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Gabriela Silva Cruz

Atividade antifúngica de fragmentos peptídicos derivados da Crotalicidina frente a *Candida* spp.

FORTALEZA -CE
2023

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Tese submetida à Coordenação do Programa de Pós-Graduação em Ciências Farmacêuticas, da Universidade Federal do Ceará como requisito parcial para obtenção do título de Doutor em Ciências Farmacêuticas.

Orientador: Prof. Dr. Gandhi Rádis-Baptista
Coorientadora: Profª Dra. Erika Helena Salles de Brito

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RESUMO

A Crotalicidina (Ctn), um peptídeo antimicrobiano relacionado à catelicidina da glândula de veneno da cascavel Sul-Americana *Crotalus durissus terrificus*, bem como seu fragmento C-terminal, Ctn [15-34], têm demonstrado importantes atividades contra microrganismos, tais como bactérias, leveduras e protozoários tripanossomatídeos e certas linhagens de células tumorais. Recentemente, o peptídeo RhoB-Ctn[1-9] demonstrou ação antimicrobiana contra leveduras e bactérias. Para melhorar a sua atividade antifúngica, ao invés da Rodamina B, foi adicionado a essa estrutura o ácido tetradecanoico (ácido mirístico), em ligação covalente com a porção N-terminal do peptídeo, sendo apelidado de Myr-Ctn[1-9]. As leveduras do gênero *Candida* possuem particularidades a depender da espécie e origem dos isolados, tais como a capacidade de produzir fatores de virulência, que contribuem para a patogenicidade e resistência aos medicamentos tradicionais, fazendo-se necessária a identificação de novas alternativas de agentes antifúngicos. Diante do exposto, este estudo teve como objetivo expandir a investigação sobre o espectro de atividade antifúngica do fragmento peptídico Ctn[15-34] derivado da Crotalicidina, frente a cepas de *Candida* spp. e comparar com a atividade anti-*Candida* do novo peptídeo Myr-Ctn[1-9]. Trata-se de um estudo analítico, com abordagem quantitativa, utilizando 35 cepas de *Candida* sp. coletadas a partir da microbiota oral de indivíduos saudáveis e isolados de indivíduos doentes. Antes dos testes de sensibilidade com os peptídeos, foi feito um levantamento sobre o perfil de sensibilidade antifúngicos convencionais e produção dos fatores de virulência (atividade hemolítica e fosfolipase). Por fim, a atividade de Ctn [15-34] sobre o biofilme de *Candida albicans* foi avaliada. Foram realizados testes de sensibilidade com 25 cepas de *Candida* spp. e constatou-se que a distribuição dos valores de Concentração Inibitória Mínima (CIM) obtidos variaram entre os grupos de diferentes espécies (valor de $p = 0,04$), com mediana de concentrações inibitórias de peptídeo menor para cepas de *Candida* não-*albicans*. Myr-Ctn [1-9] apresentou valores de CIM menores para todas as cepas, em relação ao Ctn [15-34] com variação de 0,313 a 1,25 μM (valor de $p < 0,01$). Ctn [15-34] inibiu em 50% a formação do biofilme por uma cepa de referência de *Candida albicans*, a uma concentração de 75 μM. No geral, Myr-Ctn [1-9] e Ctn [15-34] são potenciais derivações antifúngicas que exibem atividades contra *Candida* spp., demonstrando que os peptídeos derivados da Crotalicidina são modelos promissores para estudos mais aprofundados que visem o objetivo final de desenvolvimento de novos antifúngicos.

Palavras-chave: Peptídeos Antimicrobianos, *Crotalus Candida*, Biofilmes.

ABSTRACT

Crotalicidin (Ctn), an antimicrobial peptide related to cathelicidin from the venom gland of the South American rattlesnake *Crotalus durissus terrificus*, as well as C-terminal fragment, Ctn[15-34], have demonstrated important activities against microorganisms such as bacteria, yeast and trypanosomatid protozoa and certain tumor cell lines. Recently, the fragmente RhoB-Ctn[1-9] demonstrated antimicrobial action against yeasts and bacteria. To improve its antifungal activity, instead Rhodamine B, tetradecanoic acid (myristic acid) was added to this structure, in covalent bond with the N-terminal portion of the peptide, being nicknamed Myr-Ctn[1-9]. Yeasts of the genus *Candida* have particularities depending on the species and origin of the isolates, such as the ability to produce virulence factors, which contribute to pathogenicity and resistance to traditional medicines, making it necessary to identify new alternatives for antifungal agents. Given the above, this study aimed to expand the investigation into the spectrum of antifungal activity of peptide fragments Myr-Ctn[1-9] and Ctn[15-34] derived from Crotalicidin, against strains of *Candida* spp. This is an analytical study, with a quantitative approach, using 35 strains of *Candida* sp., collected from the oral microbiota of healthy individuals. Clinical isolates of *Candida* spp. from different types of clinical specimens such as blood, urine, bronchoalveolar lavage, tracheal aspirate and nail scrapings were also used for testing. Before the sensitivity tests with the peptides, a survey was carried out on the sensitivity profile of conventional antifungal agents and the production of virulence factors (hemolytic activity and phospholipase). Finally, the activity of Ctn[15-34] on *Candida albicans* biofilm was evaluated. Sensitivity tests were performed with 25 strains of *Candida* spp. and it was found that the distribution of Minimum Inhibitory Concentration (MIC) values obtained varied between groups of different species (p-value = 0.04), with median inhibitory peptide concentrations lower for non-albicans *Candida* strains. Myr-Ctn[1-9] showed lower MIC values for all strains compared to Ctn[15-34] with a range from 0.313 to 1.25 μM (p value < 0.01). Ctn[15-34] inhibited biofilm formation (50%) by a standard strain of *Candida albicans* at a concentration of 75 μM. Overall, Myr-Ctn[1-9] and Ctn[15-34] are potential antifungal derivatives that exhibit activities against *Candida* spp., demonstrating that Crotalicidin-derived peptides are promising models for further studies aimed at the ultimate goal of development of new antifungals.

Keywords: Antimicrobial Peptides, *Crotalus*, *Candida*, Biofilms.

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1. INTRODUÇÃO

Constituindo a família das Cryptococcaceae, o gênero *Candida* apresenta, em sua composição, aproximadamente 150 espécies, dispersas em diferentes ambientes, como ar, solo, água, plantas e animais. (POULAIN et al., 2009). São considerados fungos oportunistas, causando doença quando há o comprometimento da imunidade do hospedeiro (SALVATORI et al., 2016).

A forma patogênica de *Candida* spp. expressa uma série de fatores de virulência, tais como a adesão aos tecidos do hospedeiro diante da atividade de adesinas e invasinas; produção de enzimas hidrolíticas, como as proteases, fosfolipases e hemolisinas; mudança fenotípica; transição morfológica levedura-hifa e formação de biofilme (ROSSI et al, 2011; GOW; HUBE, 2012).

No tocante à formação de biofilme, a expressão deste fator de virulência pode ser observada em diversos microrganismos incluindo *Candida* spp., tendo implicações para as ciências farmacêuticas e as ciências da saúde de uma forma geral, visto que estes estão entre os microrganismos mais comuns em ambientes clínicos, com capacidade de adesão a dispositivos biomédicos, crescendo como um biofilme resistente a medicamentos (SILVA et al., 2017).

Biofilmes de *Candida* spp. estão se tornando cada vez mais resistentes a uma variedade de estresses, tais como: mecanismos de defesa imunológica do hospedeiro, estresse mecânico e ação de fármacos antifúngicos, incluindo derivados azólicos e polienos, os quais são amplamente utilizados na prática clínica para o tratamento de infecções fúngicas (POLKE; HUBE; JACOBSEN, 2015; CHANDRA; MUKHERJEE, 2015). A evolução da resistência de fungos a agentes antimicrobianos tem se tornado uma preocupação constante, visto que é limitado o número de antifúngicos disponíveis para o tratamento de infecções sistêmicas (MOUNT et al., 2018).

Todos os fatores supracitados são responsáveis pela maior dificuldade frente ao tratamento dessas doenças, havendo a necessidade de identificação de novas alternativas terapêuticas. Dessa forma os Peptídeos Antimicrobianos (PAMs) apresentam-se como uma opção.

Os peptídeos Antimicrobianos são moléculas provenientes do sistema imune inato de vertebrados e invertebrados, e que possuem a capacidade de interagir com membranas celulares, podendo apresentar atividade antimicrobiana frente a determinados agentes

infecciosos (KANG et al., 2014). Por esta razão, os PAMs têm gerado interesse considerável tendo em vista a possibilidade de serem aplicados como substitutos a antibióticos e antifúngicos, devido ao seu amplo espectro de atividade e indução de resistência reduzida (NG et al., 2018; CAVALCANTE et al., 2016).

Com base nisso, temos o fragmento peptídico Ctn[15-34], o qual pertence à família das catelicidinas, caracterizada a partir da glândula de veneno da cascavel *Crotalus durissus terrificus*, e que foi evidenciada ação antimicrobiana contra leveduras, bactérias e vírus, aplicado em substituição ou em combinações com antimicrobianos (CAVALCANTE et al., 2017; VIEIRA-GIRÃO et al., 2017; PÉREZ-PEINADO et al., 2018). Recentemente, o análogo estrutural RhoB-Ctn[1-9] (Rodamina B conjugada ao peptídeo Ctn-[1-9]), apresentou em estudo anterior, atividade antimicrobiana seletiva contra bactérias Gram-negativas infecciosas como *Escherichia coli, Pseudomonas aeruginosa* e espécies patogênicas de *Candida* com baixos efeitos hemolíticos em eritrócitos humanos, sendo capaz de permear as membranas celulares e acumular-se intracelularmente em células microbianas (LIMA et al., 2022).

Considerando o que foi mencionado, surgiram as seguintes questões de pesquisa: - Os fragmentos peptídicos modificados de Ctn[1-9] e Ctn [15-34] interferem no crescimento da forma planctônica e em biofilme de *Candida* spp.? – Há diferença da atividade antifúngica entre o análogo de Ctn[1-9] modificado e Ctn [15-34] já estudado?

2. JUSTIFICATIVA

Em virtude do limitado quantitativo de antifúngicos disponíveis no mercado e a iminente resistência de *Candida* spp. frente à antifúngicos amplamente utilizados na prática clínica, faz-se necessário o desenvolvimento de novas opções terapêuticas para o tratamento das candidíases, diminuindo as taxas de mortalidade por infecções fúngicas causadas por *Candida* sp. nos grupos de indivíduos vulneráveis (que possuem imunossupressão em decorrência de Síndrome da Imunodeficiência Adquirida, câncer, transplantes, uso crônico de corticoides, dentre outros).

A descoberta de novas moléculas com potencial biotecnológico e o desenvolvimento de novos fármacos também tem como objetivo minimizar o ônus financeiro ao paciente e aos serviços de saúde, decorrente de eventuais falhas em tratamentos convencionais. Diante da necessidade de agentes antimicrobianos de maior eficácia e que eventualmente podem levar a uma melhor resposta se associado a fármacos já conhecidos, o uso de Peptídeos Antimicrobianos (PAMs), vem sendo estudado como uma possível alternativa terapêutica para o tratamento de doenças infecciosas, com relevância diante do tratamento de infecções por *Candida* spp.

O estudo da atividade de novas substâncias, principalmente a ação dos PAMs frente às células de *Candida* spp., tanto em sua forma planctônica, como estando aderida no biofilme, é de extrema relevância para prática clínica, tendo em vista a possibilidade de realização de estudos posteriores, *in vivo*, que possam identificar uma possível utilidade como tratamento principal ou coadjuvante de infecções fúngicas sistêmicas. Ainda, este estudo contribuirá para a ampliação do conhecimento no âmbito das Ciências Farmacêuticas e avanços significativos nesta área de estudo.

3. OBJETIVOS

3.1 Objetivo Geral

Expandir a investigação sobre o espectro de atividade antifúngica dos fragmentos peptídicos Myr-Ctn [1-9] e Ctn [15-34] derivados da Crotalicidina, frente a cepas de *Candida* spp. na sua forma planctônica e em biofilme.

3.2 Objetivos específicos

- Avaliar, *in vitro*, o perfil de sensibilidade das cepas de *Candida* spp. aos antifúngicos Fluconazol, Itraconazol e Anfotericina B;
- Avaliar, *in vitro*, os fatores de virulência tais como a produção de fosfolipases, atividade hemolítica de cepas de *Candida* spp. e formação de biofilme;
- Determinar a atividade antifúngica do novo peptídeo Myr-Ctn [1-9] e comparar com a atividade antifúngica do peptídeo Ctn [15-34];
- Comparar a atividade antifúngica dos fragmentos peptídicos entre grupos de cepas de diferentes espécies;
- Determinar o perfil de sensibilidade antifúngica dos biofilmes frente ao fragmento peptídico Ctn [15-34].

4. REVISÃO DE LITERATURA

4.1 Gênero *Candida*

O gênero *Candida* é composto por fungos polimórficos os quais pertencem ao filo Ascomycota, classe Saccharomycetes, ordem Saccharomycetales (LEVETIN et al., 2016). Fazem parte deste gênero, mais de 150 espécies, que podem ser encontradas em diversos ambientes tais como no ar, solo, água, plantas e animais. Contudo, apenas 15 destas são observadas em pacientes como agentes infecciosos. São elas: *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis*, *Candida krusei*, *Candida guilliermondii*, *Candida lusitaniae*, *Candida dubliniensis, Candida pelliculosa*, *Candida kefyr*, *Candida lipolytica*, *Candida famata*, *Candida inconspicua, Candida rugosa* e *Candida norvegensis*. Em 95% das infecções por *Candida* sp. os patógenos envolvidos são *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C.tropicalis* e *C. krusei* (YAPAR, 2014). Recentemente o gênero sofreu uma mudança na classificação e espécies de importância clínica como *Candida krusei*, *Candida glabrata*, *Candida guilliermondii*, *Candida lusitaniae* e *Candida rugosa* não fazem mais parte do gênero *Candida*, sendo reclassificadas em *Pichia kudriavzevii*, *Nakaseomyces glabrata*, *Meyerozyma guilliermondii*, *Clavispora lusitaniae* e *Diutina rugosa*, respectivamente (KIDD, ABDOLRASOULI, HAGEN, 2023). Como trata-se de uma reclassificação nova, ao longo do texto, as leveduras são citadas de acordo com a antiga classificação.

Espécies do gênero *Candida* compõem a microbiota humana normal em todo o corpo, podendo estar presente na pele, trato gastrointestinal, trato geniturinário das mulheres e o trato respiratório. São comensais, podendo compor a microbiota de diversos sítios anatômicos, sem causar infecção ativa e quando há desequilíbrio da imunidade do hospedeiro, pode iniciar um quadro de Candidíase (PENDLETON et al., 2017). No contexto das doenças fúngicas, *Candida* spp. contribuem ao promover essas infecções em situações em que há comprometimento da resposta imunológica ou perda da integridade das barreiras naturais de defesa do hospedeiro, tornando-a um fungo oportunista (COLOMBO; GUIMARÃES, 2003). Podem causar infecções em variados sítios anatômicos e infecções sistêmicas em indivíduos imunocomprometidos, em casos de diabetes mellitus, neutropenia, tratamento oncológico, transplante de órgãos, hemodiálise, uso prolongado de agentes antimicrobianos e/ou hospitalização prolongada (TSUI et al., 2008, PUNITHAVATHY et

al., 2012). Ainda, com o surgimento das infecções por HIV (Vírus da Imunodeficiência Humana) e consequentemente a AIDS (Síndrome da Imunodeficiência Adquirida), em decorrência do enfraquecimento do sistema imunológico, a pessoa infectada pelo HIV/AIDS corre um risco aumentado de uma ampla variedade de infecções oportunistas, dentre elas a candidíase oral. Estima-se que 60% a 90% das pessoas vivendo com o vírus HIV apresentarão pelo menos uma manifestação oral (NUGRAHA et al., 2018).

Candida albicans é a espécie mais abundante e possui uma variedade de características microbiológicas que conferem a ela maior adaptabilidade para colonizar a mucosa ao lado das bactérias e existir em um estado de comensalismo ou tornar-se patogênico e invasivo durante a doença, tais como a sua característica de ser polimórfica, com capacidade de alterar sua morfologia entre uma levedura em brotamento e uma forma de hifa filamentosa, o que constitui um importante fator de virulência. Comparado com seu estado de levedura, quando forma hifas, *C. albicans* exibe propriedades de aderência aumentadas, invasividade e maior patogenicidade (BASMACIYAN et al., 2019).

Desde o início do novo milênio, a detecção de espécies de *Candida* não-*albicans* aumentou significativamente e superam *C. albicans* como a causa mais prevalente de infecções invasivas por *Candida*, dependendo da região geográfica, sendo observado também a crescente prevalência de espécies de *Candida* não-*albicans* associadas à diminuição da suscetibilidade aos antifúngicos mais utilizados. *C. parapsilosis*, por exemplo, apresenta-se como uma causa frequente de candidíase invasiva em neonatos e crianças (TÓTH et al., 2019). A incidência de *C. glabrata* como agente etiológico de infecções é maior em adultos do que em crianças, tendo também como característica vinculada à sua incidência, o aumento do uso de tratamento antifúngico profilático em pacientes imunossuprimidos (TURNER; BUTLER, 2014); ao contrário de *C. parapsilosis, que* tem sido frequentemente associada a infecções em recém-nascidos, receptores de transplantes e pacientes que recebem nutrição parenteral e *C. tropicalis*, que é comumente associada a pacientes com neutropenia (SILVA et al., 2012). A incidência de *C. krusei* nas infecções é baixa, no entanto, gera preocupação em decorrência de sua resistência aos azóis e outros agentes antifúngicos. *C. guilliermondii* e *C. lusitaniae* são causas relativamente raras de infecção humana (TURNER; BUTLER, 2014).

Considerado um patógeno recém evoluído, cepas de *Candida auris* foram isoladas de múltiplos locais de infecção em todo o corpo e geralmente são adquiridas no ambiente hospitalar. Já foram isoladas da urina, bile, sangue, feridas, narinas, axila, pele e reto de

indivíduos infectados. A hipótese é de que *C. auris* coloniza predominantemente a pele; no entanto, em casos raros, foi isolado da mucosa intestinal, oral e esofágica dos pacientes. Embora tenha sido relatada pela primeira vez em 1996, a partir de 2009 tornou-se alvo de frequente vigilância nos serviços de saúde, pois está presente atualmente em pelo menos 40 países. Infecções invasivas causadas por *C. auris* ocorrem mais frequentemente em pacientes críticos em UTIs, associadas a altas taxas de mortalidade global que variam de 30% a 60% (DU et al., 2020).

4.2 Fatores de virulência de *Candida* spp.

Candida spp. são um microrganismos oportunistas, sendo comensal para a maioria dos casos, mas quando há um desequilíbrio, se torna patogênica e participa ativamente da ocorrência e avanço da infecção, em decorrência dos seus fatores de virulência. A virulência de uma espécie microbiana é uma medida do resultado das interações patógeno-hospedeiro, onde existem estratégias utilizadas para combater os mecanismos naturais de defesa do hospedeiro. Dessa forma, os fatores de virulência de *Candida* spp. caracterizam-se pela expressão de moléculas de superfície como as adesinas, a formação de biofilme, a secreção de enzimas hidrolíticas, a capacidade de alterar sua morfologia, dentre outros. Esses fatores garantem uma rápida adaptação e maior patogenicidade. Um grupo de fatores de virulência causa a colonização ou o início de uma infecção, enquanto o outro grupo ajuda a espalhar a infecção (MOYES et al., 2015).

O contato inicial do fungo se dá através da adesão às células epiteliais do hospedeiro, requerendo a ação de forças passivas, incluindo efeitos atraentes (forças de Van der Waals e interações hidrofóbicas) e repulsivas (forças de eletrorrepulsão mútuas). Tendo-se o contato inicial entre célula fúngica e célula epitelial, as adesinas interagem com os receptores da célula do hospedeiro (MOYES et al., 2015). Após a adesão inicial, o contato de *C. albicans*, por exemplo, com a célula hospedeira desencadeia a transição levedura-hifa. A invasão na célula hospedeira é então facilitada por invasinas e forças físicas, ocorrendo via endocitose ou penetração ativa (CIUREA et al., 2020).

A secreção de enzimas hidrolíticas extracelulares também auxilia no rompimento e invasão de *Candida* spp. nos tecidos do hospedeiro, tais como lipases, fosfolipases e proteinases. As fosfolipases facilitam a invasão nos tecidos do hospedeiro, hidrolisando as ligações éster dos glicerofosfolipídios da membrana celular. Em *C. albicans*, quatro tipos

de fosfolipases são conhecidas, as quais foram classificadas como Fosfolipase A, B, C e D, cada uma com a capacidade de clivar uma ligação éster específica. Cepas com maior atividade de fosfolipase exibem uma maior aderência às células epiteliais e consequentemente, maior patogenicidade (MARTIN et al., 2021; KHAN et al., 2020).

De forma geral, as proteases extracelulares de *Candida* spp. são conhecidas como enzimas digestivas de ampla atividade contra muitas proteínas do hospedeiro, como albumina, imunoglobulina e proteínas da pele, e que possuem como função a degradação de tecidos e aquisição de nutrientes em diferentes nichos de acolhimento, além de estarem envolvidas na evasão do sistema imunológico e propagação do processo inflamatório no hospedeiro (RAPALA-KOZIK et al., 2018). As proteases aspárticas (Saps1-3 e Saps4-6) são secretadas em *C. albicans* e a sua função na infecção não se deve apenas às suas propriedades proteolíticas, mas também devido ao seu papel na manutenção da integridade da parede celular fúngica e capacidade de adesão. A liberação de Sap4 e Sap6, por exemplo, está associada à resposta às armadilhas extracelulares de neutrófilos do hospedeiro (STANISZEWSKA, 2015).

De acordo com Erum et al. (2020), há uma menor produção de proteinase por *Candida* não-*albicans* em comparação com *C. albicans* e em seu estudo, os achados revelaram que a atividade de fosfolipase e proteinase foi mais pronunciada em *C. albicans* em comparação com as cepas de *Candida* não-*albicans* testadas. A produção de proteinases e fosfolipases foram observadas em 90,74% e 85,18%, respectivamente, em 54 isolados de *C. albicans*, contrastando com os testes realizados com 74 cepas de espécies de *Candida* não-*albicans*, onde 70,27% e 27,02% produziram proteinases e fosfolipases, respectivamente. Contudo, segundo os autores, ainda não se sabe porque ocorre essa discrepância entre *Candida* spp.

Em relação à atividade hemolítica de *Candida* spp., também considerado um importante fator de virulência, a literatura aponta que, para tal, há a ação da Hemolisina e que existem três diferentes tipos. As toxinas formadoras de poros incluem a hemolisina que forma poros transmembrana na célula hospedeira, resultando em lise celular. As hemolisinas tensoativas incluem substâncias produzidas por microrganismos que são capazes de construir e desintegrar a membrana celular. A hemolisina enzimática inclui enzimas que danificam a bicamada fosfolipídica da membrana das células hospedeiras (ERUM et al., 2020). O fator hemolítico desregula os eritrócitos e permite a assimilação de ferro do grupo hemoglobina-heme, promovendo a sobrevivência dentro do hospedeiro através de uma maior capacidade de sequestrar o ferro. Essa capacidade hemolítica pode variar entre as espécies de *Candida*,

podendo desempenhar um papel importante na infecção da corrente sanguínea, onde eritrócitos circulantes são expostos diretamente às células fúngicas ou indicar um potencial para a geração de episódios hemolíticos agudos localizados (MIRONOV; LEONOV, 2016; FURLANETO et al., 2018).

O biofilme também representa um importante fator relacionado à virulência de espécies de *Candida* cujas células sésseis que compõe a sua estrutura exibem fenótipo e genótipo diferenciados, de forma que os biofilmes se mostram vantajosos para a sobrevivência do microrganismo, uma vez que conferem proteção contra o ambiente, resistência a estresses físicos e químicos, cooperação metabólica e regulação da expressão gênica (CARVALHO et al., 2006; RAJA et al., 2010; YANG et al., 2012).

4.3 Biofilme

Os biofilmes são definidos como comunidades microbianas organizadas, cercadas por uma matriz extracelular autoproduzida, composta de materiais exopoliméricos (WALL et al., 2019). Essas células aderentes (sésseis) têm propriedades distintas das células que estão livres e flutuantes (planctônicas). Embora estejam ligados frequentemente a superfícies sólidas, os biofilmes podem ser formados em outros ambientes, como por exemplo, interfaces líquido-ar (NOBILE; JOHNSON, 2015).

Essa matriz extracelular produzida pelas células presentes no biofilme é composta de substâncias poliméricas extracelulares (EPS) capazes de aderir a uma superfície biótica ou abiótica. As EPS são compostas principalmente por polissacarídeos, proteínas, lipídios e ácidos nucléicos (RNA e DNA extracelular), que formam uma mistura polar altamente hidratada que contribui para a arquitetura geral e a estrutura tridimensional de um biofilme (WIN et al., 2019). Dentro dessas classes de substâncias, há ampla variação nos tipos específicos e proporções de macromoléculas entre os diferentes tipos de biofilme (JAKUBOVICS et al., 2021). É considerado um importante mecanismo de sobrevivência, amplamente distribuído no meio ambiente.

A formação de biofilme por microrganismos se dá em resposta a vários estresses ambientais ou também defendido como uma forma natural de crescimento de diversos microrganismos. Sabe-se que a capacidade de formar biofilmes é importante para o crescimento desses microrganismos em ambientes extremos diversos, tais como exposição a

variações de temperatura, pH, alta salinidade, alta pressão, exposição à radiação ultravioleta e ação de antimicrobianos (WIN et al., 2019).

Os biofilmes existem em quase todos os lugares, habitando implantes médicos, tecidos vivos, canais de água, tubulações, pisos de hospitais, unidades de processamento de alimentos e outras superfícies bióticas e abióticas. Dessa forma, a formação de biofilme contribui para o desenvolvimento de resistência a antimicrobianos e para a formação de comunidades microbianas persistentes que são responsáveis pela maior dificuldade em combater infecções microbianas. Os biofilmes são responsáveis por diversas manifestações patológicas e algumas das infecções teciduais associadas incluem periodontite, osteomielite, infecção pulmonar na fibrose cística, endocardite, placa dentária, amigdalite crônica, laringite crônica, feridas crônicas e infecções do trato biliar e urinário (RATHER et al., 2021).

Ainda, foi evidenciado que os biofilmes microbianos podem ser produzidos por apenas uma espécie (biofilme monoespécie) ou pela associação de vários microrganismos (biofilme misto), podendo incluir fungos, bactérias, protozoários e algas (AZEVEDO; CERCA, 2012). Os biofilmes mistos são os mais comuns na natureza, podendo ser compostos por espécies do mesmo reino ou de reinos diferentes, podendo uma espécie auxiliar a outra na adesão, por meio da liberação de metabólitos no meio (DONLAN, 2002; AZEVEDO; CERCA, 2012).

O processo de desenvolvimento de biofilmes ocorre em várias etapas. Inicia-se com a adesão inicial do microrganismo ao substrato e fixação irreversível, seguida da sua colonização, modificação na expressão de genes/proteínas seguida e fase de crescimento exponencial. Ocorre a formação de exopolissacarídeos (EPS) e canais de água, facilitando o aporte de nutrientes que levam à maturação dos biofilmes (SHARMA et al., 2019).

O aumento da biomassa de um biofilme acontece, tanto pela divisão celular, quanto pela redistribuição de células entre as microcolônias e pela adesão de novas células planctônicas (AZEVEDO; CERCA, 2012; GULATI; NOBILE, 2016). Concomitantemente, ao aumento da biomassa, células similares às células planctônicas iniciais se desprendem do biofilme para o ambiente externo, podendo formar um novo biofilme em outro sítio, caracterizando assim, um ciclo de contaminações (FLEMING, RUMBAUGH, 2017).

4.4 Biofilmes de *Candida* spp.

Para *Candida* spp., foi observado que o desenvolvimento do biofilme segue etapas sequenciais ao longo de um período de 24 a 48 h. Inicialmente, uma única célula de levedura adere ao substrato formando uma base para a camada de células, iniciando a etapa de adesão. As leveduras *C. albicans* se fixam a uma superfície (por exemplo, epitélios, biomateriais ou agregados celulares) através de adesinas, como membros da família Als. Em seguida inicia-se a fase de proliferação celular onde as células se projetam e continuam a crescer na estrutura filamentosa através da superfície (etapa de iniciação). As leveduras transitam para hifas e este processo é regulado por muitos fatores de transcrição incluindo Tec1p e Efg1p. As hifas expressam adesinas específicas, como Hwp1p e Hyr1p. A montagem das hifas marca o início da formação do biofilme acompanhado pelo acréscimo de uma matriz extracelular (MEC) no biofilme, fornecendo suporte estrutural e proteção contra antifúngicos e o sistema imunológico do hospedeiro. A adesão é mantida e o metabolismo de aminoácidos é aumentado no biofilme. As células de levedura não aderentes se desprendem do biofilme para o ambiente externo para encontrar um local favorável de fixação (etapa de dispersão). A disseminação de células de levedura associadas ao biofilme tem um importante significado clínico, pois podem iniciar a formação de novos biofilmes e alcançar outros tecidos do hospedeiro (PONDE et al., 2021; ATRIWAL et al., 2021). Um fator importante é o *Quorum sensing*, um mecanismo de comunicação microbiana em que o acúmulo de moléculas de sinalização permite que uma célula detecte uma densidade celular. Ele regula várias características, sendo uma delas a formação de biofilme. *Quorum sensing* é um mecanismo bem conhecido e difundido de comunicação célula-célula em bactérias, em que elas se comunicam por meio de moléculas sinalizadoras chamadas autoindutores e contribuem para a regulação da expressão gênica (PADDER et al., 2018).

Para alcançar uma comunicação célula-célula eficaz, os microrganismos produzem substâncias denominadas moléculas *Quorum-Sensing* (QSM), que controlam sua resposta a estímulos externos ou internos. QSM como farnesol e tirosol. Farnesol gerencia a filamentação na levedura polimórfica patogênica *Candida albicans*. Sua função principal na fisiologia de *C. albicans* está ligada à sinalização e iniciação de consequências danosas nas células hospedeiras e outros micróbios. Após esta descoberta, o álcool aromático tirosol também revelou ser um QSM de *C. albicans*, gerenciando o crescimento, morfogênese e formação de biofilme (RODRIGUES; ČERNÁKOVÁ, 2020).

A matriz extracelular protege as células aderentes contra o sistema imunológico do hospedeiro e agentes antifúngicos, formando uma extensa estrutura. A matriz extracelular é composta por aproximadamente 55% de uma combinação de glicoproteínas e 25% de carboidratos. Os carboidratos consistem em grande parte de polissacarídeos α-manana e β-1,6-glucana com β-1,3-glucanas em menor proporção. A MEC também consiste em 15% de lipídios e apenas 5% de ácidos nucleicos. O β-1,3-glucano desempenha um papel importante na resiliência à ligação específica dos derivados azólicos (ATRIWAL et al., 2021).

Há diferenças entre as características de biofilmes de diferentes espécies de *Candida*. A secreção de proteínas em *C. albicans* é conduzida pelo tráfego mediado por vesículas entre os compartimentos celulares para fora da superfície celular. No entanto, o aparato secretor difere entre células de levedura e hifas, indicando que diferentes formas morfológicas podem desempenhar contribuições distintas para o proteoma extracelular (JAKUBOVICS et al., 2021).

Candida albicans é considerada a espécie que mais produz biofilme sendo que estes são mais confluentes do que biofilmes de outras espécies, apresentando diferentes formas morfológicas: brotamento oval, hifas septadas contínuas e pseudohifas, em tecidos infectados. Em superfícies abióticas, biofilmes de *C. albicans* exibem uma densa rede de leveduras e células filamentosas envoltas em material exopolimérico. Os biofilmes de *C. glabrata* exibem uma MEC com altos níveis de carboidratos e proteínas. A estrutura do biofilme de *C. parapsilosis* pode variar de acordo com a cepa em estudo, mas geralmente compreende morfologias de leveduras e pseudohifas, produzindo uma multicamada compacta ou agregados celulares não contíguos, com MEC composta principalmente por carboidratos e baixos níveis de proteínas. Biofilmes de *C. tropicalis* possuem uma estrutura composta principalmente por células em forma de levedura, embora algumas linhagens tenham exibido formas filamentosas em biofilmes espessos de células agregadas ou em uma monocamada descontínua de leveduras ancoradas à superfície. Embora os biofilmes *de C. tropicalis* tenham MEC com baixo teor de carboidratos e proteínas, eles são mais resistentes ao desprendimento da superfície do que os biofilmes formados por *C. albicans* (CAVALHEIRO; TEIXEIRA, 2018). Os biofilmes de *Candida* têm, portanto, uma variedade de arquiteturas possíveis, propriedades de adesão, morfologias celulares e composição da MEC.

A formação de biofilme está fortemente ligada ao desenvolvimento de resistência a fármacos antifúngicos. Esse aumento da resistência quando espécies de *Candida* crescem

como biofilmes pode ser explicado por vários fatores e um deles é o aumento da atividade metabólica que ocorre no início do desenvolvimento do biofilme. Outra questão a considerar é o papel da MEC na resistência aos antifúngicos, servindo como uma barreira para impedir a difusão de fármacos (WALL et al., 2019). Biofilmes maduros de *C. albicans* são altamente tolerantes ao fluconazol, anfotericina B e caspofungina em concentrações que são letais para as células planctônicas (PONDE et al., 2021).

4.5 Manifestações clínicas associadas à *Candida* spp.

4.5.1 Candidíase oral

A maioria dos indivíduos adultos saudáveis possuem espécies de *Candida* na cavidade oral, fazendo parte da microbiota oral normal, não estando associado a processos patológicos (RADUNOVIC et al., 2022).

De acordo com Radunovic e colaboradores (2022), a prevalência de *Candida* spp. na microbiota oral em indivíduos saudáveis é de até 70%, e *C. albicans* é a espécie frequentemente isolada. As áreas subgengivais e o dorso da língua apresentam diversas propriedades ecológicas. Primeiramente, o biofilme subgengival está aderido a uma superfície dura não descamativa, com diferentes colonizadores primários, o potencial redox, pH, e nutrientes na placa subgengival diferem da língua, bem como a disponibilidade de oxigênio, dando à área subgengival o potencial para desenvolver diferentes biofilmes da língua. O nicho oral mais investigado de *Candida* sp. foi a língua, seguida pela mucosa bucal e palatina (RADUNOVIC et a., 2022).

Por se tratar de um fungo oportunista, a colonização patológica de espécies de *Candida* está relacionada a vários fatores tais como extremos de idade, desnutrição, doença metabólica, infecções concomitantes, terapia antibacteriana, imunossupressão, radioterapia, pacientes transplantados, hipofunção das glândulas salivares, doenças de longo prazo e terapia com corticoides (HELLSTEIN et al., 2019).

A grande maioria (cerca de 90%) dos pacientes infectados pelo HIV desenvolverão candidíase em algum momento, onde a colonização oral assintomática pode levar a lesões orais ou se tornar uma fonte de infecções disseminadas. A gravidade da infecção *por Candida* spp. em pacientes infectados pelo vírus HIV pode ser atribuída à imunodeficiência

do hospedeiro; no entanto, há dados que mostram uma forte associação entre a seleção natural de cepas e o aumento da virulência das espécies de *Candida* (ORLANDI et al., 2020)

Comumente, ocorre a remoção de células de *Candida* frouxamente aderidas das superfícies da mucosa através dos efeitos do fluxo salivar e da deglutição, sendo considerado um fator importante na defesa do hospedeiro contra o crescimento excessivo de *Candida* na cavidade oral, contudo, o fungo possui a capacidade de contornar esses mecanismos de remoção, através dos fatores de virulência. Dessa forma, tem-se a descrição de atributos relacionados à *Candida albicans* que contribuem para a patogênese, tais como a capacidade de aderência à superfície epitelial, através da expressão de adesinas de superfície; formação de biofilme, o que contribui para a falha da terapia antifúngica; evasão das defesas do hospedeiro em decorrência de sua capacidade de mudança fenotípica, resistência a estresses fagocíticos (resposta ao estresse oxidativo e nitrosativo, degradação proteolítica de fatores imunológicos do hospedeiro (anticorpos, peptídeos antimicrobianos etc.); capacidade de invasão e destruição dos tecidos do hospedeiro através do desenvolvimento de hifas e tigmotropismo (penetração tecidual), secreção de enzimas hidrolíticas tais como as aspartil proteinases (SAPs), fosfolipases e lipases, secreção de toxinas e endocitose induzida (VILA et al., 2020).

A patogênese por *Candida* spp. pode estar associada a quadros agudos, crônicos, lesões na cavidade oral associadas à *Candida* e lesões primárias queratinizadas infectadas com *Candida* spp. (SINGH et al., 2014) Os quadros clínicos mais comuns são: candidíase pseudomembranosa, mais comumente conhecida como “aftas”, com a presença de placas brancas na língua, mucosa oral, palato duro, palato mole e orofaringe; a candidíase hiperplásica, que se apresenta como placas brancas bem circunscritas, levemente elevadas, mais comumente aderidas à mucosa bucal e que podem envolver as comissuras labiais; a candidíase atrófica aguda, na qual observa-se a presença de placas eritematosas, mais comumente no palato, especialmente em pacientes com HIV; a candidíase atrófica crônica, mais conhecida como estomatite protética e que está associada ao uso de próteses dentárias totais; a queilite angular ou estomatite angular, que apresenta-se como placas eritematosas e fissuradas ao longo das comissuras da boca; dentre outras diversas condições associadas à infecção por *Candida* (MILLSOP et al., 2016).

4.5.2 Candidíase esofágica

Após a deficiência funcional do sistema imunológico do hospedeiro ou a aplicação de antibióticos, a composição da microflora no trato digestivo muda e a capacidade de invasão de fungos patogênicos oportunistas é aumentada através do mecanismo de regulação gênica, levando à infecção fúngica oportunista. Além das condições já conhecidas, relacionadas à imunidade do hospedeiro, principalmente HIV/AIDS, o uso frequente de fármacos inibidores da bomba de prótons, os quais têm como efeito a redução da secreção de ácido clorídrico no estômago, ou o uso de outros fármacos que suprimem a produção de ácidos, é considerada a causa mais comum de candidíase esofágica em indivíduos imunocompetentes (MOHAMED et al., 2019), em virtude da alteração da alteração da microbiota local e maior colonização por *Candida*. Em um estudo realizado na Coreia, com indivíduos sem comorbidades, os pesquisadores identificaram que o uso recente de antibióticos, corticosteróides, medicamentos fitoterápicos e o consumo excessivo de bebidas alcoólicas foram identificados como fatores de risco significativos para a candidíase esofágica, embora o uso de inibidor da bomba de prótons estivesse também associado à essa doença (CHOI et al., 2013). No exame endoscópico, é possível observar a presença de placas brancas no esôfago, exsudatos brancos e rupturas nas mucosas, sendo a disfagia um sintoma clássico de candidíase esofágica, podendo também ser relatados: dor abdominal, pirose, náusea, vômitos, perda de peso, dentre outros sinais e sintomas (ALSOMALI et al., 2017).

Um estudo de caso realizado com dois pacientes evidenciou o surgimento de carcinoma espinocelular após quadros crônicos de candidíase esofágica. Os autores relatam que é comum o surgimento dessa doença, secundariamente ao câncer de esôfago, no entanto, devido ao dano prolongado na mucosa, há evidências crescentes de que a própria infecção por *Candida* tem propriedades carcinogênicas, devido à incidência do desenvolvimento de carcinoma em pacientes com candidíase crônica. Um mecanismo que explicaria tal fenômeno seria a atividade catalítica de *Candida*, que facilita a produção de nitrosaminas cancerígenas como a nitroso-N-metilbenzilamina (NBMA) a partir de seus precursores (DELSING et al., 2012).

4.5.3 Candida Intestinal

Candida spp. também podem ser encontradas no intestino, contudo, o alto nível de colonização de *C. albicans* vem sendo frequentemente observado em pacientes com a barreira intestinal prejudicada. A barreira da mucosa intestinal atua na proteção contra a invasão de patógenos, seu crescimento excessivo e disseminação para a corrente sanguínea e órgãos profundos. Fatores como o comprometimento da resposta imune do hospedeiro, disfunção intestinal resultante de um distúrbio da microbiota intestinal, alterações de permeabilidade ou quebra da barreira intestinal e a mudança na morfologia de *Candida,* contribuem para a disseminação do fungo. Infecções por *C. albicans* podem estar relacionadas ao uso de dispositivos médicos invasivos, como acessos intravenosos, cateteres, sondas e drenos. Esses dispositivos contornam a barreira física proporcionada pela superfície da mucosa e facilitam o acesso de microrganismos à corrente sanguínea. Em alguns indivíduos suscetíveis, acredita-se que as infecções *por C. albicans* se disseminem a partir do trato gastrointestinal (TONG; TANG, 2017). Hu et al. (2021), também demonstraram o processo inverso, quando uma infecção por *C. albicans* transmitida pelo sangue é capaz exacerbar as alterações na histologia do cólon e aumentar a permeabilidade intestinal, contribuir para a diminuição da diversidade do microbioma bacteriano intestinal, alterando a microbiota local. Dessa forma, os autores identificaram que algumas bactérias intestinais alteradas eram altamente relevantes para mudanças nos metabólitos fecais, e todas essas alterações são capazes de romper a barreira intestinal.

4.5.4 Candidíase vulvovaginal

Em relação aos quadros clínicos associados a infecções por *Candida* spp.,a Candidíase Vulvovaginal (CVV) está entre os mais comuns, pois cerca cerca de 70% de toda população feminina terá, pelo menos, um episódio da doença durante sua vida reprodutiva, sendo por isso, considerada a segunda maior causa de vulvovaginite (CRUZ et al., 2020).

A forma leveduriforme é geralmente encontrada em mulheres assintomáticas e a presença de hifas tem sido consistentemente isolada de casos de CVV grave, corroborando com a associação da forma de levedura com o comensalismo e a forma de hifa com a patogenicidade (KALIA et al., 2020). *C. albicans* durante muito tempo foi considerado o patógeno mais comum causador de CVV, no entanto, observa-se o aumento da identificação

de *Candida* não-*albicans*, principalmente de *C. glabrata*. Outras espécies que devem ser levadas em consideração são *C. tropicalis, C. parapsilosis , C. kefyr , C. krusei , C. guilliermondii , C. famata* e *C. lusitaniae* . A produção de fatores de virulência por essas cepas depende do local e do grau de invasão, bem como da natureza da resposta do hospedeiro e influencia diretamente para o dano tecidual, pois a patogênese da CVV envolve três etapas, as quais são a adesão, seguida de invasão às células epiteliais, formação de biofilme e secreção de fatores de virulência (KALIA et al., 2020).

Em relação aos sinais e sintomas, o corrimento vaginal (leucorréia), prurido, alteração do pH vaginal, edema, hiperemia, dispareunia e disúria são citados como sendo sugestivos de candidíase vulvovaginal. Quanto ao corrimento vaginal, este é descrito como de textura espessa, coloração branca, com aspecto semelhante ao "leite coalhado". No tocante aos sintomas como prurido intenso e hiperemia, isso ocorre devido à invasão das células epiteliais da mucosa genital por *Candida* spp., provocando lesões teciduais, onde a capacidade de adesão e a ação das toxinas e enzimas expressas pelo agente infeccioso estão envolvidas neste processo de patogênese (CRUZ et al., 2020).

Candida spp. faz parte da microbiota normal da vagina, entretanto, como trata-se de um fungo oportunista, fatores relacionados ao hospedeiro, tais como gravidez, desequilíbrio hormonal, diabetes mellitus descompensado, imunossupressão por diferentes causas, que pode uso de antibióticos de amplo espectro e glicocorticóides e predisposições genéticas podem estar relacionados à incidência da doença. vale ressaltar que a CVV trata-se de uma condição clínica relevante, tendo em vista o desconforto gerado às mulheres acometidas e o aumento de CVV recorrente, caracterizada por quatro ou mais episódios sintomáticos em um ano e ocorre em virtude dos fatores predisponentes, falhas no tratamento convencional e aumento do número de espécies envolvidas na infecção (RODRÍGUEZ-CERDEIRA et al., 2020).

4.5.5 Candidíase invasiva

A candidíase invasiva compreende candidemia e candidíase profunda, que podem ocorrer concomitantemente ou de forma independente uma da outra. A candidemia primária decorre mais frequentemente da translocação de espécies de *Candida* comensais, do trato gastrointestinal, ou por contaminação de dispositivos médicos, tais como cateteres intravenosos, permitindo a disseminação hematogênica. A candidíase profunda também

pode resultar da introdução não hematogênica de *Candida* em locais estéreis, como a cavidade abdominal, após ruptura de estruturas do trato gastrointestinal. Dessa forma, os autores relatam que, para o diagnóstico de candidíase invasiva, é importante considerar e identificar três tipos de entidades: candidemia na ausência de candidíase profunda, candidemia associada a candidíase profunda e candidíase profunda na ausência de candidemia (CLANCY; NGUYEN, 2018).

Tais condições são importantes causas de morbidade e mortalidade, principalmente em pacientes imunocomprometidos e hospitalizados, com milhares de casos relatados em todo o mundo, devendo ser considerada como caso suspeito em qualquer paciente com fatores de risco conhecidos e que apresentem febre persistente, que não responde a antibacterianos de amplo espectro. O choque séptico é uma possível apresentação de candidemia e pacientes podem apresentar maior incidência de insuficiência renal e hepática, além de níveis mais baixos de lactato desidrogenase do que pacientes com choque séptico bacteriano (ANTINORI et al., 2016).

As espécies mais comuns de *Candida*, isoladas desses pacientes são *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. Parapsilosis* e *C. krusei* (VASILEIOU et al., 2018). Além da tendência global do aumento de casos de candidíase invasiva por *Candida auris* (PAPPAS et al., 2018). *C. albicans* ainda é a espécie mais comumente associada a quadros diversos de candidíase, no entanto, infecções por espécies de *Candida* não-*albicans* são responsáveis por um número crescente de casos. Especificamente, *C. glabrata* tornou-se um importante patógeno na América do Norte, Europa e Austrália, enquanto *C. parapsilosis* é a espécie não-*albicans* dominante na América do Sul, Japão e Espanha. Essas espécies são frequentemente resistentes ou tolerantes ao fluconazol, o que ocasiona maiores dificuldades em relação ao tratamento (BEN-AMI, 2018).

4.6 Resistência de *Candida* spp. aos antifúngicos

O termo resistência pode ser definido como quando uma cepa responde a uma concentração inibitória mínima (CIM) para um determinado antifúngico acima de pontos de corte já preconizados de interrupção do seu crescimento, podendo essa ser comparada a uma cepa controle ou de referência e aos parâmetros pré estabelecidos nos protocolos existentes. Já o termo tolerância, está relacionado à capacidade de uma cepa fúngica suscetível a

fármacos de crescer na presença de um antifúngico em concentrações acima da CIM (LEE et al., 2020).

O uso frequente e profilático de antifúngicos têm levado ao surgimento de cepas de *Candida* spp. resistentes a esses medicamentos, através da expressão de diversos mecanismos, tais como alterações nos alvos de fármacos, superexpressão de bombas de efluxo, modulação das respostas ao estresse, modificações genômicas, além da resistência intrínseca de algumas espécies de *Candida* aos antifúngicos amplamente utilizados, como o fluconazol e equinocandinas (LEE et al., 2020). De acordo com Singh et al. (2015), as terapias atualmente disponíveis para candidíase são baseadas em antifúngicos, incluindo azóis, equinocandinas e polienos. O fluconazol é o mais utilizado no tratamento de infecções por candidíase devido à sua alta biodisponibilidade e baixa toxicidade, no entanto, o seu uso clínico excessivo e indiscriminado levou ao surgimento de cepas multirresistentes de *C. albicans*, cabendo salientar que essas cepas multirresistentes ocorrem em frequências superiores às taxas de mutação e, consistente com isso, parecem ser geneticamente idênticas ao micróbio sensível (SINGH et al., 2015).

Os fatores de virulência, tais como a capacidade de produção de biofilme por *C. albicans* são relevantes para o perfil de sensibilidade aos antifúngicos, conferindo resistência até 1.000 vezes maior em cepas formadoras de biofilme em comparação com as demais. Além dos fatores de virulência, diversos mecanismos de resistência *in vitro* e *in vivo* já foram descritos na literatura. Por exemplo, *Candida albicans* pode se tornar resistente aos azólicos aumentando o número de bombas de efluxo em sua estrutura, com função de transporte, associado à membrana, impedindo o acúmulo intracelular de fármaco e reduzindo a sua citotoxicidade. Em relação à resistência às equinocandinas, mutações nas posições de aminoácidos no gene *FKS1* de *Candida albicans* têm sido associadas à maior resistência, devido à plasticidade genômica do fungo (PRISTOV; GHANNOUM, 2019).

Candida tropicalis exibe resistência aos derivados azólicos, mostrando alta resistência especificamente ao fluconazol, com mecanismo de resistência semelhante ao de outras espécies de *Candida* (PRISTOV; GHANNOUM, 2019), tais como alterações na via de biossíntese do ergosterol e superexpressão de bombas de efluxo (WHALEY et al., 2017). *Candida glabrata* é conhecida por mostrar resistência ao fluconazol, sendo sensível a concentrações inibitórias mínimas mais altas desse fármaco, quando comparada com outras espécies de *Candida*. A falha do tratamento e o surgimento de cepas resistentes ao fluconazol ocorrem com frequência durante o tratamento de isolados inicialmente suscetíveis, fazendo

com que a heterorresistência seja um achado frequente. Considerando essas características, a escolha pelo tratamento com o fluconazol para pacientes com infecções por *Candida glabrata* deve ocorrer em casos específicos e o monitoramento deve ser constante (BEN-AMI, 2018). *C. parapsilosis* é intrinsecamente menos suscetível às equinocandinas do que outras espécies de *Candida* e *C. krusei* é intrinsecamente resistente ao fluconazol, embora o mecanismo preciso não seja completamente compreendido. Vários estudos atribuíram a resistência inata de *C. krusei* aos azólicos à atividade da bomba de efluxo, nomeadamente através do transportador de cassete de ligação de ATP Abc1p, e à redução da acumulação de antifúngico. Também há casos de cepas resistentes ao itraconazol e voriconazol (WHALEY et al., 2017).

Considerando o exposto, há a necessidade de identificação de novas alternativas terapêuticas para as infecções causadas por *Candida* spp. Dessa forma, os Peptídeos Antimicrobianos (PAMs) apresentam-se como uma opção.

4.7 Peptídeos Antimicrobianos (PAMs)

Os PAMs são peptídeos curtos, compostos por 8 a 50 aminoácidos de baixo peso molecular, a maioria dos quais são catiônicos, contêm múltiplos resíduos hidrofóbicos, são anfipáticos e exibem atividade antimicrobiana de amplo espectro (LE et al., 2017). São moléculas que fazem parte do sistema imune inato de vertebrados e invertebrados, possuindo um amplo espectro de atividades contra fungos, bactérias, vírus e parasitas (BRANDENBURG et al., 2012).

Os PAMs são bem preservados em eucariotos, tendo em vista que são componentes do sistema imune inato e a produção desses peptídeos pelas células hospedeiras requer menos tempo e energia do que a síntese de anticorpos pela imunidade adquirida. Uma outra vantagem está relacionada ao fato de que essas moléculas, por serem pequenas, podem atingir o alvo mais rapidamente do que as imunoglobulinas. Além disso, alguns eucariotos carecem de sistema imunológico baseado em linfócitos, tais como os insetos, por exemplo, que dependem principalmente da síntese de uma série de compostos antibacterianos para remover microrganismos invasores (ZHANG et al., 2021).

A existência de peptídeos antimicrobianos já foi descrita em diversos grupos de eucariotos, tais como moluscos, insetos, crustáceos, plantas, anfíbios, peixes e mamíferos

(BRANDENBURG et al., 2012; RIZZA et al., 2008; ROSA; BARRACCO, 2010). Em humanos, os PAMs podem ser suplementados por células do sistema imunológico (por exemplo, mastócitos, macrófagos e neutrófilos) em locais de lesão durante a resposta inflamatória aguda (LE et al., 2017).

A questão central na pesquisa de novos PAMs é saber como esses peptídeos são capazes de atingir especificamente o patógeno invasor enquanto poupam as células hospedeiras. Diante disso, as diferenças na composição da membrana celular entre os patógenos e as células hospedeiras são consideradas para sustentar a especificidade de direcionamento (ZHANG et al., 2021).

A capacidade dos peptídeos antimicrobianos em se associar com a membrana da célula é uma característica chave, embora a permeabilização da membrana não seja considerada um requisito essencial, tendo em vista a importância de sua atividade intracelular e imuno-moduladora (KERKIS et al., 2014). Os peptídeos antimicrobianos catiônicos exercem atividade antibacteriana interagindo com a membrana bacteriana carregada negativamente para aumentar a permeabilidade da membrana e levar à lise da membrana celular e liberação do conteúdo celular. Ao se aproximar da membrana citoplasmática através da interação eletrostática, os PAMs se ligam à membrana microbiana e interagem com os componentes aniônicos (ZHANG et al., 2021).

A interação dos peptídeos com as membranas resulta na formação de poros tipo “barrel-stave”, “carpet-like” ou “toroidal”, e essas interações ocorrem em todos os peptídeos antimicrobianos (LE et al., 2017).

No modelo de “barrel-stave” os monômeros peptídicos são dispostos paralelamente aos fosfolipídios da membrana da célula, formando um canal transmembrana hidrofílico (LE et al., 2017). Após a interação eletrostática entre o peptídeo e a membrana, as moléculas dos peptídeos se estruturam em α-hélice e se inserem através da bicamada lipídica, de modo que as regiões hidrofílicas do peptídeo formem a face interior do poro, permitindo o extravasamento de conteúdo citoplasmático. A etapa crítica na formação do poro é a inserção do monômero do peptídeo na membrana, pois sua estruturação transmembrânica é normalmente desfavorável energeticamente (SHAI, 2002).

Em relação ao modelo “carpet-like” a acumulação de peptídeos na superfície da célula, contato este mediado por interações eletrostáticas. Após a interação inicial, os peptídeos induzem fraquezas locais e dividem as bicamadas da membrana em pequenas áreas revestidas pelas unidades peptídicas, dando uma aparência de carpete. O resultado é a

desintegração da membrana em decorrência da modificação de sua curvatura (LE et al., 2017, SHAI, 2002).

