Chapter

microRNAs: Are They Important in the Development of Resistance in Leishmaniasis?

Sandra Alves de Araújo, Tatiane Aranha da Penha-Silva, Jaqueline Diniz Pinho, Marcelo de Souza Andrade and Ana Lucia Abreu-Silva

Abstract

Leishmaniasis is an infectious and parasitic disease of great importance in public health. Numerous studies indicate that biochemical and molecular mechanisms are factors that contribute to the emergence of antileishmanial drug resistance. Currently, miRNAs have been identified as targets for the invasion of pathogens to control the immune response and imply resistance to treatments. Considering the alarming growth in drug resistance, new possibilities for controlling leishmaniasis have been emerging. Natural compounds originating from medicinal plants are being increasingly explored as promising antileishmanial alternatives. The chapter aims to provide a brief review on mechanisms of action associated with traditional agents used to treat leishmaniasis, focusing mainly on molecular bases associated with the resistance of *Leishmania* spp. to current drugs and identifying the possible miRNAs involved in this process. In addition, we seek to describe some of the promising plant molecules that can be used as potential antileishmanial agents and their possible mechanisms of action.

Keywords: Leishmaniasis, drug resistance, miRNAs, mechanisms of action, natural products

1. Introduction

Leishmaniasis is an infectious and parasitic disease of major importance to public health worldwide due to its difficult control, mainly because the commonly used antileishmanial drugs have contributed to the increasing increase in parasite resistance [1, 2].

Biochemical, molecular and/or genetic mechanisms are factors that contribute to the emergence of resistance [3–5]. Among these mechanisms, we highlight microRNAs (miRNAs), small non-coding RNAs functionally involved in various biological processes of an organism [6–8]. Currently, these structures have been identified as the main targets for invasion by pathogens, including *Leishmania* spp., aiming to control the immune response and possibly implying resistance to treatments [9, 10].

Due to the alarming increase in drug resistance, the search for new drugs or natural compounds to control leishmaniasis has grown [11, 12]. Natural compounds originating from plants are increasingly being explored as promising antileishmanial alternatives, since, when compared to currently available drugs, these products can selectively act on the parasite besides that the most of them present low cytotoxicity and low market value [13, 14].

Although, researches demonstrate the importance of natural products for the treatment of leishmaniasis, little is known about the influence of miRNAs on the mechanisms of action of natural products. However, recent evidence suggests that some phytochemical substances regulate the expression of several pathological miRNAs [15, 16]. For example, in breast cancer, phytochemical substances extracted from plant foods have acted directly on the regulation of miRNA expression and these are pointed out as promising alternative chemopreventive and chemotherapeutic agents [17].

In this context, to understand the mechanisms of action of natural compounds and the emergence of resistance by parasites, this review was developed based on the following question: Is it possible that certain natural compounds also act by inhibiting or activating miRNAs that interfere with the parasitic action against *Leishmania*? The chapter aims to provide a brief review on mechanisms of action associated with traditional agents used to treat leishmaniasis, focusing mainly on molecular basis associated with resistance in *Leishmania* spp. to current drugs and identifying the possible miRNAs involved in this process. In addition, we seek to describe some of the promising plant molecules that can be used as potential antileishmanial agents and their possible mechanisms of action.

2. Traditional therapeutic agents and mechanisms of action

Leishmaniasis presents different clinical manifestations, ranging from skin lesions to severe visceral forms, depending on the species of the parasite of the genus *Leishmania* [4].

Chemotherapy is the only effective alternative to treat all forms of the disease. Therapeutic approaches to control leishmaniasis comprise five main drugs: pentavalent antimony, amphotericin B, miltefosine, paromomycin and pentamidine, however, these drugs are associated with serious problems, such as toxicity and especially with the emergence of resistant strains, making treatment difficult [18].

Although, current antileishmanial drugs have been used for decades, their mechanisms of action remain obscure, however, some assumptions are accepted. Pentavalent antimonials, for example, eliminate parasites through the process of apoptosis and also inhibit trypanothione reductase, an important enzyme for the survival of the parasite in the host [19]. Miltefosine, in turn, also induces cell death by apoptosis, in addition to inducing various immunological and inflammatory effects on macrophages [20]. The mechanism of action of amphotericin B may involve interaction with membrane sterols, resulting in membrane disorganization, increased permeability and cell damage in the parasite [21, 22]. Paromomycin inhibits protein synthesis and modifies the fluidity and permeability of the membrane and also decreases mitochondrial potential [3, 23]. While pentamidine acts as a DNA-binding drug, causing the parasite mitochondrial membrane potential collapse and induction of kinetoplast DNA destruction [24, 25].

The prolonged use of these drugs has contributed to the acceleration of leishmaniasis resistance; however, little is known about the cellular and molecular mechanisms, especially about the importance of miRNAs involved in the emergence of this resistance (**Figure 1**).

