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Seasoning to Kill: the Example of the Natural Amide Piperine and Its Potential in the Design of New Antiparasitic Drugs

Afonso S. M. M. Velez,[a] *Paulo Pitasse-Santos,*[b] *Leonardo Freire-de-Lima,*[c] *Célio G. Freire-de-Lima,*[c] *Debora Decoté-Ricardo*[d] *and Marco E. F. de Lima*[*,a]

[a]*Departamento de Química Orgânica, Instituto de Química, Universidade Federal Rural do Rio de Janeiro, 23890-000 Seropédica-RJ, Brazil*

[b]*Leicester Institute of Structural and Chemical Biology and School of Chemistry, University of Leicester, LE1 7RH Leicester, UK*

[c]*Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Ilha do Fundão, 21941-902 Rio de Janeiro-RJ, Brazil*

[d]*Departamento de Microbiologia e Imunologia Veterinária, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, 23890-000 Seropédica-RJ, Brazil*

Natural products have long been explored in the history of most cultures worldwide. They have been mainly applied in the therapy of some diseases and for food preservation due to the antimicrobial properties of some condiments. Most of this knowledge was empirically discovered and passed from generation to generation. Over the last few years, the evolution of organic chemistry and the techniques of analysis and purification of compounds from complex matrices enabled a significant increase in research to identify bioactive products isolated from plants. In this paper, we briefly discuss the relevance of natural products on drug development, mainly focusing on amide piperine, the main chemical constituent of black pepper (*Piper nigrum*). Its antiparasitic activities and the knowledge of possible mechanisms of action against different parasites provide essential information for drug development. Furthermore, its compatibility with medicinal and synthetic chemistry techniques allows the development of more effective drug candidates.

Keywords: *Piper nigrum*, black pepper, piperamides, chemotherapy, parasitic diseases, NTDs

1. Introduction

Since antiquity, the use of medicinal plants directly or in the form of infusions in the therapy of the most diverse diseases has been known in several cultures. This knowledge of traditional medicine was passed down through generations, accumulating and improving in the most diverse cultures and societies. Over the last two centuries, it has made remarkable contributions to the development of the chemistry of natural products and medicinal chemistry as we know it today.[1,2]

Natural products are the largest known source of bioactive molecules. Their great chemical diversity spans an extensive variety of structures capable of interacting with different biochemical models present in living organisms.[3] Primary metabolites present in plants, such as polysaccharides, proteins, and fatty acids, mostly have plastic, structural, and energy storage functions. Special metabolites, on the other hand, are biosynthesized by plant organisms and are not necessarily used in the maintenance of their vital functions.[4,5] These metabolites present a greater chemical diversity and are, generally, products that had their synthesis naturally selected and modulated throughout evolutionary cycles, because of a given species' adaptation mechanisms. Their function in nature includes defense against pests and pathogenic microorganisms, or as an attractive (visual or olfactory) for pollinators.[2,4,6-8] It is important to highlight the relationship of the interaction of these special metabolites with biochemical targets of

*e-mail: marcoedilson@gmail.com
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This review is dedicated to the memory of the brilliant Brazilian scientist Prof Eliezer J. Barreiro in recognition of his outstanding contribution to the development of Brazilian medicinal chemistry, mainly in training an entire generation of researchers now working in institutions both in Brazil as abroad.

pathogenic microorganisms. Many of these molecules are active against a range of pathogens, whether isolated or in complex extracts from plant matrices.[5] This chemical selectivity modulated by plants over millions of years is the result of a process of evolution and adjustment of their metabolic pathways to produce bioactive structures with anti-infective properties as a way of surviving in their environment.[4,5,9] An obvious observation, but worth mentioning, is that plants do not move around. This way, the development of a well-elaborated chemical arsenal for protection and adaptation to a variety of conditions is critical for their survival.

Plant extracts are cheap and methodologically simple to obtain. They can be obtained using solvents with different polarities, which thus enable the production of extracts with variable chemical profiles from the same plant matrix. The biological evaluation of plant extracts against pathogenic microorganisms *in vitro* is the most conventional and cheapest approach for assessing the biological potential of these matrices.[10] The evaluation of fractions of extracts of different polarities can help to infer which classes of substances may contribute to a greater activity, or even if there is a mechanism of synergy between these components.[11-13] There are several examples of natural products isolated from plant matrices with relevant *in vitro* activity against pathogens, as shown in Table 1.

The contribution of natural products on the development and discovery of new anti-infective drugs is quite expressive.[30] Their relevance is very well discussed in the series of works by Newman and Cragg,[31,32] in which they highlight that practically 50% of all drugs inserted in the market from 1981 to 2019 are from natural sources, whether the original structures, synthetic and semisynthetic derivatives of natural products or even synthetic compounds whose pharmacophore was inspired by the structure of natural products. These data illustrate the massive contribution of natural products to drug development. For example, 4 of the 5 antiparasitic drugs launched in 1987 were identified from natural products (structures **12**-**15**, Figure 1). The research involving artemisinin **12**, an antimalarial drug discovered by Tu,[33] furnished her the Nobel Prize in Physiology and Medicine in 2015. The world production of artemisinin and its derivatives does not meet the demand for the treatment of malaria. Cambié *et al*.[34] highlight in their work an efficient and inexpensive way of synthesis for the main step in the production of these derivatives, the biomimetic photooxygenation of dihydroartemisinic acid, using exclusively solar-powered photoreactors, thus being able to help in this significant demand. Ivermectin **13** is an antiparasitic drug with several applications, mainly in cases of lymphatic filariasis.[31,35-39] Another antiparasitic drug of natural origin that is worth mentioning not only for historical reasons but also for its importance even today is the alkaloid quinine **14**, isolated from the bark of the Chinchona tree, which was the first drug used to treat malaria patients. Additionally, quinine was the prototype for the development of an entire class of synthetic antimalarial drugs, such as chloroquine **15**, mefloquine **16**, primaquine **17**, amodiaquine **18**, and mefloquine **19**, which are active against *Plasmodium falciparum*.[38,40] The chemical structures of compounds **12**-**19** are shown in Figure 1.

Despite the advances in combinatorial and computational chemistry for the discovery of new bioactive molecules, the evaluation of extracts and their isolated components continues to be relevant for drug development research.[13,41] The potential of vegetal sources for obtaining bioactive compounds is far from being fully explored. It is estimated that only 15% of all plant species have been studied, which shows that there is still great chemodiversity still unknown in nature.[42,43]

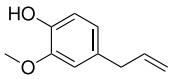
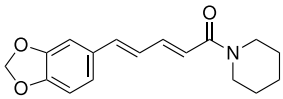
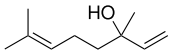
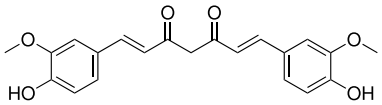
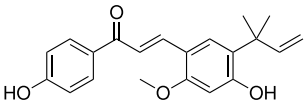
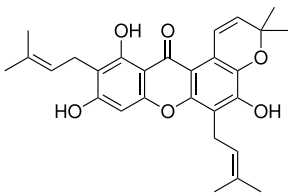
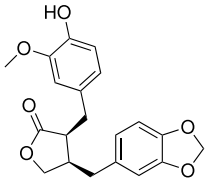
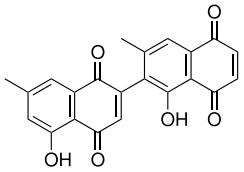
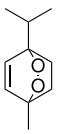
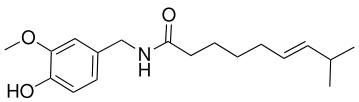
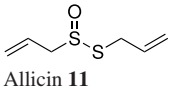
This work aims to discuss a molecule of great historical and therapeutic relevance, the natural amide piperine **2** (Table 1), abundantly found in the fruits of black pepper (*Piper nigrum*), as a potential tool in the development of new drugs to treat infectious diseases, mainly parasitic diseases labeled as neglected tropical diseases.

2. Natural Products Present in Condiments and Spices

In the same way that medicinal plants that throughout, ancient history had their empirical use as medicines, condiments, and food seasonings followed a very similar path. Empirically screened over the centuries, not only for adding flavor and color to foods but also for their preservative properties (antibacterial and antifungal), many of these condiments contain natural substances that are responsible, alone or in combination, for their biological properties (Figure 2). Then, since ancient times, humanity has benefited from the bioactive components present in plants, in different ways. Among the most important substances present in spices we can highlight the amides piperine and capsaicin (Table 1), present in plants of the genus *Piper* and *Capsicum*, respectively.[44,45] We can also mention the diaryleptanoid curcumin **4** (Table 1) from turmeric (*Curcuma longa*); the phenylpropanoid eugenol **1** (Table 1), isolated from clove oil (*Syzygium aromaticum*); in addition to allicin **11** (Table 1), a sulfur substance found in garlic (*Allium sativum* L.).[46-49]

Several scientists study what is called in the literature "Darwinian gastronomy", such as the study based on the 1998 survey done by biologists Billing and Sherman.[50]

Table 1. Some examples of natural products isolated from plant matrices with relevant *in vitro* activity against infectious disease-causing pathogens