No modelo “toroidal”, a agregação em cascata de monômeros peptídicos na membrana da célula, faz com que as partes lipídicas se dobrem para dentro, formando um canal contínuo, revestido por múltiplas unidades pépticas (poro) (LE et al., 2017).

Além de mecanismos de ação membranolíticos, os peptídeos podem apresentar modelos de ação não-membranolíticos. Após a primeira interação com a membrana, ocorre a formação de poros transitórios e em seguida, o transporte de peptídeos para o interior da célula (MAROTI et al., 2011). Os PAMs podem ter múltiplos alvos intracelulares, e podem se ligar ao DNA, RNA e proteínas, inibindo, por exemplo, a síntese da parede celular e a citocinese em células bacterianas, inibição da síntese de outras proteínas e inibição da atividade enzimática (BROWN et al., 2006).

4.8 Ctn (Crotalicidina)

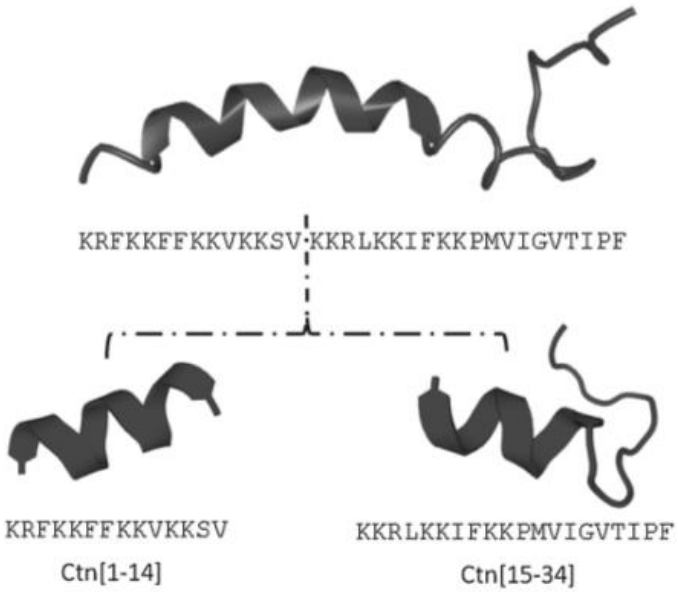
As catelicidinas (peptídeos antimicrobianos de vertebrados) são consideradas como sendo um grupo importante de peptídeos “host-defense” do hospedeiro, tendo ampla distribuição entre as espécies de animais e amplo espectro de atividade antimicrobiana (SCHWEIZER, 2009).

Recentemente, a Crotalicidina (Ctn) um peptídeo alfa-helicoidal linear com 34 resíduos de aminoácidos, que pertence ao grupo das vipericidinas e à família das catelicidinas, foi caracterizada a partir da glândula de veneno da *Crotalus durissus terrificus*, cascavel da América do Sul, e sua atividade antiproliferativa foi demonstrada *in vitro* contra bactérias (FALCÃO et al., 2014). Além disso, a atividade contra protozoários tripanossomatídeos também foi evidenciado por Ctn e seus congêneres altamente conservados, bathroxicidin, que diferem de Ctn por oito resíduos de aminoácidos não críticos (MELLO et al., 2017; BANDEIRA et al., 2017).

O Ctn foi dissecado estrutural e funcionalmente em dois fragmentos de farmacóforos, um com 14 resíduos referentes a porção N-terminal, Ctn[1-14] e a fração C-terminal com 20 resíduos Ctn[15-34], tal como ilustrado na Figura 1, mantendo a atividade antimicrobiana e antitumoral do peptídeo de comprimento total, o Ctn (FALCÃO et al., 2015). A Crotalicidina contém repetições em tandem de nove resíduos de aminoácidos (1 KRFKKFFKK 9 e 16

KRLKKIFKK 24; consenso: 1 KRhKKhFKK 9, h = aminoácido hidrofóbico) como parte integrante de sua estrutura (LIMA et al., 2022).

Figura 1. Sequências primárias e estruturas 3-D de crotalicidina (Ctn) e fragmentos projetados Ctn[1–14] e Ctn[15–34.



Fonte: FALCÃO; RÁDIS-BAPTISTA / Peptides (2020).

Os fragmentos N- e C- terminal, em comparação com a sequência completa do Ctn, mostraram atividades e seletividades diferentes *in vitro* contra bactérias Gram-negativas e algumas linhagens de células tumorais. Em bactérias Gram-negativas, tais como *Escherichia coli* e *Pseudomonas aeruginosa*, o mecanismo da ação de Ctn [15-34] envolve a perturbação de membrana, internalização citoplasmática e interação com ácidos nucléicos (PÉREZ-PEINADO et al., 2018).

Os efeitos antifúngicos destes peptídeos também foram recentemente demonstrados contra cepas de *Candida* spp. e *Cryptococcu*s spp. susceptíveis à anfotericina B (CAVALCANTE et al., 2017), e foi demonstrado que o mecanismo de morte por Ctn[15-34] envolve rompimento de membrana e indução de apoptose precoce e necrose tardia (CAVALCANTE et al., 2018). Além disso, o Ctn [15-34] também foi eficaz *in vitro* como um agente antiviral contra um tipo de vírus RNA que afeta camarões (VIEIRA-GIRÃO et al., 2017).

Recentemente, o análogo estrutural, Ctn[1-9], conjugado com Rodamina B, sendo denominado RhoB-Ctn[1-9], apresentou em estudo anterior, atividade antimicrobiana

seletiva contra bactérias Gram-negativas infecciosas como *Escherichia coli, Pseudomonas aeruginosa* e espécies patogênicas de *Candida* com baixos efeitos hemolíticos em eritrócitos humanos, sendo capaz de permear as membranas celulares e acumular-se intracelularmente em células microbianas (LIMA et al., 2022). Trata-se de uma nova estrutura, tendo em vista a observação de repetições em tandem de nove resíduos de aminoácidos (1KRFKKFFKK9 e 16KRLKKIFKK24), com o consenso 1KRhKKhFKK9, onde h é um resíduo de aminoácido hidrofóbico (Figura 2).

Figura 2. Repetições em tandem de nove resíduos de aminoácidos na estrutura da crotalicidina e seus fragmentos.

Peptide	Sequence[a]
Crotalicidin$_{1-34}$	$_{1}$KRFKKFFKKVKKSVKKRLKKIFKKPMVIGVTIPF$_{34}$
Ctn[1-14]	$_{1}$KRFKKFFKKVKKSV$_{14}$
Ctn[1-9]	$_{1}$KRFKKFFKK$_{9}$
Ctn[15-24]	$_{15}$KKRLKKIFKK$_{24}$
Ctn[15-34]	$_{15}$KKRLKKIFKKPMVIGVTIPF$_{34}$
Segment 16-24	$_{16}$KRLKKIFKK$_{24}$
Consensus	$_{1}$KRhKKhFKK$_{9}$

Fonte: LIMA et al. / Current Pharmaceutical Biotechnology (2022).

5. MATERIAIS E MÉTODO

5.1 Tipo de estudo

Trata-se de um estudo analítico com abordagem quantitativa. Nos estudos quantitativos é utilizada análise estatística para que as informações encontradas resultem em dados de formato numérico, visto que as variáveis descritas no presente projeto se apresentam desta forma. A observação da relação entre variáveis, possibilitando a realização da inferência, ou seja, concluir algo com base em informações limitadas, principalmente quando se tem uma amostra representativa da população faz referência ao estudo analítico (POLIT; BECK, 2011). Para isso, foram realizados os cruzamentos entre as variáveis independentes e dependentes descritas a seguir a fim de confirmar as hipóteses do estudo e responder às questões de pesquisa.

5.2 Local e período do estudo

Este estudo foi realizado no Laboratório de Microbiologia da Universidade da Integração Internacional da Lusofonia Afro-Brasileira (UNILAB), Redenção - Ceará. no Laboratório de Biotecnologia do Centro de Estudos Ambientais Costeiros (CEAC) da Universidade Federal do Ceará (UFC), Eusébio - Ceará e no LEPABE - Laboratório de Engenharia de Processos, Ambiente, Biotecnologia e Energia (Universidade do Porto). As atividades foram realizadas no período de 2019 a 2022, com pausa em 2020, em decorrência da pandemia de COVID-19.

5.3 Cepas utilizadas

Para este estudo, foram utilizadas cepas de *Candida* sp., coletadas a partir da microbiota oral de indivíduos saudáveis. Também foram utilizados para os testes, isolados de *Candida* spp., de diferentes tipos de amostras clínicas tais como sangue, urina, lavado broncoalveolar, aspirado traqueal e raspado de unha, correspondendo a um total de 35 cepas, listadas na Tabela 1. As mesmas foram previamente isoladas e identificadas entre 2017 e 2018 e atualmente fazem parte da Micoteca do Laboratório de Microbiologia da UNILAB.

A identificação das cepas foi realizada através de cultura em meio cromogênico (CHROMagar *Candida*®) e técnicas de biologia molecular.

Tabela 1. Banco de cepas de *Candida* spp. estocadas no Laboratório de Microbiologia da UNILAB.

Espécies	Origem das Cepas		
	Microbiota Oral [a]	Cepas Clínicas [b]	ATCC [c]
Candida albicans	n = 20	n = 6	n = 1
Candida tropicalis	n = 0	n = 2	n = 0
Candida glabrata	n = 2	n = 0	n = 0
Candida krusei	n = 1	n = 3	n = 0

[a]Isolados da microbiota oral de indivíduos saudáveis; [b]Isolados de *Candida* spp. de diferentes amostras clínicas de indivíduos doentes; [c]ATCC 90028.

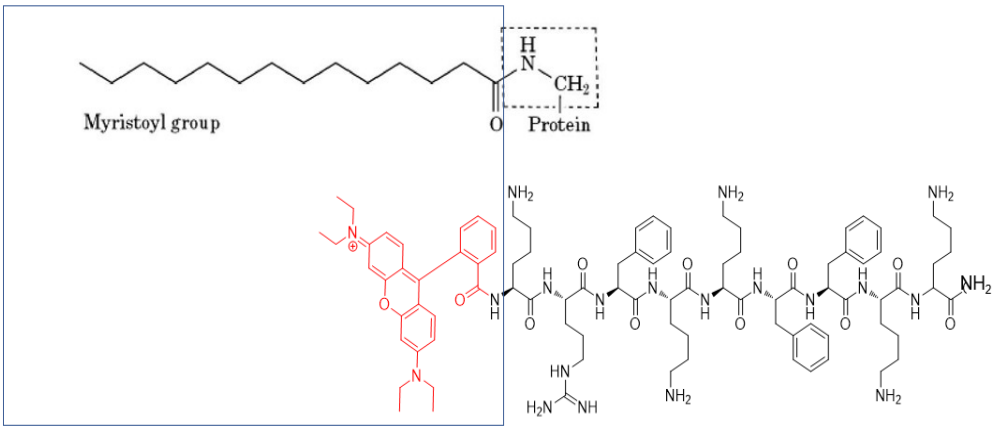
As amostras foram obtidas durante um estudo realizado anteriormente pela própria pesquisadora, aprovado pelo Comitê de Ética em Pesquisa da UNILAB conforme protocolo CAAE: 59953716.5.0000.5576, número do parecer: 1.937.092.

5.4 Peptídeos

Para este estudo, foram utilizados o Myr-Ctn[1-9] e o Ctn[15-34] os quais são fragmentos da Crotalicidina (Ctn[1-34]), derivado de uma catelicidina expressa nas glândulas de veneno da serpente sul-americana *Crotalus durissus terrificus*. Os peptídeos foram sintetizados por síntese orgânica em fase sólida e após a síntese foram purificados por cromatografia líquida de alta eficiência (HPLC) com grau de pureza maior que 95%. Em seguida caracterizados por espectrometria de massas (FALCÃO et al., 2015). Foram liofilizados e armazenados a uma temperatura de -20ºC.

Quanto ao Ctn[1-9], procedeu-se com a miristoilação, que é a adição do ácido tetradecanóico (ácido mirístico - *Myristoyl),* um ácido graxo de 14 carbonos, em ligação covalente com a porção N-terminal do peptídeo Ctn [1-9], com o objetivo de torná-lo mais hidrofóbico e melhorar a capacidade de interação com a membrana da célula fúngica (Figura 3).

Figura 3. Estrutura do peptídeo Myr-Ctn[1-9].



Dessa forma, o peptídeo foi denominado Myr-Ctn[1-9]. A sequência de aminoácidos de ambos os peptídeos se encontra no Quadro 1.

Quadro 1. Descrição dos fragmentos peptídicos de Crotalicidina utilizados nos experimentos.

Peptídeos	Tamanho (nº de resíduos)	Sequência	Massa Molecular (g/mol)
Ctn [15-34]	20	KKRLKKIFKKPMVIG VTIPF	2.372,11
Myr-Ctn [1-9]	9	Myr-KKRLKKIFKK	1.466,99

5.5 Antifúngicos

Para a realização dos testes de sensibilidade das cepas de *Candida* spp., tanto em sua forma planctônica, como após a formação do biofilme, foram utilizados os antifúngicos anfotericina B, fluconazol e Itraconazol.

Anfotericina B e Itraconazol foram diluídas em DMSO (dimetilsulfóxido) e o fluconazol foi diluído em água destilada estéril. Em seguida, foram filtradas a partir do uso de membrana filtrante de 0,22µm, para garantia de esterilidade da solução. Os medicamentos foram estocados a -20ºC e diluídos no momento do uso em meio RPMI 1640 com L-glutamina e sem bicarbonato de sódio (CLSI, 2008).

5.6 Produção de Fosfolipases

A produção de fosfolipases foi avaliada através do protocolo descrito por Price, Wilkinson e Gentry (1982), onde o meio ágar gema de ovo (ágar sabouraud dextrose 2% adicionado de 1 mol/L de cloreto de sódio, 0,05 mol/L de cloreto de cálcio e 8% de uma emulsão de gema de ovo estéril a 30%) foi distribuído em placas de Petri de 90mm estéreis que foram mantidas sob refrigeração até o momento de uso. O inóculo das cepas de *C. albicans, C. tropicalis, C. krusei, C. glabrata* e *Candida* sp. foi preparado após 24h de crescimento das mesmas em ágar batata dextrose e incubação a 35ºC, para isso foi utilizado solução salina estéril até atingir uma concentração final de quatro na escala de McFarland. Cinco microlitros de cada inóculo foram pipetados em disco de papel filtro esterilizado de aproximadamente 5mm, e estes foram depositados nas placas contendo meio ágar gema de ovo. Elas foram incubadas por 7 dias a 35ºC, e a atividade enzimática (Pz) determinada após esse período através do cálculo para obter a razão entre o diâmetro da colônia fúngica e o diâmetro total (colônia + halo da zona de precipitação). Pz=1 indicará que os isolados não produzem fosfolipases; quando $1 > Pz \geq 0{,}64$ a cepa foi considerada produtora; e $Pz < 0{,}64$ indicará forte produção (SIDRIM *et al.*, 2010).

5.7 Atividade Hemolítica

Para avaliação de atividade hemolítica por parte das cepas de *Candida*, foi utilizada a metodologia descrita por Favero et al. (2011), com modificações. Nesta os isolados a serem testados foram previamente cultivados em meio RPMI 1640 e incubados a 37ºC por 24 horas. Após esse período, os tubos foram centrifugados a 3.000 rpm por 10 minutos e o sobrenadante descartado. A solução salina estéril foi utilizada para lavagem das culturas que foram centrifugadas novamente e o sobrenadante descartado. Em seguida, 2µL da suspensão celular foram inoculadas pontualmente em placas contendo ágar sangue de carneiro enriquecido (ágar Sabouraud suplementado com 3% de glicose e 7% de sangue de carneiro desfibrinado), e estas incubadas a 37ºC por 48 horas. Após esse período, a análise se dará quanto à presença de halo translúcido e/ou esverdeado ao redor das colônias, indicando atividade hemolítica positiva. Os halos foram mensurados e a atividade hemolítica classificada como ausente (sem halo visível), fraca (halo ≤ 1 mm), moderada (halo medindo de 1,1 a 1,49 mm), ou forte (halo $\geq 1{,}5$ mm) (FAVERO et al., 2014). Uma cepa de

Streptococcus pyogenes (beta-hemolítico) e uma de *Streptococcus sanguinis* (alfa-hemolítico) foram utilizadas como controle.

5.8 Atividade antifúngica de fármacos e fragmentos peptídicos frente às células planctônicas

Para a avaliação da atividade antifúngica de fármacos e do fragmento peptídico, foi realizado o Teste de Suscetibilidade a Antifúngicos, por método de Microdiluição em Caldo, de acordo com a Norma M27-A3, a qual descreve o método preconizado pelo *Clinical Laboratory Standards* (CLSI), instituição internacional que desenvolve normas e padrões para a realização de testes de doença clínica e questões relacionadas à atenção à saúde (CLSI, 2008; SIDRIM; ROCHA, 2012).

Após a diluição seriada, as concentrações finais dos peptídeos variaram entre 0,0195 e 40 μM (CAVALCANTE et al., 2016). Cada poço da placa de 96 poços foi inoculado, no dia do teste, com 100μL a correspondente suspensão concentrada do inóculo. Os poços selecionados para o controle de crescimento continham 100μL de meio estéril, isento de fármaco, e inoculados com 100μL do inóculo. A última fileira da placa de microdiluição foi utilizada para efetuar o controle da esterilidade utilizando apenas o meio RPMI isento de fármaco.

As soluções-mãe de antifúngicos foram preparadas em concentrações de, pelo menos, 1.280 μg/mL ou dez vezes a concentração mais alta a ser testada. As concentrações dos fármacos testadas, depois de diluídas em meio RPMI, a partir de cada solução-mãe, corresponderam no primeiro poço das placas de microdiluição a: Anfotericina B – 16μg/mL; Itraconazol - 16μg/mL; Fluconazol – 64 μg/mL. Em seguida, foi realizada a diluição seriada.

As placas de microdiluição foram incubadas a 35° C por 24 horas e o valor de CIM (Concentração Inibitória Mínima) foi definido como a menor concentração capaz de inibir o crescimento visual dos microrganismos. Para Anfotericina B, determina-se CIM como a menor concentração capaz de inibir 100% do crescimento visível e para os azólicos, a CIM é definida como a menor concentração capaz de inibir 50% do crescimento fúngico quando comparado com o controle de crescimento.

Para cepas de *Candida albicans*, *Candida tropicalis* e *Candida parapsilosis*, foram aplicados os seguintes valores de corte: para o Fluconazol, com CIM ≤ 2μg/mL, 4μg/mL e ≥ 8μg/mL foram considerados como sensível, sensibilidade dose-dependente e resistente, respectivamente (MODIRI et al., 2019). Cepas de *Candida glabrata* são consideradas

sensibilidade dose-dependente quando CIM ≤ 32 µg/mL e resistentes ao Fluconazol quando CIM for ≥ 64 µg/mL (VIEIRA et al., 2018). Para o Itraconazol foram consideradas: CIM ≤ 0,125µg/mL, 0,25 - 0,5µg/mL e ≥ 1 µg/mL, podendo as cepas ser classificadas como sensível, sensibilidade dose-dependente e resistente, respectivamente (HASSANMOGHADAM et al., 2019). Para Anfotericina B, os isolados foram considerados sensíveis quando a CIM foi ≤ 1µg/mL e resistentes quando CIM > 1µg/mL.

5.9 Biofilme

5.9.1 Microrganismos

A cepa de referência, *Candida albicans* SC5314, foram adquiridas da American Type Culture Collection e cultivada em Sabouraud Dextrose Agar (SDA) (Merck, Darmstadt, Alemanha), sob condições aeróbicas por 24 h a 37°C. Optou-se pela utilização dessa cepa padrão, em detrimento dos outros isolados utilizados no estudo em razão da reconhecida capacidade de formar biofilme, sendo previamente utilizada em outros estudos (Alves et al., 2023). Trata-se de uma cepa selvagem, originalmente isolada de um paciente com infecção generalizada, virulenta em um modelo de camundongo de infecção sistêmica, três colônias foram transferidas de SDA para 50 mL de caldo líquido Sabouraud dextrose (Merck, Darmstadt, Alemanha) em um frasco de 100 mL e incubadas a 37ºC por 18 h.

5.9.2 Preparo do Inóculo

Candida sp. cepas foram cultivadas em ágar Sabouraud Dextrose e incubadas por 24 h a 37°C. Para preparar o inóculo, as células foram então inoculadas e incubadas por 18 h a 37 ◦C sob agitação a 120 rpm. Após a incubação, a densidade do inóculo foi ajustada para a turbidez de 1 × 105 células/mL com RPMI-1640 (Sigma-Aldrich, St. Louis, MO, USA) (RODRIGUES et al., 2018).

5.9.3 Antifúngico

O fluconazol foi fornecido pela Pfizer® (Nova York, NY, EUA) em sua forma pura. Alíquotas de 5.000 mg/L de fluconazol (Flu), foram preparadas com dimetilsulfóxido (DMSO). As concentrações finais utilizadas foram preparadas em RPMI-1640 (Sigma-Aldrich).

5.9.4 Análise de inibição da formação do biofilme por Ctn[15-34]

A caracterização da formação de biofilme por *Candida* albicans foi realizada de acordo com Rodrigues e Henriques (2018). Um total de 100 μL do inóculo de cada cepa foi transferido para cada poço da microplaca de 96 poços e adicionados 100 μL de RPMI-1640 suplementado ou não com antifúngicos por 48h a 37°C. O antifúngico usado como controle foi o fluconazol a uma concentração de 1.250 mg/L, previamente determinada como a concentração mínima para erradicar o biofilme (ALVES et al., 2023). O fragmento peptídeo Ctn [15-34] foi usado em três diferentes concentrações: 5 μM, 75 μM e 100 μM. Este foi escolhido por estar melhor caracterizado, levando em consideração os estudos já existentes acerca da atividade antifúngica e mecanismo de ação e também para confirmar a sua atividade antibiofilme (AGUIAR et al., 2020).

Poços contendo apenas meio de cultura sem inóculo foram usados como controle negativo. Todo o experimento foi realizado em triplicata. Após a incubação, a biomassa do biofilme foi analisada pelo ensaio do cristal violeta. Para isso, o sobrenadante foi aspirado cuidadosamente e os poços foram lavados duas vezes com 200 μL de PBS (Solução Salina Tamponada com Fosfato, 0,1M, pH=7,2). Posteriormente, o biofilme foi fixado em metanol 100%, 200 μL/poço, por 20 min. Após a secagem, o sobrenadante foi aspirado e 200 μL de cristal violeta aquoso a 1% foram adicionados a cada poço (Sigma-Aldrich, EUA). Após 5 min, a solução de corante foi aspirada e os poços foram lavados duas vezes com água destilada estéril. Posteriormente, 200 μL de uma solução de ácido acético a 33% foram adicionados a cada poço e imediatamente transferidos para uma nova placa de 96 poços. Em seguida, as placas foram lidas a 570 nm, para verificar a Densidade Óptica (DO) dos poços tratados ou não com o peptídeo.

5.10 Análise estatística

Utilizou-se, a priori, a estatística descritiva com o objetivo de sintetizar e descrever os dados quantitativos. Para tanto, foi aplicada a medida de distribuição por frequência, método utilizado para impor ordem a valores numéricos (POLIT; BECK, 2011). Foram empregadas ainda medidas de tendência central, representadas pela média aritmética (soma de todos os valores, dividida pelo número total de participantes) e mediana (ponto em que há a divisão dos escores na metade da distribuição de valores), assim como medida de dispersão,

representada pelo desvio padrão (índice de variabilidade para identificar a quantidade média de desvio dos valores em relação à média aritmética) (POLIT; BECK, 2011).

Optou-se por utilizar diretamente testes estatísticos não paramétricos, em virtude do tamanho da amostra reduzido (LEOTTI et al., 2012). Dessa forma, para a comparação envolvendo variáveis independentes quantitativas, foi aplicado o teste não-paramétrico de Kruskal-Wallis. Como alternativa ao Teste-t pareado, foi aplicado o teste de Wilcoxon. A estatística descritiva e analítica foi realizada utilizando o programa IBM SPSS *Statistics* para o sistema operacional Windows, versão 20.0. Para avaliar a inibição da formação do biofilme por Ctn[15-34], aplicou-se o teste ANOVA, no programa estatístico *GraphPad Prism* 9.

6. RESULTADOS

As cepas escolhidas para o estudo foram caracterizadas em relação ao perfil de sensibilidade a antifúngicos convencionais e a produção de enzimas hidrolíticas que atuam como fatores de virulência.

6.1 Perfil de sensibilidade aos antifúngicos

Os testes de sensibilidade *in vitro* utilizando o Fluconazol evidenciaram que a 22 cepas de *Candida* spp. se mostraram sensíveis e seis apresentando sensibilidade dose- dependente (SDD). Dentre essas últimas, duas foram identificadas como *Candida glabrata*. A cepa ATCC 90028 (*Candida albicans*) é considerada resistente ao Fluconazol (Tabela 2). Os testes com *Candida krusei* não foram realizados em decorrência da resistência intrínseca ao fluconazol e consequente ausência de pontos de corte nos documentos dos CLSI.

Tabela 2. Perfil de sensibilidade ao Fluconazol de *Candida* spp. na forma planctônica.

	Perfil de Sensibilidade* - Fluconazol		
Cepas	**Sensível**	**SDD****	**Resistente**
Candida albicans	n = 22	n = 4	n = 1
Candida tropicalis	n = 2	n = 0	n = 0
Candida glabrata	n = 0	n = 2	n = 0

*Classificação de acordo com os pontos de corte do documento M27-A3 do CLSI (Performance standards for antifungal susceptibility of yeasts, 1st Ed. CLSI supplement M60. ISBN 1-56238 – 828 – 2 [Print])
**Sensibilidade dose-dependente.

A tabela 3 traz o perfil de sensibilidade de das 35 cepas de *Candida* spp. ao Itraconazol e a cepa ATCC 90028 foi a única cepa resistente. Quanto ao perfil de sensibilidade à Anfotericina B de *Candida* spp. na forma planctônica, todas as cepas se mostraram sensíveis a esse fármaco, nas concentrações testadas.

Tabela 3. Perfil de sensibilidade ao Itraconazol de *Candida* spp. na forma planctônica.

	Perfil de Sensibilidade* - Itraconazol		
Cepas	**Sensível**	**SDD****	**Resistente**
Candida albicans	n = 26	n = 0	n = 1
Candida tropicalis	n = 2	n = 0	n = 0
Candida glabrata	n = 2	n = 0	n = 0
Candida krusei	n = 4	n = 0	n = 0

*Classificação de acordo com os pontos de corte do documento M27-A3 do CLSI (Performance standards for antifungal susceptibility of yeasts, 1st Ed. CLSI supplement M60. ISBN 1-56238 – 828 – 2 [Print])
**Sensibilidade dose-dependente.

6.2 Fatores de virulência

Dentre o total de cepas, 22 destas são produtoras de fosfolipase, sendo quatro classificadas como fortes produtoras. As cepas de *Candida tropicalis* e *Candida krusei* não produziram fosfolipase. As cepas de *C. albicans* estão em maior número e a maioria destas foi classificada como produtora de fosfolipase (Tabela 4).

Tabela 4. Produção de fosfolipase por *Candida* spp.

Cepas	**Não Produtora**	**Produtora**	**Forte Produtora**
Candida albicans	n = 5	n = 17	n = 4
Candida tropicalis	n = 2	n = 0	n = 0
Candida glabrata	n = 1	n = 1	n = 0
Candida krusei	n = 4	n = 0	n = 0

Em relação à atividade hemolítica, as cepas de *Candida* spp. demonstraram atividade ausente, moderada ou forte (Tabela 5).

Tabela 5. Atividade hemolítica de *Candida* spp.

Cepas	Ausente	Fraca	Moderada	Forte
Candida albicans	n = 11	n = 0	n = 4	n = 16
Candida tropicalis	n = 0	n = 0	n = 0	n = 2
Candida glabrata	n = 1	n = 0	n = 0	n = 1
Candida krusei	n = 3	n = 0	n = 0	n = 1

6.3 Atividade antifúngica dos fragmentos peptídicos Ctn [15-34] e Ctn [1-9]

O primeiro fragmento peptídico a ser testado foi o Ctn [15-34], com concentrações finais que variaram entre 0,0195 e 40 μM. Um total de 25 cepas foram utilizadas para os testes. As CIMs identificadas estão na faixa entre 1 a 40 μM, com valor médio que varia a depender da espécie em questão, mas que é inferior a 16 μM (Tabela 6). A CIM máxima de 40 μM foi observada frente a duas cepas de *C. albicans*, e as demais cepas foram sensíveis a concentrações mais baixas do peptídeo.

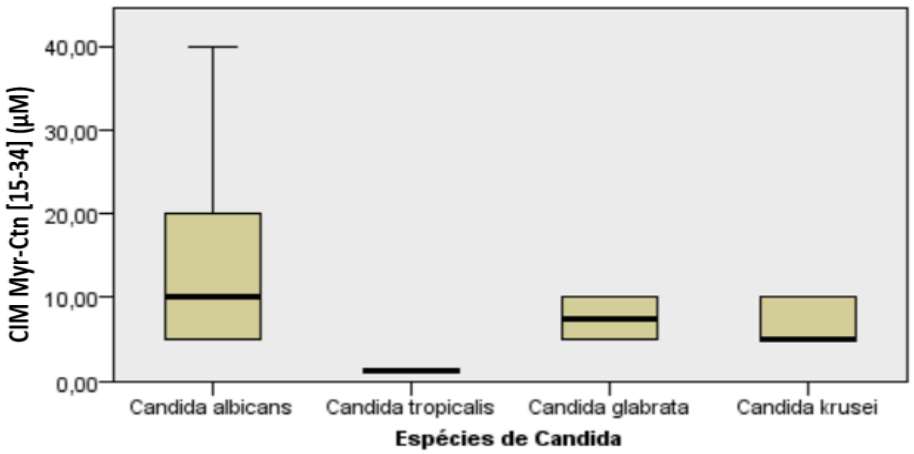
Tabela 6. Concentração Inibitória Mínima (CIM) do fragmento peptídico Ctn [15-34] frente à *Candida* spp. na forma planctônica.

	Valores de CIM* - Ctn [15-34] (μM)					
Cepas	Nº	Média	DP**	Mediana	Mínimo	Máximo
Candida albicans	n = 18	15,28	10,910	10,00	5	40
Candida tropicalis	n = 2	1,25	0,000	1,25	1	1
Candida glabrata	n = 2	7,50	3,536	7,50	5	10
Candida krusei	n = 3	6,67	2,887	5,00	5	10
TOTAL	n = 25	13,06	10,192	10,00	1	40

*CIM - Concentração Inibitória Mínima
** Desvio padrão da média

Para o fragmento peptídico Ctn [15-34], foram realizados testes de sensibilidade com 25 cepas de *Candida* spp. e constatou-se que a distribuição dos valores de CIM obtidos variaram entre os grupos de diferentes espécies (valor de p = 0,04). Considera-se então que a distribuição é diferente entre os grupos, com mediana de concentrações inibitórias de peptídeo menor para cepas de *Candida* não-*albicans* (Gráfico 1).

Gráfico 1. Valores de CIMs do fragmento peptídico Ctn [15-34] frente às cepas *Candida* de diferentes espécies. Teste de kruskal-Wallis para amostras independentes, SPSS. Valor de p < 0,05.



A CIM de Myr-Ctn [1-9] frente às diferentes espécies de *Candida* também foi verificada e os valores tiveram pouca variação, independentemente da cepa utilizada (Tabela 7).

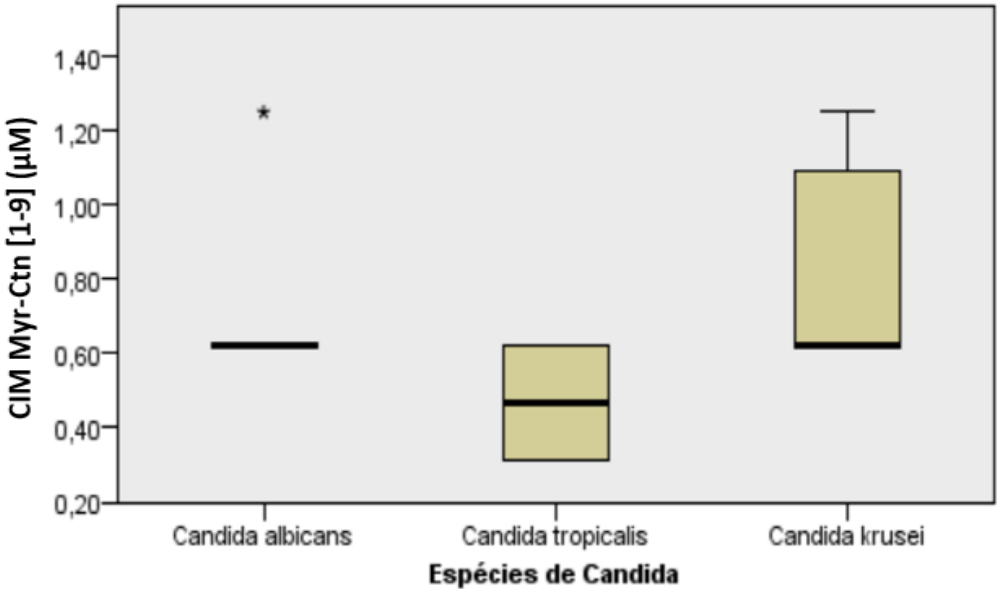
Tabela 7. Concentração Inibitória Mínima (CIM) do fragmento peptídico Myr-Ctn [1-9] frente à *Candida* spp. na forma planctônica.

	Valores de CIM* - Myr-Ctn [1-9] (µM)					
Cepas	**Nº**	**Média**	**DP****	**Mediana**	**Mínimo**	**Máximo**
Candida albicans	n = 13	0,67308	0,173344	0,62500	0,625	1,250
Candida tropicalis	n = 2	0,46875	0,220971	0,46875	0,313	0,625
Candida krusei	n = 4	0,78125	0,312500	0,62500	0,625	1,250
TOTAL	n = 19	0,69940	0,240133	0,62500	0,313	1,250

*CIM - Concentração Inibitória Mínima
** Desvio padrão da média

Para o teste, foram utilizadas um total de 19 cepas de *Candida* spp. e foi avaliada se a distribuição das CIMs de Myr-Ctn [1-9] variou entre os grupos de diferentes espécies e nesse caso, no entanto, após a aplicação do teste estatístico para a comparação entre os grupos, não houve significância (valor de p = 0,103). Considera-se então que a distribuição é a mesma entre os grupos (Gráfico 2).

Gráfico 2. Valores de CIMs do fragmento peptídico Myr-Ctn [1-9] frente às cepas *Candida* de diferentes espécies. Teste de kruskal-Wallis para amostras independentes, SPSS. Valor de p > 0,05.



De uma forma geral, considerando todos os isolados utilizados para os testes, empregando apenas estatística descritiva, observa-se menores valores de CIM referente ao menor fragmento peptídico, o Myr-Ctn [1-9], que teve variação de 0,313 a 1,25 µM. Ctn [15-34] apresentou valores de CIM que variaram entre 1 e 40µM. Todas as informações estão disponíveis na Tabela 8.

Tabela 8. Concentração Inibitória Mínima (CIM) dos fragmentos peptídicos frente a cepas de *Candida* spp.

	Valores de CIM* (µM)					
Peptídeos	**Nº**	**Média**	**DP****	**Mínimo**	**Mediana**	**Máximo**
Myr-Ctn [1-9]	n = 19	0,67434	0,215077	0,313	0,62500	1,250
Ctn [15-34]	n = 25	12,50	10,402	1	10,0	40

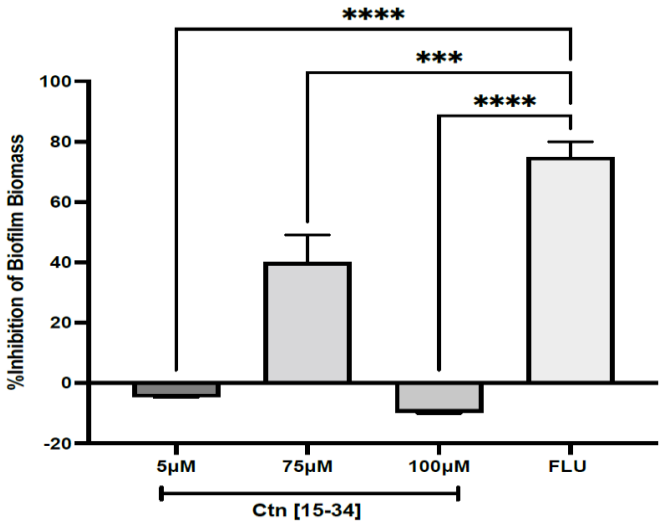
*CIM - Concentração Inibitória Mínima
** Desvio padrão da média

O teste de Wilcoxon mostrou que a CIM do fragmento peptídico Myr-Ctn [1-9] é significativamente menor do que a CIM do Ctn [15-34] frente a cepas diversas de *Candida* spp. (valor de $p < 0,01$).

6.4 Biofilme de *Candida albicans*

Para verificar a atividade do peptídeo Ctn [15-34] sobre o biofilme de uma cepa de referência de *Candida albicans* (SC5314), utilizou-se o peptídeo em questão em diferentes concentrações (5, 75 e 100 μM), partindo da concentração inibitória mínima obtida durante o teste de sensibilidade com a cepa na forma planctônica. A contração de 75 μM, quando comparada com outras concentrações de peptídeo testadas, foi responsável por um maior percentual de inibição da biomassa do biofilme, com inibição de aproximadamente 50% da formação de biomassa do biofilme (Gráfico 3). Os resultados obtidos foram comparados com a ação do fluconazol (controle), com diferença significativa (valor de $p < 0,05$).

Gráfico 3. Efeito do peptídeo Ctn[15-34] sobre a formação do biofilme de *Candida albicans*. Teste estatístico ANOVA, GraphPad Prism. Valor de $p < 0,05$.



7. DISCUSSÃO

O crescente aumento de quadros clínicos envolvendo infecções por cepas de *Candida* spp. resistentes a antifúngicos tradicionalmente utilizados, tais como por exemplo os derivados azólicos, reflete na necessidade de identificar alternativas terapêuticas aos medicamentos existentes. Dessa forma, o presente trabalho demonstra o potencial antimicrobiano do novo peptídeo Myr-Ctn [1-9] frente a diferentes isolados de fungos do gênero *Candida* e compara a atividade antifúngica deste com o peptídeo Ctn [15-34].

Sabe-se que *Candida* spp. são capazes de produzir diversos fatores de virulência, responsáveis pelo maior poder de patogenicidade de algumas cepas. Considerando esses aspectos, o presente estudo buscou primeiramente traçar o perfil de sensibilidade de diferentes isolados de *Candida* spp. e identificar os principais fatores de virulência.

Embora tenham sido consideradas cepas de diferentes origens, tais como cepas da microbiota oral de indivíduos saudáveis e isolados de amostras clínicas de indivíduos com infecção ativa, não houve resistência propriamente dita ao fluconazol, mas sensibilidade dose-dependente para alguns desses isolados. De acordo com o documento M27-A3 do CLSI (2008), o termo sensibilidade dose-dependente está relacionado a uma dose do fármaco que é maior do que a dose convencional que pode ser usada, considerando o limite máximo de concentração que pode estar presente no sangue.

O fluconazol é um dos medicamentos antifúngicos mais comumente prescritos para infecções por *Candida*. Esses antifúngicos são frequentemente preferidos para o tratamento de diferentes infecções por *Candida* spp., em decorrência da maior disponibilidade para aquisição desse medicamento, baixo custo, toxicidade limitada e possibilidade de administração por via oral, o que facilita a adesão ao tratamento. Ressalta-se que o padrão de suscetibilidade aos antifúngicos em membros do gênero *Candida* é diferente, havendo ampla utilização de antifúngicos na prática clínica (ZARRINFAR et al., 2021).

Os azóis funcionam inibindo a enzima do citocromo P450 lanosterol demetilase (14α-desmetilase), codificada por *ERG11*, na via de biossíntese do ergosterol. O fluconazol é fungistático em vez de fungicida, de modo que o tratamento oferece a oportunidade de desenvolvimento de resistência adquirida na presença deste antifúngico. Sem introdução prévia do antifúngico, a resistência ao fluconazol também pode ser inata, como é visto em *C. krusei*. Muitos isolados clínicos *de C. albicans* superexpressam *ERG11*, o gene que codifica o alvo dos azóis. Dessa forma, o surgimento de resistência aos medicamentos pode ser considerado uma

consequência inevitável das pressões seletivas impostas pelos antifúngicos (BERKOW; LOCKHART, 2017).

Embora *C. albicans* seja mais comumente isolada de pacientes com infecções invasivas, infecções por *Candida* não-*albicans* vem aumentando consideravelmente com o passar dos anos. O tratamento profilático e/ou empírico de pacientes imunocomprometidos/imunossuprimidos suscetíveis também resulta em aumento da prevalência de infecções causadas por espécies de *Candida* resistentes a medicamentos e multirresistentes (AL-BAQSAMI et al., 2020).

C. glabrata é considerada como a segunda ou terceira espécie *de Candida* mais frequentemente isolada, particularmente de pacientes críticos idosos, infecções de corrente sanguínea e outras infecções invasivas, bem como aqueles com infecções vulvovaginais e orais. Trata-se de uma espécie intrinsecamente menos suscetível a antifúngicos azólicas.

De acordo com Oliveira e Schmidt (2021) isolados de *C. glabrata* e *C. tropicalis* são frequentemente resistentes a fármacos azóicos ou requerem doses maiores para permitir o sucesso terapêutico. Ainda semelhante aos resultados encontrados no presente estudo, Sadeghi et al. (2018), ao avaliarem a distribuição e epidemiologia de *Candida* não-*albicans* isoladas de várias amostras clínicas e avaliar a suscetibilidade *in vitro* ao fluconazol, a maioria dos isolados de *C. glabrata* foi suscetível dose-dependente (SDD) (96,2%), confirmando que essa espécie é intrinsecamente mais resistente a antifúngicos, especialmente para o fluconazol.

Embora não tenhamos evidenciado resistência das cepas de *Candida* spp. aos antifúngicos testados, um estudo envolvendo análise de 87 isolados de *Candida albicans*, sendo 30 destes classificados como SDD, demonstrou que o efluxo de fármacos mediado por transportadores ABC, especialmente CDR1 é o mecanismo predominante de resistência ao fluconazol e resistência cruzada azólica em isolados clínicos de *C. albicans* da Índia. Também foram identificadas mutações no gene ERG11 em isolados suscetíveis, SDD e resistentes (MANE et al., 2016).

Em relação aos testes de sensibilidade utilizando o itraconazol, a totalidade das cepas foram sensíveis a este fármaco, sendo estes resultados condizentes com a literatura, que indicam que o itraconazol mantém importante atividade antifúngica, inclusive atividade superior contra *Candida* não-*albicans*, quando comparada com o fluconazol (GIAMARELLOS-BOURBOULIS, 2019).

Quanto à anfotericina B, a formulação convencional (anfotericina B desoxicolato) ou a de formulação lipídica, dependendo do quadro clínico do paciente, é utilizada para o tratamento

das formas mais invasivas ou disseminadas de candidíase. A resistência a esse agente antifúngico é incomum em isolados de leveduras patogênicas (BORMAN et al., 2020). Isso ocorre porque em comparação com a maioria dos outros antifúngicos, os polienos visam um componente principal da membrana celular em vez de uma enzima essencial. Vale salientar que devido à sua toxicidade e à disponibilidade dos antifúngicos tais como os triazóis e equinocandinas, o uso de anfotericina B para tratar as micoses sistêmicas mais comuns, como candidíase e aspergilose, diminuiu. Esse medicamento ainda é recomendado como tratamento de primeira linha de infecções graves como criptococose, histoplasmose disseminada e mucormicose, enquanto permanece como uma alternativa para outras infecções mediante intolerância, disponibilidade limitada ou falha de outros tratamentos. Toxicidade renal e efeitos adversos agudos relacionados à infusão, como febre e náusea, são mais comumente associados a administrações intravenosas, enquanto danos hepáticos ocorrem, mas são menos comuns (CAROLUS et al., 2020).

A produção de enzimas hidrolíticas faz-se necessária para *Candida* spp., tanto como uma forma de obtenção de nutrientes, permitindo a digestão de diversas moléculas, como também é um fator de virulência, estando diretamente relacionados com a patogênese e progressão da infecção fúngica. As enzimas facilitam a invasão tecidual e a inativação dos componentes do sistema imunológico do hospedeiro e entre os grupos mais importantes de enzimas hidrolíticas estão as proteases, hemolisinas, bem como lipases e fosfolipases (CZECHOWICZ et al., 2022).

Considerando esses aspectos, após os testes de sensibilidade aos antifúngicos, os isolados de *Candida* foram avaliados quanto à capacidade de produção de fosfolipase e atividade hemolítica, que são considerados fatores de virulência. A maioria das cepas produzem fosfolipase e possuem atividade hemolítica. Os isolados de *Candida tropicalis* e *Candida krusei* não produziram fosfolipase e considerando todas as espécies de *Candida* utilizadas para os experimentos, pelo menos um isolado de *Candida* sp. possui atividade hemolítica.

Em um trabalho realizado com isolados clínicos de *Candida albicans,* 61,42% dos 70 isolados produziram fosfolipases (MOHAMMADI et al., 2020), semelhante ao nosso estudo. Existem quatro classes de fosfolipases, dependendo da ligação éster que clivam: A, B, C e D. Quase todas as espécies de *Candida* são capazes de sintetizar várias fosfolipases pertencentes a todas essas classes. *C. albicans* produz quantidades muito maiores deles do que os representantes do grupo de espécies de *Candida* não-*albicans* (CZECHOWICZ et al., 2022), informação que corrobora com os resultados obtidos após os experimentos. Também de forma semelhante, experimentos realizados com isolados de *Candida* spp. recuperados de espécimes

vaginais identificaram que aproximadamente 42% foram capazes de produzir fosfolipase (EL-HOUSSAIN et al., 2019).

Em relação à atividade hemolítica, a absorção de ferro através da hemolisina é um requisito essencial para o crescimento e invasão de fungos. A hemolisina utiliza o ferro contido na hemoglobina e ativa o complemento para opsonizar a superfície dos glóbulos vermelhos (hemácias). Essa ação destrói as hemácias do hospedeiro e facilita a invasão das hifas em quadros de candidíase sistêmica. Singh e colaboradores (2021) identificaram que a atividade hemolítica foi maior em *C. albicans* (85,5%), seguindo de *C. glabrata* (70%) e *C. tropicalis* (59,4%) (SINGH et al., 2021). No estudo de Mohammadi et al. (2020), 66 isolados *de C. albicans* (94,28%) produziram hemolisina, corroborando com o conceito de que a atividade hemolítica é um importante fator de virulência de *C. albicans* (MOHAMMADI et al., 2020). Já outros autores, identificaram cepas de *Candida* não-*albicans* como tendo atividades hemolíticas mais altas do que *C. albicans* (MELO et al., 2019; SENEVIRATNE et al., 2016).

Embora alguns dos isolados de levedura utilizados no presente estudo tenham como origem a microbiota de indivíduos saudáveis, sabe-que que a mudança do comensalismo para a patogênese entre *Candida* spp. em vários locais do corpo é atribuída a muitos determinantes de virulência, entre os quais a adesão ao tecido do hospedeiro, resposta a estresses ambientais, secreção de hidrolases e capacidade de produzir biofilme. A virulência de uma espécie microbiana é uma medida do resultado das interações micróbio-hospedeiro, em vez de uma propriedade fixa dos microrganismos. Ao contrário de patógenos primários que não requerem um hospedeiro imunocomprometido para causar doença, patógenos oportunistas/facultativos como *Candida* spp. causam doenças principalmente em hospedeiros susceptíveis. Dessa forma, as estratégias que são utilizadas para lutar contra os mecanismos naturais de defesa dos hospedeiros são altamente influenciadas pelo ambiente e por isso a virulência não é uma propriedade constante, pois pode ser aumentada, perdida e até restaurada em várias circunstâncias (CIUREA et al., 2020). Ainda, é importante considerar que a virulência *de Candida* pode variar dependendo da espécie, origem geográfica, reação do hospedeiro e também do estágio das infecções (MROCZYŃSKA; BRILLOWSKA-DĄBROWSKA, 2021), justificando a relevância de utilizar cepas de diferentes perfis, corroborando com o método escolhido no presente estudo.

Outra característica importante a ser considerada é que a produção de enzimas hidrolíticas pode interferir nas CIMs observadas nos testes de sensibilidade. Mohammadi et al. (2020) identificaram que há correlação positiva significativa de CIMs de fluconazol e itraconazol com

a formação de biofilme e produção de fosfolipase em *C. albicans in vitro*. Além disso, houve uma associação significativa entre a CIM do fluconazol e produção de hemolisina (MOHAMMADI et al., 2020).

A partir dos testes de sensibilidade realizados com o fragmento peptídico Ctn[15-34], considerando todas as cepas de *Candida* spp., as CIMs variaram entre 1 e 40 μM, mas quando foi realizada a análise estatística considerando as diferentes espécies, constatou-se que a distribuição dos valores de CIM obtidos variaram significativamente entre esses grupos, com menores concentrações observadas entre os isolados de *Candida*-não *albicans*. Nosso grupo de pesquisa realizou estudos anteriores com metodologia semelhante, com o gênero *Candida*, mas com cepas diferentes das cepas utilizadas no presente estudo. Quando comparado com o fragmento peptídico Ctn[1-14] e com a crotalicidina em sua forma completa (Ctn[1-34]), o fragmento Ctn[15–34] apresentou CIMs que variaram entre 5 a 10 μM e foi o peptídeo de melhor desempenho contra isolados clínicos de *Candida albicans*, *Candida parapsilosis* e *Candida tropicalis* (CAVALCANTE et al., 2017), demonstrando que os resultados promissores foram mantidos.

Sabe-se que o mecanismo de morte leveduras por Ctn[15-34] envolve a ruptura de membrana e indução de apoptose precoce e necrose tardia (CAVALCANTE et al. 2018). Ainda de acordo com Cavalcante et al. (2018), parece que esses peptídeos anfipáticos carregados positivamente interagem preferencialmente com leveduras com um fenótipo de resistência a fármacos, supostamente devido à afinidade com a membrana celular modificada (AGUIAR et al., 2020).

Considerando todos os isolados utilizados para os testes, verificou-se menores valores de CIM referente ao menor fragmento peptídico, o Myr-Ctn[1-9], que teve variação de 0,313 a 1,25 μM e a análise estatística confirmou que a CIM do fragmento peptídico Myr-Ctn[1-9] é significativamente menor do que a CIM do Ctn[15-34] frente a cepas diversas de *Candida* spp.

Estudo anterior evidenciou atividade antimicrobiana de Ctn[1-9] contra bactérias e fungos. Este fragmento peptídico, derivado da crotalicidina, foi sintetizado dessa forma, com menor quantidade de resíduos de aminoácidos, tendo como pressuposto a importância da modificação estrutural de peptídeos e o subsequente estudo da seletividade do alvo correspondente, os quais são essenciais para melhorar as atividades biológicas e/ou farmacológicas dessas moléculas. O Ctn[1-9] exibiu atividade antimicrobiana seletiva contra bactérias nosocomiais e leveduras (CIM de 20 μM), com baixa citotoxicidade para eritrócitos humanos, e quando associado com antibacterianos e antifúngicos, demonstrou efeito sinérgico

(LIMA et al., 2022). A adição do Miristoil (ácido mirístico) a essa estrutura (Ctn[1-9]) o torna mais hidrofóbico, melhorando a interação com a membrana celular fúngica e após essa modificação na estrutura da molécula, observou-se menores valores de CIM para o Myr-Ctn[1-9] frente a *Candida* spp.

A busca por estruturas mínimas desses peptídeos antimicrobianos que possam manter ou aumentar suas atividades biológicas também vem crescendo, em decorrência da necessidade de reduzir os custos de produção e facilitar o desenvolvimento de novos peptídeos (FALCÃO; RÁDIS-BAPTISTA, 2020).

Considerando a capacidade de interação dos peptídeos derivados da crotalicidina com leveduras, destaca-se a relevância de estudos futuros mais aprofundados, a fim de identificar se para além a atividade inibitória, a exposição prévia aos fragmentos peptídicos aqui descritos, interferem nos fatores de virulência de *Candida* spp. Os efeitos de peptídeos antimicrobianos na inibição da transição de levedura para hifa e na redução da formação de biofilme em *C. albicans* (D'AURIA et al., 2022) bem como na produção de fosfolipases, proteases e atividade hemolítica (VAŇKOVÁ et al., 2020) já foram descritos anteriormente.

A formação de biofilme também é considerada como um fator de virulência que contribui para maior patogenicidade de *Candida* sp. e resistência aos antimicrobianos. Por razões didáticas, as análises quanto à formação de biofilme e atividade antibiofilme do peptídeo foram inseridas em um tópico à parte, separado dos outros fatores de virulência avaliados. Optou-se pela utilização da cepa de referência SC5314, em detrimento dos outros isolados utilizados no estudo em razão da reconhecida capacidade de formar biofilme, sendo previamente utilizada em outros estudos (ALVES et al., 2023). Trata-se de uma cepa selvagem, originalmente isolada de um paciente com infecção generalizada, virulenta em um modelo de camundongo de infecção sistêmica.

Quanto à capacidade do Ctn [15-34] em inibir a formação de biofilme por uma cepa de *Candida albicans* (SC5314), a concentração de 75 μM, quando comparada com outras concentrações de peptídeo testadas, foi responsável por um maior percentual de inibição da biomassa do biofilme (cerca de 50%). O fluconazol, quando utilizado na concentração padrão, mostrou atividade superior ao peptídeo, no entanto, esse resultado é satisfatório quando comparado ao estudo realizado anteriormente, onde 100 μM reduziu consideravelmente o biofilme da cepa selvagem ATCC 90028, no ensaio de inibição (AGUIAR et al., 2020).

A maioria dos antifúngicos que rompem os biofilmes de levedura atuam no estágio inicial de formação do biofilme, impedindo a adesão celular e subsequente consolidação do biofilme

(AGUIAR et al., 2020). Vale ressaltar que, para os biofilmes de *C. albicans*, a plasticidade morfológica é um importante traço de virulência em muitos contextos, pois a transição de levedura para hifa é crítica para a estabilidade do biofilme, penetração nas células epiteliais do hospedeiro e fuga das células fagocíticas do hospedeiro (SALAMA; GERSTEIN, 2022).

