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PIPLARTINE AND PIPERINE: A REVIEW OF THEIR BIOLOGICAL ACTIVITIES

PIPLARTINA E PIPERINA: UMA REVISÃO DE SUAS ATIVIDADES BIOLÓGICAS

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RESUMO

Piplartina e piperina amidas encontradas em espécies pertencentes à família Piperaceae. As plantas desta família são amplamente utilizadas em partes da região amazônica para o tratamento de algumas doenças, na culinária e como plantas ornamentais. Nesse contexto, esta revisão bibliográfica das amidas acima mencionadas teve como objetivo fornecer uma coleção de dados publicados para orientar novos estudos sobre as atividades biológicas dessas substâncias (de origem natural ou sintética). A busca bibliográfica foi realizada no portal periódico da CAPES, utilizando as palavras-chave "piplartina", "piperina" e "atividade". As referências utilizadas neste estudo foram analisadas selecionando apenas artigos revisados por pares publicados entre dezembro de 2013 e dezembro de 2018 (piplartine) e 2017-2018 (piperine). A revisão também resume as atividades antileishmanial e antiplasmodial investigadas com as substâncias (1993-2018). Na revisão, não apontamos dados como curvas dose-resposta, controles negativos e positivos. Nesse sentido, a revisão destaca as atividades biológicas, os modelos de teste e os resultados obtidos com essas substâncias, com ênfase nas atividades mais investigadas. A partir desta pesquisa, conclui-se que a piplartina e a piperina foram extensivamente investigadas. As atividades biológicas descritas aqui podem orientar futuras pesquisas que buscarão relacionar o potencial dessas amidas e seus respectivos análogos sintéticos com outras atividades biológicas. De fato, compostos que são conhecidos há muito tempo e não atingiram o status de medicina baseada em evidências requer análises adicionais.

Palavras-chave: *Piper*; alcaloides; amidas; química medicinal; parasitologia; atividade biológica.

ABSTRACT

The amides piplartine and piperine are found in species belonging to the Piperaceae family. The plants of this family are widely used in parts of the Amazon region for the treatment of some diseases, in cooking and as ornamental plants. In this context, this bibliographic review of the above-mentioned amides aimed to provide a collection of published data to guide new studies on the biological activities of these substances (of natural or synthetic origin). The bibliographic search was performed in the CAPES periodic portal, using the keywords "piplartine", "piperine" and "activity". The references used in this study were analyzed by selecting only peer-reviewed articles published between December 2013 and December 2018 (piplartine) and 2017-2018 (piperine). The review also summarizes the antileishmanial and antiplasmodial activities investigated with the substances (1993-2018). In the review, we did not point to data such as dose response curves, negative and positive controls. In this sense, the review highlights the biological activities, the test models and the results obtained with these substances, with emphasis on the most investigated activities. From this research, we conclude that piplartine and piperine have been extensively investigated. The biological activities described here may guide future research that will seek to relate the potential of these amides and their respective synthetic analogs to other biological activities. Indeed, compounds that have long been known and have not reached the status of evidence-based medicine require further analysis.

Keywords: *Piper*; alkaloids; amides; chemistry medicinal; parasitology; biological activity.

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1. INTRODUCTION

Piplartine (piperloguminine/PL) and piperine are amides described in some species of the genus *Piper* L. (Piperaceae). Piplartine was first isolated in 1962 from the stems of the species *Piper longum* L. (Piperaceae) [1]; its synthesis was first described by Boll *et al.* [2]. Piperine was first isolated in 1819 by Hans Christian Orsted in the fruits of *Piper nigrum* L. (Piperaceae) [3].

Amides are secondary metabolites belonging to the alkaloid class, a relatively large group of biomolecules present in nature. There are records of these compounds occurring mainly in plants, but they may also be present to a lesser extent in marine organisms, mosses, fungi, and bacteria. There are also non-natural alkaloids (structurally modified or synthetic analogs) [4,5].

Depending on the structure, alkaloids may have high or low molecular weights, and because they are derived from amino acids, they have a nitrogen atom in their chain, which generally provides them basicity and facilitates their process of isolation and purification. It should be noted that the basic characteristic may vary depending on the functional groups that are present in their molecular structure [6].

The main precursors involved in the synthesis of alkaloids are ornithine, lysine, nicotinic acid, tyrosine, tryptophan, anthranilic acid, and histidine. The building blocks of the acetate, shikimate or methylerythritol phosphate pathways can also be frequently incorporated into the alkaloid structures [4,5].

Considering the biological and ecological activities, chemical structures and biosynthetic pathways, alkaloids can be classified into true alkaloids, protoalkaloids (derived from amino acids) and pseudoalkaloids, which are not derived from amino acids but from terpenes and steroids. Depending on the complexity of the structures, these compounds can be subdivided into pyrrolidine, piperidine, quinoline, isoquinoline and indole alkaloids. Alkaloids also occur as acid salts, in combination with sugars; there are others that appear as esters; they may be quaternary, salts or tertiary amine oxides [6,7].

1.1 Piperaceae

The largest record on the study of the Piperaceae in Brazil is “The Piperaceae of Brazil” by Yuncker [8-10]. The family belongs to the order Piperales with approximately 4000 species, divided into 13 genera. Of these genera, four occur in Brazil (*Piper* L. 290 species), *Peperomia*

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Ruiz & Pav. (166 species), *Manekia* Trel. (one species) and *Ottonia* Spreng. (one species). It is considered one of the largest families among the basal Angiosperms, and the genera *Piper* and *Peperomia* are among the 30 most diverse genera among the Brazilian angiosperms [11]. It is distributed throughout tropical and subtropical regions of the globe presenting a great diversity in Malaysia and South and Central America [12,13].

1.2 *Piper* L.

The genus *Piper* has approximately 2000 species distributed in tropical and subtropical regions of the world [14]. They have shrub, arboreal, scandent or herbaceous habits, can reach from 2.2 to 5.5 m in height, usually growing in the interior or in the margin of forest formations, with alternating, generally asymmetric, penninerved or palminerved leaves and are common in the underbrush of tropical rain forests. Its flowers are small, perianthed, joined in spikes or less frequently in racemes, and are solitary or fasciculate. The fruit is a small drupe, with tiny seeds. They can produce an average of 40 infructescences, with an average of 472 seeds for each infructescence [14,15].

Plants of the genus *Piper* are popularly known as "pimenteiras" or "falso-jaborandis", and are of economic and medicinal importance. Some species are part of the world market, such as black pepper (*Piper nigrum* L.) and others are used in India. A mixture of *P. nigrum*, *Piper longum* L. and *Zengiber officinale* Roscoe (ginger) constitutes the so-called "trikatu", a formulation used for the treatment of various diseases by traditional populations in India; other species such as *Piper betle* L. and *Piper methysticum* G. Forst. are known masticatories [16-18].

With a great diversity of metabolites, *Piper* species may present a variety of compounds such as lignans, neolignans, terpenoids, propenylphenols, chalcones, flavones, benzopyrans and especially amides, the most outstanding being the pyrrolidine ones, with open chain metilenodioxiphenyl groups [18,19].

These natural amides can serve as prototypes for the synthesis of new molecules whose chemical properties or biological activities can be improved. This is a growing area that has directed many investigations into the search for new chemical entities that might become a new therapeutic candidate for various applications.

In this context, amides represent a class with this potential owing to a wide range of biological activities, such as antimicrobial, antifungal, antitumor, cytotoxic, antiparasitic, antioxidant, antimutagenic, antigenotoxic and hallucinogenic [20-23]. Considering the above-

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mentioned information, the present literature review aimed to carry out a survey of the investigations on the biological activities of the alkamides piplartine and piperine.

1.3 Biosynthesis of Piplartine

There are few reports in the literature about the biosynthetic pathway of piplartine. Initially, there was a dispute between the structures called piplartine and piperlongumine. Through X-ray crystallographic analysis it was shown that the two compounds were actually the same. Eventually, the substance (piplartine/piperlongumine) was synthesized to confirm its structure [24].

Citing other authors in his work, [24]; [25], discuss the biosynthesis of piplartine isolated from *Piper longum* species. The authors report that experiments with L-[U-^{14}C] phenylalanine, L-[U-^{14}C] lysine, [2-^{14}C] sodium acetate and DL-[2-^{14}C] tyrosine, demonstrate the incorporation of L-[U-^{14}C] phenylalanine and L-[U-^{14}C] lysine in the alkamides. Thus, the piperidine ring is derived from lysine and the phenyl propanoate portion is derived from phenylalanine (Fig. 1).

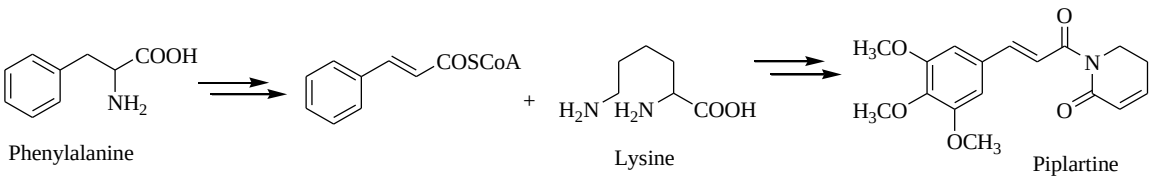

Fig. 1 Biosynthetic pathway of *Piper* alkamides (adapted [25] p. 88). Feeding experiments with L-[U-14C] phenylalanine, L-[U-^{14}C] lysine, [2-^{14}C] sodium acetate and DL-[2-^{14}C] tyrosine, demonstrate the incorporation of L-[U-^{14}C] phenylalanine and L-[U-^{14}C] lysine in the alkamides.

