote and amastigote forms of *L. amazonensis*, with IC_{50} of 8.3 and 8.7 ngmL^{-1}, respectively [49] and, finally **7** inhibited the growth of *L. amazonensis*, *L. braziliensis*, and *L. chagasi* promastigotes and *L. amazonensis* amastigotes with IC_{50} 85, 74, 75, and 67 μM, respectively [50].

Further studies are recommended to verify the occurrence of possible synergistic and/or additive effects between these compounds, which in turn could direct studies related to structure-activity relationships to develop prototypes of anti-leishmania drugs starting from scaffolds of these structures.

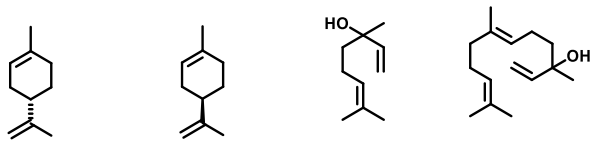

Fig. 3. Chemical structures of essential oils constituents of *C. aschersonian*.

The group of Rottini [51] evaluated the activity of essential oil of *Endlicheria bracteolata*, from the *Lauraceae* family, against *L. amazonensis*, in promastigote and intracellular amastigote forms. The Lauraceae family species are part of the vast biodiversity found in the Amazon region, where it is known as bay laurel (or *louro*). In this study, the oil was extracted from the leaves by hydrodistillation and it was analyzed by GC-MS, which resulted in the identification of 31 components. The main compounds encountered constitute sesquiterpenes (85.8%), being guaiol (**8**) the main component with 46.4% (Fig. 4).

In vitro studies began from the evaluation of the inhibitory activity against promastigote forms, which presented IC_{50} of 7.945 μg mL^{-1} (24 h) and 6.186 μg mL^{-1} (48 h), while for intracellular amastigote forms it was 3.546 μg mL^{-1} (24 h). The cytotoxic concentration (CC_{50}) in macrophages was 15.14 μg mL^{-1}, showing that *E. bracteolate* is less toxic to macrophages than to parasites. The authors performed transmission electron microscopy, which showed that treatment with essential oil of *E. bracteolata* can induce mitochondrial damage to promastigote and intracellular amastigote forms, while flow cytometry showed disruption of the mitochondrial membrane in treated parasites. The researchers concluded that these results may lead to the development of products based on *E. bracteolata* oil for the treatment of cutaneous leishmaniasis, especially for people who cannot receive conventional therapy.

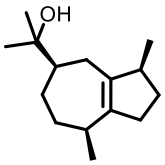

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Fig. 4. Chemical structure of guaiol.

In other emerging countries, such as in North Africa and the Middle East, the medicinal plant *Haplophyllum tuberculatum*, rich in volatile oils, is used in traditional medicine for its´s analgesic and antipyretic activities. Tea from the stem leaves is used to treat nausea, constipation, and gastric disorders as was as in parasitic infections such as malaria. The volatile oils were extracted from leaves, stems and aerial parts by hydrodistillation and analyzed by GC-FID and GC-MS. Hamdi and colleagues observed that there was variation between the quantitative and qualitative chemical composition of the oils, depending on the time of year of collection, location and stages of plant development. The main constituents encountered were sesquiterpenes, (**6**) and (**7**) and limonene. As previously mentioned, these are also found in the Brazilian plants *C. aschersoniana* and *E. bracteolate* [52]. Having established the activities of the main isolated compounds, the authors reported initial studies of both anti-leishmania activity and *in vitro* cytotoxicity [52]. The essential oils and isolated compounds were evaluated against *L. mexicana* promastigotes at different concentrations. Regarding the oils that were tested, problems related to stability were reported, and in addition, both essential oil and the pure compound **5** (Fig. 3) showed moderate anti-leishmanial activity, with an IC_{50} ranging from 6.48 to 50.28 μg mL^{-1}. Cytotoxicity assays for these volatile extracts, **4** and **5** in Chinese Hamster Ovary (CHO) cells were relatively strong cytotoxicity with CC_{50} of 75.89, 29.65μg mL^{-1} respectively, and then, the selectivity index was considered low (SI <10).

These oils could be safely used in therapeutic concentrations and short-term treatments for emergency cases, for example, in regions where there is a lack of access to first-line treatments. Finally, despite having shown the largest inhibitory activity among the isolated compounds (IC_{50} = 16.59 μg.mL^{-1}), compound limonene cannot be considered a drug candidate due to the low selectivity index.

Still exploring the bioactivity of compound [*R*]-limonene (**5**) against protozoa, Kpoviessi's group investigated its effects against *T. brucei* species which showed good inhibitory

activity (IC_{50} = 4.24 μg mL^{-1}) [53]. In this study, (**5**) and the volatile oils from *Cymbopogon giganteus*, a plant widely used in traditional medicine in Africa, were evaluated for activity and cytotoxicity. Cytotoxicity, evaluated using *in vitro* studies against CHO, showed that, in general, was low for these oils and it could be a good source of anti-trypanosomal agents. When comparing the cytotoxicity of (**5**) extracted from *Haplophyllum tuberculatum* and *C. giganteus*, the obtained values were slightly higher by a factor of approximately two (29.65 and >50 μg mL^{-1}, respectively) [52,53]. This difference was justified by the authors due to the biological variability of the cells used in the culture.

It should be noted that *Haplophyllum tuberculatum* is abundant in its native habitat; a region with a high incidence of neglected tropical diseases. In addition to its traditional use to treat various maladies, it may be an option for trypanosomosis in the absence of economically viable alternatives.

Since leishmaniasis is an endemic public health problem in North and East Africa, the Moroccan medicinal plants' *Mentha pulegium* and *Rosemarinus. officinalis*, popularly known as pennyroyal (*poejo*) and rosemary (*alecrim*), respectively, were studied to determine their abilities to combat protozoa.

This research, described by Bouyahya and co-workers [54], identified the bioactive chemical compounds and evaluated their properties against leishmaniasis. The aerial parts of plants were collected, and the essential oils were extracted by steam distillation. The hydrodistillate was analyzed by GC-MS and 29 compounds were isolated from *R. officinalis.* Of these, the main chemical components identified were: 1,8-cineole (23.6%), camphor (18.7%), borneol (15.5%) and (14.1%) of α-pinene. For *M. pulegium,* 21 compounds were identified, of which pulegone (41.0%) and menthone (21.2%) were the main constituents.

The leishmanicidal activities of both volatile oils against promastigote forms of *L. major, L. tropica* and *L. infantum* were performed by the MTT. Considerable cytotoxicity of *M. pulegium* against *L. major* (IC_{50} = 1.3 μg mL^{-1}) and *R. officinalis* against *L. infantum* (IC_{50} = 1.2 μg mL^{-1}) was observed at low concentrations, using Glucantime® (meglumine antimoniate) as the control drug which presented an IC_{50} of > 10 μg mL^{-1}. The activity of the oils can be attributed to the presence of biologically active substances which could inhibit the growth of or promote the death of the parasites. Several possible mechanisms of action can be postulated such as induction of apoptosis, disruption of the electron transport chain or inhibition of DNA topoisomerase. Further studies must elucidate the mechanisms of action, possible synergistic effects and, ideally, in *vivo* studies.

Species of the genus *Origanum* are well known for the antimicrobial properties of their essential oils. Tasdemir and co-workers have previously reported the activity of its volatile oil against protozoa [55]. The essential oil was obtained by hydrodistillation of the dry parts of *O. onites*, popularly known as Cretan oregano. The oil was analyzed by CG-FID and CG-MS, its main components were evaluated against *Trypanosoma brucei rhodesiense*, *T. cruzi*, *L.donovani* and *P. falciparum*. The main components of this oil were identified as carvacrol (70.6%), followed by linalool (9.7%), *p*-cymene (7.0%), γ-terpinene (2.1%) and thymol (1.8%). The researchers observed that the volatile oil showed significant *in vitro* activity against *T. b. rhodesiense* (IC_{50} = 180 ng mL^{-1}), however, it was inactive against *T. cruzi*, and demonstrated moderate antileishmanial and antiplasmodic effects, without toxicity to mammalian cells (up to 90 μmL^{-1}). Carvacrol (**9**), thymol (**10**), both illustrated in Fig. 5, and essential oil was tested against the same parasites, where carvacrol and thymol were maintained *in vitro* antiparasitic potency of the oil. When tested for *in vivo* trypanocidal activity in mice, only thymol extended the survival time of infected mice, while the essential oil and carvacrol were both inactive. However, the oil and (**9**) may have synergistic effects and show increased bioactivity when combined with other constituents of the oil, or with clinically used antiprotozoal drugs. The authors highlighted the already known importance of the presence of the hydroxyl group in the bioactivity of phenolic compounds, such as (**9**) and (**10**). To confirm this, the methyl ether derivatives of these compounds were found to be 40-90 times less potent against *T. b. rhodesiense* than the original compounds in the reported study. The researchers concluded that these substances present in the oils, such as thymol, can serve as a starting material to provide new semi-synthetic antiparasitic agents with improved activity profiles.

9 **10**

Fig. 5. Chemical structures of carvacrol and thymol.

1.6 Triterpenoids

Triterpenes are members of isoprenoids that are derived from the C_{30} precursor squalene. Most triterpenes found in nature have 1 to 5-ring in their structures, and these cyclic systems are later converted into several important metabolites, such as sterols and steroids, which play an important role in cellular function (Fig. 6) [56].

Triterpenoids are defined as terpenes that contain oxygen in their structure [57], and they are one of the largest subclasses with more than thirty specialized metabolites and 14,000 known structures that have potential application in the food and pharmaceutical industry [58].

Pentacyclic triterpenoids are interesting natural compounds due to their antiviral, antiparasitic, antibacterial and anti-inflammatory activity [59]. For example, oleanane, lupane and ursane are considered the most important in this class due to their plurality of bioactivities [60]. Betulinic acid is a lupane pentacyclic triterpenoid and its derivatives demonstrate activity against both *Leishmania spp.* and *Trypanosoma cruzi* [61].

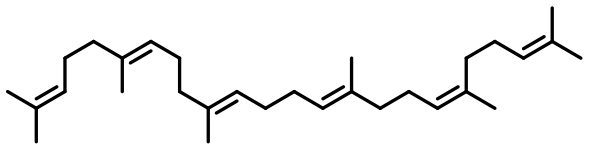
Meira and colleagues reported the synthesis of new semisynthetic amide derivatives of betulinic acid (Compounds **16**-**19**) for further evaluation of trypanocidal potential [62]. The methodology of this work has as starting material betulinic acid, which is later converted into a mixed anhydride of isobutyl chloroformate followed by the addition of secondary amines (Fig. 7).

Initially, heterocyclic piperidine, morpholine and pyrazine amides of betulinic acid were prepared to vary and investigate hydrogen bonding sites. After these structural modifications were made, semi-synthetic betulinic acid derivatives (**16**) and (**17**) showed IC_{50} values (> 100μM) inferior to those of betulinic acid (19.5μM) and the reference standard (**1**). These compounds could alter the ultrastructure of the parasite,

causing deformation of the cell structure, and inducing a necrotic process in the trypomastigotes of *T. cruzi*. This anti-parasitic effect was also observed with the combination of compound (**18**), which has the highest trypanocidal potential with benznidazole. In this case, there was also observed an IC_{50} reduction of up to 80% when compared to the individual compounds. Finally, the derivative (**19**) also presented good activity (13.7 μM). In summary, amide formation potentiated the compounds against *T. cruzi*.

Lupenone (**20**), illustrated in Fig. 8, is also a lupane triterpenoid extracted from plants of *Albizia spp.* [63]. This biomolecule shows activity against several diseases, including leishmaniasis, and can be obtained by the oxidation of lupeol with pyridinium chlorochromate (PCC) in dichloromethane [64].

The extraction and isolation of lupenone from the *Albizia inundata* plant, for further biological testing, were obtained by evaporation of the final hexane extract [65].



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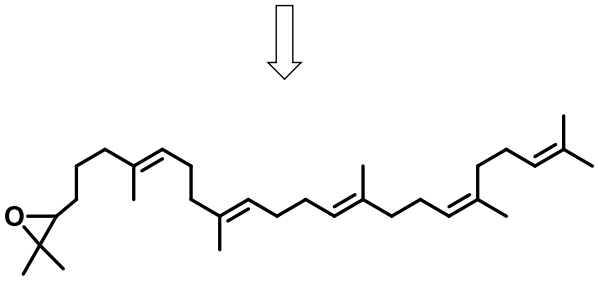
Squalene



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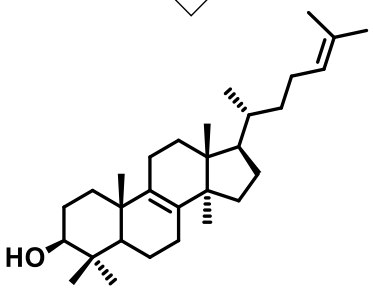
Squalene oxide



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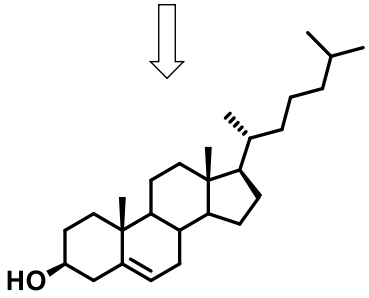
Lanosterol



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Cholesterol

Fig. 6. Biosynthesis of cholesterol from the triterpene squalene.