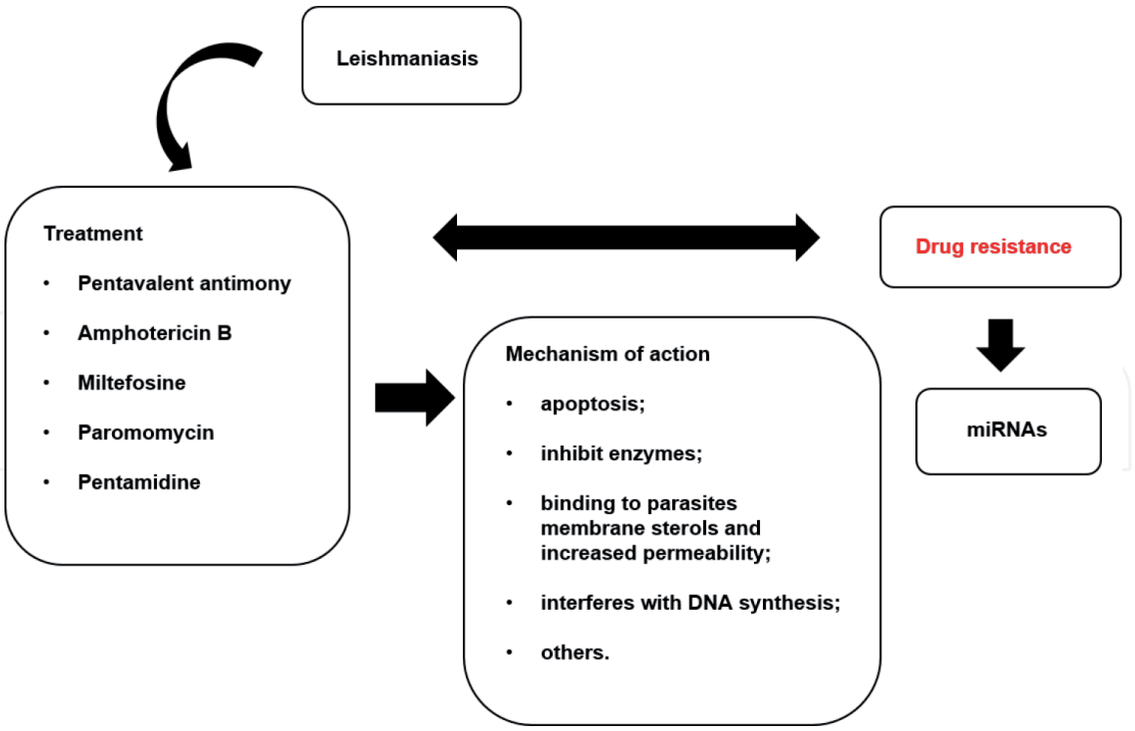

Figure 1.
Antileishmanial drugs and mechanism of action. The pentavalent antimony, amphotericin B, miltefosine, paromomycin and pentamidine drugs act mainly by inducing apoptosis; inhibiting important enzymes; binding to the parasite's membrane sterols, increasing permeability and interfering with DNA synthesis. The prolonged use of these drugs and consequently the increase in parasite resistance has been explained by molecular mechanisms, for example, the expression of miRNAs.

3. miRNAs and drug resistance

MicroRNAs (miRNAs) are a small class of non-coding RNAs composed of 18–24 nucleotides, present in a variety of organisms, including parasites, plants, animals and humans [7, 8, 26, 27]. The first description of these structures occurred after studies with the nematode parasite *Caenorhabditis elegans* [28].

The canonical biosynthesis of a miRNA starts with a primary miRNA (pri-miRNA) that is transcribed and recognized by a microprocessor complex. This process includes the double-stranded RNA-specific endoribonuclease enzymes (DROSHA) and the DiGeorge syndrome critical region gene 8 (DGCR8), which are cleaved to form a miRNA precursor (pre-miRNA). The pre-miRNAs are then exported to the cytoplasm for further processing by the DICER enzyme and cofactors such as protein kinase R activator (PACT) or transactivation response RNA binding protein (TRBP) [29].

The mature duplex miRNA is finally loaded into an RNA-induced silencing complex (RISC) that is multiprotein and a corrected miRNA strand (−5p or -3p) that binds to the Argonaute protein (AGO) guiding the complex to its mRNA target [30]. Both biogenesis and maturation of miRNAs are tightly regulated processes. The first level of adjustment is represented by single nucleotide polymorphisms (SNPs) and epigenetic control of transcription through mechanisms of acetylation, DNA methylation and histones. The biosynthesis and maturation of miRNAs can also be influenced by RNA binding proteins (RBPs), which can interact with key enzyme processes such as DROSHA, DGCR8, DICER and the complex RISC [31].

In addition to impacting the actions of miRNAs on their targets, editing pri and pre-miRNAs is also an important mechanism that modulates the biosynthesis and maturation of specific miRNAs. Degradation of the miRNA strand (−5p or -3p strand) or incorporation into the RISC complex (lead strand) determines the pool

of target mRNAs. The complementarity between the miRNA response elements (MRE) in the mRNA and the seed sequence in the miRNA strand determines the specificity of the action of the RISC complex for mRNAs. Furthermore, the degree of complementarity between the MRE and the seed sequences generally dictates whether the mRNA is degraded or its translation is blocked [32]. The mechanism of biogenesis and maturation of miRNA is described in **Figure 2**.

miRNAs are involved in important biological processes in organisms, including the control of gene and pathological expression, cell and organ development, differentiation and homeostasis, tumor suppression and stem cell regulation [26]. miRNAs are also known to be involved in the development of human parasitic diseases. For example, in *Schistosoma mansoni*, the causative agent of schistosomiasis, it was shown that miRNAs can control the development of the parasite and also of liver pathology [33]. While in Chagas disease, caused by *Trypanosoma cruzi*, there are reports of the involvement of miRNAs in cardiovascular disorders caused by illness [34–36].

In toxoplasmosis, a disease caused by the agent *Toxoplasma gondii*, some miRNAs may be related to the degree of virulence, inhibition of apoptosis and immune response [37, 38]. While in malaria (*Plasmodium* spp.), evidence has shown that the agent manipulates the host miRNA expression, supporting its growth and survival, and also regulates important genes for the host immune response [39–41].

In leishmaniasis, miRNAs play an important role in the biology of infection, pathogenicity, recognition and activation of the immune response by macrophages and dendritic cells, host–parasite interaction and drugs resistance [42–44].

