

Evaluation of the anti-*Leishmania* mechanisms of *Annona glabra* L. (Annonaceae): A brief review

Avaliação dos mecanismos anti-*Leishmania* de *Annona glabra* L. (Annonaceae): Uma breve revisão

Evaluación de los mecanismos anti-*Leishmania* de *Annona glabra* L. (Annonaceae): Una breve revisión

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Abstract

Leishmaniasis is a neglected tropical disease caused by protozoan parasites of the genus *Leishmania* and characterized by the formation of skin ulcers. Despite the large number of cases, reaching worldwide proportions, there are many factors to be discussed regarding the treatment of the disease, which although much discussed, is still poorly elucidated. The objective of this review study is to identify the main anti-*Leishmania* mechanisms of *Annona glabra*

L. (Annonaceae). This is a literature review study. To this end, we used the following descriptors in the search: natural products, leishmaniasis, mechanism of action and *Leishmania* (together and separately). In the selection criteria we opted for full articles, in the period 2012 - 2021 (last 10 years), in Portuguese and English. The searches were performed in the following databases: *Scientific Electronic Library Online* (SciELO), *Latin American and Caribbean Literature on Health Sciences* (LILACS) and Google Scholar. The articles were selected first by title, then by abstract, and finally by complete reading. The potential of natural products and their use in traditional medicine for the treatment of diseases makes them the target of research for new drugs. Different *Annona* species have demonstrated antileishmanial activity when evaluated *in vitro*. The substances that show leishmanicidal activity in *Annona glabra* extract are the alkaloids. New studies can be developed to elucidate even more specifically the effects of this natural product *in vivo*, in an attempt to obtain a new pharmacological alternative for the treatment of leishmaniasis.

Keywords: Natural products; Leishmaniasis; Mechanism of action; *Leishmania*.

Resumo

A leishmaniose é uma doença tropical negligenciada causada por parasitas protozoários do gênero *Leishmania* e caracterizada pela formação de úlceras na pele. Apesar do grande número de casos, atingindo proporções mundiais, há muitos fatores a serem discutidos com relação ao tratamento da doença, que embora muito discutido, ainda é pouco elucidado. O objetivo deste estudo de revisão é de identificar os principais mecanismos anti-*Leishmania* de *Annona glabra* L. (Annonaceae). Trata-se de um estudo de revisão da literatura. Para tanto, utilizamos na busca os descritores: produtos naturais, leishmaniose, mecanismo de ação e *Leishmania* (juntos e separados). No critério de seleção optamos por artigos completos, no período de: 2012 - 2021 (últimos 10 anos), nos idiomas: português e inglês. As buscas foram realizadas nos bancos de dados: *Scientific Electronic Library Online* (SciELO), *Literatura Latino-Americana e do Caribe em Ciências da Saúde* (LILACS) e Google Scholar. Os artigos foram selecionados primeiramente por título, posteriormente por resumo, e por fim, por leitura completa. O potencial dos produtos naturais e seu uso na medicina tradicional para o tratamento de doenças os torna alvo de pesquisas para novos fármacos. Diferentes espécies de *Annona* já demonstraram atividade antileishmania quando avaliadas *in vitro*. As substâncias que apresentam atividade leishmanicida no extrato da *Annona glabra* são os alcaloides. Novos estudos podem ser desenvolvidos no sentido de conseguir elucidar de forma ainda mais específica os efeitos desse produto natural *in vivo*, na tentativa de obter uma nova alternativa farmacológica para o tratamento da leishmaniose.

Palavras-chave: Produtos naturais; Leishmaniose; Mecanismo de ação; *Leishmania*.

Resumen

La leishmaniasis es una enfermedad tropical desatendida causada por parásitos protozoarios del género *Leishmania* y caracterizada por la formación de úlceras en la piel. A pesar del gran número de casos, que alcanzan proporciones mundiales, hay muchos factores que discutir en relación con el tratamiento de la enfermedad, que, aunque muy discutido, sigue estando poco dilucidado. El objetivo de este estudio de revisión es identificar los principales mecanismos anti-*Leishmania* de *Annona glabra* L. (Annonaceae). Se trata de un estudio de revisión de la literatura. Para ello, utilizamos en la búsqueda los descriptores: productos naturales, leishmaniasis, mecanismo de acción y *Leishmania* (juntos y por separado). En el criterio de selección se optó por artículos completos, en el período: 2012 - 2021 (últimos 10 años), en los idiomas: portugués e inglés. Las búsquedas se realizaron en las siguientes bases de datos: *Scientific Electronic Library Online* (SciELO), *Latin American and Caribbean Literature on Health Sciences* (LILACS) y Google Scholar. Los artículos se seleccionaron primero por el título, luego por el resumen y finalmente por la lectura completa. El potencial de los productos naturales y su uso en la medicina tradicional para el tratamiento de enfermedades lo convierte en el objetivo de la investigación de nuevos fármacos. Diferentes especies de *Annona* ya han demostrado su actividad antileishmania al ser evaluadas *in vitro*. Las sustancias que presentan actividad leishmanicida en el extracto de *Annona glabra* son los alcaloides. Se pueden desarrollar nuevos estudios para dilucidar aún más específicamente los efectos de este producto natural *in vivo*, en un intento de obtener una nueva alternativa farmacológica para el tratamiento de la leishmaniasis.