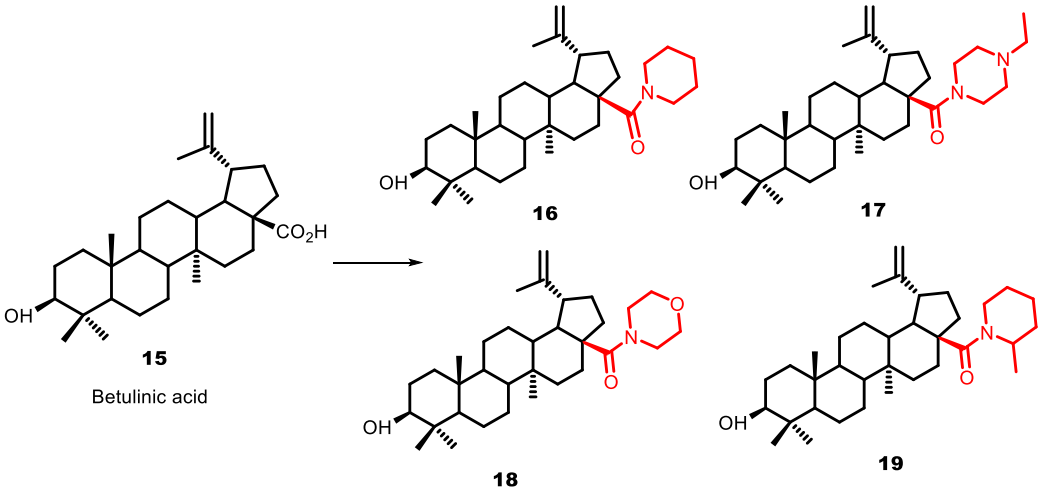

Fig. 7. Chemical structure of betulinic acid and its derivatives.

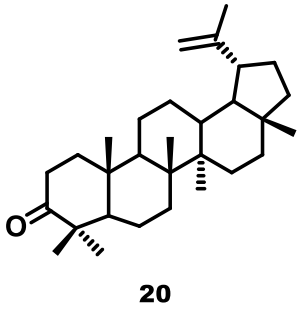

Fig. 8. Chemical structure of lupenone.

As a result, the compound showed antibacterial and antifungal activity, where the methanolic extracts of the stem bark were active against all microorganisms tested, with experimental minimum inhibitory concentration (MICs) values in the range of 3.9-12.5 μg.mL^{-1}.

The leishmanicidal effect of lupenone was also evaluated against *L. amazonensis*. There was a reduction in promastigote proliferations (IC_{50} at 20 μM), more potent than amphotericin B, which is the reference drug for the treatment of leishmaniasis. Amastigote species, challenged with lupenone presented an IC_{50} value of 20.6 μM, being more effective than lupeol.

The mechanism of action of lupenone is attributed to its lipophilicity, which facilitates penetration into the lipid bilayer of cell membranes. In the parasite, there was abnormal lipid accumulation after exposure to lupenone, suggesting that this compound affects the lipid biosynthesis pathways of the parasites, resulting in apoptosis. One of the strategies adopted by research groups in drug development is the combination of two or more compounds to achieve a better therapeutic effect [66]. With this strategy in mind, Polanco-Hernández and co-workers reported their work on the effect of different mixtures and concentrations of lupenone and β-caryophyllene oxide on leishmanicidal activity and immunomodulatory effects [67].

Tests were performed to determine the activity against the promastigote forms of *L.amazonensis; L. braziliensis; L. mexicana; L.tropica* e *L.aethipica*. The mixture containing proportions of 1:4 lupenone/β-caryophyllene oxide and β-caryophyllene were the more active combinations against all promastigotes. Regarding to *L.tropica* and *L.mexicana* studies, the mixture showed strong activities (IC_{50} = 15.1 and 14.0 μg mL^{-1}, respectively) and β-caryophyllene oxide presented IC_{50} = 19.4 and17.8 μg mL^{-1}, respectively.

Given that a compound is considered strongly active against leishmaniasis with an IC_{50}< 25 μg mL^{-1}, the authors found promising compounds for the treatment of leishmaniasis [68]. The mixture of 1:4 lupenone/β-caryophyllene oxide and pure β-caryophyllene oxide also showed a great inhibitory effect and the selectivity index (SI) for the mixture is higher, with values of 3.7 and 3.4 for *L. mexicana* e *L. tropica* respectively, when compared with pure β-caryophyllene oxide (SI < 3.0).

Finally, the immunomodulatory effect of the mixture and the pure oxide was evaluated from *in vitro* bioassays of the clinical stage of the disease after infection of THP-1 cells. Both tests were able to significantly reduce the number of infected cells. This work is important because it shows how the combination of natural products can contribute positively to the synthesis of value-added molecules.

Oleanolic acid (**21**), a pentacyclic triterpenoid, is available in fruits and vegetables, known for presenting pharmacological activities such as anti-inflammatory, antiviral, antimicrobial and antioxidant properties. Dehydroabietic acid is a tricyclic diterpenoid, also found in vegetables and has shown anticancer and antimicrobial activities and is a promising potential for the synthesis of new drugs [69,70].

Given these characteristics of oleanolic acid (**21**) and dehydroabietic acid (**22**), Pertino and co-workers analyzed the antiprotozoal activity of triazole derivatives of these acids against the promastigotes of *T. cruzi* and *Leishmania spp*. [71]. Diterpene derivatives can be extracted from *Salvia cilicica* and oleanolic acid can be found in *Fabiana imbricata* (Fig. 9).

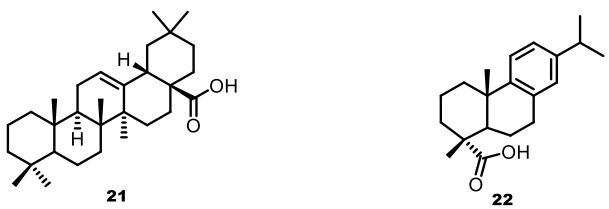

Fig. 9. Chemical structures of oleanic acid and dehydroabietic acid.

To obtain triazole derivatives, the methodology developed by Sharpless, known as *Click chemistry* was performed to evaluate the bioactivity of different biological and chemical properties (Fig. 10).

23: n = 1; R = Ph
24: n =2; S(Me)(Ph)
25: n = 2; Ph

26: n = 1; R = Ph
27: n =3; R = Ts

Fig. 10. Triazole-diterpene hybrid compounds.

The activities of **22** and several synthetic triazoles against *T. cruzi* were moderated, in which they presented IC_{50} = 46 µg mL^{-1}, 61 µg mL^{-1} and 69 µg mL^{-1} for **23**, **24** and **26** respectively, the most actives.

A structural/activity analysis was performed by the authors, where compound **23** has one methylene group (CH_2), between the diterpene and triazole, while compound **25** has two additional methylene units and its inhibitory potential is less effective (IC_{50} = 265 µg mL^{-1}). The same tendency is with compounds **26** (IC_{50} = 69 µg mL^{-1}) and **27** (IC_{50} = 199 µg mL^{-1}), which shows the importance of the chain length for activity against *T. cruzi*. When the triazole possesses a phenyl methyl sulfide substituent, the *T. cruzi* effect is more pronounced for compounds containing two CH_2 units. The same effects are observed against leishmaniasis.

Regarding the proliferation of leishmania, the following values were observed: **22** (IC_{50} = 44 µg mL^{-1}); dehydroabetinol (IC_{50} = 63 µg mL^{-1}); derivatives **23** (IC_{50} = 53 µg mL^{-1}); **24** (IC_{50} = 64 µg mL^{-1}); **25** (IC_{50} = 89 µg mL^{-1}) and **27** (IC_{50} = 71 µg mL^{-1}) presented the best results against promastigote strains *L. braziliensis*.

The compounds cited when compared with the reference drug pentamidine, which is used for the treatment of leishmaniasis but has several side effects such as nausea, hypertension, and hypoglycemia among others [72], showed a significant difference for leishmaniosis and *T. cruzi* when compared to BZN as the reference drug. These results are promising, as the compounds have good *in vivo* activity and low toxicity.

1.7 Alkaloids

Alkaloids are, in general, heterocyclic compounds containing at least one nitrogen atom and possess basic characteristics. For a long time, alkaloids have been applied in traditional medicine due to their diverse biological properties and, thus used as skeletons of molecules of industrial interest and as medicines or as starting points in the development of new drugs.

Promising sources of bioactive trypanocidal alkaloids can be found in plants of the Amaryllidaceae family and are distributed worldwide. Thus, the plants of the genus, *Narcissus*, could direct the development of new drugs with trypanocidal activity.

Regarding these studies, several alkaloids were extracted from narcissus and evaluated against *T. cruzi* by Martinez-Peinado and coworkers [73]. The *T. cruzi* growth inhibition phenotypic assay revealed that lycorine (**28**), hippeastrine (**29**), hemantamine (**30**), narcyclasine (**31**) and montanine (**32**) were active, while crinine (**33**), tazettin (**34**), sanguinine (**35**) and 1-*O*-acetylcaranine (**36**) (Fig. 11) did not show any activity against the parasite. The highest activities observed were for (**27**), IC_{50} = 0.700 µM and (**30**) with an IC_{50} of 0.495 µM, which surpassed the reference drug benznidazole. Compound (**29**) was selective and active against *T. cruzi* with an SI of 12.7 and 35.2 against Vero and HepG2 cells, respectively. Additionally, this alkaloid showed specific activity against the amastigote stage IC_{50} =3.31 µM. Although lower than the reference drug, its good selectivity suggested that it should be further studied.

An extensive chemodiversity of alkaloids can also be found in marine environments and, therefore, has also drawn the attention of researchers to develop new drug prototypes. The marine sponge *Tedania brasiliensis*, found along the Brazilian coast was studied by Parra and coworkers. They were able to isolate and identify bromopyrrolic alkaloids and synthesized 23 pseudoceratidine derivatives [74]. The compounds (Fig. 12) were evaluated against *P. falciparum*, *L. amazonensis*, *L. infantum* and *T. cruzi*. The pseudoceratidine (**37**) showed no activity against *L. amazonensis, L. infantum* and *T. cruzi*, however, it did present relevant antiplasmodial activity with an IC_{50} = 1.1 µM against *P. falciparum*. Compounds (**38**), (**39**), (**40**), (**41**) and (**42**) had moderate to low antileishmanial activities, with an EC_{50} of approximately 20 µM. Although they were active, they were less potent, against *L. amazonensis*. The authors stressed that it is necessary to explore further variations in the structure of these compounds to clarify the structure-activity relationships and improve anti-leishmanial efficacy.

Berberine (**43**) (Fig. 13) is an isoquinoline quaternary alkaloid salt extracted from the rhizomes, roots and stems of many plants. Due to its previously demonstrated biological activity, Calvo and co-workers reported *in vitro* studies of (**43**) [75]. Three different liposomal formulations were evaluated containing berberine for the treatment of visceral leishmaniasis *in vivo*. Drugs encapsulated in liposomes have several advantages over other formulations due to the colloidal nature of liposomes which facilitate absorption by cells such as macrophages. A significant reduction in liver and spleen parasitaemia was observed in mice treated with liposomes containing berberine compared to the free drug, presumably due to greater accumulation of the drug in these organs. Another finding was a significant reduction in plasma triglyceride levels, which shows the lipid-lowering effect of berberine but should be considered as a side effect in the treatment of visceral leishmaniasis.