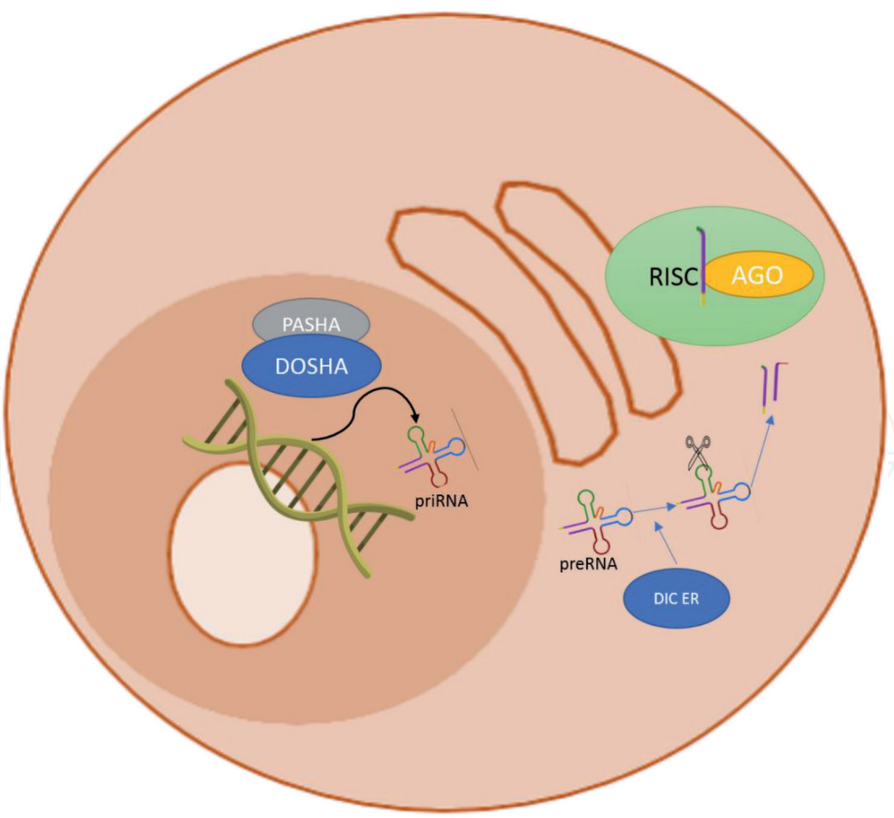

Figure 2.
Biogenesis or generation of miRNAs and the mechanisms of protein synthesis inhibition. A primary miRNA (pri-miRNA) is transcribed or produced in the nucleus. It is processed or modified by the Drosha enzyme and exported to the cytoplasm. In the cytoplasm, now called pre-miRNA, it is again processed by another enzyme, Dicer, finally forming the mature miRNA. Mature miRNA associates with a complex or set of enzymes called RISC and represses or inhibits protein synthesis by cleaving (breaking) messenger RNAs (mRNA) or may impede mRNA reading (prevent translation) by inhibiting protein production.

Although, research emphasizes the importance of studying miRNAs during infection or pathogenesis by different parasites, the role of these structures in the development of antiparasitic resistance remains unclear. Drug resistance is a growing problem worldwide. Resisting a drug means saying that there was a reduction in the drug effectiveness in the total elimination of the pathogen responsible for the disease [45]. miRNAs have been identified as targets for pathogen invasion and immune response control, in addition to resistance to chemotherapy in a variety of organisms and diseases (**Figure 3**) [6, 10].

In recent years, studies have shown that miRNAs are involved in tumor cell resistance to chemotherapy [46–48]. According to the studies, miRNAs regulate different target genes, especially genes that affect the cell response to chemotherapy drugs. In insects, some miRNAs are involved in resistance to the insecticide diamide in the control of *Plutella xylostella*, mainly miR-7a and miR-8519 [49]. Other studies have reported the importance of miR-2, miR-13, miR-7, miR-92a and miR-13,664 expression in the *Culex pipiens* insect resistance to the drug deltamethrin [50–52].

The resistance of some parasites is also related to miRNAs. In the nematode *C. elegans*, miR-1 down-regulates the expression of two nicotinic acetylcholine receptor subunits (nAChR), unc-29 and unc-63 [53]. According to the authors, when the expression of these subunits is increased, it corresponds to a decrease in muscle sensitivity to acetylcholine by levamisole, that is, the drug action is decreased. In *Toxocara* spp., the causative agent of toxocariasis in humans and other animals, computational studies predict that some miRNAs function as targets associated with drug resistance, such as Tc-miR-2861, Tc-miR-2881 and Tc-miR -5126 [54]. Additionally, miR-9551 is regulated in *Teladorsagia circumcincta*, the most prevalent parasite in United Kingdom animals with proven multiple drug resistance [55]. Recently, miR-9551 expression was also correlated with an anthelmintic resistance phenotype of *Haemonchus contortus* to the drug ivermectin [9].

The role of miRNAs in drug resistance is clear for a wide variety of organisms, for protozoan parasites, mainly *Leishmania* spp. it's still not well elucidated. However, the expression of miRNAs in leishmaniasis can be used as a strategy to escape the host immune system [13, 14]. For example, the pentavalent

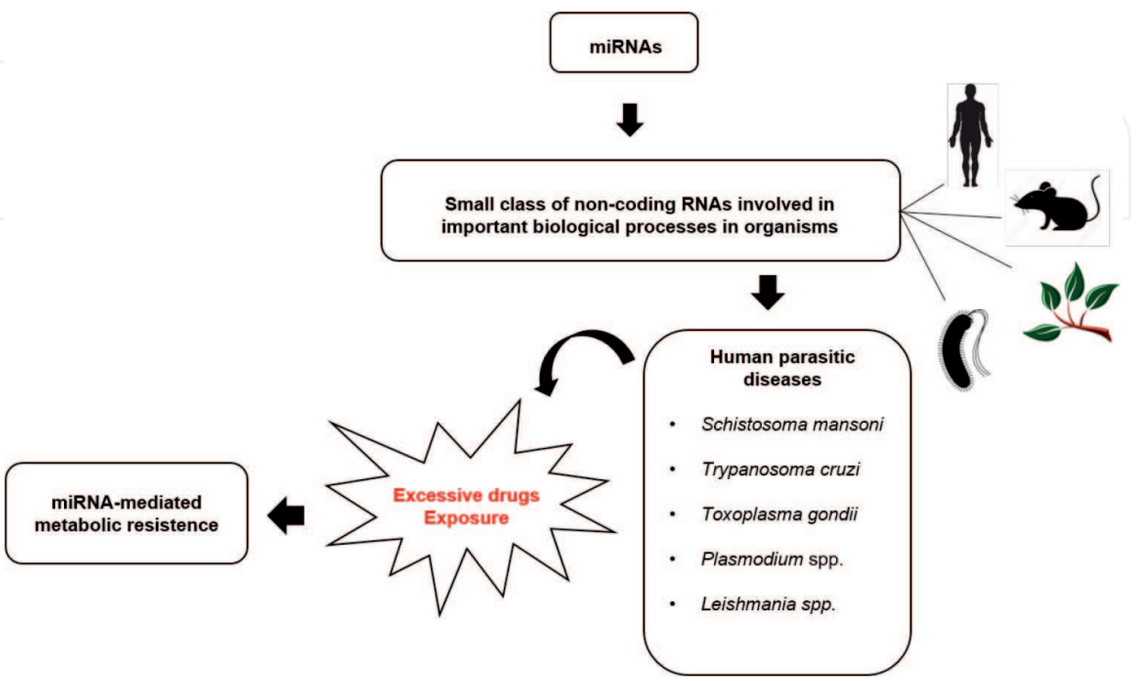

Figure 3.
miRNAs and human parasitic diseases.

antimony-resistant *Leishmania donovani* species exploits miR-466i to regulate levels of inflammatory cytokines produced by macrophages during infection, and in other cases, it decreases miR-122 expression by hepatocytes to facilitate infection, ensuring its differentiation and intracellular multiplication [56, 57].