Structure	Natural source	Biological activity	Reference
 Eugenol **1**	*Syzygium aromaticum*	antimicrobial (*Salmonella typhi*; *Staphylococcus aureus*)	14
 Piperine **2**	*Piper nigrum*	antiparasitic (*Trypanosoma cruzi*; *Leishmania amazonensis*; *Leishmania donovani*)	15-17
 Linalool **3**	*Croton cajucara*	antiparasitic (*L. amazonensis*; *T. cruzi*)	18,19
 Curcumin **4**	*Curcuma longa* L.	antiparasitic (*P. falciparum*; *Trypanosoma brucei*; *Leishmania major*; *T. cruzi*)	20-22
 Licochalcone **5**	*Glycyrrhiza* spp.	antiparasitic (*P. falciparum*)	23
 Tovophyllin A **6**	*Allanblackia monticola*	antiparasitic (*P. falciparum*)	24
 (–)-Methylpluviatolide **7**	*Zanthoxylum naranjillo* Griseb.	antiparasitic (*T. cruzi*)	25
 Diospyrin **8**	*Diospyros montana* Robx.	antiparasitic (*T. cruzi*; *T. brucei*; *L. donovani*)	26
 Ascaridole **9**	*Chenopodium ambrosioides* L.	antiparasitic (*P. falciparum*)	27
 Capsaicin **10**	*Capsicum oleoresin*	antifungal (*Candida albicans*)	28
 Allicin **11**	*Allium sativum* L.	antiparasitic (*Giardia lambia*); antifungal (*Candida albicans*)	29

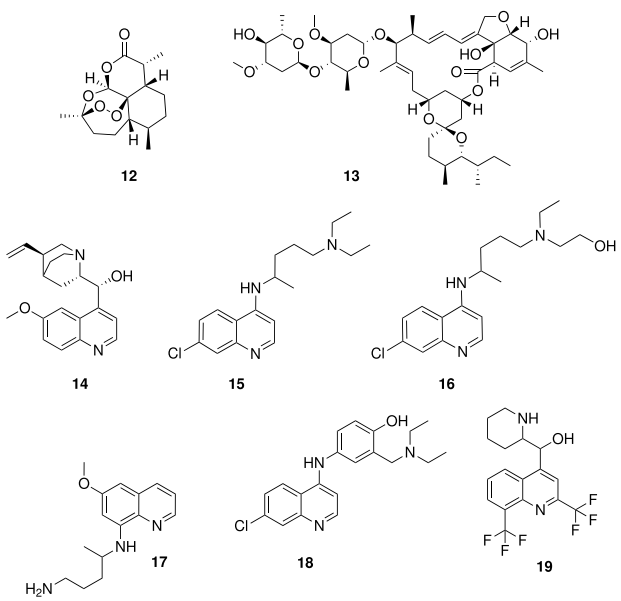


Figure 1. Natural products (artemisinin **12**, ivermectin **13**, quinine **14**) and synthetic analogs (chloroquine **15**, mefloquine **16**, primaquine **17**, amodiaquine **18**, and mefloquine **19**) with antimalarial properties.

Figure 2. Some of the main condiments and seasonings consumed worldwide and the structural diversity of the bioactive natural products in these plant materials of different shapes, tastes, aromas, and colors. In this image, we can see red pepper, black pepper, white pepper, rose pepper, nutmeg, clove, garlic, ginger, bay leaf, cinnamon, turmeric, paprika, and rosemary.

These authors discuss the evolution in the use of condiments in different cultures as an empirical strategy to avoid food-borne diseases, most often infections and/or poisonings caused by contamination by microorganisms, mainly fungi and bacteria. Based on the study of 4,570 recipes obtained from 93 different types of traditional cuisine, from countries with climates ranging from cold to tropical, the authors draw a clear connection between the use of condiments and the average temperatures of each region studied, where the higher temperatures, the greater quantities of seasonings are used, especially those with preservative properties already described today. From these observations, the authors infer that the use of spices may have been, throughout the ages, one of the most relevant key ingredients that facilitated the evolution of humanity protecting populations from diseases caused by microorganisms present in food, given the precarious conditions of the existing storage facilities. In this way, it is postulated that the selection of certain condiments is something that goes beyond simply adding color and flavor to certain recipes, but also since the components of these plant materials have food preservation properties, especially in times where domestic fridges or even conservation methods, such as pasteurization and the use of radiation, were not yet available. Comparative studies on the use of condiments in typical dishes in warmer climates compared to colder ones show the use of a greater number of condiments the higher the local average temperature. Another relevant point is highlighted when comparing traditional meat dishes and dishes with only vegetables in their preparation, where the former are notably spicier.[51] In our opinion, this issue may not be so simple. However, an important point that is extremely relevant to the matter of human selection of plants used as condiments in regional and traditional recipes is the variability of plant biodiversity in several regions with different climates. Indeed, this observation adds another relevant variable when comparing, for example, typical Indian and Nordic dishes. Nevertheless, without a shadow of a doubt, the subject is quite intriguing and arouses great interest to researchers working in the chemistry of bioactive natural products notably in the search for molecules with application in chemotherapy of infectious diseases.

3. Black Pepper: the King of Spices

Undoubtedly, black pepper (*Piper nigrum* L.) is the king of spices due to its wide use in different cultures spread across the different continents of the planet. Many authors[52,53] comment on the several applications of extracts and infusions of black pepper highlighting its wide applicability on food preservation, which is directly related to its antimicrobial potential. *Piper nigrum* can be found on the market in three different presentations: black, white and green pepper. Although the three variations are prepared from the fruits of the same plant, each one goes through different processing, generating materials with different characteristics. Black pepper is the one with the most pungent flavor, green pepper has a milder flavor than black or white pepper. White pepper is derived from the fully ripened fruits of the *P. nigrum* plant by removing the outer pericarp before drying. Black pepper is obtained from the fruits of *P. nigrum*, washed in

boiling water and dried in the sun or in ovens for several days, producing the dark color observed. White pepper is also made from the fruits of *P. nigrum*, however, in the production of white pepper, the fruits are soaked in water and left to ferment for a few days. Finally, the outer layer of the fruits is removed, leaving only the inner seeds. As the fruit skins are removed, white pepper tends to have a milder flavor than black pepper. Green pepper is obtained from *P. nigrum* green fruits, harvested approximately 10 to 15 days before ripening. After harvesting, the fruits are processed to maintain their green color. The quality factors of the three different types of pepper are based on their physical and chemical characteristics, which impact their odors, flavors and colors.[54,55]

Over the years, black pepper has encouraged several researchers. Many studies[45,52,56-58] have reported the isolation of different classes of compounds from *Piper nigrum*, such as phenolic compounds, flavonoids, alkaloids, several amides, steroids, lignans, neolignanes, terpenes, chalcones, among many other compounds. Undoubtedly, amides are the main constituents of *P. nigrum* (Figure 3).

Among the components of *Piper nigrum*, the natural amide piperine **2** deserves special attention, which has a privileged chemical structure and activities described in works that show the antiparasitic activity of this natural product.

Piperine **2** can be found in several species of the genus *Piper*, being abundantly present in the fruits of *P. nigrum*. Its extraction from the dried fruits is easily set up using a Soxhlet extractor, involves the use of inexpensive solvent ethanol, and can yield up to 7%, pure piperine (Figure 4).[59]

The methodology currently used in our research group is an adaptation of Ikan,[59,60] using Soxhlet and ethanol as extractor solvent, followed by the removal of tannins by precipitation with basic ethanolic solution (tannins are the main contaminants present in the extract of dry and grounded fruits of *P. nigrum*). Then, piperine crystallizes as yellow needles, in high purity. Figure 5 shows the chromatogram and UV curve of piperine obtained in 97% purity after recrystallization from ethanol.

The great popularity of this condiment, as well as its widespread use in all regions of the planet, means that black pepper is currently a commodity. According to data from Food and Agriculture Organization of the United Nations (FAO),[61] the world production of black pepper, which in 2021 was around 794 thousand tons, is concentrated in just nine countries, among which Vietnam and Brazil stand out as the main world producers with 288 and 118 thousand tons of the condiment, respectively. However, the potential for expansion of black pepper production is immense, as the vine has adapted to almost all regions with a temperate climate on the globe. These data, combined with the fact of the greater occurrence of piperine in yields of up to 7% in the plant's fruits, and the ease of its isolation, make black pepper a renewable source of this natural amide, making piperine an extremely accessible and easily scalable input to the pharmaceutical industry. Piperine has also been in the spotlight in many pharmaceutical research studies in recent years.[62]

Extracts and essential oils of *P. nigrum* possess anti-inflammatory, antioxidant, and antimicrobial profiles, and such biological activity is attributed to piperine **2**.[63] Furthermore, piperine **2** is associated with increased bioavailability of medicines in combined treatments, which is one of the most interesting biological properties of this natural amide. The natural amide **2** has important effects in increasing the absorption of nutrients and drugs in the gastrointestinal (GI) tract, in addition to exhibiting potent effects in inhibiting the metabolism of different classes of drugs, acting mainly in modulating the activity of

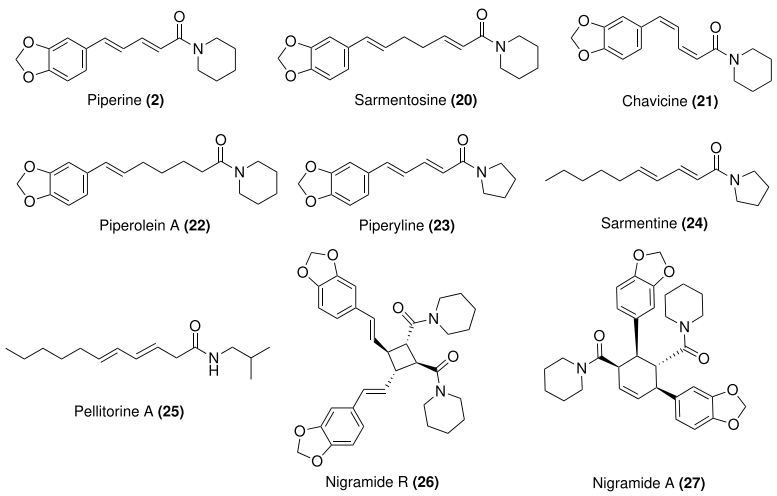


Figure 3. Natural amides (**2**, **20**-**27**) isolated from *P. nigrum*.