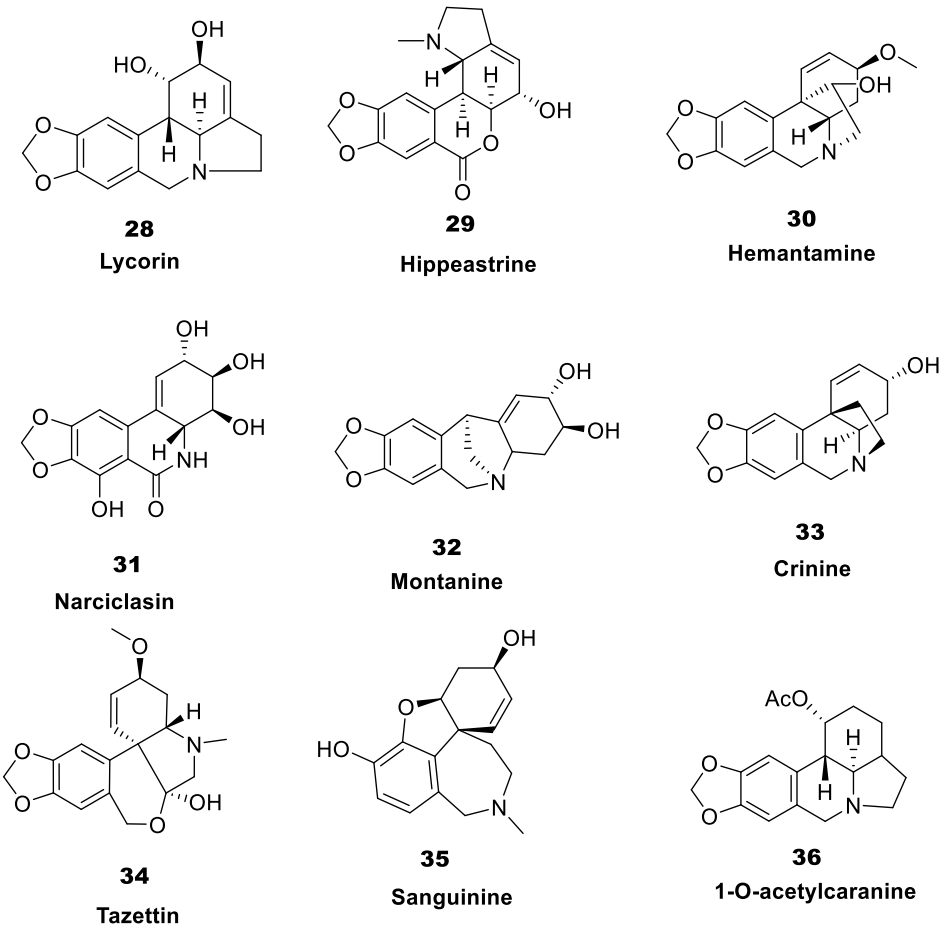

Fig. 11. Chemical structures of alkaloids extracted from Narcissus.

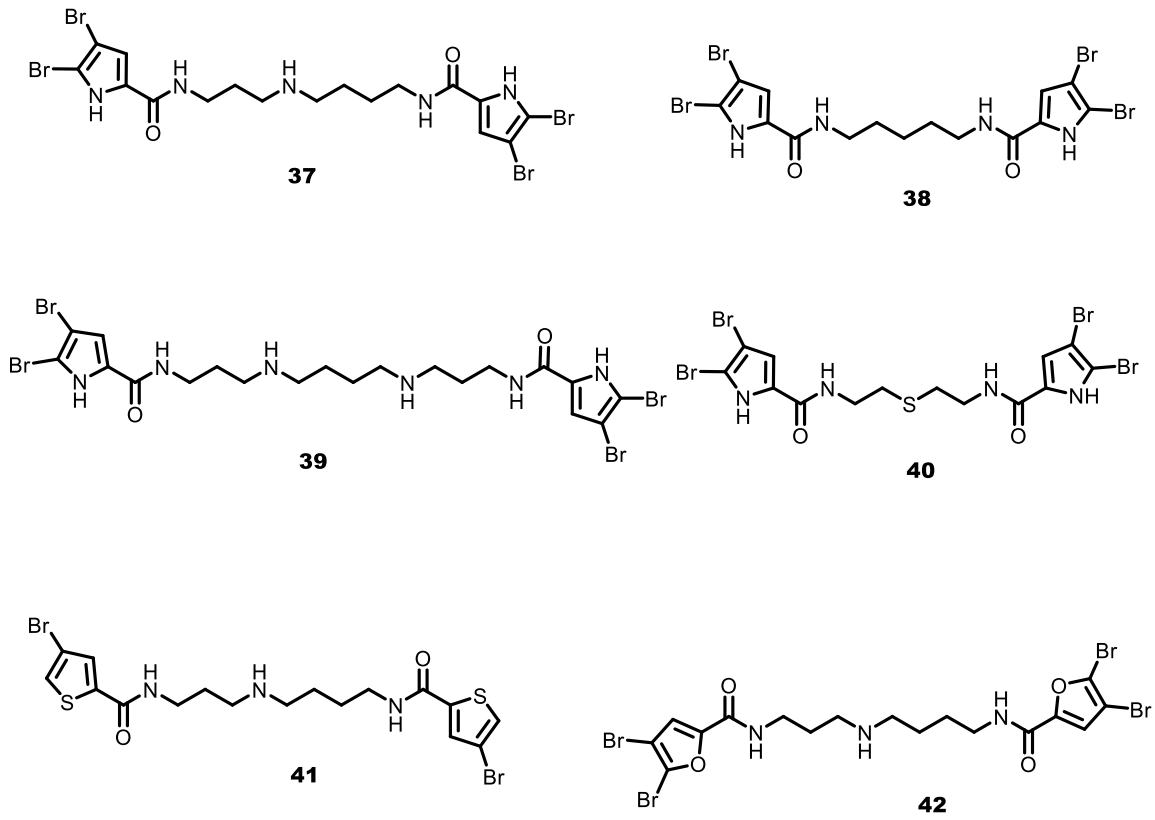

Fig. 12. Chemical structure of pseudoceratidine (**37**) and its derivatives.

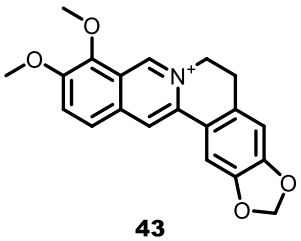

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Fig. 13. Chemical structure of berberine.

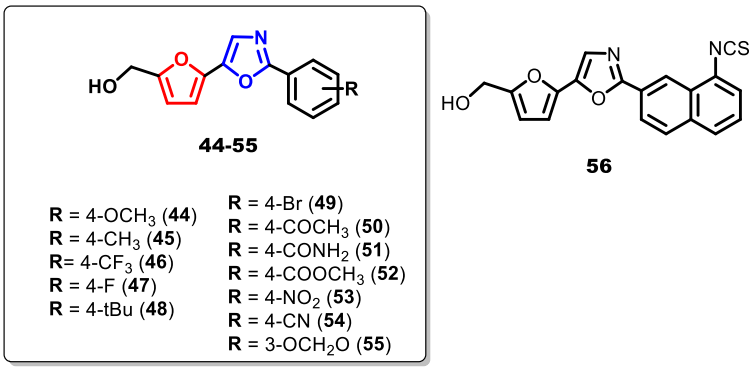


Fig. 14. Chemical structure of alkaloid derivatives from oxazole (blue) and tetrahydrofuran (red) skeletons (**44-55**) and (**56**).

In the search for novel compounds active against *T. cruzi*, Rosa and co-workers [76] synthesized 46 derivatives based on the structure of two classes of natural products: oxazole alkaloids and tetrahydrofuran lignans. Oxazole-containing alkaloids (Fig. 14) have multiple biological activities and have demonstrated activity against *T. cruzi*. The authors sought to optimize the potency and chemical properties by replacing one of the aryl rings attached to the oxazole nucleus with a hydroxymethylfurfuryl moiety. In the series of alkaloid derivatives, 17 compounds were active at a concentration of 50 μM; the most powerful compounds (**44**), (**46**), (**48**), (**50**), (**56**), (**60**) and (**64**) (Fig. 14) presented EC_{50} values ranging from 24.2 to 49.1 μM. The presence of bulky and nonpolar substituents on the aryl ring of compounds (**45**), (**46**), (**48**), (**50**), (**53**) and (**64**) improved cell permeability due to the increase in hydrophobicity. *In silico* calculations and principal component analysis have shown that the active compounds share common chemical characteristics with other trypanocidal molecules and are predicted to have a good ADMET profile. Therefore, the results suggested that the compounds are important candidates to be further studied for potential activity against *T. cruzi*.

1.8 Chalcones

Chalcones are naturally occurring aromatic ketones, bioprecursors of flavanones, which have previously demonstrated a broad spectrum of pharmacological activities [77]. These compounds are ubiquitous and can be found in petal pigments, bark, roots and leave of plants of the genera *Angelica, Glycyrrhiza, Piper* and *Ruscus* among many others. Their structures are characterized by the presence of two aromatic rings linked by an α,β-unsaturated ketone (Fig. 15) [78].

Licochalcone A (LicoA, **57**) presents anticancer, antibacterial and antiviral activities [79]. Souza and co-workers explored the leishmanicidal effect of LicoA and two analogues (Fig. 15) against *L. amazonensis* and *L. infantum in vitro* and *in vivo* in mice [80].

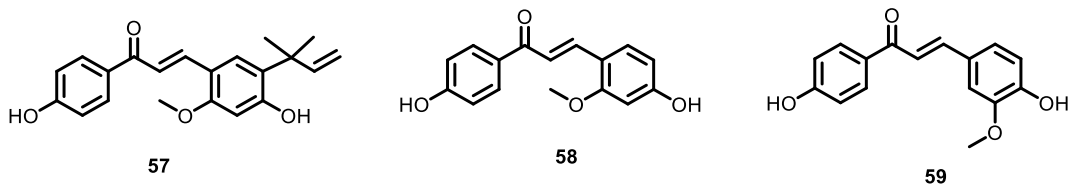


Fig. 15. Chemical structures of chalcone LicoA and some analogues.

In the respective work, the leishmanicidal activities of LicoA and its analogues were evaluated against promastigote and amastigote forms of the pathogen, in which (**57**) exhibited promising leishmanicidal activity *in vitro* with an IC_{50} of 20.26 μM in 24 hours and 3.88 μM in 48 hours against the promastigote.

Compound (**58**) also showed promising activity against the promastigote forms of *L. amazonensis* with an IC_{50} = 74.94 μM in 24 h and 67.16 μM in 48 h. Considering that (**57**) is more active, it was evaluated against amastigote and promastigote forms of *L.infantum*. It showed *in vitro* leishmanicidal activity against promastigote forms with values of IC_{50} at 41.10 μM in 24 h and 12.77 μM after 48 h. In tests on the amastigote form of the parasite, the IC_{50} result after 48 h was 29.58 μM. These results illustrate that (**57**) is more active in the promastigote stage of the parasite.

The cytotoxicity of (**57**) was moderate (CC_{50} = 123.21μM) and no hepatotoxicity was observed at the dosage tested guaranteeing its safety in an animal model. The *in vivo* model was also tested with hamsters infected with the parasite, in which the total parasite load in the host was reduced by 96%. This observation justifies further studies to verify the possibility of compound **(57)** becoming a drug.

Plants of the genus *Poligonum* have demonstrated antiparasitic and antiprotozoal properties and, due to this observation, some of the active compounds have been compound extracted. As such, their activities against leishmaniasis and trypanosomiasis were determined [81]. Different chalcones present in *P. salicifolium* were isolated and evaluated against *T.brucei, T. congolense and L. mexicana* (Fig. 16) [82].

Inhibition was observed for all tested parasites and the highest activity was assigned to compound (**60**), which showed a promising half maximal effective concentration (EC_{50}) of 2.04 μM against kinetoplastid strains of *T. brucei* with the chalcone (**61**) being the second most active with EC_{50} of 14.6 μM.

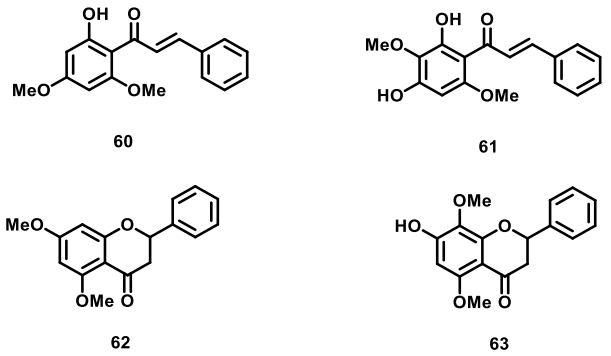


Fig. 16. Chemical structures of chalcones (**60** and **61**) and their derivatives (**62** and **63**).

The trypanocidal effect according to the authors may be related to the position of hydroxyl and methoxy groups in the aromatic rings of chalcones (**60**) and (**61**), in addition to the trypanocidal effect against *T. brucei,* which also showed moderate activity against *T. congolense* and a reasonable activity against *L. mexicana,* with an SI>172.

1.9 Flavonoids

Flavonoids are secondary metabolites with varied medicinal properties, found in fruits, flowers and leaves of various plants. They have a phenolic structure containing a skeleton with 15 carbon atoms in which an aromatic ring is fused with a heterocyclic ring attached to another heterocyclic ring, and are subdivided into six main groups: flavonols, flavanones, flavones, flavonoids, isoflavones and anthocyanins [83, 84].