For years, pentavalent antimony was the main treatment against leishmaniasis and with this, the parasites acquired resistance and the drug lost its effectiveness [58]. The resistance mechanism of *Leishmania donovani* to pentavalent antimonals can be explained by the increased production of IL-10 and TGF-β inducing the upregulation of the multiresistant protein-1 (MDR1) and causing the expulsion of the drug by macrophages [58]. Also, according to the authors, during infection by the resistant parasite, miRNAs control the expression of pro and anti-inflammatory cytokines in macrophages internally regulated by the proteins Ago2, PP2A and HuR. HuR positively inhibits PP2A expression by activating the modulatory function of HuR and PP2A antagonist miRNAs and acting as a balancing factor for immune responses in macrophages to interrupt or prevent infection by pentavalent antimonal resistant *L. donovani* [59].

Tiwari et al. [10] pointed out that *Leishmania* parasites possibly induce macrophages to express miRNA regulatory proteins that help expel drugs from cells. This drug resistance has been associated with the downregulation of miRNA-763, –1264, and -3473f by parasitized macrophages that induced upregulation of ABC transporters [10]. Recent studies have revealed that miRNA deficiency can also influence the emergence of host resistance to leishmaniasis. According to Varikuti et al. [60], miR-21 is positively expressed in dendritic cells and macrophages and in organs such as the spleen and liver infected by *L. donovani*. During infection, the lack of miR-21 increases IL-12 production and the Th1 immune response and consequently increases host resistance. miR-21 plays an essential role in the pathogenesis of the disease and is considered a potential target in the treatment of leishmaniasis. miRNAs alter transporters, receptors and ion channels drug, reducing sensitivity and consequently developing resistance [61, 62]. The change in the miR profile may be a strategy of infection or drug resistance of parasites.

Thus, these studies highlight the importance of these structures in the development of leishmaniasis resistance to currently available drugs and indicate that the identification of miRNAs can provide strategies for controlling leishmaniasis.

To contain the acceleration of resistance by parasites, the search for new treatments has emerged. The discovery of new drugs with fewer side effects and more advantageous cost-benefits than current treatments has gained strength, in this sense, the use of natural products in the treatment of leishmaniasis has become an excellent alternative.

4. Natural products as an anti-*Leishmania* source

Natural products originating from plants are being increasingly explored as promising therapeutic alternatives, and the action of these products is mainly attributed to existing bioactive phytochemicals [63, 64].

The concentration of these compounds depends on the nature of the chemical used as a solvent during the extraction and handling process, as well as the storage conditions [65]. Terpenoids, phenolic compounds, alkaloids and flavonoids are the most important phytochemical groups. Terpenoids from essential oils are extracted by hydrodistillation and mechanical pressing techniques, while phenolic compounds, alkaloids and flavonoids are extracted using organic solvents, mainly dichloromethane, methanol, ethanol, ethyl acetate, n-butanol, chloroform, ether and benzene [65–67].

After extraction, the phytochemical analysis to identify the main classes of compounds is performed using chromatographic and colorimetric techniques [68]. Approximately 10,000 phytochemicals have been identified so far and their numerous medicinal properties are being investigated. Leaves, seeds, fruits and flowers are the plant organs richest in phytochemicals [69, 70].

Currently, these groups of phytochemicals have anti-*Leishmania* properties and mechanisms of action already described. Terpenoids, aromatic and lipophilic substances, possibly can cross the plasma membrane, interfering with cell composition, inducing mitochondrial rupture and chromatin condensation and causing parasite death by apoptosis [71, 72]. The glycyrrhizic acid, a triterpene extracted from the root of *Glycyrrhiza glabra*, was found to be responsible for the change in the host immune system, inducing an increase in the release of inflammatory cytokines in macrophages infected by *Leishmania*, facilitating a more efficient response against the parasite [73].

Phenolic compounds primarily function as antioxidants and free radical scavengers [74]. Its action on *Leishmania* is attributed to its ability to induce cell death through apoptosis and inhibition of cell replication through iron chelation [75–77]. Recently, Antwi et al. [78] also suggested that rosmarinic acid exerts an antileishmanial effect through iron chelation, however, its mechanism of action results in morphological alterations and interruption of the parasite cell cycle.

Alkaloids are alkaline compounds and can be classified based on the presence and activity of specific amino acids [79]. Many alkaloids have been described as having biological activities against *Leishmania* spp., however, the mechanism of action of these compounds is not fully understood. According to Fournet et al. [80] alkaloids can inhibit an essential antioxidant enzyme in the parasite, trypanothione reductase. Additionally, a study showed that different synthetic quinoline alkaloids caused ultrastructural changes in *Leishmania*, such as loss of cell membrane integrity in the parasites, in addition to inducing apoptosis [81].

Flavonoids, in turn, are a class of natural compounds with several known biological activities, including anti-*Leishmania*. Flavonoids subdivide into classes such as chalcones, flavones, isoflavones, flavonols and anthocyanidins [82]. Quercetin, a flavonol extracted from *Kalanchoe pinnata*, showed activity against *Leishmania amazonensis* promastigotes [83]. According to the authors, the compound increased the levels of reactive oxygen species, causing mitochondrial damage and leading to parasite death. Recently, Araújo et al. [84] demonstrated that flavonoids isolated from *Solanum paludosum* had an antiproliferative effect on *Leishmania* parasites inducing cell cycle blockage and autophagy.

In general, plants contain biomolecules with high active potential against leishmaniasis. However, the process of discovering these molecules is considered complex and time-consuming, since it involves phases such as isolation, identification, optimization of their properties and selection of safe and effective compounds for drug development [64, 70].

Studies have identified that the expression of miRNAs in different types of cancer is specifically regulated by phytochemicals [15]. In the treatment of breast cancer, several phytochemicals have been identified as tumor suppressors, directly controlling the expression of miRNAs [15–17]. According to Baselga-Escudero et al. [85] polyphenols can bind directly to miRNAs and that the chemical structure of these compounds influences the expression of miRNAs. Recently, through *in vitro* and *in vivo* studies, quercetin was able to modulate miRNAs and, consequently, suppress oncogenes or stimulate tumor suppressor genes by altering the expression of miRNAs [86].

These studies show the importance of natural compounds in the regulation of miRNAs and the possible influence of these in their action on diseases. However,

it remains unclear whether miRNAs can interact with phytochemicals so that they are not absorbed and/or metabolized by the parasites and thus contribute to the resistance mechanism of leishmaniasis (**Figure 3**).

5. Conclusions

In conclusion, miRNAs have a wide range of important biological functions for parasites, including leishmaniasis. Although, the role of these structures in the development of antiparasitic resistance remains unclear, this work provides suggestive information about the strong contribution of miRNAs in this process. Furthermore, natural compounds with anti-*Leishmania* activity may play an important role in the regulation of miRNAs, influencing their action on the parasite. Therefore, the identification and characterization of specific miRNAs, as well as the clarification of their interaction with natural compounds are crucial for understanding the resistance mechanism of leishmaniasis.