A matriz polissacarídica extracelular compreende polímeros extracelulares e DNA extracelular envolvidos na manutenção da estrutura do biofilme. O DNA extracelular também desempenha um papel vital na ligação do biofilme ao substrato. Outra parte essencial da matriz extracelular são os β-1,3-glucanos, que contribuem significativamente para a resistência do biofilme aos antifúngicos, pois impedem o contato com as células-alvo, dessa forma, as células de *C. albicans* no biofilme liberam mais β-1,3-glucanos na matriz extracelular do que as células planctônicas (TALAPKO et al., 2021). No trabalho desenvolvido por Alves e colaboradores (2023), quando avaliada a composição da matriz extracelular do biofilme da cepa SC5314, o valor médio da quantidade de proteínas foi de 1,24 ± 0,05 mg/g e o valor médio da quantidade de polissacarídeos foi de 0,03 ± 0,05 mg/g. Os polissacarídeos da matriz extracelular formam um complexo que sequestra fármacos, provavelmente por meio de interações não covalentes, restringindo a ação dos antimicrobianos sobre as células presentes no biofilme maduro.

Os efeitos inibitórios na formação do biofilme são avaliados de uma forma diferente, quando comparado com os testes de susceptibilidade de células planctônicas. Dessa forma, o efeito inibitório pode ser descrito como a menor concentração de uma substância antimicrobiana na qual não há aumento dependente do tempo, no número médio de células viáveis do biofilme (*Minimal Biofilm Inhibitory Concentrations* - MBIC) (THIEME et al., 2019). Isso deve ser considerado, pois embora a capacidade de matar micróbios seja rotineiramente determinada como a concentração inibitória mínima de um composto usando micróbios planctônicos, isso não corresponderia à concentração que pode prevenir ou erradicar os biofilmes. Dessa forma, Peptídeos com MBIC menor, próximas do valor de CIM são mais atraentes porque reduzem o risco de citotoxicidade para o hospedeiro (LI et al., 2021). Diante disso, o Ctn[15-34] se mantém promissor, havendo a necessidade de identificar estratégias para aumentar a atividade antibiofilme, tais como a identificação de compostos que possam promover efeito sinérgico com o peptídeo, mantendo respostas satisfatórias mesmo baixas concentrações utilizadas.

8. CONCLUSÃO

A partir dos resultados obtidos, é possível concluir que:

- Os isolados de *Candida* spp. avaliados, em sua maioria, são suscetíveis aos antifúngicos fluconazol, itraconazol e anfotericina B;

- Dentre as cepas de *Candida albicans*, a maioria destas foi considerada produtora de fosfolipase, mas *Candida tropicalis* e *Candida krusei* não produziram. *C. albicans*, *C. tropicalis*, *C. krusei* e *Candida glabrata* possuem atividade hemolítica;

- Frente às diversas cepas de *Candida* spp., o fragmento peptídico Ctn[15-34] manteve valores de Concentração Inibitória Mínima (CIM) semelhantes aos valores relatados na literatura e o Myr-Ctn[1-9] apresentou atividade antifúngica superior (*in vitro*), pois foram observados menores valores de CIM contra *Candida* spp., sendo valioso para estudos posteriores com cepas resistentes a antifúngicos e produtoras de biofilme;

- O Ctn [15-34] inibiu em 50% a formação do biofilme por uma cepa padrão de *Candida albicans*, a uma concentração de 75 μM;

- No geral, Myr-Ctn[1-9] e Ctn[15-34] são moléculas que exibem atividade contra *Candida* spp., demonstrando que os peptídeos derivados da Crotalicidina são modelos promissores para estudos mais aprofundados que visem o objetivo final de desenvolvimento de novos fármacos antifúngicos.

9. REFERÊNCIAS

AGUIAR, Francisca Lidiane Linhares de et al. Antibiofilm activity on *Candida albicans* and mechanism of action on biomembrane models of the antimicrobial peptide Ctn [15–34]. **International journal of molecular sciences**, v. 21, n. 21, p. 8339, 2020.

AGUIAR, Francisca Lidiane Linhares et al. The antiproliferative peptide Ctn [15-34] is active against multidrug-resistant yeasts *Candida albicans* and *Cryptococcus neoformans*. **Journal of applied microbiology**, v. 128, n. 2, p. 414-425, 2020.

AL-BAQSAMI, Zahraa F.; AHMAD, Suhail; KHAN, Ziauddin. Antifungal drug susceptibility, molecular basis of resistance to echinocandins and molecular epidemiology of fluconazole resistance among clinical *Candida glabrata* isolates in Kuwait. **Scientific reports**, v. 10, n. 1, p. 1-10, 2020.

ALSOMALI, Mohammed I. et al. Challenges to "classic" esophageal candidiasis: looks are usually deceiving. **American journal of clinical pathology**, v. 147, n. 1, p. 33-42, 2017.

ALVES, Anelise Maria Costa Vasconcelos et al. Characterization of Oral *Candida* spp. Biofilms in Children and Adults Carriers from Eastern Europe and South America. **Antibiotics**, v. 12, n. 5, p. 797, 2023.

ANTINORI, Spinello et al. Candidemia and invasive candidiasis in adults: A narrative review. **European journal of internal medicine**, v. 34, p. 21-28, 2016.

ANTUNES, Francisco et al. *Candida auris*: the recent emergence of a Multiresistant Pathogenic Fungi. **Acta Médica Portuguesa**, v. 33, n. 10, p. 680-684, 2020.

ATRIWAL, Tanu et al. Mechanistic understanding of *Candida albicans* biofilm formation and approaches for its inhibition. **Frontiers in Microbiology**, v. 12, p. 638609, 2021.

AZEVEDO, N. S.; CERCA, N. **Biofilmes na saúde, no ambiente e na indústria.** 1. ed. Porto: Publindustria, 2012.

BANDEIRA, I.C.J., BANDEIRA-LIMA, D., MELLO, C.P., PEREIRA, T.P.; MENEZES, R.P.B.; SAMPAIO, T.L.; FALCÃO, C.B.; MARTINS, A.M.C. Antichagasic effect of crotalicidin, a cathelicidin-like vipericidin, found in *Crotalus durissus terrificus* rattlesnake's venom gland. **Parasitology**, p. 1-6, 2017.

BASMACIYAN, Louise et al. *Candida albicans* interactions with the host: crossing the intestinal epithelial barrier. **Tissue Barriers**, v. 7, n. 2, p. 1612661, 2019.

BEN-AMI, Ronen. Treatment of invasive candidiasis: A narrative review. **Journal of Fungi**, v. 4, n. 3, p. 97, 2018.

BERKOW, Elizabeth L.; LOCKHART, Shawn R. Fluconazole resistance in *Candida* species: a current perspective. **Infection and drug resistance**, p. 237-245, 2017.

BIZERRA, F. C.; NAKAMURA, C. V.; DE POERSCH, C.; ESTIVALET SVIDZINSKI, T. I.; BORSATO QUESADA, R. M.; GOLDENBERG, S.; KRIEGER, M. A.; YAMADA-OGATTA, S.F. Characteristics of biofilm formation by *Candida tropicalis* and antifungal resistance. **FEMS yeasts research**, v. 8, n. 3, p. 442-50, 2008.

BORMAN, Andrew M. et al. MIC distributions for amphotericin B, fluconazole, itraconazole, voriconazole, flucytosine and anidulafungin and 35 uncommon pathogenic yeast species from the UK determined using the CLSI broth microdilution method. **Journal of Antimicrobial Chemotherapy**, v. 75, n. 5, p. 1194-1205, 2020.

BORMANGOW, A.R.; HUBE, B. Importance Of the *Candida albicans* cell wall during commensalism and infection. **Current Opinion in Microbiology**, v. 15, p. 406-12, 2012.

BRANDENBURG, L.; MERRES, J.; ALBRECHT, L.; VAROGA, D. PUFE, T. Antimicrobial Peptides: Multifunctional Drugs for Different Applications. **Polymers**, v. 4, p. 539-560, 2012.

BROWN, KL.; HANCOCK, RE. Cationic host defense (antimicrobial) peptides. **Curr Opin Immunol**, v. 18, p. 24–30, 2006.

CAROLUS, Hans et al. Amphotericin B and other polyenes—Discovery, clinical use, mode of action and drug resistance. **Journal of Fungi**, v. 6, n. 4, p. 321, 2020.

CARVALHO, F.G.; SILVA, D.S.; HEBLING, J.; SPOLIDORIO, L.C.; SPOLIDORIO, D.M. P. Presence of mutans streptococci and *Candida* spp. in dental plaque/dentine of carious teeth and early childhood caries. **Arch Oral Biol**, v. 51, n. 11, p. 1024–1028, 2006.

CAVALCANTE, C.S.P.; FALCÃO, C.B.; FONTENELLE, R.O.; RÁDIS-BAPTISTA, G.; Anti-fungal activity of Ctn[15-34], the C-terminal peptide fragmente of crotalicidin, a rattlesnake venom gland cathelicidin. **J. Antibiot.**, v. 70, n. 3, p. 231-237, 2017.

CAVALCANTE, C.S.P.; FALCÃO, C.B.; FONTENELLE, R.O.S.; ANDREU, D.; RÁDIS-BAPTISTA, G. . Anti-fungal activity of Ctn[15-34], the C-terminal peptide fragment of crotalicidin, a rattlesnake venom gland cathelicidin. **Journal of antibiotics**, v. 70, p. 231-237, 2016.

CAVALCANTE, Carolina Sidrim P. et al. Insights into the candidacidal mechanism of Ctn [15–34] – a carboxyl-terminal, crotalicidin-derived peptide related to cathelicidins. **Journal of medical microbiology**, v. 67, n. 1, p. 129-138, 2018.

CAVALHEIRO, Mafalda; TEIXEIRA, Miguel Cacho. *Candida* biofilms: threats, challenges, and promising strategies. **Frontiers in medicine**, v. 5, p. 28, 2018.

CHANDRA, J.; KUHN, D.M.; MUKHERJEE, P.K.; HOYER, L.L.; MCCORMICK, T.; GHANNOUM, M.A. Biofilm formation by the fungal pathogen *Candida albicans*:

development, architecture, and drug resistance. **Journal of bacteriology**, v. 183, n. 18, p. 5385- 5394, 2001.

CHANDRA, J.; MUKHERJEE, P.K. *Candida* biofilms: development, architecture and resistance. **Microbiology Spectr**., v. 3, n. 4, 2015.

CHOI, Jae Hyeuk et al. Prevalence and risk factors of esophageal candidiasis in healthy individuals: a single center experience in Korea. **Yonsei medical journal**, v. 54, n. 1, p. 160-165, 2013.

CIUREA, Cristina Nicoleta et al. *Candida* and candidiasis—opportunism versus pathogenicity: a review of the virulence traits. **Microorganisms**, v. 8, n. 6, p. 857, 2020.

CLANCY, Cornelius J.; NGUYEN, M. Hong. Diagnosing invasive candidiasis. **Journal of clinical microbiology**, v. 56, n. 5, p. e01909-17, 2018.

CLSI. **Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard-Third Edition.** CLSI document M27-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.

COLOMBO, A. L.; GUIMARÃES, T. Epidemiologia das infecções hematogênicas por *Candida* spp. **Rev Soc Bras Med Trop**, v. 36. n. 5, p. 599-607, 2003.

CORDEIRO, R. DE A.; TEIXEIRA, C. E. C.; BRILHANTE, R. S. N.; CASTELO-BRANCO, D. S. C. M.; ALENCAR, L. P.; DE OLIVEIRA, J. S.; MONTEIRO, A. J.; BANDEIRA, T. J. P. G.; SIDRIM, J. J. C.; MOREIRA, J. L. B. Exogenous tyrosol inhibits planktonic cells and biofilms of *Candida* species and enhances their susceptibility to antifungals. **FEMS Yeast Res.**, v. 15, n. 4, 2015.

COSTA, M.F.L.; BARRETO, S.M. Tipos de estudos epidemiológicos: conceitos básicos e aplicações na área do envelhecimento. **Epidemiologia e Serviços de Saúde**; v. 12, n. 4, p. 189- 201, 2003.

CRUZ, Gabriela Silva et al. Candidíase vulvovaginal na Atenção Primária à Saúde: diagnóstico e tratamento. **Revista Enfermagem Atual In Derme**, v. 94, n. 32, 2020.

CUÉLLAR-CRUZ, M.; LÓPEZ-ROMERO, E.; VILLAGÓMEZ-CASTRO, J. C.; RUIZ-BACA, E. *Candida* species: new insights into biofilm formation. **Future Microbiol.**, v. 7, n. 6, p. 755–771, 2012.

CZECHOWICZ, Paulina; NOWICKA, Joanna; GOŚCINIAK, Grażyna. Virulence factors of *Candida* spp. and host immune response important in the pathogenesis of vulvovaginal candidiasis. **International Journal of Molecular Sciences**, v. 23, n. 11, p. 5895, 2022.

D'AURIA, Felicia Diodata et al. Antifungal Activity of the Frog Skin Peptide Temporin G and Its Effect on *Candida albicans* Virulence Factors. **International Journal of Molecular Sciences**, v. 23, n. 11, p. 6345, 2022.

DELSING, C. E. et al. Association of esophageal candidiasis and squamous cell carcinoma. **Medical mycology case reports**, v. 1, n. 1, p. 5-8, 2012.

DONLAN, R.M. Biofilms: microbial life on surfaces. **Emerging Infectious Diseases**, v. 8, n. 9, p. 881–890, 2002.

DOS SANTOS, A. L. S. Protease expression by microorganisms and its relevance to crucial physiological/pathological events. **World J. Biol. Chem.**, v. 2, n. 3, p. 48, 2011.

DU, Han et al. Candida auris: Epidemiology, biology, antifungal resistance, and virulence. **PLoS pathogens**, v. 16, n. 10, p. e1008921, 2020.

EL-HOUSSAINI, Houdaii H. et al. Correlation between antifungal resistance and virulence factors in *Candida albicans* recovered from vaginal specimens. **Microbial pathogenesis**, v. 128, p. 13-19, 2019.

ELLEPOLA, A. N.; SAMARANAYAKE, L. P.; KHAN, Z. U. Extracellular phospholipase production of oral *Candida albicans* isolates from smokers, diabetics, asthmatics, denture wearers and healthy individuals following brief exposure to polyene, echinocandin and azole antimycotics. **Brazilian journal of microbiology**, v. 47, n. 4, p. 911–916, 2016.

ERUM, Rakhshanda et al. A comparative study on production of extracellular hydrolytic enzymes of *Candida* species isolated from patients with surgical site infection and from healthy individuals and their co-relation with antifungal drug resistance. **BMC microbiology**, v. 20, n. 1, p. 1-12, 2020.

FALCÃO, C. B.; PÉREZ-PEINADO, C.; LA TORRE, B.G.; MAYOL, X.; ZAMORA-CARRERAS, H.; JIMÉNEZ, M.A.; RÁDIS-BAPTISTA, G.; ANDREU, D. Structural Dissection of Crotalicidin, a Rattlesnake Venom Cathelicidin, Retrieves a Fragment with Antimicrobial and Antitumor Activity. **J Med Chem**, v. 58, n. 21, p. 8553-8563, 2015.

FALCÃO, C.B.; LA TORRE, B.G.; PÉREZ-PEINADO, C.; BARRON, A.E.; ANDREU, D.; RÁDIS-BAPTISTA, G. Vipericidins: a novel family of cathelicidin- related peptides from the venom gland of South American pit vipers. **Amino Acids**, v. 46, n. 11, p. 2561-71, 2014.

FALCAO, Claudio Borges; RADIS-BAPTISTA, Gandhi. Crotamine and crotalicidin, membrane active peptides from *Crotalus durissus terrificus* rattlesnake venom, and their structurally-minimized fragments for applications in medicine and biotechnology. **Peptides**, v. 126, p. 170234, 2020.

FAVERO, D. et al. Production of haemolytic factor by clinical isolates of *Candida tropicalis*. **Mycoses**, v. 54, n. 6, p. e816-e820, 2011.

FAVERO, Daniel et al. Hemolytic factor production by clinical isolates of *Candida* species. **Current microbiology**, v. 68, p. 161-166, 2014.

FLEMING, D.; RUMBAUGH, K.P. Approaches to dispersing medical biofilms. **Microorganisms**, v. 5, n. 2, 2017.

FONTENELLE, R.O.S.; MORAIS, S.M. ; BRITO, E.H.S. ; MENDONÇA, M.R.K.; BRILHANTE, R.S.N. ; CORD EIRO, R.A.; NASCIMENTO, N.R.F.; SIDRIM, J.J.C.; ROCHA, M.F.G . Chemical composition, toxicological aspects and antifungal activity of essential oil from *Lippia sidoides* Cham. **Journal of Antimicrobial Chemotherapy**, v. 59, p. 934-940, 2007.

FURLANETO, Márcia C. et al. How much do we know about hemolytic capability of pathogenic *Candida* species?. **Folia microbiologica**, v. 63, p. 405-412, 2018.

GULATI, M.; ENNIS, C.L.; RODEIGUEZ, D.L.; NIBILE, C.J. Visualizations oh biofilm formation in *Candida albicans* using na automated microfluid device. **J. Vs. Exp**., v. 130, 2018.

HASSANMOGHADAM, Fozieh et al. High prevalence of itraconazole resistance among *Candida parapsilosis* isolated from Iran. **Current Medical Mycology**, v. 5, n. 3, p. 43, 2019.

HELLSTEIN, John W.; MAREK, Cindy L. Candidiasis: red and white manifestations in the oral cavity. **Head and neck Pathology**, v. 13, p. 25-32, 2019.

HOIBY, N.; BJARNSHOLT, T.; GIVSKOV, M.; MOLIN, S.; CIOFU, O. Antibiotic resistance of bacterial biofilms**. International Journal of Antimicrobial Agents**, v. 35, n. 4, p. 322–332, 2010.

HU, Wanchao et al. Alterations in the gut microbiota and metabolic profiles coincide with intestinal damage in mice with a bloodborne *Candida albicans* infection. **Microbial Pathogenesis**, v. 154, p. 104826, 2021.

JAKUBOVICS, Nicholas S. et al. The dental plaque biofilm matrix. **Periodontology 2000**, v. 86, n. 1, p. 32-56, 2021.

KALIA, Namarta; SINGH, Jatinder; KAUR, Manpreet. Microbiota in vaginal health and pathogenesis of recurrent vulvovaginal infections: a critical review. **Annals of clinical microbiology and antimicrobials**, v. 19, n. 1, p. 1-19, 2020.

KANG, S.J.; PARK, S.J.; MISHIG-OCHIR, T.; LEE, B.J. Antimicrobial peptides: therapeutic potentials. **Expert Review of Anti-infective Therapy**, v. 12, n. 12, p. 1477-86, 2014.

KERKIS, I; HAYASHI M.A.; SILVA, A.R.B.P.; PEREIRA, A.; SÁ JUNIOR, P.L.; ZAHARENKO, A.J.; RÁDIS-BAPTISTA, G.; KERKIS, A.; YAMANE, T. State of the Art in the Studies on Crotamine, a Cell Penetrating Peptide from South American Rattlesnake. **BioMed Research International**, 2014.

KHAN, Mohd Sajjad et al. Virulence and biofilms as promising targets in developing antipathogenic drugs against candidiasis. **Future science OA**, v. 6, n. 2, p. FSO440, 2020.

KOJIC, E. M.; DAROUICHE, R. O. *Candida* infections of medical devices. **Clin. Microbiol. Rev.**, v. 17, n. 2, p. 255–267, 2004.

LE, C. F.; FANG, C. M.; SEKARAN, S. D. Intracellular Targeting Mechanisms by Antimicrobial Peptides. **Antimicrobial agents and chemotherapy**, v. 61, n. 4, 2017.

LEE, Yunjin et al. Antifungal drug resistance: molecular mechanisms in *Candida albicans* and beyond. **Chemical reviews**, v. 121, n. 6, p. 3390-3411, 2020.

LEOTTI, Vanessa Bielefeldt; COSTER, Rodrigo; RIBOLDI, João. Normalidade de variáveis: métodos de verificação e comparação de alguns testes não-paramétricos por simulação. **Revista HCPA. Porto Alegre. Vol. 32, no. 2 (2012), p. 227-234**, 2012.

LEVETIN, E.; HORNER, W. E.; SCOTT, J. A.; WORKGROUP, E. A. Taxonomy of allergenic fungi. **J. Allergy Clin. Immunol. Pract.**, v. 4, n. 3, p. 375–385, 2016.

LI, Jing; CHEN, Dongru; LIN, Huancai. Antibiofilm peptides as a promising strategy: comparative research. **Applied Microbiology and Biotechnology**, v. 105, p. 1647-1656, 2021.

LIM, C. S.; ROSLI, R.; SEOW, H.F.; CHONG, P.P. *Candida* and invasive candidiasis: back to basics. **European Journal of Clinical Microbiology Infectious Diseases**, v. 31, n. 1, p. 21- 31, 2012.

LIMA, Hilania VD et al. The Rhodamine B-encrypted Vipericidin Peptide, RhoB-Ctn [1-9], Displays *In vitro* Antimicrobial Activity Against Opportunistic Bacteria and Yeasts. **Current Pharmaceutical Biotechnology**, v. 23, n. 2, p. 172-179, 2022.

LOHSE, M.B.; GULATI, M.; JOHNSON, C.J. Development and regulation of single- and multi-species *Candida albicans* biofilms**. Nature Reviews Microbiology**, v.16, p. 19–31, 2018.

MANE, Arati et al. Molecular mechanisms associated with fluconazole resistance in clinical *Candida albicans* isolates from India. **Mycoses**, v. 59, n. 2, p. 93-100, 2016.

MARÓTI, G.; KERESZT, A.; KONDOROSI, E.; MERGAERT, P. Natural roles of antimicrobial peptides in microbes, plants and animals. **Res Microbiol**, v. 162, p. 363–74, 2011.

MARTIN, Harlei; KAVANAGH, Kevin; VELASCO-TORRIJOS, Trinidad. Targeting adhesion in fungal pathogen *Candida albicans*. **Future Medicinal Chemistry**, v. 13, n. 03, p. 313-334, 2021.

MELLO, C.P.; LIMA, D.B.; MENEZES, R.R.; BANDEIRA, I.C.; TESSAROLO, L.D.; SAMPAIO, T.L.; FALCÃO, C.B.; RÁDIS-BAPTISTA, G.; MARTINS, A.M. Evaluation of the antichagasic activity of batroxicidin, a cathelicidin-related antimicrobial peptide found in *Bothrops atrox* venom gland. **Toxicon: official journal of the International Society on Toxinology**, v. 130, p. 56-62, 2017.

MELO, AP Vieira et al. Iconographies supplémentaires de l'article: Virulence factors of *Candida* spp. obtained from blood cultures of patients with candidemia attended at tertiary hospitals in Northeast Brazil. **Journal de Mycologie Médicale**, v. 29, n. 2, p. 132-139, 2019.

MILLSOP, Jillian W.; FAZEL, Nasim. Oral candidiasis. **Clinics in dermatology**, v. 34, n. 4, p. 487-494, 2016.

MIRONOV, A. Y. U.; LEONOV, V. V. Iron, virulence and intermicrobial interactions of opportunistic pathogens. **Biology Bulletin Reviews**, v. 136, n. 3, p. 301-310, 2016.

MODIRI, Mona et al. Antifungal susceptibility pattern and biofilm-related genes expression in planktonic and biofilm cells of *Candida parapsilosis* species complex. **Current Medical Mycology**, v. 5, n. 4, p. 35, 2019.

MOHAMED, Abdimajid Ahmed et al. Diagnosis and treatment of esophageal candidiasis: current updates. **Canadian Journal of Gastroenterology and Hepatology**, v. 2019, 2019.

MOHAMMADI, Faezeh et al. Relationship between antifungal susceptibility profile and virulence factors in *Candida albicans* isolated from nail specimens. **Revista da Sociedade Brasileira de Medicina Tropical**, v. 53, 2020.

MONOD, M.; CAPOCCIA, S.; LÉCHENNE, B.; ZAUGG, C.; HOLDOM, M.; JOUSSON, O. Secreted proteases from pathogenic fungi. **Int. J. Med. Microbiol.**, v. 292, n. 5–6, p. 405–419, 2002.

MOUNT, H. O.; REVIE, N. M.; TODD, R. T.; ANSTETT, K.; COLLINS, C.; COSTANZO, M.; BOONE, C.; ROBBINS, N.; SELMECKI, A.; COWEN, L. E. Global analysis of genetic circuitry and adaptive mechanisms enabling resistance to the azole antifungal drugs. **Plos genetics**, v. 14, n. 4, 2018.

MOYES, D. L.; RICHARDSON, J. P.; NAGLIK, J. R. *Candida albicans*-epithelial interactions and pathogenicity mechanisms: scratching the surface. **Virulence**, v. 6, n. 4, p. 338–346, 2015.

MROCZYŃSKA, Martyna; BRILLOWSKA-DĄBROWSKA, Anna. Virulence of clinical *Candida isolates*. **Pathogens**, v. 10, n. 4, p. 466, 2021.

NAGLIK, J.R.; MOYES, D.L.; WACHTLER, B.; HUBE, B. *Candida albicans* interactions with epithelial cells and mucosal immunity. **Microbes and infection / Institut Pasteur,** v. 13, n. 1, p. 963–976, 2011.

NG, S.M.S.; YAP, J.M; LAU, Q.Y.; NG, F.M.; ONG, E.H.Q.; BARKHAM, T.; TEO, J.W.P.; ALFATAH, M.; KONG, K.W.; HOON, S.; ARUMUGAN, P.; HILL, J.; CHIA, C.S.B. Structure-activity relationship studies of ultra-short peptides with potential activities agaibst fluconazole-resistant *Candida albicans*. **European Journal of Medical Chemistry,** v. 150, 2018.

NOBILE, C.J.; JOHNSON, A.D. *Candida albicans* biofilms and human disease. **Annual Review of Microbiology**, v. 69, n. 1, p. 71-92, 2015.

NUGRAHA, Alexander Patera et al. Prevalence of candida species in oral candidiasis and correlation with CD4+ count in HIV/AIDS patients at Surabaya, Indonesia. **Journal of International Dental and Medical Research**, v. 11, n. 1, p. 81-85, 2018.

OLIVEIRA, Diogo Luan; SCHMIDT, Juliana Cristina. *Candida* species cause vulvovaginitis and resistance to antifungals used for treatment. **Saúde e Pesquisa**, v. 14, 2021.

ORLANDINI, Renata Klemp et al. Are *Candida albicans* isolates from the oral cavity of HIV-infected patients more virulent than from non-HIV-infected patients? Systematic review and meta-analysis. **Microbial Pathogenesis**, v. 149, p. 104477, 2020.

PADDER, Sajad Ahmad; PRASAD, Rajendra; SHAH, Abdul Haseeb. Quorum sensing: A less known mode of communication among fungi. **Microbiological research**, v. 210, p. 51-58, 2018.

PAPPAS, P. G.; LIONAKIS, M. S. Arendrup MCet al. **Invasive candidiasis. Nat Rev Dis Primers**, v. 4, p. 18026, 2018.

PENDLETON, Kathryn M.; HUFFNAGLE, Gary B.; DICKSON, Robert P. The significance of *Candida* in the human respiratory tract: our evolving understanding. **Pathogens and disease**, v. 75, n. 3, p. ftx029, 2017.

PEREIRA, R.; MATOS NETO, F.A.; AGUIAR, F.L.L.; SOUZA, E.B.; SANTOS, H.S.; CAVALCANTE, C.S.P.; FONTENELLE, R.O.S. Atividade antifúngica, sinergismo e citotoxicidade do extrato etanólico da casca de *Myroxylon peruiferum* L.f. **Revista da Universidade Vale do Rio Verde,** v. 16, n. 1, jan./jul., 2018.

PÉREZ-PEINADO, C.; DIAS, S.A.; DOMINGUES, M.M.; BENFIELD, A.H.; FREIRE, J.M.; RÁDIS-BAPTISTA, G.; GASPAR, D.; CASTANHO, M.A.R.B.; CRAIK, D.J.; HENRIQUES, S.T.; VEIGA, A.S.; ANDREU, D. Mechanisms of bacterial membrane permeabilization by crotalicidin (Ctn) and its fragment Ctn(15–34), antimicrobial peptides from rattlesnake venom. **J.Biol. Chem**., v. 293, n. 5, 2018.

POLIT, D. F.; BECK, C.T. **Fundamentos de pesquisa em enfermagem: avaliação de evidências para a prática da enfermagem**. Porto Alegre: Artmed, 2011.

POLKE, M.; HUBE, B.; JACOBSEN, I. D. Chapter Three - *Candida* Survival Strategies. **Adv. Appl. Microbiol.**, v. 91, p. 139–235, 2015.

PONDE, Nicole O. et al. *Candida albicans* biofilms and polymicrobial interactions. **Critical reviews in microbiology**, v. 47, n. 1, p. 91-111, 2021.

POULAIN, D.; SENDID, B., STANDAERT-VITSE, A., FRADIN, C., JOUAULT, T., JAWHARA, S., et al. Yeasts: neglected pathogens. **Dig Dis.** v. 27, n. 1, p. 104-110, 2009.

PRICE, Margaret F.; WILKINSON, Ian D.; GENTRY, Layne O. Plate method for detection of phospholipase activity in *Candida albicans*. **Sabouraudia: Journal of Medical and Veterinary Mycology**, v. 20, n. 1, p. 7-14, 1982.

PRISTOV, K. E.; GHANNOUM, M. A. Resistance of *Candida* to azoles and echinocandins worldwide. **Clinical Microbiology and Infection**, v. 25, n. 7, p. 792-798, 2019.

PUNITHAVATHY, P.M.; NALINA, K.; MENON, T. Antifungal susceptibility testing of *Candida tropicalis* biofilms against fluconazole using calorimetric indicator resazurin**. Indian Journal of Pathology & Microbiology**, v. 55, n. 1, p. 72–74, 2012.

RADUNOVIC, Milena et al. Antifungal Susceptibility of *Candida albicans* Isolated from Tongue and Subgingival Biofilm of Periodontitis Patients. **Antibiotics**, v. 11, n. 6, p. 802, 2022.

RAJA, M.; HANNAN, A.; ALI, K. Association of oral candidal carriage with dental caries in children. **Caries Res**, v. 44, n. 3, p. 272–276, May. 2010.

RAMAGE, G.; RAJENDRAN, R.; SHERRY, L.; WILLIAMS, C. Fungal biofilm resistance. **Int. J. Microbiol.**, v. 2012, 2012.

RAPALA-KOZIK, M. et al. Extracellular proteinases of *Candida* species pathogenic yeasts. **Molecular oral microbiology**, v. 33, n. 2, p. 113-124, 2018.

RATHER, Muzamil Ahmad; GUPTA, Kuldeep; MANDAL, Manabendra. Microbial biofilm: formation, architecture, antibiotic resistance, and control strategies. **Brazilian Journal of Microbiology**, p. 1-18, 2021.

RIZZA, M.D.; DELLAYALLE, P.D.; NARANCIO, R.; CABRERA, A.; FERREIRA, F. Biomolecules as Host Defense Weapons Against Microbial Pathogens. **Recent Patents on DNA & Gene Sequences**, v. 2, n. 2, p. 82-96, 2008.

ROCHA, F.; ALVES, A.; ROCHA, M.; CORDEIRO, R. A.; BRILHANTE, R.; PINTO, A.; NUNES, R. M.; GIRÃO, V.; SIDRIM, J. Tumor necrosis factor prevents *Candida albicans* biofilm formation. **Scientific reports**, v. 7, n. 1, 2017.

RODRIGUES, C.F.; RODRIGUES M.E.; SILVA, S.; HENRIQUES, M. *Candida glabrata* Biofilms: How Far Have We Come? ***J. Fungi***, v. 3, n. 1, p. 11, 2017.

RODRIGUES, Célia F. et al. The MNN2 gene knockout modulates the antifungal resistance of biofilms of *Candida glabrata*. **Biomolecules**, v. 8, n. 4, p. 130, 2018.

RODRIGUES, Célia F.; ČERNÁKOVÁ, Lucia. Farnesol and tyrosol: secondary metabolites with a crucial quorum-sensing role in *Candida* biofilm development. **Genes**, v. 11, n. 4, p. 444, 2020.

RODRIGUES, Célia F.; HENRIQUES, Mariana. Portrait of matrix gene expression in *Candida glabrata* biofilms with stress induced by different drugs. **Genes**, v. 9, n. 4, p. 205, 2018.

RODRÍGUEZ-CERDEIRA, Carmen et al. Pathogenesis and clinical relevance of *Candida* biofilms in vulvovaginal candidiasis. **Frontiers in Microbiology**, v. 11, p. 544480, 2020.

ROSA, R.D.; BARRACCO, M.A. Antimicrobial peptides in crustaceans. **Invertebrate Survival Journal,** v. 7, p. 262-284, 2010.

ROSSI, T.; LOZOVOY, A.B.; SILVA, R.V.; FERNANDES, E.V.; GERALDINO, T.H.; COSTA, I.C.; SARIDAKIS, H.O.; EATANABE, M.A.E.; FELIPE, I. Interações entre *Candida albicans* e hospedeiro. **Semina: Ciências biológicas e da saúde**, v. 32, n. 1, p. 15-28, 2011.

ROSSONI, R. D. et al. Comparison of the hemolytic activity between *C. albicans* and non-albicans Candida species. **Braz. oral res.**, v. 27, n. 6, p. 484-489, 2013.

SADEGHI, G. et al. Emergence of non-Candida albicans species: epidemiology, phylogeny and fluconazole susceptibility profile. **Journal de mycologie medicale**, v. 28, n. 1, p. 51-58, 2018.

SALAMA, Ola E.; GERSTEIN, Aleeza C. Differential response of *Candida* species morphologies and isolates to fluconazole and boric acid. **Antimicrobial Agents and Chemotherapy**, v. 66, n. 5, p. e02406-21, 2022.

SCHWEIZER, F. Cationic amphiphilic peptides with cancer-selective toxicity. **Eur. J. Pharmacol**, v. 625, p. 190−194. 2009.

SENEVIRATNE, Chaminda J. et al. Antifungal susceptibility in serum and virulence determinants of *Candida* bloodstream isolates from Hong Kong. **Frontiers in microbiology**, v. 7, p. 216, 2016.

SHAI, Y. Mode of action of membrane active antimicrobial peptides. **Biopolymers**, v. 66, p. 236- 4, 2002.

SHARMA, Divakar; MISBA, Lama; KHAN, Asad U. Antibiotics versus biofilm: an emerging battleground in microbial communities. **Antimicrobial Resistance & Infection Control**, v. 8, n. 1, p. 1-10, 2019.

SIDRIM, José Júlio Costa et al. *Candida* species isolated from the gastrointestinal tract of cockatiels (*Nymphicus hollandicus*): in vitro antifungal susceptibility profile and phospholipase activity. **Veterinary microbiology**, v. 145, n. 3-4, p. 324-328, 2010.

SIDRIM, J. J. C.; ROCHA, M. F. G. **Micologia Médica à Luz de Autores Contemporâneos**. 2. ed. Rio de Janeiro: Guanabara Koogan, 2012.

SILVA, S.; RODRIGUES, C.F.; ARAÚJO, D.; RODRIGUES, M.E.; HENRIQUES, M. *Candida* Species Biofilms' Antifungal Resistance. **Journal of Fungi**, v. 3, n. 8, p. 1, 2017.

SILVA, Sónia et al. *Candida glabrata*, *Candida parapsilosis* and *Candida tropicalis*: biology, epidemiology, pathogenicity and antifungal resistance. **FEMS microbiology reviews**, v. 36, n. 2, p. 288-305, 2012.

SINGH, Arun et al. Oral candidiasis: An overview. **Journal of oral and maxillofacial pathology: JOMFP**, v. 18, n. Suppl 1, p. S81, 2014.

SINGH, B. N. et al. Quercetin sensitizes fluconazole-resistant *Candida albicans* to induce apoptotic cell death by modulating quorum sensing. **Antimicrobial agents and chemotherapy**, v. 59, n. 4, p. 2153-2168, 2015.

SINGH, Dharmendra Prasad et al. Non-*Candida albicans* species: virulence factors and species identification in India. **Current Medical Mycology**, v. 7, n. 2, p. 8, 2021.

STANISZEWSKA, Monika. Virulence factors in *Candida* species. **Current Protein and Peptide Science**, v. 21, n. 3, p. 313-323, 2020.

TALAPKO, Jasminka et al. *Candida albicans*—the virulence factors and clinical manifestations of infection. **Journal of Fungi**, v. 7, n. 2, p. 79, 2021.

THIEME, Lara et al. MBEC versus MBIC: the lack of differentiation between biofilm reducing and inhibitory effects as a current problem in biofilm methodology. **Biological procedures online**, v. 21, p. 1-5, 2019.

TONG, Yiqing; TANG, Jianguo. *Candida albicans* infection and intestinal immunity. **Microbiological research**, v. 198, p. 27-35, 2017.

TÓTH, Renáta et al. *Candida parapsilosis*: from genes to the bedside. **Clinical microbiology reviews**, v. 32, n. 2, p. e00111-18, 2019.

TSUI, C.K.; DANIEL, H.M.; ROBERT, V.; MEYER, W. Re-examining the phylogeny of clinically relevant *Candida* species and allied genera based on multigene analyses. **FEMS Yeast Research**, v.8, n. 4, p. 651–659, 2008.

TURNER, Siobhán A.; BUTLER, Geraldine. The *Candida* pathogenic species complex. **Cold Spring Harbor perspectives in medicine**, v. 4, n. 9, p. a019778, 2014.

VAŇKOVÁ, Eva et al. Combined effect of lasioglossin LL-III derivative with azoles against *Candida albicans* virulence factors: biofilm formation, phospholipases, proteases and hemolytic activity. **FEMS yeast research**, v. 20, n. 3, p. foaa020, 2020.

VASILEIOU, Eleni et al. Invasive candidiasis and candidemia in pediatric and neonatal patients: A review of current guidelines. **Current medical mycology**, v. 4, n. 3, p. 28, 2018.

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VIEIRA, Camila Aoyama et al. Estudo comparativo das espécies de *Candida*: sensibilidade antifúngica e genes de virulência. **Multitemas**, p. 169-182, 2018.

VIEIRA-GIRÃO, P.R.N.; FALCÃO, C.B.; ROCHA, I.; LUCENA, H.M.R.; COSTA, F.H.F.; RÁDIS-BAPTISTA, G. Antiviral Activity of Ctn[15-34], A Cathelicidin-Derived Eicosapeptide, Against Infectious Myonecrosis Virus in Litopenaeus vannamei Primary Hemocyte Cultures. **Food and environmental virology**, v. 9, n. 3, p. 277-286, 2017.

VILA, T.; SULTAN, A. S. Montelongo-Jauregui Det al. **Oral candidiasis: A disease of opportunity. J Fungi (Basel)**, v. 6, 2020.

WALL, Gina et al. *Candida albicans* biofilm growth and dispersal: contributions to pathogenesis. **Current opinion in microbiology**, v. 52, p. 1-6, 2019.

WHALEY, Sarah G. et al. Azole antifungal resistance in *Candida albicans* and emerging non-albicans *Candida* species. **Frontiers in microbiology**, v. 7, p. 2173, 2017.

WHITE, R.L.; BURGESS, D.S.; MANDURU, M.; BOSSO, J.A. Comparison of three different *in vitro* methods of detecting synergy: Time-kill, checkerboard and E-test. **Antimicrobial agents and chemotherapy.** v. 40, p. 1914-1918, 1996.

YANG, X. Q.; ZHANG, Q.; LU, L.Y.; YANG, R.; LIU, Y.; ZOU, J. Genotypic distribution of *Candida albicans* in dental biofilm of Chinese children associated with severe early childhood caries. **Arch Oral Biol**, v. 57, n. 8, p. 1048–1053, Aug. 2012.

YAPAR, N. Epidemiology and risk factors for invasive candidiasis. **Ther. Clin. Risk Manag.**, v. 10, p. 95-105, 2014.

YIKE, I. Fungal proteases and their pathophysiological effects. **Mycopathologia**, v. 171, n. 5, p. 299–323, 2011.

YIN, Wen et al. Biofilms: the microbial “protective clothing” in extreme environments. **International journal of molecular sciences**, v. 20, n. 14, p. 3423, 2019.

ZARNOWSKI, R.; WESTLER, W.M.; LACMBOUH, G.A.; MARITA, J.M.; BOTHE, J.R,; BERNHARDT, J.; SAHRAOUI, L-HA.; FONTAINE, J.; SANCHEZ, H.; HATFIELD, R.D.; NTAMBI, J.M.; NETT, J.E.; MITCHELL, A.P.; ANDES, D.R. Novel entries in a fungal biofilm matrix encyclopedia. **mBio**. v. 5, n. 4, p. 1333-14, 2014.

ZARRINFAR, Hossein; KORD, Zahra; FATA, Abdolmajid. High incidence of azole resistance among *Candida albicans* and *C. glabrata* isolates in Northeastern Iran. **Current Medical Mycology**, v. 7, n. 3, p. 18, 2021.

ZHANG, Qi-Yu et al. Antimicrobial peptides: mechanism of action, activity and clinical potential. **Military Medical Research**, v. 8, p. 1-25, 2021.

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10. ANEXOS

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Review

Cell-Penetrating Antimicrobial Peptides with Anti-Infective Activity against Intracellular Pathogens

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Abstract: Cell-penetrating peptides (CPPs) are natural or engineered peptide sequences with the intrinsic ability to internalize into a diversity of cell types and simultaneously transport hydrophilic molecules and nanomaterials, of which the cellular uptake is often limited. In addition to this primordial activity of cell penetration without membrane disruption, multivalent antimicrobial activity accompanies some CPPs. Antimicrobial peptides (AMPs) with cell-penetrability exert their effect intracellularly, and they are of great interest. CPPs with antimicrobial activity (CPAPs) comprise a particular class of bioactive peptides that arise as promising agents against difficult-to-treat intracellular infections. This short review aims to present the antibacterial, antiparasitic, and antiviral effects of various cell-penetrating antimicrobial peptides currently documented. Examples include the antimicrobial effects of different CPAPs against bacteria that can propagate intracellularly, like *Staphylococcus* sp., *Streptococcus* sp., *Chlamydia trachomatis*, *Escherichia coli*, *Mycobacterium* sp., *Listeria* sp., *Salmonella* sp. among others. CPAPs with antiviral effects that interfere with the intracellular replication of HIV, hepatitis B, HPV, and herpes virus. Additionally, CPAPs with activity against protozoa of the genera *Leishmania*, *Trypanosoma*, and *Plasmodium*, the etiological agents of Leishmaniasis, Chagas' Disease, and Malaria, respectively. The information provided in this review emphasizes the potential of multivalent CPAPs, with anti-infective properties for application against various intracellular infections. So far, CPAPs bear a promise of druggability for the translational medical use of CPPs alone or in combination with chemotherapeutics. Moreover, CPAPs could be an exciting alternative for pharmaceutical design and treating intracellular infectious diseases.

Keywords: antimicrobial peptide; cell-penetrating peptide; cell-penetrating antimicrobial peptide; intracellular pathogen; intracellular infection

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1. Introduction

Intracellular pathogens can grow and reproduce within their host cells, and this feature helps them evade the immune system and makes treatment difficult. Accessible and efficacious drugs targeting these pathogens are challenging, as many antimicrobials cannot accumulate in intracellular spaces to reach optimal therapeutic concentrations in infected cells [1]. Treatment options generally involve long-term therapy with a combination of medications. However, the drug's inherent toxicity and prolonged exposure time of treatment usually cause undesired side effects. Therefore, strategies targeting intracellular infection and eradicating intracellular pathogens are advantageous since therapeutic agents penetrate and concentrate into infected cells, reaching the pathogens, increasing efficacy, decreasing drug toxicity, and sustaining and releasing the drugs [2]. Faced with these challenges, cell-penetrating peptides (CPPs) have emerged as an exciting alternative for pharmaceutical drug design and formulations. CPPs are known for their great potential

for delivering peptides and proteins into cells and across epithelial barriers, both in vitro and in vivo [3]. Having the unique ability to transport various payloads within cells with low toxicity, CPPs hold the promise of a powerful tool for medical applications. They can transport molecules for which intracellular supply is often limited due to hydrophilic character, net negative charge, and high molecular weight. In addition, they can mediate the delivery of drugs, diagnostic agents, nanoparticles, and therapeutic proteins [4].

Notably, CPPs share several physicochemical characteristics with another class of biologically active peptides, antimicrobial peptides (AMPs). CPPs with antimicrobial activities and reciprocally AMPs with cell-penetrating activities are generally short sequences of amino acids, amphipathic, and display a net positive charge due to a high proportion of arginine and lysine residues. The sources of CPPs with antimicrobial activity are diverse: they might be natural, synthetic, or chimeric. The spectrum of action of cell-penetrating peptides with anti-infective properties includes activity against intracellular Gram-positive and Gram-negative bacteria, parasites, viruses, and fungi, as reviewed and discussed herein. Considering this initial conception, CPPs with anti-infective activities arise as an exciting alternative for pharmaceutical drug design to treat intracellular infections. This short review aims to call attention to and identify in the scientific literature previously published studies and references regarding the action of CPPs that are effective against intracellular infectious pathogenic agents.

2. Intracellular Pathogens

2.1. Bacteria

Many microorganisms are obligate or facultative intracellular, which allows growth and replication in different biological niches. When initiating the process of internalization in the host cells, some microbial pathogens can be detected by the host defense mechanisms, activating the recruitment of macrophages. However, as is the case, intracellular bacteria can protect themselves from the host's immune response and the killing effects of antimicrobials inside the infected cells. In this way, intracellular bacteria reside in the cytoplasm or vacuoles of the mammalian host cell, coopting the endocytic or secretory pathways to recruit the necessary supplements to ensure their replication [5]. Pathogens can enter different cell types via phagocytic and non-phagocytic routes. Phagocytosis is a mechanism related to the host's response to injuries and infections. Pathogens or scavengers larger than 0.5 μm are recognized by phagocytic cells through specific pathogen recognition receptors. Then, phagosomes that contain the microorganisms and scavengers form, usually in macrophages, that fuse with lysosomes to form mature phagolysosomes, where the bacterium is degraded due to lysozymes and oxidizing agents. *Mycobacterium tuberculosis* is one of the most successful pathogens in escaping this process. It has evolved to prevent phagolysosome maturation and can live in macrophages, masking pathogen-associated molecular patterns (PAMPs) [6]. A latent state may occur for bacterial and viral infections, such as those caused by *M. tuberculosis* and HIV.

Blocking the progression of the disease may not only result from the annihilation of pathogens but may also involve the death of the host cell. The disease process may involve interrupting bacterial growth in a subset of cells by innate immune pathways. However, it may trigger an inflammatory response that does not interfere with pathogen replication, as is seen with the enteric bacteria *Salmonella* during intestinal growth [7]. Another example of an intracellular microorganism, *Listeria* spp. can induce epigenetic and miRNA modifications in the host to modulate immune defense. *Listeria monocytogenes* is a Gram-positive facultative pathogen that causes the foodborne illness called Listeriosis. Upon entry into epithelial cells, *L. monocytogenes* are internalized into a vacuole. It physically disrupts the vacuolar membrane to escape this subcellular niche, using Listeriolysin O (LLO) and phospholipase A and B (PlcA and PlcB). Then, *L. monocytogeneses* can survive and replicate in the cytosol of host cells and modify the processes of cellular organelles. Intracellular bacteria can also grow an actin tail to move between epithelial cells evading host defense [8].

Chlamydiae, an obligate intracellular Gram-negative bacterium, has a unique developmental cycle of replication consisting of extracellular and intracellular life forms. *Chlamydia trachomatis* and *C. pneumonia* cause sexually transmitted diseases, eye infections, and atypical pneumonia. *Chlamydiae* have developed strategies for chromatin regulation through epigenetic modifications [9]. In addition to the classic examples of intracellular bacteria such as the case of *Listeria* and *Chlamydia*, cumulative evidence suggests that known extracellular bacteria such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* also can invade and localize inside the host cells [10].

2.2. Parasites

Regarding parasitic infections, intracellular obligate parasites use diverse strategies to deceive host cell immune responses and persist in the host cells and organism. One common strategy in clinically relevant infectious diseases is the formation of vacuoles containing pathogens within host cells after the pathogen has been internalized. Included in this category are the trypanosomatids, of which *Trypanosoma cruzi* and *Leishmania* spp. are intracellular parasites of mammals [11]. In the case of the causative agent of Leishmaniasis, the first hours after infection are crucial to the obligated intracellular *Leishmania* parasites. *Leishmania* parasites benefit from the pro-inflammatory properties of the mosquito vector's saliva, which also plays an essential role in the chemoattraction of phagocytes.

Additionally, the complement system affects the initial phases of *Leishmania* infection, as opsonization promotes the parasite uptake by phagocytes, favoring the intracellular stage of infection. Once the parasite is phagocytosed, innate immune cells react by producing cytokines that, in its turn, activate the adaptive immunity, thus generating a protective or harmful response. Adaptive immune system cells have also participated in Leishmaniasis's pathogenesis, causing tissue destruction and disease relapse [12]. For *Plasmodium falciparum*, one of the etiological agents of malaria, free sporozoites and intrahepatic parasites overcome the obstacle of the host's immune response to entering the erythrocytic stage, actively passing through Kupffer cells and endothelial cells. Once inside the hepatocyte, the parasitophorous vacuole prevents lysosomal degradation. Host heme oxygenase-1 (HO-1) also enhances the development of intrahepatic parasites by modulating the host's inflammatory response [13].

2.3. Viruses

As for viruses, such as those caused by Human Immunodeficiency Virus (HIV) from type HIV-1 and HIV-2, Hepatitis B Virus, Influenza Virus, to Middle East Respiratory Syndrome Coronavirus, SARS-CoV-2 and others, the high number of cases of these viral diseases are related to several environmental and behavioral factors, which contribute to the spreading of these highly persistent and replicative viruses. Mutations constantly appear in the genomes of all species, making it difficult to treat and control some viral diseases [14].

3. Challenge to Treat Intracellular Infections

Efficient treatment of intracellular infections with antimicrobials is challenging due to the evasion of intracellular infectious pathogens from the host's phagocytic killing mechanisms, the establishment of intracellular survival machinery, and the misuse of antimicrobials that cause an increase of multidrug resistance phenotypes in pathogens. Once inside the cell, antimicrobial activity can be influenced by enzymatic inactivation and changes in pH and chemical environment [15]. Moreover, the intracellular concentration of antibiotics in host cells is lower than their minimum inhibitory and lethal concentrations, which may lead to the emergence of drug resistance. The increase and spread of multidrug-resistant bacteria and infectious agents reinforce the urgent need to develop effective intracellularly active antibacterial drugs and chemotherapeutics capable of improving the low cellular permeability of currently used antimicrobials.

In this context, CPPs with anti-infective activities have been envisioned as an alternative to overcome the difficulty-to-treat intracellular infectious microbes and to potentiate the targeted-driven antimicrobial action to defeat intracellular microorganisms [16,17].

4. Cell-Penetrating and Antimicrobial Peptides: The Basics

Cell-penetrating peptides are natural or synthetic peptide sequences, usually displaying selective cytotoxicity, high cell-uptake efficiency, and penetrability of lipid membranes and accumulation in intracellular compartments. Another notable feature of CPPs is that cell membrane translocation can occur at low concentrations, both in vivo and in vitro, without causing significant membrane damage. Cell-penetrating peptides usually consist of 5 to 30 amino acid residues. They group into different categories according to their origin, sequence, and structure [18]. One of the first CPPs ever described was the HIV transcriptional transactivator protein (TAT) which was proven to translocate cell membranes and effectively internalize into the cells in vitro. The TAT peptide becomes a paradigmatic CPP for basic and applied research [19,20]. Endocytosis and direct translocation are essential mechanisms contributing to membrane translocation by different CPPs. These events are related to the specific CPP sequence, peptide concentration, cell type, and cell differentiation state, among other factors [21]. Several CPPs adopt a well-defined secondary structure upon encountering the cell plasma membrane, which seems to contribute to translocation propensity across the membrane [22]. Some CPPs and AMPs share structural and physicochemical characteristics that intriguingly call attention to their subtle differences and eventual similar functionalities (e.g., cell penetration and anti-infectivity) [23,24]. Native AMPs are widely found in organisms' cells, tissues, and biological fluids and are essentially involved in the innate defense of the host against invading pathogens. They kill microbes by diverse but convergent mechanisms of cell disruption that essentially compromise the integrity of the cytoplasmic membranes [25,26], which serve as a portal for the synergic action of chemotherapeutics [27]. A handful of exciting reviews about the structures, activities, and mechanisms of action of CPPs and AMPs [28–30] are available. Some suggested reviews on the biological activity of AMPs against cancer cells [31–33] and CPPs as delivery systems [34–37] could be of interest.

The cellular penetrability of some AMPs seems to reinforce their antimicrobial efficacy and performance due to the interaction and interference with intracellular components of target microorganisms, such as macromolecules and organelles [31,32]. Consequently, AMPs interacting with cell membranes can exhibit antimicrobial activity at the membrane level or intracellularly through cell-penetrability. The positively-charged amino acid residues that facilitate the electrostatic interactions of CPPs and AMPs with cell membranes and their components are common to sequences of both peptide types. Interspaced positively charged amino acid residues in these two peptide families contribute to the first step of membrane interaction and aggregation, leading to membrane insertion and cellular uptake. Components on the cell surface with an overall net negative charge facilitate binding the positively charged and amphipathic peptides. These are the anionic phospholipids or phosphate groups of lipopolysaccharides in Gram-negative bacteria and acidic polysaccharides, teichoic acids, and lipoteichoic acids in Gram-positive bacteria [33].

The antiviral mechanisms of AMPs mainly involve targeting and disrupting viral membrane envelopes and inhibiting different stages of the viral life cycle [33,34]. As for the majority of therapeutic peptides, the physiological instability, lack of selectivity, and limited efficacy comprise drawbacks that should be considered during the development and medicinal use of CPPs with antimicrobial activity and AMPs with cell-penetrating properties. Proteolytic degradation, hepatic and renal clearance, and inherent instability of CPPs and AMPs in physiological fluids are reasons for the large discrepancy of their performance in vitro and in vivo [35,36].