For example, the polyphenol epigallocatechin-3-gallate (EGCG, (**64**), Fig. 17), found in green tea, was screened by Reis and co-workers [85] for arginase (ARG) inhibition, which is the first enzyme of the polyamine synthesis pathway. The enzymes of this pathway are highly important in the development of drugs against leishmaniasis and *Leishmania* arginase (ARG-L) is considered essential for the parasite rhythm. Furthermore, previous research has reported the potential inhibitions of flavonoids against protozoa. EECG showed strong inhibition against recombinant arginine from *L. amazonensis* (ARG-L), as well as (+)-catechin (**65**) and (-)-epicatechin (**66**) (Fig. 17). In high concentrations, they also inhibit mouse liver arginase (ARG-1), however, they are more active against the parasite enzyme. It was observed by the enzymatic kinetics that (**65**) and (**66**) are competitive inhibitors of ARG-L, whereas EGCG is a mixed inhibitor and gallic acid (**67**) is a non-competitive inhibitor. The most potent arginase inhibitor is (**65**) (IC_{50} = 0.8 μM) followed by (**66**) (IC_{50} =1,8 μM), (**67**) (IC_{50} = 2,2 μM) and (**64**) (IC_{50} = 3,8 μM). Docking analysis showed interactions of different amino acids with the arginase inhibitors ARG-L and ARG-1. This study helps to explain the mechanism of action of EGCG against *L. amazonensis*, through selective and mixed inhibition of arginase, and suggests further studies regarding the use as a possible drug in association with other antileishmanial agents.

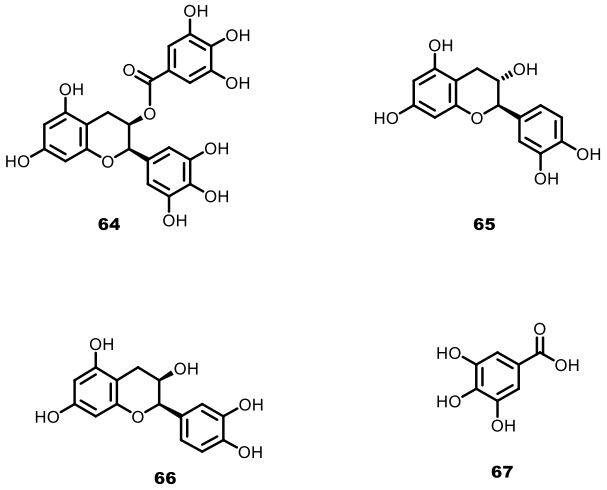


Fig. 17. Chemical structure of EGCG, (+)-catechin, (-)-epicatechin and gallic acid.

In a study by Wong's group [86], flavonoid dimers linked to aminoethylene glycol were prepared and evaluated against leishmaniasis. One of the flavonoid compounds (**68),** containing a hydroxymethylpyridinyl substituent (Fig. 18) showed antipromastigote activity with an IC_{50} ranging from 0.19 to 0.69 μM and anti-amastigote activity with an IC_{50} ranging from 0.17 to 2.2 μM against different *Leishmania* species that cause cutaneous leishmaniasis, including *L. amazonensis*, *L. braziliensis*, *L. tropica* and *L. major*. Compound (**68**) was not toxic to macrophages (IC_{50}> 88 μM). In mouse models of cutaneous leishmaniasis induced by subcutaneous inoculation of *L. amazonensis*, intra-lesional administration of (**68**) in saline solution reduced the lesion thickness by 36% when compared to controls. In addition, the amastigote load on the lesions was reduced 20-fold. The study suggested that this flavonoid derivative represents a new class of safe and effective leishmanicidal agents against visceral and cutaneous leishmaniasis.

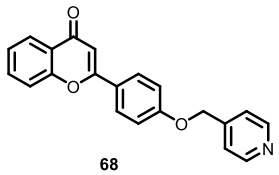
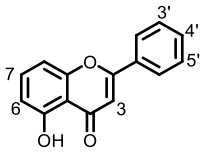


Fig. 18. Chemical structure of flavonoid (**68**).

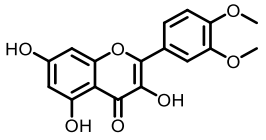
Vitex simplicifolia (Verbenaceae) commonly used for the

treatment of toothache, edema, skin diseases, gout and trypanosomiasis in Nigeria, showed pronounced trypanocidal activity against *T. brucei rhodesiense*. Its methanolic extract was studied by Nwodo and co-workers [87] for identification and assessment of the component responsible for the activity. Isolated compounds (**69**), (**70**), **(71**) and (**72**) (Fig. 19) showed good trypanocidal activity with IC_{50} values ranging from 4.7-12.3 μg mL^{-1} and cytotoxicity in the range of 1.58 - 46.20 μg mL^{-1}. Compound (**71**), however, showed the most selective trypanocidal activity with a SI of 9.8. The authors suggest carrying out further studies with structural modifications to obtain molecules with better trypanocidal activity and selectivity.



Flavon-5-ol

	3	6	7	8	3'	4'	5'
69	OCH_3	H	OH	H	H	OH	OCH_3
70	OCH_3	OCH_3	OCH_3	H	H	OH	H
71	OCH_3	H	OCH_3	H	H	OH	H
72	OH	H	OH	H	H	OH	H
73	OCH_3	OCH_3	OCH_3	H	H	OCH_3	OCH_3



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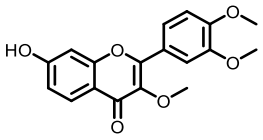
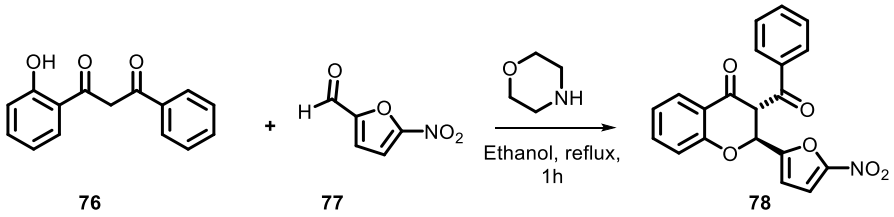


75

Fig. 19. Chemical structures of seven flavonoids derived from *Vitex simplicifolia*.

The group of Diogo [88] executed the synthesis of novel 3-benzoyl-flavanone derivatives and evaluated their trypanocidal activity, one of which was shown to have biological activity. The *in vitro* evaluation of the activity of derivative (**78**), with a nitrofuran function (Fig. 20) against *T. cruzi* was carried out against the amastigote and trypomastigote forms of the parasite. The results were promising since this synthetic flavanone exhibited an IC_{50} = 2.6 μM, lower than the reference drug, benznidazole, whose IC_{50} was 3.8 μM. In general, nitrofuryl derivatives are potent compounds that exhibit trypanocidal activity, as highlighted in the study. Therefore, the authors recommend the *in vivo* evaluation of the compound in question.



76 **77** **78**

Fig. 20. Synthesis of a nitrofuranyl flavanone derivative.

1.10 Quinones and naphtoquinones

Quinones are a class of organic compounds found in several natural products, including plants, fungi and bacteria [89]. These molecules are derived from hydroquinone (**79**) and are divided into classes known as benzoquinones (**80**), naphthoquinones (**81**), anthraquinones (**82**) and polyquinones (**83**), as illustrated in Fig. 21. Many quinones show antifungal, anticancer and antibacterial activities [90].

These classes of compounds are very versatile chemical species due to their biological activities and have been used for the synthesis of new drugs, with activities against cancer, HIV-1, leishmaniasis, and trypanosomiasis [91,92].

In the context of DNT, β-Lapachone (**81**) shows anti-trypanosomal and anti-leishmanicidal activities [93]. In general, naphthoquinones, such as lapachone, have been studied for 40 years since they are promising prototypes of new leishmanicidal and trypanocidal agents mainly due to their oxidizing properties. In addition, the bioactivities have been associated with redox cycling, DNA fragmentation, DNA topoisomerase I and II inhibition, bioreductive alkylation via generation of quinone metides, arylation of thiol groups of proteins and generation of free radicals [94].

To increase the bioavailability and biological activity of quinone derivatives, Vázques and collaborators reported new derivatives as potential anti-Chagas agents [95]. In this work, different 2-aryloxy-naphthoquinones (Series I), 7-aryloxy-quinones (Series II) and 6-aryloxy-furanonaphthoquinones (Series III) were prepared (Fig. 22), for subsequent evaluation of their *in vitro* activities against *T. cruzi*.

To obtain the IC_{50} values of the compounds, nifurtimox

was used as a reference drug, which is an effective drug in the human therapy of trypanosomiasis, caused by *T. cruzi*. As a result, it was concluded that all aryloxyquinones exhibited potent and more active trypanosomal activity than nifurtimox. In addition, the insertion of electron-withdrawing groups in the aryloxy portion intensifies the trypanosomicidal effect. As a comparison, compounds that contain a pyridine ring (series II) have a greater trypanosomicidal effect and the insertion of an oxygenated heterocyclic ring (series III) reduces the trypanosomicidal effect.

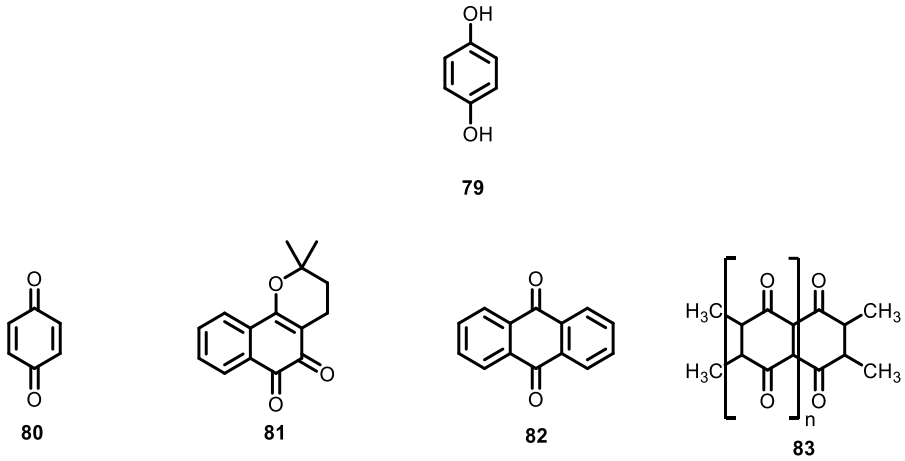


Fig. 21. Examples of quinones according to their class.

Another strategy currently implemented in organic synthesis consists of using metallic catalysts, due to: 1) their ability to form σ and π C-C bonds, 2) the wide variety of available ligands 3) the ligand effect 4) oxidation state variability and 5) the coordination number [96].

The use of rhodium-catalyzed [2+2+2] cycloaddition is reported in the literature as a good option for the synthesis of new substrates derived from quinones [97]. In this work, several naphthoquinones with different substitution patterns in the A ring portion were obtained (Fig. 23). From the biological tests, they concluded that such modifications increase the ability of the compounds to kill the parasite that causes Chagas disease with the compounds synthesized in this work being more potent than the reference drug (benznidazole). This methodology also extends to the synthesis of anthraquinone derivatives which is a natural product with leishmanicidal action [98].

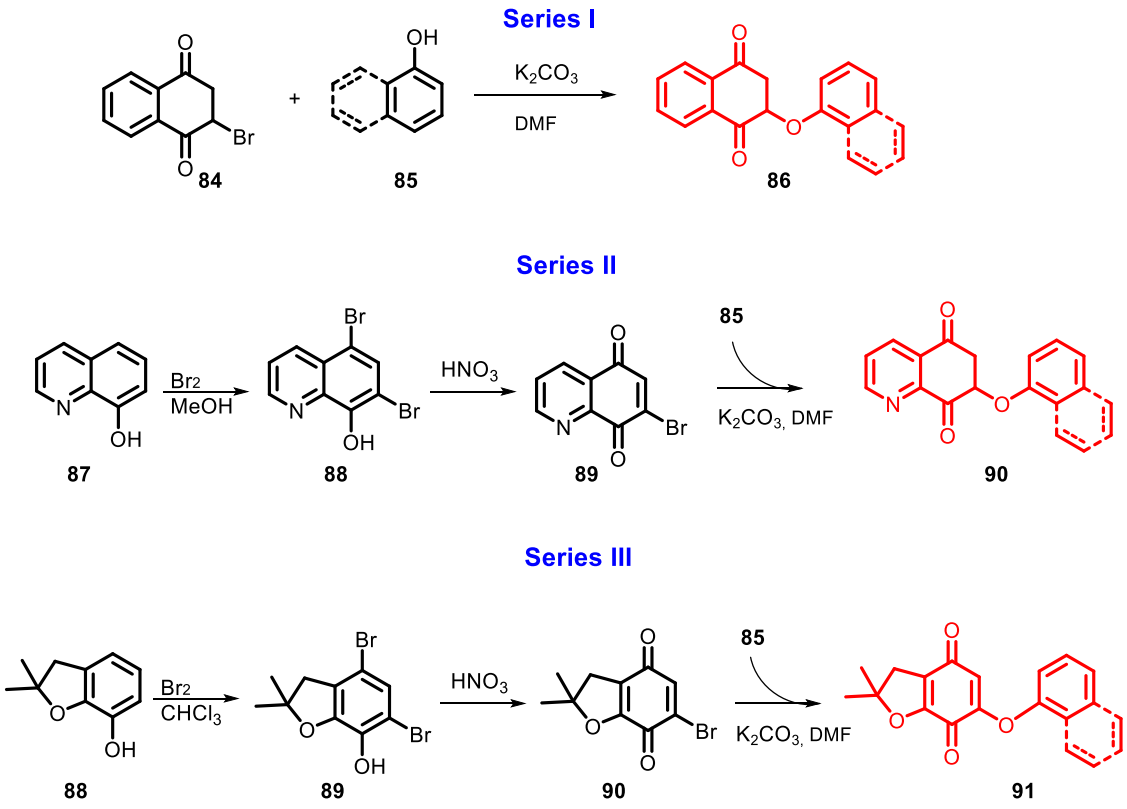


Fig. 22. Synthesis of several aryloxyquinones with trypanosomicidal activity.