Acknowledgements

The authors would like to thank CAPES (the Brazilian Federal Agency for support and evaluation of graduate education) for the scholarship to S.A. Araujo.

Conflict of interest

The authors declare no conflict of interest.

Author details

Sandra Alves de Araújo[1,2,3], Tatiane Aranha da Penha-Silva[4*], Jaqueline Diniz Pinho[2], Marcelo de Souza Andrade[1,3*] and Ana Lucia Abreu-Silva[1,2]

1 Postgraduate Program in Biotechnology, RENORBIO, São Luís, Brazil

2 State University of Maranhão, São Luís, Brazil

3 Federal University of Maranhão, São Luís, Brazil

4 Faculdade Pitágoras de São Luis, Brazil

*Address all correspondence to: tatianearanha@hotmail.com and marcelo.andrade@ufma.br

References

[1] Ponte-Sucre A, Gamarro F, Dujardin JC, Barrett MP, López-Vélez R, García-Hernández R, et al. Drug resistance and treatment failure in leishmaniasis: A 21st century challenge. PLoS Neglected Tropical Diseases. 2017;**11**(12):e0006052. DOI: 10.1371/journal.pntd.0006052

[2] Sundar S, Chakravarty J, Meena LP. Leishmaniasis: Treatment, drug resistance and emerging therapies. Expert Opinion on Orphan Drugs. 2019;**7**(1):1-10. DOI: 10.1080/21678707.2019.1552853

[3] Maltezou HC. Drug resistance in visceral leishmaniasis. Journal of Biomedicine & Biotechnology. 2010;**2010**:617521. DOI: 10.1155/2010/617521

[4] Ouellette M, Drummelsmith J, El-Fadili A, Kündig C, Richard D, Roy G. Pterin transport and metabolism in Leishmania and related trypanosomatid parasites. International Journal for Parasitology. 2002;**32**(4):385-398. DOI: 10.1016/s0020-7519(01)00346-0

[5] Capela R, Moreira R, Lopes F. An overview of drug resistance in protozoal diseases. International Journal of Molecular Sciences. 2019;**20**(22):5748. DOI: 10.3390/ijms20225748

[6] Britton C, Winter AD, Gillan V, Devaney E. microRNAs of parasitic helminths - Identification, characterization and potential as drug targets. International Journal for Parasitology: Drugs and Drug Resistance. 2014;**4**(2):85-94. DOI: 10.1016/j.ijpddr.2014.03.001

[7] Judice CC, Bourgard C, Kayano AC, Albrecht L, Costa FT. MicroRNAs in the Host-Apicomplexan Parasites Interactions: A review of immunopathological aspects. Frontiers in Cellular and Infection Microbiology. 2016;**6**:5. DOI: 10.3389/fcimb.2016.00005

[8] Acuña SM, Floeter-Winter LM, Muxel SM. MicroRNAs: Biological regulators in pathogen-host interactions. Cell. 2020;**9**(1):113. DOI: 10.3390/cells9010113

[9] Gillan V, Maitland K, Laing R, Gu H, Marks ND, Winter AD, et al. Increased expression of a MicroRNA correlates with anthelmintic resistance in parasitic nematodes. Frontiers in Cellular and Infection Microbiology. 2017;**7**:452. DOI: 10.3389/fcimb.2017.00452

[10] Tiwari N, Kumar V, Gedda MR, Singh AK, Singh VK, Gannavaram S, et al. Identification and characterization of miRNAs in response to leishmania donovani infection: Delineation of their roles in macrophage dysfunction. Frontiers in Microbiology. 2017;**8**:314. DOI: 10.3389/fmicb.2017.00314

[11] Hefnawy A, Berg M, Dujardin JC, De Muylder G. Exploiting knowledge on leishmania drug resistance to support the quest for new drugs. Trends in Parasitology. 2017;**33**(3):162-174. DOI: 10.1016/j.pt.2016.11.003

[12] Raj S, Sasidharan S, Balaji S, Dubey VK, Saudagar P. Review on natural products as an alternative to contemporary anti-leishmanial therapeutics. Journal of Proteins and Proteomics. 2020;**11**:135-158. DOI: 10.1007/s42485-020-00035-w

[13] Geraci NS, Tan JC, McDowell MA. Characterization of microRNA expression profiles in Leishmania-infected human phagocytes. Parasite Immunology. 2015;**37**(1):43-51. DOI: 10.1111/pim.12156

[14] Bayer-Santos E, Marini MM, da Silveira JF. Non-coding RNAs in

host-pathogen interactions: Subversion of mammalian cell functions by protozoan parasites. Frontiers in Microbiology. 2017;**8**:474. DOI: 10.3389/fmicb.2017.00474

[15] Kang H. MicroRNA-mediated health-promoting effects of phytochemicals. International Journal of Molecular Sciences. 2019;**20**(10):2535. DOI: 10.3390/ijms20102535

[16] Ganapathy A, Ezekiel U. Phytochemical modulation of MiRNAs in colorectal cancer. Medicines (Basel). 2019;**6**(2):48. DOI: 10.3390/medicines6020048

[17] Abbasi BA, Iqbal J, Mahmood T, Khalil AT, Ali B, Kanwal S, et al. Role of dietary phytochemicals in modulation of miRNA expression: Natural swords combating breast cancer. Asian Pacific Journal of Tropical Medicine. 2018;**11**(9):501. DOI: 10.4103/1995-7645.242314

[18] Tiwari N, Gedda MR, Tiwari VK, Singh SP, Singh RK. Limitations of current therapeutic options, possible drug targets and scope of natural products in control of leishmaniasis. Mini Reviews in Medicinal Chemistry. 2018;**18**(1):26-41. DOI: 10.2174/1389557517666170425105129

[19] Singh N. Drug resistance mechanisms in clinical isolates of Leishmania donovani. The Indian Journal of Medical Research. 2006;**123**(3):411-422

[20] Pérez-Victoria FJ, Sánchez-Cañete MP, Seifert K, Croft SL, Sundar S, Castanys S, et al. Mechanisms of experimental resistance of Leishmania to miltefosine: Implications for clinical use. Drug Resistance Updates. 2006;**9**(1-2):26-39. DOI: 10.1016/j.drup.2006.04.001

[21] Mohapatra S. Drug resistance in leishmaniasis: Newer developments. Trop Parasitol. 2014;**4**(1):4-9. DOI: 10.4103/2229-5070.129142