1.4 Biosynthesis of Piperine

[4] suggests that the biosynthesis of piperine derives from the cinnamoyl-CoA precursor. It undergoes an extension of the chain using malonyl-CoA, then the reduction of the dehydration reactions as in the fatty acid biosynthesis giving rise to the piperic acid (piperoyl-CoA). The piperidine ring (tertiary amide structure) is Δ^1-piperideine reduction product. The piperoyl-CoA moiety is incorporated by piperidine itself forming the alkamide piperine (Fig. 2).

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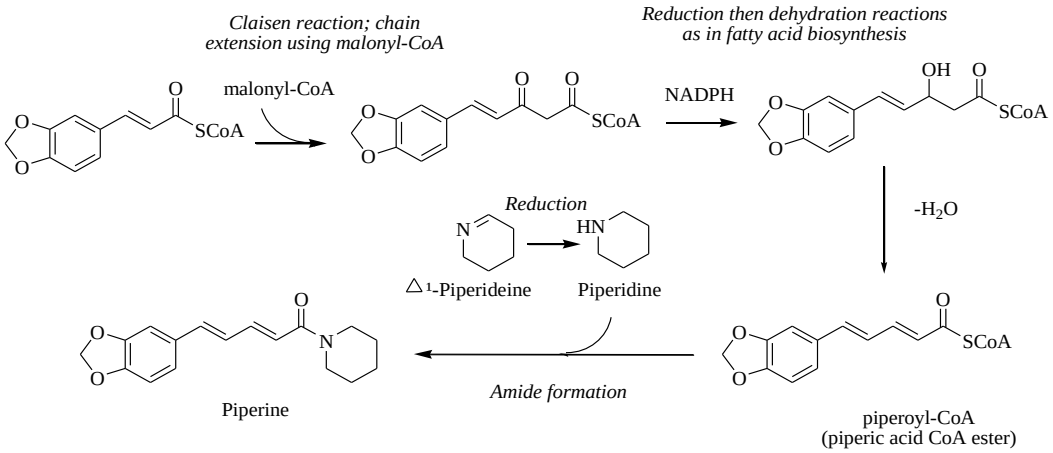

Fig. 2 Biosynthetic pathway of Piperine. [4] p. 328. The piperidine ring (tertiary amide structure) is Δ^1-piperideine reduction product. The piperoyl-CoA moiety is incorporated by piperidine itself forming the alkamide piperine.

[25] also suggest a mechanism for the biosynthesis of piperine. For the authors, phenylalanine promotes a chain extension using malonyl-CoA transforming into piperoyl-CoA (piperic acid CoA ester). N-piperoyltransferase catalyzes the formation of the amide by attaching piperoyl-CoA to a piperidine, pyrrolidine or isobutylamine to provide the series of *Piper* alkaloids (Fig. 3).

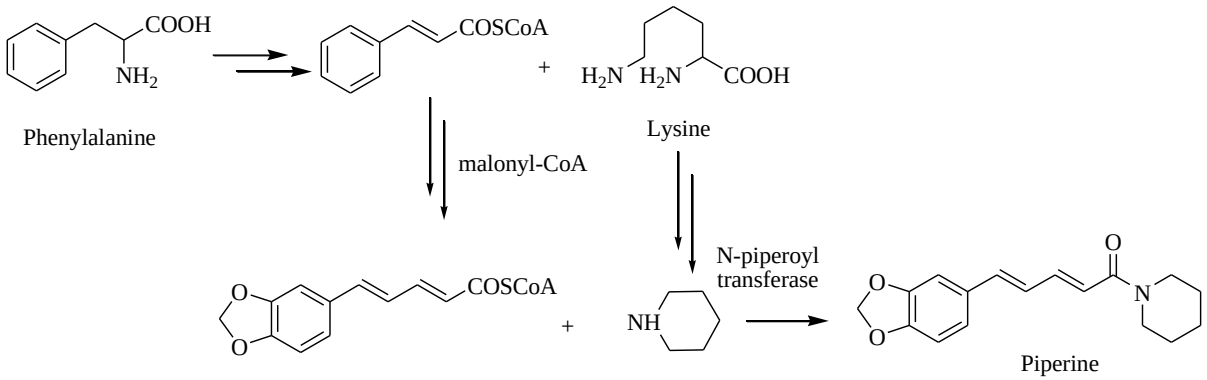

Fig. 3 Biosynthetic pathway of *Piper* alkamides (adapted [25] p. 88). Phenylalanine promotes a chain extension using malonyl-CoA transforming into piperoyl-CoA (piperic acid CoA ester). N-piperoyltransferase catalyzes the formation of the amide by attaching piperoyl-CoA to a piperidine, pyrrolidine or isobutylamine to provide the series of *Piper* alkaloids.

2. METHODOLOGY

The literature review obeyed the following inclusion criteria: peer-reviewed articles from 2013 to 2018 for piplartine; for Piperine, published researches in 2018 were included, as

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well as other studies of 2016 and 2017 that were not mentioned in previous studies, since the biological activity was documented in the studies by [26]; [27]; [28] and [29]. For the antiparasitic activities (*Leishmania* ssp. and *Plasmodium* ssp.), articles from 1993 to 2018 were selected since in the initial surveys it was identified that in the period prior to 2013 there is a scarcity of publications describing these activities.

The consultation was carried out in the CAPES periodic portal using the following terms: 'piplartine', 'piperine', 'biological activity', 'leishmanicidal activity', 'antiplasmodial activity' and 'antiparasitic activity'. The selection of the articles was done by selecting and reading the documents in three stages: the titles, the abstracts and finally the complete manuscripts; 73 articles were selected.

3. RESULTS AND DISCUSSION

3.1 Biological activities of Piplartine

Sixty-three studies were found which were related to the present survey, divided into 21 biological activities. The anticancer/cytotoxic activity has been extensively investigated, followed by leishmanicidal, anticoagulant/antiplatelet, anthelmintic (Tables 1 and 2) and other less investigated activities (Fig. 4).

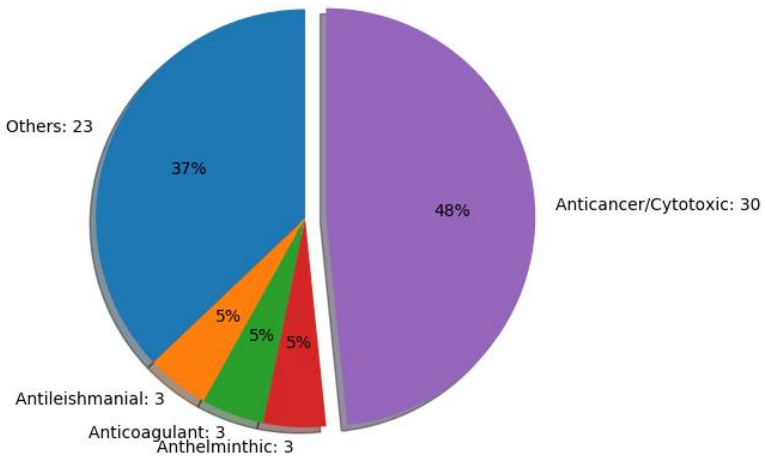

Fig. 4 Biological activities of piplartine. We found 63 studies, divided into twenty-one biological activities. Anticancer/cytotoxic activity has been extensively investigated, followed by leishmanicidal, anticoagulant/antiplatelet, anthelmintic and other less investigated activities.

We highlighted the most investigated activities in the period from 2013 to 2018, with most of these studies being carried out with pure synthetic substances and/or obtained

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commercially. Piplartine has a wide variety of biological activities, some of which were summarized in Table 1.

Table 1 Biological activities of piplartine.

Bioactivity	Source	Test Model	Main Findings	References
Anticancer/ cytotoxic	Commercially obtained	Determination of cytotoxicity on Burkitt lymphomas cells DG-75, Daudi, Raji and Ramos by the PMS (1-methoxy phenazine methosulfate) assay; Evaluation of cell viability by the trypan blue exclusion assay (TBE); Evaluation of programmed cell death by DNA fragmentation assays; Western Blot analysis; Gene expression. Analysis of oncogenes through RT-PCR (Real-Time Reverse Transcription-PCR) and qPCR.	Piplartine was shown to inhibit the growth of Epstein-Barr virus + B-lymphoma cell lines and Epstein-Barr virus - B-lymphoma, with IC_{50} values of 2.8 and 8.5 μM, respectively. The substance was also observed to interfere with the expression of some genes that play a crucial role in many blood cancers.	[30]
Anticancer/ cytotoxic	Not informed	Cell growth inhibition assay against MDA-MB-231 cells; Cell cycle analysis; Detection of apoptosis by Annexin-V/PI flow cytometry (FCM); Intracellular ROS (reactive oxygen species) analysis; SDS-polyacrylamide gel electrophoresis and Western blot analysis (measurement of Bcl-2 and Bax levels).	Piperlongumine was found to inhibit proliferation, arrest cells at G0/G1 phase and induce apoptosis of MDA-MB-231 cells. The authors stated that these effects seem to be associated with the increased generation of intracellular reactive oxygen species as well as the down-regulation of Bcl-2 and up-regulation of Bax.	[31]
Anticancer/ cytotoxic	Commercially obtained	AlamarBlue cytotoxicity assay on HT-29, HCT 116, and NCM460 cells; Trypan blue exclusion assay on HT-29 cells; Apoptosis assay on HT-29 cells; Determination of Phospho-ERK; levels through Western Blotting in HT-29 cells.	The study found that piplartine caused colon-cancer cell death in a dose-dependent manner. The mechanistic assays suggested that piplartine induce cell death, at least partially, through the MEK/ERK pathway.	[32]
Anticancer/ cytotoxic	Not informed	Determination of PC-3 and DU-145 prostate cancer cell growth inhibition through CellTitier Blue assay; Measurement of NF-κB Activity Using Luciferase Reporter Assay;	The study demonstrated that piplartine affects cell proliferation, adhesion, and invasiveness of prostate cancer cells.	[33]