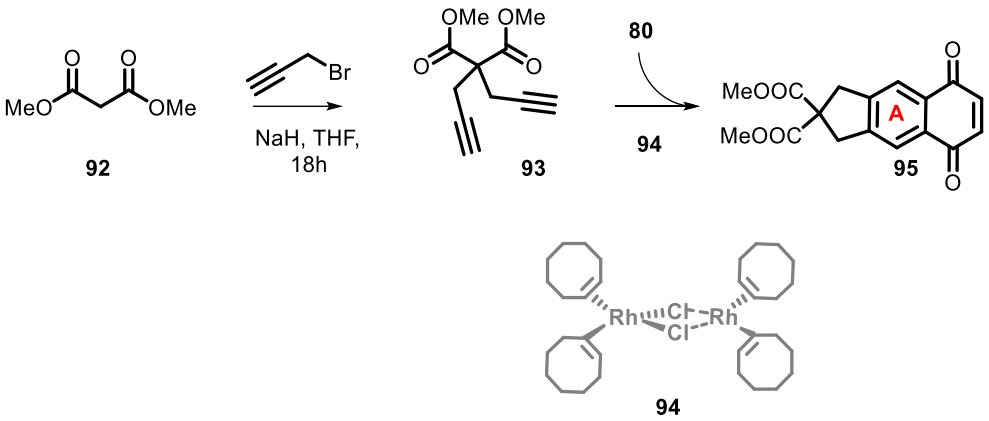


Fig. 23. Synthesis of naphthoquinones from malonates using a rhodium catalyst.

Considering current trends in "green chemistry" which seek to reduce the number of steps in synthesis, increase yields, consideration of environmental sustainability, chemoselectivity and the "atomic" economy of reagents, catalysis employing transition metals for C-H activation is an attractive method [99].

For the synthesis of quinone derivatives, the first example of the application of C-H activation catalyzed by palladium and ruthenium for the functionalization of these compounds has recently been reported in the literature [100]. In this work, a variety of 2-aryl-1,4-naphthoquinones (**97**-**98**) was prepared using the protocol of Baran and coworkers [101]. After arylation, a methodology was sought for the insertion of a hydroxyl group in the A ring of the quinoidal system, both catalyzed by ruthenium and palladium, in which the methodologies were regioselective and complementary (Fig. 24).

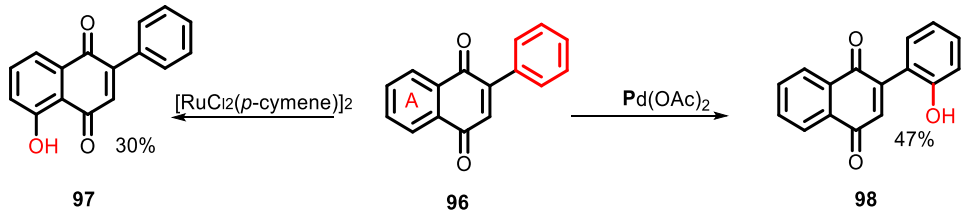


Fig. 24. Methodology for the synthesis of hydroxy-aryl-1,4-naphthoquinones using ruthenium and palladium catalysts.

As the oxidizing agent, the compound iodosobenzene bis(trifluoroacetate), PIFA, was used, and the yields obtained for the ruthenium-catalyzed compounds were low and the palladium-catalyzed ones led to C-H activation in the aryl ring of 1,4-naphthoquinones. Finally, as a result of biological tests, it was observed that the insertion of methyl and electron-withdrawing groups in the aryl portion significantly increases the trypanocidal activity, four times more than benznidazole [101].

1.11 Saponins

Saponins are important active substances in Chinese medicine and are characterized by having glycosylated steroids or triterpenoid groups in their structure [102]. Triterpenoids such as saponins (**99**) (Fig. 25), where R is the sugar portion, are the most abundant and have their characteristics, such as hemolytic activity and foaming property, imparting astringency and bitter taste to materials containing high concentrations of saponins [103].

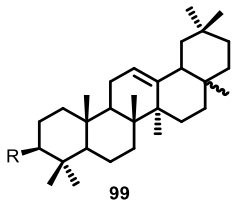


Fig. 25 General skeleton of saponin triterpenoid.

Considering plants that have saponins in their composition, one can cite the species *Albizia gummifera,* which is a plant naturalized in Brazil and is also known as the peacock flower [104]. Recent work described extracts from *Albizia gummifera* that were carried out *in vitro* studies against *T. brucei brucei* species [105].

In this study, it was revealed that the plant has antibacterial and antitrypanosomal activities related to the presence of alkaloids, saponins and triterpenes. The results were obtained from the ethanolic and aqueous extracts of the plant *A. gummifera*, which showed a high concentration of saponins (126.66 mg g^{-1}), while the aqueous extracts showed a moderate concentration of saponins (44.33 mg g^{-1}). When using the ethanolic extracts against *T. brucei brucei*, the earlier total cessation of total mortality occurs within 30 minutes of incubation and in 45 minutes complete mortality was observed, the aqueous extracts presented similar data as both resulted in complete parasite mortality in no more than 60 minutes.

The IC_{50} value found for the ethanolic extract is 15.5-25 µg mL^{-1} whereas the IC_{50} found for the aqueous extract is 23.4-25 µg mL^{-1}, indicating a higher potency of the ethanolic extract compared to the aqueous extract, and similar to the standard drugs Centre-Diminal Plus® (diminazine diaceturate, antipyrine and vitamin B12) with IC_{50} = 15.73-20 mg mL^{-1}.

Assays using plant species to determine anti-leishmanial activity are also described, such as the use of *Glinus oppositifolius*, in the study reported by Banerjee and

collaborators on the intracellular effect of spergulin-A (**100**) (Fig. 26) against leishmaniasis [106].

The tests were carried out against the promastigote and axenic amastigote forms of *L. donovani* at intervals of 24 hours to 72 hours, where the viability at 24 hours was 87.6% for the promastigote form and 77.8% for the amastigote at 100 μg mL^{-1}. After 72 hours at the exposed dose of 100 μg mL^{-1} of (**100**), the viability of 74.79% and 73.55% were obtained for promastigote and amastigote, respectively. The authors observed a plateau of the anti-leishmania effect in 30 μg mL^{-1} of (**100**), and the experiments were carried out at this concentration, utilizing time as a function of efficacy. This resulted in a parasite reduction of 86.2% after 24 hours of exposure compared to baseline.

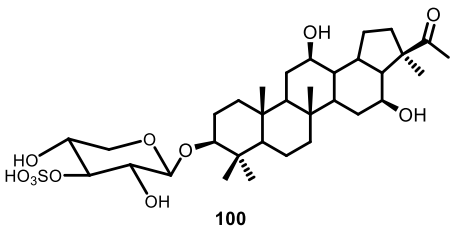
The Lethal Concentration 50 (LC50) values of **100** against the amastigote stage of *L. donovani* were 15.15 μg mL^{-1}after 24 h, 9.32 μg mL^{-1} after 48 h and 6.22 μg mL^{-1} after 72 h, indicating that the median lethal dose is more toxic against *L. donovani* at 72 h of exposure. Miltefosine was used as a reference drug and showed promising results for the treatment of several Leishmania strains [107]. LC_{50} values presented by this drug, according to the authors, were 23.59, 16.64 and 6.73 μg mL^{-1} for 24, 48 and 72 h, respectively. When comparing the LC_{50} of the reference drug to the values of spergulin-A, a saponin functionalized derivative, presented more promising results, with LC_{50}values lower and consequently more potent against the amastigote stage of *L. donovani*.

Methods aimed at the functionalization of bioactive molecules are useful for increasing the bioavailability of antileishmanial agents. Anderson and collaborators took advantage of this strategy to evaluate the effect of semisynthetic saponins extracted from the species *Hedera helix* [108].

The compounds of interest are hederagenins, which are triterpenoid saponins that have biological, hemolytic, antiviral, fungicidal and cytotoxic activities [109]. However, it is also known that hederagenins can deregulate mammalian cell membranes resulting in toxicity [110]. This limiting factor against mammalian cells was found when 128 compounds derived from hederaginin were tested against axenic amastigotes of *L. mexicana* and only 12 presented $EC_{50} \leq 10$, which is the standard imposed by the industry for the development of anti-leishmania drugs [111].

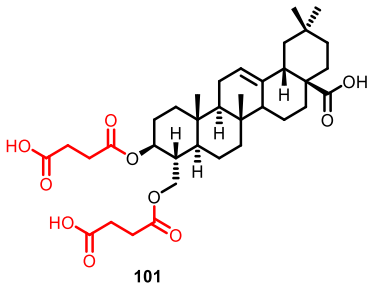
From the 12 selected compounds, cytotoxicity tests were performed against RAW 467.4 macrophages, where only one compound derived from the hederagenin skeleton (**101**) with a triacid portion (Fig. 27) did not show toxicity, selectivity index (SI) > 10.

The compound (**101**) is not effective against infected cells, but the ability to synthesize a non-toxic hederagenin derivative would be a great achievement for medicinal chemistry and open the way for deeper studies of this class of compounds.



100

Fig. 26. Chemical structure of spergulin-A.



101

Fig. 27. Chemical structure of hederagenin disuccinate.

1.12 Lignans

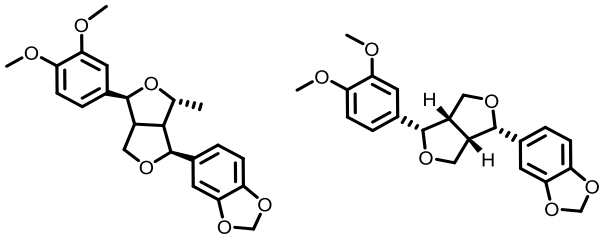
Lignans are a class of secondary metabolites, known to show anti-inflammatory and antioxidant activity, in addition to presenting excellent ADMET profiles. This class of compounds is widely found in plants, in several locations, such as roots, seeds and flowers. Classic lignans are phenylpropane dimers that have a β-β' bond, whereas neolignans are phenylpropanoid dimers that do not contain a β-β' bond [112].

Maia and co-workers [113] performed a computational analysis to create a database to predict protein functions and model their structures. This knowledge would assist in performing simulations of the kinetics of the metabolic pathway to predict biological activity, toxicity, affinity and flexibility between receptors and ligands. In this manner, it was possible to facilitate the development and identification of lignans with low toxicity and selective activity against different leishmania targets. A set of 160 lignans selected for testing showed that the analysis of the results was able to select 11 lignans with potential activity against *L. major* and 21 lignans against *L. braziliensis*, with low or no toxicity. Of these compounds, 4 were isolated from the species *Justicia aequilabris* (Nees) Lindau, popularly known as eucalyptus and were studied *in vitro*. The epipinoresinol-4-*O*-β-*D*-glucopyranoside lignan was the only one to demonstrate antileishmanial activity against promastigote forms of *L. major*, with an IC_{50} = 36,51 μM. In *L. braziliensis*, the compounds secoisolariciresinol, pinoresinol-4-*O*-β-*D*-glucopyranoside, epipinoresinol-4-*O*-β-*D*-glucopyranoside and pinoresinol-4-*O*-β-*D*-apiofuranosyl-(1→2)-β-*D*-glucopyranoside inhibited growth, with IC_{50} values of 9.28, 36.35, 5.39 and 13.77 μM respectively. Secoisolariciresinol, epipinoresinol-4-*O*-β-*D*-glucopyranoside and pinoresinol-4-*O*-β-*D*-apiofuranosyl compounds-(1→2)-β-*D*-glucopyranoside proved to be excellent growth inhibitors for the promastigote stage of the parasite. When compared with the values obtained in the prediction of biological activity, using the random forest (RF) models, it was noted that the probability of activity for *L. major* ranged from 50% to 60%, while the values for *L. braziliensis* ranged from 50% to 75%. The authors suggested testing compounds with activity probabilities above 60% to obtain improved results and reported that the computational approach can be employed to guide experimental research in the development of new drugs.