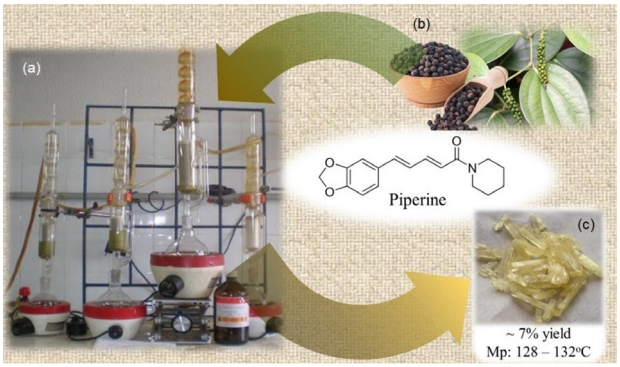


Figure 4. Methodology and results currently used in our research group to extract piperine: (a) extraction system with Soxhlet apparatus; (b) dried black pepper fruits; (c) piperine crystals obtained after precipitation from the ethanolic extract and recrystallization from ethanol/water.

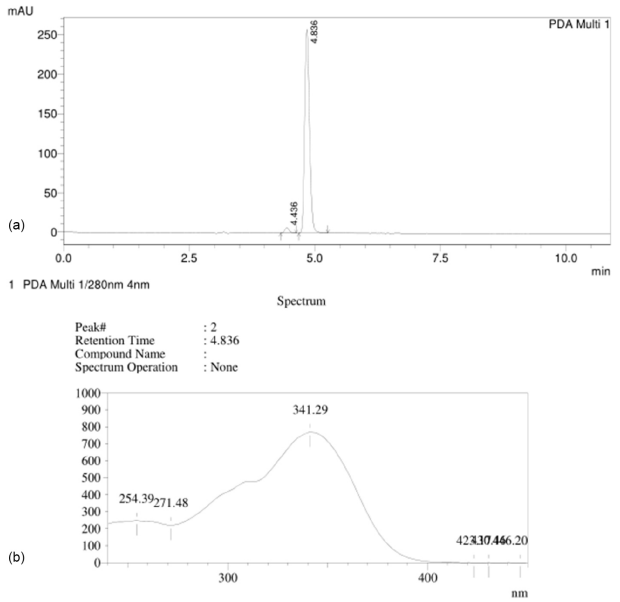


Figure 5. Liquid chromatography analysis (a) and the UV spectrum (b), of piperine **2** (retention time: 4.836 min) extracted from dry and grounded fruits of *P. nigrum* through the methodology adapted from the work of Ikan.[59] Analysis conditions: reverse phase: BetaSil column (25 cm × 4.6 mm × 5 µm) Thermo Scientific. Elution: (B) MeOH with 1% AcOH; (A) H_2O with 1% AcOH (90:10).

important metabolizing enzymes, both phase I and phase II of xenobiotic metabolism, such as human P-glycoproteins, CYP3A4, UDP-glucose dehydrogenase (UDP-GDH), aryl hydrocarbon hydroxylase (AAH) and UDP-glucuronyl transferase.[64-70] Theoretical studies[71] indicate the interaction of piperine **2** with the CYP3A4, an enzyme associated with the oxidative metabolism of more than half of the drugs and xenobiotics in humans. Results show the formation of a stable piperine:CYP3A4 complex, suggesting a mechanism of selective interaction with CYP3A4, which would explain the increase in the bioavailability of the drugs when co-administered with piperine.[72]

Furthermore, piperine **2** possesses antiparasitic activity, especially against *T. cruzi*, *L. donovani*, and *L. amazonensis*.[73] This set of characteristics can be explored to support the utilization of this natural amide, alone or in combination with other bioactive molecules and drugs, to the development of new therapeutic strategies to treat parasitic mainly related to the neglected tropical diseases.[15-17]

3.1. Potential applications of piperine against protozoa infections

Kinetoplastid protozoa of the genera *Leishmania* and *Trypanosoma*, as well as those of the genus *Plasmodium*, cause widespread diseases in humans: leishmaniasis, Chagas disease (or American trypanosomiasis), sleeping sickness (or African trypanosomiasis), and malaria. These infectious microorganisms are eukaryotic and form a unique group among human pathogens, especially if we consider the harmful impacts caused by them on public health in many countries. Although they have been recognized by medical science for over a century, as is the case with Chagas disease caused by the hemoflagellate protozoan *Trypanosoma cruzi*, and described by Brazilian scientist Carlos Justiniano Chagas,[74] there is still a limited availability of therapeutic alternatives for treating infected individuals, in addition to the total absence, to date, of accessible and effective vaccination strategies for the prophylaxis of these infections. These limitations still apply today, despite all ongoing efforts and the huge burden imposed by these parasitic infections, mainly in developing countries.[75] In fact, this perverse logic opposes the necessary development of new and effective therapeutic alternatives, applicable to the treatment of the most diverse parasitic infections. Large pharmaceutical corporations, despite the pressing humanitarian need, do not envisage a consumer market capable of providing the financial return they seek. This scenario is due to the fact that parasitic diseases, for the most part, affect population groups that live in a situation of great economic vulnerability, and therefore do not have the financial resources to pay for their treatments.

The natural amide piperine stands as an interesting alternative for the development of new alternatives for the chemotherapy of these parasitic infections.

3.1.1. Piperine against *Trypanosoma cruzi*

In a pioneering work by our research group, the trypanocidal activity of piperine **2** and its semisynthetic analogs (e.g., **28**-**30**, Figure 6) were carried out against amastigote and epimastigote forms of *T. cruzi in vitro*. The results indicated a promising activity profile against *T. cruzi*. The chemical structures of compounds **2**, **28**-

30, as well as for reference drug, benznidazole **31**, are shown in Figure 6, along with the respective half maximal effective concentration (EC_{50}) values calculated on the study indicated for each evolutive form of *T. cruzi*.[15] Further investigations provided valuable clues about the mechanism of action of piperine **2** against *T. cruzi*. It was observed that the treatment with the natural product resulted in the reversible interruption of the cytokinesis of the parasite, which characterizes the trypanostatic activity of piperine **2**. In this context, a viable hypothesis is the disruption of the microtubule polymerization mechanism.[76] However, the presence of various vacuolated membranous structures in parasites treated with sublethal concentrations of piperine **2**, observed by transmission electron microscopy (TEM) images, suggests that the effects of its treatment are not restricted to only one mechanism of action against the parasite.

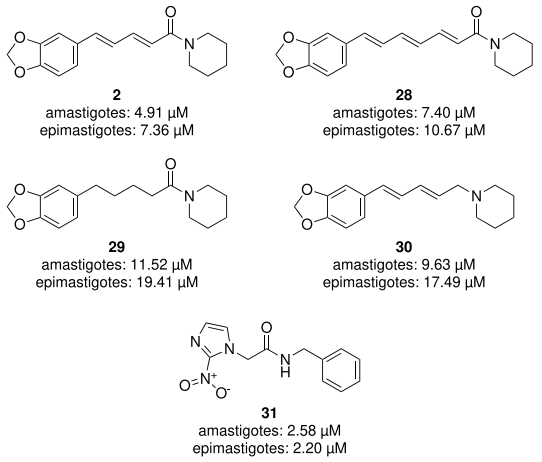


Figure 6. Chemical structures and EC_{50} values obtained for piperine **9** and some structural analogs (**28**, **29**, and **30**), in addition to the reference drug benznidazole **31** against amastigote and epimastigote forms of *T. cruzi* (Y strain).

The chemical scaffold of piperine **2** was explored in the design of potentially bioactive compounds using classic medicinal chemistry strategies, such as bioisosterism of the amide function and molecular hybridization. A series of 1,3,4-thiadiazolium-2-phenylamine chlorides (**32**-**34**, Figure 7) derived from piperine **2** hybrids was synthesized.[45] The molecular design of the new compounds was based on the leishmanicidal activity of a cinnamic series of 1,3,4-thiadiazolium-2-phenylamine chlorides described by Echevarria and co-workers[77] and our results with piperine against *T. cruzi*. As the 1,3-benzodioxole moiety present in piperine was previously demonstrated to be important to activity, it was preserved in the structure of the planned new derivatives. It was observed an increase in activity against *T. cruzi* (Y strain) compared to the natural prototype. Compounds **32** and **34** were able to inhibit the parasite growth even more effectively than the reference drug benznidazole *in vitro*.[78] The piperine scaffold was also explored in the design of a series of 1,2,4-triazoles-3-thiones (e.g., **35** and **36**, Figure 7). These structures were planned to use both strategies of bioisosterism and molecular hybridization. The 1,2,4-triazoline-thione group acts as a bioisoster of amide group. At the same time, it is the pharmacophore group present on prothioconazole, an antifungal agent that acts through inhibition of the biosynthesis of membrane steroids such as ergosterol, also present on the parasite's cytosolic membrane.[79] The chemical structure of compounds **32**-**36** and the respective EC_{50} values calculated for different evolutive forms of *T. cruzi* (Y strain) are shown in Figure 7.[78]

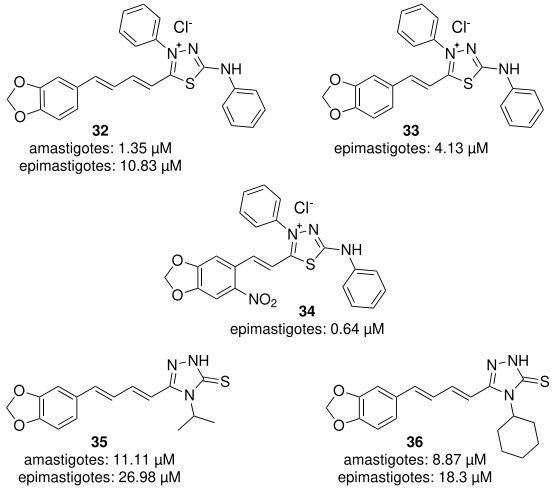


Figure 7. Chemical structures and EC_{50} values of piperine derivatives (**32**-**36**) against amastigote and epimastigote forms of *T. cruzi* (Y strain).