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		Measurement of IL-6, IL-8, and MMP-9 Proteins; Measurement of ICAM-1; Expression via Immunocytometry; Analysis of Cancer Cells Invasiveness; Adhesion Assay.		
Anticancer/ cytotoxic	Not informed	Various head-and-neck cancer (HNC) cell lines were used both *in vitro* and *in vivo;* ***In vitro* studies:** All assays tested piplartine alone and in combination with cisplatin; Determination of cell viability through trypan blue exclusion, crystal violet staining, MTT and clonogenic assays; Measurement of total cellular glutathione and glutathione disulfide; Cell cycle and cell death assays through flow cytometry; Measurement of ROS production by flow cytometry; Measurement of p53, p21 and apoptotic proteins through Western Blot analyzes; ***In vivo* studies:** Six-week-old athymic male nude mice were used; *In situ* apoptosis assays in mice on AMC-HN9 tumor cells (mice were treated intraperitoneally).	Piplartine selectively induces cell death by targeting regulators of ROS homeostasis. It was observed to trigger HNC cell death through the activation of the JNK and PARP pathways. Piplartine was also able to enhance the toxicity of cisplatin against p53-wild-type and p53-mutant HNC cells.	[34]
Anticancer/ cytotoxic	Not informed	Human epithelial ovarian cancer (EOC) lines A2780, OVCAR3, and SKOV3 and human embryonic kidney cell line HEK293T were used; Cells were treated with piplartine alone or in combination with paclitaxel or cisplatin; Determination of cytotoxicity by the MTT assay; Evaluation of cell apoptosis through flow cytometry assay; Measurement of ROS Production; Cell cycle analysis; Determination of apoptosis markers through Western Blot.	Piplartine inhibited cell growth, induced ROS-dependent apoptosis and arrested the cell cycle in human ovarian cancer cells. It was also observed to present synergistic effects when combined with DDP and TX	[35]

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Anticancer/ cytotoxic	Commercially obtained	U266 cells, human embryonic kidney A293 cells, human breast MCF-7 cells and human T-cell leukemia Jurkat cells were used; Determination of cytotoxicity by the MTT cytotoxicity assay; Determination of apoptosis induced by TNF-α through LIVE/DEAD assay; Determination of early apoptosis through Annexin V/PI assay; Invasion assay on H1299 cells; Detection of IL-6 on U266 through ELISA; Assessment of NF-κB activation through EMSA; Determination of TNF-α–induced IKK activation by Kinase assay; NF-κB–Dependent Reporter Gene Expression Assay; Western Blot analysis.	Piplartine was found to suppress NF-κB activation pathways induced by inflammatory stimuli, growth factors, carcinogens, and tumor promoters through the direct inhibition of cysteine 179 (Cys179) of IKKβ, leading to the inhibition of IκBα, the suppression of NF-κB–regulated gene products, and the enhancement of apoptosis in tumor cells.	[36]
Anticancer/ cytotoxic	Commercially obtained	Human glioblastoma cell lines LN229 and U87 MG (U87) were used; Cell migration assay; Measurement of ROS Production; Measurement of reduced glutathione; Transwell Cell Migration Assay; Determination of expression of cdc2, cdc25c, and cyclin D1 through Western Blot.	The study found that piplartine is able to suppress cancer cell migration, which is caused by its effects in the ROS-p38/JNK-NF*κ*B signaling pathway	[37]
Anticancer/ cytotoxic	Commercially obtained	***In silico***: Molecular docking simulations on PI3 K/Akt/mTOR and NF-Kb; ***In vitro***: Cell lines used: MCF-7 (ER +ve), MDA-MB-231(ER −ve), MDA-MB-453 (HER +ve) and BT-549 (ER −ve) were used; **PI3K110α/85α enzyme assay;** Determination of cytotoxicity by the MTT assay;	The study demonstrated that piplartine inhibited the growth of different human triple-negative breast cancer, which was mainly attributed to G1-phase cell cycle arrest and downregulation of the NF-kB pathway. It was also found to alter the expression of some genes, such as p-Akt, p70S6K1, 4E-BP1, cyclin D1, Bcl-2, p53, Bax, and cytochrome c in human triple-negative breast cancer cells	[38]

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		Evaluation of LDH activity by Lactate Dehydrogenase (LDH) release assay; **Colony formation assay;** **Assessment of cell morphology by contrast phase microscopy;** **Annexin V binding assay;** **Cell cycle analysis;** **Determination of apoptosis by DNA fragmentation analysis;** **Detection of intracellular ROS by DCF-DA method;** Determination of caspases activity; **Analysis of mitochondrial membrane potential (MMP);** RT-PCR studies on the expression of cyclin D1, p21, Bcl-2, Bax, p53 and human beta-actin; **Determination of protein levels and phosphorylation by Western blotting.**		
Anticancer/ cytotoxic	Commercially obtained	Human patient-derived HGG (high-grade glioma) sphere cultures were used for the assays; Determination of cytotoxicity by the MTS assay; Peroxiredoxin 4 Activity Assay.	Piplartine was found to selectively kill HGG cells, with little effects on normal brain cells. The underlying mechanisms of this effect were inactivation of PRDX4 and exacerbated endoplasmic reticulum (ER) stress in HGG cells	[39]
Anticancer/ cytotoxic	Roots of *Piper tuberculatum* Jacq	***In vitro* studies:** SF-295 (glioblastoma) and HCT-8 (colon carcinoma) human cells were used; Determination of cytotoxicity by the MTT assay; ***In vivo* studies:** Female BALB/c nude (nu/nu) mice were used; Hollow fiber assay (HFA).	Piplartine displayed cytotoxicity, with a tumor growth inhibition rate of 33.7–62.2%. No signs of toxicity were observed	[40]
Anticancer/ cytotoxic	Commercially obtained	HeLa cell lines were used; Determination of cytotoxicity by the MTT assay; Immunofluorescence microscopy assay; Nuclear transport functional assays; Immunoprecipitation assay; Streptavidin-biotin pull-down assay; Protein expression analysis through Western Blot; Analysis of covalent attachment to CRM1 peptide through mass spectrometry.	The study suggests that piplartine may act against cancer cells by modulating nuclear traffic, retaining tumor suppressor proteins	[41]

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Anticancer/ cytotoxic	Commercially obtained	HCT116 cells were used; Cells were pretreated with JNK inhibitors; Determination of cytotoxicity by the MTS assay; Trypan blue dye exclusion assay; Cell cycle analysis by flow cytometry; Analysis of cell apoptosis by flow cytometry; Protein expression analysis through Western blotting.	The study demonstrated that piplartine reduced cell viability and triggered cell death in HCT116 cells through the JNK pathway in a dose-dependent manner	[42]
Anticancer/ cytotoxic	Commercially obtained	***In vitro* studies:** Human and mice HCC, normal hepatic L-02, HepG2, Huh7 and HCC LM3 cell lines were used; Determination of cytotoxicity by MTT assay; Determination of cell death by trypan exclusion assay; Analysis of apoptosis and cell cycle through Flow cytometry assay; Scratch-wound healing assay; Transwell migration and invasion assays; Measurement of intracellular ROS and reduced glutathione (GSH); Protein analysis through Western blotting analysis and fluorescent cytoimmunostaining; Intracellular Ca^{2+} measurements; ***In vivo* studies:** Male Kunming mice with H22-derived solid hepatocarcinoma were used.	Piplartine was able to kill and suppress migration/invasion of HCC cells, preferentially through the via ROS-ER-MAPKs-CHOP axis signaling.	[43]
Anticancer/ cytotoxic	Commercially obtained	Determination of cytotoxicity against the human cancer cell lines 518A2, FADU, HT-29, MCF-7, A549 and A2780 by SRB assay.	Piplartine exhibited a strong cytotoxicity against melanoma, colon adenocarcinoma, breast adenocarcinoma alveolar basal epithelial adenocarcinoma and ovarian carcinoma cells.	[44]
Anticancer/ cytotoxic	Not informed	Quantification of induction of caspase-3 cleavage by ELISA; Determination of Inhibition of COX-2 by ELISA; Determination of cytotoxicity by the MTT assay.	Piplartine caused growth inhibition of NB4 cells, with an IC_{50} of 8.97 uM, but has little effect toward Jurkat and Mec1 cells regardless of the concentrations used. Piplartine was found to induce apoptosis through caspase-3 cleavage in Mec-1 and NB4 cells. Piplartine alone slightly decreased the levels of COX-2 in Nb4, while a combination of piplartine and ALA increased COX-2 levels in Mec-1 and Jurkat	[45]

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			cells and decreased COX-2 levels in Nb4 cells	
Anticancer/ cytotoxic	Commercially obtained	Determination of cytotoxicity against human gastric cancer cell lines SGC-7901, BGC-823, KATO III and normal Human Gastric Epithelial Cell Line (GES-1) by the MTT assay; Measurement of ROS production; Cell apoptosis analysis on SGC-7901, BGC-823 and KATO III cells; Western Blot analysis; Analysis of thioredoxin reductase 1 (TrxR) overexpression; Evaluation of TrxR activity by the DTNB assay; Evaluation of TrxR activity in tumor tissues and cells; Analysis of binding with TrxR through surface plasmon resonance; Analysis of molecular docking interaction with TrxR; Determination of caspase-3/9 activity; Evaluation of mitochondrial membrane potential ($\triangle\psi m$); Determination of antitumoral activity on mice; MDA (lipid peroxidation) assay.	The study showed that piplartine may interact with TrxR1 to induce ROS-mediated apoptosis in gastric cancer cells. Piplartine was also found to be synergistically lethal when combined with GSH inhibitors. *In vivo* analyses showed that piplartine reduced tumor cell burden and TrxR1 activity.	[46]
Anticancer/ cytotoxic	Commercially obtained	MKN45 and AGS gastric cancer cell lines were used; Cell proliferation assay by MTT method; Cell cycle assay; Cell invasion and migration assays; Protein expression analysis through Western Blot; Gene expression analysis through RT-qPCR.	The study found that piplartine presented inhibitory on gastric cancer cells. Furthermore, it was demonstrated that piplartine inhibited proliferation, cell cycle progression as well as invasion and migration of two GC cell lines. The underlying mechanisms discussed by the authors include the suppression of the JAK1,2/STAT3 signaling pathway as well as the inhibition of the expression of downstream genes.	[47]
Anticancer/ cytotoxic	Fruits of *Piper Tuberculatum* Jacq	MDA-MB-231 cells were used; Determination of cell migration inhibition by Wound-healing assay; Evaluation of the effects of piperlongume on cell migration by Boyden chamber assay;	Piplartine displayed selective toxicity toward the normal breast cell line MCF10A and inhibited migration of the breast cancer cell line MDA-MB-231, presenting an $EC_{50}= 3 \pm 1$ µM when evaluated by the Boyden chamber assay.	[48]