Palabras clave: Productos naturales; Leishmaniasis; Mecanismo de acción; *Leishmania*.

1. Introduction

Leishmaniasis is a neglected tropical disease caused by protozoan parasites of the genus *Leishmania* and characterized by the formation of skin ulcers. It is considered a neglected disease because there is little interest from the pharmaceutical industry to design and develop new drugs for the chemotherapy of patients affected by this disease; this is probably due to the fact that there would be little financial return for the industries. It is associated with socioeconomic factors, such as poverty and malnutrition, poor access to adequate sanitation, and a malfunctioning immune system. It has an incidence of 220,000 cases per year, and is responsible for affecting more than 1 million people in the last 5 years. It is estimated that about 1 billion

people live in endemic areas at risk for leishmaniasis. Countries that concentrate the highest numbers of cases include Afghanistan, Algeria, Colombia, Islamic Republic of Iran, Pakistan, Peru, Saudi Arabia and Syrian Arab Republic, and Brazil (Who, 2018).

Despite the large number of cases, reaching worldwide proportions, there are many factors to be discussed regarding the treatment of the disease, which although much discussed, is still poorly elucidated. Currently, there are first choice (pentavalent antimonials) and second choice (Amphotericin B and Pentamidine) drugs available for the treatment of leishmaniasis. However, the use of these drugs presents some problems. One of them is related to parenteral administration, which requires collaboration from the patient, often resulting in abandonment of treatment. These drugs also have limited and variable efficacy among *Leishmania* species, besides being expensive and having numerous adverse reactions, which can cause renal failure and hypotension (Lage, 2019). In addition to these factors, parasite resistance to antimonials has been gaining prominence, resulting in treatment failure in up to 60% of patients.

Due to the lack of better therapies, the identification of new drugs is urgent, and medicinal plants are an important source of bioactive molecules that can fulfill this need. Related to *Leishmania*, the use of natural products is an alternative strategy for treatment. In Brazil, the genus *Annona* is the most representative of the Annonaceae family, as it has a wide variety of species, many used in traditional medicine for the treatment of leishmaniasis (Hellman, 2018).

The potential of natural products and their use in traditional medicine for the treatment of diseases makes them a target for research for new drugs (Rocha, et al. 2013). Related to parasitic diseases, this issue is no different. Several studies have shown that species of the Annonaceae family are sources of secondary metabolites with anti-*Leishmania* activity, moreover, the use of these species is comprehensive in folk medicine (Vila-Nova et al., 2011).

In view of the information already described, the aim of this review study is to identify the main anti-*Leishmania* mechanisms of *Annona glabra* L. (Annonaceae).

2. Methodology

To achieve the proposed objective, an integrative literature review was used. The integrative review is the analysis of relevant research that provides support for decision making and the improvement of clinical practice (PEREIRA et al., 2018). To operationalize the development of this review, six steps were followed: development of the research question, sampling or literature search of primary studies, data extraction, evaluation of the included primary studies, analysis and synthesis of the results, and presentation of the review.

To guide the integrative review, the following guiding question was formulated, "What is the scientific evidence in the literature on the anti-*Leishmania* mechanism of *Annona glabra* L.?" The search for primary studies was conducted according to the criteria and manuals of each database. Controlled descriptors were used: natural products, leishmaniasis, mechanism of action and *Leishmania* (together and separately), combined with Boolean operators (AND and OR). The descriptors, as well as the articles selected to compose this study were searched between January and March 2021, in the following databases: *Scientific Electronic Library Online* (SciELO), *Latin American and Caribbean Literature on Health Sciences* (LILACS) and Google Scholar.

The inclusion criteria for the delimited studies were articles that addressed the proposed theme, published between January 2012 and February 2021, and with the following classifications: studies with experimental design, studies with non-experimental design, such as descriptive correlational and qualitative research or case studies, case reports or systematically obtained data, and literature reviews, published in Portuguese and English. The exclusion criteria established were: articles that did not address the topic in question, that were written in languages other than those chosen for inclusion, studies published before 2013, and duplicates in divergent databases.

Given the established criteria, a number of 12 articles were selected to compose the theoretical foundation for the study in question.