5. Peptides with Cell-Penetrating and Anti-Infective Activity against Intracellular Pathogens

In the current scientific literature, cumulative data about cell-penetrating antimicrobial peptides (CPAPs) with anti-infective activities to treat infections caused by obligatory or facultative intracellular pathogens are accessible. This particular class of bioactive peptides is also called antimicrobial and cell-penetrating peptides (ACPPs) [37]. Antimicrobial peptides that penetrate the target cells without causing membrane disruption exert their biocide effects intracellularly figure in the subset of CPAPs [38,39]. CPPs that intrinsically translocate eukaryotic cell membranes and cross the cytoplasmic membrane of bacteria can kill pathogens by distributing and accumulating within the vacuolar compartments of cells, as is the case of penetratin analogs [40]. The design and synthesis of chimeric CPP-AMP peptides with a cleavable link also comprise an attractive and efficient mechanistic option to kill intracellular pathogens [41,42]. Another exciting mechanism to kill intracellular pathogens is via a cooperative action of the AMPS α-defensins and humanized θ-defensin with macrophages that entrapped the bacteria in the phagosome, where they are annihilate [43]. The following sections present examples of these cell-penetrating antimicrobial peptides with antibacterial, antiviral, and antiparasitic activities.

5.1. Cell-Penetrating Antimicrobial Peptides Active against Bacteria

Essentially, bacteria evolved diverse strategies to escape from the antimicrobial arsenal of macrophages, and once bacteria internalized into macrophages, they evade the host's immune response and the action of antimicrobial drugs [44]. The first barrier for a therapeutic compound to reach intracellular bacteria is crossing the host cell membrane at non-toxic concentrations. The second barrier is the challenge of accessing bacteria located in the host cell cytosol or sequestered inside membrane-bound vesicles and maintaining an effective antimicrobial activity. Several CPAPs have been investigated for their applicability against intracellular bacteria in this context.

The Gram-positive bacterium *S. aureus* is considered an extracellular pathogen. However, it can survive in primary human macrophages until bacterial proliferation occurs. Replication begins hours after the initial phagocytosis, while the bacteria reside in mature phagolysosomes and may remain for several days. The lysis of infected phagocytes allows them to escape to the extracellular medium, where dissemination occurs [45]. Budagavi et al. [46] evaluated the antimicrobial properties of cell-penetrating peptides derived from Latarcin1 (LDP) toxin against methicillin-resistant *S. aureus* (MRSA) strains. Latarcins are a group of antimicrobials and cytolytic peptides, 20 to 35 amino acids in length, from the venom of the *Lachesana tarabaevi* spider. Human cervical cancer epithelial (HeLa) cells were used for the invasion assay by MRSA strains and *Bacillus subtilis*, *E. coli*, *S. typhimurium*, *M. smegmatis,* and *Xanthomonas oryzae*. The peptide exhibited antimicrobial activity against all microorganisms tested, within a range of 5 to 20 μM, showing a 75% inhibition of intracellular MRSA at different concentrations. However, the peptide's mechanism of action was not fully described [46]. Other studies have also attempted to identify peptides with activity against intracellular MRSA infections, such as the TAT peptide (47–58) (YGRKKRRQRRRD) derived from the TAT protein of the HIV-1 virus, responsible for activating viral replication. The authors studied this paradigmatic peptide because of its ability to penetrate the fungal cell membrane in a time- and temperature-independent manner, leading to cell cycle arrest and death. Infected HeLa cells were treated with TAT (47–58) and its enantiomer at a concentration of 50 μM and observed an antibacterial activity against MRSA without affecting the viability of mammalian host cells [47]. Wang and colleagues [48] investigated the antibacterial activity of the H2 peptide against MRSA strains causing bovine mastitis. They confirmed that the peptide enters into mammary epithelial cells by clathrin-mediated endocytosis in a dose-dependent manner, killing the intracellular MRSA and clinical isolates of *S. aureus* with a performance superior to vancomycin. Another Gram-positive coccus, *Streptococcus agalactiae*, can survive within macrophages for prolonged periods, contributing to pathogen spread and disease progression. To defeat *S. agalactiae*, peptides L2 and L10 were developed and exhibited more

potent antimicrobial activity against bacteria inside the cell in an intracellular environment. The results of the localization of peptides in macrophages indicated that after endocytosis, L2 and L10 probably entered the compartments containing *S. agalactiae* by endosomal traffic and killing the bacteria [42]. Nepal et al. [49] attempted to demonstrate that increasing the length of CAPH peptides (i.e., CPAPs based on a cationic amphiphilic proline helix structure) increases their ability to enter cells and the antimicrobial activity compared to shorter cationic amphiphilic proline helix structures. This peptide has a dual mode of action: effective cell penetration capability into human macrophages and potent antimicrobial activity in vitro against Gram-positive and Gram-negative pathogens. Several pathogenic bacteria were effectively killed with the Fl-PRPLPL-5 peptide, including MRSA, *Acinetobacter baumannii*, *E. coli* O157, and VRSA (Vancomycin-resistant *S. aureus*). Significantly, this CPAP could deal with the problem of intracellular bacteria and the elimination of pathogens in macrophages [49]. Three similar peptides, also of the same class, derived from the initial peptide P14LRR, have specific subcellular locations that allow the targeting of pathogenic bacteria in their intracellular niches. This unique feature successfully eliminates *Salmonella*, *Shigella*, and *Listeria pathogens*, which reside in macrophages. More effective subcellular localization of P14-5C within endosomes may allow this CAPH to localize to endosome-resident *Salmonella*, thus resulting in improved intracellular clearance of the pathogen. The P14-5L peptide accumulates in the cytosol of macrophages and significantly reduces the population of *Listeria* and *Shigella* residing in the cytosol of J774A cells. The selected CAPHs could substantially reduce *Listeria* bacterial infection in an in vivo model of *Caenorhabditis elegans* with minimal toxicity to the worms [50]. The Fl-PLPRPR-4 peptide, another proline-rich synthetic peptide, displayed activity to kill intracellular bacterial pathogens, *Salmonella* and *Brucella*, infecting J774A cells. While intracellular *Salmonella* was reduced by approximately 62% with the addition of the peptide, intracellular *Brucella* showed a significant reduction of 90%. This difference in inhibition between *Salmonella* and *Brucella* may be related to several factors but the subcellular location of *Salmonella* and compartmentalization inside phagosomes. These synthetic peptides could internalize cells by endocytosis, direct translocation, or a combination of both pathways, depending on concentration, cell type, and charge. The entry of Fl-PLPRPR-4 in both endosomes and the mitochondria of J774A.1 cell may be related to both penetration mechanisms, by which peptide reach bacteria residing in infected J774A.1 cells, but with a limited impact on mitochondrial function [51]. Another example of peptide, TPk was investigated to verify its ability to internalize in eukaryotic cells, the intracellular location, the level of growth inhibition of bacterial growth, the influence of eukaryotic cell viability, and the destabilization of membranes through the use of liposomes that mimic *S. aureus* and mammalian cell membranes. TPk is transported to lysosomes and is considered a candidate for future investigations as an AMP capable of targeting intracellular infections, as it can potentially be optimized for cellular penetration, resulting in an effective tool for eradicating bacteria residing within epithelial cells [16].

Chlamydia trachomatis can survive and replicate in an intracellular or extracellular environment. The synthetic peptide Pep-1 contains a hydrophobic tryptophan-rich motif and a hydrophilic lysine-rich domain for efficiently targeting cell membranes. Pep-1 had a concentration-dependent action against intracellular *C. trachomatis* growth with 100% inhibition of inclusion formation at a concentration of 8 mg/L, with a window of susceptibility during the developmental cycle with maximum effect when treatment was initiated 12 h after infection. This anti-chlamydial activity may directly affect the bacterium or indirectly by targeting the inclusion of *C. trachomatis* or host cell processes essential for growth [52]. Amiss and colleagues [53] identified two horseshoe crab β-hairpin peptides, tachyplesin I and polyphemusin I, with broad antimicrobial activity against *E. coli* UPEC (Uropathogenic *E. coli*), strain EC958. They also developed the peptide analogs [I11A]tachyplesin I and [I11S]tachyplesin I that maintained activity against bacteria but were less toxic to mammalian cells than native tachyplesin I. For the in vitro tests, macrophages derived from the bone marrow of mice were used. Polyphemusin I, tachy-

plesin I, and analogs [I11A]tachyplesin I and [I11S]tachyplesin I inhibit bacterial growth at concentrations at least ten-fold lower than concentrations that are toxic to mammalian cells (8 μM). EC958 were observed within intracellular vesicle compartments, and after treatment of infected macrophages with eight μM of the marked peptides for one hour, especially [I11S]tachyplesin I produced diffuse fluorescence throughout the cytosol and strong co-localization with intracellular EC958 bacteria. This study demonstrated that peptides could enter macrophages without damaging their membrane at concentrations that kill co-localized bacteria. The mechanistic studies using bacterial cells, model membranes, and cell membrane extracts, indicated tachyplesin I and polyphemusin I peptides could kill UPEC by selectively binding and disrupting bacterial cell membranes.

Table 1 summarizes active CPAPs against intracellular bacteria, their amino acid sequences, origins, and targeted bacteria.

Table 1. CPAPs with anti-infective activity against intracellular bacteria.

Peptide	Source	Sequence	Target	Study Model	Ref.
TPk	Neutrophil granules	VRRFkWWWkFLRR	*S. aureus*	in vitro	[16]
PenShuf	Synthetic peptide	RWFKIQMQIRRWKNKK	*S. aureus* and *E. coli*	in vitro	[16]
L2 and L10	A synthetic peptide with a fragment of the pheromone (DILIIVGG) of *Streptococcus agalactiae*	DILIIVGGGSGKERKKRRR DILIIVGGKRRR	*Streptococcus agalactiae*	in vitro and in vivo	[42]
LDP	*Lachesana tarabaevi* (Spider)	KWRRKLKKLR	MRSA, *Bacillus subtilis, Escherichia coli, Salmonella typhimurium, Xanthomonas oryzae, Mycobacterium smegmatis*	in vitro	[46]
TAT (47–58)	TAT protein of HIV-1	YGRKKRRQRRRD	MRSA	in vitro	[47]
H2	Derived from Plectasin, isolated from *Pseudoplectania nigrella*	-	MRSA	in vitro and in vivo	[48]
Fl-PRPLPL-5	Synthetic peptide	-	*A. baumannii*, *E. coli* O157, MRSA e VRSA	in vitro	[49]
P14-5L, P14-5B and P14-5C	Synthetic peptide	-	*Salmonella* sp., *Shigella* sp. and *Listeria* sp.	in vitro in vivo	[50]
Fl-PLPRPR-4	Synthetic peptide	-	*Salmonella typhimurium* and *Brucella abortus*	in vitro	[51]
Pep-1	Synthetic peptide	KETWWETWWTEWSQPKKKRKV	*Chlamydia trachomatis*	in vitro	[52]
[I11A]tachyplesin I and [I11S]tachyplesin I	Analogs from Tachyplesin I, a horseshoe crab peptide.	KWCFRVCYRGACYRRCR KWCFRVCYRGSCYRRCR	*Escherichia coli*	in vitro	[53]

5.2. Cell-Penetrating Antimicrobial Peptides Active against Virus

Usually, the penetration of hydrophilic drugs that act intracellularly has their effect impaired due to the lipophilic barrier of the cell membranes, resulting in poor efficacy on their targets. Many techniques have been developed to find a non-invasive carrier to circumvent this problem and increase drug availability. Antiviral CPAPs can enter cells without causing appreciable damage, increasing molecules' cellular uptake and internalization [54].

HIV has been the subject of numerous studies for a long time to seek a cure for carriers of this virus. Recently, a synthetic 18-residue cationic peptide capable of neutralizing the DH12 and SF162 strains of the HIV1 virus showed inhibition of 80% for both virus strains. In addition, its action was potentiated when using the bacterial toxins LT-IB or LT-IIaB derived from *E. coli*. This peptide was found to home in the cytoplasm and nucleus,

demonstrating that it can act and influence the stages of production and maturation of the HIV-1 virus [55]. In another example, the 10-residue TAT peptide derived from the stretch comprising residues 48 to 57 of the TAT protein found in the HIV-1 virus can inhibit HIV-1 viral infection in a dose-dependent manner.

Moreover, a change in the peptide net charge can increase its activity with the replacement of arginine by non-cationic residues, and the conjugation of deca-arginine potentiated the action of the TAT peptide [56]. RNA plays a role in regulating several processes in the biological system. In HIV-1, it is possible to find TAR, a short hairpin of RNA. This hairpin interacts with the TAT protein, responsible for the viral gene transcription, and this interaction has been the research subject in developing drugs that act at this point. The LK-3 peptide was synthesized with leucine (L) and lysine (K) residues, and results showed that in nanomolar concentrations, the LK dimer effectively inhibits TAR transcription, thus being a promising agent with strong HIV-1 inhibitory potential [57]. A determining agent for the assembly of the HIV-1 virus is the GAG protein, an envelope of this virus, thus being considered a critical pharmaceutical target. A peptide called CAI was discovered from bacteriophages and in vitro studies showed its ability to inhibit the formation of the GAG capsid. However, it was ineffective in HIV-1. After structural analysis, this peptide underwent modifications to make it cell penetrable, thus creating NYAD-1. This peptide demonstrated significant effects, such that it enters cells without the aid of a carrier protein, stops the development of HIV-1 particles, and effectively inhibits the HIV-1 infection in cell cultures [58]. Another molecule acting on HIV-1 was discovered after genomic analyses: the TOE1 protein, a target of the Egr1 gene, found in the nucleoli and Cajal's bodies. In vitro studies, this protein has been shown to act at the transcriptional level, preventing replication of HIV-1 so that this protein can be released by CD8+ cells penetrating cells, thus decreasing the action of HIV [59].

The Human Papillomavirus (HPV) is responsible for causing an average of 5% of cervical cancer, and although vaccines are available, they are efficacious against only one set of HPV. Understanding the life cycle and performance of HPV can lead to the production of new pharmacological strategies that reduce its viral load and other HPV-related diseases. HPV is a DNA virus with 360 L1 capsid residues and 72 L2 molecules. The L2 protein is essential for the assembly and infectious viral process. A 29-residue peptide was synthesized, containing a binding site for L2 and a sequence for cell penetration into cells. Peptide P16/16 has been shown to inhibit the release of the virus from the endosome, causing a reduction of viral constituents in cells which is dose-dependent, thus being a potential antiviral target [60].

The herpes virus (*Herpes simplex* type—HSV-1) can trigger diseases in the human body ranging from cold sores to encephalitis and blindness. Two peptides (Hp1036 and Hp1239) derived from the scorpion *Heterometrus petersii* significantly inhibit the cell entry and proliferation of the HSV-1 virus. The amphipathic characteristic of peptides and their α-helix structure helped insert the virus into the membrane and reduce its replication after infection. Based on these data, the peptides Hp1036 and Hp1239 appeared promising candidates to combat viral activity [61]. Belonging to the Hepadnaviridae family with a size of 3.2 kb, the hepatitis B virus (HBV) is one of the leading infectious human viruses, affecting millions worldwide annually. HBV infection can cause chronic hepatitis and liver cancer. Therapy for hepatitis B is limiting, and most macromolecular antivirals cannot cross the impermeable plasma membrane. Based on the necessity to treat this viral disease, a peptide was synthesized that comprises an oligoarginine and a core capsid binding sequence (NBS). Such a peptide effectively penetrated the plasma membrane, inhibiting the release of the hepatitis B virus, proving to be an efficient transducing agent with intrinsic activity for delivering antiviral peptides to cells [62].

Table 2 lists some examples of cell-penetrating antimicrobial peptides with antiviral activity.

Table 2. Cell-penetrating antimicrobial peptides with antiviral activity.

Peptide	Source	Sequence	Target	Study Model	Ref.
MFK	Synthetic peptide	MFKLRAKIKVRLRAKIKL	HIV-1 Virus	in vitro	[55]
TAT (48–57)	TAT protein of HIV-1 (Strain SF2)	GRKKRRQRRR	HIV-1 Virus	in vitro	[56]
LK-3	Synthetic peptide	LKKLCKLLKKLCKLAG	HIV-1 Virus	in vitro	[57]
NYAD-1	Synthetic peptide	-	HIV-1 Virus	in vitro	[58]
Target of Egr1 (TOE1)	Nuclear protein in nucleoli and Cajal bodies	335-KRRRRRRREKRKR-347	HIV-1 Virus	in vitro	[59]
P16/16	Synthetic peptide	CSPQYTI-IADAGDFYLHPSYYMLRKRRKR	HPV Virus	in vivo and in vitro	[60]
Hp1036 and Hp1239	Venom of scorpion *Heterometrus petersii*	ILGKIWEGIKSIF ILSYLWNGIKSIF	Herpes simplex virus type 1	in vitro	[61]
P4	Synthetic peptide	LDPAFR	Hepatitis B virus	in vitro	[62]
Deca-$(Arg)_8$	Synthetic peptide	Decanoic acid (C10:0)-D(WRRRRRRRRG)-NH2	Hepatitis B Virus	in vitro	[63]

5.3. Cell-Penetrating Antimicrobial Peptides Active against Intracellular Parasites

The search for antimicrobial, cell-penetrating peptides with antiparasitic effects is of great interest since some infectious parasites hide inside host cells, and drug resistance increases considerably and concomitantly with drug ineffectiveness.

Among diseases caused by parasites, malaria is a potentially severe infectious disease transmitted by the bite of the Anopheles mosquito, which affects an average of one million persons annually. Of particular interest, antimalarial peptides have been discovered in recent years. Crotamine, a 42-amino acid, a multivalent peptide derived from the venom of South American rattlesnake *Crotalus durissus terrificus*, was investigated as an inhibitor of the *Plasmodium falciparum* parasite, and it was found that this substance prevents the parasite development in a dose-dependent manner. Its action may be related to the arrest of homeostasis in the acidic compartments of *Plasmodium*. In addition, the peptide decreased the fluorescence of dye tracker in the parasite's organelle, indicating a compromise of the parasite's metabolism [64].

Moreover, crotamine internalizes into erythrocytes infected by *P. falciparum* as confirmed with exposed cultured cells harboring blood-stage development of the parasite and the peptide in different incubation periods. Crotamine has no appreciable hemolytic activity and is selectively internalized only by infected erythrocytes [65]. Another peptide, derived from *P. falciparum* itself, demonstrated an antiparasitic effect. *Plasmodium falciparum* dihydrofolate reductase-thymidylate synthase (pfDHFR-TS) is a bifunctional enzyme responsible for folate production and thymidylate (dTMP). The pfDHFR-TS comprises 231 residues of the DHFR domain in the N-terminal region, followed by a junctional region (JR) with 89 residues and ending with the C-terminal end of 238 residues [66]. Peptide sequences from the junctional region and DHFR domain five peptides were designed and synthesized. Among these, the peptides JR21 and rR8-JR21 were effective in inhibiting the growth of *P. falciparum* in vitro. The rR8-JR21 peptide effectively delayed parasite development and caused minimal hemolysis of red blood cells, thus being a substance capable of affecting the development and growth of the parasite [67].

The transportan 10 peptide (TP10) is a 27-residue chimeric CPP derived from the venom peptide of the wasp *Vespula lewisii*. This peptide can penetrate the cell independently of the receptor and transport several types of cargo across cell membranes [68]. TP10 was tested for its anti-plasmodial and anti-trypanocidal potential. In vitro cell culture, this peptide reduced parasitemia by more than 99% and decreased oocyst infection, thus

being an active substance against malaria in the blood and vector stages. Against the vector, *Trypanosoma brucei*, TP10 was also detrimental to the parasite in the blood phase, thus emerging as an anti-plasmodial and anti-trypanocidal peptide [69]. A developed mitochondria-penetratin peptide (MPP) with the ability to enter human cells and specifically target the mitochondria effectively controlled intracellular *Plasmodium* parasite. In some instances, mitochondria are considered an exciting drug target because they play a crucial role in energy production and programmed cell death of eukaryotic cells. The combination of two significant characteristics, cationic and lipophilic, drives the permeation of MPPs through the hydrophobic mitochondrial membrane. A short peptide, (L-cyclohexyl alanine-D-arginine)^3 or (Fxr)^3 exhibited efficient cell penetration and mitochondrial localization with activity against *P. falciparum*, including chloroquine-resistant K1 strain. This peptide can pass through the red blood cell membrane without disruption or destruction and subsequently kill the blood stage of *P. falciparum*. (Fxr)3 showed more potent antimalarial activity toward late-stage (trophozoite and schizont) parasites, consistent with the high intensity of (Fxr)3 localized in the parasites' mitochondria observed by confocal microscopy. The antimalarial action may be related to a collapse of the mitochondrial membrane potential [70].

Trypanosomiasis, popularly known as Chagas' disease, is caused by the protozoan *T. cruzi* and is among the 13 most neglected diseases worldwide, affecting about 10 million people annually. Currently, the pharmacotherapy used in this pathology works only in the acute phase, being ineffective in the chronic phase and eliminating the vector. Batroxycidin (BatxC), an α-helix peptide derived from the venom gland of the lancehead pit viper *Bothrops atrox*, was evaluated against the *T. cruzi* strain. BatxC inhibited the development of the benznidazole-resistant strains of the protozoan. In addition, it could lead to protozoan necrosis, observed by the loss of the cell membrane [71].

Among the parasitic diseases, Leishmaniasis is the seventh most important and represents a severe public health problem, affecting about 15 million people annually. This parasitosis is caused by the bite of the sand fly, mainly of the genera *Phlebotomus* and *Lutzomyia* [72]. Notably, histatin 5 (Hst5), an AMP isolated from human saliva that targets fungal mitochondria, showed anti-leishmania effects against strains of *Leishmania donovani*. In protozoa, Hst5 exerts its anti-parasite effects against promastigote and amastigote phases. This peptide proved capable of causing damage to the plasma membrane of the parasite, translocating to the *Leishmania* cytoplasm independently of a receptor for internalization. The peptide still has the potential to reduce ATP synthesis, leading to a subsequent drop in ATP content and causing a bioenergetic breakdown. Thus, Hst5, in addition to exhibiting anti-leishmanial effects, acts directly on the mitochondrial ATP synthesis of this protozoan [73]. Tachypesin is a peptide derived from the horseshoe crab *Tachypleus tridentatus* of marine origin, containing 17 residues in its structure with a positive charge and molecular weight of 2.36 kDa. When evaluated in the same strain mentioned above, this substance demonstrated efficacy in the promastigote and amastigote forms of *L. donovani*, damaging the membrane in a way that makes it difficult for the parasite to acquire resistance [74]. Phospholipases A2 (PLA2) are potent active toxins with multiple biological activities from enzymatic to neurotoxic, so their structure is used as a model for the structural design of several molecules with numerous pharmacological properties.

The PLA2-derived p-AclR7 peptide and analogs were synthesized and evaluated against strains of *Leishmania amazonensis*. The peptide p-AclR7 was designed from the C-terminal p-Acl region of a segment of Lys49 from the PLA2 from the venom of the broad-banded copperhead snake *Agkistrodon contortrix laticinctus*. An analog prepared by replacing all its lysine residues with arginine combined the characteristics of its original p-Acl and oligoarginine. The replacement of lysine for arginine residues in the peptide sequence potentiated the antiparasitic effect compared to the original peptide. The p-AclR7 was most active against the amastigote and promastigote stages of the protozoa [75]. Peptides with antimicrobial activities were evaluated in two other *Leishmania* species, namely *L. panamensis* and *L. major*. From the waxy monkey leaf frog *Phyllomedusa sauvagei*,

it was possible to isolate dermaseptin, a potent AMP with a broad spectrum of action against fungi and bacteria. Dermaseptin promoted an increase in nitric oxide levels and acted on intracellular forms of the *Leishmania* parasite [76]. Cecropin A is a peptide isolated from various insects with sequences with 35–37 amino acids in length. Cecropin A and andropin prevented the development of intracellular forms of *Leishmania* in a dose-dependent fashion, demonstrating a direct activity of these peptides and an action related to the activation of some cellular functions of the parasitized phagocyte [77]. Derived from cecropin, CM11 is a hybrid peptide consisting of 11 residues, 4 of which are in the domain five of melittin (6 to 9 residues) C-terminal and cecropin A (2 to 8 residues) in the N-terminal region. The anti-leishmanicidal activity was observed in the *Leishmania* significant strain and exhibited a dose-dependent effect on promastigotes, in addition to demonstrating substantial effects on amastigotes [77]. As recently reviewed, the effectiveness of the members of the principal families of AMPs exhibiting a potential anti-leishmanial activity is discussed [78].

The examples listed in Table 3 give a glimpse of cell-penetrating peptides with anti-parasite activity and AMPs with cell-penetrability to kill intracellular disease-causing protozoa.

Table 3. Cell-penetrating antimicrobial peptides against intracellular parasites.

Peptide	Source	Sequence	Target	Study Model	References
Crotamine	*Crotalus durissus terrificus* (Snake)	YKQCHKKGGHCFP KEKICLPPSSDFGK MDCRWRWKCCKKGSG	*Plasmodium falciparum*	in vitro	[64]
Crotamine	*Crotalus durissus terrificus* (Snake)	YKQCHKKGGHCFP KEKICLPPSSDFGK MDCRWRWKCCKKGSG	*Plasmodium falciparum*	in vitro	[65]
rR8-JR21	Derived from the junctional region of *Plasmodium falciparum*	rRrRrRRR-KKKKKKKKKYYKYKKKEKEKK	*Plasmodium falciparum*	in vitro	[67]
TP10	Synthetic peptíde	AGYLLGKINLKALAALAKKIL	*Plasmodium falciparum* and *Trypanosoma brucei brucei*	in vitro	[69]
(L-cyclohexyl alanin-D-arginine)$_3$	Synthetic peptíde		*Plasmodium falciparum*	in vitro	[70]
Batroxicidin (BatxC)	*Bothrops atrox* venom gland	KRFKKFFKKLK NSVKKRVKKFFRK PRVIGVTFPF	*Trypanosoma cruzi*	in vitro	[71]
Histatin 5	Human saliva	DSHAKRHHGYKRK FHEKHHSHRGY	*Leishmania donovani*	in vitro	[73]
Tachyplesin	Marine-sourced Japanese horseshoe crab (Tachypleus tridentatus)	KWCFRVCYRGICYRRCRGK	*Leishmania donovani*	in vitro	[74]
p-AclR7	Synthetic peptide	RRYRAYFRFRCRR	*Leishmania (L.) amazonensis*	in vitro	[75]
Andropin and Cecropin A	Hemolymph of the giant silkworm *Hyalophora cecropia*	VFIDILDKMENAIHKAAQAGIG KWKLFKKIE KVGQNIRDGIIKAG PAVAWVGQATQIAK	*Leishmania panamensis*	in vitro	[76]
CM11	Chimeric peptide	WKLFKKILKVL	*Leishmania major*	in vitro	[77]

6. Discussion

The cumulative data in the current scientific literature place the CPAPs in a particular group of biological multivalent active peptides with a high potential of druggability. The cell-penetrating peptides with anti-infective activity and AMPs with cell penetrability behavior offer uncountable possibilities for pharmaceutical development to care for intracellular infection caused by difficult-to-treat pathogenic microorganisms. Whether on one side, microorganisms evolved strategies to evade the host immune by coopting the cellular machinery and hiding inside the cell and subcellular compartments, on another side, the drugs have limited efficacy to translocate across cellular membranes and act intracellularly, consequently contributing to increasing microbial drug-resistance [8,10]. The primary mechanism of action of AMPs is disrupting the cytoplasmic membrane of microorganisms and even cancer cells [79–82]. However, a handful of AMPs exert their effect intracellular by homing to organelles like the mitochondria and the nucleus and interacting with protein and nucleic acids [16,32,83].

In contrast, CPPs translocate across cytoplasmic and lipid membranes by different mechanisms, like endocytosis-dependent and energy-independent, without causing membrane damage [84,85]. In the translocation process, CPPs can transport hydrophilic substances and nanoparticles, serving as an intracellular shuttle and delivery system. By homing in the cytoplasm or other cytoplasmic compartments of target cells where, for instance, infectious intracellular microorganisms temporally reside or conditions in which the cell cycle is altered, as in cancer cells, CPPs can serve as molecular scalpels to interrupt cellular processes coopted by intracellular microorganisms.

Making evident the growing list of multivalent CPAPs, that is, cell-penetrating with anti-infective (antibacterial, anti-parasite, and antiviral) and anti-proliferative (anticancer) activities and antimicrobial peptides with cell-penetrating capabilities, the clinical arsenal to target-driven intracellular infection is substantially improved. CPPs covalently conjugated with antibiotics and antivirals also appear as an excellent pharmaceutical strategy to deal with intracellular infectious agents [17], while chimeric peptide sequences emerging as a rational design of hybrid CPAPs [78]

It is a matter of debate that AMPs and CPPs display low selectivity toward diseased and healthy cells, narrowing the therapeutic index. However, some critical level of selectivity does exist, notably, the composition and charge of membranes of bacteria, fungi, and parasite, the lipid envelope of the virus, and the membrane of cancer cells, which facilitate the interaction of bioactive peptides [80,86,87]. Interestingly, some CPAPs internalize preferentially infected and diseased cells, as inferred from the abovementioned examples. This fact is evidenced by the low level of cytotoxicity caused by CPAPs to healthy host cells in contrast with cells loaded with parasites in vitro models. As one can observe, this particular class of bioactive peptides, CPAPs, bears two essential functionalities in a single molecule: cell penetrability and antibiosis. These functionalities can be combined with other properties of the CPPs and the AMPs, like receptor-mediated endocytosis and conjugation [88,89] and interconnection of innate and adaptative immunity [90,91].

7. Conclusions

This review summarizes cell-penetrating antimicrobial peptides with anti-infective activity against intracellular microorganisms. It covers a list of compelling studies demonstrating the promising antimicrobial effects of peptides that internalize infected cells and kill intracellular pathogens. Although CPAPs are druggable and amenable to be translated from the bench to the clinical sets, strategies of structural design and peptide engineering of promising CPAPs are advisable to increase their selectivity and stability for in vivo use.

8. Materials and Methods

This systematic review followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) guidelines [92]. The PubMed, ScienceDirect, and Google Scholar electronic databases were inquired for published articles on cell-penetrating

peptides with antimicrobial activities against intracellular infections. The bibliographic search on the database was up to October 2022. The interrogation of databases was without the restriction of the date of publication. The publications were analyzed using a search string containing the terms: "Cell-penetrating peptides" in combinations such as "Intracellular infections", "Antimicrobial peptide", "Intracellular parasite", "Antiviral", "Malaria", "Leishmania", and "Trypanosoma". The selection of works was carried out with the collaboration of the reviewers and through Mendeley software (version 1803, 2020) and verified, ensuring the review work's quality. The selected literature followed the criteria: full research articles conducted in vitro or in vivo experimental studies and evaluated cell-penetrating peptides as an antimicrobial agent. In addition, other studies containing reports related to the topic of this review were selected that were found outside of the original search. The criteria used to exclude studies were repeated articles, reviews, editorials, letters to the editor, theses, dissertations, reports, and articles that do not agree with the subject of this review. The papers selected for inclusion in the systematic review were chosen by the authors who added studies that followed the required criteria. The information collected from the literature contains the following information: authors, year of publication, peptides used in the study, amino acid sequences, target microorganism, study model, and main results. Thirty-three original articles were initially selected from the databases search using the primary keywords. References were further complemented with essential information on the topics covered in this review.

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References

1. Cillóniz, C.; Torres, A.; Niederman, M.; van der Eerden, M.; Chalmers, J.; Welte, T.; Blasi, F. Community-acquired pneumonia related to intracellular pathogens. *Intensive Care Med.* **2016**, *42*, 1374–1386. [CrossRef] [PubMed]
2. Armstead, A.L.; Li, B. Nanomedicine as an emerging approach against intracellular pathogens. *Int. J. Nanomed.* **2011**, *6*, 3281–3293. [CrossRef]
3. Kristensen, M.; Birch, D.; Mørck Nielsen, H. Applications and Challenges for Use of Cell-Penetrating Peptides as Delivery Vectors for Peptide and Protein Cargos. *Int. J. Mol. Sci.* **2016**, *17*, 185. [CrossRef] [PubMed]
4. Habault, J.; Poyet, J.-L. Recent Advances in Cell Penetrating Peptide-Based Anticancer Therapies. *Molecules* **2019**, *24*, 927. [CrossRef] [PubMed]
5. Yeh, Y.-C.; Huang, T.-H.; Yang, S.-C.; Chen, C.-C.; Fang, J.-Y. Nano-Based Drug Delivery or Targeting to Eradicate Bacteria for Infection Mitigation: A Review of Recent Advances. *Front. Chem.* **2020**, *8*, 286. [CrossRef]
6. Rodriguez, D.C.; Ocampo, M.; Salazar, L.M.; Patarroyo, M.A. Quantifying intracellular Mycobacterium tuberculosis: An essential issue for in vitro assays. *MicrobiologyOpen* **2018**, *7*, e00588. [CrossRef]
7. Mitchell, G.; Isberg, R.R. Innate Immunity to Intracellular Pathogens: Balancing Microbial Elimination and Inflammation. *Cell Host Microbe* **2017**, *22*, 166–175. [CrossRef]
8. Bourdonnay, E.; Henry, T. Catch me if you can. *eLife* **2016**, *5*, e14721. [CrossRef]
9. Fol, M.; Włodarczyk, M.; Druszczyńska, M. Host Epigenetics in Intracellular Pathogen Infections. *Int. J. Mol. Sci.* **2020**, *21*, 4573. [CrossRef]

10. Kamaruzzaman, N.F.; Kendall, S.; Good, L. Targeting the hard to reach: Challenges and novel strategies in the treatment of intracellular bacterial infections. *Br. J. Pharmacol.* **2017**, *174*, 2225–2236. [CrossRef]
11. Batista, M.F.; Nájera, C.A.; Meneghelli, I.; Bahia, D. The Parasitic Intracellular Lifestyle of Trypanosomatids: Parasitophorous Vacuole Development and Survival. *Front. Cell Dev. Biol.* **2020**, *8*, 396. [CrossRef] [PubMed]
12. Rossi, M.; Fasel, N. How to master the host immune system? Leishmania parasites have the solutions! *Int. Immunol.* **2018**, *30*, 103–111. [CrossRef] [PubMed]
13. Belachew, E.B. Immune Response and Evasion Mechanisms of *Plasmodium falciparum* Parasites. *J. Immunol. Res.* **2018**, *2018*, 6529681. [CrossRef] [PubMed]
14. Baker, R.E.; Mahmud, A.S.; Miller, I.F.; Rajeev, M.; Rasambainarivo, F.; Rice, B.L.; Takahashi, S.; Tatem, A.J.; Wagner, C.E.; Wang, L.F.; et al. Infectious disease in an era of global change. *Nat. Rev. Microbiol.* **2022**, *20*, 193–205. [CrossRef] [PubMed]
15. Sánchez, A.; Mejía, S.P.; Orozco, J. Recent Advances in Polymeric Nanoparticle-Encapsulated Drugs against Intracellular Infections. *Molecules* **2020**, *25*, 3760. [CrossRef] [PubMed]
16. Bahnsen, J.S.; Franzyk, H.; Sayers, E.J.; Jones, A.T.; Nielsen, H.M. Cell-Penetrating Antimicrobial Peptides—Prospectives for Targeting Intracellular Infections. *Pharm. Res.* **2015**, *32*, 1546–1556. [CrossRef] [PubMed]
17. Zeiders, S.M.; Chmielewski, J. Antibiotic-cell-penetrating peptide conjugates targeting challenging drug-resistant and intracellular pathogenic bacteria. *Chem. Biol. Drug Des.* **2021**, *98*, 762–778. [CrossRef]
18. Xu, J.; Khan, A.R.; Fu, M.; Wang, R.; Ji, J.; Zhai, G. Cell-penetrating peptide: A means of breaking through the physiological barriers of different tissues and organs. *J. Control. Release* **2019**, *309*, 106–124. [CrossRef]
19. Brooks, H.; Lebleu, B.; Vivès, E. Tat peptide-mediated cellular delivery: Back to basics. *Adv. Drug Deliv. Rev.* **2005**, *57*, 559–577. [CrossRef]
20. Zou, L.; Peng, Q.; Wang, P.; Zhou, B. Progress in Research and Application of HIV-1 TAT-Derived Cell-Penetrating Peptide. *J. Membr. Biol.* **2017**, *250*, 115–122. [CrossRef]
21. Ruseska, I.; Zimmer, A. Internalization mechanisms of cell-penetrating peptides. *Beilstein J. Nanotechnol.* **2020**, *11*, 101–123. [CrossRef] [PubMed]
22. Gong, Z.; Ikonomova, S.P.; Karlsson, A.J. Secondary structure of cell-penetrating peptides during interaction with fungal cells. *Protein Sci.* **2018**, *27*, 702–713. [CrossRef] [PubMed]
23. Splith, K.; Neundorf, I. Antimicrobial peptides with cell-penetrating peptide properties and vice versa. *Eur. Biophys. J. EBJ* **2011**, *40*, 387–397. [CrossRef] [PubMed]
24. Neundorf, I. Antimicrobial and Cell-Penetrating Peptides: How to Understand Two Distinct Functions Despite Similar Physicochemical Properties. *Adv. Exp. Med. Biol.* **2019**, *1117*, 93–109. [CrossRef] [PubMed]
25. Talapko, J.; Meštrović, T.; Juzbašić, M.; Tomas, M.; Erić, S.; Horvat Aleksijević, L.; Bekić, S.; Schwarz, D.; Matić, S.; Neuberg, M.; et al. Antimicrobial Peptides-Mechanisms of Action, Antimicrobial Effects and Clinical Applications. *Antibiotics* **2022**, *11*, 1417. [CrossRef] [PubMed]
26. Chakraborty, S.; Chatterjee, R.; Chakravortty, D. Evolving and assembling to pierce through: Evolutionary and structural aspects of antimicrobial peptides. *Comput. Struct. Biotechnol. J.* **2022**, *20*, 2247–2258. [CrossRef]
27. Duong, L.; Gross, S.P.; Siryaporn, A. Developing Antimicrobial Synergy with AMPs. *Front. Med. Technol.* **2021**, *3*, 640981. [CrossRef]
28. Zhang, R.; Xu, L.; Dong, C. Antimicrobial Peptides: An Overview of their Structure, Function and Mechanism of Action. *Protein Pept. Lett.* **2022**, *29*, 641–650. [CrossRef]
29. Dini, I.; De Biasi, M.G.; Mancusi, A. An Overview of the Potentialities of Antimicrobial Peptides Derived from Natural Sources. *Antibiotics* **2022**, *11*, 1483. [CrossRef]
30. Yu, L.; Li, K.; Zhang, J.; Jin, H.; Saleem, A.; Song, Q.; Jia, Q.; Li, P. Antimicrobial Peptides and Macromolecules for Combating Microbial Infections: From Agents to Interfaces. *ACS Appl. Bio Mater.* **2022**, *5*, 366–393. [CrossRef]
31. Rádis-Baptista, G. Cell-Penetrating Peptides Derived from Animal Venoms and Toxins. *Toxins* **2021**, *13*, 147. [CrossRef] [PubMed]
32. Le, C.F.; Fang, C.M.; Sekaran, S.D. Intracellular Targeting Mechanisms by Antimicrobial Peptides. *Antimicrob. Agents Chemother.* **2017**, *61*, e02340-16. [CrossRef] [PubMed]
33. Pärn, K.; Eriste, E.; Langel, Ü. The Antimicrobial and Antiviral Applications of Cell-Penetrating Peptides. In *Cell-Penetrating Peptides: Methods and Protocols*; Langel, Ü., Ed.; Springer: New York, NY, USA, 2015; pp. 223–245. [CrossRef]
34. Lee, Y.-C.J.; Shirkey, J.D.; Park, J.; Bisht, K.; Cowan, A.J. An Overview of Antiviral Peptides and Rational Biodesign Considerations. *BioDesign Res.* **2022**, *2022*, 9898241. [CrossRef]
35. Kim, G.C.; Cheon, D.H.; Lee, Y. Challenge to overcome current limitations of cell-penetrating peptides. *Biochim. Biophys. Acta (BBA)-Proteins Proteom.* **2021**, *1869*, 140604. [CrossRef] [PubMed]
36. Chen, C.H.; Lu, T.K. Development and Challenges of Antimicrobial Peptides for Therapeutic Applications. *Antibiotics* **2020**, *9*, 24. [CrossRef]
37. Hadjicharalambous, A.; Bournakas, N.; Newman, H.; Skynner, M.J.; Beswick, P. Antimicrobial and Cell-Penetrating Peptides: Understanding Penetration for the Design of Novel Conjugate Antibiotics. *Antibiotics* **2022**, *11*, 1636. [CrossRef]
38. del Rio, G.; Trejo Perez, M.A.; Brizuela, C.A. Antimicrobial peptides with cell-penetrating activity as prophylactic and treatment drugs. *Biosci. Rep.* **2022**, *42*, BSR20221789. [CrossRef]

39. Nooranian, S.; Oskuee, R.K.; Jalili, A. Antimicrobial Peptides, a Pool for Novel Cell Penetrating Peptides Development and Vice Versa. *Int. J. Pept. Res. Ther.* **2021**, *27*, 1205–1220. [CrossRef]
40. Bahnsen, J.S.; Franzyk, H.; Sandberg-Schaal, A.; Nielsen, H.M. Antimicrobial and cell-penetrating properties of penetratin analogs: Effect of sequence and secondary structure. *Biochim. Biophys. Acta* **2013**, *1828*, 223–232. [CrossRef] [PubMed]
41. Li, Z.; Teng, D.; Mao, R.; Wang, X.; Hao, Y.; Wang, X.; Wang, J. Improved Antibacterial Activity of the Marine Peptide N6 against Intracellular Salmonella Typhimurium by Conjugating with the Cell-Penetrating Peptide Tat(11) via a Cleavable Linker. *J. Med. Chem.* **2018**, *61*, 7991–8000. [CrossRef] [PubMed]
42. Li, J.; Shang, L.; Lan, J.; Chou, S.; Feng, X.; Shi, B.; Wang, J.; Lyu, Y.; Shan, A. Targeted and Intracellular Antibacterial Activity against S. agalactiae of the Chimeric Peptides Based on Pheromone and Cell-Penetrating Peptides. *ACS Appl. Mater. Interfaces* **2020**, *12*, 44459–44474. [CrossRef] [PubMed]
43. Arnett, E.; Lehrer, R.I.; Pratikhya, P.; Lu, W.; Seveau, S. Defensins enable macrophages to inhibit the intracellular proliferation of Listeria monocytogenes. *Cell. Microbiol.* **2011**, *13*, 635–651. [CrossRef] [PubMed]
44. Leseigneur, C.; Lê-Bury, P.; Pizarro-Cerdá, J.; Dussurget, O. Emerging Evasion Mechanisms of Macrophage Defenses by Pathogenic Bacteria. *Front. Cell. Infect. Microbiol.* **2020**, *10*, 577559. [CrossRef] [PubMed]
45. Flannagan, R.S.; Heit, B.; Heinrichs, D.E. Intracellular replication of Staphylococcus aureus in mature phagolysosomes in macrophages precedes host cell death, and bacterial escape and dissemination. *Cell. Microbiol.* **2016**, *18*, 514–535. [CrossRef]
46. Budagavi, D.P.; Chugh, A. Antibacterial properties of Latarcin 1 derived cell-penetrating peptides. *Eur. J. Pharm. Sci.* **2018**, *115*, 43–49. [CrossRef]
47. Jung, H.J.; Jeong, K.S.; Lee, D.G. Effective antibacterial action of tat (47–58) by increased uptake into bacterial cells in the presence of trypsin. *J. Microbiol. Biotechnol.* **2008**, *18*, 990–996.
48. Wang, X.; Teng, D.; Wang, X.; Hao, Y.; Chen, H.; Mao, R.; Wang, J. Internalization, distribution, and activity of peptide H2 against the intracellular multidrug-resistant bovine mastitis-causing bacterium Staphylococcus aureus. *Sci. Rep.* **2019**, *9*, 7968. [CrossRef]
49. Nepal, M.; Thangamani, S.; Seleem, M.N.; Chmielewski, J. Targeting intracellular bacteria with an extended cationic amphiphilic polyproline helix. *Org. Biomol. Chem.* **2015**, *13*, 5930–5936. [CrossRef]
50. Nepal, M.; Mohamed, M.F.; Blade, R.; Eldesouky, H.E.; Anderson, T.N.; Seleem, M.N.; Chmielewski, J. A Library Approach to Cationic Amphiphilic Polyproline Helices that Target Intracellular Pathogenic Bacteria. *ACS Infect. Dis.* **2018**, *4*, 1300–1305. [CrossRef]
51. Kuriakose, J.; Hernandez-Gordillo, V.; Nepal, M.; Brezden, A.; Pozzi, V.; Seleem, M.N.; Chmielewski, J. Targeting Intracellular Pathogenic Bacteria with Unnatural Proline-Rich Peptides: Coupling Antibacterial Activity with Macrophage Penetration. *Angew. Chem. Int. Ed.* **2013**, *52*, 9664–9667. [CrossRef]
52. Park, N.; Yamanaka, K.; Tran, D.; Chandrangsu, P.; Akers, J.C.; de Leon, J.C.; Morrissette, N.S.; Selsted, M.E.; Tan, M. The cell-penetrating peptide, Pep-1, has activity against intracellular chlamydial growth but not extracellular forms of Chlamydia trachomatis. *J. Antimicrob. Chemother.* **2008**, *63*, 115–123. [CrossRef] [PubMed]
53. Amiss, A.S.; von Pein, J.B.; Webb, J.R.; Condon, N.D.; Harvey, P.J.; Phan, M.D.; Schembri, M.A.; Currie, B.J.; Sweet, M.J.; Craik, D.J.; et al. Modified horseshoe crab peptides target and kill bacteria inside host cells. *Cell. Mol. Life Sci. CMLS* **2021**, *79*, 38. [CrossRef] [PubMed]
54. Vasconcelos, L.; Pärn, K.; Langel, Ü. Therapeutic potential of cell-penetrating peptides. *Ther. Deliv.* **2013**, *4*, 573–591. [CrossRef] [PubMed]
55. Samuels, S.; Alwan, Z.; Egnin, M.; Jaynes, J.; Connell, T.D.; Bernard, G.C.; Nashar, T. Novel Therapeutic Approach for Inhibition of HIV-1 Using Cell-Penetrating Peptide and Bacterial Toxins. *J. AIDS Clin. Res.* **2017**, *8*, 737. [CrossRef]
56. Keogan, S.; Passic, S.; Krebs, F.C. Infection by CXCR4-Tropic Human Immunodeficiency Virus Type 1 Is Inhibited by the Cationic Cell-Penetrating Peptide Derived from HIV-1 Tat. *Int. J. Pept.* **2012**, *2012*, 349427. [CrossRef]
57. Jang, S.; Hyun, S.; Kim, S.; Lee, S.; Lee, I.-S.; Baba, M.; Lee, Y.; Yu, J. Cell-Penetrating, Dimeric α-Helical Peptides: Nanomolar Inhibitors of HIV-1 Transcription. *Angew. Chem. Int. Ed.* **2014**, *53*, 10086–10089. [CrossRef] [PubMed]
58. Zhang, H.; Zhao, Q.; Bhattacharya, S.; Waheed, A.A.; Tong, X.; Hong, A.; Heck, S.; Curreli, F.; Goger, M.; Cowburn, D.; et al. A Cell-penetrating Helical Peptide as a Potential HIV-1 Inhibitor. *J. Mol. Biol.* **2008**, *378*, 565–580. [CrossRef] [PubMed]
59. Sperandio, S.; Barat, C.; Cabrita, M.A.; Gargaun, A.; Berezovski, M.V.; Tremblay, M.J.; de Belle, I. TOE1 is an inhibitor of HIV-1 replication with cell-penetrating capability. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, E3392–E3401. [CrossRef]
60. Zhang, P.; Moreno, R.; Lambert, P.F.; DiMaio, D. Cell-penetrating peptide inhibits retromer-mediated human papillomavirus trafficking during virus entry. *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 6121–6128. [CrossRef]
61. Hong, W.; Li, T.; Song, Y.; Zhang, R.; Zeng, Z.; Han, S.; Zhang, X.; Wu, Y.; Li, W.; Cao, Z. Inhibitory activity and mechanism of two scorpion venom peptides against herpes simplex virus type 1. *Antivir. Res.* **2014**, *102*, 1–10. [CrossRef]
62. Pan, X.-B.; Wei, L.; Han, J.-C.; Ma, H.; Deng, K.; Cong, X. Artificial recombinant cell-penetrating peptides interfere with envelopment of hepatitis B virus nucleocapsid and viral production. *Antivir. Res.* **2011**, *89*, 109–114. [CrossRef]
63. Abdul, F.; Ndeboko, B.; Buronfosse, T.; Zoulim, F.; Kann, M.; Nielsen, P.E.; Cova, L. Potent Inhibition of Late Stages of Hepadnavirus Replication by a Modified Cell Penetrating Peptide. *PLoS ONE* **2012**, *7*, e48721. [CrossRef] [PubMed]
64. El Chamy Maluf, S.; Dal Mas, C.; Oliveira, E.B.; Melo, P.M.; Carmona, A.K.; Gazarini, M.L.; Hayashi, M.A.F. Inhibition of malaria parasite Plasmodium falciparum development by crotamine, a cell penetrating peptide from the snake venom. *Peptides* **2016**, *78*, 11–16. [CrossRef] [PubMed]

65. El Chamy Maluf, S.; Hayashi, M.A.F.; Campeiro, J.D.; Oliveira, E.B.; Gazarini, M.L.; Carmona, A.K. South American rattlesnake cationic polypeptide crotamine trafficking dynamic in Plasmodium falciparum-infected erythrocytes: Pharmacological inhibitors, parasite cycle and incubation time influences in uptake. *Toxicon* **2022**, *208*, 47–52. [CrossRef]
66. Chaianantakul, N.; Sirawaraporn, R.; Sirawaraporn, W. Insights into the role of the junctional region of Plasmodium falciparum dihydrofolate reductase-thymidylate synthase. *Malar. J.* **2013**, *12*, 91. [CrossRef] [PubMed]
67. Chaianantakul, N.; Sungkapong, T.; Supatip, J.; Kingsang, P.; Kamlaithong, S.; Suwanakitti, N. Antimalarial effect of cell penetrating peptides derived from the junctional region of Plasmodium falciparum dihydrofolate reductase-thymidylate synthase. *Peptides* **2020**, *131*, 170372. [CrossRef]
68. Yandek, L.E.; Pokorny, A.; Florén, A.; Knoelke, K.; Langel, Ü.; Almeida, P.F.F. Mechanism of the Cell-Penetrating Peptide Transportan 10 Permeation of Lipid Bilayers. *Biophys. J.* **2007**, *92*, 2434–2444. [CrossRef]
69. Arrighi, R.B.G.; Ebikeme, C.; Jiang, Y.; Ranford-Cartwright, L.; Barrett, M.P.; Langel, Ü.; Faye, I. Cell-Penetrating Peptide TP10 Shows Broad-Spectrum Activity against both *Plasmodium falciparum* and *Trypanosoma brucei brucei*. *Antimicrob. Agents Chemother.* **2008**, *52*, 3414–3417. [CrossRef]
70. Somsri, S.; Mungthin, M.; Klubthawee, N.; Adisakwattana, P.; Hanpithakpong, W.; Aunpad, R. A Mitochondria-Penetrating Peptide Exerts Potent Anti-Plasmodium Activity and Localizes at Parasites' Mitochondria. *Antibiotics* **2021**, *10*, 1560. [CrossRef]
71. Mello, C.P.; Lima, D.B.; Menezes, R.R.P.P.B.d.; Bandeira, I.C.J.; Tessarolo, L.D.; Sampaio, T.L.; Falcão, C.B.; Rádis-Baptista, G.; Martins, A.M.C. Evaluation of the antichagasic activity of batroxicidin, a cathelicidin-related antimicrobial peptide found in Bothrops atrox venom gland. *Toxicon* **2017**, *130*, 56–62. [CrossRef]
72. Torres-Guerrero, E.; Quintanilla-Cedillo, M.; Ruiz-Esmenjaud, J.; Arenas, R. Leishmaniasis: A review [version 1; peer review: 2 approved]. *F1000Research* **2017**, *6*. [CrossRef]
73. Luque-Ortega, J.R.; van't Hof, W.; Veerman, E.C.I.; Saugar, J.M.; Rivas, L. Human antimicrobial peptide histatin 5 is a cell-penetrating peptide targeting mitochondrial ATP synthesis in Leishmania. *FASEB J.* **2008**, *22*, 1817–1828. [CrossRef] [PubMed]
74. Kumar, V.; Chugh, A. Peptide-mediated leishmaniasis management strategy: Tachyplesin emerges as an effective anti-leishmanial peptide against Leishmania donovani. *Biochim. Biophys. Acta (BBA)-Biomembr.* **2021**, *1863*, 183629. [CrossRef] [PubMed]
75. Mendes, B.; Almeida, J.R.; Vale, N.; Gomes, P.; Gadelha, F.R.; Da Silva, S.L.; Miguel, D.C. Potential use of 13-mer peptides based on phospholipase and oligoarginine as leishmanicidal agents. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2019**, *226*, 108612. [CrossRef] [PubMed]
76. Pérez-Cordero, J.J.; Lozano, J.M.; Cortés, J.; Delgado, G. Leishmanicidal activity of synthetic antimicrobial peptides in an infection model with human dendritic cells. *Peptides* **2011**, *32*, 683–690. [CrossRef]
77. Khalili, S.; Ebrahimzade, E.; Mohebali, M.; Shayan, P.; Mohammadi-Yeganeh, S.; Moosazadeh Moghaddam, M.; Elikaee, S.; Akhoundi, B.; Sharifi-Yazdi, M.K. Investigation of the antimicrobial activity of a short cationic peptide against promastigote and amastigote forms of Leishmania major (MHRO/IR/75/ER): An in vitro study. *Exp. Parasitol.* **2019**, *196*, 48–54. [CrossRef]
78. El-Dirany, R.; Shahrour, H.; Dirany, Z.; Abdel-Sater, F.; Gonzalez-Gaitano, G.; Brandenburg, K.; Martinez de Tejada, G.; Nguewa, P.A. Activity of Anti-Microbial Peptides (AMPs) against Leishmania and Other Parasites: An Overview. *Biomolecules* **2021**, *11*, 984. [CrossRef] [PubMed]
79. Zhang, Q.-Y.; Yan, Z.-B.; Meng, Y.-M.; Hong, X.-Y.; Shao, G.; Ma, J.-J.; Cheng, X.-R.; Liu, J.; Kang, J.; Fu, C.-Y. Antimicrobial peptides: Mechanism of action, activity and clinical potential. *Mil. Med. Res.* **2021**, *8*, 48. [CrossRef]
80. Deslouches, B.; Di, Y.P. Antimicrobial peptides with selective antitumor mechanisms: Prospect for anticancer applications. *Oncotarget* **2017**, *8*, 46635–46651. [CrossRef]
81. Jafari, A.; Babajani, A.; Sarrami Forooshani, R.; Yazdani, M.; Rezaei-Tavirani, M. Clinical Applications and Anticancer Effects of Antimicrobial Peptides: From Bench to Bedside. *Front. Oncol.* **2022**, *12*, 819563. [CrossRef]
82. Kardani, K.; Bolhassani, A. Antimicrobial/anticancer peptides: Bioactive molecules and therapeutic agents. *Immunotherapy* **2021**, *13*, 669–684. [CrossRef] [PubMed]
83. Juretić, D. Designed Multifunctional Peptides for Intracellular Targets. *Antibiotics* **2022**, *11*, 1196. [CrossRef] [PubMed]
84. Kardani, K.; Milani, A.; Shabani, S.H.; Bolhassani, A. Cell penetrating peptides: The potent multi-cargo intracellular carriers. *Expert Opin. Drug Deliv.* **2019**, *16*, 1227–1258. [CrossRef] [PubMed]
85. Futaki, S. Functional Peptides That Target Biomembranes: Design and Modes of Action. *Chem. Pharm. Bull.* **2021**, *69*, 601–607. [CrossRef] [PubMed]
86. Bobone, S.; Stella, L. Selectivity of Antimicrobial Peptides: A Complex Interplay of Multiple Equilibria. *Adv. Exp. Med. Biol.* **2019**, *1117*, 175–214. [CrossRef] [PubMed]
87. Reissmann, S.; Filatova, M.P. New generation of cell-penetrating peptides: Functionality and potential clinical application. *J. Pept. Sci. Off. Publ. Eur. Pept. Soc.* **2021**, *27*, e3300. [CrossRef] [PubMed]
88. Martín, I.; Teixidó, M.; Giralt, E. Building Cell Selectivity into CPP-Mediated Strategies. *Pharmaceuticals* **2010**, *3*, 1456–1490. [CrossRef] [PubMed]
89. Szabó, I.; Yousef, M.; Soltész, D.; Bató, C.; Mező, G.; Bánóczi, Z. Redesigning of Cell-Penetrating Peptides to Improve Their Efficacy as a Drug Delivery System. *Pharmaceutics* **2022**, *14*, 907. [CrossRef]
90. Brunetti, J.; Carnicelli, V.; Ponzi, A.; Di Giulio, A.; Lizzi, A.R.; Cristiano, L.; Cresti, L.; Cappello, G.; Pollini, S.; Mosconi, L.; et al. Antibacterial and Anti-Inflammatory Activity of an Antimicrobial Peptide Synthesized with D Amino Acids. *Antibiotics* **2020**, *9*, 840. [CrossRef]

91. Luo, Y.; Song, Y. Mechanism of Antimicrobial Peptides: Antimicrobial, Anti-Inflammatory and Antibiofilm Activities. *Int. J. Mol. Sci.* **2021**, *22*, 11401. [CrossRef]
92. Page, M.J.; Moher, D.; Bossuyt, P.M.; Boutron, I.; Hoffmann, T.C.; Mulrow, C.D.; Shamseer, L.; Tetzlaff, J.M.; Akl, E.A.; Brennan, S.E.; et al. PRISMA 2020 explanation and elaboration: Updated guidance and exemplars for reporting systematic reviews. *BMJ* **2021**, *372*, n160. [CrossRef] [PubMed]

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Anti-inflammatory activities of arthropod peptides: a systematic review

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Abstract

Peptides obtained from different animal species have gained importance recently due to research that aims to develop biopharmaceuticals with therapeutic potential. In this sense, arthropod venoms have drawn attention, not only because of their toxicity but mainly for the search for molecules with various bioactivities, including anti-inflammatory activity. The purpose of the present study is to gather data available in the literature on new peptides derived from arthropod species with anti-inflammatory potential. This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. Studies on peptides from arthropods that display anti-inflammatory activity were retrieved from PubMed, Scopus, Web of Science, and Google Scholar databases. The bibliographic research started in 2020 and searched papers without a limit on the publication date. The articles were analyzed using a search string containing the following terms: "Peptides" and "Anti-inflammatory", in combinations such as "Ant", "Bee", "Wasp", "Crab", "Shrimp", "Scorpion", "Spider", "Tick" and "Centipedes". Besides, a search was carried out in the databases with the terms: "Peptides", "Antitumor", or "Anticancer", and "Arthropods". Articles that met the inclusion and exclusion criteria totalized 171, and these served for data extraction. Additionally, the present review included anti-inflammatory peptides with anticancer properties. Peptides with confirmed anti-inflammatory activity were from insects (ants, bees, and wasps), crustaceans (shrimp and crabs), arachnids (scorpions, spiders, and ticks), and centipedes. These arthropod peptides act mainly by decreasing pro-inflammatory cytokines as analyzed *in vitro* and *in vivo*. Some showed significant antineoplastic activity, working in essential cellular pathways against malignant neoplasms.