In their subsequent work, Franklim *et al.*[80] explored the synthesis of *S*-alkylated derivatives of the piperine-triazole derivative **36** from their previous work possessing a best trypanocidal profile. The results obtained highlighted a promising improvement in the antiparasitic profile when the new series was tested against the main evolutive forms of *T. cruzi*. Derivatives **37**-**39** (Figure 8) were at least twice as active as their precursor **36**, with cell viability in murine macrophages ranging from 90-95% at the highest concentration evaluated, showing an important selectivity index. The chemical structure of compounds **37**-**39** and the respective EC_{50} values calculated for different evolutive forms of *T. cruzi* (Y strain) are shown in Figure 8.[80]

In Scheme 1 we show in detail the synthetic sequences developed for the preparation of the two series of heterocycles, highlighting piperic acid, obtained by the basic hydrolysis of piperine, as a common precursor to both. Piperic acid generates the corresponding acyl chloride **41** through the action of oxalyl chloride and,

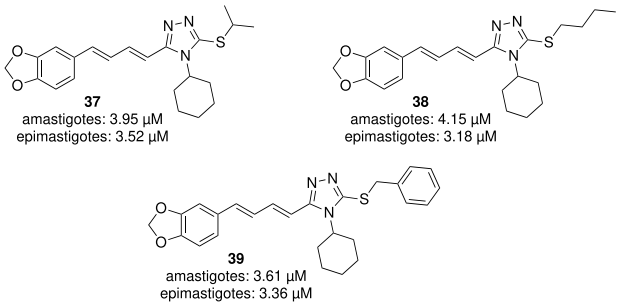


Figure 8. Chemical structures and EC_{50} values of piperine derivatives (**37**-**39**) against amastigote and epimastigote forms of *T. cruzi* (Y strain).

depending on the conditions to which it is exposed, leads to the formation of two series of heterocycles, passing through acylhydrazide **42**, which then reacts with suitable isothiocyanates, followed by microwave-assisted cyclization, as in the case of 1,2,4-triazole-3-thione **36**.[81] Alternatively, the acid chloride **41** can react with 1,4-diphenyl thiosemicarbazide, generating the mesoionic derivative **32** and a series of analogs.[78]

3.1.2. Piperine against *Leishmania* spp.

Piperine **9** was also investigated for its leishmanicidal activity. The natural product was tested against different forms of *L. amazonensis* and they were found EC_{50} values of 14.2 μM against promastigotes and 28 μM against amastigotes, in addition to natural amide, some analogues and derivatives of piperine were evaluated (Figure 9). Investigations on possible mechanisms of action evidenced that piperine **2** does not act through the induction of nitric oxide (NO) production as described in the work of Ferreira *et al*.[16] and Chouhan *et al*.[82] Investigations on possible mechanisms of action evidenced that piperine **2** does not act through the induction of NO production. On the other hand, TEM images of the parasite treated with piperine **2** showed significant alterations, such as an increase in mitochondria size and loss of organization of mitochondrial matrix. These observations suggest that the mitochondria of *L. amazonensis* are a potential target for piperine **2**. These findings corroborate data in the literature[83,84] that describe mitochondrial alterations induced by the treatment of *L. amazonensis* with extracts and natural products isolated from *Piper* spp. Additionally, piperine is an inhibitor of lipid droplet blockage in mouse macrophages, particularly inhibiting the cholesterol ester (EC) scheme through inhibition of acyl-CoA:cholesterol acyltransferase (ACAT) activity. It is hypothesized that a similar mode of action might be observed on the parasite, remaining in the sequence of another enzyme that is also involved in the parasite's lipid metabolism.[16,85]

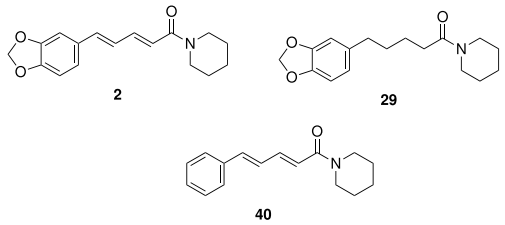
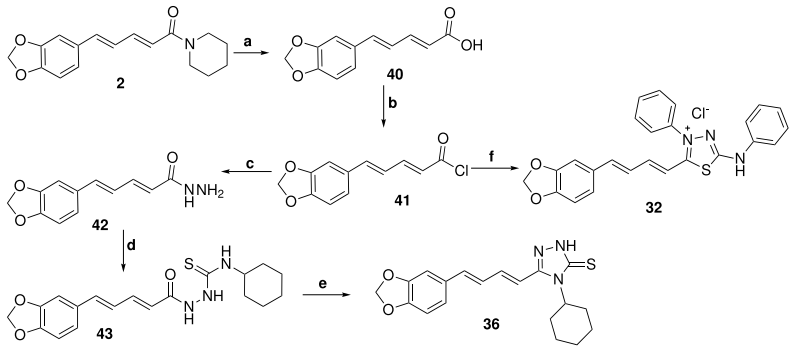


Figure 9. Chemical structures of piperine **2** and its derivatives (**29** and **40**) that showed inhibition profile over 50% against promastigotes of *L. amazonensis* at 50 μM after 48- or 72-h incubation.

Vieira-Araújo *et al*.[86] described the potential of two natural amides from spices, capsaicin and piperine, when used in association with pentavalent antimony-based drugs (Sb^{+5}), as meglumine antimoniate, which are available drugs for human use only in Brazil. In this study an isobolographic study was performed using different proportions of natural amides and antimoniate in which the proportion of 25% piperine and 75% meglumine antimoniate showed the best activity profile against *L. infantum*, including synergistic action of these compounds. This association was made with the aim of increasing the drug's leishmanicidal activity, which would make it possible to reduce the doses



Scheme 1. Synthetic paths to the synthesis of mesoionic 1,3,4-thiadiazolium-2-phenylamine chloride **32** and 1,2,4-triazole-3-thione **36**. (a) Ethanolic KOH, reflux; HCl to pH 3.0 (80%); (b) $(COCl)_2$, 30 min; (c) $NH_2NH_2.H_2O$, CH_2Cl_2, 0 °C to rt (71%); (d) cyclohexyl isothiocyanate, ethanol, microwave (MW), 30 min (91%); (e) aqueous NaOH, MW, 30 min; then acidification with aqueous HCl up to pH 3.0 (91%); (f) 1,4-diphenylthiosemicarbazide, 1,4-dioxane, 25 °C, 24-48 h (57%).

of antimonials used in the treatment of infections caused by *Leishmania*. The possibility of the dose reduction is extremely important, due to the toxic effects caused by these substances.[87] The authors observed a significant reduction in the necessary concentration of meglumine antimoniate to achieve the same response when the drug was associated with capsaicin and piperine (EC_{50} = 4.31 ± 0.44 and 7.25 ± 4.84 μg mL^{-1} against promastigote and amastigote forms of *Leishmania infantum*, respectively).

3.1.3. Piperine against *Plasmodium* spp.

Malaria is a serious acute infectious disease caused by protozoa of the genus *Plasmodium*, transmitted by the bite of an infected female *Anopheles* mosquito, also known as "sandfly". Once installed in the human host, the infection causes fever and can progress to its severe forms, especially if it is not diagnosed and the patient is not treated in a timely and appropriate manner. In Brazil, most malaria cases are concentrated in the Amazon region, due to the favorable conditions for the vector's development (clean, non-running waters, located in shaded areas). Asymptomatic *Plasmodium* carriers can develop episodes of malaria when their immune systems are compromised. Therefore, the importance of controlling asymptomatic carriers of malaria parasites becomes increasingly important. These actions can prevent the spread of infection to non-endemic areas and can even prevent the reintroduction of the disease in regions where malaria was still under control.[88] Primate species can also function as reservoirs for the parasite, increasing the prevalence of the disease in some regions.[89]

The emergence and rapid spread of multidrug-resistant strains of *Plasmodium falciparum* is one of the greatest threats to effective malaria control in many regions.[90] These resistant strains make treatment more difficult, and often more expensive and difficult, as they greatly limit the possibilities of drugs that can be used to treat patients affected by infections with these characteristics, in addition to putting at risk individuals who are occasionally exposed to the possibility of contracting the infection, using prophylactic medications. Without a doubt, the emergence of resistant strains is the main problem in efforts to control malaria in endemic areas. Therefore, the discovery and development of new therapeutic alternatives for the treatment and prophylaxis of *Plasmodium* infection becomes urgent, with the need for new molecules that have innovative mechanisms of action, in order to circumvent current resistance mechanisms. In this scenario, the structural diversity offered by natural products of plant origin stands as an attractive alternative, with those present in the composition of plant parts used as condiments and seasonings being one of the most promising ones.[91]