3. Results and Discussion

3.1 Information about the species *Annona glabra* L.

Annona glabra (Figure 1) is characterized by its subcoriaceous, glabrous leaves with long petioles varying from 1.2 to 2 cm in length. Its popular names are: "araticum", "araticum-do-rio", "araticum-panã", "araticum do brejo", "araticum-cortiça", "araticum-da-praia", "araticum-de-jangada". Studies on *A. glabra* have demonstrated a large amount of compounds of diverse chemical nature present in extracts prepared from bark, stem, leaves and fruit with anti-inflammatory potential on HIV virus replication in lymphocytes, as a cytotoxic agent, trypanocidal, larvicidal, antimicrobial, vermifuge, sporicidal, analgesic, contraceptive, anti-inflammatory and anti-*Leishmania* (Rocha et al., 2017; Amarasingue et al., 2020).

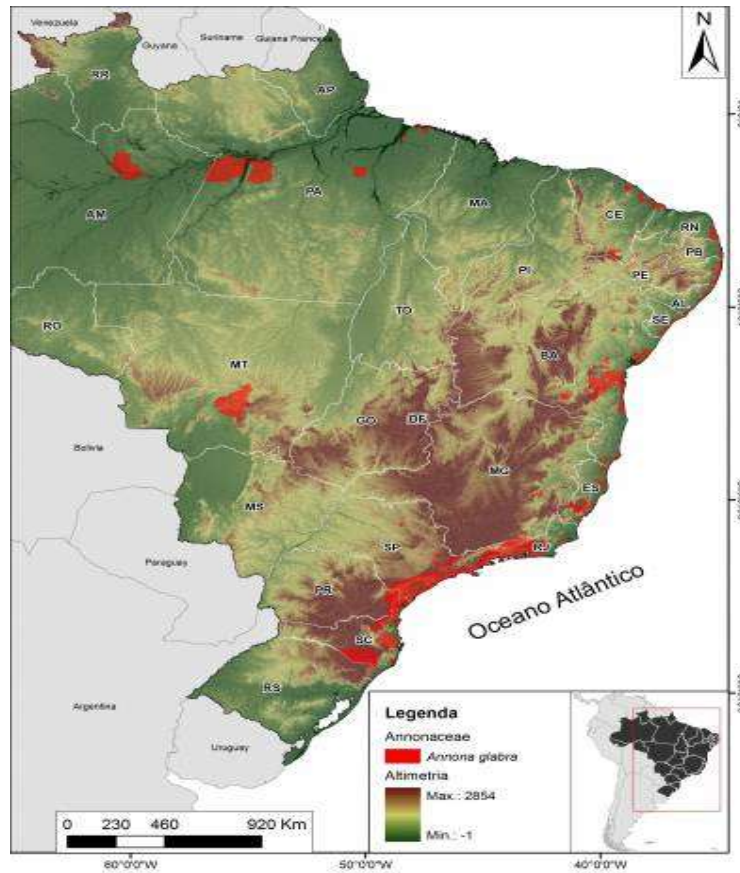
Figure 1. *Annona glabra* L.



Source: National Center for Flora Conservation (2012).

The species is abundant and common in Brazilian forests, but with few population data. It is a species of wide distribution in the states of Amapá, Pará, Maranhão, Ceará, Pernambuco, Bahia, Alagoas, Sergipe, Minas Gerais, Espírito Santo, São Paulo, Rio de Janeiro, Paraná, and Santa Catarina (Figure 2). It is also distributed in other Latin American and African countries.

Figure 2. Geographical distribution of the *Annona glabra* species.