The trypanocidal activity of *Piper jericoense* was studied by the García-Huertas group [114]. The researchers prepared extracts from the leaves of *P. jericoense* using different solvents including ethyl acetate, benzene, dichloromethane and methanol and these extracts were evaluated against *T. cruzi*. All extracts showed activity against *T. cruzi* strains, however, the ethyl acetate extract fractions showed good activity and selectivity, with an IC_{50} = 14.3 μg mL^{-1}.

The chemical analysis performed showed that the fraction

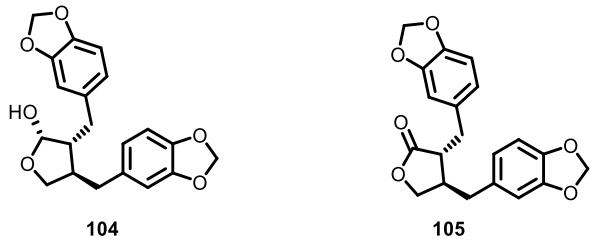
consisted of only one compound, a furofuran-type lignan (**102**) (Fig. 28). This lignan was tested against all forms of the parasite (epimastigote, amastigote and trypomastigote) and was active against all of them. In addition, the fraction from ethyl acetate showed less toxicity than the reference drug, benznidazole, with an SI of 18.4 and 6.7 respectively. Another finding was that this featured compound inhibited the infectious process and was active in acutely infected mice. The authors also evaluated the inhibition of the enzyme iron superoxide dismutase (Fe-SOD) of *T. cruzi* by lignan, which showed a significant result, while Cu/Zn-SOD of human erythrocytes was not affected. The study proposed that the mechanism of action of the researched lignan may be related to alterations in the parasite's antioxidant defences, which leads to cell death. The chemical structure of lignan is similar to kobusin (**103**) which is active against *P. falciparum* [115].



102 **103**

Fig. 28. Chemical structures of lignans furofuran and kobusin.

The lignans cubebin (**104**) and hynokinin (**105**) (Fig. 29), isolated from several plant species have been investigated for their antitrypanosomal activities [116]. This study was carried out using the dried seeds of *Piper cubeba*, popularly known as cubeb, for the isolation of (**104**), which by an oxidation reaction with pyridinium chlorochromate (PCC) yielded (**105**). Lignans were evaluated during the acute phase of infection by *T. cruzi* and there was a significant reduction in parasitemia in animals treated orally with lignans and an increase in life span, as the animals in the negative control group died between 23 and 26 days, while those treated with cubebin and hynokinin died between 28 and 32 days. days. In addition, the lignans were more efficient than the control drug, benzimidazole.

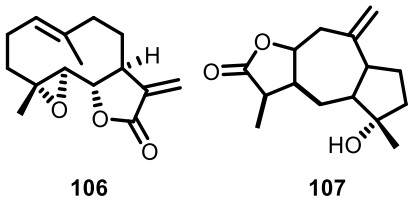


104 **105**

Fig. 29. Chemical structure of cubebin and hinokinin.

Cubebin was also researched by Neres and co-workers [117], in which the researchers used both encapsulated with Poly(D,L-lactide-co-glycolide) (PLGA) and unencapsulated cubebin in BALB mice. As a result, a reduction of 61.3% and 58.5% in the number of circulating trypomastigotes was observed for encapsulated and non-encapsulated cubebin, respectively, in addition to a longer survival time in which the animals treated with encapsulated cubebin survived for 20 days, those treated with benznidazole 18 days, while those who received unencapsulated cubebin and the negative control PLGA had shorter survival (16 days). Thus, it was evidenced that the particles encapsulated with lignan were more active than the number of circulating parasites. Furthermore, molecular docking simulations indicated that cubebin interacts hydrophobically with α-tubulin residues and, by hydrogen bonding with β-tubulin residues, which helps in understanding the trypanocidal activity of this lignan.

The sesquiterpene lactones, parthenolide (**106**) and guaianolide (**107**) (Fig. 30), obtained from the aerial parts of the *Tanacetum parthenium* plant were studied *in vitro* and *in vivo* against leishmaniasis [118]. The lactone-rich dichloromethane extract was used. For the *in vitro* studies, the extract presented an IC_{50} of 2.40 μg mL^{-1} against the promastigote form and 1.76 μg mL^{-1} against the axenic amastigote form of *L.amazonensis*. The *in vivo* intramuscular treatment with the lactone-rich extract decreased the growth and size of the paw lesions in mice. There was also a significant decrease in the parasite population compared to the reference drug, Glucantime®. Plasma levels of malondialdehyde were evaluated to detect possible changes in oxidative stress and it was observed that the extract slightly increased these levels, which can be attributable to its composition rich in parthenolide lactone. This compound causes cellular apoptosis and demonstrates efficacy in treatment without toxicity or genotoxicity.



106 **107**

Fig. 30. Chemical structures of partenolide e guaianolide.

The plant *Haplophyllum tuberculatum* was also studied by the group of Mahmoud [119] regarding the lignans and saponins present. The compounds (Fig. 31) were extracted using ethanol and three partitions were obtained, in petroleum ether, chloroform and ethyl acetate, which were purified and subsequently identified subfractions were obtained. The antiprotozoal activity of the compounds was determined against amastigote forms of *L. donovani, P. falciparum* and *T. brucei rhodesiense*. The lignan, nectandrin B (**108**) exhibited the highest activity against L. donovani (IC_{50} = 4.5 μM) and the highest SI of 25.5. The comparison between (**108**) and tetrahydrofuroguaiacin B (**109**) has shown that (**108**) was less active and more toxic. The two compounds differ only in their configuration in the tetrahydrofuran ring, which appears to play a crucial role in the antileishmanial activity. Therefore, to more easily understand the role of the stereochemistry in the central ring and the contribution of substituents on aromatic rings, structurally related lignans (**110**) and (**111**) were also tested. However, both compounds were significantly less active than nectrandrin B. The compound 3,3'-dimethoxy-4,4'-dihydroxylignan-9-ol (**112**) was the most active against *P. falciparum* (IC_{50} of 9.3 μM; 13.7 SI), steroidal saponins showed activity with $IC_{50} < 8.0$ μg mL^{-1} in all parasites, however, they were less selective (SI < 2). The activity of nectandrin B against axenic amastigotes of *L. donovani* was not relevant when tested against intracellular amastigotes, possibly due to the lack of penetration or stability of nectandrin B in macrophages. However, the authors pointed out that the specific antileishmanial activity of nectandrin B could serve as a starting point for the development of a novel anti-leishmanial drug target.

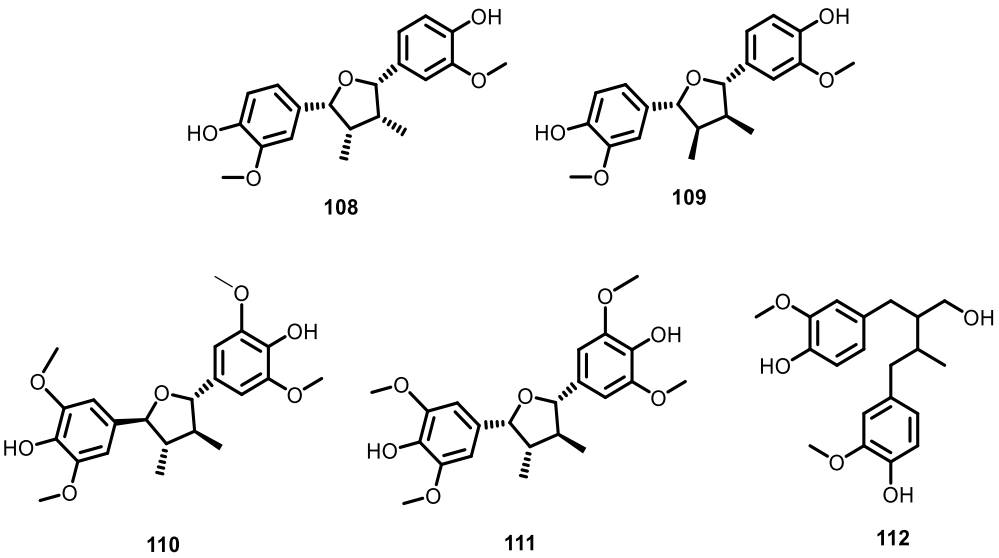


Fig. 31. Chemical structures of constituents present in the extract of *H. tuberculatum*.

2. Conclusions

This work highlights recent research concerning the applications of natural products as candidates for treatments against leishmaniasis and Chagas Disease. The classes of compounds addressed essential oils, triterpenoids, synthetic and semi-synthetic derivatives of flavonoids, quinones and chalcones. The results of this research are summarized to demonstrate the state of the art in the area and to direct the reader to the sources.

Although further clinical studies are ongoing, continued research is still necessary. Some of the NPs could provide for populations in vulnerable socioeconomic groups. The lack of access to conventional allopathic treatments to combat these diseases, NPs offer a relatively inexpensive treatment and have been used traditionally for various pathologies. The biodiversity of tropical biomes encountered in Latin America and Africa provides an extensive source of biomolecules which have never previously been studied.

Based on recent data, the essential oils from *M. pulegium* and *R. officinalis,* which are distributed worldwide, could be an option for alternative, a natural product-derived, leishmaniasis treatments.

Although several natural products and their derivatives show broad-spectrum activity against leishmaniasis and Chagas disease, there is still much to be desired for improvements and investments in this area of research. It is of primordial importance to develop novel synthetic and semi-synthetic molecules. This would permit the modification of the physical, chemical and biological properties of the pharmaceutical agents. These modifications could conceivably alter the pharmacokinetic; absorption, distribution, metabolism, elimination and toxicity profiles, as well as the pharmacodynamic alterations offering possibly new mechanisms of action. These improvements could conceivably result in the development of improved therapeutic agents and treatments for leishmaniasis and Chagas disease.

For regions with socio-economic difficulties, the development of effective treatments based upon renewable natural product resources represents an opportunity to combat these diseases while simultaneously advancing medical research.

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Author Contributions

AFS Fuzaro, CS Ponciano, JC Barros, EP Ávila: Conceptualization, Data curation, Writing – original draft, Writing – review & editing. RM Grazul, MV Almeida: Supervision, Writing – review & editing.

References and Notes

[1] Available from: https://www.dndial.org/wp-content/uploads/2021/10/DNDi_Caderno_Institucional_PORT_2018.pdf?x65733. Access April, 2022.

[2] Hotez, P. J.; Molyneux, D. H.; Fenwick, A.; Ottesen, E.; Sachs, S. E.; Sachs, J. D. *PLoS Med.* **2006**, *3*, 576. [Crossref]

[3] Available from: http://www.oim.tmunicipal.org.br/?pagina=detalhe_noticia&noticia_id=28312. Access April, 2022.

[4] Dias, L. C.; Dessoy, M. A.; Guido, R. V. C.; Oliva, G.; Andricopulo, A. D. *Quim. Nova* **2013**, *36*, 1552. [Crossref]

[5] Hotez, P. J.; Aksoy, S.; Brindley, P. J.; Kamhawi, S. *PLoS Negl. Trop. Dis*. **2020**, *14*, 6. [Crossref]

[6] Available from: https://www.who.int/neglected_diseases/diseases/en/. Access May, 2022.

[7] Costa, J. M. L. *Gaz. Méd. Bahia* **2005**, *75*, 3. [Link]

[8] Lukeš, J.; Skalický, T.; Týč, J.; Votýpka, J.; Yurchenko, V. *Mol. Biochem. Parasitol*. **2014**, 195, 115. [Crossref]

[9] Votýpka, J.; d'Avila-Levy, C. M.; Grellier, P.; Maslov, D. A.; Lukes, J.; Yurchenko, V. *Trends Parasitol*. **2015**, *31*, 460. [Crossref]

[10] Guerin, P. J.; Olliaro, P.; Sundar, S.; Boelaert, M.; Croft, S. L.; Desjeux, P.; Wasunna, M. K.; Bryceson, A. D. M.