Keywords:
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Background

The use of enzymes and polypeptides for medicinal purposes has attracted considerable interest due to their high specificity and selectivity. They are also less likely to interfere with cellular processes that are not the aimed therapeutic targets. Protein drugs are composed of bioactive polypeptides with significant therapeutic potential [1]. Although animal venoms have toxic effects, they are extensively studied to find pharmacologically active molecules [2]. A known example of an isolated venom component that served as a template for developing the anti-hypertensive drug captopril belongs to the bradykinin-potentiating peptide (BPP) family found in the venom of *Bothrops jararaca* [3].

Arthropods comprise one of the largest groups of animals on Earth, with diverse species being venomous. These species contain complex mixtures of components in their venoms with various families of toxins that exert numerous biological effects on target organisms and systems, testified by a growing number of reported studies available in public databases. This kind of natural chemical and peptide library provides excellent potential for discovering new compounds and activities for alternative or adjuvant therapies based on the mimetic modulation of pharmacological activities of endogenous (poly)peptides in the body [4–6]. More than 400 toxins from various animals have activities reported in the literature, and around 3400 reported proteins are from arthropods [7].

Natural products comprise an essential source of bioactive substances, and they have contributed significantly to the manufacture of old and new drugs for diverse therapeutic purposes. In recent years, of all the molecules approved by the U. S. Food and Drug Administration (FDA), a third of them are natural products and derivatives from mammals and microbes [8]. However, arthropod venoms as sources of new pharmaceutically functional molecules are yet to be deeply explored [9]. Many arthropod venom peptides represent an opportunity by which venom components could be converted into "pharmaceutical gold" [10,11,12]. The production of a drug derived from venoms also includes the characterization of synthetic or recombinant peptide forms. Examples include peptides capable of modulating and/or regulating pain [13].

This review presents examples of peptides from various arthropod species, mainly focused on biologically active peptides found in arthropod venom with anti-inflammatory potential.

Methods

Investigation plan

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [14]. The search of published articles on the topic of arthropod-derived peptides with anti-inflammatory activity was through PubMed, Scopus, Web of Science, and Google Scholar electronic databases. The bibliographic retrieval started in August 2020 and finished in March 2021. The search did not limit the date of publication. The publications were analyzed using a search string containing terms: "Peptides" and "Anti-inflammatory", in combinations such as "Ant", "Bee", "Wasp", "Crab", "Shrimp", "Scorpion", "Spider", "Tick" and "Centipede". In addition, a search was carried out in the databases with the terms: "Peptides", "Antitumor", or "Anticancer", and "Arthropods".

Selection of the literature

The studies were selected by the coauthors' ATS and GSC through Mendeley software (version 1803, 2020) and verified by GRB, ensuring the review work's inclusion. The selected literature adhered to the following criteria: full research articles that have been conducted *in vitro* or *in vivo* experimental studies and evaluated the anti-inflammatory effects of peptides derived from arthropod venoms or their crude extract. Besides, included in this review are ethnopharmacological data related to the topic covered. The criteria used to exclude studies were: repeated articles, editorials, letters to the editor, thesis, dissertations, reports, and articles that are out of the scope of this review.

Data collection

According to the required criteria, the studies selected for inclusion in this systematic review were chosen by the authors' ATS and GSC. The information collected from the literature contains the following information: authors, affiliation, year of publication, applied methodology, characterized compound, and main results.

Results

After searching the databases, 171 original and review articles were selected out of 769 published papers and utilized to prepare the current review. The flow diagram (Figure 1) depicts the details of the selection process in the databases. Also, general information was obtained, referring to the article's title, authorship, and publication year.

Reading the material in its entirety made it possible to identify specific information about the animal species involved in the study, the peptide structure identified as a potential anti-inflammatory agent, and the anti-inflammatory activity described more precisely. Table 1 summarizes the collection of this information.

Insect peptides

Ants

Insects possess a multitude of unexplored toxins with presumed potent biological activities. For instance, ants (Insecta class, Hymenoptera order, Formicidae family) are mostly venomous and express several types of peptides in their venoms, therefore emerging as an essential source of bioactive peptides [15]. Not so long ago, investigating the biological effects of isolated peptide toxins from insects was hampered by the size of these majorly tiny animals. With the advent of omics technology, the discovery and characterization of novel peptides progressed [16]. Initial studies aimed to unveil a way to alleviate the secondary effects

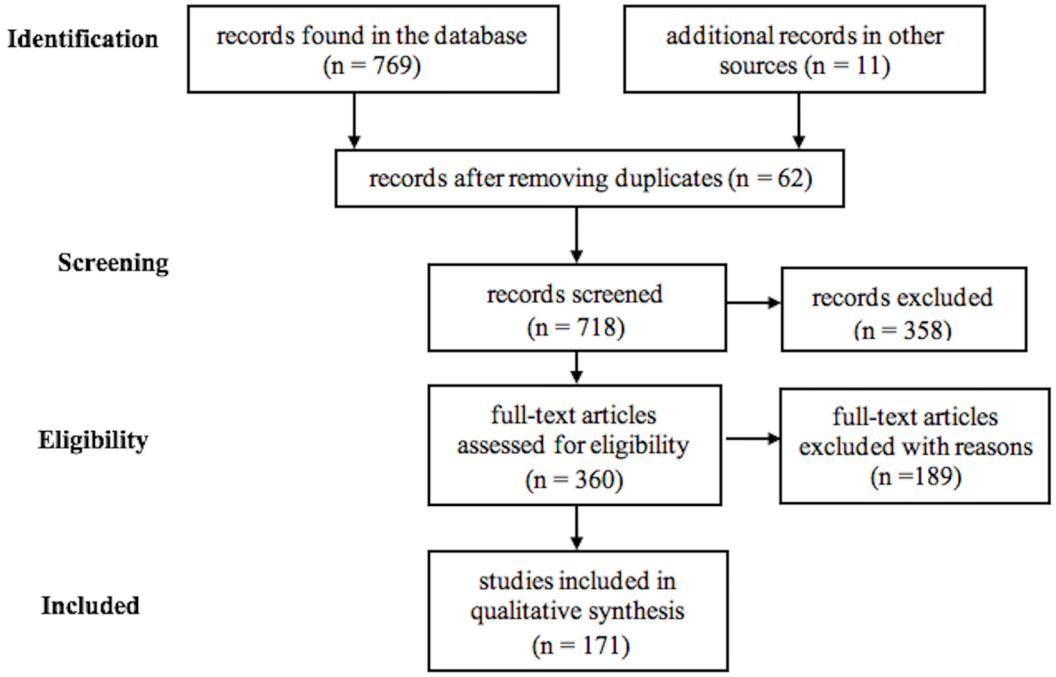

Figure 1. PRISMA flowchart showing the research design process of the study.

Table 1. Examples of peptides from the Uniprot database with anti-inflammatory activies.

Animal (Source)	Peptide	Access number	Activity as inflammatory mediator	Ref.
Insect				
Pseudomyrmex triplarinus	Pseudomyrmecitoxin-Pt1 subunit LS1	P0DSL7	Antidematogenic effect	[19–21]
	Pseudomyrmecitoxin-Pt1 subunit SS3	P0DSM1		
	Pseudomyrmecitoxin-Pt1 subunit LS2	P0DSL8		
	Pseudomyrmecitoxin-Pt1 subunit SS2	P0SDM0		
	U1-pseudomyrmecitoxin-Pt1 subunit SS1	P0DSL9		
Paraponera clavata	Delta-paraponeritoxin-Pc1a	P41736	Edema reduction, antinociceptive	[22]
Dinoponera quadríceps	Venom peptides (Extract)	C0HJK0	Suppression of inflammatory mediators	[23–26]
Brachyponera sennaarensisare	Venom peptides (Extract)	–	Regulate the expression of MHC-II, CD80 y CD-86, IFN-γ and IL-17	[28,29]
Pachycondyla sennaarensis	Venom peptides (Extract)	–	Regulate NF-kB, kinase IkB, TNF-α and Fas	[39]
Apis melífera	Venom peptides (Extract)	–	Reduction the levels of inflammatory mediators	[40–46]
	Phospolipase A2	P00630	Reduction of apoptotic levels mediated by Bcl-2 and Bcl-xL	[55–57]
	Melittin	P01501	Inactivation of NF-kB	[58–67]
	Apamin	P01500	Suppression Th2-related chemokines/Regulation the activation of the NF-kB, STATS 1 and 2 pathways	[69–71]
	Adolapin	–	Reduction of paw edema, the levels of prostaglandins, cyclooxygenase 2, in addition to inhibiting PLA2 activity	[72–74]

Table 1. Cont.

Animal (Source)	Peptide	Access number	Activity as inflammatory mediator	Ref.
Crustacean				
Protopolybia exígua	Mastoporan-1	P69034	Inhibition Toll-like receptor 4 (TLR4) mRNA, suppressionTNF-α and interleukin-6 (IL-6)	[80]
Nasonia vitripennis	Venom peptides (Extract)	–	ReductionIL-1β, IL-6 and NF-kB	[82,83]
Vespa magnificca	–	P0CH47	Inhibition of the NF-kB pathway	[84]
Limulus polyphemus	Anti-lipopolysaccharide factor	P07086	Immunomodulatory activity	[67,68]
Penaeus monodon	Anti-lipopolysaccharide factor	B1NMC7	Disruption of the mitogen-activated protein (MAP) pathway by regulating and reducing the release of pro-inflammatory cytokines	[90–91]
Portunus trituberculatus	Anti-lipopolysaccharide factor	C0KJQ4	Immunomodulatory activity	[98–102]
	Anti-lipopolysaccharide factor isoform 4	H9MYY2		
	Anti-lipopolysaccharide factor isoform 5	–		
	Antilipopolysaccharide factor isoform 8	–		
Scylla paramamosain	Catalase	D0EVW7	Antioxidant potential	[105,106]
Scylla serrata	Anti-lipopolysaccharide factor	B5TTX7		
Charybdis natator	Crab leg	–	Modulating the NF-kB pathway	[107]
Arachnid				
Titius obscurus	Toxin To3	P60213	Suppression of TNF-α and IL-1β	[112]
	Toxin To4	P60215		
Tityus stigmurus	Hyaluronidase	P0C8X3	Reduction the migration of leukocytes and TNF-α release	[113]
Tityus serrulatus	Antimicrobial peptide TsAP-2	S6D3A7		
Mesobuthus martensii	Makatoxin-1	P56569	Reduction the production of inflammatory mediators such as nitric oxide (NO), TNF -α, IL-6 and IL-1β	[116]
	Potassium channel toxin alpha-KTx 3.6	Q9NII7	Suppress cytokine secretion	[119, 121, 122]
Heterometrus laoticus	Hetlaxin	C0HJN0	Act on Kv1.3 potassium channel	[123]
Heterosodra maculata	Delta-theraphotoxin-Hm1a	P60992	To control thehypersensitivity and chronic visceral pain	[125]
Phlogiellus sp.	Phlotoxin 1	P0DM14	Antinociceptive activity	[128]
Phoneutria nigriventer	Phα1β	P81789	Anti-inflammatory and antinociceptive	[132–135]
	Tx3-3	–		
	PnTx4			
	PhKv			
Pardosa astrigera	Lycotoxin-Pa4a	–	Suppresses nitric oxide, nitric oxide-induced synthase (iNOS), IL-1β, TNF-α	[136]
Ornithodoros savignyi	OsDef2	–	Inhibits the production of TNF-α and NO-induced	[139]
Hyalomma asiaticum	Hyalomin-A1 Hyalomin-B1	–	Inhibits the secretion of pro-inflammatory cytokines and increasing the secretion of IL-10	[140,141]
Rhipicephalus sanguineus	Evasin-1 Evasin 3 Evasin 4	P0C8E7 P0C8E8 P0C8E9	Inhibits cell of chemokines CCL3, CCL3L1, and CCL4 and CCL5	[142]
Amblyomma variegatum	Amphiregulin	–	Inhibitis the secretion of TNF-α, IL-1, IL-8, and IFN-γ	[143]
Chilopod				
Scolopendra subspinipes	Formyl peptide receptor 2	–	Inhibitis the release of pro-inflammatory cytokines and the recruitment of neutrophils in the joint	[147]
	Scolopendrasin IX	–	down-regulate the expression of pro-inflammatory mediators such as TNF-α and IL-6	

Source: Uniprot database.

caused by these animals' bites, with ants belonging to the genera Solenopsis, Pachycondyla spp, and Myrmecia the most studied [17, 18]. In crude and isolated forms, the characterization and verification of several bioactive peptides from the venom of *Pseudomyrmex* species, such as the mirmexin peptide, proved to have a potent antidematogenic activity [19–21]. As observed *in vivo,* poneratoxin, a 25-residue peptide from the bullet ant *Paraponera clavate*, and some Formicidae peptides, can reduce edema, besides their antinociceptive activity [22]. In the context of ethnopharmacology, there are reports about the topical use of macerated giant ants *Dinopera quadriceps* for the treatment of back pain and rheumatic cases [23]. These studies have shown that the crude extracts reduced paw edema, leukocyte migration, malonaldehyde, and nitrite content, ameliorating acute peritonitis *in vivo* and *in vitro*. This extract contained modulator molecules of cellular oxidant/antioxidant mechanisms involved in acute inflammation elicited by zymosan, but more specific mechanisms of action have not been described [24,25]. The crude venom of this species has the potential to reduce nociception and interleukin-1β (IL-1β), which suggests that it suppresses inflammatory mediators such as cyclooxygenase-2 (COX-2) and prostaglandin-2 (PGE-2) involved with pain [26,27]. The *Brachyponera sennaarensisare* (Samsum ant) ant-derived toxins modulate not only pain but also the immune response. The *B. sennaarensisare* toxins regulate the expression of MHC-II, CD80, and CD-86, as well as interferon- γ (IFN-γ) and interleukin-17 (IL-17), mediators that are involved in various chronic pathologies and cancer as demonstrated after in vivo tests [28]. Furthermore, these peptides can regulate the nuclear factor kappa B (NF-kB), kinase IkB upward, and suppress nuclear transcription factor-α (TNF-α) and the cell surface death receptor (Fas), although the mechanism involved in anti-inflammatory activity has not been fully elucidated [29,30].

Bees

Bees are part of the class Insecta, order Hymenoptera, family Apoidea, and clade Anthophilia. In Brazil, bee venom is commonly found and consists of various bioactive agents that induce allergic reactions when injected into the human body [31]. However, its use for medicinal purposes was documented approximately 6,000 years ago [32]. Bee venom therapy (BV) is a form of medicine native to ancient Greece and China [33]. In recent years, bee-based therapy has become a new treatment option. An increasing body of scientific evidence has demonstrated the therapeutic potential of bee venom [34]. In traditional medicine in Asia, BV was used in conjunction with acupuncture to treat some anti-inflammatory diseases. Furthermore, combination therapy can reduce inflammation in amyotrophic lateral sclerosis (ALS) due to the disease's side effects on the liver, kidney, and spleen [35]. Combination acupuncture and BV therapy (i.e., *Apis mellifera* crude venom) were also favorable to treat respiratory inflammation accompanied by leukocyte, myeloperoxidase (MPO), and IL-1 suppression, using a carrageenan-induced pleurisy mouse model [36].

The inflammation suppression mechanism of *European honey bee Apis mellifera* BV, observed in previous studies with animal models, also reduces the formation of atherosclerotic lesions by decreasing the intercellular adhesion molecule 1 (ICAM-1), vascular adhesion molecule- 1 (VCAM -1), and transforming growth factor-β1 (TGF-β1) [37]. Furthermore, the reduction of inflammation induced by apitoxins - a venom bee peptide (*A. mellifera*) component, is due to the decrease in apoptotic levels mediated by Bcl-2 and Bcl-xL and activating BCL2-associated X protein (Bax) and caspase-3 [38]. The application of bee venoms (*A. mellifera*) extends to reduce inflammatory lesions caused by the bacteria *Propionibacterium acnes* through decreasing TNF-α, interleukin-8 (IL-8), and IFN-γ, while also blocking the expression of Toll-like receptor 2 (TLR2) in human keratinocytes and monocytes [39].

Based on previous studies, bee venom toxins from *A. mellifera* and *A. cerana indica* act by regulating NF-kB signaling; the antiarthritic effect has been explored to reduce the levels of inflammatory mediators directly involved in the pathophysiology of rheumatoid arthritis, similarly to standard drugs such as methotrexate [40–46]. The compound bee venom's potential extends to reducing pain, acting as an antinociceptive agent by modulating the α2-adrenergic receptor and cyclooxygenase-2, accompanied by suppressing edema [47–51]. BV has a broad spectrum of activities. Its effects are not limited only to joint diseases and respiratory diseases, promoting an improvement in the allergic condition by suppressing inflammatory cytokines when tested in an allergic chronic rhinosinusitis mouse model [52].

Bee venom is a complex mixture that includes proteins and peptides such as melittin, apamin, phospholipase A2, phospholipase B, hyaluronidase, phosphatase, α-glucosidase, MDC peptide, and adolapin, among other minor components [53,54]. Secretory phospholipase A2 (PLA2- *Apis mellifera*), a prototype enzyme in bee venom, hydrolyze fatty acids while also having a role in protecting liver damage by producing anti-inflammatory cytokines in mice and reducing neuroinflammation by reducing phosphorylation of STAT3 and inflammatory mediators, including p-STAT3 [55,56]. Bee venom phospholipase A2 ameliorates amyloidogenesis and neuroinflammation by inhibiting signal transducer and activating the transcription-3 pathway in Tg2576 mice [57].

Melittin (*Apis mellifera*), one of the main peptides in bee venom, comprises 26 amino acid residues with an overall amphipathic character. Administration in high doses of this apitoxin can trigger an allergic reaction, causing local itching and pain. In low doses, it may have an anti-inflammatory role by inhibiting the enzymatic activity of PLA2. Synthetic melittin inhibited the enzymatic activity of secretory phospholipase A2 (PLA2) from various sources, including bee and snake venoms, bovine pancreas, and synovial fluid from rheumatoid arthritis patients. Based on melittin's hydrophobic nature and its capacity to bind to PLA2, melittin could act as a carrier for PLA2 to translocate it to the membrane. Melittin inhibits the bee venom PLA2 noncompetitively by binding to the enzyme

domain other than the catalytic site. [58]. The protective effect of melittin on inflammation and apoptosis was also observed in acute liver failure; the treatment with melittin attenuated the increase of inflammatory cytokines and significantly inhibited caspase expression Bax protein levels, as well as cytochrome *c* release in vivo [59,60].

Moreover, the JNK-dependent inactivation of NF-kB caused by melittin may prevent the release of inflammatory mediators involved in oxidative stress and the generation of pain [61]. Melittin-induced inhibition of this signaling pathway, which included the ERK and AkT cascade, and suppression of the inflammatory mediators upregulated in periodontitis, a chronic inflammatory disease, was observed in *P. gingivalis* LPS-stimulated human keratinocytes [62]. Melittin also reduced the release of pro-inflammatory cytokines by monocytes after contact with *P. acnes*. It is also an effective agent that prevents liver fibrosis by inhibiting inflammation by interrupting the NF-κB signaling pathway [63–64]. Moreover, melittin modulated inflammation, having better activity and less toxicity when associated with glutathione S-transferase while *in vitro*. When using doses that exceed the toxic concentration, it still retains its inflammatory properties [65]. A study reports its beneficial effect in treating inflammatory diseases, including skin inflammation, neuroinflammation, atherosclerosis, arthritis, and liver inflammation [66].

Apamine is another toxin that constitutes bee venom. It is an 18 amino acid-residue neurotoxic peptide. Despite its neurotoxicity, apamine helps treat Parkinson's disease or learning deficits [67]. Moreover, apamine, as an anti-inflammatory peptide, reduced the paw's volume and the haptoglobin and seromucoid contents *in vivo* [68,69]. This bee venom peptide was efficient in treating atopic dermatitis. The Apamin inhibits TNF-α- and IFN-γ-induced inflammatory cytokines and chemokines via suppressions of NF-κB signaling pathway and STAT in human keratinocytes [70]. Apamine showed anti-inflammatory effects in mice with gouty arthritis by inhibiting pro-inflammatory cytokine production and inflammasome formation [71].

Adolapin, from *A. mellifera* venom, is another bee venom peptide with potent anti-inflammatory effects but not as well studied as melittin. It reduces the edema of the paw in mice, the levels of prostaglandins, cyclooxygenase 2, in addition to inhibiting PLA2 activity. The anti-inflammatory activity of adolapin is evident in carrageenin models, prostaglandin, rat hind paw edemas, and adjuvant polyarthritis. The adolapin effects are presumably due to its capacity to inhibit the prostaglandin synthase system, following a biphasic dose-response relationship. Likely, among the central mechanisms, one involved an analgesic action of adolapin [72]. Peptide 401 (mast cell degranulating peptide – MCD peptide), with 22 amino acid residues, considered a potent degranulation factor for bee venom mast cells, substantially inhibited the edema caused in rats and attenuated the inflammatory process at the affected site [73,74].

Wasps

Like bees, wasps (Insecta, Hymenoptera, Apocrita) have complex mixtures of toxins in their venoms and have attracted interest as a potential arthropod source of bioactive substances. Wasps belong to the family Vespidae, and members include the genus Dolichovespula (wasp), Vespula (yellow wasps), and Polistes (paper wasps) [75]. When injected, the wasp toxins trigger local adverse effects such as pain, edema, erythema, and immune reactions such as anaphylaxis [76,77]. In general, wasps' venom comprises a cocktail of hydrophobic peptides, including amines, peptides, enzymes, allergens, and toxins [78,79]. For example, mastoparan is an amphipathic, 14-amino acid residue, and it was the first peptide isolated from wasps. This toxin is found in the genera *Vespa, Parapolybia, Protonectarina, Polistes, Protopolybia* [80].

Like bee venom, wasps' venoms have a considerable anti-inflammatory effect, shown in *in vitro* studies. These contain toxins that have the potential to inhibit Toll-like receptor 4 (TLR4) mRNA expression, in addition to suppressing TNF-α and interleukin-6 (IL-6) [81]. Although crude venoms contain several toxins that can trigger a toxic reaction, wasp venoms have powerful anti-inflammatory complexes, as is the case of the crude venom of the wasp *Nasonia vitripennis* (jewel wasp). The *N. vitripennis* crude venom reduced the expression of inflammatory cytokines directly involved in inflammatory processes mediated by IL-1β, IL-6, and NF-kB [82,83]. In an arthritis model, crude wasp venoms caused the inhibition of the NF-kB pathway. Likewise, *Vespa magnifica* (murder hornet) and other wasp species' crude venoms suppressed the expression of mediators involved in hyperalgesia and rheumatoid arthritis [84–88].

A study dealing with *Vespa tropica* (Greater banded hornet) showed that crude venom significantly reduced oxidative stress and the mouse microglial cell line activation, previously stimulated by LPS. Moreover, the peptides purified from the crude venom exhibited potential anti-inflammatory properties, targeting the p38 and MAPK pathways, causing the suppression of NF-κB phosphorylation in LPS-stimulated cells [89].

Crustacean peptides

Prawns/shrimps

Despite not being poisonous, shrimps (Crustacea, Malacostraca, Decapoda) were included here because they do not have an adaptive immune system and therefore rely on their innate immunity bioactive peptide components to deter invading pathogens. Antimicrobial peptides (AMP) are responsible for the immediate host response against invading bacteria, fungi, parasites, and, in some cases, they connect the innate and the adaptive immune response by modulating the expression and release of cytokines. The primary AMPs found in shrimp are grouped into three families of cationic peptides, namely, penaeidins, crustines, and anti-lipopolysaccharide factor (ALF) [90]. The ALF, firstly discovered in the horseshoe crab (*Limulus*

polyphemus), was followed by the identification in other crustacean species, like in the black tiger prawn *Penaeus monodon*, being designated SALF (Shrimp Anti-Factor Lipopolysaccharide) [90,91]. It is a precursor molecule with a signal sequence of 22 to 28 residues, followed by a mature peptide that contains two conserved cysteine residues. ALF's functional domain is named lipopolysaccharide-binding domain (LPS-BD) and contains the primary amino acids involved in recognizing and binding LPS and other components of Gram-positive bacteria and fungi [92].

P. monodon shrimp contain eleven ALF isoforms distributed in seven groups (Group A to Group G). Likewise, these isoforms can be found in the shrimp species *Farfantepenaeus aztecus* (brown shrimp), *L. vannamei* (pacific white shrimp or king prawn), and *Marsupenaeus japonicus* (known as the kuruma shrimp, kuruma prawn, or Japanese tiger prawn) [91,92]. LPS is an endotoxin present in the outer cell membrane of Gram-negative bacteria. When in contact with the host, it binds to pathogen recognition receptors that recognize this pathogen-associated molecular pattern (PAMP) and activates the signaling pathways that initiate the inflammatory process [93]. Recent studies show that SALF, besides antimicrobial activity, plays an essential role in neutralizing LPS and preventing its binding to the TLR-4 type Toll-like receptor (TLR). This peptide could inhibit or reduce the inflammatory response, disrupting the mitogen-activated protein (MAP) pathway by regulating and reducing the release of pro-inflammatory cytokines after *in vitro* tests with different cell lines [93–96].

Among studies about the efficacy of SALF as an anti-inflammatory agent, the effects of *Penaeus monodon* (giant tiger prawn) SALF on the production and release of tumor necrosis factor (TNF) were reported. This peptide showed suppression of inflammation in a dose-dependent manner in LPS-stimulated cervical cancer HeLa cells. Although the results have been promising, the mechanism involved in anti-inflammatory activity has not been fully elucidated [93]. The SALF peptides' protective role includes an anti-inflammatory effect in response to LPS, as observed in cervical cancer epithelial cells (HELA cells). SALF fragments inhibited inflammatory cytokines production, including TNF, interleukin IL-1 β, IL-6, IL-1, and monocyte chemoactive protein (MCP-1). SALF also suppressed IL-6, IL-8, IL-1, and MPC-1e mRNA levels and regulated vaginal epithelial cell immune responses through MAPK (mitogen-activated protein kinases) and NF-κβ (nuclear factor kappa B) pathways [93].

In addition to the SALF response to bacterial LPS, this peptide modulates the inflammatory responses provoked by the protozoan *Trichomonas vaginalis*, an etiological agent of Trichomoniasis that affects the cervicovaginal mucosa. When vaginal cells were subjected to stimulation by *T. vaginalis*, SALF inhibited the release of pro-inflammatory cytokines such as TNF-α, IL-6, IL-8, and MCP-1 through the MAPK pathways and NF-κβ [96]. These reports exemplify the promising profile of SALF as an anti-inflammatory agent.

Crabs

In recent years, marine organisms have attracted great interest due to their unique constituents with diverse bioactivities. These animals have hemolymph with potent antimicrobial peptides essential for their innate immunity. These peptides are valuable for biomedical applications [97]. Crabs (Crustacea, Malacostraca, Decapoda, Pleocyemata) have been investigated for the peptides' antimicrobial activity and their immunomodulatory effects. Purified peptides from various species of crabs such as LALF (The Atlantic horseshoe crab-*Limulus polyphemus*), M-ALF (kuruma shrimp-*Marsupenaeus Japonicus*), PtALF, PtALF4, PtALF5, and PtALF8 (horse crab-*Portunus trituberculatus*) showed an anti-lipopolysaccharide activity [98–103]. In another example, the β-1,3-glucan binding protein (β-GPB) from the rice paddy crab *Paratelphusa hydrodromus* can trigger an immune response against external aggressors. Additionally, β-GPB also exerts an antioxidant effect, reducing DPPH radicals, in a model of restraining the albumin's denaturation [104]. Regarding the antioxidant enzymatic profile, enzymes purified from distinct crab species showed an effective antioxidant potential by increasing the activity of superoxide dismutase (SOD) and catalase (CAT) [105,106]. Moreover, crab-derived peptides can restrain the inflammatory process by reducing inflammatory mediators' levels and modulating the NF-kB pathway, implicated in various inflammatory diseases [107]. Besides their role as an anti-inflammatory substance, these crustacean-derived peptides can exert antinociceptive effects, consequently playing a role in pain control as potent COX-2 reducers *in vitro* [108].

Arachnida peptides

Scorpions

Venom peptides from scorpion (Chelicerata, Arachnida, Scorpiones) distribute into two main groups: DBPs (disulfide-bridged peptides) and NDBPs (non-disulfide-bridged peptides). DBPs generally target ion channels. Most scorpion DBPs contain three to four disulfide bridges and interact with the Na+, K+, Ca2+, and Cl− channels. In comparison, the NDBP peptides are less abundantly distribute among scorpion venoms and exhibit multiple activities, such as insecticide, antiviral, antimicrobial, hemolytic, antiproliferative, bradykinin-enhancing, and immunomodulatory [109,110].

Dias and collaborators [111] analyzed 320 non-disulfide bond-containing peptides, of which 27 had their sequences assigned. Among them, thirteen peptides constituting novel toxins in *Tityus obscurus* venom (Amazonian black scorpion). As examples, ToAP3 (FIGMIPGLIGGLISAIK-NH2) and ToAP4 (FFSLIPSLIGGLVSAIK-NH2) NDBPs exerted their effect on immunomodulation and suppression of inflammatory mediators, such as TNF-α and IL-1β. Furthermore, ToAP3 and ToAP4 were associated with the modulation of antigen presentation. They reduced TNF-α and IL-1β at transcriptional and translational levels in bone marrow-derived macrophages (BMDM) and dendritic cells (BMDC). The reduction of TNF-α secretion

before LPS-inflammatory stimuli is associated with peptide interaction with TLR-4. ToAP4 increased MHC-II expression in BMDC, while ToAP3 decreased co-stimulatory molecules such as CD80 and CD86 [112]. Stigmurin, a cationic peptide from the scorpion *Tityus stigmurus* venom (scorpion from the family Buthidae found in Brazil) and TsAP-2 from the scorpion *Tityus serrulatus* venom (Brazilian yellow scorpion) both reduced the migration of leukocytes and TNF-α release, reducing the inflammatory process. Additionally, the fractions extracted from their respective crude venoms could modulate the expression of the cytokines IL-4, IL-6, IL-13, and IL-13, which are pro and anti-inflammatory [113].

The peptide Ts14 from *T. serrulatus* modulates critical events occurring in the fibrovascular tissue, i.e., it causes neovascularization, inflammatory cell recruitment, and extracellular matrix deposition induced by polyether-polyurethane sponge implants in mice. Consequently, Ts14 has therapeutic potential in wound healing and ischemic and inflammatory conditions. Furthermore, Ts14 reduced TNF-α levels and neutrophil infiltration, although stimulated macrophage infiltration into implants, as determined by myeloperoxidase (MPO) and N-acetyl-β-d-glucosaminidase (NAG) enzyme activities, respectively [114]. BotAF is a peptide derived from *Buthus occitanus tunetanus* (common yellow scorpion), another yellow scorpion species that comprises a long chain of 64 amino acid residues, with potent analgesic activity in rodents [115]. From the Chinese scorpion *Mesobuthus martensii* (Chinese scorpion), 35 scorpion oligopeptides (CMOs) were studied. Specifically, the peptide CMO-1 suppressed inflammation by reducing the production of inflammatory mediators such as nitric oxide (NO), TNF-α, IL-6, and IL-1β in RAW264.7 macrophages cells. Moreover, CMO-1 inhibited the degradation of IkBα and the nuclear translocation of p65. It also suppressed NF-kβ activation and inhibited MAPK phosphorylation of ERK, JNK, and p38 [116]. The venom of another species of *Mesobuthus* (*Mesobuthus eupeus*- lesser Asian scorpion, the lesser Asian scorpion, or the mottled scorpion) was effective in treating CFA-induced arthritis, in which the edema reduction correlated with the reduction of arthritis [117].

Sc20 from the venom of *Scorpiops tibetanus* is also a potent anti-inflammatory and immunosuppressor. This peptide modulated two important pro-inflammatory factors: the secretion of TNF-α and IFN-γ, displaying a positive effect in delayed hypersensitivity. Similar peptide St20, the first disulfide-bridged toxin peptide from the scorpion *S. tibetanus*, showed immunosuppressive and anti-inflammatory activities, suggesting that it may be a novel source of venom peptides to treat human disease [118].

The voltage-gated Kv1.3 channel, expressed in memory-efficient T cells, is presently a recognized targeted drug for treating various autoimmune diseases. Scorpion venom possesses Kv1.3 channel peptide blockers that suppress cytokine secretion and alleviate disease in animal models of T-cell-mediated autoimmune diseases [119]. Thus, to improve the selectivity and activity of these scorpion venom peptides directed at regulating Kv1.3 potassium channels are currently undertaken. A remarkable example is the study of the scorpion toxin BmKTX, isolated from *M. martensii* [120]. Recently, BmKTX analogs such as ADWX-1, BmKTX-D33H, BmKTX-19, and BmKTX-196 demonstrated specific inhibition of the Kv1.3 channel. Most venom-derived peptides have not evolved to target specific mammalian receptors of therapeutic interest; therefore, preparing peptide analogs with higher potency toward specific targets is customary [119,120,121]. The Vm24 scorpion toxin also showed similar activity to the venom-peptides above, which are blockers of Kv1.3 channels, acting without affecting the T cells' viability and inhibiting the activation of CD25 and CD40L, as well as the cytokine secretion of pro-inflammatory IFN-γ and TNF [122].

Hetlaxin (ISCTGSKQCYDPCKKKTGCPNAKCMNKS-CKCYGC) is a DBPs, belonging to the scorpion alpha-toxin family, isolated from the *Heterometrus laoticus* venom (Vietnam forest scorpion), which possesses a high affinity to the Kv1.3 potassium channel. This isolated *H. laoticus* venom peptide exerted an anti-inflammatory effect similar or slightly superior to ketoprofen [123].

Spiders

Spiders (Chelicerata, Arachnida, Araneae) comprise one of the oldest living animals on Earth that surged approximately 300 million years ago and comprise the most significant number of living species (> 40,000) [124]. As in other arthropods, inoculation of their venom causes local discomfort, such as edema, and more severe deleterious effects, like ulcerations, acute renal failure, and even death in the worse cases [125,126]. Although arachnids venoms are harmfully toxic to humans, some venom peptides have beneficial bioactivities applicable to biomedicine. In general, arthropod-derived venom's biochemical targets are excitable neuronal receptors; these include ion channels like voltage-gated sodium channels (Nav) found in neurons, which allow the modulating of pain. Spider peptides that modulate such pharmacological targets serve as molecular templates for the development of analgesic drugs. For example, the Hm1a peptide purified from the venom of *Heterosodra maculate* (togo starburst baboon spider) can control the hypersensitivity in chronic visceral pain [127].

Phlotoxin 1 (Ph1Tx1) is a 34-residue toxin purified from *Phlogiellus spider* venom, a promising antinociceptive peptide with a high affinity for Pav [128]. The crude venom of *Phoneutria nigriventer* (armed spiders), besides its antineoplastic activity, can suppress the IFN-γ release and increase the expression of the anti-inflammatory cytokine IL-10. Phα1β, a peptide purified from the venom of *P. nigriventer*, has a significant role in the control of the CFA-induced chronic arthritis model. The Phα1β suppressed the inflammatory agent's side effects while the antinociceptive role acted as the antagonist of the TRAP1 channel [129–131]. Furthermore, other peptides such as Tx3-3, PnTx4, PhKv, and PhTx3-5 from the *P. nigriventer* venom have important antinociceptive properties as observed in the animal neuropathic inflammatory pain model [132–135].

Lycotoxin-Pa4a peptide from *Pardosa astrigera* venom displays immunomodulatory activity by increasing the expression of IL-10 and suppressing pro-inflammatory mediators such as nitric oxide, nitric oxide-induced synthase (iNOS), IL-1β, TNF-α, in addition to reducing COX-2. *In vitro* studies with an LPS-stimulated model demonstrated that this peptide could act as a potential antinociceptive modulator [136].

Ticks

Ticks are hematophagous arthropods that rely only on the innate defense to protect themselves against invading microorganisms. Biologically active molecules are also necessary to keep blood fluid during feeding and eliminate the host's defense mechanisms, such as vasoconstriction, forming a hemostatic plug, activating the coagulation cascade, and initiating inflammatory responses that lead to wound healing and tissue remodeling. Thus, some bioactive molecules have anticoagulant, antiplatelet, vasodilatory, anti-inflammatory, and immunomodulatory activity and are crucial to overcoming the host's hemostatic and immunological responses, allowing ticks to feed and develop [137].

Ornithodoros savignyi (sand tampan, African-eyed tampan, or Kalahari sand tampan) is a tick that parasites cattle and is endemic in arid and semi-arid regions of the African continent. This tick species express antimicrobial peptides (defensins) constitutively in various tissues at low levels and inductively during blood-feeding or in response to bacterial challenge. Defensins are cationic molecules with molecular masses of approximately 4 kDa containing cysteine residues forming three disulfide bonds [138]. Studies on *O. savignyi* resulted in the cloning and sequencing of defensin isoforms, OsDef1 and OsDef2, derived from the terminal carboxy region. Due to the bactericidal activity isoform 2, this peptide served as a model for the synthesis of the peptide Os (KGIRGYKGGYCKGAFKQTCKCY) and its analog Os-C (KGIRGYKGGY- KGAFKQT- K-Y), with 22 and 19 residues of amino acids, respectively [139]. Os peptides' mechanisms of action in bacterial cells' membrane involve their penetration into the cell and action on intracellular targets. As a result of these findings, Malan et al. [139] evaluated these peptides' effects in inflammatory conditions resulting from gram-negative bacteria infection. Thus, Os and Os-C's showed anti-inflammatory properties on Raw 264.7 macrophages stimulated by LPS and IFN-γ *in vitro*. Both peptides inhibited the production of TNF-α and NO-induced by LPS in RAW 264.7 cells without appreciable cytotoxic effects. In addition to anti-endotoxin activity and anti-inflammatory properties, Os eliminated NO directly, and both Os and Os-C peptides exhibited antioxidant activity, which together can reduce oxidative stress associated with inflammation [139].

Wu et al. identified two families of immunoregulatory peptides, hyalomin-A1 and hyalomin-B1, from the salivary glands of the *Hyalomma asiaticum* tick. The amino acid sequences of hyalomin-A1 and B1 correspond to the sequences QTPRTIGPPYT and TLRTTTGYWTTVEKGNGTTPAANSTEKGNRPYGR, respectively. Hyalomin-A1 and B1 act as immunoregulators, inhibiting the secretion of pro-inflammatory cytokines induced by LPS in vitro and increasing immunosuppressive cytokine, IL-10 [140]. Both hyalomin-A1 and B1 could quickly eliminate oxidants in a few seconds. Such antioxidant activities can contribute to immunoregulatory and anti-inflammatory abilities.

Furthermore, the results indicated that both hyalomin-A1 and B1 significantly suppressed the LPS-induced activation of the JNK subgroup of the MAPK signaling pathway by blocking JNK phosphorylation and, consequently, led to a reduction in MCP-1, IFN-γ, and tumor necrosis factor-α genes. The *in vivo* experiments identified that these peptides could inhibit the hind paw's inflammation in mice depending on the dose administered. These anti-inflammatory functions were significantly present after nine days of administration. At a dose of 5 mg/kg of body weight, the mice could recover to a normal state after 21 days of administration of hyalomin-A1 or B1 [141].

Ticks have another mechanism of escape from the host's defenses related to the presence of evasins, small cysteine-rich binding proteins secreted in their saliva. To neutralize chemokines and their signaling, ticks, such as *Rhipicephalus sanguineus* (commonly called the brown dog tick, kennel tick, or pantropical dog tick), secrete evasins [142]. Evasin-1 (P0C8E7) inhibits cell recruitment of chemokines CCL3, CCL3L1, and CCL4-mediated chemotaxis in L1.2/CCR5 transfectants *in vivo* and *in vitro*. Besides, it also inhibited CCL3-induced granulocyte recruitment in mice. Evasin-3 (P0C8E8) inhibits neutrophil recruitment and reduces inflammation. Treatment with this peptide resulted in inhibiting total cell accumulation in the synovial cavity in a mouse-induced arthritis model. Inhibition of neutrophil infiltration in the knee joint reduced induced hypernociception, reduced production of TNF-α in the periarticular tissues, and inhibition of leukocyte adhesion [142]. The peptide derived from the N-terminal region of evasin-4 (P0C8E9), which had an affinity with the chemokine CCL5, inhibited the activity of CCL5 in monocyte migration assays. This result suggests that evasin-4 derivatives can serve as a starting point for developing anti-inflammatory drugs [142].

Tian et al. [143] investigated the immunosuppressive peptide amphiregulin from the tick *Amblyomma variegatum* (the tropical bont tick). This peptide is composed of 40 amino acid residues (HLHMHGNGATQVFKPRLVLKCPNAAQLIQ-PGKLQRQLLLQ). In rat splenocytes, amphiregulin exerted significant anti-inflammatory effects by inhibiting the secretion of TNF-α, IL-1, IL-8, and IFN-γ *in vitro*. Compared to LPS, these inflammatory mediators' inhibition was significant in all tested peptide concentrations (2, 4, and 8 μg/mL). Amphiregulin showed substantial elimination of free radicals and antioxidant activities in specific concentrations (5, 10, and 20 μg/mL) *in vitro* and also significantly inhibited the paw inflammation induced by adjuvant mice *in vivo* [143].

Chilopod peptides

Centipede

Centipedes are part of the subphylum Myriapoda (class Chilopoda). *Scolopendra subspinipes mutilans* (Chinese red-

headed centipede) is a component of natural extract formulation widely used in traditional Chinese and Korean medicine to treat various conditions due to its anti-inflammatory, antimicrobial, and analgesic effects [144]. It is a stable extract of which studies report its neuroinflammatory activity and efficacy as a mitigating agent of inflammation in rheumatoid arthritis, as well as antitumor and immunostimulant [145,146]. From the venom of *Scolopendra subspinipes mutilans* (Chinese redhead), the formyl peptide receptor 2 (FPR2) peptide with a chemo-attractive property for FRP2 on the neutrophils' surface was isolated. Results evidenced the therapeutic effects of this peptide on rheumatoid arthritis by inhibiting the release of pro-inflammatory cytokines and the recruitment of neutrophils in the joint [147]. Scolopendrasin IX, another peptide isolated from the same centipede species, can down-regulate the expression of pro-inflammatory mediators such as TNF-α and IL-6, also having therapeutic effects against rheumatoid arthritis. In mouse neutrophils, peptides from this centipede species' venom have a high potential to control the inflammatory process due to their targeted effects. However, the mechanism of action has not been clarified yet [147].

Discussion

Peptides and antitumor activities

When there is a failure in the inflammatory process's control mechanism, the condition can evolve into chronic inflammation with consequent mutation and cell proliferation, thus creating an environment conducive to cancer development. In this context, numerous treatments rely on antineoplastic therapy, including chemotherapy, radiotherapy, and immunotherapy [148]. These therapeutic options can cause serious side effects and increase resistance to neoplastic cells, therefore continuous research intent to find new therapeutical options. Animal venoms have become an object of interest because they have specific and structurally stable components that can interact with and modulate their molecular targets, making them good therapeutic candidates [149].

Among the drugable candidates, peptides from different arthropod species can potentially control inflammatory processes and control malignant neoplasms [150]. For instance, among the various ant toxins, solenopsin A (derived from red imported fire ant- *Solenopsis invicta*) is a potent anti-angiogenic agent that inhibits the phosphorylation of Akt-1 and FOXO1a, a substrate of Akt, thus modulating the Akt signal transduction, phosphatidylinositol-3-kinase in mouse embryos (3T3-L1 and NIH3T3) and zebrafish [151]. In cell cultures of HepG2, MCF-7, and LoVo lines, this peptide proved to be an anti-angiogenic toxin that can reduce the levels of cytokines such as interleukin (IL) -1β, IL-6, IL-8, and NF-κB) [152]. Table 2 summarizes information regarding some venom peptides with antitumoral and anti-inflammatory activity.

In this line, the centipede glycosphingolipid peptide-7 from the millipede – *Parafontaria laminata armigera* exerts an antiproliferative effect on neoplastic cells and inhibits the focal adhesion kinase (FAK) pathway in addition to the signal-regulated kinase (Erk) 1 and 2, both involved in the proliferation of melanoma cells. This same peptide reduced proteins' expression related to oral squamous cell carcinoma (cyclin D1) [153]. Regarding bee venom, melittin (*Apis mellifera*) is undoubtedly one of the most multifunctional toxins. In the fight against neoplastic cells, melittin can bind calmodulin and prevent cell proliferation, inducing the death of neoplastic cells through the activation of caspases and metalloproteinases (MMPs) [154,155]. In cells transformed by an oncogene, melittin activates PLA2, which destroys cancer cells and comprises another mechanism that acts as an antineoplastic agent. Through the PLA2-dependent mechanism of activation, melittin is effective in leukemic cell lines that are even resistant to TNF-α [156, 157].

PLA2 (*Apis mellifera*) is a toxin that negatively regulates transduction pathways related to cell survival and tumor invasion. Moreover, treatment with this peptide decreased epidermal growth factor (EGFr) [158]. BV is efficient in killing K1735M2 and B16 melanoma cells, halting the cell cycle at the G1 stage and, therefore, inhibiting cancer cells' proliferation in a dose-dependent manner. Furthermore, BV treatment stimulated Bax production, a pro-apoptotic protein, and reduced the expression of Bcl-2, resulting in the formation of dimers with Bax and the consequent cell death [159, 160].

Mastoparan is a peptide isolated from wasp *Polybia paulista*, which alone can induce mitochondrial permeability; however, it does not have specificity in malignant cells. Though, when encapsulated in a liposome, this peptide could release cytochrome in human chronic myeloid leukemia cells [161]. Isolated from *Polybia paulista*, the Polybia MPI peptide has cytotoxicity against leukemic T lymphocytes, in addition to being able to reach the cells of the lipid membranes creating channels that provoke ionic permeabilization, depolarization, and consequent cell death [162].

Although spiders are a widespread species within the arthropod group, toxins that act as antineoplastic agents are understudied. Research has shown that the crude venom from *Macrothele raven* (Araneae, Hexathelidae) can arrest cancer cells via caspase 3 in treated cells, leading to the HeLa cell's cell death. In breast cancer cells, the crude venom of this species caused cell death, in addition to causing a cell arrest in the G2/M and G0/ G1 cycles [163, 164].

The toxins obtained from the Chinese bird spider *Haplopelma hainanum* showed antitumor activity in a liver cancer cell line, decreasing cell growth, mitochondrial membrane potential, in addition to stimulating the production of caspase 3 and 9 and inducing apoptosis through a dependent mitochondrial pathway [165].

Scorpion venoms have been a promising target in cancer treatment, the most interesting being the long-chain toxins that act on K +, Cl-, and ion channels. For example, human breast cancer MCF-7 cells treated with *Buthus matensii karsch* toxin extract could induce apoptosis by producing caspase 3 and down-regulating Bcl-2. In *in vitro* studies, gonoearrestide, a peptide found in the fat-tailed scorpion *Androctonus mauritanicus* and *A. australis*, was able to kill neoplastic cells by arresting the cell

Table 2. Examples of peptides from the Uniprot database with antineoplastic activities.

Animal (Source)	Peptide	Access number	Antitumoral activity	Ref.
Insect				
Solenopsis invicta	Solenopsin	–	Inhibits PIK3 activation, Akt and FOXO1 phosphorylation	[150–152]
	Melitin	P01501	Activation of caspases, metalloproteinases and PLA2	[155–157]
Apis mellifera	Phospholipase	P00630	Epidermal growth factor receptor (EGFr) reduction	[158]
	Bee venom	–	Reduction of Bcl-2 expression	[159,160]
Polybia paulista	Mastoparan 1	P0C1Q4	Induces mitochondrial permeability and cytochrome release	[161]
	Polybia MPI	–	Cytotoxicity against leukemic T lymphocytes	
Arachnid				
Macrothele raven	Macrothele raven venom	–	Antitumoral activity	[163,164]
Haplopelma haunanum	*Haplopelma haunanum* venom	–	Reduced cell growth and stimulation of the production of caspase 3 and 9	[167]
Crustacean				
Buthus matensii karsch	*Buthus matensii karsch* venom	–	Induce apoptosis by producing caspase 3 and down-regulating Bcl-2	[168]
Androctonus mauritanicus e *Androctonus australis*	Gonearrestide	–	Inhibition of cyclin-dependent kinase 4 (CDK4) and increased cell expression of cycle regulators and inhibitors (cyclin D3, p27, and p21)	[169]
Leiurus quinquestriatus	Chlorotoxin	P45639	Can bind endogenously to MMP-2 expressed in glioma cells	[170,171]
Parabuthus schlechteri	PBITx1	P60271	Selective toxin of the Na^+ channel	[172]
Chilopod				
Parafontaria laminata			Suppressive activity of the focal adhesion kinase pathway (FAK) and the kinase pathway regulated by the extracellular signal (ERK)	[153–154]

cycle in the G1 phase due to inhibition of cyclin-dependent kinase 4 (CDK4) and increased cell expression of cycle regulators and inhibitors cyclin D3, p27, and p21 [166]. Also, this species' venom was able to block the cell cycle from the G0/G1 phase to the S phase [167].

