Drug Candidates for Chagas Disease: A Mini Review on Natural Products in Brazil.

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Abstract. Chagas disease (CD), caused by the protozoan *Trypanosoma cruzi*, is a neglected tropical disease that affects more than 6 million people worldwide, mainly in areas of Latin America. Currently, there is no vaccine against Chagas disease and the available drugs (benznidazole and nifurtimox) are poorly effective and have been associated with several side effects. Therefore, research initiatives to identify new lead compounds for treating Chagas disease are required. Concomitant, natural products have stood out as a source of potential bioactive compounds and Brazilian research groups have been conducting studies on the identification of new molecules. In this context, this study reviews Brazilian publications on Web of Science database in terms of natural product chemical diversity, identification of potential compounds with trypanocidal effect and characterization of hit compounds identified through phenotypic-based screening. From 2012 to 2022, fifty three papers reported four hundred and forty nine active molecules to fight different strains and life stages of the parasite. Plant derived extract, marine derived extract and microorganisms were the classes of compounds, which had the required IC_{50} potency and SI selectivity to be a hit compound, indicating that natural products can be an important source of potential new drugs to treat Chagas disease.

Keywords. *Trypanosoma cruzi*, natural product, drug development.

1. Introduction

Chagas disease (CD) is an infectious disease caused by a group of parasites called *Trypanosoma cruzi*. It has been classified as one of the Neglected Tropical Diseases (NTDs) by the World Health Organization (WHO), which refers to diseases that affect the low and middle income population and receive little attention and investment in research from government and private agencies.[1][2] According to the WHO, approximately 6–7 million people are infected with *T. cruzi* worldwide and it is mostly distributed among the 21 countries of Latin America.[1]

Infection occurs mainly through infected bloodsucking insects (Triatomine), but other ways of transmission include blood transfusion, organ transplants or contaminated food (e.g., açaí berry, sugar cane, fruit juice, undercooked meat).[3] After the initial infection, around 60% to 70% of patients remain asymptomatic for a long time and the remaining 30% to 40% of them will evolve to a chronic disease with cardiac and/or digestive damages, often with a latency of 10 to 30 years.[4]

T. cruzi presents a complex life cycle with three main morphological forms: the epimastigote (replicative, noninfective), which is found in the midgut of the invertebrate vector; the trypomastigote (nonreplicative, infective), which is the form of the parasite found in both hosts; and the amastigote (replicative, infective), which is the intracellular form found only in mammals, and the most difficult to reach by drugs.[5]

Currently, only two nitroheterocyclic drugs are available for the treatment of Chagas disease: benzonidazole and nifurtimox. These drugs are effective mainly in the early infection stage, and they can cause side effects during their use, such as anorexia, nausea, vomiting, headache, central nervous system depression or maniacal symptoms, seizures, vertigo, paresthesia, peripheral polyneuropathies, and dermatitis, which lead to therapy discontinuation in 10–30% of treated patients.[6] As a consequence, there is an urgent need to discover new compounds that are efficient and less toxic for drug development.

In this scenario, natural products have stood out as an alternative source for new bioactive molecules

with trypanocidal potential. About 35 percent of drugs available in the market came directly or indirectly from natural products.[7] Therefore, natural products can inspire the development of molecules to fight protozoan parasites. In this study, the purpose of the short review is to provide an overview of reported projects over a period of 10 years (2012 - 2022) in the identification of natural anti-chagasic compounds in Brazil, a nation that has a rich biodiversity.

2. Research Methods

A literature search was conducted using the Web of Science database and the keywords "*Trypanosoma cruzi* AND natural product AND IC_{50}". To these search criteria, the database returned 150 publications as an initial result.

In order to have significant result, some inclusion criteria were established: papers published between 2012 – 2022 and papers published in Brazil. A total of 67 publications were found and these papers were carefully analyzed to ensure that all of them were related to research on natural compounds with in vitro phenotypic screening against Chagas disease.

The final set of 449 bioactive compounds identified in 53 publications were used in this mini-review.

3. Drug Development

The development of efficient drugs for treating Chagas disease depends on the identification of new hits compounds. Currently, there are two types of drug discovery screening: (i) phenotypic-based screening, which tests the compound in the whole cell organism ,and (ii) target-based screening, which tests the compound in specific molecular of the disease.[8] In the case of CD drug discovery, phenotypic based screening has historically been widely used over target-based screening due to the advantage of addressing the problem in the whole organism, and therefore, identifying hits that are active against the whole cell.[9] In this study, this tendency also remained in the second filtering of publications (n = 67), in which phenotypic-based screening articles were the most prevalent.

One of the greatest difficulties for researchers in the field of Chagas disease is the lack of standardization of experimental protocol, criteria and results. Therefore, in the Drug Discovery process, several experts have recently published a target product profile (TPPT), which defines the properties required for identifying hit and lead compounds. [6] Although these criteria are not strictly applied, they are very important to guide the CD drug development and will be used in this mini review.