One of the first works that described the antiplasmodial activity of *P. nigrum* extract was described by Kamaraj *et al.*,[92] where the authors carried out a study of the anti-malarial potential of strata obtained from parts of six plants, popularly used in the treatment of malaria cases, including the ethyl acetate extract of *P. nigrum* seeds. The authors observed, in addition to reduced cytotoxicity against mammalian cells, a promising toxic activity against cells in the erythrocytic stages of chloroquine (CQ)-sensitive 3D7 and CQ-resistant INDO strains of *Plasmodium falciparum* in culture using the fluorescence-based SYBR Green I assay, with half maximal inhibitory concentration (IC_{50}) values of 12.5 μg mL^{-1} against both strains and a selectivity index of 7.[92] Thiengsusuk *et al.*[91] described the toxic activity of crude extracts of *Piper chaba*, which has piperine as its main chemical constituent. The work was performed against two strains of *P. falciparum*, both chloroquine-sensitive (3D7) and chloroquine-resistant (K1) *P. falciparum* clones. The crude extracts assessed showed IC_{50} values of 5.3 and 4.1 μg mL^{-1}, against 3D7 and K1 clones, respectively. In a subsequent study carried out by the same research group, some constituents isolated from *P. chaba* were assessed against the same clones of *P. falciparum*, including the amide piperine **2**. Despite piperine being the main chemical constituent present in the crude extract, the natural amide showed lower antiplasmodial activity than the complex mixture of components presents in the extract, with IC_{50} values of 59 and 111.5 μM for K1 and 3D7 clones, respectively. These experimental observations suggest a synergistic or additive effect between the components of the crude extract. The antimalarial activity of piperine is due to its ability to inhibit the modulation of expression of all *P. falciparum* resistance genes, which makes the natural amide a promising structure for the development of new anti-malarial drugs or even to be used in association with other drugs or even natural and synthetic drug candidates having anti-plasmoidal activity.[93,94]

More recently, Khairani *et al.*[95] described the results obtained *in vivo*, with the oral administration of piperine in mice infected with *Plasmodium berghei* ANK. The natural amide was administered in doses of 10, 20, and 40 mg kg^{-1}. As a control drug, the authors used artesunate 5 mg kg^{-1}, and dimethyl sulfoxide (DMSO) as a negative control. Four groups of Swiss Webster mice were used, in treatments that lasted four days. The animals were monitored for parasitemia, body weight, rectal temperature, and survival rate, in addition to some clinical parameters. Animals treated with piperine, at a dose of 40 mg kg^{-1}, in curative and prophylactic tests, showed maximum parasitemia of 79.21 and 58.8% ($p < 0.05$), respectively, with a significant effect on the survival rate, compared to control untreated

animals. In the curative test, piperine at a dose of 40 mg kg^{-1} reduced the average clinical score compared to the control group. Additionally, the natural amide showed the ability to protect organs (lungs, liver, spleen, and kidneys) from some damage in a dose-dependent manner. The results obtained in this study can be used as a basis for the use of piperine in the planning of new drugs with chemotherapeutic or chemoprophylactic activity against malaria.[95]

3.2. Potential applications of piperine against nematode infections

Diseases caused by nematodes in humans are still responsible for serious public health problems in different parts of the world, mainly affecting children living in pockets of poverty with low sanitation levels. Currently, it is estimated that more than a billion people carry one or several species of these parasites in their bodies, most of which are long-lived animals that cause long-term damage. Nematode infections are normally acquired in the environment, from person to person, or through the consumption of contaminated food, especially those consumed fresh. There are also transmissions mediated by insect vectors, such as different species of mosquitoes capable of transmitting filariasis through their bites. Another form of transmission is mediated by flies that become contaminated when they come into contact with waste and then mechanically transport eggs and larvae to utensils and food. Improving sanitation and vector control are important long-term solutions for eliminating human nematode infections. The main treatment strategy is chemotherapy.[96-99] An interesting point in the area of anthelmintic drugs is the fact that all substances approved for the treatment of infections in humans, except diethylcarbamazine, were first developed for application in veterinary medicine, and later adapted for human use.

Among the diseases caused by helminths, lymphatic filariasis stands out, which is considered by the World Health Organization (WHO) to be a neglected disease. Three different filarial species can cause lymphatic filariasis in humans. Most of the infections worldwide are caused by *Wuchereria bancrofti*. In Asia, the disease can also be caused by *Brugia malayi* and *Brugia timori*. The infection spreads from person to person by mosquito bites. In Africa, the most common vector is Anopheles and, in the Americas, it is *Culex quinquefasciatus*. *Aedes* and *Mansonia* can transmit the infection in the Pacific and Asia. This infection causes major disorders, both physical and psychological, as the worms lodge in the patient's lymphatic system, leading to the formation of lymphedema and elephantiasis. In males, hydroceles may appear, which are permanent edemas in the scrotum. Lymphatic filariasis affects over 120 million people in 72 countries throughout the tropics and sub-tropics of Asia, Africa, the Western Pacific, and parts of the Caribbean and South America.[100] To our knowledge, one of the first reports on the potential activity of piperine against nematodes was the work of Tiner,[101] where the author describes the development of a methodology for determining the nematocidal activity of both natural and synthetic substances against nematodes of the genus *Tricostrongylus*, which are intestinal parasites of medical and veterinary interest. Studies on the activity of piperine against filariasis worms were conducted by Joardar *et al.*,[102] which evaluated piperine and 20 of its direct derivatives (secondary and tertiary amides) against different evolutionary forms of *Setaria cervi*, which causes infections in cattle. Figure 10 shows the structures of the most active derivatives evaluated. Experiments carried out in this work indicate that the activity of piperine and derivatives against the filaria species is related to the changes caused by these compounds in the redox homeostasis of the nematode. Being one of the compounds capable of binding to proteins involved in redox balance (thioredoxin and CED-3). The detoxification mechanisms of oxidizing species are the main targets studied for the treatment of filariasis since the maintenance of worms in the mammalian host's body for long periods requires an effective redox homeostasis system. Azeez *et al.*[103] described theoretical studies, validated by enzyme inhibition data, on the effects of piperine and other natural products, present in ten plant species, on the inhibition of glutathione-*S*-transferase(s) (GST) from *Brugia malayi*.

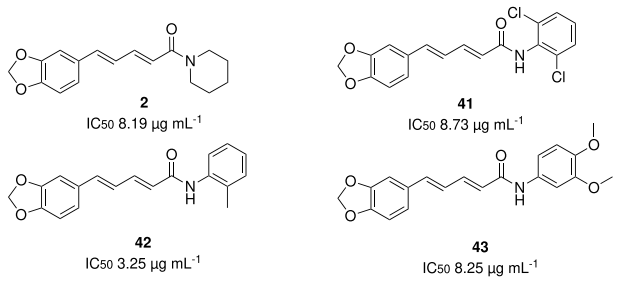


Figure 10. Structures of the most active piperine derivatives against filarial worm *Setaria cervi*.

As far as we know this is the first work evaluating piperine and its direct derivatives against worms that cause filariasis. We could find works[104-106] evaluating the natural amide against free-living nematodes used as models for testing antihelmintic activity, such as *Tubifex tubifex*; against nematodes of medical-veterinary interest and even against worms of the class of phytopathogens, as *Bursaphelenchus xylophilus* which is a notorious pest of

pine trees known to cause the pine-wilt disease. Although still scarce, these observations highlight the nematocidal potential of the natural amide piperine and its derivatives.

4. Conclusions and Perspectives

The abundance and availability of piperine **2** in nature, its extraction from a renewable source, and its facile isolation make this molecule a very interesting alternative as a prototype for the development of new antiparasitic drugs. This potential has been explored in different studies focused on *T. cruzi*, *L. amazonensis*, *Plasmodium* ssp., and also against nematodes. The observed antiparasitic activities *in vitro* and the indications of possible modes of action on unicellular and multicellular parasites highlight the relevance of this compound for the development of novel antiparasitic drugs. The design of analogs based on structural modifications on piperine **2** has also been explored. The use of the natural product's chemical scaffold as a platform along with the employment of medicinal chemistry tools, such as bioisosterism and molecular hybridization, have proven promising. Compounds chemically related to piperine were able to inhibit the parasites' growth even more effectively than the reference drug benznidazole.[78,80,81] The example of drugs used in the treatment of malaria, in which some of the main options are based on naturally occurring products, serves as a stimulus to insist on the planning of new derivatives of the natural amide, to develop new molecules with therapeutic potential to treat patients affected by the neglected tropical diseases.

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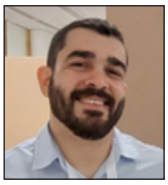
Author Contributions

A. S. M. M. V. was responsible for the visualization, writing original draft, and editing; P. P.-S. for the writing-review, and editing; C. G. F. D. L. for the data curation, visualization, and writing-review; L. F. D. L. for the data curation, visualization, and writing-review; D. D. R. L. for the data curation, visualization, and writing-review; M. E. F. L. for the conceptualization, validation, data curation, editing, and writing-review.