Chlorotoxin (Cltx) is found in the venom of the Palestine yellow scorpion *Leiurus quinquestriatus*. *In vitro* studies showed that Cltx binds to glioma cells without affecting normal cells; Cltx can bind endogenously to MMP-2 expressed in glioma cells, thus generating a loss of the gelatinase activity of the glioma and decreasing the expression of MMP2. PBITx1, extracted from the burrowing thick tail scorpion *Parabuthus schlechteri*, is a selective toxin of the Na+ channel and structurally similar to Cltx, suggesting that it could act on chloride channels and arrest cancer cells [168–170]. This synthesized peptide showed low toxicity in clinical trials, inhibiting angiogenesis, a possible candidate to combat gliomas [171].

Work limitations

Arthropods comprise a large phylum of invertebrate animals, and their particular biological and ecological characteristics vary according to each species. It is worth mentioning that numerous species have bioactive peptides in their venoms with anti-inflammatory activity, as observed in studies conducted *in vivo* and *in vitro*. Thus, we selected certain arthropods groups that provided more publications related to the theme when inquiring databases. We expected the present review to glimpse the theme and attract the audience's attention to this exciting research topic. A limitation of the study is about some elusive mechanisms of action of venom peptides reported by different laboratories that can be further explored for peptide drug development. Despite this, a handful of information allowed describing the peptides' significant "anti-inflammatory effects" from venom components of numerous arthropod species.

Conclusion

Considerable diversity of bioactive molecules under investigation can be developed as therapeutic agents to treat numerous human diseases. Various research groups have studied different peptides identified in arthropod venoms to unravel their potential as anti-inflammatory agents. The selected examples listed herein comprise peptides found in the venom and hemolymph of diverse species of arthropods. Included in this review were arthropods related to insects (ants, bees, and wasps), crustaceans (shrimp and crabs), arachnids (scorpions and spiders), and chilopods (centipedes), all of them containing in their venom peptides with important anti-inflammatory activity. Peptides derived from arthropod venoms act on different inflammatory pathways,

reducing pro-inflammatory cytokines both in *in vitro* and *in vivo* models. It is known that inflammation at an advanced stage can trigger malignant neoplasms and contribute to their exacerbation. Thus, multifunctional venom peptides that act on inflammatory pathways and pathways related to cancer deserve considerable attention in the present and future natural drug development programs. Consequently, arthropod venom peptides, which evolved over millions of years, comprise a rich source for discovering and developing peptides with potent pharmacological efficacy to treat inflammatory and malignant diseases. The disclosure of their specific mechanisms of action and application potential as therapeutic agents should continue in the years to come.

Abbreviations

ALF: anti-lipopolysaccharide factor; ALS: amyotrophic lateral sclerosis; BAX: BCL2-associated X protein; BCL: B-cell lymphoma; BV: bee venom therapy; CAT: catalase; CD: cluster of differentiation; CMO: scorpion oligopeptides; COX: cyclooxygenase; DBPs: disulfide-bridged peptides; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ERK: extracellular signal-regulated kinase; FAZ: cell surface death receptor; FDA: U.S. Food and Drug Administration; FPR-2: formyl peptide receptor-2; ICAM-1: intercellular adhesion molecule 1; IFN- γ: interferon gama; IL-1: interleukin 1; IL-1β: interleukin beta; IL-4: interleukin 4; IL-6: interleukin 6; IL-8: interleukin 8; IL-10: interleukin 10; IL-13: interleukin 13; iNOS: nitric oxide-induced synthase; JNK: c-Jun N-terminal kinases; LALF: limulus anti-lipopolysaccharide factor; LPS-BD: lipopolysaccharide-binding domain; LPS: anti-lipopolysaccharide; M-ALF: marsupenaeus anti-lipopolysaccharide factor; MAP: mitogen-activated protein; MAPK: mitogen-activated protein kinase; MHC-II: major histocompatibility complex 2; MPC: monocyte chemoactive protein; MPO: myeloperoxidase; NDBPs: non-disulfide-bridged peptides; NF-kβ: nuclear factor kappa beta; PAM: antimicrobial peptides; PAMP: pathogen-associated molecular pattern; PRISMA: preferred reporting items for systematic reviews and meta-analysis; PGE: prostaglandin; PLA2: phospholipase A2; PtALF: portunus trituberculatus anti-lipopolysaccharide factor; SALF: shrimp anti-lipopolysaccharide factor; SOD: superoxide dismutase; TGF-β1: transforming growth factor-β1; TLR: toll-like receptor; TNF- α: nuclear transcription factor-alpha; TRAP1: transient receptor potential ankyrin; VCAM: vascular adhesion molecule; β-GPB: guanine nucleotide-binding protein subunit beta.

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Competing interests

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Authors' contributions

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References

1. Perumal Samy R, Stiles BG, Franco OL, Sethi G, Lim LHK. Animal venoms as antimicrobial agents. Biochem Pharmacol. 2017 Jun 15;134:127-38. doi: 10.1016/j.bcp.2017.03.005. PubMed PMID: 28288817.
2. Harvey AL. Toxins and drug discovery. Toxicon. 2014 Dec 15;92:193-200. doi: 10.1016/j.toxicon.2014.10.020. PubMed PMID: 25448391.
3. Waheed H, Moin SF, Choudhary MI. Snake venom: from deadly toxins to life-saving therapeutics. Curr Med Chem. 2017;24(17):1874-91. doi: 10.2174/0929867324666170605091546. PubMed PMID: 28578650.
4. Calderon LA, Sobrinho JC, Zaqueo KD, de Moura AA, Grabner AN, Mazzi MV, Marcussi S, Nomizo A, Fernandes CF, Zuliani JP, Carvalho BMA, da Silva SL, Stábeli RG, Soares AM. Antitumoral activity of snake venom proteins: new trends in cancer therapy. Biomed Res Int. 2014;2014:203639. doi: 10.1155/2014/203639. PubMed PMID: 24683541; PMCID: PMC3943284.
5. Zhang, Y. Why do we study animal toxins? Dongwuxue Yanjiu. 2015;36 (4):183–222. doi: 10.13918/j.issn.2095-8137.2015.4.183. PubMed PMID: 26228472.
6. Bondaryk M, Staniszewska M, Zielińska P, Urbańczyk-Lipkowska Z. Natural antimicrobial peptides as inspiration for design of a new generation antifungal compounds. J Fungi (Basel). 2017 Aug 26;3(3):46. doi: 10.3390/jof3030046. PubMed PMID: 29371563; PMCID: PMC5715947.
7. Uniprot.Universal Protein [Internet]. 2020 [cited 2020 Aug 30]. Available from: https://www.uniprot.org/.
8. Patridge E, Gareiss P, Kinch MS, Hoyer D. An analysis of FDA-approved drugs: natural products and their derivatives. Drug Discov Today. 2016 Feb;21(2):204-7. doi: 10.1016/j.drudis.2015.01.009. PubMed PMID: 25617672.
9. Bonfanti AP, Barreto N, Munhoz J, Caballero M, Cordeiro G, Rocha-E-Silva T, Sutti R, Moura F, Brunetto S, Ramos CD, Thomé R, Verinaud L, Rapôso C. Spider venom administration impairs glioblastoma growth and modulates immune response in a non-clinical model. Sci Rep. 2020 Apr 03;10(1):5876. doi: 10.1038/s41598-020-62620-9. PubMed PMID: 32246025.
10. Trim SA, Trim CM. Venom: the sharp end of pain therapeutics. Br J Pain. 2013 Nov;7(4):179-88. doi: 10.1177/2049463713502005. PubMed PMID: 265165; PMCID: PMC4590164.
11. Pountos I, Panteli M, Lampropoulos A, Jones E, Calori GM, Giannoudis PV. The role of peptides in bone healing and regeneration: a systematic review. BMC Med. 2016 Jul 11;14:103. doi: 10.1186/s12916-016-0646-y. PubMed PMID: 27400961; PMCID: PMC4940902.

12. Lamiyan AK, Dalal R, Kumar NR. Venom peptides in association with standard drugs:a novel strategy for combating antibiotic resistance– an overview. J Venom Anim Toxins incl Trop Dis. 2020 Aug 10;26: e20200001 doi: 10.1590/1678-9199-JVATITD-2020-0001. PubMed PMID: 32843888.
13. Monge-Fuentes V, Arenas C, Galante P, Gonçalves JC, Mortari MR, Schwartz EF. Arthropod toxins and their antinociceptive properties: from venoms to painkillers. Pharmacol Ther. 2018 Aug;188:176-85. doi: 10.1016/j.pharmthera.2018 Aug.03.007. PubMed PMID: 29605457.
14. Liberati A, Altma DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, Clarke M, Devereaux PJ, Kleijnen J, Moher D. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. PLoS Med. 2009 Jul 21;6(7): e1000100. doi: 10.1371/journal.pmed.1000100. PubMed PMID: 19621070.
15. Heep J, Skaljac M, Grotmann J, Kessel T, Seip M, Schmidtberg H, Vilcinskas A. Identification and functional characterization of a novel insecticidal decapeptide from the myrmicine ant manica rubida. Toxins. 2019 Sep 25;11(10):562. doi: 10.3390/toxins11100562. PubMed PMID: 31557881.
16. Gonçalves Peterson Fox E, Solis DR, Delazari dos Santos L, dos Santos Pinto JRA, da Silva Menegasso AR, Costa Silva RCM, Palma MS, Correa Bueno O, de Alcântara Machado E. A simple, rapid method for the extraction of whole fire ant venom (insecta: *Formicidae*: *Solenopsis*). Toxicon. 2012 Apr;65:5–8. doi: 10.1016/j.toxicon.2012.12.009. PubMed PMID: 23333648.
17. Nelder MP, Paysen ES, Zungoli PA, Benson EP. Emergence of the introduced ant *Pachycondyla* chinensis (*Formicidae*: *Ponerinae*) as a public health threat in the southeastern United States. J Med Entomol. 2006 Sep 1;43(5):1094-5. doi: 10.1093/jmedent/43.5.1094.
18. Wanandy T, Wilson R, Gell D, Rose HE, Gueven N, Davies NW, Brown SGA, Wiese MD. Towards complete identification of allergens in Jack Jumper (*Myrmecia pilosula*) ant venom and their clinical relevance: an immunoproteomic approach. Clin Exp Allergy. 2018 Sep;48(9):1222–34. doi: 10.1111/cea.13224. PubMed PMID: 29975807.
19. Pan J, Hink WF. Isolation and characterization of myrmexins, six isoforms of venom proteins with anti-inflammatory activity from the tropical ant, *Pseudomyrmex triplarinus*. Toxicon. 2000 Oct;38(10):1403–13. doi: 10.1016/s0041-0101(99)00233-0. PubMed PMID: 10758275.
20. Cologna CT, Cardoso JS, Jourdan E, Degueldre M, Upert G, Gilles N, Uetanabaro APT, Costa Neto EM, Thonart P, de Pauw E, Quinton L. Peptidomic comparison and characterization of the major components of the venom of the giant ant *Dinoponera quadriceps* collected in four different areas of Brazil. J Proteomics. 2013 Dec 6;94:413-22. doi: 10.1016/j.jprot.2013.10.017. PubMed PMID: 24157790.
21. Barassé V, Touchard A, Téné N, Tindo M, Kenne M, Klopp C, Dejean A, Bonnafé E, Treilhou M. The peptide venom composition of the fierce stinging ant *Tetraponera aethiops* (*Formicidae: Pseudomyrmecinae*). Toxins (Basel). 2019 Dec 14;11(12):732. doi: 10.3390/toxins11120732. PMID: 31847368; PubMed PMCID: PMC6950161.
22. Rykaczewska-Czerwińska M, Radosz A, Konopinska D. , Wrobel M. , Plech A. Antinociceptive effect of poneratoxin [PoTX] in rats. Pestycydy. 2008;1–2:135–41.
23. Costa Neto EM, Ramos-Elorduy J, Pino JM. Los insectos medicinales de Brasil: primeros resultados. Bol Soc Entomol Aragonesa. 2006;1(38):395–414.
24. Lopes KS, Rios ERV, Lima CNC, Linhares MI, Torres AFC, Havt A, Quinet YP, Fonteles MMF, Martins AMC. The effects of the brazilian ant *Dinoponera quadriceps* venom on chemically induced seizure models. Neurochem Int. 2013 Sep;63(3):141–5. doi: 10.1016/j.neuint.2013.06.001. PubMed PMID: 23747963.
25. Madeira JC, Quinet YP, Nonato DTT, Sousa PL, Chaves EMC, Honório Júnior JER, Pereira MG, Assreuy AMS. Novel pharmacological properties of *Dinoponera quadriceps* giant ant venom. Nat Prod Commun. 2015 Sep;10(9):1607–9. doi: 10.1177/1934578X1501000930. PubMed PMID: 26594770.
26. Sousa PL, Quinet Y, Ponte EL, do Vale JF, Torres AFC, Pereira MG, Assreuy MAS. Venom's antinociceptive property in the primitive ant *Dinoponera quadriceps*. J Ethnopharmacol. 2012 Oct 31;144(1):213-6. doi: 10.1016/j.jep.2012.08.033. PubMed. PMID: 22960549.
27. Sousa PL, Quinet YP, Brizeno LAC, Sampaio TL, Torres AFC, Martins AMC, Assreuy AMS. The acute inflammatory response induced in mice by the venom of the giant ant *Dinoponera quadriceps* involves macrophage and interleukin-1β. Toxicon. 2016 Jul;117:22-9. doi: 10.1016/j.toxicon.2016.03.009. PubMed PMID: 27018043.
28. Ebaid H, Abdel-Salam B, Alhazza I, Al-Tamimi J, Hassan I, Rady A, Mashaly A, Mahmoud A, Sammour R. Samsum ant venom modulates the immune response and redox status at the acute toxic dose in vivo. J Venom Anim Toxins incl Trop Dis. 2019 Dec 2;25: e20190020. doi: 10.1590/1678-9199-JVATITD-2019-0020. PubMed PMCID: PMC6892565.
29. Al-Tamimi J, Alhazza IM, Al-Khalifa M, Metwalli A, Rady A, Ebaid H. Potential effects of samsum ant, *Brachyponera sennaarensis*, venom on TNF-α/NF-κB mediated inflammation in CCL4-toxicity in vivo. Lipids Health Dis. 2016 Nov 18;15(1):198. doi: 10.1186/s12944-016-0364-7. PubMed PMID: 27863485; PMCID: PMC5116174.
30. Ebaid H, Al-Khalifa M, Isa AM, Gadoa S. Bioactivity of samsum ant (*Pachycondyla sennaarensis*) venom against lipopolysaccharides through antioxidant and upregulation of Akt1 signaling in rats. Lipids Health Dis. 2012 Jul 23;11:93. doi: 10.1186/1476-511X-11-93. PubMed PMID: 22824368; PMCID: PMC3416678.
31. Czarnetzki BM, Thiele T, Rosenbach T. Immunoreactive leukotrienes in nettle plants (*Urtica urens*). Int Arch Allergy Appl Immunol. 1990;91(1):43–6. doi: 10.1159/000235087. PubMed PMID: 2155878.
32. Raghuraman H, Chattopadhyay A. Melittin: a membrane-active peptide with diverse functions. Biosci Rep. 2007 Oct;27(4-5):189-223. doi: 10.1007/s10540-006-9030-z. PubMed PMID: 17139559.
33. Hwang DS, Kim SK, Bae H. Therapeutic effects of bee venom on immunological and neurological diseases. Toxins (Basel). 2015 Jun 29;7(7): 2413-21. doi: 10.3390/toxins7072413. PubMed PMID: 26131770; PMCID: PMC4516920.
34. Romero-Curiel A, López-Carpinteyro D, Gamboa C, De la Cruz F, Zamudio S, Flores G. Apamin induces plastic changes in hippocampal neurons in senile Sprague–Dawley Rats. Synapse. 2011 Oct;65(10):1062–72. doi: 10.1002/syn.20938. PubMed PMID: 21465567.
35. Fan AY, Lao L, Zhang RX, Zhou AN, Wang LB, Moudgil KD, Lee DYW, Ma ZZ, Zhang WY, Berman BM. Effects of an acetone extract of *Boswellia carterii* Birdw. (*Burseraceae*) gum resin on adjuvant-induced arthritis in lewis rats. J Ethnopharmacol. 2005 Oct 3;101(1-3):104-9. doi: 10.1016/j.jep.2005.03.033. PubMed PMID: 15970410.
36. Choi HS, Kang SY, Roh DH, Choi SR, Ryu Y, Lee JH. Bee venom stimulation of a lung meridian acupoint reduces inflammation in carrageenan-induced pleurisy: an alternative therapeutic approach for respiratory inflammation. J Vet Sci. 2018 Sep 30;19(5):708–15. doi: 10.4142/jvs.2018.19.5.708. PubMed PMID: 29929357.
37. Lee WR, Kim SJ, Park JH, Kim KH, Chang YC, Park YY, Lee KG, Han SM, Yeo JH, Pak SC, Park KK. Bee venom reduces atherosclerotic lesion formation via anti-inflammatory mechanism. Am J Chin Med. 2010;38(6):1077-92. doi: 10.1142/S0192415X10008482. PubMed PMID: 21061462.
38. Chung KS, An HJ, Cheon SY, Kwon KR, Lee KH. Bee venom suppresses testosterone-induced benign prostatic hyperplasia by regulating the inflammatory response and apoptosis. Exp Biol Med (Maywood). 2015 Dec;240(12):1656-63. doi: 10.1177/1535370215590823. PubMed PMID: 26085572; PMCID: PMC4935329.
39. Kim JY, Lee WR, Kim KH, An HJ, Chang YC, Han SM, Park YY, Pak SC, Park KK. Effects of bee venom against *Propionibacterium* acnes-induced inflammation in human keratinocytes and monocytes. Int J Mol Med. 2015 Jun;35(6):1651-6. doi: 10.3892/ijmm.2015.2180. PubMed PMID: 25872535.
40. Darwish SF, El-Bakly WM, Arafa HM, El-Demerdash E. Targeting TNF-α and NF-κB activation by bee venom: role in suppressing adjuvant induced arthritis and methotrexate hepatotoxicity in rats. PLoS One. 2013 Nov 20;8(11):e79284. doi: 10.1371/journal.pone.0079284. PubMed PMID: 24278124; PMCID: PMC3835890.
41. Kocyigit A, Guler EM, Kaleli S. Anti-inflammatory and antioxidative properties of honey bee venom on Freund's Complete Adjuvant-induced arthritis model in rats. Toxicon. 2019 Apr 1;161:4-11. doi: 10.1016/j.toxicon.2019.02.016. PubMed PMID: 30825463.

42. El-Tedawy DM, Abd-Alhaseeb MM, Helmy MW, Ghoneim AI. Systemic bee venom exerts anti-arthritic and anti-inflammatory properties in a rat model of arthritis. Biomed Rep. 2020 Oct;13(4):20. doi: 10.3892/br.2020.1327. PubMed PMID: 32765859; PMCID: PMC7403832.
43. Kwon GL, Koh HK. An experimental study with bee venom therapy on anti-inflammatory and analgesic effects. J Acupunct Res. 1998 Dec;15(2):97–103.
44. Lee KG, Cho HJ, Bae YS, Park KK, Choe JY. Bee venom suppresses LPS-mediated NO/iNOS induction through inhibition of PKC-α expression. J Ethnopharmacol. 2009 May 4;123(1):15-21. doi: 10.1016/j.jep.2009.02.044.
45. Cho HJ, Jeong YJ, Park KK, Park YY, Chung IK, Lee KG, Yeo JH, Han SM, Bae YS, Chang YC. Bee venom suppresses PMA-mediated MMP-9 gene activation via JNK/p38 and NF-kappaB-dependent mechanisms. J Ethnopharmacol. 2010 Feb 17;127(3):662-8. doi: 10.1016/j.jep.2009.12.007. PubMed PMID: 19969058.
46. Nipate SS, Bhandarkar AA. Anti-inflammatory, antinociceptive and antiarthritic potential of apis cerana indica bee venom by reducing pain and degeneration of articular cartilage in adjuvant and collagen induced rat models of arthritis. J Orthop Muscular Syst. 2020 Mar 25;3(1):1-10.
47. Calixto MC, Trichês KM, Calixto JB. Analysis of the inflammatory response in the rat paw caused by the venom of *Apis melifera* bee. Inflamm Res. 2003 Mar;52(3):132-9. doi: 10.1007/s000110300026. PubMed PMID: 12755378.
48. Jang MH, Shin MC, Lim S, Han SM, Park HJ, Shin I, Lee JS, Kim KA, Kim EH, Kim CJ. Bee venom induces apoptosis and inhibits expression of cyclooxygenase-2 mRNA in human lung cancer cell line NCI-H1299. J Pharmacol Sci. 2003 Feb;91(2):95-104. doi: 10.1254/jphs.91.95. PubMed PMID: 12686753.
49. Baek YH, Huh JE, Lee JD, Choi DY, Park DS. Antinociceptive effect and the mechanism of bee venom acupuncture (Apipuncture) on inflammatory pain in the rat model of collagen-induced arthritis: mediation by alpha2-Adrenoceptors. Brain Res. 2006 Feb 16;1073-1074:305-10. doi: 10.1016/j.brainres.2005.12.086. PubMed PMID: 16457792.
50. Kim JH, Lee HY, Kim MH, Han TS, Cho KR, Kim GH, Choi SH. Antinociceptive efficacy of korean bee venom in the abdominal pain of the mouse. J Vet Clin. 2007;24(3):320-4.
51. Jeong CH, Cheng WN, Bae H, Lee KW, Han SM, Petriello MC, Lee HG, Seo HG, Han SG. Bee venom decreases LPS-induced inflammatory responses in bovine mammary epithelial cells. J Microbiol Biotechnol. 2017 Oct 28;27(10):1827-36. doi: 10.4014/jmb.1706.06003. PubMed PMID: 28813781.
52. Shin SH, Ye MK, Choi SY, Park KK. Anti-inflammatory effect of bee venom in an allergic chronic rhinosinusitis mouse model. Mol Med Rep. 2018 May;17(5):6632-8. doi: 10.3892/mmr.2018.8720. PubMed PMID: 29532888.
53. Son DJ, Lee JW, Lee YH, Song HS, Lee CK, Hong JT. Therapeutic application of anti-arthritis, pain-releasing, and anti-cancer effects of bee venom and its constituent compounds. Pharmacol Ther. 2007 Aug;115(2):246-70. doi: 10.1016/j.pharmthera.2007.04.004. PubMed PMID: 17555825.
54. Ye X, Guan S, Liu J, Ng CCW, Chan GHH, Sze SCW, Zhang KY, Naude R, Rolka K, Wong JH, Ng TB. Activities of venom proteins and peptides with possible therapeutic applications from bees and WASPS. Protein Pept Lett. 2016;23(8):748-55. doi: 10.2174/0929866523666160618120824. PubMed PMID: 27323949.
55. Monti MC, Casapullo A, Santomauro C, D'Auria MV, Riccio R, Gomez-Paloma L. The molecular mechanism of bee venom phospholipase A2 inactivation by bolinaquinone. Chembiochem. 2006 Jun;7(6):971-80. doi: 10.1002/cbic.200500454. PubMed PMID: 16671124.
56. Kim H, Keum DJ, Kwak Jw, Chung HS, Bae H. Bee venom phospholipase A2 protects against acetaminophen-induced acute liver injury by modulating regulatory T cells and IL-10 in mice. PLoS One. 2014 Dec 5;9(12):e114726. doi: 10.1371/journal.pone.0114726. PubMed PMID: 25478691; PMCID: PMC4257707.
57. Ham HJ, Han SB, Yun J, Yeo IJ, Ham YW, Kim SH, Park PH, Choi DY, Hong JT. Bee venom phospholipase A2 ameliorates amyloidogenesis and neuroinflammation through inhibition of signal transducer and activator of transcription-3 pathway in Tg2576 mice. Transl Neurodegener. 2019 Oct 2;8:26. doi: 10.1186/s40035-019-0167-7. PubMed PMID: 31592103; PMCID: PMC6774221.
58. Saini SS, Peterson JW, Chopra AK. Melittin binds to secretory phospholipase A2 and inhibits its enzymatic activity. Biochem Biophys Res Commun. 1997 Sep 18;238(2):436-42. doi: 10.1006/bbrc.1997.7295. PubMed PMID: 9299527.
59. Park JH, Kim KH, Lee WR, Han SM, Park KK. Protective effect of melittin on inflammation and apoptosis in acute liver failure. Apoptosis. 2012 Jan;17(1):61-9. doi: 10.1007/s10495-011-0659-0. PubMed PMID: 21928088.
60. Nam KW, Je KH, Lee JH, Han HJ, Lee HJ, Kang SK, Mar W. Inhibition of COX-2 activity and proinflammatory cytokines (TNF-alpha and IL-1beta) production by water-soluble sub-fractionated parts from bee (*Apis mellifera*) venom. Arch Pharm Res. 2003 May;26(5):383-8. doi: 10.1007/BF02976695. PubMed PMID: 12785734.
61. Park HJ, Lee HJ, Choi MS, Son DJ, Song HS, Song MJ, Lee JM, Han SB, Kim Y, Hong JT.JNK pathway is involved in the inhibition of inflammatory target gene expression and NF-kappaB activation by melittin. J Inflamm (Lond). 2008 May 29;5:7. doi: 10.1186/1476-9255-5-7. PubMed PMID: 18507870; PMCID: PMC2442592.
62. Kim WH, An HJ, Kim JY, Gwon MG, Gu H, Jeon M, Kim MK, Han SM, Park KK. Anti-inflammatory effect of melittin on *Porphyromonas gingivalis* LPS-stimulated human keratinocytes. Molecules. 2018 Feb 5;23(2):332. doi: 10.3390/molecules23020332. PubMed PMID: 29401750; PMCID: PMC6017529.
63. Lee WR, Kim KH, An HJ, Kim JY, Han SM, Lee KG, Park KK. Protective effect of melittin against inflammation and apoptosis on *Propionibacterium acnes*-induced human THP-1 monocytic cell. Eur J Pharmacol. 2014 Oct 5;740:218-26. doi: 10.1016/j.ejphar.2014.06.058.
64. Park JH, Kum YS, Lee TI, Kim SJ, Lee WR, Kim BI, Kim HS, Kim KH, Park KK. Melittin attenuates liver injury in thioacetamide-treated mice through modulating inflammation and fibrogenesis. Exp Biol Med (Maywood). 2011 Nov;236(11):1306-13. doi: 10.1258/ebm.2011.011127. Epub 2011 Oct 3. PMID: 21969711.
65. Rayahin JE, Buhrman JS, Gemeinhart RA. Melittin-glutathione S-transferase fusion protein exhibits anti-inflammatory properties and minimal toxicity. Eur J Pharm Sci. 2014 Dec 18;65:112-21. doi: 10.1016/j.ejps.2014.09.012. Epub 2014 Sep 21. PMID: 25240321; PMCID: PMC4253680.
66. Lee G, Bae H. Anti-inflammatory applications of melittin, a major component of bee venom: detailed mechanism of action and adverse effects. Molecules. 2016 May 11;21(5):616. doi: 10.3390/molecules21050616. PMID: 27187328; PMCID: PMC6273919.
67. Ovcharov R, Shkenderov S. Anti-inflammatory effects of apamin. Toxicon. 1976; 14,441–7. doi: 10.1016/0041-0101(76)90060-x. PubMed PMID: 1014033.
68. Messier C, Mourre C, Bontempi B, Sif J, Lazdunski M, Destrade C.. Effect of apamin, a toxin that inhibits Ca(2+)-dependent K+ channels, on learning and memory processes. Brain Res. 1991 Jun 14;551(1-2):322-6. doi: 10.1016/0006-8993(91)90950-z. PubMed PMID: 1913161.
69. Alvarez-Fischer D, Noelker C, Vulinović F, Grünewald A, Chevarin C, Klein C, Oertel WH, Hirsch EC, Michel PP, Hartmann A. Bee venom and its component apamin as neuroprotective agents in a Parkinson disease mouse model. PLoS One. 2013 Apr 18;8(4):e61700. doi: 10.1371/journal.pone.0061700. PubMed PMID: 23637888; PMCID: PMC3630120.
70. Kim WH, An HJ, Kim JY, Gwon MG, Gu H, Lee SJ, Park JY, Park KD, Han SM, Kim MK, Park KK. Apamin inhibits TNF-α- and IFN-γ-induced inflammatory cytokines and chemokines via suppressions of NF-κB signaling pathway and STAT in human keratinocytes. Pharmacol Rep. 2017 Oct;69(5):1030-5. doi: 10.1016/j.pharep.2017.04.006. PubMed PMID: 28958612.
71. Lee YM, Cho SN, Son E, Song CH, Kim DS. Apamin from bee venom suppresses inflammation in a murine model of gouty arthritis. J Ethopharmacol. 2020 Jul 15;257(15): 112860. doi: 10.1016/j.jep.2020.112860.
72. Shkenderov S, Koburova K. Adolapin--a newly isolated analgetic and anti-inflammatory polypeptide from bee venom. Toxicon. 1982;20(1):317-21. doi: 10.1016/0041-0101(82)90234-3. PubMed PMID: 7080045.
73. Banks BE, Dempsey CE, Vernon CA, Warner JA, Yamey J. Anti-inflammatory activity of bee venom peptide 401 (mast cell degranulating peptide) and compound 48/80 results from mast cell degranulation in vivo. Br J Pharmacol. 1990 Feb;99(2):350-4. doi: 10.1111/j.1476-5381.1990.tb14707.x. PMID: 2328399; PMCID: PMC1917405.

74. Hanson JM, Morley J, Soria-Herrera C. Anti-inflammatory property of 401 (MCD-peptide), a peptide from the venom of the bee *Apis mellifera* (L.). Br J Pharmacol. 1974 Mar;50(3):383-92. doi: 10.1111/j.1476-5381.1974.tb09613.x. PMID: 4152780; PMCID: PMC1776661.

75. Chen L, Chen W, Yang H, Lai R. A novel bioactive peptide from wasp venom. J Venom Res. 2010 Sep 30;1:43-7. PubMed PMID: 21544181; PMC: 3086190.

76. Chen DM, Lee PT, Chou KJ, Fang HC, Chung HM, Chen DM, Chang LK. Descending aortic thrombosis and cerebral infarction after massive wasp stings. Am J Med. 2004 Apr 15;116(8):567-9. doi: 10.1016/j.amjmed.2003.08.036. PubMed PMID: 15063823.

77. Yuan H, Lu L, Gao Z, Hu F. Risk factors of acute kidney injury induced by multiple wasp stings. Toxicon. 2020 Jul 30;182:1–6. doi: 10.1016/j.toxicon.2020.05.002. PubMed PMID: 32387349.

78. Yang H, Xu X, Ma D, Zhang K, Lai R. A phospholipase A1 platelet activator from the wasp venom of vespa magnifica (smith). Toxicon. 2008 Feb;51(2):289–96. doi: 10.1016/j.toxicon.2007.10.003. PubMed PMID: 18023835.

79. Graaf DC, Aerts M, Danneels E, Devreese B. Bee, wasp and ant venomics pave the way for a component-resolved diagnosis of sting allergy. J Proteomics. 2009 Mar 6;72(2):145-54. doi: 10.1016/j.jprot.2009.01.017. PubMed PMID: 19344653.

80. Moreno M, Giralt E. Three valuable peptides from bee and wasp venoms for therapeutic and biotechnological use: melittin, apamin and mastoparan. Toxins (Basel). 2015 Apr 1;7(4):1126-50. doi: 10.3390/toxins7041126. PubMed PMID: 25835385; PMCID: PMC4417959.

81. Yibin G, Jiang Z, Hong Z, Gengfa L, Liangxi W, Guo W, Yongling L. A synthesized cationic tetradecapeptide from hornet venom kills bacteria and neutralizes lipopolysaccharide *in vivo* and in vitro. Biochem Pharmacol. 2005 Jul 15;70(2):209-19. doi: 10.1016/j.bcp.2005.04.040. PubMed PMID: 15935330.

82. Danneels EL, Gerlo S, Heyninck K, Craenenbroeck KV, De Bosscher K, Haegeman G, de Graaf DC. How the venom from the ectoparasitoid *Wasp nasonia vitripennis* exhibits anti-inflammatory properties on mammalian cell lines. PLoS One. 2014 May 12;9(5):e96825. doi: 10.1371/journal.pone.0096825. PMID: 24821138; PMCID: PMC4018385.

83. Danneels EL, Formesyn EM, de Graaf DC. Exploring the potential of venom from *Nasonia vitripennis* as therapeutic agent with high-throughput screening tools. Toxins (Basel). 2015 Jun 3;7(6):2051-70. doi: 10.3390/toxins7062051. PubMed PMID: 26046700; PMCID: PMC4488689.

84. Mortari M R, Cunha AOS, Carolino ROG, Coutinho-Netto J, Tomaz JC, Lopes NP, Coimbra NC, Dos Santos WF. Inhibition of acute nociceptive responses in rats after i.c.v. injection of Thr6 -bradykinin , isolated from the venom of the social wasp , *Polybia occidentalis*. Br J Pharmacol. 2007 Jul;151(6),860–9. doi: 10.1038/sj.bjp.0707275. PubMed PMID: 17533426.

85. Gomes PC, de Souza BM, Dias NB, Brigatte P, Mourelle D, Arcuri HA, dos Santos Cabrera MP, Stabeli RG, Neto JR, Palma MS. Structure–function relationships of the peptide paulistine: a novel toxin from the venom of the social wasp *Polybia paulista*. Biochim Biophys Acta. 2014 Jan 1;1840(1):170-83.

86. Gao Y, Yu WX, Duan XM, Ni LL, Liu H, Zhao HR, Xiao H, Zhang CG, Yang ZB. Wasp venom possesses potential therapeutic effect in experimental models of rheumatoid arthritis. Evid Based Complement Alternat Med. 2020 Apr 6;2020: 1-10. doi: 10.1155/2020/6394625.

87. dos Anjos LC, Gonçalves JC, de Araujo BR, Mortari MR. Antinociceptive activity of the peptide fraction from the venom of social wasp *Pseudopolybia vespiceps* testacea. 2019;15(61): 322-6.

88. Brigatte P, Cury Y, de Souza BM, Baptista-Saidemberg NB, Saidemberg DM, Gutierrez VP, Palma MS.. Hyperalgesic and edematogenic effects of peptides isolated from the venoms of honeybee (*Apis mellifera*) and neotropical social wasps (*Polybia paulista* and *Protonectarina sylveirae*). Amino Acids. 2011 Jan;40(1):101-11. doi: 10.1007/s00726-010-0512-8. PubMed PMID: 20177946.

89. Kaushik DK, Thounaojam MC, Mitra A, Basu A. Vespa tropica venom suppresses lipopolysaccharide-mediated secretion of pro-inflammatory cyto-chemokines by abrogating nuclear factor-κB activation in microglia. Inflamm Res. 2014 Aug;63(8):657–65. doi: 10.1007/s00011-014-0738-0.

90. Tassanakajon A, Amparyup P, Somboonwiwat K, Supungul P. Cationic antimicrobial peptides in penaeid shrimp. Mar Biotechnol (NY). 2010 Oct;12(5):487-505. doi: 10.1007/s10126-010-9288-9. PubMed PMID: 20379756.

91. Zhou L, Li G, Jiao Y, Huang D, Li A, Chen H, Liu Y, Li S, Li H, Wang C. Molecular and antimicrobial characterization of a group G anti-lipopolysaccharide factor (ALF) from *Penaeus monodon*. Fish Shellfish Immunol. 2019 Nov;94,149–56. doi: 10.1016/j.fsi.2019.08.066. PubMed PMID: 31465873.

92. Matos GM, Schmitt P, Barreto C, Farias ND, Toledo-Silva G, Guzmán F, Destoumieux-Garzón D, Perazzolo LM, Rosa RD. Massive gene expansion and sequence diversification is associated with diverse tissue distribution, regulation and antimicrobial properties of anti-lipopolysaccharide factors in shrimp. Mar Drugs. 2018 Oct 11;16(10):381. doi: 10.3390/md16100381. PubMed PMID: 30314303; PMC: 6213531.

93. Lin MC, Pan CY, Hui CF, Chen JY, Wu JL. peptides shrimp anti-lipopolysaccharide factor (SALF), an antimicrobial peptide, inhibits proinflammatory cytokine expressions through the MAPK and NF-κB pathways in LPS-induced HeLa cells. Peptides. 2013 Feb;40,42–8. doi: 10.1016/j.peptides.2012.11.010. PubMed PMID: 23247147.

94. Lin MC, Lin SB, Lee SC, Lin CC, Hui CF, Chen JY. Peptides antimicrobial peptide of an anti-lipopolysaccharide factor modulates of the inflammatory response in RAW264.7 cells. Peptides 2010 Jul;31(7),1262–72. doi: 10.1016/j.peptides.2010.04.004. PubMed PMID: 20385189.

95. Ravipati AS, Zhang L, Koyyalamudi SR, Jeong SC, Reddy N, Bartlett J, Smith PT, Shanmugam K, Münch G, Wu MJ, Satyanarayanan M, Vysetti B. Antioxidant and anti-inflammatory activities of selected chinese medicinal plants and their relation with antioxidant content. BMC Complement Altern Med. 2012 Oct 6;12:173. doi: 10.1186/1472-6882-12-173. PubMed PMID: 23038995; PMCID: PMC3534023.

96. Lin MC, Hui CF, Chen JY, Wu JL. The antimicrobial peptide, shrimp anti-lipopolysaccharide factor (SALF), inhibits proinflammatory cytokine expressions through the MAPK and NF-κB pathways in *Trichomonas vaginalis* adherent to HeLa cells. Peptides. 2012 Dec;38(2):197-207. doi: 10.1016/j.peptides.2012.10.003. PubMed PMID: 23088922.

97. Marggraf MB, Panteleev PV, Emelianova AA, Sorokin MI, Bolosov IA, Buzdin AA, Kuzmin DV, Ovchinnikova TV. Cytotoxic potential of the novel horseshoe crab peptide polyphemusin III. Mar Drugs. 2018 Nov 26;16(12):466. doi: 10.3390/md16120466. PubMed PMID: 30486233; PMCID: 6315362.

98. Hoess A, Watson S, Siber GR, Liddington R. Crystal structure of an endotoxin-neutralizing protein from the horseshoe crab, Limulus anti-LPS factor, at 1.5 A resolution. EMBO J. 1993 Sep;12(9):3351-6. PubMed PMID: 8253062; PMCID: 413608.

99. Kadowaki T, Inagawa H, Kohchi C, Nishizawa T, Takahashi Y, Soma GI. Anti-lipopolysaccharide factor evokes indirect killing of virulent bacteria in kuruma prawn. In Vivo. 2011 Sep-Oct;25(5):741-4. PubMed PMID: 21753127.

100. Liu Y, Cui Z, Li X, Song C, Li Q, Wang S. A new anti-lipopolysaccharide factor isoform (PtALF4) from the swimming crab *Portunus trituberculatus* exhibited structural and functional diversity of ALFs. Fish Shellfish Immunol. 2012 May;32(5),724–31. doi: 10.1016/j.fsi.2012.01.021. PubMed PMID: 22333564.

101. Li S, Guo S, Li F, Xiang J. Functional diversity of anti-lipopolysaccharide factor isoforms in shrimp and their characters related to antiviral activity. Mar Drugs. 2015 Apr 27;13(5):2602-16. doi: 10.3390/md13052602. PubMed PMID: 25923317; PMCID: PMC4446596.

102. Wu X, Huang Y, Yu Z, Mu C, Song W, Li R, Liu L, Ye Y, Shi C, Wang C. An MBT domain containing anti-lipopolysaccharide factor (PtALF8) from *Portunus trituberculatus* is involved in immune response to bacterial challenge. Fish Shellfish Immunol. 2019 Jan;84,252–8. doi: 10.1016/j.fsi.2018.10.016. PubMed PMID: 30300736.

103. Vallespi MG, Glaria LA, Reyes O, Garay HE, Ferrero J, Araña MJ. A *Limulus antilipopolysaccharide* factor-derived peptide exhibits a new immunological activity with potential applicability in infectious diseases. Clin Diagn Lab Immunol. 2000 Jul;7(4):669-75. doi: 10.1128/CDLI.7.4.669-675.2000. PMID: 10882670; PMCID: PMC95932.

104. Iswarya A, Anjugam M, Vaseeharan B. Role of purified β-1, 3 glucan binding protein (β-GBP) from *Paratelphusa hydrodromus* and their anti-inflammatory, antioxidant and antibiofilm properties. Fish Shellfish Immunol. 2017 Sep;68:54-64. doi: 10.1016/j.fsi.2017.07.007. PubMed PMID: 28684323.

105. Liu HP, Chen FY, Gopalakrishnan S, Qiao K, Bo J, Wang KJ. Antioxidant enzymes from the crab *Scylla paramamosain*: gene cloning and gene/protein expression profiles against LPS challenge. Fish Shellfish Immunol; 2010 May-Jun;28(5-6):862–71. doi: 10.1016/j.fsi.2010.02.008. PubMed PMID: 20153435.

106. Soundarapandian P, Roy S, Varadharajan D. Antioxidant activity in hard and soft shell crabs of *Charybdis lucifera* (Fabricius, 1798). J Aquac Res Development. 2014; 5(7):1–5. doi: 10.4172/2155-9546.1000288.

107. Sharma S, Yedery RD, Patgaonkar MS, Selvaakumar C, Reddy KVR. Antibacterial activity of a synthetic peptide that mimics the LPS binding domain of Indian mud crab, *Scylla serrata* anti-lipopolysaccharide factor (SsALF) also involved in the modulation of vaginal immune functions through NF-kB signaling. Microb Pathog. 2011 Mar-Apr;50(3-4):179-91. doi: 10.1016/j.micpath.2010.12.007. PubMed PMID: 21195157.

108. Narayanasamy A, Balde A, Raghavender P, Shashanth D, Abraham J, Joshi I, Nazeer RA. Isolation of marine crab (*Charybdis natator*) leg muscle peptide and its anti-inflammatory effects on macrophage cells. Bio Agricul Biotec. 2020 May;25:101577. doi: 10.1016/j.bcab.2020.101577.

109. Almaaytah A, Albalas Q. Scorpion venom peptides with no disulfide bridges: a review. Peptides. 2014 Jan;51:35-45. doi: 10.1016/j.peptides.2013.10.021. PubMed PMID: 24184590.

110. Daniele-Silva A, Machado RJA, Monteiro NKV, Estrela AB, Santos ECG, Carvalho E, Araújo Júnior RF, Melo-Silveira RF, Rocha HAO, Silva-júnior AA, Fernandes-Pedrosa MF. Stigmurin and TsAP-2 from *Tityus stigmurus* scorpion venom: assessment of structure and therapeutic potential in experimental sepsis. Toxicon. 2016 Oct;121,10–21. doi: 10.1016/j.toxicon.2016.08.016. PubMed PMID: 27567704.

111. Dias NB, de Souza BM, Cocchi FK, Chalkidis HM, Dorce VAC, Palma MS. Profiling the short, linear, non-disulfide bond-containing peptidome from the venom of the scorpion *Tityus obscurus*. J Proteomics. 2018 Jan 6;170:70-79. doi: 10.1016/j.jprot.2017.09.006. PMID: 28918200.

112. Veloso Júnior PHH, Simon KS, de Castro RJA, Coelho LC, Erazo FAH, de Souza ACB, das Neves RC, Lozano VF, Schwartz EF, Tavares AH, Mortari MR, Junqueira-Kipnis AP, Silva-Pereira I, Bocca AL. Peptides ToAP3 and ToAP4 decrease release of inflammatory cytokines through TLR-4 blocking. Biomed Pharmacother. 2019 Oct;118:109152. doi: 10.1016/j.biopha.2019.109152. PubMed PMID: 31376652.

113. Yaqoo R, Tahir HM, Rasheed A, Ahshan MM, Arshad M. Anti-inflammatory role peptides isolated from venom of *Androctonus finitimus* (Pocock 1897) (scorpiones: *Buthidae*). J Anim Plant Sci. 2019; 29(1):334-42.

114. Cassini-Vieira P, Felipetto M, Prado LB, Verano-Braga T, Andrade SP, Santos RAS, Teixeira MM, de Lima ME, Pimenta AMC, Barcelos LS. Ts14 from *Tityus serrulatus* boosts angiogenesis and attenuates inflammation and collagen deposition in sponge-induced granulation tissue in mice. Peptides. 2017 Dec; 98:63-9. doi: 10.1016/j.peptides.2016.10.002. Epub 2016 Oct 11. PMID: 27732900.

115. Maatoug R, Jebali J, Guieu R, Waard M, Kharrat R. BotAF, a new *Buthus occitanus tunetanus* scorpion toxin, produces potent analgesia in rodents. Toxicon. 2018 Jul;149:72-85.

116. Zheng M, Ju W, Chen J, Yuan Y, Zhang C, Liu F, Zhang F. Structural characterization and anti-inflammatory potency of *Mesobuthus martensii* Karsch oligopeptides in lipopolysaccharide (LPS)-induced RAW264. 7 macrophages. Royal Soc Chem. 2019;9(43):24822–32. doi:10.1039/C9RA01623F.

117. Ahmadi M, Zare MA, Hashemlou M, Hejazi M. Study on anti inflammatory effect of scorpion (*Mesobuthus eupeus*) venom in adjuvant-induced arthritis in rats. Arch Razi Insti. 2009 Jun;64(1):51-6.

118. Xiao M, Ding L, Yang W, Chai L, Sun Y, Yang X, Li D, Zhang H, Li W, Cao Z, Wu Y, Li J, Li S, Chen Z. St20, a new venomous animal derived natural peptide with immunosuppressive and anti-inflammatory activities. Toxicon. 2017;127:37–43. doi: 10.1016/j.toxicon.2017.01.005. PubMed PMID: 28077339.

119. Chen Z, Hu Y, Hu J, Yang W, Sabatier JM, De Waard M, Cao Z, Li W, Han S, Wu Y. Unusual binding mode of scorpion toxin BmKTX onto potassium channels relies on its distribution of acidic residues. Biochem Biophys Res Commun. 2014 Apr 25;447(1):70-6. doi: 10.1016/j.bbrc.2014.03.101. PubMed PMID: 24704423.

120. Renisio JG, Romi-Lebrun R, Blanc E, Bornet O, Nakajima T, Darbon H. Solution structure of BmKTX , a K^+ blocker toxin from the chinese scorpion *Buthus martensi*. Proteins. 2000 Jan 1;38(1),70–7. doi: 10.1002/(sici)1097-0134(20000101)38:1<70::aid-prot8>3.0.co;2-5. PubMed PMID: 10651040.

121. Ye F, Hu Y, Yu W, Xie Z, Hu J, Cao Z, Li W, Wu Y. The scorpion toxin analogue BmKTX-D33H as a potential Kv1.3 channel-selective immunomodulator for autoimmune diseases. Toxins (Basel). 2016 Apr 19;8(4):115. doi: 10.3390/toxins8040115. PubMed PMID: 27104568; PMCID: PMC4848641.

122. Veytia-Bucheli JI, Jiménez-Vargas JM, Melchy-Pérez EI, Sandoval-Hernández MA, Possani LD, Rosenstein Y. Kv1.3 channel blockade with the Vm24 scorpion toxin attenuates the $CD4^+$ effector memory T cell response to TCR stimulation. Cell Commun Signal. 2018 Aug 14;16(1):45. doi: 10.1186/s12964-018-0257-7. PMID: 30107837; PMCID: PMC6092819.

123. Hoang AN, Vo HDM, Vo NP, Kudryashova KS, Nekrasova OV, Feofanov AV, Kirpichnikov MP, Andreeva TV, Serebryakova MV, Tsetlin VI, Utkin YN. Vietnamese *Heterometrus laoticus* scorpion venom: evidence for analgesic and anti-inflammatory activity and isolation of new polypeptide toxin acting on Kv1.3 potassium channel. Toxicon. 2014 Jan;77:40–4. doi: 10.1016/j.toxicon.2013.10.027.

124. Zhang H, Liu H, Chen Y, Yang X, Wang P, Liu T, Deng M, Qin B, Correia C, Lee S, Kim J, Sparks M, Nair AA, Evans DL, Kalari KR, Zhang P, Wang L, You Z, Kaufmann SH, Lou Z, Pei H. A cell cycle-dependent BRCA1-UHRF1 cascade regulates DNA double-strand break repair pathway choice. Nat Commun. 2016 Jan 5;7:10201. doi: 10.1038/ncomms10201. PMID: 26727879; PMCID: PMC4728409.

125. Okamoto CK, van den Berg CW, Masashi M, Gonçalves-de-Andrade RM, Tambourgi DV. tetracycline reduces kidney. Toxins (Basel). 2017 Mar 2;9(3):90. doi: 10.3390/toxins9030090. PMID: 28257106; PMCID: PMC5371845.

126. Manzoni-de-Almeida D, Squaiella-Baptistão CC, Lopes PH, van den Berg CW, Tambourgi DV. Loxosceles venom sphingomyelinase D activates human blood leukocytes: role of the complement system. Mol Immunol. 2018 Feb;94:45-53. doi: 10.1016/j.molimm.2017.12.009. PubMed PMID: 29257998.

127. Dongol Y, Cardoso FC, Lewis RJ. Spider Knottin Pharmacology at Voltage-Gated Pain Pathways. Toxins (Basel). 2019 Oct 29;11(11),1–39. doi: 10.3390/toxins11110626. PMID: 31671792.

128. Gonçalves TC, Lesport P, Kuylle S, Stura E, Ciolek J, Mourier G, Servent D, Bourinet E, Benoit E, Gilles N. Evaluation of the spider (*Phlogiellus genus*) phlotoxin 1 and synthetic variants as antinociceptive drug candidates. Toxins (Basel). 2019 Aug 22;11(9):484. doi: 10.3390/toxins11090484. PubMed PMID: 31443554; PMCID: PMC6784069.

129. Tonello R, Fusi C, Materazzi S, Marone IM, De Logu F, Benemei S, Gonçalves MC, Coppi E, Castro-Junior CJ, Gomez MV, Geppetti P, Ferreira J, Nassini R. The peptide Phα1β, from spider venom, acts as a TRPA1 channel antagonist with antinociceptive effects in mice. Br J Pharmacol. 2017 Jan;174(1):57-69. doi: 10.1111/bph.13652. PubMed PMID: 27759880; PMCID: PMC5341489.

130. de Souza AH, Lima MC, Drewes CC, da Silva JF, Torres KCL, Pereira EMR, de Castro Junior CJ, Vieira LB, Cordeiro MN, Richardson M, Gomez RS, Romano-Silva MA, Ferreira J, Gomez MV. Antiallodynic effect and side effects of Phα1b, a neurotoxin from the spider *Phoneutria nigriventer*: comparison with ω-conotoxin MVIIA and morphine. Toxicon. 2011 Dec 1;58:626–33. doi: 10.1016/j.toxicon.2011.09.008. PubMed PMID: 21967810.

131. Tenza-Ferrer H, Magno LAV, Romano-Silva MA, da Silva JF, Gomez MV. Phα1β spider toxin reverses glial structural plasticity upon peripheral inflammation. Front Cell Neurosci. 2019 Jul 10;13:306. doi: 10.3389/fncel.2019.00306. PubMed PMID: 31354431; PMCID: PMC6635560.

132. Grishin EV, Savchenko GA, Vassilevski AA, Korolkova YV, Boychuk YA, Viatchenko-Karpinski VY, Nadezhdin KD, Arseniev AS, Pluzhnikov KA, Kulyk VB, Voitenko NV, Krishtal OO. Novel peptide from spider venom inhibits P2X3 receptors and inflammatory pain. Ann Neurol. 2010 May;67(5):680-3. doi: 10.1002/ana.21949. PubMed PMID: 20437566.

133. Dalmolin GD, Silva CR, Rigo FK, Gomes GM, Cordeiro MN. Antinociceptive effect of brazilian armed spider venom toxin Tx3-3 in animal models of neuropathic pain. Pain. 2011 Oct;152(10):2224-32. doi: 10.1016/j.pain.2011.04.015. Epub 2011 May 13. PMID: 21570770.

134. Emerich BL, Ferreira RCM, Cordeiro MN, Borges MH, Pimenta AMC, Figueiredo SG, Duarte IDG, de Lima ME. δ-Ctenitoxin-Pn1a, a peptide from *Phoneutria nigriventer* spider venom, shows antinociceptive effect involving opioid and cannabinoid systems, in rats. Toxins (Basel). 2016 Apr 12;8(4):106. doi: 10.3390/toxins8040106. PubMed PMID: 27077886.

135. Dalmolin GD, Bannister K, Gonçalves L, Sikandar S, Patel R, Cordeiro MN, Gomez MV, Ferreira J, Dickenson AH. Effect of the spider toxin Tx3-3 on spinal processing of sensory information in naive and neuropathic rats: an in vivo electrophysiological study. Pain Rep. 2017 Jul 6;2(4):e610. doi: 10.1097/PR9.0000000000000610. PubMed PMID: 29392225; PMCID: PMC5741365.

136. Shin MK, Hwang IW, Kim Y, Kim ST, Jang W, Lee S, Bang WY, Bae CH, Sung JS. Antibacterial and anti-inflammatory effects of novel peptide toxin from the spider *Pardosa astrigera*. Antibiotics (Basel). 2020 Jul 19;9(7):422. doi: 10.3390/antibiotics9070422. PMID: 32707636; PMCID: PMC7400607.

137. Kazimírová M, Štibrániová I. Tick salivary compounds: their role in modulation of host defences and pathogen transmission. Front Cell Infect Microbiol. 2013 Aug 20;43(3):43. doi: 10.3389/fcimb.2013.00043. PMID: 23971008; PMCID: PMC3747359.

138. Prinsloo L, Naidoo A, Serem J, Taute H, Sayed Y, Bester M, Neitz A, Gaspar A. Structural and functional characterization of peptides derived from the carboxy-terminal region of a defensin from the tick *Ornithodoros savignyi*. J Pept Sci. 2013 May;19(5):325-32. doi: 10.1002/psc.2505. PMID: 23553969.

139. Malan M, Serem JC, Bester MJ, Neitz AWH, Gaspar ARM. Anti-inflammatory and anti-endotoxin properties of peptides derived from the carboxy-terminal region of a defensin from the tick *Ornithodoros savignyi*. J Pept Sci. 2016 Jan;22(1):43-51. doi: 10.1002/psc.2838. PMID: 26662999.

140. Wu J, Wang Y, Liu H, Yang H, Ma D, Li J, Li D, Lai R, Yu H. Two immunoregulatory peptides with antioxidant activity from tick salivary glands. J Biol Chem. 2010 May 28;285(22):16606-13. doi: 10.1074/jbc.M109.094615. PMID: 20178988; PMCID: PMC2878058.