Lancet Infect. Dis. **2002**, *2*, 494. [Crossref]

[11] Moraes, C. S.; Seabra, S. H.; Castro, D. P.; Brazil, R. P.; de Souza, W.; Garcia, E. S.; Azambuja, P. *Exp. Parasitol*. **2008**, *118*, 561. [Crossref]

[12] David, C. V.; Craft, N. *Dermatol. Ther*. **2009**, *22*, 491. [Crossref]

[13] Moafi, M.; Rezvan, H.; Sherkat, R.; Taleban, R. *Int. J. Prev. Med*. **2019**, *10*, 95. [Crossref]

[14] Available from: https://www.gov.br/saude/pt-br/assuntos/noticias/saude-lanca-nova-estrategia-para-controle-da-leishmaniose-visceral. Access April, 2022.

[15] Dujardin, J.-C.; Gonzalez-Pacanowska, D.; Croft, S. L.; Olesen, O. F.; Spath, G. F. *Trends Parasitol*. **2010**, *26*, 395. [Crossref]

[16] Dorlo, T. P. C.; Balasegaram, M.; Beijnen, J. H.; de Vries, P. J. *J. Antimicrob. Chemother*. **2012**, *67*, 2576. [Crossref]

[17] Barrett, M. P.; Croft, S. L. *Br. Med. Bull*. **2012**, *104*, 175. [Crossref]

[18] Minodier, P.; Parola, P. *Travel Med. Infect. Dis*. **2007**, *5*, 150. [Crossref]

[19] Testasicca, M.; Santos, M. S.; Machado, L. M.; Serufo, A. V.; Doro, D.; Avelar, D.; Tibúrcio, A. M. L.; Abrantes, C.F.; Machado-Coelho, G. L. L.; Grimaldi, G.; Gazzinelli, R. T.; Fernandes, A. P. *Vet. Parasitol*. **2014**, *204*, 169. [Crossref]

[20] Grimaldi, G.; Teva, A.; Santos, C. B.; Santos, F. N.; Pinto, I. D.; Fux, B.; Leite, G. R.; Falqueto, A. *PLoS One* **2017**, *12*, 18. [Crossref]

[21] Cotrina, J. F.; Iniesta, V.; Monroy, I.; Baz, V.; Hugnet, C.; Maranon, F.; Fabra, M.; Gómez-Nieto, L. C.; Alonso, C. *Vaccine* **2018**, *36*, 1972. [Crossref]

[22] Jansen, A. M.; Roque, A. L. R. Domestic and wild mammalian reservoirs. In: American Trypanosomiasis. Telleria, J.; Tibayrenc, M., eds. London: Elsevier; 2010. p. 249-276. [Crossref]

[23] Patino, L. H.; Ramirez, J. D. *Infect. Genet. Evol*. **2017**, *49*, 273. [Crossref]

[24] Souza, W., Carvalho, T. M., Barrias, E. S. *Int. J. Cell. Biol*. **2010**, 295394. [Crossref]

[25] Dias, J. C. P.; Coura, J. R. Clínica e terapêutica da doença de Chagas: uma abordagem prática para o clínico geral, 1st ed. Rio de Janeiro: Fiocruz, 1997.

[26] Yoshida, N.; Cortez, M. *Subcell. Biochem*. **2008**, *47*, 82. [Crossref]

[27] Coura, J. R. *Mem. Inst. Oswaldo Cruz* **2009**, *104*, 549. [Crossref]

[28] Available from: https://ufmg.br/comunicacao/noticias/faculdade-de-medicina-lanca-site-com-historico-do-combate-a-doenca-de-chagas. Access May, 2022.

[29] Available from: https://revistapesquisa.fapesp.br/um-mapa-da-resposta-a-doenca-de-chagas/. Access April, 2022.

[30] Freitas, E.; Melo, M. N.; da Costa-Val, A. P.; Michalick, M. S. M. *Vet. Parasitol*. **2006**, *137*, 159. [Crossref]

[31] Coutinho, M. T. Z.; Linardi, P. M. *Vet. Parasitol*. **2007**, *147*, 320. [Crossref]

[32] Naucke, T. J.; Lorentz, S. *Parasit. Vectors*. **2012**, *5*, 67. [Crossref]

[33] Schmunis, G. A.; Yadon, Z. E. *Acta Trop*. **2010**, *115*, 14. [Crossref]

[34] Bern, C.; Kjos, S.; Yabsley, M. J.; Montgomery, S. P. *Clin. Microbiol. Rev*. **2011**, *24*, 655. [Crossref]

[35] Manderson, L.; Aagaard-Hansen, J.; Allotey, P.; Gyapong, M.; Sommerfeld, J. *PLoS Negl. Trop. Dis*. **2009**, *3*, 6. [Crossref]

[36] Buschiazzo, A.; Amaya, M. F.; Cremona, M. L.; Frasch, A. C.; Alzari, P. M. *Mol. Cell*. **2002**, *10*, 757. [Crossref]

[37] Amaya, M. F.; Watts, A. G.; Damager, I.; Wehenkel, A.; Nguyen, T.; Buschiazzo, A.; Paris, G.; Frasch, A. C.; Withers, S. G.; Alzari, P. M. *Structure* **2004**, *12*, 775. [Crossref]

[38] Azeredo, C. M. O.; Avila, E. P.; Pinheiro, D. L. J.; Amarante, G. W.; Soares, M. J. *FEMS Microbiol. Lett*. **2017**, *364*, 7. [Crossref]

[39] Santos, M. M. M.; Moreira, R. *Mini Rev. Med. Chem*. **2007**, *7*, 1040. [Crossref]

[40] Available from: https://www.bio.fiocruz.br/index.php/br/noticias/2794-dia-mundial-das-doencas-tropicais-negligenciadas. Access April, 2022.

[41] Wadood, A. *Biochem. Anal. Biochem*. **2013**, 02. [Crossref]

[42] Sangi, D. P. *Quim. Nova*. **2016**, *39*, 995. [Crossref]

[43] Cheuka, P. M.; Mayoka, G.; Mutai, P.; Chibale, K. *Molecules* **2017**, *22*, 41. [Crossref]

[44] Ndjonka, D.; Rapado, L. N.; Silber, A. M.; Liebau, E.; Wrenger, C. *Int. J. Mol. Sci*. **2013**, *14*, 3395. [Crossref]

[45] Varela, M. T.; Fernandes, J. P. S. *Curr. Med. Chem*. **2020**, *27*, 2133. [Crossref]

[46] Shaaban, H. A. E.; El-Ghorab, A. H.; Shibamoto, T. *J. Essent. Oil Res*. **2012**, *24*, 203. [Crossref]

[47] Estevam, E. B. B.; Miranda, M. L. D.; Alves, J. M.; Egea, M. B.; Pereira, P. S.; Martins, C. H. G.; Esperandim, V. R.; Magalhães, L. G.; Bolela, A. C.; Cazal, C. M.; Souza, A. F.; Alves, C. C. F. *Rev. Virtual Quim*. **2016**, *8*, 1842. [Crossref]

[48] Andrade, P. M. D.; Melo, D. C. D.; Alcoba, A. E. T.; Ferreira Jr, W. G.; Pagotti, M. C.; Magalhães, L. G.; Santos, T. C. L.; Crotti, A. E. M.; Alves, C. C.F.; Miranda, M. L. D. *An. Acad. Bras. Cienc*. **2018**, *90*, 2671. [Crossref]

[49] Rosa, M. S. S.; Mendonça-Filho, R. R.; Bizzo, H. R.; Rodrigues, I. A.; Soares, R. M. A.; Souto-Padrón, T.; Alviano, C. S.; Lopes, A. H. C. S. *Antimicrob. Agents Chemother*. **2003**, *47*, 1895. [Crossref]

[50] Arruda, D. C.; D'Alexandri, F. L.; Katzin, A. M.; Uliana, S. R. B. *Antimicrob. Agents Chemother*. **2005**, *49*, 1679. [Crossref]

[51] Rottini, M. M.; Amaral, A. C. F.; Ferreira, J. L. P.; Oliveira, E. S. C.; Silva, J. R. A.; Taniwaki, N. N.; Santos, A. R.; Almeida-Souza, F.; Souza, C. S. F.; Calabrese, K. S. *Molecules* **2019**, *24*, 2525. [Crossref]

[52] Hamdi, A.; Bero, J.; Beaufay, C.; Flamini, G.; Marzouk, Z.; Heyden, Y. V.; Quetin-Leclercq, J. *BMC Complement. Med. Ther*. **2018**, *18*, 1. [Crossref]

[53] Kpoviessi, S.; Bero, J.; Agbani, P.; Gbaguidi, F.; Kpadonou-Kpoviessi, B.; Sinsin, B.; Accrombessi, G.; Frédérich, M.; Moudachirou, M.; Quetin-Leclercq, J. *J. Ethnopharmacol*. **2014**, *151*, 652. [Crossref]

[54] Bouyahya, A.; Et-Touys, A.; Bakri, Y.; Talbaui, A.; Fellah, H.; Abrini, J.; Dakka, N. *Microb. Pathog.* **2017**, *111*, 41. [Crossref]

[55] Tasdemir, D.; Kaiser, M.; Demirci, B.; Demirci, F.; Baser, K. *Molecules* **2019**, *24*, 4421. [Crossref]

[56] Kushiro, T.; Ebizuka, Y. Triterpenes. In: Comprehensive Natural Products II. Liu, H.-W.; Mander, L., eds. Oxford: Elsevier, 2010, p. 673-708. [Crossref]

[57] Bakkali, F.; Averbeck, S.; Averbeck, D.; Waomar, M. *Food Chem. Toxicol.* **2008**, *46*, 446. [Crossref]

[58] Cárdenas, P. D.; Almeida, A.; Bak, S. *Front. Plant Sci.* **2019**, *10*, 1. [Crossref]

[59] Hodon, J.; Borkova, L.; Pokorny, J.; Kazakova, A.; Urban, M. *Eur. J. Med. Chem.* **2019**, *182*, 25. [Crossref]

[60] Cháirez-Ramirez, M. H.; Moreno-Jimenez, M. R.; Gonzalez-Laredo, R. F.; Gallegos-Infante, J. A.; Rocha-Guzman, N. E. *Excli J*. **2016**, *15*, 758. [Crossref]

[61] Duarte, N.; Ramalhete, C.; Lourenço, L. Plant terpenoids as lead compounds against malaria and leishmaniasis. In: Studies in Natural Products Chemistry. Atta-ur-Rahman, ed. Amsterdam: Elsevier, 2019, p. 243-306. [Crossref]

[62] Meira, C. S.; Barbosa, J. M.; Lanfredi-Rangel, A.; Guimaraes, E. T.; Moreira, D. R. M.; Soares, M. B. P. *Exp. Parasitol.* **2016**, *166*, 108. [Crossref]

[63] Sobeh, M.; Rezq, S.; Sabry, O. M.; Abdelfattah, M. A. O.; El Raey, M. A.; El-Kashak, W. A.; El-Shazly, A. M.; Mahmoud, M. F.; Wink, M. *Biomed. Pharmacother*. **2019**, *115*, 10. [Crossref]

[64] Xu, F.; Huang, X. L.; Wu, H. M.; Wang, X. P. *Biomed. Pharmacother.* **2018**, *103*, 198. [Crossref]

[65] Andrade, E. M. J.; Teixeira, J. S.; Silva, D. K. C.; Santos, T. B.; Korn, M. G. A.; Brandão, H. N.; Ferraz, C. G.; Meira, C. S.; Soares, M. B. P.; Guimarães, E. T.; Neta, L. C. S.; Júnior, A. F. S. *J. Braz. Chem. Soc.* **2020**, *31*, 1825. [Crossref]

[66] Wagner, H.; Ulrich-Merzenich, G. *Phytomedicine* **2009**, *16*, 97. [Crossref]

[67] Polanco-Hernández, G. M.; Giménez-Turba, A.; Salamanca, E.; Getti, G.; Rai, R.; Acosta-Viana, K. Y.; Arana-Argáez, V. E.; Torres-Romero, J. C.; Fernández-Martín, K. G.; Segura-Campos, M. R.; Moo-Puc, R. E.; Peña-Rodríguez, L. M. *Rev. Bras. Farmacogn*. **2021**, *31*, 199. [Crossref]

[68] Arevalo-Lopez, D.; Nina, N.; Ticona, J. C.; Limachi, I.; Salamanca, E.; Udaeta, E.; Paredes, C.; Espinoza, B.; Serato, A.; Garnica, D.; Limachi, A.; Coaquira, D.; Salazar, S.; Flores, N.; Sterner, O.; Giménez, A. *J. Ethnopharmacol.* **2018**, *216*, 120. [Crossref]

[69] Madkour, L. H. Endoplasmic reticulum stress-induced cell death mechanism. In: Reactive Oxygen Species (ROS), nanoparticles, and Endoplasmic Reticulum (ER) stress-induced cell death mechanisms. Madkour, L. H., ed. London: Academic Press, 2020, p. 299-342. [Crossref]