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		Determination of cytotoxicity by the MTS assay; Determination of EC_{50} of effects of tubulin polimeryzation by tubulin polymerization assay.		
Anticancer/ cytotoxic	Commercially obtained	Determination of cytotoxicity against the cancer cell lines (HCT116, HepG2, HSC-3, SCC-4, SCC-9, HL-60, K-562 and B16-F10) and the non-cancer cells MRC-5 and PBMC by alamarblue assay; Evaluation of piplartine against a 3D model *in vitro* model of cancer spheroids of HCT116 cells; Analysis of caspase-dependent and mitochondrial intrinsic apoptosis on HCT116 cells by flow cytometry; Determination of the effect of piplartine on ROS levels on HCT116 cells; Assessment of DNA-intercalation induction; Gene expression analysis of HCT116 cells by qPCR.	Piplartine was shown to have activity against a broad range of cancer cell lines. It was also demonstrated to induce apoptosis, induce ROS production, and alter the expression of genes related to cell cycle.	[49]
Anticancer/ cytotoxic	Commercially obtained	Cell Migration, Invasion, and Lateral Migration Assay (HCT-116 cells); MTT Assay; Measurement of ROS Generation; ***In vivo*** Antitumor Effects of 4. Adhesion Assay; Anticolon Cancer Lung metastases Test *in vivo*; Western Blotting.	Piplartine was found to inhibit the growth of HCT-116 and non-tumor CCD-841 cells, with IC_{50} values of 8.13 µM and 44.3 µM, respectively. It was also observed to inhibit HCT-116 tumors in mice by 60.6%. Mechanistically, it increased reactive oxygen species (ROS) levels and preferably induces cancer cell apoptosis by triggering different pathways.	[50]
Anticancer/ cytotoxic	Commercially obtained	Melanoma cell lines A375, A875, and B16-F10 were used; Determination of cytotoxicity by the MTT assay; Cell cycle analysis by flow cytometry.	Piplartine was found to be toxic towards A375, A875, and B16-F10 melanoma cell lines and to inhibit the growth of A375 melanoma cells in a dose-dependent manner. Mechanistically, it was found to induce G2/M arrest, trigger apoptosis, increase ROS levels, disrupt mitochondrial membrane potential and interfere with the expression of proliferation, apoptosis and ROS regulators, such as p21, p27, cleaved caspases-3, Bax, Bcl-2, and p-Jun N-terminal kinase (JNK).	[51]
Anticancer/ cytotoxic	Commercially obtained	***In vitro* studies:** Oral cancer cell lines SAS and CGHNC8 were used;	Piplartine was found to increase therapeutic sensitivity and suppress the malignant phenotype by	[52]

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		Determination of cell migration and cell invasion assays; Radio and chemosensitivity assays; Cell growth and colony formation assays; Gene expression analysis by RT- qPCR; Protein expression; ***In vivo* studies:** BALB/c mice were used; Antitumoral assay.	supressing migration, invasion, EMT and proliferation *in vitro* and *in vivo*.	
Anticancer/ cytotoxic	Commercially obtained	***In vitro* studies:** MIA PaCa-2 and PANC-1 cell lines were used; Determination of cytotoxicity by the MTT assay; Evaluation of synergistic effects of piplartine + gemcitabine; Clonogenic survival assay; Matrigel cell growth assay; Cell cycle arrest assay; Viability assay by flow cytometry; Measurement of ROS levels by the 2,7-dichlorodihydrofluorescein diacetate (DCFDA) assay; ***In vivo* studies:** Female athymic nude mice were used; Antitumoral assays; Tumor protein expression analysis by immunohistochemistry; Transcriptome analysis by RNA-Seq of tumors.	For *in vitro* assays, the study found that PDAC cells treated with PL + GEM showed reduced cell viability, clonogenic survival, and growth on Matrigel compared to control and individually-treated cells. *In vivo* investigations found that the combination piplartine (5 mg/kg) + GEM (25 mg/kg) was able to reduce tumor weight and volume. Gene expression analyses showed that this combination caused changes in the expression of p53-responsive genes that play a role in cell death, cell cycle, oxidative stress, and DNA repair pathways.	[53]
Anticancer/ cytotoxic	Commercially obtained	***In vitro* studies:** Pancreatic cancer cells (Panc1, L3.6pL), kidney (786-O), lung (A549), and breast (SKBR3) cancer cell lines were used; Determination of cytotoxicity by XTT assay; Measurement of ROS production; Measurement of apoptosis; Chromatin immunoprecipitation (ChIP) assay on Panc1 cells; Expression of miR-17, miR20a and miR-27 by RT-PCR; Proteome analysis by Western Blotting; ***In vivo* studies:**	The study demonstrated that piplartine exerts its action by inducing the ROS-dependent downregulation of cMyc and a cMyc-regulated pathway, leading to downregulation of Sp1, Sp3, Sp4 and pro-oncogenic Sp-regulated genes.	[54]

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		Female athymic nu/nu mice were used; Antitumoral assays.		
Anticancer/ cytotoxic	Commercially obtained	MCF-7, Jurkat-T lymphocites and MCF-10a cells were used; Determination of cytotoxicity by Alamar Blue assay; Cell cycle analysis by propidium iodide staining and flow cytometry on MCF-7 cells; Assessment of Tubulin Polymerization on MCF-7 cells; Sedimentation assay and western blotting (tubulin polymerization) on MCF-7 cells; Evaluation of expression levels of anti-apoptotic proteins Bcl-2 and Mcl-1 on MCF-7 cells; Evaluation of the effects os piplartine on MCF-7 cytoskeleton by Immunofluorescence and confocal microscopy on MCF-7 cells; Colchicine-binding site assay on MCF-7 cells; Cell viability assay including pre-treatment with N-acetyl cysteine or Trolox on MCF-7 cells.	Piplartine caused growth inhibition in MCF-7 and Jurkat cells, with IC_{50} values of 1.2 and 1.4 µM, respectively. The study also demonstrated that piplartine targeted tubulin by destabilizing microtubules in the above-mentioned cell lines.	[55]
Anticancer/ cytotoxic	Commercially obtained	Human lung adenocarcinoma A549 cells were used for the assays; Determination of cell proliferation by trypan blue dye exclusion assay; Determination of ROS levels; Cell cycle analysis; Analysis of cell cycle protein expression by Western Blot.	The results suggested that piplartine exerted inhibitory effects against A549 cells by inhibiting the expression of proteins related to cell cycle. During the antiproliferative process, cellular events such as production of ROS, decreased Akt phosphorylation and deactivation of NF-κB were identified	[56]
Anticancer/ cytotoxic	Commercially obtained	***In vitro* studies** Determination of cytotoxicity on A375 and B16-F0 cells by (Sulforhodamine B) SRB assay; ***In vivo* studies** Female C57-BL/6 mice were used; Antitumoral efficacy evaluation of piplartine nanoemulsions.	Nanoemulsions of piplartine did not obstruct its uptake in Caco-2 cells. In mice, the nanoemulsions showed a 1.5-fold increase in oral bioavailability when compared to piplartine alone.	[57]
Anticancer/ cytotoxic	Not informed	PC12 cells (rat adrenal pheochromocytoma cell line) and L02 cells (the normal	Piplartine exhibited the highest toxicity towards L02 and PC12	[58]

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		liver cell line) were used for *in vitro* assays; Determination of cytotoxicity by MTT Assay; Determination of cytotoxicity by Lactate Dehydrogenase (LDH) Release Assay; Measurement of Caspase-3 Activity; Determination of Intracellular ROS; Gene expression analysis by RT-PCR; Measurement of Total Glutathione; Determination of NQO1 Activity; Measurement of cellular Trx and TrxR activity.	cells. It was also able to alleviate cell damage.	
Anticancer/ cytotoxic	Leaves of *Piper cernuum* Vell.	Cytotoxic evaluation by MTT assay.	Piplartine displayed no *in vitro* cytotoxicity against cancer cell lineages B16F10-Nex2, U87, HeLa, HL-60, HCT, and A2058.	[59]
Anticancer/ cytotoxic	Fruits of *Piper tuberculatum* Jacq	Proteasome activity on human constitutive 20S proteasome cCP and human 20S immunoproteasome iCP; Determination of cytotoxicity on HeLa cells by XTT assay.	Piplartine inhibited the human immunoproteasome (IC_{50} = 15 µM), with no inhibition for the constitutive proteasome. Furthermore, piplartine displayed cytotoxicity against HeLa cells, with an EC_{50} = 2.7 µM	[60]
Antirheumatic	Commercially obtained	Fibroblast-like synoviocytes (FLS) for assays; Determination of cytotoxicity by the MTT assay; Determination of apoptosis by flow cytometry; Proliferation assay (Assessment of 5-Ethynyl-2′-deoxyuridine incorporation); Cell migration and invasion assays (chemotaxis assay); Wound closure assay; Determination of intracellular ROS; Protein analysis of p-p38/p38, p-JNK/JNK, p-ERK/ERK, p-p65/p65 and p-STAT3/STAT3 by Western Blot; Evaluation of FLS actin organization by confocal fluorescence microscopy.	Piplartine induced apoptosis in FLS at 15 and 20 µM. Additionally, it was shown to inhibit/decrease the intensity of the following cellular events: FLS proliferation at 1,5 and 10 µM; cytoskeletal organization in FLS; intracellular ROS production; TNF-α-induced secretion of MMP-1, MMP-3 and MMP-13; activation of the p38 and JNK pathways.	[61]
Gastroprotective	Fruits of *Piper tuberculatum* Jacq	Evaluation of hippocratic screening and acute oral toxicity; Effect of piplartine on acute gastric lesion induced by ethanol;	Piplartine was able to inhibit the incidence of ethanol-induced gastric ulcers by 80% at 4.5 mg/kg (p.o). Additionally, it partially restored GSH levels in ethanol-induced gastric ulcers. It also inhibited H^+, K^+ ATPase activity, with an EC_{50} of	[62]