141. Denisov SS, Ramírez-Escudero M, Heinzmann ACA, Ippel JH, Dawson PE, Koenen RR, Hackeng TM, Janssen BJC, Dijkgraaf I. Structural characterization of anti-CCL5 activity of the tick salivary protein evasin-4. J Biol Chem. 2020 Oct 16;295(42):14367-78. doi: 10.1074/jbc.RA120.013891. PMID: 32817341; PMCID: PMC7573271.

142. Déruaz M, Frauenschuh A, Alessandri AL, Dias JM, Coelho FM, Russo RC, Ferreira BR, Graham GJ, Shaw JP, Wells TNC, Teixeira MM, Power CA, Proudfoot AE. Ticks produce highly selective chemokine binding proteins with antiinflammatory activity. J Exp Med. 2008 Sep 1;205(9):2019-31. doi: 10.1084/jem.20072689. PMID: 18678732; PMCID: PMC2526197.

143. Tian Y, Chen W, Mo G, Chen R, Fang M, Yedid G, Yan X. An immunosuppressant peptide from the hard tick *Amblyomma variegatum*. Toxins (Basel). 2016 May 3;8(5):133. doi: 10.3390/toxins8050133.

144. Hwang L, Ko IG, Jin JJ, Kim SH, Kim CJ, Jeon JW, Han JH. *Scolopendra subspinipes mutilans* extract suppresses inflammatory and neuropathic pain in vitro and in vivo. Evid based Complement Alternat Med. 2018 Dec 17;2018: 5057372. doi: 10.1155/2018/5057372. PubMed PMID: 30647762.

145. Liu DY, Zhao HM, Cheng SM, Rao Y, Huang XY, Zuo ZQ, Lei M, Guan YM, Liu HN, Lu AP. Scorpio and *Scolopendra* attenuate inflammation and articular damage in rats with collagen-induced arthritis. J Ethnopharmacol. 2012 Jun 1;141(2):603–7. doi: 10.1016/j.jep.2011.08.056. PubMed PMID: 21911049.

146. Zhao H, Li Y, Wang Y, Zhang J, Ouyang X, Peng R, Yang J. Antitumor and immunostimulatory activity of a polysaccharide–protein complex from *Scolopendra subspinipes mutilans* L. Koch in tumor-bearing mice. Food Chem Toxicol. 2012 Aug;50(8):2648–55. doi: 10.1016/j.fct.2012.05.018. PubMed PMID: 22613217.

147. Park YJ, Park B, Lee M, Jeong YS, Lee HY, Sohn DH, Song JJ, Lee JH, Hwang JS, Bae YS. A novel antimicrobial peptide acting via formyl peptide receptor 2 shows therapeutic effects against rheumatoid arthritis. Sci Rep. 2018 Oct 2;8(1):14664. doi:10.1038/s41598-018-32963-5.

148. Singh N, Baby D, Rajguru JP, Patil PB, Thakkannavar SS, Pujari VB. Inflammation and cancer. Ann Afr Med. 2019 Jul-Sep;18(3):121-6. doi: 10.4103/aam.aam_56_18. PMID: 31417011; PMCID: PMC6704802.

149. Roy A, Jauhari N, Bharadvaja N. medicinal plants as a potential source of chemopreventive agents. In: Akhtar M, Swamy M, editors. Anticancer plants: natural products and biotechnological implements. Springer, Singapura;2018. p. 109–39. doi: 10.1007/978-981-10-8064-7_6.

150. Tomar V, Kumar N, Tomar R, Sood D, Dhiman N, Dass SK, Prakash S, Madan J, Chandra R. Biological evaluation of noscapine analogues as potent and microtubule-targeted anticancer agents. Sci Rep. 2019 Dec 20;9(1):19542. doi: 10.1038/s41598-019-55839-8.

151. Arbiser JL, Kau T, Konar M, Narra K, Ramchandran R, Summers SA, Vlahos CJ, Ye K, Perry BN, Matter W, Fischl A, Cook J, Silver PA, Bain J, Cohen P, Whitmire D, Furness S, Govindarajan B, Bowen JP. Solenopsin, the alkaloidal component of the fire ant (*Solenopsis invicta*), is a naturally occurring inhibitor of phosphatidylinositol-3-kinase signaling and angiogenesis. Blood. 2007 Jan 15;109(2):560-5. doi: 10.1182/blood-2006-06-029934. PMID: 16990598; PMCID: PMC1785094.

152. Al-Tamimi J, Semlali A, Hassan I, Ebaid H, Alhazza IM, Mehdi SH, Al-Khalifa M, Alanazi MS. Samsum ant venom exerts anticancer activity through immunomodulation in vitro and in vivo. Cancer Biother Radiopharm. 2018 Mar;33(2):65-73. doi: 10.1089/cbr.2017.2400. PMID: 29634416.

153. Sonoda Y, Hada N, Kaneda T, Suzuki T, Ohshio T, Takeda T, Kasahara T. A synthetic glycosphingolipid-induced antiproliferative effect in melanoma cells is associated with suppression of FAK, Akt, and Erk activation. Biol Pharm Bull. 2008 Jun;31(6):1279-83. doi: 10.1248/bpb.31.1279. PMID: 18520069.

154. Holle L, Song W, Holle E, Wei Y, Wagner T, Yu X. A matrix metalloproteinase 2 cleavable melittin/avidin conjugate specifically targets tumor cells in vitro and in vivo. Int J Oncol. 2003 Jan;22(1):93-8. doi: 10.3892/ijo.22.1.93 PMID: 12469190.

155. Moon DO, Park SY, Heo MS, Kim KC, Park C, Ko WS, Choi YH, Kim GY. Key regulators in bee venom-induced apoptosis are Bcl-2 and caspase-3 in human leukemic U937 cells through downregulation of ERK and Akt. Int Immunopharmacol. 2006 Dec 5;6(12):1796-1807. doi: 10.1016/j.intimp.2006.07.027. PMID: 17052670.

156. Arora AS, de Groen PC, Croall DE, Emori Y, Gores GJ. Hepatocellular carcinoma cells resist necrosis during anoxia by preventing phospholipase-mediated calpain activation. J Cell Physiol. 1996 Jun;167(3):434-42. doi: 10.1002/(SICI)1097-4652(199606)167:3<434::AID-JCP7>3.0.CO;2-Q. PMID: 8655597.

157. Wu YL, Jiang XR, Newland AC, Kelsey SM. Failure to activate cytosolic phospholipase A2 causes TNF resistance in human leukemic cells. J Immunol. 1998 Jun 15;160(12):5929-35. PMID: 9637506.

158. Putz T, Ramoner R, Gander H, Rahm A, Bartsch G, Thurnher M. Antitumor action and immune activation through cooperation of bee venom secretory phospholipase A2 and phosphatidylinositol-(3,4)-bisphosphate. Cancer Immunol Immunother. 2006 Nov;55(11):1374-83. doi: 10.1007/s00262-006-0143-9. PMID: 16485125.

159. Wolff H, Saukkonen K, Anttila S, Karjalainen A, Vainio H, Ristimäki A. Expression of cyclooxygenase-2 in human lung carcinoma. Cancer Res. 1998 Nov 15;58(22):4997-5001. PMID: 9823297.

160. Jang MH, Shin MC, Lim S, Han SM, Park HJ, Shin I, Lee JS, Kim KA, Kim EH, Kim CJ. Bee venom induces apoptosis and inhibits expression of cyclooxygenase-2 mRNA in human lung cancer cell line NCI-H1299. J Pharmacol Sci. 2003 Feb;91(2):95-104. doi: 10.1254/jphs.91.95. PMID: 12686753.

161. de Souza BM, da Silva AVR, Resende VMF, Arcuri HA, dos Santos Cabrera MP, Ruggiero Neto J, Palma MS. Characterization of two novel polyfunctional mastoparan peptides from the venom of the social wasp *Polybia paulista*. Peptides. 2009 Aug;30(8):1387-95. doi: 10.1016/j.peptides.2009.05.008. Epub 2009 May 20. PMID: 19463874.

162. Pal P, Roy S, Chattopadhyay S, Pal TK. Medicinal value of animal venom for treatment of cancer in humans—a review. World Sci News. 2015;22: 91-107.

163. Gao L, Shan BE, Chen J, Liu JH, Song DX, Zhu BC. Effects of spider *Macrothele raven* venom on cell proliferation and cytotoxicity in HeLa cells. Acta Pharmacol Sin. 2005 Mar;26(3):369-76. doi: 10.1111/j.1745-7254.2005.00052.x. PMID: 15715936.

164. Gao L, Yu S, Wu Y, Shan B. Effect of spider venom on cell apoptosis and necrosis rates in MCF-7 cells. DNA Cell Biol. 2007 Jul;26(7): 485-9. doi: 10.1089/dna.2007.0579. PMID: 17630852.

165. Lian W, Lian H, Li Q, Hu A, Liu S. The venom of spider *Haplopelma hainanum* suppresses proliferation and induces apoptosis in hepatic cancer cells by caspase activation in vitro. J Ethnopharmacol. 2018 Oct 28;225:169-177. doi: 10.1016/j.jep.2018.06.022. PMID: 29928971.

166. Li W, Li Y, Zhao Y, Yuan J, Mao W. Inhibition effects of scorpion venom extracts (*Buthus matensii* Karsch) on the growth of human breast cancer MCF-7 cells. Afr J Tradit Complement Altern Med. 2014 Aug 23;11(5):105-10. doi: 10.4314/ajtcam.v11i5.17. PMID: 25395713; PMCID: PMC4202526.

167. Li B, Lyu P, Xi X, Ge L, Mahadevappa R, Shaw C, Kwok HF. Triggering of cancer cell cycle arrest by a novel scorpion venom-derived peptide-gonearrestide. J Cell Mol Med. 2018 Sep;22(9):4460-73. doi: 10.1111/jcmm.13745. PMID: 29993185; PMCID: PMC6111814.

168. Mamelak AN, Rosenfeld S, Bucholz R, Raubitschek A, Nabors LB, Fiveash JB, Shen S, Khazaeli MB, Colcher D, Liu A, Osman M, Guthrie B, Schade-Bijur S, Hablitz DM, Alvarez VL, Gonda MA. Phase I single-dose study of intracavitary-administered iodine-131-TM-601 in adults with recurrent high-grade glioma. J Clin Oncol. 2006 Aug 1;24(22):3644-50. doi: 10.1200/JCO.2005.05.4569. PMID: 16877732.

169. Mamelak AN, Jacoby DB. Targeted delivery of antitumoral therapy to glioma and other malignancies with synthetic chlorotoxin (TM-601). Expert Opin Drug Deliv. 2007 Mar;4(2):175-86. doi: 10.1517/17425247.4.2.175. PMID: 17335414.

170. Tytgat J, Debont T, Rostoll K, Müller GJ, Verdonck F, Daenens P, van der Walt JJ, Possani LD. Purification and partial characterization of a 'short' insectotoxin-like peptide from the venom of the scorpion *Parabuthus schlechteri*. FEBS Lett. 1998 Dec 28;441(3):387-91. doi: 10.1016/s0014-5793(98)01589-0. PMID: 9891977.

171. El-Ghlban S, Kasai T, Shigehiro T, Yin HX, Sekhar S, Ida M, Sanchez A, Mizutani A. Chlorotoxin-Fc fusion inhibits release of MMP-2 from pancreatic cancer cells. Biomed Res Int. 2014;2014:152659. doi: 10.1155/2014/152659. Epub 2014 Jan 6. PMID: 24511528; PMCID: PMC3910484.

172. Jacoby DB, Dyskin E, Yalcin M, Kesavan K, Dahlberg W, Ratliff J, Johnson EW, Mousa SA. Potent pleiotropic anti-angiogenic effects of TM601, a synthetic chlorotoxin peptide. Anticancer Res. 2010 Jan;30(1):39-46. PMID: 20150615.

10.3 Artigo qualis A2 publicado durante o doutorado, como critério para obtenção de aprovação na disciplina "Peptídeos e proteínas antimicrobianos: aspectos estruturais e mecanismos de ação".

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Systematic review of antiprotozoal potential of antimicrobial peptides

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ABSTRACT

Protozoa is a group of microorganisms that cause neglected tropical diseases, such as malaria, Chagas disease, and Leishmaniasis. Due to the growing demand for new therapeutic agents, antimicrobial peptides (AMPs) have gained attention for antiprotozoal action. A systematic literature review described the current scenario of plant and animal AMPs with action antiprotozoal. The terms "antimicrobial peptides", "plant", and "animal" combined with the names of the etiological agents were used in the search. Boolean and Operator were used to connect the terms. The search found 4,825 articles. However, 79 articles were excluded because they were duplicates, and 4,627 were excluded based on title and abstract. Therefore, 119 were evaluated and included here. Of these, the use of antimicrobial peptides of animal origin was predominant. Still, the works with plant peptides focused on the genus Leishmania. Only antimicrobial peptides of animal origin were described for the other genera of protozoa (*Toxoplasma* spp, *Trypanosoma* spp, *Plasmodium* spp). Antimicrobial peptides are an excellent option as a pharmacological tool to fight these infections due to their aggregation and extravasation of cellular content through the formation of pores in the cell membrane of these microorganisms.

1. Introduction

Antimicrobial Peptides (AMPs) represent an essential defense mechanism for most living organisms, being active against several types of pathogens. They are part of the innate immunity of vertebrates and invertebrates (Ferreira et al., 2019). Among invertebrates, AMPs can be found in insects and marine animals. In vertebrate animals, such as fishes, mammals, and amphibians, AMPs have been isolated from a variety of cells and tissues, such as white blood cells, epithelial tissue of the oral cavity, lung, skin, and body fluids (Ferreira et al., 2019; Kumar et al., 2019). Plants do not have an immune system like animals. Therefore, AMPs play a fundamental role in protecting against infection by bacteria and fungi found in the leaves, flowers, seeds, and tubers (Kumar et al., 2019). AMPs also are produced by microorganisms (Browne et al., 2020).

Most natural AMPs are shorts (10 to 50 amino acids), have positive net charges (ranging from +2 to +11), and contain a significant portion of hydrophobic residues, being classified based on their secondary structures, corresponding to three categories: (1) α-helix (the most abundant among the AMPs), (2) β-sheet or (3) extended structure (Mahlapuu et al., 2020). Due to their biochemical features, the main action mechanism of AMPs involves damage to the cell membrane of bacteria, viruses, fungi, and protozoa (Browne et al., 2020; Ferreira et al., 2019). The increased demand for the development of new agents with antiparasitic activity, as well as the broad spectrum of antimicrobial activity and the possibility of selecting or modifying peptide sequences for specific biological pathways, have positioned AMPs as attractive compounds for the development of new drugs (Giovati et al., 2018a).

The urgency in the identification of new compounds with antiprotozoal activity is related to the growing increase of neglected tropical diseases, such as Chagas disease (caused by *Trypanosoma cruzi*), American Tegumentary and Visceral Leishmaniasis (caused by *Leishmania* spp.) and malaria (caused by *Plasmodium* spp.) (Ferreira et al., 2022; Moraes et al., 2019). Besides, there is little funding for the development of studies in this area because these diseases affect regions with low socioeconomic status. The treatment options for Chagas disease and Leishmaniasis are limited, increasing cases of resistance to available drugs (Parthasarathy and Kalesh, 2020). In the case of malaria, resistance has been reported due to the selection of parasites with genetic

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mutations or gene amplifications that confer reduced susceptibility to antimalarial drugs (Shibeshi et al., 2020). Another protozoan agent of medical importance is *Toxoplasma gondii*, the etiologic agent of Toxoplasmosis. Although the current therapies for the disease, such as sulfadiazine and pyrimethamine, are reasonably effective, studies show that drug resistance is ongoing, posing a concern for treatment failure and possible increased clinical severity in immunocompromised patients (Liu et al., 2019; Montazeri et al., 2018).

Due to the growing problem involving the treatment of infectious diseases caused by protozoa, studies are being conducted to identify new bioactive molecules and develop new drugs that can overcome the existing deficiencies. Based on that, this study aimed to conduct a systematic review of the literature to identify studies on AMPs of plant and animal origins with activity against medically important protozoa.

2. Methods

A systematic review was prepared following PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) (Page et al., 2021). The electronic databases PubMed and ScienceDirect were selected to search for published articles on antimicrobial peptides with antiprotozoal activity, considering Protozoa of medical importance.

The bibliographic study began in July 2021 and ended in August 2021, with no publication date limit. Publications were selected using the following terms: "Antimicrobial Peptides", in combination with "animal", "plant", "*Leishmania*", "*Trypanosoma cruzi*", "*Plasmodium*" and "*Toxoplasma gondii*". A set of articles related to plant and animal origin peptides were retrieved from the databases.

Only complete research articles *were selected to evaluate* the *in vitro, in vivo*, and *in silico* antiprotozoal effects of antimicrobial peptides derived from plants or animals. In addition, reviews containing clinical, epidemiological, or other reports related to the topic of this review were chosen. The criteria used for exclusion were: repeated articles, editorials, letters to the editor, theses, dissertations, reports, and articles not related to the topic of this review. The information collected in the literature contained the following information: authors, year of publication, study model, the antimicrobial peptide used, amino acid sequences, source, target protozoan species, and mechanism of action.

3. Results and discussion

After searching the PubMed and ScienceDirect databases, 4.825 published articles were found. After removing the duplicate manuscripts (79) and those whose titles and abstracts (4.627) or the content (16) were not related to this review, 103 full texts were selected for further

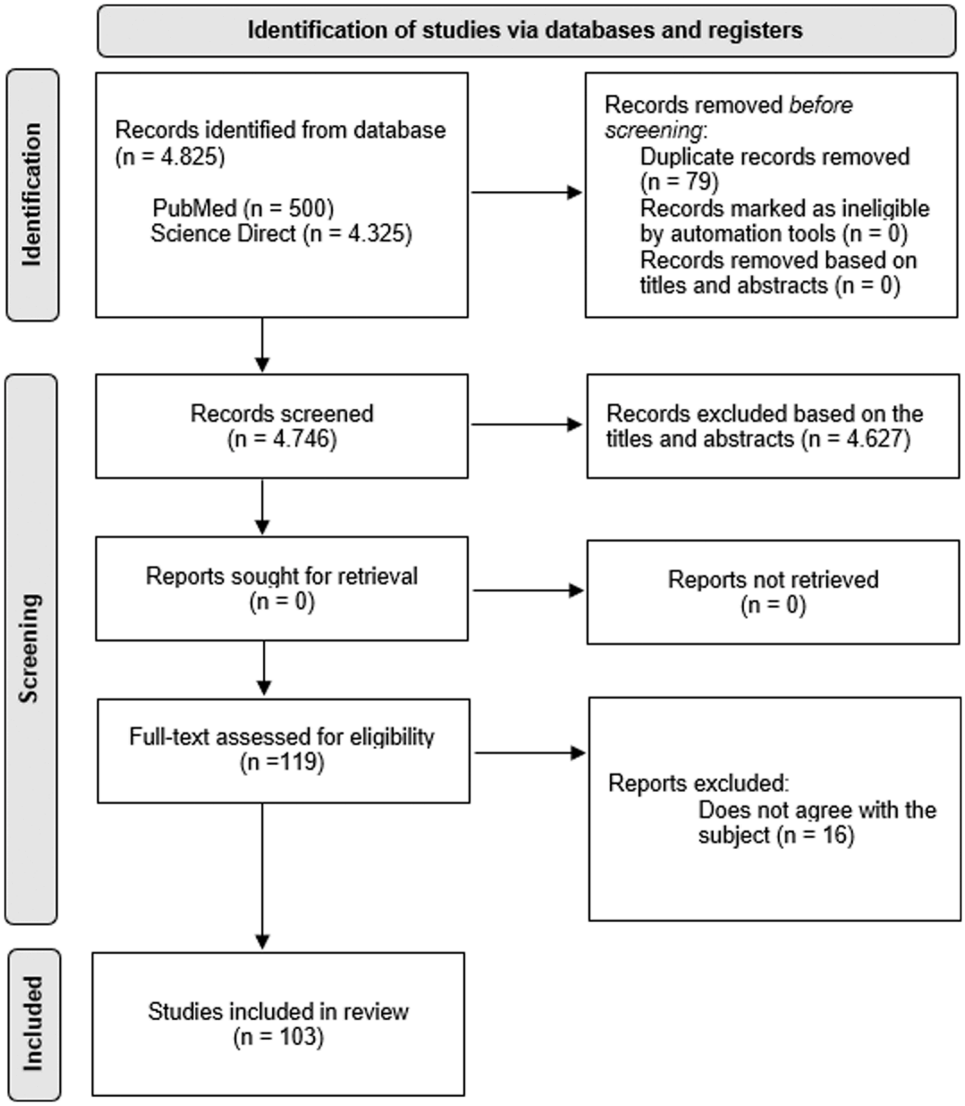

Fig. 1. Flowchart of studies considered for inclusion in this review.

analysis. The details of the selection process are illustrated in Fig. 1.

3.1. Leishmania spp

About 1 billion people live in endemic regions, subject to infection by the parasite *Leishmania* spp (World Health Organization, n.d.). The disease, named Leishmaniasis, can be manifested in three forms: tegumentary Leishmaniasis (TL), visceral Leishmaniasis (VL), and mucocutaneous Leishmaniasis (LCM). WHO data estimated that between 700 thousand to 1 million new cases are registered annually (World Health Organization, 2022). Therefore, these parasitoses represent an important zoonosis for public health services worldwide. In Brazil, for example, VL cases corresponded to 97% of the cases registered in the WHO Americas (World Health Organization, 2022), with the North and Northeast regions presenting the highest numbers of VL cases (Brasil, 2017). The drug market for the treatment of Leishmaniasis varies from US$ 4 million to 7.1 million per year (Choiid et al., 2021). The drugs involved in the treatment are commonly antimonials, amphotericin B, miltefosine, or paromomycin (Saha et al., 2021). Some studies have reported resistance to these treatments (Croft et al., 2006; Saha et al., 2021). Therefore, searching to find new molecules to address the problem has been considered, including the prospection of AMPs, which are the target molecules of this review. Several types of AMPs have shown leishmanicidal activity for different species, such as L. *amazonenses* (Nascimento et al., 2015; Souza et al., 2019a), L. *donovani* (Berrocal-Lobo et al., 2009; Paik et al., 2020), L. *major,* (Savoia et al., 2008), L. *mexicana,* and L. *tropica* (Campos-Salinas et al., 2013) L. *chagasi* (Zampa et al., 2009), L. *pifanoi* (Chicharro et al., 2001), L. *infantum* (Mendes et al., 2019), and L. *tarentolae* (Abdossamadi et al., 2017b).

The L. *amazonensis* causes skin ulcers, and eventually, those infected may manifest a classic form of Diffuse Cutaneous Leishmaniasis (DCL) (Brasil, 2017). L. *donovani* exclusively causes fatal systemic visceral leishmaniasis (Fernández-Arévalo et al., 2020). The species L. *mexicana* causes cutaneous and eventually diffuse lesions, while L. *chagasi* leads to the development of the visceral form. The species *L. major* L. *infantum, and* L. *tropica* cause the mucous forms (Shirian et al., 2013). L. *tarentolae* is considered non-pathogenic to humans (Klatt et al., 2019), while L. *pifanoi* is responsible for the tegumentary form. (Alves, 2009). The vector for this protozoan is *Lutzomyia flaviscutellata,* and animal reservoirs are rodents belonging to the genus *Proechymis* and *Oryzomys* (Brasil, 2017).

Sandflies transmit this parasite and their reservoirs can be humans and dogs (Fernández-Arévalo et al., 2020). The life cycle of the genus *Leishmania* is characterized by two main morphologies in its development, the amastigote, an intracellular stage found in mammalian hosts, and the promastigote found in the invertebrate host (Wheeler et al., 2011). The infection process is initiated by the bite of the sandfly infected with metacyclic promastigotes. When accessing the bloodstream of vertebrates, promastigotes are phagocytosed by defense cells, the macrophages. Inside macrophages, the promastigote forms transform into amastigotes, a tissue stage observed in vertebrates. Amastigotes undergo an intense process of cell division, leading to the lysis of the infected cells. The release of the amastigote forms allows a new process of infection and circulation of these forms in the bloodstream (Fig. 2). During the blood meal, sandflies absorb the amastigote forms initiating the process of migration to the midgut to assume the promastigote form reaching proboscis (Fig. 2) (Wheeler et al., 2011).

In general, it is understood that vector surveillance and control are important strategies in the control of this parasitosis, as well as the development of new chemical formulations, as suggested by the Pan-American Health Organization for the elimination of neglected infectious diseases for the period 2016–2022 (OPAS, 2016). To improve this issue, a set of investigations has been carried out to offer alternative forms of intervention to overcome this problem. Thus, the efforts using *in vitro, in vivo,* and *silico* tests verified the action of several AMPs on different *Leishmania* species (Table 1). The first work on the subject data from 1998 (Díaz-Achirica et al., 1998), whose focus was the analysis of damage caused by peptides on *Leishmania* promastigote membranes. Since then, around 79 surveys have been developed, testing different peptides from different biological sources, including amphibians (Mangoni et al., 2005), tunicate (Donia et al., 2008), and plants (Nascimento et al., 2015). In addition, these peptides have been inspired to improve their leishmanicidal activities by creating analogs (Campos-Salinas et al., 2014) or coupling biomolecules such as lauric acid (Zahedifard et al., 2020).

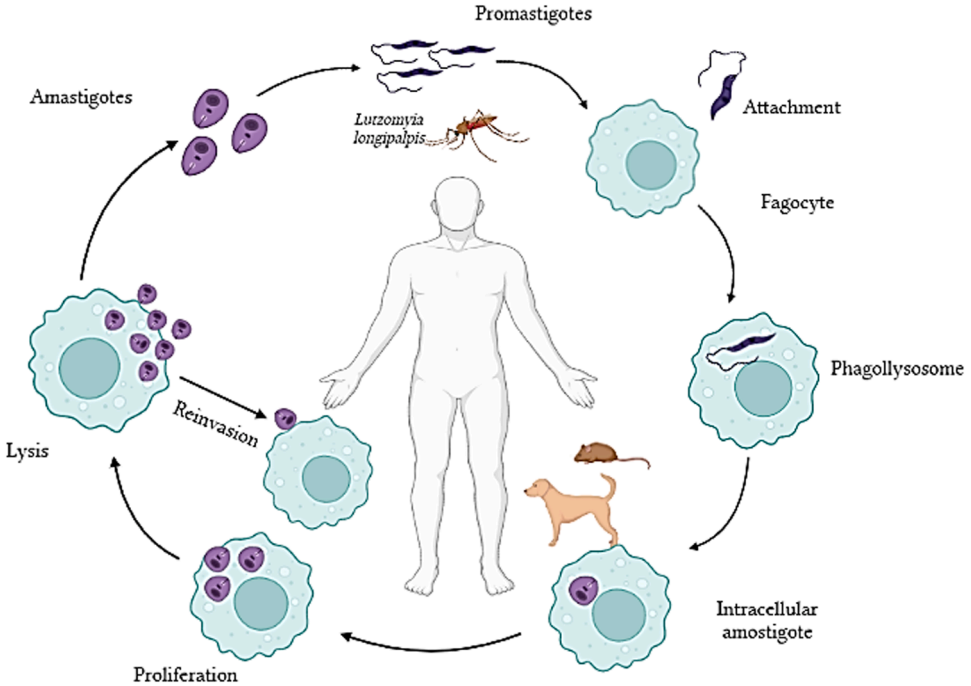

Fig. 2. Simplified diagram of the *Leishmania* infection cycle with man as the reservoir.

Table 1
Studies involving AMPs against Leishmania species.

Selected Articles	Peptide	Source	Sequence	Study Model	IC50 (μM)	
(Pinto et al., 2013)	Dermaseptin 4	*Phyllomedusa*	GLWSTIKQKGKEAAIAAAKAAGKAALNAASEAL-NH2	*In vitro*	*n.a*	
	Dermaseptin 1	*nordestina*	GLWSTIKNVGKEAAIAAGKAALGAL-NH2		*n.a*	
	Phylloseptin 7		FLSLIPHAINAVSAIAKHF-NH2		*10.06 (7.86–12.85)*	
	Phylloseptin 8		FLSLIPTAINAVSALAKHF-NH2		*n.a*	
(Berrocal-Lobo et al., 2009)	Tioninas a-1, a-2	*Triticum*		*In vitro*	*0,2 (0,1-0,5)*	
	LTP2	*aestivum*	******		*+ 100*	
	Defensina PTH1	*Hordeum*			*33,4 (21,5-52,4)*	
	Snakin1	*vulgare*			*+ 100*	
		Solanum				
		tuberosum				
(Bittencourt et al., 2016)	DRS 01	*Phyllomedusa*	GLWSTIKQKGKEAAIAAA-KAAGQAALGAL-NH2	*In vitro*	***	
(Löfgren et al., 2008b)	Tach	*Tachypleus tridentatus*	KWCFRVCYRGICYRRC	*In vitro*	***	
	MSI-94	*Xenopus laevis*	GIGKFLKKAKKFGKAFVKMKK			
	Clavanin-A	*Styela clava*	VFQFLGKIIHHVGNFVHGFSHVF			
	Mytilin-A	*Mytilus edulis*	CASRCKAKCAGRRCKGWASASFRGRCYCKCFR			
(Souza et al., 2013)	Vu-Defr	*Vigna unguiculata*	******	*In vitro*	***	
(Kückelhaus et al., 2009)	PS-1	*Phyllomedusa azurea*	******	*In vitro*	***	
(Zampa et al., 2009)	DS01	*Phyllomedusa hypochondrialis*	******	*In vitro*	***	
(Isabel A. Patiño-Márquez et al., 2018)	Moricina-B	*Galleria*	MSIILVVVMMVMMVMAMFVSSGDAAPGKIPVKAIKKGGQIIGKALRGINIASTAHDIISQFKPKKKK	*In vitro*	***	
	Moricina-C4	*mellonella*	MKLTGLFLMIMAVIALFIDVGQADPKVPVGAIKKGGKAIKTGLGVVGAAGTAHEVYSHIRNRH			
	Cecropin-D		ENFFKEIERAGQRIRDAIISAAPAVETLAQAQKIIKGGD			
	Peptíde		ETESTPDYLKNIQQQLEEYTKNFNTQVQNAFDSDKIKSE			
	Aniônico2		VNNFIESLGKILNTEKKEAPK			
(Mendes et al., 2019)	p-Acl	*Agkistrodon contortrix*	KKYKAYFKFKCKK	*In vitro*	***	
	p-AclR7	laticinctus	RRYRAYFRFRCRR			
(Savoia et al., 2008)	DS1	*Phyllomedusinae*	ALWKTMLKKLGTMALHAGKAALGAAADTISQGTQ	*In vitro*	***	
	DS1(1–29)-NH2		ALWKTMLKKLGTMALHAGKAALGAAADTI-NH2			
(Konno et al., 2007)	Decoralin	*Oreumenes*	SLLSLIRKLIT	*In vitro*	*72*	
	Decoralin - NH2	*decoratus*	SLLSLIRKLIT-NH_2		*11*	
(Rangel et al., 2011)	Eumenitin-R	*Eumenes rubrofemoratus*	LNLKGLIKKVASLLN	*In vitro*	*>62*	
	Eumenitina-F	*E. fraterculus*	LNLKGLFKKVASLLT		*52*	
	EMP-ER		FDIMGLIKKVAGAL-NH2		*20*	
	EMP-EF		FDVMGIIKKIAGAL-NH2		*40*	
(Isabel Andrea Patiño-Márquez et al., 2018)	Anionic Peptide2	*Galleria*	TKNFNTQVQNAFDSDKIKSEVNNFIESLGKILNTEKKEAPK	*In vitro*	***	
	Cecropin D-like peptide	*mellonella*	ENFFKEIERAGQRIRDAIISAAPAVETLAQAQKIIKGGD			
(Pitale et al., 2020)	Halictine-2	*Halictus*	P5S GKWMSLLKHILK-NH2	*In vitro*	***	
		sexcinctus	P5T GKWMTLLKHILK-NH2			
(Lynn et al., 2011)	BMAP-28	Bovine	GGLRSLGRKILRAWKKYGPIIVPIIRIG	*In vitro*	*17,1,*	
(Iorns et al., 2014)	D-BMAP-28	myeloid	******		*4,6*	
	RI-BMAP-28		******		*3,6*	
(Oliveira et al., 2016)					*Promastigote*	*Amastigote*
	Ocellatin-PT1	*Leptodactylus pustulatus*	******	*In vitro*	*63.4/23.9*	*n.a*
	Ocellatin-PT2				*≫128/≫*	*n.a*
	Ocellatin-PT3				*49.1*	*n.a*
	Ocellatin-PT4				*34/13.4*	*75/28.9*
	Ocellatin-PT5				*25.6/9.8*	*n.a*
	Ocellatin-PT6				*62.7/25.5*	*67.519.8*
	Ocellatin-PT7				*42.6/12.7*	*n.a*
	Ocellatin-PT8				*42.2/72.8*	*n.a*
					51.6/15.5	
(Cao et al., 2019)	KDEL	*Pseudomonas aeruginosa*	******	*In vitro*	***	

(*continued on next page*)

Table 1 (*continued*)

Selected Articles	Peptide	Source	Sequence	Study Model	IC50 (μM)
(Guerra et al., 2017)	BH2				***
	PH1	******	******	*In silico*	
	TA				
	TB				
	DCN				
	DEC				
(Erfe et al., 2012)	RP-1	α-helical quinocidine C-terminals	N-ALYKKFKKKLLKSLKRLG	*In vitro*	***
	AA-RP-1	CXCL4	******	*In vivo*	
(Marr et al., 2016)	LL-37	Synthetic	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES-NH2	*In vitro*	***
	E6		RRWRIVVIRVRR-NH2		
	L-1018		VRLIVAVRIWRR-NH2		
	RI-1018		RRWIRVAVILRV-NH2		
(Khalili et al., 2018)	CM11	Cecropine-Mellitin Hybrid	******	*In vitro*	*9.58*
(Zahedifard et al., 2020)	Jellein	Honey bee royal jelly	PFKISIHL	*In vitro*	400
	Lauric acid – L-Jellein		PFKISIHL acid – CLIP		n.a
	CLIP		LPKPPKPVSKMRMATPLLMQALPM		n.a
	Lauric acid – CLIP (L-CLIP)		Lauric acid - LPKPPKPVSKMRMATPLLMQALPM		280
(Bera et al., 2003)	IL	indolicidin	ILPWKWPWWPWRR	*In vitro*	***
	SPFK	Seminalplasmin Derivatives	PKLLKTFLSKWK		
	27RP		SLSRYAK LANRLANPKLLETFLSKWIG		
(Kulkarni et al., 2011)	mCAMP	Synthetic	NH2-LLRKGGEKIGEKLKKIGQKIKNFFQKLVPQPEQCOOH	*In vitro* *In vivo*	***
(Pérez-Cordero et al., 2011)	Andropin	Synthetic	VFIDILDKMENAIHKAAQAGIG	*In vitro*	***
	Cecropin A		KWKLFKKIEKVGQNIRDGIIKAGPAVAWVGQATQIAK		
	Cecropin B		KWKVFKKIEKNGRNIRNGIVKAGPAIAVLGEAKAL		
	Cecropin P1		SWLSKTAKKLENSAKKRISEGIAIAQGPR		
	Dermaseptin		ALWKTMLKKLGTMALHAGKAALGAAADTISQGTQ		
	Melittin		GIGAVLTTGLPALISWIKRKRQQ		
	Tachyplesin		KWCFRVCYRGICYRRCR		
	Pr-1		PRRRRSSSRP		
	Pr-2		VRRRRRPR		
	Pr-3		VSRRRRRRGGRRRR		
	CLIP		LPKPPKPVSKMRMATPLLMQALPM		
(Mangoni et al., 2006)	Bombinins H2 Bombinins H4	*Bombina Variegata*	IIGPVLGLVGSALGGLLKKI-NH2 ******	*In vitro*	***
(Téné et al., 2016)	Bicarinalina	*Tetramorium*	KIKIPWGKVKDFLVGGMKAV-NH2	*In vitro*	***
	Bicarinalin (4-20)	*bicarinatu*	VVMKLGKAFVPIGKWKKDGI-NH2 IPWGKVKDFLVGGMKAV-NH2		
(Costa et al., 2020)	RP1	Synthetic	ALYKKFKKKLLKSLKRLG-COOH	*In vitro*	*1,25*
	Fc- RP1		ALYKKFKKKLLKSLKRLG-COOH+ ferrocene carboxylic acid		*0.25*
(Keller et al., 2014)	MPGa		Ac-GALFLAFLAAALSLMGLWSQPKKKRKV-NH-CH2-CH2-SH	*In vitro*	***
	MPGb	******	Ac-GALFLGFLGAAGSTMGAWSQPKKKRKV-NH-CH2-CH2-SH		
	CAD-2 penetratin,		GLWRALWRLLRSLWRLLWKA-NH-CH2-CH2-SH		
	HIV-Tat (47–57),		RQIKIWFQNRRMKWKK		
	CPPP-2		YGRKKRRQRRR-amide KLPVM		
(Mendes et al., 2020)	CZS-1	*Cruziohyla*	GFLDIVKGVGKVALGAVSKLF-NH2,	*In vitro*	***
	CZS-2		GFLDVIKHVGKAALGVVTHLINQ-NH2,		
	CZS-3		GFLDVVKHIGKAALGAVTHLINQ-NH2		
(Alberola et al., 2004)	Cecropina A-melitina AP Oct-CA (1-7) M (2-9)	Synthetic	******	*In vivo*	***

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Table 1 (*continued*)

Selected Articles	Peptide	Source	Sequence	Study Model	IC50 (μM)
(Mangoni et al., 2005)	Temporinas A Temporinas B	*Rana temporaria*	FLPLIGRVLSGIL-NH2 LLPIVGNLLKSLL-NH2	*In vitro*	***
(Nascimento et al., 2015)	PvD1	*Phaseolus vulgaris*	******	*In vitro*	***
(Mijiddorj et al., 2018)	Bombinin H4	*Bombina variegata*	I-(D-allo-Ile)-GPVLGLVGSALGGLLKI- NH2	*In vitro* *In silico*	***
(Raja et al., 2013)	PLS-S1 PLS-S2 PLS-S3 PLS-S4 PLS-S5	*Phyllomedusa sauvagii*	FLSLIPHIVSGVASIAKHF FLSLIPHIVSGVASLAKHF FLSLIPHIVSGVASLAIHF FLSMIPHIVSGVAALAKHL LLGMIPVAISAISALSKL	*In vitro* *In silico*	*16,5; 12,6; 15.3* *18,5; 13,3; 15,0;* *NA* *22.0; 18.0; 17.2* *NA*
(Fern Andez-Reyes et al., 2010)	Cecropin A-melittin CA(1-7)M(2-9 K_1 (Me_3) K_3 (Me_3) K_6 (Me_3) K_7 (Me_3) K_{13} (Me_3) $K_{1,3}$ $(Me_3)_2$ $K_{3,6}$ $(Me_3)_2$ $K_{6,7}$ $(Me_3)_2$ $K_{1,13}$ $(Me_3)_2$ $K_{1,3,6,7,13}$ $(Me_3)_5$	Synthetic	KWKLFKKIGAVLKVL-amida KWKLFKKIGAVLKVL KWKLFKKIGAVLKVL KWKLFKKIGAVLKVL KWKLFKKIGAVLKVL KWKLFKKIGAVLKVL KWKLFKKIGAVLKVL KWKLFKKIGAVLKVL KWKLFKKIGAVLKVL KWKLFKKIGAVLKVL KWKLFKKIGAVLKVL KWKLFKKIGAVLKVL	*In vitro*	1.8 (±0.0) 3.9 (±0.3) 3.3 (±0.0) 3.7 (±0.0) 3.7 (±0.2) 3.5 (±0.1) 12.8 (±0.4) 5.1 (±0.2) 6.1 (±0.1) 8.8 (±0.3) >50
(khalili et al., 2019)	Híbrido CM11	Synthetic	WKLFKKILKVL-NH2	*In vitro*	*6,92* *9,015*
(Kückelhaus et al., 2020)	PSN-1	*Phyllomedusa azurea = (Pithecopus azureus)*	FLSLIPHAINAVSAIAKHN-NH2	*In vitro*	***
(Kulkarni et al., 2009)	Defensin Magainin Cathelicidin	******	******	*In vitro*	***
(Radzishevsky et al., 2005)	Magainina MSI-78 Catelicidina LL37	Synthetic	ALWKTLLKKVLKA$_{CONH2}$ GIGKFLKKAKKFGKAFVKILKK$_{CONH2}$ LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES	*In vitro*	***
(Eaton et al., 2014)	DRS 01	*Phyllomedusa oreades* P. ypochondrialis	******	*In vitro*	*10,8*
(Silva et al., 2000)	Gomesina	*Acanthoscurria gomesiana*	ZCRRLCYKQRCVTYCRGR)	*In vitro*	***
(Crauwels et al., 2019)	CAMP	Human	******	*In vitro*	***
(Kulkarni et al., 2014)	Lys-Pex: Arg-Pex	Synthetic	GIGKPLKKALLPGAKPVKILKK GIGRPLRRARRPGARPVRILRR	*In vitro*	***
(Kückelhaus et al., 2009)	PS-1	*Phyllomedusa azurea*	FLSLIPHAINAVSAIAKHN-NH2	*In vitro*	***
(Souza et al., 2018)	Vu-Defr	*Vigna unguiculata*	MKTCENLADTYRGPCFTTGSCDDHCKNKEHLLSGRCRDDVRCWCTRN	*In vitro*	***
(Allane et al., 2018)	Disintegrin_Cc	*Cerastes cerastes*	NSAHPCCDPVTCKP	*In vitro*	***
(Yang et al., 2019)	DS1 Cry3Aa-DS1	******	******	*In vitro* *In vivo*	*>20* *0,67*

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Table 1 (*continued*)

Selected Articles	Peptide	Source	Sequence	Study Model	IC50 (μM)	
(Brand et al., 2006)	Dshypo 01	*Phyllomedusa hypochondrialis*	GLWSTIKNVGEAAIAAGKAALGAL-NH_2	*In vitro*	***	
	Dshypo 02		GLWKSLLKNVGVAAGKAALNAVTDMVNQ			
	Dshypo 03		ALWKDVLKKIGTVALHAGKAAFGAAADTISQGGS			
	Dshypo 04		GLWSTIKQKGKEAAIAAAKAAGKAVLNAASEAL- NH_2			
	Dshypo 06		GLWSTIKQKGKEAAIAAAKAAGQAVLNSASEAL- NH_2			
	Dshypo 07		GLWSTIKQKGKEAAIAAAKAAGQAALNAASEAL- NH_2			
(André et al., 2020)	Temporin-SHd	*Pelophylax saharicus*	FLPAALAGIGGILGKLFamide	*In vitro*	*4.6; 16.5*	
	Temporin-She		SHe FLP-ALAGIAGLLGKIFamide		*10.5; 17.9*	
					11.6; 14.6	
(Dabirian et al., 2013)	HNP-1	Human neutrophil	******	*In vitro*	***	
(Zahedifard et al., 2019)	L- Brevinin	Syntetic	lauric acid- KLKNFAKGVAQSLLNKASCKLSGQC	*In vitro*	*40 a 50*	
	2R lauric acid-			*In vivo*		
(Campos-Salinas et al., 2013)	UCNI	Syntetic	******	*In vitro*	***	
	UCNII					
(Raja et al., 2017)	Temporin-SHa	Syntetic	$FLSGIVGMLGKLF_{NH2}$	*In vitro*	18	10
	Temporin-[K^3]SHa		$FLKGIVGMLGKLF_{NH2}$		14	9
					13	10
					7	5
					13	8
					20	20
					9	5
(Campos-Salinas et al., 2014)	VIP	Intestinal peptide	HSDAVFTDNYTRLRKQMAVKKYLNSILN	*In vitro*	***	
	VIP51		HSDAVFTANYTRLRRQLAVRRYLAAILGRR	*In vivo*		
	$VIP51_{(6–30)}$		FTANYTRLRRQLAVRRYLAAILGRR			
(Luque-Ortega et al., 2008)	L-Hst5	Human salivary	DSHAKRHHGYKRKFHEKHHSHRGY	*In vitro*	***	
(Borges et al., 2006)	******	*Tityus discrepans*	******	*In vitro*	***	
(Silva et al., 2012)	LFcin17–30	Bovine lactoferrin	FKCRRWQWRMKKLG	*In vitro*	21.9 ± 1.1(4)	>50 (3)
	LFampin 265-284		DLIWKLLSKAQEKFGKNKSR		30.9 ± 1.0(4)	>50 (3)
	LFchimera		FKCRRWQWRMKKLG-K-RSKNKGFKEQAKSLLKWILD		3.7 ± 0.2 (7)	6.3 ± 0.2 (5)
	LFchimera-R		DLIWKLLSKAQEKFGKNKSR-K-GLKKMRWQWRRCKF		3.5 ± 0.1 (2)	4.5 ± 0.2 (2)
	Di-LFcin		FKCRRWQWRMKKLG-K-GLKKMRWQWRRCKF		2.4 ± 0.1 (2)	3.8 ± 0.4 (2)
	Di-LFampin		DLIWKLLSKAQEKFGKNKSR-K-RSKNKGFKEQAKSLLKWILD		4.6 ± 0.1 (2)	5.4 ± 0.5 (2)
	LFcin–LFampin		FKCRRWQWRMKKLG-DLIWKLLSKAQEKFGKNKSR		4.1 ± 0.1 (2)	5.4 ± 0.3 (2)
	LFampin–LFcin		DLIWLLSKAQEKFGKNKSR-FKCRRWQWRMKKLG		1.7 ± 0.1 (2)	1.6 ± 0.2 (2)
(Fragiadaki et al., 2018)	Antiamoebin I	Syntetic	Ac-Phe-Aib-Aib-Aib-A-b-D-Iva-Gly-Leu-Aib-Aib-Hyp-Gln-D-Iva-Hyp-Aip-Pro-Pheol	*In vitro*	7.5 ± 0.2	8.7 ± 0.1
	Suzukacilin-A4		Ac-Aib-Ala-Aib-Ala-Aib-Alana- Gln-Ainb-Lxx-Aib-Gly-Aib-Aib-Pro-Vxx-Aib-Vxx-Gln-Gln-Pheol		7.6 ± 1.2	8.2 ± 0.1
(Haines et al., 2009)	BMAP-18	Bovine Myeloid	GRFKRFKKFKKLFKKLS	*In vitro*	***	
(Guerrero et al., 2004)	MG-H1	*Xenopus laevis*	GIKKFLHIIWKFIKAFVGEIMNS IIKKFLHSIWKFGKAFVGEIMNI	*In vitro*	***	
	MG-H2					
(Abbassi et al., 2013)	Temporin-SHd	Synthetic	$FLPAALAGIGGILGKLF_{amide}$	*In vitro*	16,5 -23,5	
(Philippe et al., 2004)	Fragmentos B, D, E, P e Q	*Mytilus galloprovinciali*	******	*In vitro*	***	
(Abdossamadi et al., 2017b)	HNP1	Mammalian	******	*In vitro*	***	
(Das et al., 2017)	LL 37	Mammalian	******	*In vitro*	***	
(Abdossamadi et al., 2017a)	HNP1	Human neutrophil	******	*In vivo*	*27*	

(*continued on next page*)

Table 1 (*continued*)

Selected Articles	Peptide	Source	Sequence	Study Model	IC50 (μM)
(Abengózar et al., 2017)	AS-48	*Enterococcus faecalis*	******	*In vitro*	*3,9 ± 1,1* *10,2 ± 1,2*
(Abbassi et al., 2008)	Temporin-1Sa Temporin - 1Sb Temporin -1Sc	*Pelophylax (Rana) saharica*	FLSGIVGMLGKLFamide FLPIVTNLLSGLLamide FLSHIAGFLSNLFamide	*In vitro*	18,1 22,8 n.a
(Boumaiza et al., 2015)	Hepcidin Met-HepcD Hepcidin [A11,A19]	Syntetic	 MDTHFPICVFCCGCCHKSKCGMCCKT DTHFPICVFCAGCCHKSKAGMCCKT	*In vitro*	***
(Sardar et al., 2013)	Spinigerin	*Pseudacanthotermes spiniger*	HVDKKVADKVLLLKQLRIMRLLTRL	*In vitro*	*150 μM*
(Mirzaei et al., 2019)	LLO	******	******	*In vitro*	*1.72 ±0.07*
(Boulanger et al., 2004)	Defensin	*Phlebotomus duboscqi*	ATCDLLSAFGVGHAACAAHCIGHGYRGGYCNSKAVCTCRR	*In vitro*	*68-85*
(Souza et al., 2019b)	Vu –Def-γ	*Vigna unguiculata*	LSGRARDDVRAWATR	*In vitro*	***
(Donia et al., 2008)	Mollamides B (1)	*Didemnum molle*	******	*In vitro*	18
(Gaidukov et al., 2003)	S1 S4 K4K20-S4 K4-S4(1-16)a K4-S4(1-13)a K4-S4(1-10)a	Syntetic	ALWKTMLKKLGTMALHAGKAALGAAADTISQGTQ ALWMTLLKKVLKAAAKAALNAVLVGANA ALWKTLLKKVLKAAAKAALKAVLVGANA ALWKTLLKKVLKAAAK-NH_2 ALWKTLLKKVLKA-NH_2 ALWKTLLKKV-NH_2	*In vitro*	***
(Kustanovich et al., 2002)	S4 S4(9–28) S4(5–16) K4K20-S4 K4-S4(1–16)a K4-S4(1–13)a K4-S4(1–10)a	Syntetic	ALWMTLLKKVLKAAAKAALNAVLVGANA KVLKAAAKAALNAVLVGANA TLLKKVLKAAAK ALWKTLLKKVLKAAAKAALKAVLVGANA ALWK TLLKKVLKAAAK-NH_2 ALWKTLLKKVLKA-NH_2 ALWKTLLKKV-NH_2	*In vitro*	***
(Díaz-Achirica et al., 1998)	Cecropina A-melitina (CA (1-8)M(1-18)	Syntetic	KWKLFKKIGIGAVLKVLTTGLPALIS-NH2	*In vitro*	***
(Chicharro et al., 2001)	CA(1-7)M(2-9)	Syntetic	KWKLFKKIGAVLKVL-NH2	*In vitro*	***

*** absent, n.a., not active

3.2. Trypanosoma cruzi

Chagas disease is a chronic condition caused by the protozoan *T. cruzi*; being considered a neglected tropical disease and endemic mainly in Latin America. However, this disease has been observed in non-endemic countries due to human migrations from endemic to non-endemic areas, making the disease a global public health problem (Lidani et al., 2019).

An acute phase with high parasitemia characterizes the disease. The patients are often asymptomatic or with symptoms such as prolonged fever, headache, myalgia, lymphadenitis, hepatomegaly, and splenomegaly. In the chronic phase, which occurs between 10 and 30 years after infection, 30 to 40% of asymptomatic patients develop clinical manifestations, including neurological, digestive, and cardiac manifestations (Lidani et al., 2019).

T. cruzi can assume three different morphological forms throughout its life cycle: (1) the epimastigote form, which is present in the insect vector; (2) metacyclic trypomastigotes, which originates after the differentiation of epimastigotes into trypomastigotes (in the final portion of the intestine of the triatomine) and that infects the bloodstream of the human host; and (3) amastigotes, which are intracellular proliferative forms that differentiate again into trypomastigotes, with the ability to disrupt host cells and continue infecting new cells (Mello et al., 2017). In the absence of adequate drug treatment, the infection persists throughout the life of the mammalian host. In addition to transmission via vectors, it can occur orally, through the ingestion of contaminated pulp and fruit juices, blood transfusion, organ transplantation, and congenital transmission (Bern et al., 2020).

The drugs currently available for the treatment of Chagas disease, nifurtimox, and benznidazole, are effective in the acute phase but do not eradicate the intracellular form of the parasites in the chronic phase (Mello et al., 2017). Benznidazole has been a classic drug against chronic Chagas disease for decades. Still, some issues can make treatment difficult, such as its prolonged duration, frequent side effects, and the need for high concentration gradients (Pedron et al., 2020). Considering these questions, different studies bring the development of new compounds, such as peptides with antiprotozoal action.

Our searches in the databases did not detect studies involving the analysis of AMPs of plant origin with activity against *T. cruzi*. However, many studies were carried out using AMPs of animal origin. All selected articles and information about the studied peptides are listed in Table 2.

The first condition of interest for a peptide to have activity against *T. cruzi* is related to its physicochemical characteristics. The literature indicates that most of the peptides active against this parasite are peptides with an α-helical amphipathic structure, consisting mainly of positively charged amino acid residues (Pinto et al., 2013). The selectivity of AMPs for these pathogens is related to their cationic, which is an important feature since it is the initial electrostatic interaction between the peptide and the phospholipids present in the membranes of microorganisms (Pedron et al., 2020). Thus, they have a greater potential against *T. cruzi*, mainly because long-range electrostatic attraction forces guide the peptides towards the parasite membrane, which has negative charges (Löfgren et al., 2008a; Pinto et al., 2013).

Positively charged peptides are also responsible for the ability to discriminate between mammalian and protozoa cells, in addition to conferring a differentiated antimicrobial potential when considering the different forms of the parasite (Brand et al., 2002). Some of the tested peptides were more potent against the trypomastigote form (Jacobs et al., 2003). Trypomastigote form has a more negative surface charge than amastigote and epimastigote forms, thus demonstrating excellent selectivity and more potent peptide activity (Löfgren et al., 2008a). For example, Polybia-CP peptides showed greater selectivity for infected mammalian cells (Freire et al., 2020). The mechanism of action involved in selectivity has not been identified. However, it is believed that the parasitic infection modifies the host cell membrane, so the host membrane becomes more negative, promoting the peptides' better attraction (Jacobs et al., 2003).

Finally, the studies cite the tested peptides as a promising therapeutic alternative to benznidazole due to the ability to reduce the number of intracellular amastigotes in a short incubation period, with peptide concentration not showing toxicity after *in vitro* tests. Thus, there could be a solution to the existing gap due to the limitation of conventional treatment in the chronic phase of the disease (Freire et al., 2020).

3.3. Plasmodium spp

Plasmodium is a protozoan parasite that causes malaria, which has infected over 3 billion people in 90 countries, causing more than 400,000 deaths annually, despite several decades of efforts to control the disease (Murray et al., 2014). Among the five species of *Plasmodium, P. falciparum* is the most lethal and responsible for the severe form of the disease, especially in sub-Saharan Africa (Meibalan and Marti, 2017). *P. vivax* typically causes milder infections than *P. falciparum* infection; however, its geographic distribution is much more comprehensive (Gething et al., 2012).