[22] Légaré D, Ouellette M. Drug resistance in leishmania. In: Gotte MB, Matlashewski A, Wainberg G, Mark A, Donald S, editors. Handbook of Antimicrobial Resistance. New York, NY: Springer; 2017. pp. 313-341. DOI: 10.1007/978-1-4939-0694-9_17

[23] Jhingran A, Chawla B, Saxena S, Barrett MP, Madhubala R. Paromomycin: uptake and resistance in Leishmania donovani. Molecular and Biochemical Parasitology. 2009;**164**(2):111-117. DOI: 10.1016/j.molbiopara.2008.12.007

[24] Thomas JA, Baker N, Hutchinson S, Dominicus C, Trenaman A, Glover L, et al. Insights into antitrypanosomal drug mode-of-action from cytology-based profiling. PLoS Neglected Tropical Diseases. 2018;**12**(11):e0006980. DOI: 10.1371/journal.pntd.0006980

[25] Baker CH, Welburn SC. The long wait for a new drug for human African trypanosomiasis. Trends in Parasitology. 2018;**34**(10):818-827. DOI: 10.1016/j.pt.2018.08.006

[26] Bartel DP. MicroRNAs: Genomics, biogenesis, mechanism, and function. Cell. 2004;**116**(2):281-297. DOI: 10.1016/s0092-8674(04)00045-5

[27] Kim YK, Kim VN. Processing of intronic microRNAs. The EMBO Journal. 2007;**26**(3):775-783. DOI: 10.1038/sj.emboj.7601512

[28] Lee RC, Ambros V. An extensive class of small RNAs in Caenorhabditis elegans. Science. 2001;**294**(5543):862-864. DOI: 10.1126/science.1065329

[29] Sousa MC, Gjorgjieva M, Dolicka D, Sobolewski C, Foti M. Deciphering miRNAs' Action through miRNA

Editing Int. J. Mol. Sci. 2019;**20**:6249. DOI: 10.3390/ijms20246249

[30] Van den Berg A, Mols J, Han J. RISC-target interaction: Cleavage and translational suppression. Biochimica et Biophysica Acta. 2008;**1779**:668-677. DOI: 10.1016/j.bbagrm.2008.07.005

[31] Ota H, Sakurai M, Gupta R, Valente L, Wul BE, Ariyoshi K, et al. ADAR1 forms a complex with Dicer to promote microRNA processing and RNA-induced gene silencing. Cell. 2013;**153**:575-589. DOI: 10.1016/j.cell.2013.03.024

[32] Sobolewski C, Calo N, Portius D, Foti M. MicroRNAs in fatty liver disease. Seminars in Liver Disease. 2015;**35**:12-25. DOI: 10.1055/s-0034-1397345

[33] Zhu L, Liu J, Cheng G. Role of microRNAs in schistosomes and schistosomiasis. Frontiers in Cellular and Infection Microbiology. 2014;**4**:165. DOI: 10.3389/fcimb.2014.00165

[34] Ferreira LRP, Frade AF, Santos RHB, Teixeira PC, Baron MA, Navarro IC, et al. MicroRNAs miR-1, miR-133a, miR-133b, miR-208a and miR-208b are dysregulated in Chronic Chagas disease Cardiomyopathy. International Journal of Cardiology. 2014;**175**(3):409-417. DOI: 10.1016/j.ijcard.2014.05.019

[35] Linhares-Lacerda L, Palu CC, Ribeiro-Alves M, Paredes BD, Morrot A, Garcia-Silva MR, et al. Differential expression of microRNAs in thymic epithelial cells from trypanosoma cruzi acutely infected mice: Putative role in thymic atrophy. Frontiers in Immunology. 2015;**6**:428. DOI: 10.3389/fimmu.2015.00428

[36] Navarro IC, Ferreira FM, Nakaya HI, Baron MA, Vilar-Pereira G, Pereira IR, et al. MicroRNA transcriptome profiling in heart of trypanosoma cruzi-infected mice: Parasitological and cardiological outcomes. PLoS Neglected Tropical Diseases. 2015;**9**(6):e0003828

[37] Braun L, Cannella D, Ortet P, Barakat M, Sautel CF, Kieffer S, et al. A complex small RNA repertoire is generated by a plant/fungal-like machinery and effected by a metazoan-like Argonaute in the single-cell human parasite Toxoplasma gondii. PLoS Pathogens. 2010;**6**(5):e1000920. DOI: 10.1371/journal.ppat.1000920

[38] Cai Y, Chen H, Jin L, You Y, Shen J. STAT3-dependent transactivation of miRNA genes following Toxoplasma gondii infection in macrophage. Parasites & Vectors. 2013;**6**:356. DOI: 10.1186/1756-3305-6-356

[39] Rathjen T, Nicol C, McConkey G, Dalmay T. Analysis of short RNAs in the malaria parasite and its red blood cell host. FEBS Letters. 2006;**580**(22):5185-5188. DOI: 10.1016/j.febslet.2006.08.063

[40] Taganov KD, Boldin MP, Chang KJ, Baltimore D. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proceedings of the National Academy of Sciences of the United States of America. 2006;**103**(33):12481-12486. DOI: 10.1073/pnas.0605298103

[41] Hakimi MA, Ménard R. Do apicomplexan parasites hijack the host cell microRNA pathway for their intracellular development? F1000 Biol Rep. 2010;**2**:42. DOI: 10.3410/B2-42

[42] Frank B, Marcu A, de Oliveira Almeida Petersen AL, Weber H, Stigloher C, Mottram JC, et al. Autophagic digestion of Leishmania major by host macrophages is associated with differential expression of BNIP3, CTSE, and the miRNAs miR-101c, miR-129, and miR-210. Parasites & Vectors. 2015;**8**:404. DOI: 10.1186/s13071-015-0974-3

[43] Muxel SM, Laranjeira-Silva MF, Zampieri RA, Floeter-Winter LM. Leishmania (Leishmania) amazonensis induces macrophage miR-294 and miR-721 expression and modulates infection by targeting NOS2 and L-arginine metabolism. Scientific Reports. 2017;**7**:44141. DOI: 10.1038/srep44141

[44] Bragato JP, Melo LM, Venturin GL, Rebech GT, Garcia LE, Lopes FL, et al. Relationship of peripheral blood mononuclear cells miRNA expression and parasitic load in canine visceral leishmaniasis. PLoS One. 2018;**13**(12): e0206876. DOI: 10.1371/journal.pone.0206876