Afonso S. M. M. Velez *received his BS degree in Chemistry from the Federal Rural University of Rio de Janeiro (2019); a master's degree in chemistry from the same University (2021). Nowadays, he is a PhD student (PPGQ-UFRRJ) working on the design, synthesis and antichagasic evaluation of new piperine-nitroimidazole hybrids, under the supervision of Prof Marco E. F. Lima and co-supervision of Prof Débora Decoté-Ricardo.*



Paulo P. Santos *received a BS degree in Chemical Engineering (2014) and a master's degree in Chemistry (2017) from the Federal Rural University of Rio de Janeiro (UFRRJ, Brazil). He got a PhD degree in Chemistry from the PPGQ-UFRRJ (2022) working with cell-penetrating antimicrobial peptides and peptide-drug conjugates under the supervision of Prof Marco E. F. Lima, with a sandwich period at Purdue University (Indiana, USA), under the supervision of Prof Jean Chmielewski. He is currently working as a Postdoctoral Research Associate with the design and synthesis of molecular glues related to 14-3-3 protein-protein interactions, under the supervision of Dr Richard Doveston, at the University of Leicester (United Kingdom).*

Leonardo Freire-de-Lima *is assistant professor at Federal University of Rio de Janeiro. During his scientific career, he worked in projects related to cellular interactions mediated by carbohydrates, glycoimmunology of infectious diseases, as well as glycobiology of cancer cells. So far, the obtained results and the ongoing experiments in his lab aim to determine the role of oncofetal glycoproteins and/or glycosyltransferases in many events related to cancer progression, opening the possibility of discovering new targets and/or therapeutic strategies for the treatment of cancer.*

Celio G. Freire-de-Lima *is a professor in the Program de Imunobiologia at the Instituto de Biofísica Carlos Chagas Filho at Universidade Federal do Rio de Janeiro, has a PhD from the Instituto de Microbiologia Paulo de Goes at UFRJ and a Post-Doctorate from the National Jewish Medical and Research Center in Denver, Colorado. Dr Freire-de-Lima is the*

head of the Immunomodulation Laboratory (LABIM) of the IBCCF/UFRJ. The Lab's thematic lines have been concentrated in the areas of Immunology, Microbiology and Experimental Medicine, evaluating the immunomodulatory mechanisms triggered by parasitic infection and/or purified molecules. Briefly, the Laboratory investigates the importance of cellular components of the innate response in regulating immunity. For example, the phagocytosis of apoptotic cells by macrophages and the involvement of B-1 cells and their secreted products in models of infectious and inflammatory diseases. Furthermore, our group described the involvement of capsular components of the opportunistic fungus Cryptococcus neoformans in modulating the immune response and consequently in the course of infection. The Immunomodulation Laboratory also collaborates with different research groups. Furthermore, it is important to highlight that the laboratory is committed to training future professionals who can contribute in a relevant way to the progress of science.

Débora Decoté-Ricardo *is an associate professor responsible for the disciplines of Basic Immunology, Clinical Immunology and Immunotherapy in the Department of Microbiology and Veterinary Immunology of Veterinary Institute at the Federal Rural University of Rio de Janeiro (Brazil). She worked as a visiting researcher at the Integrated Department of Immunology at National Jewish Health, Denver, Colorado, USA (2005-2006) under the supervision of Dr John Cambier. She received her PhD in science (2008) at the Federal University of Rio de Janeiro. Her area of expertise is the modulation of the immune response in models of infection and autoimmunity. Among the infection models, she has long experience in the study of parasitic diseases such as those caused by protozoa and fungi, always focusing on immunomodulation strategies by regulating the immune system through humoral, cellular therapies or the use of natural products. Likewise, studies have been carried out on the modulation of inflammatory responses resulting from autoimmunity.*

Marco E. F. de Lima *is a full professor in the Department of Organic Chemistry of the Institute of Chemistry at the Federal Rural University of Rio de Janeiro (Seropédica, RJ, Brazil). He worked as a visiting researcher at Prof Victor Hruby's group (2015-2016) at the University of Arizona (Tucson, AZ, USA) on the design and synthesis of peptides for medicinal purposes. Prof Lima received his PhD in sciences (1994) from IQ-UFRJ working under the supervision of Prof Andrew E. Greene at LEDSS-3 (University of Grenoble-France, 1991-1993) in the total synthesis of natural products using the [2 + 2] cycloaddition reaction of dichloroketene to olefins. He got a master's in sciences degree (1989) from IQ-UFRJ working on the design and synthesis of new anti-inflammatory drugs under the supervision of Prof Eliezer J. Barreiro; and he got a BS degree in Pharmacy from the School of Pharmacy of the Federal University of Rio de Janeiro (1986). Prof Lima's research interests include the medicinal chemistry of natural products, focusing the design and synthesis of new chemical entities potentially useful as antiparasitic drugs with a major emphasis on neglected tropical diseases.*

References

1. Viegas Jr., C.; Bolzani, V. S.; Barreiro, E. J.; *Quim. Nova* **2006**, *29*, 326. [Link] accessed in May 2024
2. Bernardini, S.; Tiezzi, A.; Masci, V. L.; Ovidi, E.; *Nat. Prod. Res.* **2018**, *32*, 1926. [Crossref]
3. Atanasov, A. G.; Zotchev, S. B.; Dirsch, V. M.; Orhan, I. E.; Banach, M.; Rollinger, J. M.; Barreca, D.; Weckwerth, W.; Bauer, R.; Bayer, E. A.; Majeed, M.; Bishayee, A.; Bochkov, V.; Bonn, G. K.; Braidy, N.; Bucar, F.; Cifuentes, A.; D'Onofrio, G.; Bodkin, M.; Diederich, M.; Dinkova-Kostova, A. T.; Efferth, T.; El Bairi, K.; Arkells, N.; Fan, T. P.; Fiebich, B. L.; Freissmuth, M.; Georgiev, M. I.; Gibbons, S.; Godfrey, K. M.; Gruber, C. W.; Heer, J.; Huber, L. A.; Ibanez, E.; Kijjoa, A.; Kiss, A. K.; Lu, A.; Macias, F. A.; Miller, M. J. S.; Mocan, A.; Müller, R.; Nicoletti, F.; Perry, G.; Pittalà, V.; Rastrelli, L.; Ristow, M.; Russo, G. L.; Silva, A. S.; Schuster, D.; Sheridan, H.; Skalicka-Woźniak, K.; Skaltsounis, L.; Sobarzo-Sánchez, E.; Bredt, D. S.; Stuppner, H.; Sureda, A.; Tzvetkov, N. T.; Vacca, R. A.; Aggarwal, B. B.; Battino, M.; Giampieri, F.; Wink, M.; Wolfender, J. L.; Xiao, J.; Yeung, A. W. K.; Lizard, G.; Popp, M. A.; Heinrich, M.; Berindan-Neagoe, I.; Stadler, M.; Daglia, M.; Verpoorte, R.; Supuran, C. T.; *Nat. Rev. Drug Discovery* **2021**, *20*, 200. [Crossref]
4. Vizzotto, M.; Krolow, A. C.; Weber, G. E. B.; *Documento 316: Metabólitos Secundários Encontrados em Plantas e sua Importância*; Embrapa Clima Temperado: Pelotas, 2010. [Link] accessed in May 2024
5. Zaynab, M.; Fatima, M.; Abbas, S.; Sharif, Y.; Umair, M.; Zafar, M. H.; Bahadar, K.; *Microb. Pathog.* **2018**, *124*, 198. [Crossref]
6. Weng, J.-K.; Philippe, R. N.; Noel, J. P.; *Science* **2012**, *336*, 1667. [Crossref]
7. Salam, A. M.; Quave, C. L.; *Curr. Opin. Microbiol.* **2018**, *45*, 189. [Crossref]