[70] Eksi, G.; Kurbanoglu, S.; Erdem, S. A. Analysis of diterpenes and diterpenoids. In: Recent Advances in Natural Products Analysis. Silva, A. S.; Nabavi, S. F.; Saeedi, M., Nabavi, S. M., eds. Amsterdam: Elsevier, 2020, p. 313-345. [Crossref]

[71] Pertino, M. W.; Vega, C.; Rolón, M.; Coronel, C.; de Arias, A. R.; Schmeda-Hirschmann, G. *Molecules* **2017**, *22*, 10. [Crossref]

[72] Paula, C. D. R.; Sampaio, J. H. D.; Cardoso, D. R.; Sampaio, R. N. R. *Rev. Soc. Bras. Med. Trop.* **2003**, *36*, 365. [Crossref]

[73] Martinez-Peinado, N.; Cortes-Serra, N.; Torras-Claveria, L.; Pinazo, M. J.; Gascon, J.; Bastida, J.; Alonso-Padilla, J. *Parasit. Vectors* **2020**, *13*, 10. [Crossref]

[74] Parra, L. L. L.; Bertonha, A. F.; Severo, I. R. M.; Aguiar, A. C. C.; Souza, G. E.; Oliva, G.; Guido, R. V. C.; Grazzia, N.; Costa, T. R.; Miguel, D. C.; Gadelha, F. R.; Ferreira, A. G.; Hajdu, E.; Romo, D.; Berlinck, R. G. S. *J. Nat. Prod.* **2018**, *81*, 188. [Crossref]

[75] Calvo, A.; Moreno, E.; Larrea, E.; Sanmartin, C.; Irache, J. M.; Espuelas, S. *Pharmaceutics* **2020**, *12*, 1. [Crossref]

[76] Rosa, R.; Dambrós, B. P.; Höehr de Moraes, M.; Grand, L.; Jacolot, M.; Popowycz, F.; Steindel, M.; Schenkel, E. P.; Bernardes, L. S. C. *Bioorg. Chem*. **2022**, *119*, 105492. [Crossref]

[77] Ferreira, M. K. A.; Fontenelle, R. O. S.; Magalhães, F. E. A.; Bandeira, P. N. S.; Menezes, J. E. S. A.; Santos, H. *Rev. Virtual Quim.* **2018**, *10*, 1455. [Crossref]

[78] Rozmer, Z.; Perjési, P. *Phytochem. Rev*. **2016**, *15*, 87. [Crossref]

[79] Friis-Møller, A.; Chen, M.; Fuursted, K.; Christensen, S. B.; Kharazmi, A. *Planta Med*. **2002**, *68*, 416. [Crossref]

[80] Souza, J. M.; Carvalho, E. A. A.; Candido, A.; Mendonca, R. P.; Silva, M. F.; Parreira, R. L. T.; Dias, F. G. G.; Ambrósio, S. R.; Arantes, A. T.; Silva Filho, A. A.; Nascimento, A. N.; Costa, M. R.; Sairre, M. I.; Veneziani, R. C. S.; Magalhães, L. G. *Front. Vet. Sci*. **2020**, *7*, 10. [Crossref]

[81] Zhou, S.-Y.; Liu, Y.-M.; Zhang, Q.-Z.; Fu, Y.-W.; Lin, D.-J. *Vet. Parasitol*. **2018**, *253*, 22. [Crossref]

[82] Zheoat, A. M.; Alenezi, S.; Elmahallawy, E. K.; Ungogo, M. A.; Alghamdi, A. H.; Watson, D. G.; Igoli, J. O.; Gray, A. I.; Koning, H. P.; Ferro, V. A. *Pathogens* **2021**, *10*, 9. [Crossref]

[83] Mendes, L. A. O.; Ponciano, C. S.; Cataneo, A. H. D.; Wowk, P. F.; Bordignon, J.; Silva, H.; Almeida, M. V.; Ávila, E. P. *Chem. Biol. Interact.* **2020**, *331*, 12. [Crossref]

[84] Ponciano, C. S.; Ávila, E. P.; Grazul, R. M.; Mendes, L. A. D.; Almeida, M. V. *Med. Chem. Res*. **2021**, *30*, 1056. [Crossref]

[85] Reis, M. B. G.; Manjolin, L. C.; Maquiaveli, C. D.; Santos, O. A.; Silva, E. R. *PLoS One* **2013**, *8*, 9. [Crossref]

[86] Wong, I. L. K.; Chan, K. F.; Chen, Y. F.; Lun, Z. R.; Chan, T. H.; Chow, L. M. C. *Antimicrob. Agents Chemother.* **2014**, *58*, 3379. [Crossref]

[87] Nwodo, N.; Okoye, F.; Lai, D.; Debbab, A.; Kaiser, M.; Brun, R.; Proksch, P. *BMC Complement. Altern. Med.* **2015**, *15*, 5. [Crossref]

[88] Diogo, G. M.; Andrade, J. S.; Sales Junior, P. A.; Murta, S. M. F.; Santos, V. M. R.; Taylor, J. G. *Molecules* **2020**, *25*, 16. [Crossref]

[89] Obach, R. S.; Kalgutkar, A. S. Reactive electrophiles and metabolic activation. In: Comprehensive Toxicology. McQueen, C. A, ed, 2nd ed. Oxford: Elsevier, 2010, p. 309-47. [Crossref]

[90] Eyong, K. O.; Kuete, V.; Efferth, T. Quinones and benzophenones from the medicinal plants of Africa. In:

Medicinal Plant Research in Africa. Kuete, V., ed. Oxford: Elsevier, 2013, p. 351-391. [Crossref]

[91] Pérez-Sacau, E.; Díaz-Peñate, R. G.; Estévez-Braun, A.; Ravelo, A. G.; García-Castellano, J. M.; Pardo, L.; Campillo, M. *J. Med. Chem.* **2007**, *50*, 696. [Crossref]

[92] Eyong, K.O.; Kumar, P. S.; Kuete, V.; Folefoc, G. N.; Nkengfack, E. A.; Baskaran, S. *Bioorg. Med. Chem. Lett*. **2008**, *18*, 5387. [Crossref]

[93] Ferreira, V. F.; Ferreira, S. B.; Silva, F. D. *Org. Biomol. Chem*. **2010**, *8*, 4793. [Crossref]

[94] Barbosa, J. M. C.; Nicoletti, C. D.; Silva, P. B.; Melo, T. G.; Futuro, D. O.; Ferreira, V. F.; Salomão, K. *PLoS One* **2021**, *16*, e0246811. [Crossref]

[95] Vázquez, K.; Espinosa-Bustos, C.; Soto-Delgado, J.; Tapia, R. A.; Varela, J.; Birriel, E.; Segura, R.; Pizarro, J.; Cerecetto, H.; González, M.; Paulino, M.; Salas, C. O. *RSC Adv.* **2015**, *5*, 65153. [Crossref]

[96] Victor, M. M.; da Silva, G. S. B. *Quim Nova* **2016**, *39*, 859. [Crossref]

[97] Wood, J. M.; Satam, N. S.; Almeida, R. G.; Cristani, V. S.; Lima, D. P.; Dantas-Pereira, L.; Salomão, K.; Menna-Barreto, R. F. S.; Namboothiri, I. N. N.; Bower, J. F.; Silva Júnior, E. N. *Bioorg. Med. Chem*. **2020**, *28*, 13. [Crossref]

[98] Pérez, J. A.; Boluda, C.; López, H.; Trujillo, J. M. *Chem. Pharm. Bull*. **2004**, *52*, 130. [Crossref]

[99] Meyer, T. H.; Finger, L. H.; Gandeepan, P.; Ackermann, L. *Trends Chem*. **2019**, *1*, 63. [Crossref]

[100] Gontijo, T. B.; Carvalho, R. L.; Dantas-Pereira, L.; Menna-Barreto, R. F. S.; Rogge, T.; Ackermann, L.; Silva Júnior, E. N. *Bioorg. Med. Chem.* **2021**, *40*, 11. [Crossref]

[101] Fujiwara, Y.; Domingo, V.; Seiple, I. B.; Gianatassio, R.; Del Bel, M.; Baran, P. S. *J. Am. Chem. Soc.* **2011**, *133*, 3292. [Crossref]

[102] Zhang, C.; Wang, N.; Feng, Y. Oxidative stress, Chinese herbals and toxicity: A focused review with examples. In: Toxicology. Patel VB, Preedy VR, eds. London: Academic Press, 2021, p. 215-224. [Crossref]

[103] Mohan, V. R.; Tresina, P. S.; Daffodil, E. D. Antinutritional factors in legume seeds: Characteristics and determination. In: Encyclopedia of Food and Health. Caballero, B.; Finglas, P. M.; Toldrá, F., eds. London: Academic Press, 2016, p. 211-220. [Crossref]

[104] Available from: **https://www.worldagroforestry.org/output/agroforestree-database**. Access April, 2022.

[105] Oloruntola, D. A.; Dada, E. O.; Oladunmoye, M. K. *Open Vet. Sci.* **2021**, *2*, 33. [Crossref]

[106] Banerjee, S.; Mukherjee, N.; Gajbhiye, R. L.; Mishra, S.; Jaisankar, P.; Datta, S.; Das Saha, K. *Infect. Drug Resist*. **2019**, *12*, 2933. [Crossref]

[107] Soto, J.; Soto, P. *Biomédica* **2006**, *26*, 207. [Crossref]

[108] Anderson, O.; Beckett, J.; Briggs, C. C.; Natrass, L. A.; Cranston, C. F.; Wilkinson, E. J.; Owen, J. H.; Williams, R. M.; Loukaidis, A.; Bouillon, M. E.; Pritchard, D.; Lahmann, M.; Baird, M. S.; Denny, P. W. *RSC Med. Chem.* **2020**, *11*, 833. [Crossref]

[109] Chwalek, M.; Lalun, N.; Bobichon, H.; Ple, K.; Voutquenne-Nazabadioko, L. *Biochim. Biophys. Acta – Gen. Subj.* **2006**, *1760*, 1418. [Crossref]

[110] Delmas, F.; Di Giorgio, C.; Elias, R.; Gasquet, M.; Azas, N.; Mshvildadze, V.; Dekanosidze, G.; Kemertelidze, E.; Timon-David, P. *Planta Med*. **2000**, *66*, 343. [Crossref]

[111] Norcliffe, J. L.; Mina, J. G.; Alvarez, E.; Cantizani, J.; de Dios-Anton, F.; Colmenarejo, G.; Gonzalez-Del Valle, S.; Marco, M.; Fiandor, J. M.; Martin, J. J.; Steel, P. G.; Denny, P. W. *Sci. Rep.* **2018**, *8*, 10. [Crossref]

[112] Pilkington, L. I. *Molecules* **2018**, *23*, 24. [Crossref]

[113] Maia, M. D.; Silva, J. P. E.; Nunes, T. A. D.; Sousa, J. M. S.; Rodrigues, G. C. S.; Monteiro, A. F. M.; Tavares, J. F.; Rodrigues, K. A. F.; Mendonça-Junior, F. J. B.; Scotti, L.; Scotti, M. T. *Molecules* **2020**, *25*, 33. [Crossref]

[114] García-Huertas, P.; Olmo, F.; Sánchez-Moreno, M.; Dominguez, J.; Chahboun, R.; Triana-Chavez, O. *Exp. Parasitol*. **2018**, *189*, 34. [Crossref]

[115] Ortet, R.; Prado, S.; Regalado, E. L.; Valeriote, F. A.; Media, J.; Mendiola, J.; Thomas, O. P. *J. Ethnopharmacol*. **2011**, *138*, 637. [Crossref]

[116] Esperandim, V. R.; Ferreira, D. D.; Rezende, K. C. S.; Cunha, W. R.; Saraiva, J.; Bastos, J. K.; Silva, M. L. A.; Albuquerque, S. *Exp. Parasitol.* **2013**, *133*, 442. [Crossref]

[117] Neres, N. B. R.; Montagnini, D.; Ferreira, D. S.; Parreira, R. L. T.; Orenha, R. P.; Lima, T. C.; Molina, E. F.; Cunha, W. R.; Silva, M. L. A.; Esperandim, V. R. *Chem. Biodivers*. **2021**, *18*, 8. [Crossref]

[118] Rabito, M. F.; Britta, E. A.; Pelegrini, B. L.; Scariot, D. B.; Almeida, M. B.; Nixdorf, S. L.; Nakamura, C. V.; Ferreira, I. C. P. *Exp. Parasitol*. **2014**, *143*, 18. [Crossref]

[119] Mahmoud, A. B.; Danton, O.; Kaiser, M.; Khalid, S.; Hamburger, M.; Mäser, P. *Front. Pharmacol*. **2020**, *11*, 1. [Crossref]

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