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		Effect of piplartine on gastric mucus; Determination of glutathione levels; *In vitro* determination of H+, K+ -ATPase activity; Effect of piplartine on gastric acid secretion.	5.1 µg/mL. It significantly reduced gastric lesions.	
Neuroprotective	Fruits of *Piper longum* L.	***In vitro* studies:** HEK293 cells were used; Measurement of the NAD^+/NADH ratio; Determination of Sirt1 deacetylation in a Sirt1 enzyme-based assay; Sirt1 deacetylation assay by luciferase reporter cell-based assay; Antioxidant response element (ARE)-reporter gene assay; ***In vivo* studies:** Tg-APPswe/PS1dE9 transgenic mice were used; Novel object recognition test; Y-maze alternation test; Protein expression analysis through Western Blot.	Piplartine attenuated the cytotoxicity induced by intraneuronal Aβ1-42 expression at 0.1 to 1 µM. Its oral administration significantly reduced the occupied area of β-amyloid in the parietal cortex of APP/PS1 mice at 50 mg/kg. It also improved cognitive deficits in APP/PS1 mice, ameliorating novel object recognition and working memory impairment at 50 mg/kg.	[63]
Antiinflammatory	Commercially obtained	Determination of cell viability of human chondrocytes by ELISA; Detection of Nitric Oxide (NO), prostaglandin E2 (PGE2) and MMP-3/13 by ELISA; Gene expression analysis by Quantitative real-time - PCR (qRT-PCR); Protein production analysis by Western Blot.	Piplartine slightly decreased chondrocyte viability at 100 µg/mL. It inhibited NO and PGE-2 production in IL-1β-treated cells in a dose-dependent manner. It also inhibited iNOS and COX-2 expression, NF-kB signaling and MMP-3 and MMP-13 production in chondrocytes.	[64]
Anticoagulant/ Antiplatelet	Fruits of *Piper longum* L.	***In vitro* studies:** Platelet aggregation assay; Assessment of Thrombin or reptilase-catalyzed fibrin polymerization; Determination of Factor Xa generation on the surfaces of HUVEC's; Thrombin activity assay Factor Xa (FXa) activity assay; Determination of PAI-1 and t-PA by ELISA. ***In vivo* studies:** Male ICR mice were used; Effect of piplartine on bleeding time.	*In vitro*, piplartine was found to change coagulation properties, prolonging aPTT and PT. It was shown that it inhibited Fxa and thrombin generation. *In vivo*, piplartine significantly prolonged tail bleeding time in mice when compared to control groups.	[65]
Anticoagulant/ Antiplatelet	Not informed	Inhibition of platelet aggregation induced by Arachidonic acid;	Piplartine exhibited IC_{50} values of 5.489 and 11.121 mM for platelet	[66]

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		Inhibition of platelet aggregation induced by Adenosine diphosphate.	aggregation induced by arachidonic acid and ADP, respectively.	
Anticoagulant/ Antiplatelet	Commercially obtained	Determination of inhibitory effects of piplartine on collagen-induced platelet aggregation; Determination of the effect of PL on collagen-induced platelet activation; Effect of piplartine on collagen-induced microparticle production; Effect of piplartine on thrombus formation; Determination of phosphorylated and total JAK2, STAT3, Syk, and PLCγ2 in platelet lysates by immunoblotting (assessment of protein activation); Effect of piplartine on ROS production.	Piplartine inhibited collagen-induced platelet aggregation in a dose-dependent manner. Additionally, it was found to block the STAT3 pathway to exert its antiplatelet effect and induced the release of ROS in platelets.	[67]
Trypanocidal	Leaves of *Piper tuberculatum* Jacq	Evaluation of the effect of piplartine on the proteome of *Trypanosoma cruzi* epimastigotes using 2D-DIGE.	Piplartine caused significant changes in the expression of enzymes involved in parasite protection against oxidative stress, such as tryparedoxin peroxidase (TXNPx) and methionine sulfoxide reductase (MSR).	[68]
Enzyme inhibition/binding	Not informed	Inhibition of Hsp90-Mediated Refolding of Denatured Luciferase; Determination of cell growth inhibition on MCF-7 cells by MTS assay; Effect of piplartine on HRI's Hsp90-dependent maturation.	Piplartine inhibited Hsp90-mediated refolding of luciferase (IC_{50} = 80.7 µM), inhibited maturation of HRI, and caused depletion of Hsp90-dependent client proteins	[69]
Enzyme inhibition/binding	---	Molecular docking simulations against: -Glutathione S-transferase pi 1 (GSTP1); -Carbonyl reductase (CBR1); -Wild type p53 protein; -Mutant p53 protein; -Aldose reductase; -G-protein-coupled receptors $P2Y_1$ and $P2Y_{1;2}$.	The molecular docking simulations estimated that piplartine may interact with proteins linked to cancer, diabetes and platelet aggregration, with the most prominent predicted activity being anticancer.	[70]
Hemocytotoxicity	Commercially obtained	Effect of piplartine on phosphatidylserine exposure; Measurement of Intracellular Ca^{2+}; Determination of ROS Production; Determination of Ceramide Formation; Measurement of Hemolysis.	Piplartine promoted eryptosis in red blood cells, as observed by the presence of phosphatidylserine by flow cytometry, and cell shrinkage, with both effects being observed at 15 µM. Piplartine was shown to have little hemolytic effects at concentrations up to 30 µM. It also induced the generation of ROS and increased ceramide abundance at 30 µM	[71]

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Senolytic	Not informed	Effect of piplartine in reducing the viability of IR-induced WI-38 senescent cells (IR-SCs) compared to non-senescent WI-38 cells (NCs) by flow cytometry; Analysis of ROS production by flow cytometry; Assessment of apoptosis induction in WI-38 cells; Determination of senolytic activity of piplartine +ABT-263; Western Blot analysis of cleaved-poly(ADP-ribose) polymerase (cPARP), procaspase-3 (Procasp- 3), cleaved caspase-3 (cCasp-3), and β-actin.	Piplartine was shown to preferentially target senolytic cells, with EC_{50} values of 7.97, 6.24 and 7.09 µM for IR induced senescent cells, replication-exhausted senescent cells and Ras-induced senescent cells. Additionally, it was shown to induce apoptosis in senescent cells and kill them through a ROS-independent mechanism.	[72]
Senolytic	Not informed	Effect of piplartine in reducing the viability of IR-induced WI-38 senescent cells (IR-SCs) compared to non-senescent WI-38 cells (NCs) by flow cytometry; Induction of OXR1 degradation in senescent cells by Western Blotting; Evaluation of piplartine for its ability to induce ROS production in senescent cells.	Piplartine was senolytic for non-senescent WI38 cells and ionizing radiation-induced WI-38 senescent cells, with EC_{50} values of 20.3 and 8 µM, respectively.	[73]
Antistress	Fruits of *Piper longum* L.	Adult male swiss albino mice were used; Foot Shock Stress-Induced Hyperthermia Test; Tail Suspension Test; Determination of Plasma Glucose, Insulin and Cortisol Level, Organs Weights and Stomach Ulcer Scoring.	At 5 mg/kg, piplartine prevented stress-induced weight loss and transient hyperthermia in mice. The results also showed that it shortened immobility time in the tail suspension test and reduced stress-induced gastric ulcers as compared to control.	[74]
Antistress	Roots of *Piper longum* L.	Adult male Swiss albino mice were used; Foot shock stress-induced hyperthermia (FSIH) test; Tail suspension test; Potentiation of pentobarbitone induced hypnosis; Hot plate test; Acetic acid induced writhing test; Determination of plasma glucose, insulin, and cortisol level and organs weights.	Piplartine at concentrations as low as 1mg/kg prevented the animals from suffering from foot shock induced weight loss and elevation of body temperatures. The molecule also shortened the immobility period of mice in the tail suspension test at 4 mg/kg.	[75]
Antibacterial	Not informed	*In vitro* activity by Mueller Hinton broth microdilution method against *Staphylococcus* aureus,	Piplartine showed potential against Gram-positive *S. aureus*, but not against *P. aeruginosa* and *E. coli.* It was shown to be to BHK-21 cells.	[76]