8. Hüther, C. M.; Ferreira, P. G.; Forezi, L. S. M.; da Silva, F. C.; Ferreira, V. F.; *Rev. Virtual Quim.* **2023**, *15*, 12. [Crossref]
9. Wright, G. D.; *Microb. Biotechnol.* **2019**, *12*, 55. [Crossref]
10. Paloque, L.; Triastuti, A.; Bourdy, G.; Haddad, M. In *Natural Antimicrobial Agents*, vol. 19; Mérillon, J.-M.; Riviere, C., eds.; Springer: Cham, 2018, p. 215. [Crossref]
11. Sitarek, P.; Merecz-Sadowska, A.; Kowalczyk, T.; Wieczfinska, J.; Zajdel, R.; Śliwiński, T.; *Int. J. Mol. Sci.* **2020**, *21*, 5105. [Crossref]
12. de Oliveira, J. R.; de Castro, V. C.; Vilela, P. G. F.; Camargo, S. E. A.; Carvalho, C. A. T.; Jorge, A. O. C.; de Oliveira, L. D.; *BMC Complementary Altern. Med.* **2013**, *13*, 208. [Crossref]
13. Thomford, N. E.; Senthebane, D. A.; Rowe, A.; Munro, D.; Seele, P.; Maroyi, A.; Dzobo, K.; *Int. J. Mol. Sci.* **2018**, *19*, 1578. [Crossref]
14. Marchese, A.; Barbieri, R.; Coppo, E.; Orhan, I. E.; Daglia, M.; Nabavi, S. F.; Izadi, M.; Abdollahi, M.; Nabavi, S. M.; Ajami, M.; *Crit. Rev. Microbiol.* **2017**, *43*, 668. [Crossref]
15. Ribeiro, T. S.; Freire-de-Lima, L.; Previato, J. O.; Mendonça-Previato, L.; Heise, N.; de Lima, M. E. F.; *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3555. [Crossref]
16. Ferreira, C.; Soares, D. C.; Barreto-Junior, C. B.; Nascimento, M. T.; Freire-De-Lima, L.; Delorenzi, J. C.; Lima, M. E. F.; Atella, G. C.; Folly, E.; Carvalho, T. M. U.; Saraiva, E. M.; Pinto-da-Silva, L. H.; *Phytochemistry* **2011**, *72*, 2155. [Crossref]
17. Kapil, A.; *Planta Med.* **1993**, *59*, 474. [Crossref]
18. 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]
19. Villamizar, L. H.; Cardoso, M. G.; de Andrade, J.; Teixeira, M. L.; Soares, M. J.; *Mem. Inst. Oswaldo Cruz* **2017**, *112*, 131. [Crossref]
20. Saleheen, D.; Ali, S. A.; Ashfaq, K.; Siddiqui, A. A.; Agha, A.; Yasinzai, M. M.; *Biol. Pharm. Bull.* **2002**, *25*, 386. [Crossref]
21. Cui, L.; Miao, J.; Cui, L.; *Antimicrob. Agents Chemother.* **2007**, *51*, 488. [Crossref]
22. Sueth-Santiago, V.; Moraes, J. B. B.; Alves, E. S. S.; Vannier-Santos, M. A.; Freire-de-Lima, C. G.; Castro, R. N.; Mendes-Silva, G. P.; Del Cistia, C. N.; Magalhães, L. G.; Andricopulo, A. D.; Sant'Anna, C. M. R.; Decoté-Ricardo, D.; de Lima, M. E. F.; *PLoS One* **2016**, *11*, e0162926. [Crossref]
23. Mi-Ichi, F.; Miyadera, H.; Kobayashi, T.; Takamiya, S.; Waki, S.; Iwata, S.; Shibata, S.; Kita, K.; *Ann. N. Y. Acad. Sci.* **2005**, *1056*, 46. [Crossref]
24. Azebaze, A. G. B.; Dongmo, A. B.; Meyer, M.; Ouahouo, B. M. W.; Valentin, A.; Nguemfo, E. L.; Nkengfack, A. E.; Vierling, W.; *Ann. Trop. Med. Parasitol.* **2007**, *101*, 23. [Crossref]
25. Bastos, J. K.; Albuquerque, S.; Silva, M. L. A.; *Planta Med.* **1999**, *65*, 541. [Crossref]
26. Yardley, V.; Snowdon, D.; Croft, S.; Hazra, B.; *Phytother. Res.* **1996**, *10*, 559. [Crossref]
27. Pollack, Y.; Segal, R.; Golenser, J.; *Parasitol. Res.* **1990**, *76*, 570. [Crossref]
28. Behbehani, J. M.; Irshad, M.; Shreaz, S.; Karched, M.; *Int. J. Mol. Sci.* **2023**, *24*, 1046. [Crossref]
29. Ankri, S.; Mirelman, D.; *Microbes Infect.* **1999**, *1*, 125. [Crossref]
30. Ali, H. S.; Mishra, S. In *Drugs from Nature: Targets, Assay Systems and Leads*; Haridas, M.; Abdulhameed, S.; Francis, D.; Kumar, S. S., eds.; Springer Nature: Singapore, 2024, p. 367. [Crossref]
31. Newman, D. J.; Cragg, G. M.; *J. Nat. Prod.* **2020**, *83*, 770. [Crossref]
32. Cragg, G. M.; Newman, D. J.; Snader, K. M.; *J. Nat. Prod.* **1997**, *60*, 52. [Crossref]
33. Tu, Y.; *Angew. Chem., Int. Ed.* **2016**, *55*, 10210. [Crossref]
34. Cambié, D.; Dobbelaar, J.; Riente, P.; Vanderspikken, J.; Shen, C.; Seeberger, P. H.; Gilmore, K.; Debije, M. G.; Noël, T.; *Angew. Chem., Int. Ed.* **2019**, *58*, 14374. [Crossref]
35. Mathew, N.; Kalyanasundaram, M.; *Expert Opin. Ther. Pat.* **2007**, *17*, 767. [Crossref]
36. WHO, Filariose Linfática, https://mectizan.org/wp-content/uploads/2018/06/Learners-Guide-Portuguese.pdf, accessed in May 2024.
37. Hertweck, C.; *Angew. Chem., Int. Ed.* **2015**, *54*, 14622. [Crossref]
38. Gribble, F. M.; Davis, T. M. E.; Higham, C. E.; Clark, A.; Ashcroft, F. M.; *Br. J.Pharmacol.* **2000**, *131*, 756. [Crossref]
39. The Nobel Prize in Physiology or Medicine 2015, https://www.nobelprize.org/prizes/medicine/2015/summary/, accessed in May 2024.
40. Boulos, M.; Dutra, A. P.; DiSanti, S. M.; Shiroma, M.; Amato Neto, V.; *Rev. Soc. Bras. Med. Trop.* **1997**, *30*, 211. [Crossref]
41. Feher, M.; Schmidt, J. M.; *J. Chem. Inf. Comput. Sci.* **2003**, *43*, 218. [Crossref]
42. Li, J. W.-H.; Vederas, J. C.; *Science* **2009**, *325*, 161. [Crossref]
43. Saklani, A.; Kutty, S. K.; *Drug Discovery Today* **2008**, *13*, 161. [Crossref]
44. Srinivasan, K.; *Crit. Rev. Food Sci. Nutr.* **2016**, *56*, 1488. [Crossref]
45. Takooree, H.; Aumeeruddy, M. Z.; Rengasamy, K. R. R.; Venugopala, K. N.; Jeewon, R.; Zengin, G.; Mahomoodally, M. F.; *Crit. Rev. Food Sci. Nutr.* **2019**, 59, S210. [Crossref]
46. Praditya, D.; Kirchhoff, L.; Brüning, J.; Rachmawati, H.; Steinmann, J.; Steinmann, E.; *Front. Microbiol.* **2019**, *10*, 912. [Crossref]
47. Sueth-Santiago, V.; Mendes-Silva, G. P.; Decoté-Ricardo, D.; de Lima, M. E. F.; *Quim. Nova* **2015**, *38*, 538. [Crossref]
48. Haro-González, J. N.; Castillo-Herrera, G. A.; Martínez-Velázquez, M.; Espinosa-Andrews, H.; *Molecules* **2021**, *26*, 6387. [Crossref]