[45] Loke MF, Hanafi A. Molecular Mechanisms Responsible for Drug Resistance. In: Ranganathan S, Gribskov M, Nakai K, Schönbach C, editors. Encyclopedia of Bioinformatics and Computational Biology, Academic Press, 2019;**3**:926-931. DOI: 10.1016/B978-0-12-809633-8.20467-6

[46] Hu S, Yuan Y, Song Z, Yan D, Kong X. Expression profiles of microRNAs in drug-resistant non-small cell lung cancer cell lines using microRNA sequencing. Cellular Physiology and Biochemistry. 2018;**51**(6):2509-2522. DOI: 10.1159/000495921

[47] Si W, Shen J, Zheng H, Fan W. The role and mechanisms of action of microRNAs in cancer drug resistance. Clinical Epigenetics. 2019;**11**(1):25. DOI: 10.1186/s13148-018-0587-8

[48] Duan L, Yang W, Feng W, Cao L, Wang X, Niu L, et al. Molecular mechanisms and clinical implications of miRNAs in drug resistance of colorectal cancer. Ther Adv Med Oncol. 2020;**12**: 1758835920947342. DOI: 10.1177/1758835920947342

[49] Li X, Guo L, Zhou X, Gao X, Liang P. miRNAs regulated overexpression of ryanodine receptor is involved in chlorantraniliprole resistance in Plutella xylostella (L.). Scientific Reports. 2015;**5**(1):1-9. DOI: 10.1038/srep14095

[50] Guo Q, Huang Y, Zou F, Liu B, Tian M, Ye W, et al. The role of miR-2~13~71 cluster in resistance to deltamethrin in Culex pipiens pallens. Insect Biochemistry and Molecular Biology. 2017;**84**:15-22. DOI: 10.1016/j.ibmb.2017.03.006

[51] Ma K, Li X, Hu H, Zhou D, Sun Y, Ma L, et al. Pyrethroid-resistance is modulated by miR-92a by targeting CpCPR4 in Culex pipiens pallens. Comparative Biochemistry and Physiology. Part B, Biochemistry & Molecular Biology. 2017;**203**:20-24. DOI: 10.1016/j.cbpb.2016.09.002

[52] Sun XH, Xu N, Xu Y, Zhou D, Sun Y, Wang WJ, et al. A novel miRNA, miR-13664, targets CpCYP314A1 to regulate deltamethrin resistance in Culex pipiens pallens. Parasitology. 2019;**146**(2):197-205. DOI: 10.1017/S0031182018001002

[53] Simon DJ, Madison JM, Conery AL, Thompson-Peer KL, Soskis M, Ruvkun GB, et al. The microRNA miR-1 regulates a MEF-2-dependent retrograde signal at neuromuscular junctions. Cell. 2008;**133**(5):903-915. DOI: 10.1016/j.cell.2008.04.035

[54] Ma G, Luo Y, Zhu H, Korhonen PK, Young ND, Gasser RB, et al. MicroRNAs of Toxocara canis and their predicted functional roles. Parasites & Vectors. 2016;**9**:229. DOI: 10.1186/s13071-016-1508-3

[55] Sargison ND, Jackson F, Bartley DJ, Wilson DJ, Stenhouse LJ, Penny CD. Observations on the emergence of multiple anthelmintic resistance in sheep flocks in the south-east of Scotland. Veterinary Parasitology. 2007;**145**(1-2):65-76. DOI: 10.1016/j.vetpar.2006.10.024

[56] Ghosh J, Bose M, Roy S, Bhattacharyya SN. Leishmania donovani targets Dicer1 to downregulate miR-122, lower serum cholesterol, and facilitate murine liver infection. Cell Host & Microbe. 2013;**13**(3):277-288. DOI: 10.1016/j.chom.2013.02.005

[57] Mukherjee B, Paul J, Mukherjee S, Mukhopadhyay R, Das S, Naskar K, et al. Antimony-resistant leishmania donovani exploits miR-466i to deactivate host MyD88 for regulating IL-10/IL-12 levels during early hours of infection. Journal of Immunology. 2015;**195**(6):2731-2742. DOI: 10.4049/jimmunol.1402585

[58] Mukherjee B, Mukherjee K, Nanda P, Mukhopadhayay R, Ravichandiran V, Bhattacharyya SN, et al. Probing the molecular mechanism of aggressive infection by antimony resistant *Leishmania donovani*. Cytokine. 2021;**145**:155245. DOI: 10.1016/j.cyto.2020.155245

[59] Goswami A, Mukherjee K, Mazumder A, Ganguly S, Mukherjee I, Chakrabarti S, et al. MicroRNA exporter HuR clears the internalized pathogens by promoting pro-inflammatory response in infected macrophages. EMBO Molecular Medicine. 2020;**12**(3):e11011. DOI: 10.15252/emmm.201911011

[60] Varikuti S, Verma C, Holcomb E, Jha BK, Viana A, Maryala R, et al. MicroRNA-21 Deficiency Promotes the Early Th1 Immune Response and Resistance toward Visceral Leishmaniasis. Journal of Immunology. 2021;**207**(5):1322-1332. DOI: 10.4049/jimmunol.2001099

[61] Ren N, Gao G, Sun Y, Zhang L, Wang H, Hua W, et al. MicroRNA signatures from multidrug-resistant Mycobacterium tuberculosis. Molecular Medicine Reports. 2015;**12**(5):6561-6567. DOI: 10.3892/mmr.2015.4262

[62] Nawaz M, Malik MI, Hameed M, Zhou J. Research progress on the composition and function of parasite-derived exosomes. Acta Tropica. 2019;**196**:30-36. DOI: 10.1016/j.actatropica.2019.05.004

[63] Carter NS, Stamper BD, Elbarbry F, Nguyen V, Lopez S, Kawasaki Y, et al. Natural products that target the arginase in. Microorganisms. 2021;**9**(2):267. DOI: 10.3390/microorganisms9020267

[64] Oliveira RM, Melo SA, Penha-Silva TA, Almeida-Souza F, Abreu-Silva AL. Alternative treatment for leishmaniasis. In: Afrin F, Hemeg H, editors. Leishmaniases as Re-emerging Diseases. Rijeka: IntechOpen; 2018. DOI: 10.5772/intechopen.75895

[65] Martins N, Barros L, Henriques M, Silva S, Ferreira IC. In vivo anti-candida activity of phenolic extracts and compounds: Future perspectives focusing on effective clinical interventions. BioMed Research International. 2015;**2015**:247382. DOI: 10.1155/2015/247382