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		Escherichia coli and *Pseudomonas eruginosaI*; Determination of cytotoxicity on BHK-21 cells (kidney cells of hamster) by MTT method.		
Antibacterial	Roots of *Piper tuberculatum* Jacq.	Determination of the Minimum Inhibitory Concentration against the gram-positive bacterial strains: -*Staphylococcus aureus* (UFPEDA 02); -*Bacillus subtilis* (UFPEDA 86); -*Enterococcus faecalis* (UFPEDA 138); And the gram-negative bacteria: -*Escherichia coli* (UFPEDA 224); -*Klebsiella pneumoniae* (UFPEDA 396); -*Pseudomonas aeruginosa* (UFPEDA 416).	Piplartine presented MIC values ranging from 312-1250 µg/mL against the evaluated bacteria.	[77]
Antifungal	Not informed	*In vitro* activity by Mueller Hinton broth microdilution method against *Candida albicans, C. tropicalis, C. krusei, C. glabrata* and *C. parapsilosi;* Determination of cytotoxicity on BHK-21 cells (kidney cells of hamster) by MTT method.	Piplartine presented moderate activity against the evaluated fungal strains, with IC_{50} values ranging from 94.6 to189.20 µM, except for *C. tropicalis* ATCC 750, where the IC_{50} value was not determined. However, piplartine was observed to be toxic for BHK-21 cells culture with the lowest selective index range, between 0.2 and 0.4.	[76]
Antifungal	Roots of *Piper tuberculatum* Jacq.	Determination of the Minimum Inhibitory Concentration against *Microsporum gypseum* (UFPEDA 2565).	Piplartine presented a minimum inhibitory concentration of 625 µg/mL against *M. gypseum*.	[77]
Molluscicidal	Roots of *Piper tuberculatum* Jacq.	Determination of molluscicidal activity against *Biomphalaria glabrata;* Determination of ovicidal activity against eggs of *B. glabrata* at the blastula, gastrula, trochophore and veliger stages by stereomicroscopy; Acute toxicity against Daphnia similis; Acute toxicity against Danio renio.	Piplartine killed adults of *B. glabrata*, with an LC_{50} = 4.19 µg/mL and is lethal to the embryos inside the eggs.	[78]
Anthelminthic	*Piper tubeculatum* Jacq.	Determination of lethal (LC_{100}) and median effect concentrations (LC_{50}) on *Schistosoma mansoni* schistosomula and adults *in vitro;*	Piplartine was found to have anti-schistosomal effects, with LC_{50} values of 7.87, 11.02 and 70.87 µM for adults, 7-day-old schistosomula and 3 hour-old schistosomula, respectively. Combinations of	[79]

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		Determination of the effect of the combination of piplartine+dermaseptin on adult worms; Determination of the effect of the combination of piplartine+dermaseptin on schistosomula; Assessment of the effect of piplartine/dermaseptin combinations on sexual fitness of *S. mansoni* adults.	piplartine + dermaseptin were found to have a synergistic effect, with a Combination index of 0.9 Additionally, *in vitro* piplartine/dermaseptin combinations caused changes on the reproductive fitness of adult worms by irreversibly inhibiting egg laying.	
Anthelmintic	Not informed	***In vitro*** evaluation of the combination of piplartine + praziquantel against *S. mansoni;* ***In vitro*** evaluation of the combination of piplartine+epiisopiloturine against *S. mansoni;* Morphological analysis of parasite tegument by laser confocal microscopy and scanning electron microscopy.	Piplartine alone was shown to be lethal for *S. mansoni*, with an IC_{50} value of 7.87 µM. Combinations of PZQ-PPT and EPI-PPT were observed to act synergistically against *S. mansoni*.	[80]
Anthelmintic	Roots of *Piper tuberculatum* Jacq.	Murine fibroblast (NIH3T3) cells were used; Cytotoxic evaluation by MTT assay; Evaluation of motor activity and mortality of adult worms of *S. mansoni* by microscopy or magnifying glass.	Piplartine was shown to have anti-Schistosomal effects *in vitro*, killing 100% of adults at 10 µM (48h of incubation).	[59]
Insecticidal	Seeds and leaves of *Piper tiberculatum* Jacq.	Determination of the effect of piplartine on larval development and mortality; Morphological analysis of the digestive system of L4 larvae through Transmission Electron Microscopy.	Piplartine showed toxicity against L3 larvae of *Ae. Aegypti*. It delayed the development and caused high mortality of *Aedes aegypti* larvae. Moreover, piplartine caused changes to the digestive tube cells, as revealed by transmission electron microscopy.	[81]
Pharmacokinetic Evaluation	Not informed	Evaluation of microsomal metabolism of piplartine; Determination of metabolic products of piplartine by mass spectrometry; Screening of the CYP enzymes involved in the metabolism of piplartine by treatment with specific CYP inhibitors; Determination of P450 isoforms involved in the metabolism of piplartine; Inhibition of CYP1A2 by high-performance liquid chromatography; Prediction of *in vivo* parameters [Intrinsic	Kinetic data suggest that piplartine binds in enzymes in multiple sites. The low hepatic extraction (E= 0.09) suggests a negligible first-pass metabolism catalyzed by CYP450 enzymes. The small CL value implies an elimination pathway performed by CYP450 enzymes. The CL_{int}, *in vivo* indicate the hepatic metabolism as the main route of elimination. Four metabolites were shown to be generated in the metabolism of piplartine. It also has the potential to inhibit P450-mediated metabolism through competitive inhibition. It causes dose-, time-and NADPH-dependent inhibition on CYP1A2	[82]

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		Clearance (CL_{int}), Unbounded Intrinsic Clearance (CL_{uint}), Predicted *in vivo* Clearance (CL), Hepatic Clearance (CL_H) and Hepatic Extraction Ratio (E)].	isoenzyme, evidencing a possible drug-drug interaction *in vivo.*	
Pharmacokinetic Evaluation	Commercially obtained	***In vitro* studies** Murine melanoma cell line B16-F0, Human melanoma cell line A375, and Human colon cancer Caco-2 cells were used for the assays; ***In vitro*** dissolution study; Caco-2 permeability study of piplartine-loaded nanoemulsions; Determination of cellular uptake of piplartine nanoemulsions (A375 and B16-F0 cells); Cytotoxic evaluation of piplartine nanoemulsions (efficacy of intracellular release of PL from nanoemulsions); ***In vivo* studies** Toxicologial evaluation of piplartine nanoemulsions on CD-1 mice; Determination of pharmacokinetic parameters of piplartine nanoemulsions on CD-1 mice.	Nanoemulsions of piplartine increased permeability in Caco-2 cells by 2-fold compared to piplartine alone. The nanoemulsions were not found to obstruct cellular uptake of piplartine by A375 and B1F0 cells. The various pharmacokinetic parameters obtained indicated that the nanoemulsions increased the bioavailability of piplartine.	[57]

3.2 Anticancer/Cytotoxic

There are several studies reporting cytotoxic activity against a broad spectrum of tumor cell lines in different *in vitro* and *in vivo* models in literature. The study by [47] was the first one to reveal the inhibitory effects of piplartine on GC cells, and to evaluate the underlying mechanisms of action of this substance against these cells. Piplartine was found to inhibit proliferation, cell cycle progression, and invasion and migration of two GC cell lines. The underlying mechanisms discussed by the authors include suppression of the JAK1,2/STAT3 signaling pathway and the inhibition of the expression of downstream genes.

Piplartine showed extensive activity against the HCT116, HepG2, HSC-3, SCC-4, SCC-9, HL-60, K-562, and B16-F10 cancer cell lines. Furthermore, it was found to induce apoptosis, ROS production and alteration of the expression of genes related to cell cycle [49]. Song *et al.* [51] report that piplartine interferes with the expression of p21, p27, cleaved caspase-3, Bax,

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Bcl-2, and p-Jun N-terminal kinase (JNK), which are typical regulators associated with cell proliferation, intrinsic apoptosis and JNK pathways.

The study by [55] showed for the first time that piplartine is able to target tubulin, destabilizing the microtubules in MCF-7 breast cancer cells and Jurkat lymphocytes. This effect was also confirmed by the sedimentation, polymerization and western blotting of tubulin, in addition to immunofluorescence and confocal microscopy.

There are studies that also investigate the association of piplartine with other substances in order to assess its possible anticancer and cytotoxic activity. [53] observed that PDAC cells treated with the combination of piplartine + GEM showed alterations in some parameters when compared to control groups and individually treated cells, such as reduced cell viability, clonogenic survival and growth in Matrigel. The authors observed some changes *in vivo* as well, such as reduction of tumor weights and volumes in nude mice with orthotopically implanted tumor cells after treatment with this combination, when compared to control and individually treated groups. The combination piplartine+GEM was also observed to increase ROS levels, disrupt the G0/G1 phase of the cell cycle and induce death in PDAC cells.

3.3 Leishmanicidal activity

For the leishmanicidal activity, only studies investigating the species *Leishmania donovani*, *L. amazonensis* and *L. infantum* were found in the period from 2007 to 2018 (Table 2). No studies investigating the activity piplartine against *L. (V.) braziliensis* were found, a species of great importance, responsible for cutaneous leishmaniasis in Latin America. Of the three references, only one investigates the growth inhibition on both the promastigote and amastigote forms [83]; One study proposes a mechanism of action [84]; and only one investigate the *in vivo* antileishmanial activity [85].

For *L. donovani*, piplartine was shown to have leishmanicidal activity against promastigotes, with IC_{50} of 7.5 μM. Piplartine exhibited an IC_{50} of 3.3 μM for promastigote forms of *L. amazonensis* and 7.9 μM for those of *L. infantum*. The substance also reduced the rates of infection by amastigote forms in a dose-dependent manner with an IC_{50} of 0.4 μM [83]. The *in vivo* evaluation revealed that this molecule was able to reduce the spleen weight by 50% and the parasitic burden by 36% at a dose of 30 mg/kg [85].