49. Salehi, B.; Zucca, P.; Orhan, I. E.; Azzini, E.; Adetunji, C. O.; Mohammed, S. A.; Banerjee, S. K.; Sharopov, F.; Rigano, D.; Sharifi-Rad, J.; Armstrong, L.; Martorell, M.; Sureda, A.; Martins, N.; Selamoğlu, Z.; Ahmad, Z.; *Trends Food Sci. Technol.* **2019**, *86*, 502. [Crossref]
50. Billing, J.; Sherman, P. W.; *Q. Rev. Biol.* **1998**, *73*, 3. [Crossref]
51. Sherman, P. W.; Hash, G. A.; *Evol. Hum. Behav.* **2001**, *22*, 147. [Crossref]
52. Joshi, D. R.; Shrestha, A. C.; Adhikari, N.; *Int. J. Pharm. Sci. Res.* **2018**, *9*, 4089. [Crossref]
53. Damanhouri, Z. A.; Ahmad, A.; *Med. Aromat. Plants* **2014**, *3*, 1000161. [Link] accessed in May 2024
54. Food and Agricultural Organization of the United Nations, *Codex Alimentarius*, https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B326-2017%252FCXS_326e.pdf, accessed in March 2024.
55. Zeiner, M.; Fiedler, H.; Cindrić, I. J.; Nemet, I.; Toma, D.; Habinovec, I.; *Foods* **2023**, *12*, 3132. [Crossref]
56. Parmar, V. S.; Jain, S. C.; Bisht, K. S.; Jain, R.; Taneja, P.; Jha, A.; Tyagi, O. D.; Prasad, A. K.; Wengel, J.; Olsen, C. E.; Boll, P. M.; *Phytochemistry* **1997**, *46*, 597. [Crossref]
57. Jeon, H.-J.; Kim, K.; Kim, Y.-D.; Lee, S.-E.; *Appl. Biol. Chem.* **2019**, *62*, 63. [Crossref]
58. Wei, K.; Li, W.; Koike, K.; Chen, Y.; Nikaido, T.; *J. Org. Chem.* **2005**, *70*, 1164. [Crossref]
59. Ikan, R.; *Natural Products, A Laboratory Guide*, 2nd ed.; Academic Press: San Diego, 1991.
60. Ternes, W.; Krause, E. L.; *Anal. Bioanal. Chem.* **2002**, *374*, 155. [Crossref]
61. FAO, Black Pepper Production by Country 2024, https://worldpopulationreview.com/country-rankings/black-pepper-production-by-country, accessed in May 2024.
62. França, A. A. C.; Pereira, A. D.; Fernandes, K. A.; Costa, A. K. B.; Ramos, M. A. B.; Martins, F. A.; Batista, N. C.; Matos, J. M. E.; Sá, J. L. S.; *Rev. Virtual Quim.* **2021**, *13*, 310. [Crossref]
63. Carnevalli, D. B.; de Araújo, A. P. S.; *UNICIÊNCIAS* **2013**, *17*, 41. [Link] accessed in May 2024
64. Han, H.-K.; *Expert Opin. Drug Metab. Toxicol.* **2011**, *7*, 721. [Crossref]
65. Mhaske, D. B.; Sreedharan, S.; Mahadik, K. R.; *Pharm. Anal. Acta* **2018**, *09*, 1000591. [Crossref]
66. Deferme, S.; Augustijns, P.; *J. Pharm. Pharmacol.* **2010**, *55*, 153. [Crossref]
67. Cui, T.; Wang, Q.; Tian, X.; Zhang, K.; Peng, Y.; Zheng, J.; *Drug Metab. Dispos.* **2020**, *48*, 123. [Crossref]
68. Singh, J.; Dubey, R. K.; Atal, C. K.; *J. Pharmacol. Exp. Ther.* **1986**, *236*, 488. [Link] accessed in May 2024
69. Singh, J.; Reen, R. K.; Wiebel, F. J.; *Cancer Lett.* **1994**, *86*, 195. [Crossref]
70. Reen, R. K.; Jamwal, D. S.; Taneja, S. C.; Koul, J. L.; Dubey, R. K.; Wiebel, F. J.; Singh, J.; *Biochem. Pharmacol.* **1993**, *46*, 229. [Crossref]
71. Jayakanthan, M.; Chandrasekar, S.; Muthukumaran, J.; Mathur, P. P.; *J. Mol. Graph. Model.* **2010**, *28*, 455. [Crossref]
72. de Oliveira, R. G.; Alencar-Filho, E. B.; Vasconcellos, M. L. A. A.; *Quim. Nova* **2014**, *37*, 69. [Crossref]
73. Ferreira, W. S.; Franklim, T. N.; Lopes, N. D.; de Lima, M. E. F.; *Rev. Virtual Quim.* **2012**, *4*, 208. [Crossref]
74. Chagas, C.; *Mem. Inst. Oswaldo Cruz* **1909**, *1*, 159. [Crossref]
75. Filardy, A. A.; Guimarães-Pinto, K.; Nunes, M. P.; Zukeram, K.; Fliess, L.; Pereira, L.; Nascimento, D. O.; Conde, L.; Morrot, A.; *Front. Immunol.* **2018**, *9*, 1493. [Crossref]
76. Freire-de-Lima, L.; Ribeiro, T. S.; Rocha, G. M.; Brandão, B. A.; Romeiro, A.; Mendonça-Previato, L.; Previato, J. O.; de Lima, M. E. F.; de Carvalho, T. M. U.; Heise, N.; *Parasitol. Res.* **2008**, *102*, 1059. [Crossref]
77. da Silva, E.; Canto-Cavalheiro, M. M.; Braz, V. R.; Cysne-Finkelstein, L.; Leon, L. L.; Echevarria, A.; *Eur. J. Med. Chem.* **2002**, *37*, 979. [Crossref]
78. Ferreira, W. S.; Freire-de-Lima, L.; Saraiva, V. B.; Alisson-Silva, F.; Mendonça-Previato, L.; Previato, J. O.; Echevarria, A.; de Lima, M. E. F.; *Bioorg. Med. Chem.* **2008**, *16*, 2984. [Crossref]
79. Urbina, J. A.; *Mem. Inst. Oswaldo Cruz* **2009**, *104*, 311. [Crossref]
80. Franklim, T. N.; Freire-de-Lima, L.; Chaves, O. A.; LaRocque-de-Freitas, I. F.; da Silva-Trindade, J. D.; Netto-Ferreira, J. C.; Freire-de-Lima, C. G.; Decoté-Ricardo, D.; Previato, J. O.; Mendonça-Previato, L.; de Lima, M. E. F.; *J. Braz. Chem. Soc.* **2019**, *30*, 1378. [Crossref]
81. Franklim, T. N.; Freire-de-Lima, L.; Diniz, J. N. S.; Previato, J. O.; Castro, R. N.; Mendonça-Previato, L.; de Lima, M. E. F.; *Molecules* **2013**, *18*, 6366. [Crossref]
82. Chouhan, G.; Islamuddin, M.; Want, M. Y.; Ozbak, H. A.; Hemeg, H. A.; Sahal, D.; Afrin, F.; *Front. Microbiol.* **2015**, *6*, 1368. [Crossref]
83. Torres-Santos, E. C.; Moreira, D. L.; Kaplan, M. A. C.; Meirelles, M. N.; Rossi-Bergmann, B.; *Antimicrob. Agents Chemother.* **1999**, *43*, 1234. [Crossref]
84. Vendrametto, M. C.; dos Santos, A. O.; Nakamura, C. V.; Dias Filho, B. P.; Cortez, D. A. G.; Ueda-Nakamura, T.; *Parasitol. Int.* **2010**, *59*, 154. [Crossref]
85. Matsuda, D.; Ohte, S.; Ohshiro, T.; Jiang, W.; Rudel, L.; Hong, B.; Si, S.; Tomoda, H.; *Biol. Pharm. Bull.* **2008**, *31*, 1063. [Crossref]
86. Vieira-Araújo, F. M.; Rondon, F. C. M.; Vieira, Í. G. P.; Mendes, F. N. P.; de Freitas, J. C. C.; de Morais, S. M.; *Exp. Parasitol.* **2018**, *188*, 79. [Crossref]
87. Frézard, F.; Demicheli, C.; Kato, K. C.; Reis, P. G.; Lizarazo-Jaimes, E. H.; *Rev. Inorg. Chem.* **2013**, *33*, 1. [Crossref]
88. Sato, S.; *J. Physiol. Anthropol.* **2021**, *40*, 1. [Crossref]

89. de Abreu, F. V. S.; Santos, E.; Mello, A. R. L.; Gomes, L. R.; de Alvarenga, D. A. M.; Gomes, M. Q.; Vargas, W. P.; Bianco-Júnior, C.; de Pina-Costa, A.; Teixeira, D. S.; Romano, A. P. M.; Manso, P. P. A.; Pelajo-Machado, M.; Brasil, P.; Daniel-Ribeiro, C. T.; de Brito, C. F. A.; Ferreira-da-Cruz, M. F.; Lourenço-de-Oliveira, R.; *PLoS Negl. Trop. Dis.* **2019**, *13*, e0007906. [Crossref]
90. Thu, A. M.; Phyo, A. P.; Landier, J.; Parker, D. M.; Nosten, F. H.; *FEBS J.* **2017**, *284*, 2569. [Crossref]
91. Thiengsusuk, A.; Chaijaroenkul, W.; Na-Bangchang, K.; *Parasitol. Res.* **2013**, *112*, 1475. [Crossref]
92. Kamaraj, C.; Kaushik, N. K.; Rahuman, A. A.; Mohanakrishnan, D.; Bagavan, A.; Elango, G.; Zahir, A. A.; Santhoshkumar, T.; Marimuthu, S.; Jayaseelan, C.; Kirthi, A. V.; Rajakumar, G.; Velayutham, K.; Sahal, D.; *J. Ethnopharmacol.* **2012**, *141*, 796. [Crossref]
93. Alkandahri, M. Y.; Yuniarsih, N.; Berbudi, A.; Subarnas, A.; *Pharmacogn. J.* **2022**, *14*, 245. [Crossref]
94. Thiengsusuk, A.; Muhamad, P.; Chaijaroenkul, W.; Na-Bangchang, K.; *J. Trop. Med.* **2018**, *2018*, 9486905. [Crossref]
95. Khairani, S.; Fauziah, N.; Wiraswati, H. L.; Panigoro, R.; Setyowati, E. Y.; Berbudi, A.; *J. Trop. Med.* **2022**, *2022*, 5721449. [Crossref]
96. Knopp, S.; Steinmann, P.; Keiser, J.; Utzinger, J.; *Infect. Dis. Clin. North Am.* **2012**, *26*, 341. [Crossref]
97. Knopp, S.; Steinmann, P.; Hatz, C.; Keiser, J.; Utzinger, J.; *Infect. Dis. Clin. North Am.* **2012**, *26*, 359. [Crossref]
98. Molyneux, D. H.; *Adv. Parasitol.* **2006**, *61*, 1. [Crossref]
99. Montresor, A.; Gabrielli, A. F.; Chitsulo, L.; Ichimori, K.; Mariotti, S.; Engels, D.; Savioli, L.; *Expert Rev. Anti-Infect. Ther.* **2012**, *10*, 237. [Crossref]
100. Epidemiology & Risk Factors; https://www.cdc.gov/parasites/lymphaticfilariasis/epi.html, accessed in May 2024.
101. Tiner, J. D.; *Exp. Parasitol.* **1958**, *7*, 292. [Crossref]
102. Joardar, N.; Shit, P.; Halder, S.; Debnath, U.; Saha, S.; Misra, A. K.; Jana, K.; Babu, S. P. S.; *Free Radical Biol. Med.* **2021**, *169*, 343. [Crossref]
103. Azeez, S.; Babu, R. O.; Aykkal, R.; Narayanan, R.; *J. Mol. Model.* **2012**, *18*, 151.[Crossref]
104. Paul, A.; Adnan, M.; Majumder, M.; Kar, N.; Meem, M.; Rahman, M. S.; Rauniyar, A. K.; Rahman, N.; Chy, M. N. U.; Kabir, M. S. H.; *Clin. Phytosci.* **2018**, *4*, 17. [Crossref]
105. da Silva, G. D.; de Lima, H. G.; de Sousa, N. B.; Genipapeiro, I. L. J.; Uzêda, R. S.; Branco, A.; Costa, S. L.; Batatinha, M. J. M.; Botura, M. B.; *Vet. Parasitol.* **2021**, *296*, 109505. [Crossref]
106. Rajasekharan, S. K.; Raorane, C. J.; Lee, J.; *J. Asia-Pac. Entomol.* **2020**, *23*, 863. [Crossref]

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