[66] Santos HLLR, Souza N, Macedo NR, Ramos JPS, Dias AF, Nascimento A. Phytochemical analysis of extracts from the atlantic forest wood species. Floresta e Ambiente. 2019;**26**. DOI: 10.1590/2179-8087.038118

[67] Wilkin PJ, Al-Yozbaki M, George A, Gupta GK, Wilson CM. The Undiscovered Potential of Essential Oils for Treating SARS-CoV-2 (COVID-19). Current Pharmaceutical Design. 2020;**26**(41):5261-5277. DOI: 10.2174/1381612826666201015154611

[68] Acquavia MA, Pascale R, Foti L, Carlucci G, Scrano L, Martelli G, et al. Analytical methods for extraction and identification of primary and secondary metabolites of apple (Malus domestica) fruits: A review. Separations. 2021;**8**(7):91. DOI: 10.3390/separations8070091

[69] Reddy MV, Reddy MK, Gunasekar D, Caux C, Bodo B. A flavanone and a dihydrodibenzoxepin from Bauhinia variegata. Phytochemistry. 2003;**64**(4):879-882. DOI: 10.1016/s0031-9422(03)00416-3

[70] Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants (Basel). 2017;**6**(4):42. DOI: 10.3390/plants6040042

[71] Corpas-López V, Merino-Espinosa G, Díaz-Sáez V, Morillas-Márquez F, Navarro-Moll MC, Martín-Sánchez J. The sesquiterpene (−)-α-bisabolol is active against the causative agents of Old World cutaneous leishmaniasis through the induction of mitochondrial-dependent apoptosis. Apoptosis. 2016;**21**(10):1071-1081. DOI: 10.1007/s10495-016-1282-x

[72] Hajaji S, Sifaoui I, López-Arencibia A, Reyes-Batlle M, Jiménez IA, Bazzocchi IL, et al. Leishmanicidal activity of α-bisabolol from Tunisian chamomile essential oil. Parasitology Research. 2018;**117**(9): 2855-2867. DOI: 10.1007/s00436-018-5975-7

[73] Bhattacharjee S, Bhattacharjee A, Majumder S, Majumdar SB, Majumdar S. Glycyrrhizic acid suppresses Cox-2-mediated anti-inflammatory responses during Leishmania donovani infection. The Journal of Antimicrobial Chemotherapy. 2012;**67**(8):1905-1914. DOI: 10.1093/jac/dks159

[74] Verruck S, Prudencio ES, da Silveira SM. Compostos bioativos com capacidade antioxidante e antimicrobiana em frutas. Revista do Congresso Sul Brasileiro de Engenharia de Alimentos; 2018;**4**(1):111. DOI: 10.5965/24473650412018111.

[75] Montrieux E, Perera WH, García M, Maes L, Cos P, Monzote L. In vitro and in vivo activity of major constituents from Pluchea carolinensis against Leishmania amazonensis. Parasitology Research. 2014;**113**(8):2925-2932. DOI: 10.1007/s00436-014-3954-1

[76] Fonseca-Silva F, Canto-Cavalheiro MM, Menna-Barreto RF, Almeida-Amaral EE. Effect of apigenin on leishmania amazonensis is associated with reactive oxygen species production followed by mitochondrial dysfunction. Journal of Natural Products. 2015;**78**(4): 880-884. DOI: 10.1021/acs.jnatprod.5b00011

[77] Belkhelfa-Slimani R, Djerdjouri B. Caffeic acid combined with autoclaved Leishmania major boosted the protection of infected BALB/c mice by enhancing IgG2 production, IFN-γ/TGF-β and iNO synthase/arginase1 ratios, and the death of infected phagocytes. Inflammopharmacology. 2018;**26**(2):621-634. DOI: 10.1007/s10787-017-0399-z

[78] Antwi CA, Amisigo CM, Adjimani JP, Gwira TM. In vitro activity and mode of action of phenolic compounds on Leishmania donovani. PLoS Neglected Tropical Diseases. 2019;**13**(2):e0007206. DOI: 10.1371/journal.pntd.0007206

[79] Gervazoni LFO, Barcellos GB, Ferreira-Paes T, Almeida-Amaral EE. Use of natural products in leishmaniasis chemotherapy: An overview. Frontiers in Chemistry. 2020;**8**:579891. DOI: 10.3389/fchem.2020.579891

[80] Fournet A, Rojas de Arias A, Ferreira ME, Nakayama H, Torres de Ortiz S, Schinini A, et al. Efficacy of the bisbenzylisoquinoline alkaloids in acute and chronic Trypanosoma cruzi murine model. International Journal of Antimicrobial Agents. 2000;**13**(3):189-195. DOI: 10.1016/s0924-8579(99)00117-x

[81] Torres Suarez E, Granados-Falla DS, Robledo SM, Murillo J, Upegui Y, Delgado G. Antileishmanial activity of synthetic analogs of the naturally occurring quinolone alkaloid N-methyl-8-methoxyflindersin. PLoS One. 2020;**15**(12):e0243392. DOI: 10.1371/journal.pone.0243392

[82] Seleem D, Pardi V, Murata RM. Review of flavonoids: A diverse group of natural compounds with anti-Candida albicans activity in vitro. Archives of Oral Biology. 2017;**76**:76-83. DOI: 10.1016/j.archoralbio.2016.08.030

[83] Fonseca-Silva F, Inacio JD, Canto-Cavalheiro MM, Almeida-Amaral EE. Reactive oxygen species production by quercetin causes the death of Leishmania amazonensis intracellular amastigotes. Journal of Natural Products. 2013;**76**(8):1505-1508. DOI: 10.1021/np400193m

[84] Araújo MV, Queiroz AC, Silva JFM, Silva AE, Silva JKS, Silva GR, et al. Flavonoids induce cell death in Leishmania amazonensis: in vitro characterization by flow cytometry and Raman spectroscopy. The Analyst. 2019;**144**(17):5232-5244. DOI: 10.1039/c9an00948e

[85] Baselga-Escudero L, Blade C, Ribas-Latre A, Casanova E, Suárez M, Torres JL, et al. Resveratrol and EGCG bind directly and distinctively to miR-33a and miR-122 and modulate divergently their levels in hepatic cells. Nucleic Acids Research. 2014;**42**(2):882-892. DOI: 10.1093/nar/gkt1011

[86] Ramos YAL, Souza OF, Novo MCT, Guimarães CFC, Popi AF. Quercetin shortened survival of radio-resistant B-1 cells. Oncotarget. 2021;**12**(4):355-365. DOI: 10.18632/oncotarget.27883