Table 2 Antileishmanial activity of piplartine

Species	Bioactivity	Main Findings	References

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Leishmania donovani	***In vitro*** growth inhibition by piplartine against promastigotes; ***In vivo*** evaluation of the antileishmanial activity of piplartine on Golden Hamsters.	Piplartine was shown to possess leishmanicidal action against promastigote forms, with an IC_{50} of 7.5 µM. The *in vivo* evaluation revealed that this molecule was able to reduce the spleen weight by 50% and parasite burden by 36% at 30 mg/ kg.	[85]
Leishmania amazonensis	***In vitro*** growth inhibition assays against promastigote forms by piplartine.	For 6h of incubation, piplartine obtained an IC_{50} of 179 µg/mL.	[84]
Leishmania infantum and *Leishmania amazonensis*	***In vitro*** growth inhibition assays against promastigote forms by piplartine; ***In vitro*** assays of inhibition of amastigote forms by piplartine; Promastigotes (*L. infantum* and *L. amazonensis*) and amastigotes (*L. amazonensis*).	Piplartine exhibited an IC_{50} of 3.3 µM for promastigote forms of *L. amazonensis* and 7.9 µM for *L. infantum*; Piplartine reduced the rates of infection by amastigote forms in a dose-dependent manner, with an IC_{50} of 0.4 µM.	[83]

3.4 Anticoagulant/antiplatelet activity

Studies have shown (Table 1) that piplartine inhibits both the extrinsic and intrinsic pathways of blood coagulation by inhibiting the generation of FXa and thrombin and that both inhibit TNF-α induced secretion of the PAI-1 protein via the HUVECs [65]. Piplartine exhibited IC_{50} values of 5.489 and 11.121 mM for platelet aggregation induced by AA and ADP, respectively [66]. The work conducted by Yuan *et al.* [67] demonstrates that piplartine inhibits collagen-induced platelet reactivity, targeting the JAK2-STAT3 pathway. This study also provides experimental evidence that piplartine and collagen induce different oxidants that have differential effects on platelets [67].

3.5 Anthelminthic activity

Piplartine has been shown to have anti-schistosomal effects *in vitro*, although also being toxic towards the evaluated mammal cells [59]. The combined action of piplartine with other substances is also highlighted for this activity. [79] describe that the *in vitro* treatment of piplartine and dermaseptin exhibited a synergistic behavior for larval stages (schistosomula) and adult worms of *S. mansoni*. They also observed that *in vitro* combinations of piplartine and dermaseptin caused changes in the reproductive fitness of adult worms by inhibiting egg laying. [80] report that synergistic combinations of PZQ-piplartine and EPI-piplartine create the possibility of reduced doses to be used against *S. mansoni*.

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3.6 Other studies

Other biological activities which were less investigated (with only one or two studies - Table 1) between 2013 and 2018 include studies that investigate the trypanocidal [68], pharmacokinetic evaluation [57, 82], insecticidal [81], molluscicidal [78], antifungal and antibacterial [76-77], anti-stress [74-75], senolytic [72-73], hemocytotoxicity [71-70], enzyme inhibition/binding [69] and antiplasmodial activity [84].

Of these studies, one that stands out is the work conducted by Go *et al.* [63], which investigated the neuroprotective action of piplartine, a natural substance isolated from fruits of *Piper longum*. They found evidences that piplartine activated the protein sirt1, attenuating the pathogenesis of Alzheimer disease in hippocampal neurons by reducing the Aβ-induced cytotoxicity in these cells. Additionally, it was able to decrease the percent area occupied by amyloid plaques in the cortex of APP/PS1 mice. These results suggest that piplartine may serve as a tool for the treatment of Alzheimer's disease.

[84] investigated the antiplasmodial activity (*P. falciparum*) action of piplartine. It was shown to inhibit parasitic growth in a dose-dependent manner, presenting IC_{50} values of 19.5 μM (6.2 μg/mL) for 48h of 10.1 μM (3.2 μg/mL) for 72h. Evaluation of the *in vitro* parasite growth inhibition in human erythrocytes; It does not discuss the mechanism of action (only hypothesizes in the discussion, but does not investigate it).

3.7 Biological activities of Piperine

Considering the period mentioned in the methodology, we found 14 studies comprising seven biological activities, with emphasis on leishmanicidal, antiplasmodial and bioenhancer activities, followed by antivenom, anticancer, neuroprotective and antiasthmatic activities. Most of these studies were performed with the pure synthetic substance, which was commercially obtained (Fig. 5).

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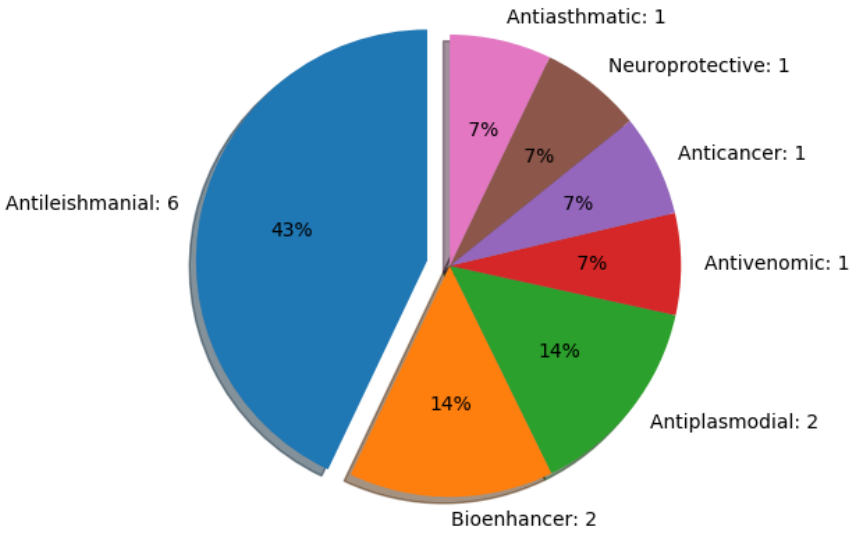

Fig. 5 Biological activities of piperine. We found 14 studies comprising seven biological activities, with emphasis on leishmanicidal, antiplasmodial and bioenhancer activities, followed by antivenom, anticancer, neuroprotective and antiasthmatic activities.

The biological importance of piperine is wide and has been documented in the publications of [26]; [27]; [28] and . [29]. Thus, this study sought to include studies published in the year of 2018 and other studies published in 2016 and 2017 that were not mentioned in previous studies (Table 3).

Table 3 Biological activity of piperine.

Bioactivity	Source	Bioassay Model	Main Findings	References
Bioenhancer	Commercially obtained	Inhibition of CYP3A4 by piperine; Determination of Rh2 permeability in intestinal Caco-2 cells in the presence of piperine; Studies of the pharmacokinetic behavior of Rh2 in Sprague-Dawley rats by piperine: Cmax, Tmax and AUC were calculated.	Piperine markedly increased the permeability of Rh2 and inhibited its metabolism. The pharmacokinetic study demonstrated that Rh2 AUC was significantly higher in combination with piperine at 20 mg/kg when compared to the control (relative bioavailability = 196.8%); Piperine can be used as a bioenhancer to improve the pharmacological effect of Rh2 when given orally.	[87]
	Commercially obtained	***In vivo*** determination of doxorubicin distribution by inhibition of P-gp by piperine in Balb/c mice by through LC-MS.	The plasma concentration profiles of doxorubicin over time were not significantly affected by	[88]

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			piperine. In contrast, accumulation of doxorubicin was observed in the tissues after pretreatment with piperine. The data demonstrated that piperine modulated the distribution of doxorubicin in tissues, suggesting its potential to induce interactions between drugs and foods and to act with a strategy for delivering drugs that act as a substrate of P-gp to target tissues and tumors.	
Antivenom	Commercially obtained	Simulation of the interaction between piperine and EcPLA2 by molecular dynamics; Kinetics of the interaction between piperine and EcPLA2 by surface plasmon resonance (SPR).	The results of molecular mechanics showed that the strong hydrophobic and electrostatic interactions favored the formation of the EcPLA2-Piperine complex in the absence and presence of calcium, respectively. Experimental results of affinity kinetics showed that the dissociation constant of piperine with EcPLA2 was 1.708 pM. Piperine improved the pharmacological and catalytic activity of PLA2's by its binding mechanism and inhibition kinetics.	[89]
Anticancer	Not informed	Evaluation of the cytotoxic effect of piperine on Caco-2 and CEM/ADR 5000 multiresistant cancer cells; and non-multidrug resistant cells: HCT 116, CCRF-CEM); Evaluation of the interaction between piperazine+doxorubicin in the viability of Caco-2 and CEM/ADR 5000 cells; Evaluation of the interaction between piperine+doxorubicin+digitonin in the viability of Caco-2 and CEM/ADR 5000 cells; Analysis of P-gp activity through the intracellular accumulation of rhodamine and calcein.	Piperine acted synergistically, increasing the cytotoxicity of doxorubicin in Caco-2 and CEM/ADR 5000 cells. Additionally, piperine increased the intracellular concentration of the fluorescent P-glycoprotein (P-gp) substrates and calcein and inhibited their cell efflux multiresistant, demonstrating that piperine is a substrate of P-gp and potentially has a chemosensitizing action, which is of interest in the development of multidrug modulators.	[90]

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Neuroprotective	*Piper nigrum* L.	Determination of cell viability by the WST-8 assay; Analysis of the expression of the BDNF-IV (brain-derived neurotrophic factor) promoter by the reporter gene assay; Measurement of neurite growth; Protein analysis by Western Blot.	In both assays, remarkable activity related to reporter genes was observed for piperine, which suggests its effect on disorders associated with the dysregulation of BDNF expression, such as depression.	[91]
Antiasthmatic	Not informed	Analysis of airway inflammation; Histological analysis of the lungs; Effect of piperine on eosinophil peroxidase (EPO) levels; Effect of piperine on IgE levels; Assessment of ROS and NO (Nitric Oxide) levels after treatment with piperine.	Piperine has been shown to significantly reduce ovalbumin-induced inflammation and EPO activity levels, and to cause a decrease in serum IgE levels and ROS levels. However, it did not cause a significant decrease in NO levels.	[92]

3.8 Antileishmanial activities

For the activity of piperine, articles were selected from 1993 to 2018, because we identified that in the period before 2013 there is a shortage of articles describing these activities (Table 4). Of the six articles cited, four investigate the activity of piperine against the species *L. donovani*, one against *L. amazonensis* and one against *L. infantum*. Studies investigating piperine against *L. (V.) braziliensis* were not found. Piperine was shown inactive with IC_{50} values as high as 0.752 mM for amastigote forms and 2.558 mM for *L. donovani* promastigote forms [93]. None of the studies performed with the species *L. donovani* describe any mechanism of action.