Among the classification criteria based on the in vitro evaluation, Kei Katsuno and other researchers defined that a promising Chagas hit should have a half-maximal inhibitory concentration (IC_{50})<10 µM against intracellular amastigotes and a selectivity index (SI) > 10. [10]

4. Literature Review

Considering the number of papers found in the Web of Science database, the final sample with the criteria defined in the methodology provided 53 publications. All these publications are scientific papers published in Brazil during the period of 10 years (2012-2022) that aim to identify natural products as active compounds against Chagas disease through phenotypic screening.

The data extracted from each of the studies were analyzed and divided according to "classes of natural products" (Figure 1), "in vitro IC_{50} values" (Figure 2), and "life stage of the parasite used in the study" (Figure 3).

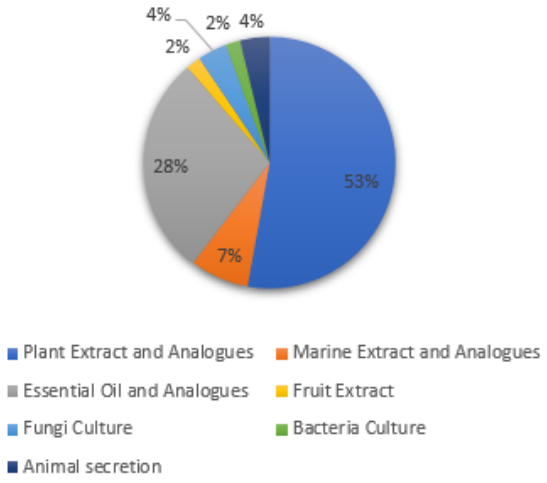

Fig. 1 - Classification of natural products papers published between 2012-2022 in the Web of Science database (n=53 papers).

As depicted in Figure 1, it is not surprising that the highest number of research are from plant-derived extracts (53%), since Brazil has one of the richest floras in the world. Within this group, essential oil and plants from the Cerrado were frequently present in studies of potential drugs for Chagas disease.

When considering either a compound as "active" or "inactive" in Chagas disease, IC_{50} value is one of the parameters to be considered. Although there are no accepted standardized IC_{50} values to evaluate promising extract or compound, it was observed that many papers selected in this review, evaluate natural products with benznidazole and/or nifurtimox, using them as a positive control. Thus, compounds that present an IC_{50} value similar or inferior to benznidazole or nifurtimox will move to the next screening phase.

For the in vitro IC_{50} values, four hundred and forty nine compounds were evaluated to fight different life stages of *T. cruzi* (epimastigote, trypomastigote and amastigotes forms). (Figure 2)

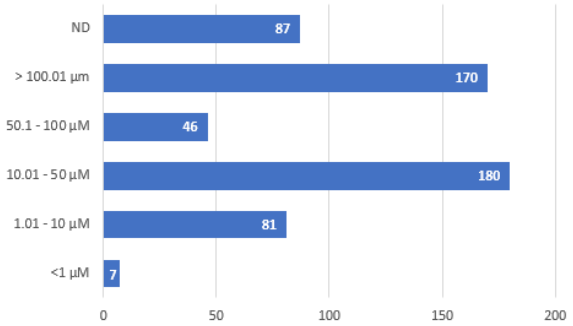

Fig. 2 - In vitro IC_{50} of natural compounds against *Trypanosoma cruzi* trypomastigotes, amastigotes or epimastigotes (n= 571 analyses) reported from 2012 – 2022 in the Web of Science database. ND = not determined.

According to the hit criteria defined before, a desirable hit compound should present considerable efficacy (IC_{50} < 10 µM). Therefore, from the analysed papers, a considerable number of compounds (n= 78) have high in vitro trypanocidal activity, showing that natural products can be an important source of potential new drugs to treat Chagas disease.

Within IC_{50}, trends in cell assays used in the studies were investigated (Figure 3) and highlighted below:

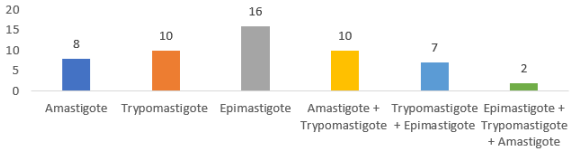

Fig. 3 – Life stages of *T. cruzi* used to investigate trypanocidal activity in the papers reported between 2012 – 2022 in the Web of Science database (n=53 papers).

- Around one-third (n = 19 papers) of the studies screened compounds with more than one life stage of *T. cruzi*.
- Almost half (n=25 papers) of the studies used epimastigote of *T. cruzi*.
- There are few studies (n = 10 papers) dealing with in vitro assays in amastigotes of *T. cruzi*.