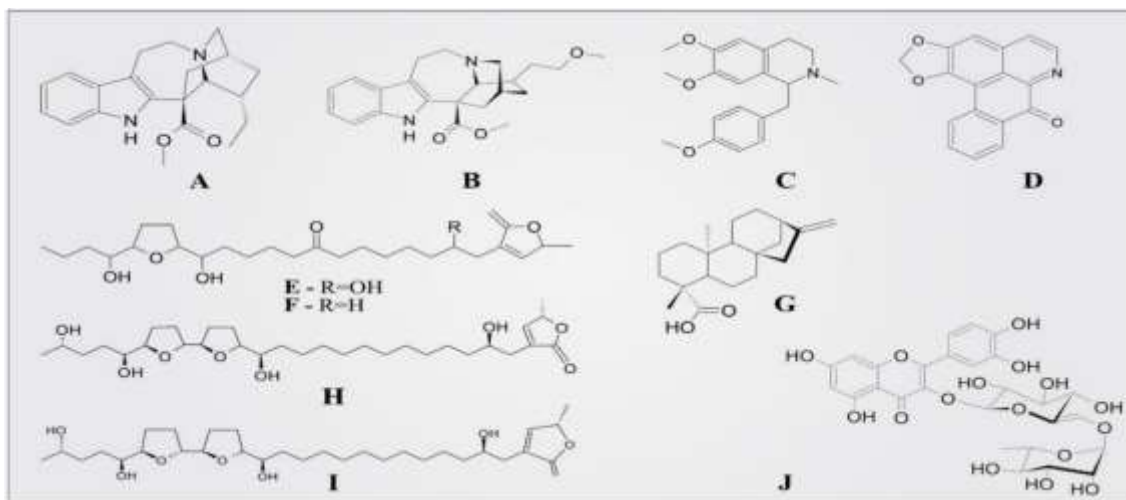


Source: National Center for Flora Conservation (2012).

3.2 Anti-*Leishmania* mechanisms of *Annona glabra* L.

Different *Annona* species have demonstrated antileishmanial activity when evaluated *in vitro*. The substances that show leishmanicidal activity in *Annona glabra* extract are the alkaloids (figure 3). Among the alkaloids isolated from the genus species that have already demonstrated anti-*Leishmania* activity are coronaridine (Figure 1.A), 18-methoxycoronaridine (Figure 1.B), O-methylarmepavine (Figure 1.C), and liriodenine (Figure 1.D). In addition, acetogenins anonacinone (Figure 1.E) and corosolone (Figure 1.F) were also promising as leishmanicides. Among terpenes, kaurenoic acid (Figure 1.G) showed activity against *Trypanosoma cruzi* and Human Immunodeficiency Virus (HIV), besides showing antimicrobial activity.

Figure 3. Isolated compounds from *Annona* species.



Source: Da Silva et al. (2020).

From the studies available in the literature regarding the anti-*Leishmania* activity of *Annona* species, it was possible to verify that there is a lack of research evaluating the activity against the intracellular form (amastigote). One of the few studies showed that extracts obtained from *Annona mucosa* were tested on *L. donovani*, *L. amazonensis* and *L. braziliensis*. Most of the extracts showed activity against promastigotes of *L. amazonensis* ($IC_{50} > 50 \mu\text{g/mL}$) (IC = Inhibitory Concentration), and no significant reduction in the infection rate of macrophages by amastigotes was observed (Lima et al., 2012).

Another study demonstrated that flavonoid dimers exhibit antipromastigote (CI_{50} : 0.19 to 0.69 μM) and antiamastigote (CI_{50} : 0.17 to 2.2 μM) activity against *L. amazonensis*, *L. braziliensis*, *L. tropica*, and *L. major* (Wong et al., 2014). In addition, synthetic flavonoid dimers can inhibit the pumping activity of ATP-binding cassette (ABC) transporters, resulting in increased intracellular drug concentration and reversal of parasite drug resistance in *Leishmania* (Wong et al., 2014). This synergism may be related to inhibition of the pumping activity of ATP-binding cassette (ABC) transporters, resulting in increased intracellular drug concentration. In this sense, thorough investigation on such mechanism of action is a must, especially because one of the problems related to drug therapy of LC is the resistance of parasites to available drugs (Sundar et al., 2012; Frezard et al., 2014).

In a study conducted by Da Silva and co-workers in 2020, the samples obtained from *A. glabra* (EE = Ethanolic Extract; FH = Hexane Fraction; FM = Methanol and Rutin Fraction) were evaluated for antipromastigote activity and cytotoxicity. The results show that none of the samples were found to be active on the promastigote form of *L. amazonensis*. They were also not considered toxic to THP-1 cells that underwent the induction process. All samples were submitted to activity evaluation against the amastigote form of *Leishmania amazonensis*. Although the ethanolic extract, FM and Rutin did not reduce the infection rate in macrophages, FH reduced infection in a dose-dependent manner (250 $\mu\text{g/mL}$ = 39.1%; 125 $\mu\text{g/mL}$ = 18.7% and 62.5 $\mu\text{g/mL}$ = 4.6% (Da Silva et al., 2020).

It is true that one of the weaknesses observed by the authors to obtain references for theoretical foundation of this work is the scarcity of scientific articles about *in vivo* experiments that could prove the accuracy of this method in question; in this sense, this finding leads us to reflect to seek in the near future, the possibility of performing these tests so that there is a contribution to the scientific community.

4. Conclusion

The data presented here suggest that species of the genus *Annona* are promising for the treatment of leishmaniasis,

considering the importance of extract fractionation and isolation of alkaloids and other phytoconstituents. It is worth noting that, flavonoids can be an important tool for the treatment of leishmaniasis, because besides decreasing the protozoan's trypanothione production and increasing the therapeutic effect on host cells, they can be an alternative for parasites resistant to existing drugs for chemotherapy against *Leishmania*.

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