A study by [94], analyzed the *in vitro* growth inhibition of promastigotes and axenic amastigotes by piperine; The study also analyzed the effect of piperine on mitochondrial swelling, kDNA and mitochondrial membrane potential; Finally, they analyzed the combination of different concentrations of piperine and phenylamide on *L. amazonensis* growth, for which the isobologram analysis showed a synergistic action between the two compounds, and discussed its mechanism of action

[95] carried out an *in vitro* study of growth inhibition against promastigotes and axenic amastigotes of *L. infantum*. The study investigated the combination of capsaicin/piperine/meglumine antimoniate at different concentrations, reporting a synergistic effect between piperine and meglumine antimoniate (25%+75%) for promastigote and amastigote forms of *L. infantum*. The study do not discusses the mechanism of action.

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Table 4 Antileishmanial activity of piperine

Species	Bioassays Performed	Main Findings	References
Leishmania donovani	***In vitro*** growth inhibition assays against promastigotes by piperine.	Piperine showed promastigote growth inhibition at concentrations equal or higher than 800 μg/ml.	[96]
Leishmania donovani	Inhibition of the ***in vivo*** growth of *L. donovani* by piperine alone, piperine with a liposomal formulation, and piperine with mannose-coated liposomes using golden hamsters;	Piperine alone, and encapsulated in mannose-coated liposomes was able to reduce 29.77 and 90% of spleen parasitemia, respectively, with a dose of 6 mg/k.	[97]
Leishmania donovani	***In vivo*** evaluation of parasite burden reduction in mice by piperine and liposomal piperine;	Piperine reduced parasite burden in the liver and spleen in 38 and 31% after 15 days of infection, respectively. The results demonstrated that a single dose of piperine (5 mg/kg) in lipid nanospheres can significantly reduce the parasite burden in the liver and in the spleen.	[98]
Leishmania donovani	***In vitro*** inhibition assays against promastigote forms by piperine; ***In vitro*** growth inhibition assays against axenic amastigotes by piperine.	Piperine was inactive, with IC_{50} of 0.752 mM for amastigote forms and 2.558 mM for promastigote forms.	[93]
Leishmania amazonensis	***In vitro*** inhibition assays against promastigote forms by piperine; ***In vitro*** growth inhibition assays against axenic amastigotes by piperine; Analysis of the effect of piperine on the cell cycle of promastigote forms; Analysis of the effect of piperine on mitochondrial swelling, kDNA and membrane potential of mitochondria; Analysis of the effect of piperine on promastigote lipids; Analysis of the combination of different concentrations of piperine and phenylamide on *L. amazonensis* growth.	Piperine was found to be active against promastigotes and amastigotes in infected macrophages; It induced mitochondrial swelling, loosened kDNA, and led to loss of the mitochondrial membrane potential; The cell cycle of promastigote forms was also affected, with an increase in G1 cell frequency and a decrease in S phase cells, respectively, after treatment with piperine; Lipid asnalyses of promastigote forms showed that piperine reduced the content of triglycerides, diacylglycerol, and monoacylglycerol; Piperine induced the production of nitric oxide and reduced its production in activated macrophages; Analyses showed that piperine and phenylalanine acted synergistically on the parasites, suggesting that they act on different molecular targets.	[94]
Leishmania infantum	***In vitro*** growth inhibitory assays of promastigote forms by combinations of meglumine and piperine; ***In vitro*** growth inhibition assays against axenic amastigote forms by combinations of meglumine and piperine; The study evaluates the combination of drugs between alkaloids capsaicin/piperine and meglumine	The combination of piperine in combination with meglumine antimoniate showed a synergistic effect against *Leishmania infantum*; Piperine alone was found to have leishmanicidal action; Piperine was more active than the reference drug.	[95]

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	antimoniate at different concentrations. CAP; PIP and MEG.		

3.9 Antiplasmodial activity

Two studies investigated the antiplasmodial activity of piperine (Table 5). [99] carried out the *in vivo* evaluation of the interaction between curcumin/piperine in combination with artemisinin in the suppression of parasitemia of an artemisinin-resistant *P. chabaudi* clone. The results indicated that piperine combined with curcumin had a modest antimalarial effect and was not able to reverse the phenotype or significantly affect the growth of the resistant clone when compared to artemisinin.

[100] evaluated *in vivo* the interaction between curcumin/piperine in combination with chloroquine/artemisinin in the suppression of parasitemia of artemisinin and chloroquine-resistant clones in BALB/c mice expression of the profile of genes encoding proteasome/ubiquitin pathways in *P. chabaudi* after treatment, owing to the putative involvement of these enzymes in artemisinin resistance. A real-time PCR analysis of the *P. chabaudi* genes encoding the deubiquitinating enzymes was performed and the mechanism of action was discussed. The Curcumin/piperine/artemisinin combination was not shown to be advantageous in the murine model of malaria.

Table 5 Antiplasmodial activity of piperine

Species	Bioassays	Main Findings	References
P. chabaudi	***In vivo*** evaluation of the interaction between curcumin/piperine in combination with artemisinin in the suppression of parasitemia of an artemisinin-resistant *P. chabaudi* clone.	The results indicated that piperine combined with curcumin had only a modest antimalarial effect, with the greatest difference between this treatment and control being on day 8, with a parasitemia of 50-60% for control and 30-40% for the curcumin-piperine combination. Furthermore, it was not able to reverse the phenotype or significantly affect the growth of the resistant clone when compared to artemisinin.	[99]
P. chabaudi	***In vivo*** evaluation of the interaction between curcumin/piperine in combination with chloroquine/artemisinin in suppressing parasitemia of chloroquine and artemisinin-resistant clones in BALB / c mice; Expression of the profile of genes encoding enzymes of the proteasome/ubiquitin pathway in *P. chabaudi*; Real-time PCR analysis of *P. chabaudi* genes encoding deubiquinating enzymes.	The curcumin/chloroquine/piperine combination reduced parasitemia to 37% seven days after treatment against 65% of the control group, and an additive interaction was found; The combination of curcumin/ piperine/artemisinin did not show a favorable pharmacological interaction in this murine model of malaria.	[100]

3.10 Bioenhancer activity

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Two studies were conducted to investigate the bioenhancer activity of piperine. [87] showed that piperine markedly increased the permeability of Rh2 and inhibited its metabolism. The pharmacokinetic study demonstrated that the AUC (area under the curve) of Rh2 was significantly higher in combination with piperine at 20 mg/kg when compared to the control (relative bioavailability = 196.8%). The authors concluded that piperine might be used as a bioenhancer to improve the pharmacological effect of Rh2 when administered orally.

The study by [88] demonstrate that the plasma concentration profiles of doxorubicin over time were not significantly affected by piperine. In contrast, accumulation of doxorubicin was observed in the tissues after pretreatment with piperine. The data demonstrated that piperine modulated the distribution of doxorubicin in tissues, suggesting its potential to induce interactions between drugs and foods and to act as a strategy for delivering drugs that act as a substrate of P-gp to target tissues and tumors.

3.11 Other studies

Other biological activities of piperine which were less investigated between 2013 and 2018 include studies investigating the antivenom [89], anticancer [90], neuroprotective [91] and antiasthmatic activity [92].

In one of these studies, [92] investigated the antiasthmatic activity of piperine. The authors examined airway inflammation, lung histology, the effect of piperine on eosinophil peroxidase (EPO) levels and the effect of piperine on IgE levels. The ROS and NO levels were also evaluated after treatment with piperine. The substance (piperine) has been shown to significantly reduce ovalbumin-induced inflammation and EPO activity levels and to cause a decrease in serum IgE levels and ROS levels. However, the substance did not cause a significant decrease in NO levels.

CONCLUSION AND FUTURE PERSPECTIVE

The review highlights the biological activities, the test models and the results obtained with these substances, with emphasis on the most investigated activities. From this research, we conclude that piplartine and piperine have been extensively investigated. Piplartine has an anticancer / cytotoxic potential, with well-established mechanisms of action. However, there are few *in vivo* studies that can contribute to the validation of piplartine against this set of diseases. It has been observed that the substances have some studies on antiparasitic activity.

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Few leishmanicidal studies have been found to investigate its activity against amastigote and *in vivo* forms. Indeed, compounds that have long been known and have not reached the status of evidence-based medicine require analysis.

Some studies were not concerned to meet certain quality requirements that are suggested in the literature to investigate biological activities such as: dose response curves, and appropriate negative and positive controls. Still, in this sense, [101] brings in his research recommendations on how to develop a stronger *in vitro* "proof of concept" to assess the biological potential of natural substances against infectious diseases. The authors highlight parameters, requirements and potency criteria of natural substances to be tested, namely: use of reference strains or characterized clinical isolates, *in vitro* cell-based models; selectivity index assessment, sufficiently wide dose range allowing dose-response curves; restrictive criteria with IC_{50} values generally below 100 μg/mL for extracts and essential oils and below 25 μM for pure compounds; inclusion of appropriate controls in each replication of the *in vitro* test, among others. Thus, this research synthesizes the biological activities already performed and can guide future researches that seek to relate the potential of these amides and their respective synthetic analogues with other biological activities, taking into consideration stricter criteria for evaluating these activities.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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