These results are also supported by the finding that a relevant number of laboratories use epimastigotes forms in cell assays, due to financial resources and feasibility. Epimastigote screening is much less expensive and easier to implement. However, the results may be questionable as epimastigotes, found in the invertebrate vector, are quite different from the trypomastigote and amastigotes, found in mammalian tissues. [11] While trypomastigotes can easily break into immune mechanisms and remain in the host blood [5], amastigotes forms are predominantly found during the chronic Chargas disease and are primarily responsible for causing the symptoms associated with this stage.[12]

For this reason, considering the relevance of the amastigote forms for Chagas disease, in the following section, the studied compounds that meet the hit criteria[10] for intracellular amastigotes was highlighted.

5. Anti-trypanosomal candidates

From the total of 53 papers, compounds of 8 papers met hit selection criteria in the discovery of potential antichagasic compounds.

Among these 8 papers, four studies were performed considering toxicity in mammalian cells, since toxicity has been a major impediment in the development of new drugs candidates for parasitic disease. Therefore the early knowledge about it allows the compounds to become a successful drug.[13]

5.1 Dehydrodieugenol B Neolignans

A series of analogues of Neolignan dehydrodieugenol B, extracted from *Nectandra leucantha*, showed properties against *T. cruzi*. Among the 21 analogues, five met the hit criteria displaying high potency (IC_{50} of 4–7.7 µM), selectivity over 10-fold (SI of 16,7 - 50) and no mammalian toxicity (CC_{50} of >200 µM). From SAR and in vitro studies, the chemical structure of these analogues has a wide tolerance of modification without decreasing activity against amastigote forms of the parasite.[14]

5.2 Acid meroditerpene

Acid meroditeroene, isolated from brown algae *Stypopodium zonale*, presented a potent trypanocidal effect against amastigotes forms, exhibiting IC_{50} = 2,4 µM and SI = 16. [15]

5.3 3,5-disubstituted isoxazoles

A set of 26 isoxazoles, analogues of the natural lignans grandisin and veraguensin, was synthesized and the trypanocidal activity was tested. Among the synthesizes compounds, three of them were the most active with IC_{50} of 1.13–5.267 µM and SI of 13.3 - 160.9 in intracellular amastigotes of *T. cruzi*. [16]

5.4 Trixikingolides

The crude extract from *Trixis vauthieri DC*, a plant from family *Asteraceae*, resulted an IC_{50} value of 2.7 µg and a selectivity index of 15 against the trypomastigote and amastigote forms of *T. cruzi*. A mixture of two new trixikingolides (1 and 2), fractionated from the extract, showed a higher activity against amastigote forms of the parasite. The mixture 4:1 of 1 and 2 displayed an IC_{50} = 0.053 µM and SI = 68. [17]

5.5 T. Ignis

From five species of marine sponges, *T. ignis* was the only one that exhibited the highest efficacy (IC_{50} of 7.2 µM) and selectivity (SI of 24) against amastigote forms and it does not induce toxicity in mammalian cells (CC50 of >177 µM). [18]

5.6 Arrabidaea brachypoda

Halogenated derivatives of *Arrabidaea brachypoda* showed a higher biological activity against *T. cruzi* than the original natural product. Four halogenated derivatives exhibited IC_{50} of 1.4 - 1.6 μM and SI > 63 against amastigote forms of *T. cruzi* and no mammalian toxicity (CC_{50} of >100 μM). [19]

5.7 Licarin A

A set of semi-synthetic analogues of licarin A, isolated from leaves of *Nectandra oppositifolia*, was designed by the molecular simplification approach. This molecular simplification improved water solubility, decreased lipophilicity and reduced toxicity for the mammals cells. Vanillin and its acetyl derivative displayed activity against intracellular amastigotes of *T. cruzi* with IC_{50} values of 5.5 and 5.6 μM and SI > 36,4 and > 35.6.[20]

5.8 Nectandra leucantha

Inspired by the extract of the flower, *Nectandra leucantha*, new neolignan-based compounds were designed through molecular modeling techniques with the aim of improving biological activity against *T. cruzi.* Eight new compounds displayed activity against intracellular amastigotes of *T. cruzi* with IC_{50} values of 4.2 – 9.5 μM and SI > 21.[21]

6. Conclusion

This mini review shows that between 2012 and 2022, a significant number of natural products papers reported studies on Chagas disease in Brazil. Most of these papers used phenotypic screening to discover new potential compounds to treat CD and a considerable number of analyzed natural products have shown high in vitro trypanocidal activity (n= 78/499 compounds). During the hit identification phase, more than half of the researches have focused on analyzing trypanocidal effects on plants, using benznidazole or nifurtimox as a positive control and approximately half of the studies used epimastigote forms of *T. cruzi* in the cell assays.

During papers reviewing, it was noticed a difficulty in comparing the biological activity between extracts and compounds due to the lack of standardization of results and parameters. Therefore, even at the early stage of drug development, standardization should be adapted to improve the selection of hit compounds, eliminating false positives.

In conclusion, the hit natural compounds reviewed in this studiy have shown a promising effect in their potency and high selectivity against *T. cruzi*. For this reason, the results of these compounds certainly have the potential to aid and inspire future design for new bioactive analogues to fight Chagas disease.

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