The infectious process begins when a mosquito bite releases the protozoan in the form of sporozoites into the bloodstream of the vertebrate host. The sporozoites then infect hepatocytes, where they proliferate and develop into merozoites. Merozoites are released into the bloodstream and invade erythrocytes. The parasites develop in the form of a ring and later develop into proliferative (and morphologically

Table 2
Studies involving antimicrobial peptides against *Trypanosoma cruzi*.

Selected Articles	Peptide	Source	Sequence	Study Model	IC_{50}μg/ml.
(Jacobs et al., 2003)	NK-2	Synthetic peptide	KILRGVCKKIMRTFLRRISKDILTGKK	*In vitro*	***
(Souza et al., 2016)	Temporizin-1	Synthetic peptide	FLPLWLWLWLWLWKLK	*In vitro*	***
(Kleschenko et al., 2010)	Defensin-α1	Human	ACYCRIPACIAGERRYGTCIYQGRLWAFCC	*In vitro*	
(Pinto et al., 2013)	Phylloseptin 7	*Phyllomedusa nordestina* (Frog)	FLSLIPHAINAVSAIAKHF	*In vitro*	0.34
(Brand et al., 2002)	DS 01	*Phyllomedusa oreades* (Frog)	GLWSTIKQKGKEAAIAAAKAAGQAALGAL	*In vitro*	***
(Adade et al., 2013)	Melittin	*Apis mellifera* (Bee)	–	*In vitro*	*2.44 ± 0.23*
(Freire et al., 2020)	Polybia-CP	*Polybia paulista* (Wasp)	ILGTILGLLSKL	*In vitro*	***
(Monteiro et al., 2020)	Hmc 364–382	*Penaeus monodon* (Shrimp)	NVQYYGALHNTAHIVLGRQ	*In vitro*	4,79
(Löfgren et al., 2008a)	Tachyplesin-I	*Tachypleus tridentatus* (Crab)	KWCFRVCYRGICYRRC	*In vitro*	***
(Mello et al., 2017)	Batroxicidin	*Bothrops atrox* (Snake)	KRFKKFFKKLKNSVKKRVKKFFRKPRVIGVTFPF	*In vitro*	0,44μM
(Izabel Cristina Justino Bandeira et al., 2017)	Crotalicidin	*Crotalus durissus terrificus* (Snake)	KRFKKFFKKVKKSVKKRLKKIFKKPMVIGVTIPF	*In vitro*	4,47 (±0,9)
(Pedron et al., 2020)	$[Arg]^{11}$-VmCT1	*Vaejovis mexicanus* (Scorpion)	PLGALTAVALAVPN	*In vitro*	***
(Amorim-Carmo et al., 2019)	Stig A25	*Tityus stigmurus* (Scorpion)	FFSLIPSLVKKLIKAFK	*In vitro*	***

*** absent.

distinct) stages of trophozoites and schizonts. When the schizont undergoes lysis, new merozoites are released, which starts a new asexual cycle in the blood. A low proportion of ring-shaped parasites develop into gametocytes (sexual stages), which the mosquito absorbs during a blood meal. In the mosquito's midgut, male and female gametes emerge and fuse to form zygotes, which differentiate into oocysts. Each oocyst divides to produce and release thousands of haploid sporozoites into the mosquito's body cavity. These sporozoites travel to and invade the mosquito's salivary glands, from where they are injected into the human host. The trophozoite and schizont stages are sequestered in host tissue to cause severe disease (Haldar et al., 2007; Milner et al., 2015). The *P. falciparum* life cycle is illustrated in Fig. 3.

Different classes of antimalarial drugs have been implemented to treat this infection, but in most cases, the effectiveness of the drugs is hampered by the development of drug-resistant strains (Haldar et al., 2018). Several antimicrobial peptides of natural origin have been tested as a potential therapeutic agent against *Plasmodium* infection, including dermaseptin S3 and S4, magainin 2, cecropin B, and defensins (Gwadz et al., 1989), as well as synthetic peptides, such as P2WN, ILF, Vida 1–3, SM1, SB-37 and SHIVA-1 and 3 (Arrighi et al., 2002; Carmen Rodriguez et al., 1995; Ghosh et al., 2001; Jaynes et al., 1988a; Possani et al., 1998; Jaynes et al., 1988b). Magainins were initially isolated from the skin of the African frog *Xenopus laevis* (Zasloff, 1987). Two closely related forms containing 23 amino acids have been described. An analog (Z-12) of the peptide magainin 2, in which all Lys-and Phe-residues were replaced by D enantiomers (Soravia et al., 1988) was also tested. Cecropins are a group of inducible antimicrobial peptides derived from insects of the giant silk moth *Hyalophora cecropia* (Steiner et al., 1981; van Hofsten et al., 1985). Defensins have also shown activity against oocysts in mosquitoes (Hoffmann and Hetru, 1992). Studies involving AMPs of animal sources against *Plasmodium* spp. are listed in Table 3.

3.4. *Toxoplasma gondii*

T. gondii is an obligate intracellular parasite belonging to the phylum Aplicomplexa, capable of parasitizing warm-blooded animals. It is responsible for Toxoplasmosis, a disease that can be life-threatening in immunosuppressed and developing fetuses (Zhang et al., 2019). It is the most prevalent infection in humans, infecting around 30 to 50% of the world population, but in most general hosts a latent infection throughout life in tissues such as skeletal muscle, cardiac, and central nervous system (Flegr et al., 2014; Mendez and Koshy, 2017)

In Brazil, 50 to 83% of the adult population is seropositive for *T. gondii,* and the prevalence varies from 54% in the Center-West to 75% in the North. Infection occurs mainly by ingesting oocysts found in soil, sand, and food, from tissue cysts found in meat, and via the placental route (Oréfice et al., 2010). The infective forms that the parasite presents during the biological cycle are tachyzoites, bradyzoites, and sporozoites. Trachizoite is the form found during the acute phase of the infection; being also called the proliferative form, free form, or trophozoite. Bradizoite is found in several tissues (skeletal muscle and cardiac, nervous, and retina), usually during the chronic phase of the infection, also called cystozoite. An oocyst is a form of resistance with a double wall quite resistant to environmental conditions, which are expelled by feline feces (Prado et al., 2011).

T. gondii can disrupt the intrinsic (mitochondrial) and extrinsic (death receptor-mediated) pathways of apoptosis in the invaded cells. This helps the parasite to preserve its intracellular niche, replicate and

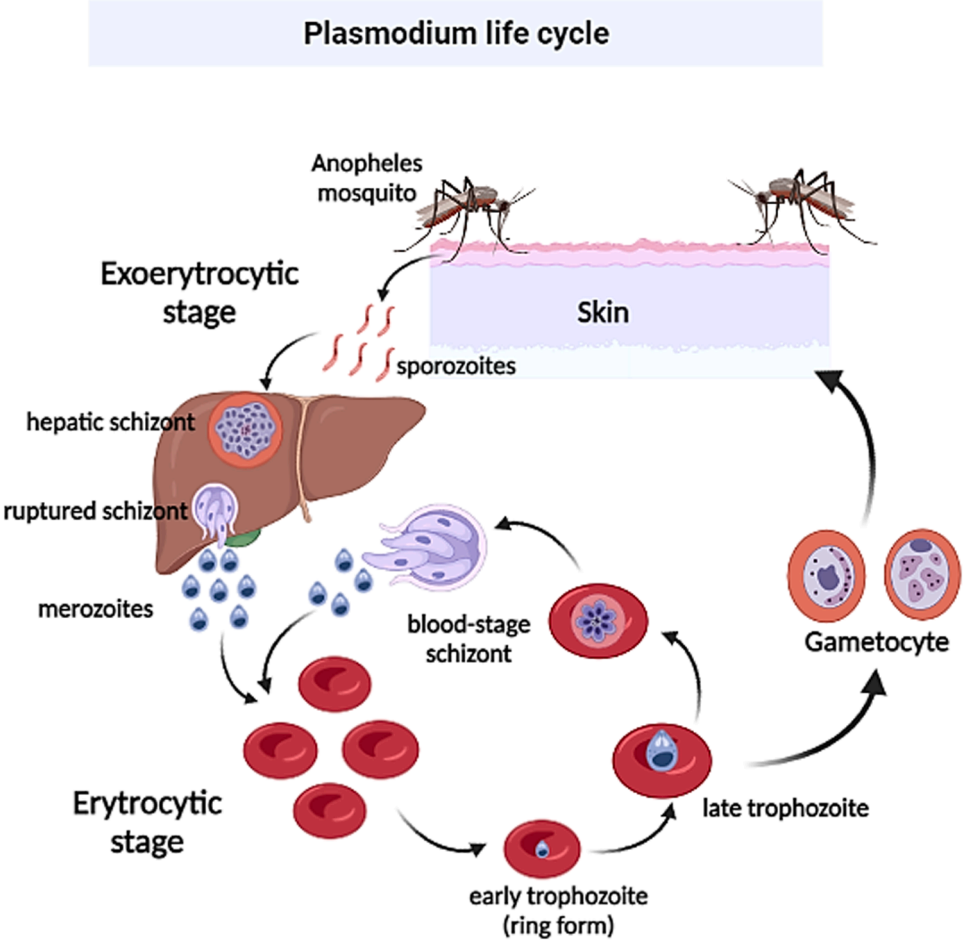

Fig. 3. *Plasmodium falciparum* life cycle.

Table 3
Studies involving antimicrobial peptides against *Plasmodium* spp.

Selected articles	Peptide	Source	Sequence	Study model	$IC_{50}\mu M$
	$PF4P_{1–34}$	Human	PRHITSLEVIKAGP	*In vitro*	8,8
(Lawrence et al., 2018)	$PF4P_{35–70}$	Human	HCPTAQLIATLKNGRLICLDLQ	*In vitro*	5
	$PF4P_{57–70}$	Human	APLYKKIIKKLLES	*In vitro*	4,3
(Moreira et al., 2007)	Gomesin	*Acanthoscurria gomesiana*	******	*In vitro*	76–87

avoid elimination by humoral immunity (Lima and Lodoen, 2019). For the reasons mentioned above, the high prevalence of Toxoplasmosis and the limitations of conventional treatments lead to the search for new drugs capable of neutralizing the parasite as well as resources are currently being invested in faster and more effective diagnostic tests to detect the parasite (Wang et al., 2021) and DNA vaccine cocktails for host immunization (Pagheh et al., 2021). Studies show the efficacy of alpha-toxin from *Clostridium septicum* against *T. gondii*, through the formation of pores on the cell surface of the parasite (Wichroski et al., 2002), in addition to the use of spider venom, which showed a decrease in the rate of invasion and proliferation of tachyzoites in host cells (Hou et al., 2019). AMPs have shown activity against *T. gondi*. For instance, peptide HPRP-A1/2 showed a reduction in adhesion, infection, and proliferation of the *T. gondii* tachyzoite in the host cells, in addition to the inhibition of *T. gondii* growth and a pro-inflammatory response against this infection (Liu et al., 2019). Thus, AMPs are a critical biotechnological tool against this parasite. Table 4 shows articles published using AMPs against this parasite.

In the literature review of the present study, only peptides of animal origin were found to show activity against *T. gondii*, presenting aggregation mechanisms that lead to destruction or decreasing the invasiveness of tachyzoites in concentrations ranging from 10 to 250 μM of the peptides. Although plant-derived AMPs have not been found with activity against *T. gondii*, this result is interesting since plant-derived AMPs have relevant activity against several microorganisms (Tam et al., 2015), and the interaction of these peptides with the protozoan may be an area to be investigated.

3.5. Mechanism of action

Interaction with the cell membrane is the most common mechanism of action of peptides with antiprotozoal activity. Most of the peptides that interact with the cell membrane act by damaging the integrity of the membrane, inducing osmotic lysis. In addition, they can interact with the membrane and do not cause lysis but alter the fluidity of the protozoan membrane, affecting the activity of membrane proteins (Torrent et al., 2012). The ability of cationic antimicrobial peptides to distinguish between the protozoan cell membrane and the host cell membrane depends on the composition of anionic phospholipids in the outer membrane of the target cell. When infected by intracellular protozoa, host cell membranes undergo significant changes induced by the parasite. *P. falciparum* is an example that causes changes in the membrane of infected erythrocytes, whose composition becomes similar to that of protozoa. This explains the specific interaction of infected erythrocytes with some small cationic antimicrobial peptides with antimalarial activity, such as the NK-2 derivative of mammalian NK-lysine (Pretzel et al., 2013).

Other peptides can cause depletion of energy activity due to the reduction of the electrochemical gradient necessary for producing adenosine triphosphate (ATP) in *Leishmania* spp. (Pitale et al., 2020). In this context, morphological changes are sought after peptide treatment, such as membrane roughness (Oliveira et al., 2016) and pore formation (Pitale et al., 2020); cytoplasmic vacuolization (Bera et al., 2003); reduction of flagella and short and rounded forms (Allane et al., 2018). Other damage pathways can be triggered, such as DNA fragmentation (Bera et al., 2003; Raja et al., 2017) and apoptosis (Bera et al., 2003; Raja et al., 2017; Kulkarni et al., 2014). Some antimicrobial peptides can kill protozoa by inducing autophagic and apoptotic processes, as demonstrated in *Leishmania* spp. (Pretzel et al., 2013) and *Trypanosoma cruzi* (Adade et al., 2013). The death of necrotic and apoptotic cells of *T. cruzi* and T. *gondii* after exposure to different antimicrobial peptides has recently been observed (Bandeira et al., 2017a; Giovati et al., 2018b) as outlined in Fig. 4.

Regarding the mechanism of action involved in antichagasic peptides, most studies report the ability of antimicrobial peptides to permeate the plasma membrane and consequent release of intracellular content, followed by death (Jacobs et al., 2003; Pinto et al., 2013; Mello et al., 2017; Monteiro et al., 2020; Pedron et al., 2020). In addition, other cellular events can also be observed, such as mitochondrial damage (Adade et al., 2013; Souza et al., 2016; Mello et al., 2017; Freire et al., 2020), formation of reactive species of oxygen (Mello et al., 2017; Freire et al., 2020), chromatin condensation (Souza et al., 2016) and trypomastigote DNA fragmentation (Kleschenko et al., 2010).

The primary mechanism of action through the formation of pores in the cell membrane of the protozoan is known as Barrel-stave (Jacobs et al., 2003), which is illustrated in Fig. 5. At this stage, there is an electrostatic interaction between the peptides and the membrane. These are inserted perpendicularly to the plane of the lipid bilayer. Then, the peptide's hydrophilic regions from the pore's inner face create a water-filled channel that is completely lined with aggregated peptide monomers, allowing the extravasation of cytoplasmic content and, consequently, cell death. Login P4 is a peptide with a molecular mass of 2,6 kDa and an isoelectric point of 9.51, originating from the Asian tick *Haemphysalis longicornis* that showed more effects on *T. gondii* tachyzoites. The peptides mentioned above can form pores in the cell membrane, disorganizing the membrane and leading to extravasation of cell content and cytoplasmic vacuolization. These results were observed in infected J774A.1 cells exposed for 60 min at a concentration of 50 μM of

Table 4
Studies involving antimicrobial peptides against *Toxoplasma gondii*.

Selected Articles	Peptide	Source	Sequence	Study Model	$IC_{50}\mu M$.
(Naoyoshi et al., 1990)	Obiopeptide-1 (GpG)	Synthetic peptide	GEEEEE	*In vitro*	***
(Silva et al., 2002)	PW2	Synthetic peptide	HPLKQYWWRPSI	*In vitro*	***
(Tanaka et al., 2010)	Defensin-α-5	Human	ATCYCRTGRCATRESLSGV CEISGRLYRLCCR	*In vitro*	***
(Tanaka et al., 2012)	Longicin P4	Synthetic peptide	FITC-SIGRRGGYCAGIIKQTCTCYR	*In vitro*	***
(Tang et al., 2019)	Lycosin-I, XYP1	*Lycosa singoriensis* *Lycosa coelestis* (Spiders)	******	*In vivo / In vitro*	*10,08*

*** absent.

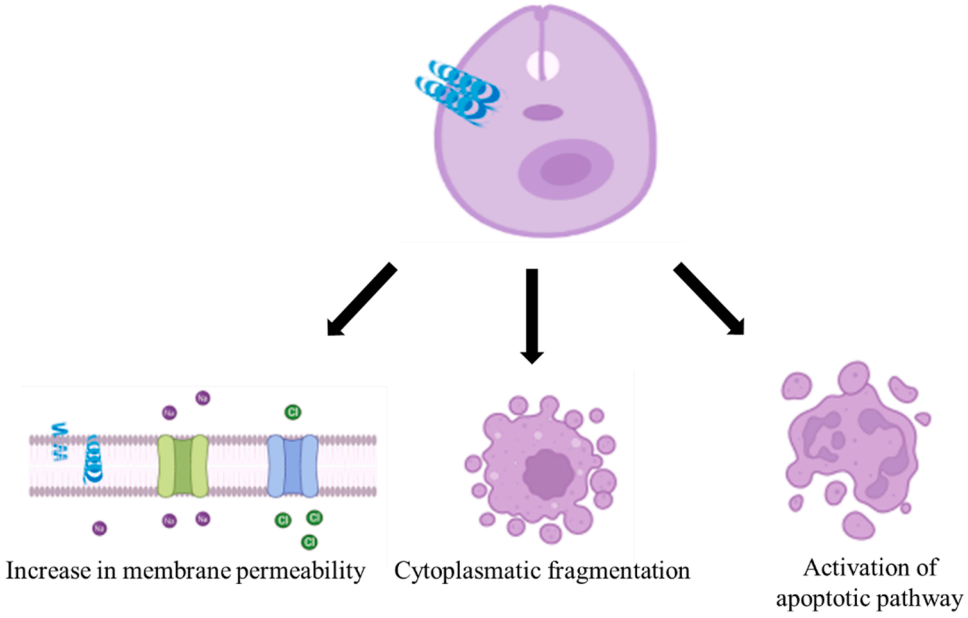

Fig. 4. Scheme of the mechanism of action of some AMPs studied.

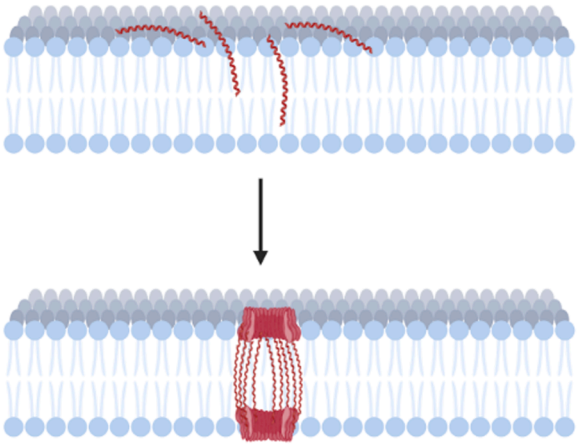

Fig. 5. Schematic representation of AMPs interacting with protozoan plasma membranes and leading to pore formation Barrel-stave model.

the peptide (Tanaka et al., 2012).

Tang et al. (2019) showed the antiparasitic effect on *T. gondii* induced by the lycosine-I peptide of the spider *Lycosa singoriensis*, which was analyzed in vitro, showing changes in the cell membrane with the formation of invaginations, and *in vivo*, extending the survival time of mice and controlling the proliferation of *T. gondii* (Tang et al., 2019). The results could be observed at the concentration of 10 μM of the peptide. Despite the promising results, further research is needed to assess the effectiveness of the action in animal models (Yang et al., 2019; Alberola et al., 2004). It is also worth noting that the absence of investigations can be interpreted as a field eager for new works, given the global health impact that this protozoan represents on health issues. The little investment in studies related to the area may result from the fact that it is a neglected and predominant disease in underdeveloped countries. As reported by the WHO, there is resistance to developing new drugs to treat these diseases.

4. Conclusion

After analyzing all these data, it was possible to conclude the animal peptides were employed the most as antiparasitic. However, in some cases, plant-based peptides were the most effective. The problem with those peptides was the toxicity presented to them. It seems that synthetic peptides emerged as possible alternatives to this toxicity presented by other peptides. Despite the origin, all peptides present the exact mechanisms of aggregation and extravasation of cell content through forming pores in the cell membranes. The mechanism presented by peptides can be used as a strategy to control these parasites and be used as a therapeutic alternative to drugs already on the market.

Based on what was discussed, as perspective is possible to suggest antiparasitic synthetic peptides are safe and have high potential application to develop a new antiparasitic drug. Synthetic peptides are safe and present high activity against parasites. However, one question arises: is the employment of synthetic peptides cost-effective? There are already two synthetic peptides applied in clinical treatments. FuzeonR, with 36-amino acid residues, is applied to treat HIV, and RybelsusR, a glucagon-like synthetic peptide used to treat type II diabetes, was recently approved by US FDA. These peptides proved that synthetic peptides could be applied as antiparasitic molecules, opening the perspectives to their applications.

CRediT authorship contribution statement

Francisco A. Santos: Conceptualization, Writing – original draft. **Gabriela S. Cruz:** Conceptualization, Writing – original draft. **Filipe A. Vieira:** Conceptualization, Writing – original draft. **Bruno R.S. Queiroz:** Conceptualization, Writing – original draft. **Cleverson D.T. Freitas:** Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Felipe P. Mesquita:** Conceptualization, Data curation, Formal analysis. **Pedro F.N. Souza:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare there are no conflicts of interest, including financial, personal, or any other relationships with other people or organizations.

Data availability

No data was used for the research described in the article.

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References

Abbassi, F., Raja, Z., Oury, B., Gazanion, E., Piesse, C., Sereno, D., Nicolas, P., Foulon, T., Ladram, A., 2013. Antibacterial and leishmanicidal activities of temporin-SHd, a 17-residue long membrane-damaging peptide. Biochimie 95, 388–399. https://doi.org/10.1016/J.BIOCHI.2012.10.015.

Abbassi, F., Oury, B., Blasco, T., Sereno, D., Bolbach, G., Nicolas, P., Hani, K., Amiche, M., Ladram, A., 2008. Isolation, characterization and molecular cloning of new temporins from the skin of the North African ranid Pelophylax saharica. Peptides 29, 1526–1533. https://doi.org/10.1016/J.PEPTIDES.2008.05.008 (N.Y.).

Abdossamadi, Z., Seyed, N., Zahedifard, F., Taheri, T., Taslimi, Y., Montakhab-Yeganeh, H., Badirzadeh, A., Vasei, M., Gharibzadeh, S., Rafati, S., 2017b. Human neutrophil peptide 1 as immunotherapeutic agent against Leishmania infected BALB/c mice. PLoS Negl.Trop. Dis. 11, e0006123 https://doi.org/10.1371/JOURNAL.PNTD.0006123.

Abdossamadi Z., Taheri T., Seyed N., Montakhab-Yeganeh H., Zahedifard F., Taslimi Y., Habibzadeh S., Gholami E., Gharibzadeh S., Rafati S., Live leishmania tarentolae secreting HNP1 as an immunotherapeutic tool against leishmania infection in BALB/c mice, 9 (2017) 1089–1102. 10.2217/IMT-2017-0076.

Abengózar, M.Á., Cebrián, R., Saugar, J.M., Gárate, T., Valdivia, E., Martínez-Bueno, M., Maqueda, M., Rivas, L., 2017. Enterocin AS-48 as evidence for the use of bacteriocins as new leishmanicidal agents. Antimicrob. Agents Chemother. 61 https://doi.org/10.1128/AAC.02288-16/SUPPL_FILE/ZAC004176079S1.PDF.

Adade, C.M., Oliveira, I.R.S., Pais, J.A.R., Souto-Padrón, T., 2013. Melittin peptide kills Trypanosoma cruzi parasites by inducing different cell death pathways. Toxicon 69, 227–239. https://doi.org/10.1016/J.TOXICON.2013.03.011.

Alberola, J., Rodríguez, A., Francino, O., Roura, X., Rivas, L., Andreu, D., 2004. Safety and efficacy of antimicrobial peptides against naturally acquired leishmaniasis. Antimicrob. Agents Chemother. 48, 641–643. https://doi.org/10.1128/AAC.48.2.641-643.2004/ASSET/15172F5E-E2DB-4B78-ACEB-C2D43D7FDE05/ASSETS/GRAPHIC/ZAC0020407280001.JPEG.

Allane, D., Oussedik-Oumehdi, H., Harrat, Z., Seve, M., Laraba-Djebari, F., 2018. Isolation and characterization of an anti-leishmanial disintegrin from Cerastes cerastes venom. J. Biochem. Mol. Toxicol. 32, e22018. https://doi.org/10.1002/JBT.22018.

André, S., Raja, Z., Humblot, V., Piesse, C., Foulon, T., Sereno, D., Oury, B., Ladram, A., 2020. Functional characterization of temporin-SHe, a new broad-spectrum antibacterial and leishmanicidal temporin-SH paralog from the sahara frog (Pelophylax saharicus). Int. J. Mol. Sci. 21, 6713. https://doi.org/10.3390/IJMS21186713, 2020, Vol. 21, Page 6713.

Amorim-Carmo, B., Daniele-Silva, A., Parente, A.M.S., Furtado, A.A., Carvalho, E., Oliveira, J.W.F., Santos, E.C.G., Silva, M.S., Silva, S.R.B., Silva-Júnior, A.A., Monteiro, N.K., Fernandes-Pedrosa, M.F., 2019. Potent and broad-spectrum antimicrobial activity of analogs from the scorpion peptide stigmurin. Int. J. Mol. Sci. 20, 623. https://doi.org/10.3390/IJMS20030623, 2019, Vol. 20, Page 623.

Arrighi, R.B.G., Nakamura, C., Miyake, J., Hurd, H., Burgess, J.G., 2002. Design and activity of antimicrobial peptides against sporogonic-stage parasites causing murine malarias. Antimicrob. Agents Chemother. 46, 2104. https://doi.org/10.1128/AAC.46.7.2104-2110.2002.

Bandeira, I.C.J., Bandeira-Lima, D., Mello, C.P., Pereira, T.P., De Menezes, R.R.P.P.B., Sampaio, T.L., Falcão, C.B., Rádis-Baptista, G., Martins, A.M.C., 2017. Parasitology Antichagasic effect of crotalicidin, a cathelicidin-like vipericidin, found in Crotalus durissus terrificus rattlesnake's venom gland. Parasitology 145, 1059–1064. https://doi.org/10.1017/S0031182017001846.

Bera, A., Singh, S., Nagaraj, R., Vaidya, T., 2003. Induction of autophagic cell death in Leishmania donovani by antimicrobial peptides. Mol. Biochem. Parasitol. 127, 23–35. https://doi.org/10.1016/S0166-6851(02)00300-6.

Bern, C., Messenger, L.A., Whitman, J.D., Maguire, J.H., 2020. Chagas disease in the United States: a public health approach. Clin. Microbiol. Rev. 33 https://doi.org/10.1128/CMR.00023-19.

Berrocal-Lobo, M., Molina, A., Rodríguez-Palenzuela, P., García-Olmedo, F., Rivas, L., 2009. Leishmania donovani: thionins, plant antimicrobial peptides with leishmanicidal activity. Exp. Parasitol. 122, 247–249. https://doi.org/10.1016/J.EXPPARA.2009.03.019.

Bittencourt, C.R., de Oliveira Farias, E.A., Bezerra, K.C., Véras, L.M.C., Silva, V.C., Costa, C.H.N., Bemquerer, M.P., Silva, L.P., de Souza De Almeida Leite, J.R., Eiras, C., 2016. Immobilization of cationic antimicrobial peptides and natural cashew gum in nanosheet systems for the investigation of anti-leishmanial activity. Mater. Sci. Eng. C 59, 549–555. https://doi.org/10.1016/J.MSEC.2015.10.059.

Borges, A.J., Silva, S., den Camp, H.O., Velasco, E., Alvarez, M., Alfonzo, M.J.M., Jorquera, A., De Sousa, L., Delgado, O., 2006. *In vitro* leishmanicidal activity of Tityus discrepans scorpion venom. Parasitol. Res. 99, 167–173. https://doi.org/10.1007/s00436-006-0133-z.

Boumaiza, M., Jaouen, M., Deschemin, J.C., Ezzine, A., ben Khalaf, N., Vaulont, S., Marzouki, M.N., Sari, M.A., 2015. Expression and purification of a new recombinant camel hepcidin able to promote the degradation of the iron exporter ferroportin1. Protein Expr. Purif. 115, 11–18. https://doi.org/10.1016/J.PEP.2015.04.016.

Boulanger, N., Lowenberger, C., Volf, P., Ursic, R., Sigutova, L., Sabatier, L., Svobodova, M., Beverley, S.M., Späth, G., Brun, R., Pesson, B., Bulet, P., 2004. Characterization of a defensin from the sand fly Phlebotomus duboscqi induced by challenge with bacteria or the protozoan parasite Leishmania major. Infect. Immun. 72, 7140–7146. https://doi.org/10.1128/IAI.72.12.7140-7146.2004.

Brand, G.D., Leite, J.R.S.A., de Sá Mandel, S.M., Mesquita, D.A., Silva, L.P., Prates, M.v., Barbosa, E.A., Vinecky, F., Martins, G.R., Galasso, J.H., Kuckelhaus, S.A.S., Sampaio, R.N.R., Furtado, J.R., Andrade, A.C., Bloch, C., 2006. Novel dermaseptins from Phyllomedusa hypochondrialis (Amphibia). Biochem. Biophys. Res. Commun. 347, 739–746. https://doi.org/10.1016/J.BBRC.2006.06.168.

Brand, G.D., Roberto A Leite, J.S., Silva, L.P., rgio Albuquerque, S., v Prates, M., Azevedo, R.B., Carregaro, V., Silva, J.S., Sá, V.C.L, Brandã, R.A., Bloch, C., 2002. Dermaseptins from Phyllomedusa oreades and Phyllomedusa distincta. Anti-Trypanosoma cruzi activity without cytotoxicity to mammalian cells. J. Biol. Chem. 277, 49332–49340. https://doi.org/10.1074/jbc.M209289200.

Brasil, Manual de vigilância da leishmaniose tegumentar, Ministério Da Saúde, Secretaria de Vigilância Em Saúde, Departamento de Vigilância Das Doenças Transmissíveis. 1 (2017) 1–189. http://bvsms.saude.gov.br/bvs/publicacoes/manual_vigilancia_leishmaniose_ (accessed April 21, 2022).

Browne, K., Chakraborty, S., Chen, R., Willcox, M.D.P., Black, D.S., Walsh, W.R., Kumar, N., 2020. A new era of antibiotics: the clinical potential of antimicrobial peptides. Int. J. Mol. Sci. 21, 7047. https://doi.org/10.3390/IJMS21197047.

Cao, L., Jiang, W., Cao, S., Zhao, P., Liu, J., Dong, H., Guo, Y., Liu, Q., Gong, P., 2019. *In vitro* leishmanicidal activity of antimicrobial peptide KDEL against Leishmania tarentolae. Acta Biochim. Biophys. Sin. 1286–1292. https://doi.org/10.1093/abbs/gmz128.

Campos-Salinas, J., Cavazzuti, A., O'Valle, F., Forte-Lago, I., Caro, M., Beverley, S.M., Delgado, M., Gonzalez-Rey, E., 2014. Therapeutic efficacy of stable analogues of vasoactive intestinal peptide against pathogens. J. Biol. Chem. 289, 14583–14599. https://doi.org/10.1074/JBC.M114.560573.

Campos-Salinas, J., Caro, M., Cavazzuti, A., Forte-Lago, I., Beverley, S.M., O'Valle, F., Gonzalez-Rey, E., 2013. Protective role of the neuropeptide urocortin II against experimental sepsis and leishmaniasis by direct killing of pathogens. J. Immunol. 191, 6040–6051. https://doi.org/10.4049/JIMMUNOL.1301921.

Chicharro, C., Granata, C., Lozano, R., Andreu, D., Rivas, L., 2001. N-terminal fatty acid substitution increases the leishmanicidal activity of CA(1-7)M(2-9), a cecropin-melittin hybrid peptide. Antimicrob. Agents Chemother. 45, 2441–2449. https://doi.org/10.1128/AAC.45.9.2441-2449.2001.

Choiid, H.L., Jainid, S., Ruiz Postigo, J.A., Borisch, B., Dagne, D.A., 2021. The global procurement landscape of leishmaniasis medicines. PLoS Negl.Trop. Dis. 15, e0009181 https://doi.org/10.1371/journal.pntd.0009181.

Croft, S.L., Sundar, S., Fairlamb, A.H., 2006. Drug resistance in Leishmaniasis. Clin. Microbiol. Rev. 19, 111–126. https://doi.org/10.1128/CMR.19.1.111-126.2006/ASSET/CD5FD0DE-752F-4D65-A727-AF11693FA2C8/ASSETS/GRAPHIC/ZCM0010621630003.JPEG.

Costa, N.C.S., Piccoli, J.P., Santos-Filho, N.A., Clementino, L.C., Fusco-Almeida, A.M., de Annunzio, S.R., Fontana, C.R., Verga, J.B.M., Eto, S.F., Pizauro-Junior, J.M., Graminha, M.A.S., Cilli, E.M., 2020. Antimicrobial activity of RP-1 peptide conjugate with ferrocene group. PLoS One 15, e0228740. https://doi.org/10.1371/JOURNAL.PONE.0228740.

Crauwels, P., Bank, E., Walber, B., Wenzel, U.A., Agerberth, B., Chanyalew, M., Abebe, M., König, R., Ritter, U., Reiling, N., van Zandbergen, G., 2019. Cathelicidin contributes to the restriction of leishmania in human host macrophages. Front. Immunol. 10, 2697. https://doi.org/10.3389/FIMMU.2019.02697/BIBTEX.

da Silva, A., Kawazoe, U., Freitas, F.F.T., Gatti, M.S.v., Dolder, H., Schumacher, R.I., Juliano, M.A., da Silva, M.J., Leite, A., 2002. Avian anticoccidial activity of a novel membrane-interactive peptide selected from phage display libraries. Mol. Biochem. Parasitol. 120, 53–60. https://doi.org/10.1016/S0166-6851(01)00439-X.

Dabirian, S., Taslimi, Y., Zahedifard, F., Gholami, E., Doustdari, F., Motamedirad, M., Khatami, S., Azadmanesh, K., Nylen, S., Rafati, S., 2013. Human neutrophil peptide-1 (HNP-1): a new anti-leishmanial drug candidate. PLoS Negl.Trop. Dis. 7, e2491. https://doi.org/10.1371/JOURNAL.PNTD.0002491.

Das, S., Sardar, A.H., Abhishek, K., Kumar, A., Rabidas, V.N., Das, P., 2017. Cathelicidin augments VDR-dependent anti-leishmanial immune response in Indian Post-Kala-Azar Dermal Leishmaniasis. Int. Immunopharmacol. 50, 130–138. https://doi.org/10.1016/J.INTIMP.2017.06.010.

del Carmen Rodriguez, M., Zamudio, F., Torres, J.A., Gonzalez-Ceron, L., Possani, L.D., Rodriguez, M.H., 1995. Effect of a cecropin-like synthetic peptide (Shiva-3) on the sporogonic development of plasmodium berghei. Exp. Parasitol. 80, 596–604. https://doi.org/10.1006/EXPR.1995.1075.

Díaz-Achirica, P., Ubach, J., Guinea, A., Andreu, D., Rivas, L., 1998. The plasma membrane of Leishmania donovani promastigotes is the main target for CA(1–8)M (1–18), a synthetic cecropin A–melittin hybrid peptide. Biochem. J. 330, 453–460. https://doi.org/10.1042/BJ3300453.

Donia, M.S., Wang, B., Dunbar, D.C., Desai, P.v., Patny, A., Avery, M., Hamann, M.T., 2008. Mollamides B and C, cyclic hexapeptides from the indonesian tunicate Didemnum molle. J. Nat. Prod. 71, 941–945. https://doi.org/10.1021/NP700718P/ASSET/IMAGES/LARGE/NP-2007-00718P_0003.JPEG.

Eaton, P., Bittencourt, C.R., Costa Silva, V., Véras, L.M.C., Costa, C.H.N., Feio, M.J., Leite, J.R.S.A., 2014. Anti-leishmanial activity of the antimicrobial peptide DRS 01 observed in Leishmania infantum (syn. Leishmania chagasi) cells. Nanomedicine 10, 483–490. https://doi.org/10.1016/J.NANO.2013.09.003.

Erfe, M.C.B., David, C.V., Huang, C., Lu, V., Maretti-Mira, A.C., Haskell, J., Bruhn, K.W., Yeaman, M.R., Craft, N., 2012. Efficacy of synthetic peptides RP-1 and AA-RP-1 against Leishmania species *in vitro* and *in vivo*. Antimicrob. Agents Chemother. 56, 658–665. https://doi.org/10.1128/AAC.05349-11.

Flegr, J., Prandota, J., Sovičková, M., Israili, Z.H., 2014. Toxoplasmosis-A global threat. Correlation of latent toxoplasmosis with specific disease burden in a set of 88 countries. PLOS ONE 9, 90203. https://doi.org/10.1371/journal.pone.0090203.

Fragiadaki, I., Katogiritis, A., Calogeropoulou, T., Brückner, H., Scoulica, E., 2018. Synergistic combination of alkylphosphocholines with peptaibols in targeting Leishmania infantum *in vitro*. Int. J. Parasitol. Drugs Drug Resist. 8, 194–202. https://doi.org/10.1016/J.IJPDDR.2018.03.005.

Freire, K.A., Torres, M.D.T., Lima, D.B., Monteiro, M.L., Bezerra de Menezes, R.R.P.P., Martins, A.M.C., Oliveira, V.X., 2020. Wasp venom peptide as a new antichagasic agent. Toxicon 181, 71–78. https://doi.org/10.1016/J.TOXICON.2020.04.099.

Fernández-Arévalo, A., el Baidouri, F., Ravel, C., Ballart, C., Abras, A., Lachaud, L., Tebar, S., Lami, P., Pratlong, F., Gállego, M., Muñoz, C., 2020. The leishmania donovani species complex: a new insight into taxonomy*. Int. J. Parasitol. 50, 1079–1088. https://doi.org/10.1016/J.IJPARA.2020.06.013.

Fern Andez-Reyes, M., Dıáz, D., de La Torre, B.G., Cabrales-Rico, A., Vall Es-Miret, M., Us, J., Enez-Barbero, J., Andreu, D., Rivas, L., 2010. Lysine N ε-trimethylation, a tool for improving the selectivity of antimicrobial peptides. J. Med. Chem. 53, 5587–5596. https://doi.org/10.1021/jm100261r.

Ferreira, L.L.G., de Moraes, J., Andricopulo, A.D., 2022. Approaches to advance drug discovery for neglected tropical diseases. Drug Discovery Today. https://doi.org/10.1016/J.DRUDIS.2022.04.004.

Ferreira, E., Júnior, S., Felipe, L., Menezes, S., Flor, I., de Araújo, S., Schwartz, E.F., 2019. Toxins natural occurrence in venomous arthropods of antimicrobial peptides active against protozoan parasites. Toxins 11, 563. https://doi.org/10.3390/toxins11100563.

Gaidukov, L., Fish, A., Mor, A., 2003. Analysis of membrane-binding properties of dermaseptin analogues: relationships between binding and cytotoxicity †. Biochemistry 42, 12866–12874. https://doi.org/10.1021/bi034514x.

Gething, P.W., Elyazar, I.R.F., Moyes, C.L., Smith, D.L., Battle, K.E., Guerra, C.A., Patil, A. P., Tatem, A.J., Howes, R.E., Myers, M.F., George, D.B., Horby, P., Wertheim, H.F.L., Price, R.N., Mü Eller, I., Baird, J.K., Hay, S.I., 2012. A Long Neglected World Malaria Map: plasmodium vivax Endemicity in 2010. PLOS Negl. Trop. Di. 6 https://doi.org/10.1371/journal.pntd.0001814.

Ghosh, A.K., Ribolla, P.E.M., Jacobs-Lorena, M., 2001. Targeting Plasmodium ligands on mosquito salivary glands and midgut with a phage display peptide library. Proc. Natl. Acad. Sci. 98, 13278–13281. https://doi.org/10.1073/pnas.241491198.

Giovati L., Ciociola T., Magliani W., Conti S., Antimicrobial peptides with antiprotozoal activity: current state and future perspectives, (2018) 2569–2572. 10.4155/FMC-2018-0460.

Giovati, L., Santinoli, C., Mangia, C., Vismarra, A., Belletti, S., D'Adda, T., Fumarola, C., Ciociola, T., Bacci, C., Magliani, W., Polonelli, L., Conti, S., Kramer, L.H., 2018b. Novel activity of a synthetic decapeptide against Toxoplasma gondii Tachyzoites. Front. Microbiol. 9, 753. https://doi.org/10.3389/FMICB.2018.00753/BIBTEX.

Guerra, M.E.R., Fadel, V., Maltarollo, V.G., Baldissera, G., Honorio, K.M., Ruggiero, J.R., dos Santos Cabrera, M.P., 2017. MD simulations and multivariate studies for modeling the antileishmanial activity of peptides. Chem. Biol. Drug Des. 90, 501–510. https://doi.org/10.1111/CBDD.12970.

Guerrero, E., Saugar, J.M., Matsuzaki, K., Rivas, L., 2004. Role of positional hydrophobicity in the leishmanicidal activity of magainin 2. Antimicrob. Agents Chemother. 48, 2980–2986. https://doi.org/10.1128/AAC.48.8.2980-2986.2004.

Gwadz, R.W., Kaslow, D., Lee, J.Y., Maloy, W.L., Zasloff, M., Miller, L.H., 1989. Effects of magainins and cecropins on the sporogonic development of malaria parasites in mosquitoes. Infect. Immun. 57, 2628–2633. https://doi.org/10.1128/IAI.57.9.2628-2633.1989.

Haldar, K., Bhattacharjee, S., Safeukui, I., 2018. Drug resistance in Plasmodium. Nat. Rev. Microbiol. 16, 156–170. https://doi.org/10.1038/nrmicro.2017.161, 2018 16: 3.

Haldar, K., Murphy, S.C., Milner, D.A., Taylor, T.E., 2007. Malaria: mechanisms of erythrocytic infection and pathological correlates of severe disease. Annu. Rev. Pathol. 2, 217–249. https://doi.org/10.1146/annurev.pathol.2.010506.091913.

Haines, L.R., Thomas, J.M., Jackson, A.M., Eyford, B.A., Razavi, M., Watson, C.N., Gowen, B., Hancock, R.E.W., Pearson, T.W., 2009. Killing of trypanosomatid parasites by a modified bovine host defense peptide, BMAP-18. PLoS Negl.Trop. Dis. 3, e373. https://doi.org/10.1371/JOURNAL.PNTD.0000373.

Hoffmann, J.A., Hetru, C., 1992. Insect defensins: inducible antibacterial peptides. Immunol. Today 13, 411–415. https://doi.org/10.1016/0167-5699(92)90092-L.

Hou, S., Liu, Y., Tang, Y., Wu, M., Guan, J., Li, X., Wang, Z., Jiang, J., Deng, M., Duan, Z., Tang, X., Han, X., Jiang, L., 2019. Anti-Toxoplasma gondii effect of two spider venoms *in vitro* and *in vivo*. Toxicon 166, 9–14. https://doi.org/10.1016/J.TOXICON.2019.05.003.

Iorns, E., Gunn, W., Erath, J., Rodriguez, A., Zhou, J., Benzinou, M., Reproducibility Initiative, T., Carlos Pizarro, J., 2014. Effect of BMAP-28 antimicrobial peptides on leishmania major promastigote and amastigote growth: role of leishmanolysin in parasite survival. PLoS One 9, 1–13. https://doi.org/10.1371/journal.pone.

Jacobs, T., Bruhn, H., Gaworski, I., Fleischer, B., Leippe, M., 2003. NK-lysin and its shortened analog NK-2 exhibit potent activities against Trypanosoma cruzi. Antimicrob. Agents Chemother. 47, 607–613. https://doi.org/10.1128/AAC.47.2.607-613.2003.

Jaynes, J.M., Burton, C.A., Barr, S.B., Jeffers, G.W., Julian, G.R., White, K.L., Enright, F. M., Klei, T.R., Laine, R.A., 1988b. *In vitro* cytocidal effect of novel lytic peptides on *Plasmodium falciparum* and Trypanosoma cruzi1. FASEB J. 2, 2878–2883. https://doi.org/10.1096/FASEBJ.2.13.3049204.

Jaynes, J.M., Burton, C.A., Barr, S.B., Jeffers, G.W., Julian, G.R., White, K.L., Enright, F. M., Klei, T.R., Laine, R.A., 1988a. *In vitro* cytocidal effect of novel lytic peptides on *Plasmodium falciparum* and Trypanosoma cruzi1. FASEB J. 2, 2878–2883. https://doi.org/10.1096/FASEBJ.2.13.3049204.

Keller, A.-A., Breitling, R., Hemmerich, P., Kappe, K., Braun, M., Wittig, B., Schaefer, B., Lorkowski, S., Reissmann, S., 2014. Transduction of proteins into leishmania tarentolae by formation of non-covalent complexes with cell-penetrating peptides. J. Cell. Biochem. 115, 243–252. https://doi.org/10.1002/jcb.24654.

Khalili, S., Mohebali, M., Ebrahimzadeh, E., Shayan, P., Mohammadi-Yeganeh, S., Moghaddam, M.M., Elikaee, S., Akhoundi, B., Sharifi-Yazdi, M.K., 2018. Antimicrobial activity of an antimicrobial peptide against amastigote forms of Leishmania major. Vet. Res. Forum 9, 323–328. https://doi.org/10.30466/VRF.2018.33107.

khalili, S., Ebrahimzade, E., Mohebali, M., Shayan, P., Mohammadi-Yeganeh, S., Moosazadeh Moghaddam, M., Elikaee, S., Akhoundi, B., Sharifi-Yazdi, M.K., 2019. Investigation of the antimicrobial activity of a short cationic peptide against promastigote and amastigote forms of Leishmania major (MHRO/IR/75/ER): an *in vitro* study. Exp. Parasitol. 196, 48–54. https://doi.org/10.1016/J.EXPPARA.2018.11.006.

Klatt, S., Simpson, L., Maslov, D.A., Konthur, Z., 2019. Leishmania tarentolae: taxonomic classification and its application as a promising biotechnological expression host. PLoS Negl.Trop. Dis. 13 https://doi.org/10.1371/JOURNAL.PNTD.0007424.

Kleschenko Y.E., Karpenko L.P., Villalta F., Effects of Human Defensin-α 1 on Trypanosoma cruzi Trypomastigotes *in vitro*, 2010.

Konno, K., Rangel, M., Oliveira, J.S., dos Santos Cabrera, M.P., Fontana, R., Hirata, I.Y., Hide, I., Nakata, Y., Mori, K., Kawano, M., Fuchino, H., Sekita, S., Neto, J.R., 2007. Decoralin, a novel linear cationic alpha-helical peptide from the venom of the solitary eumenine wasp Oreumenes decoratus. Peptides 28, 2320–2327. https://doi.org/10.1016/J.PEPTIDES.2007.09.017 (N.Y.).

Kulkarni, M.M., Barbi, J., Mcmaster, W.R., Gallo, R.L., Satoskar, A.R., Mcgwire, B.S., 2011. Mammalian antimicrobial peptide influences control of cutaneous Leishmania infection. Cell. Microbiol. 13, 913–923. https://doi.org/10.1111/J.1462-5822.2011.01589.X.

Kulkarni, M.M., McMaster, W.R., Kamysz, W., McGwire, B.S., 2009. Antimicrobial peptide-induced apoptotic death of Leishmania results from calcium-dependent, caspase-independent mitochondrial toxicity. J. Biol. Chem. 284, 15496–15504. https://doi.org/10.1074/JBC.M809079200.

Kulkarni, M.M., Karafova, A., Kamysz, W., Mcgwire, B.S., 2014. Design of protease-resistant pexiganan enhances antileishmanial activity. Parasitol. Res. 113, 1971–1976. https://doi.org/10.1007/s00436-014-3847-3.

Kückelhaus, S.A.S., de Aquino, D.S., Borges, T.K., Moreira, D.C., Leite, L.de M., Muniz-Junqueira, M.I., Kückelhaus, C.S., Sierra Romero, G.A., Prates, M.v., Bloch, C., Leite, J.R.S.A., 2020. Phylloseptin-1 is leishmanicidal for amastigotes of leishmania amazonensis inside infected macrophages. Int. J. Environ. Res. Public Health 17, 4856. https://doi.org/10.3390/IJERPH17134856, 2020, Vol. 17, Page 4856.

Kumar, P., Kizhakkedathu, J.N., Straus, S.K., 2019. Antimicrobial peptides: diversity, mechanism of action and strategies to improve the activity and biocompatibility *in vivo*. Biomolecules 19, 1–24. https://doi.org/10.3390/biom8010004.

Kustanovich, I., Shalev, D.E., Mikhlin, M., Gaidukov, L., Mor, A., 2002. Structural requirements for potent versus selective cytotoxicity for antimicrobial dermaseptin S4 derivatives. J. Biol. Chem. 277, 16941–16951. https://doi.org/10.1074/JBC.M111071200.

Lawrence, N., Dennis, A.S.M., Lehane, A.M., Ehmann, A., Harvey, P.J., Benfield, A.H., Cheneval, O., Henriques, S.T., Craik, D.J., McMorran, B.J., 2018. Defense peptides engineered from human platelet factor 4 kill plasmodium by selective membrane disruption. Cell Chem. Biol. 25, 1140–1150. https://doi.org/10.1016/J.CHEMBIOL.2018.06.009. .e5.

Lidani, K.C.F., Andrade, F.A., Bavia, L., Damasceno, F.S., Beltrame, M.H., Messias-Reason, I.J., Sandri, T.L., 2019. Chagas disease: from discovery to a worldwide health problem. J. Phys. Oceanogr. 49, 166. https://doi.org/10.3389/FPUBH.2019.00166/BIBTEX.

Lima, T.S., Lodoen, M.B., 2019. Mechanisms of human innate immune evasion by Toxoplasma gondii. Front. Cell. Infect. Microbiol. 9, 103. https://doi.org/10.3389/FCIMB.2019.00103/BIBTEX.

Liu, R., Ni, Y., Song, J., Xu, Z., Qiu, J., Wang, L., Zhu, Y., Huang, Y., Ji, M., Chen, Y., 2019. Research on the effect and mechanism of antimicrobial peptides HPRP-A1/A2 work against Toxoplasma gondii infection. Parasite Immunol. 41, e12619. https://doi.org/10.1111/PIM.12619.

Löfgren, S.E., Miletti, L.C., Steindel, M., Bachère, E., Barracco, M.A., 2008. Trypanocidal and leishmanicidal activities of different antimicrobial peptides (AMPs) isolated from aquatic animals. Exp. Parasitol. 118, 197–202. https://doi.org/10.1016/J.EXPPARA.2007.07.011.

Luque-Ortega, J.R., van't Hof, W., Veerman, E.C.I., Saugar, J.M., Rivas, L., 2008. Human antimicrobial peptide histatin 5 is a cell- penetrating peptide targeting mitochondrial ATP synthesis in Leishmania. FASEB J. 22, 1817–1828. https://doi.org/10.1096/FJ.07-096081.

Lynn, M.A., Kindrachuk, J., Marr, A.K., Jenssen, H., Panté, N., Elliott, M.R., Napper, S., Hancock, R.E., McMaster, W.R., 2011. Effect of BMAP-28 antimicrobial peptides on

leishmania major promastigote and amastigote growth: role of leishmanolysin in parasite survival. PLoS Negl.Trop. Dis. 5, e1141. https://doi.org/10.1371/JOURNAL.PNTD.0001141.

M. Mahlapuu, C. Björn, J. Ekblom, Antimicrobial peptides as therapeutic agents: opportunities and challenges, 40 (2020) 978–992. 10.1080/07388551.2020.1796576.

Mangoni, M.L., Papo, N., Saugar, J.M., Barra, D., Shai, Y., Simmaco, M., Rivas, L., 2006. Effect of natural L- to D-amino acid conversion on the organization, membrane binding, and biological function of the antimicrobial peptides bombinins H. Biochemistry 45, 4266–4276. https://doi.org/10.1021/bi052150y.

Mangoni, M.L., Saugar, J.M., Dellisanti, M., Barra, D., Simmaco, M., Rivas, L., 2005. Temporins, small antimicrobial peptides with leishmanicidal activity. J. Biol. Chem. 280, 984–990. https://doi.org/10.1074/JBC.M410795200.

Marr, A.K., Cen, S., Hancock, R.E.W., McMaster, W.R., 2016. Identification of synthetic and natural host defense peptides with leishmanicidal activity. Antimicrob. Agents Chemother. 60, 2484–2491. https://doi.org/10.1128/AAC.02328-15.

Mello, C.P., Lima, D.B., de Menezes, R.R.P.P.B., Bandeira, I.C.J., Tessarolo, L.D., Sampaio, T.L., Falcão, C.B., Rádis-Baptista, G., Martins, A.M.C., 2017. Evaluation of the antichagasic activity of batroxicidin, a cathelicidin-related antimicrobial peptide found in Bothrops atrox venom gland. Toxicon 130, 56–62. https://doi.org/10.1016/J.TOXICON.2017.02.031.

Meibalan, E., Marti, M., 2017. Biology of Malaria transmission. Cold Spring Harb. Perspect. Med. 7 https://doi.org/10.1101/cshperspect.a025452.

Mendes, B., Almeida, J.R., Vale, N., Gomes, P., Gadelha, F.R., da Silva, S.L., Miguel, D.C., 2019. Potential use of 13-mer peptides based on phospholipase and oligoarginine as leishmanicidal agents. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 226, 108612 https://doi.org/10.1016/J.CBPC.2019.108612.

Mendes, B., Proaño-Bolaños, C., Gadelha, F.R., Almeida, J.R., Miguel, D.C., 2020. Cruzioseptins, antibacterial peptides from Cruziohyla calcarifer skin, as promising leishmanicidal agents. Pathog. Dis. 78, 53. https://doi.org/10.1093/FEMSPD/FTAA053.

Mendez, O.A., Koshy, A.A., 2017. Toxoplasma gondii: entry, association, and physiological influence on the central nervous system. PLoS Pathog. 12, 1006351 https://doi.org/10.1371/journal.ppat.1006351.

Milner, D.A., Lee, J.J., Frantzreb, C., Whitten, R.O., Kamiza, S., Carr, R.A., Pradham, A., Factor, R.E., Playforth, K., Liomba, G., Dzamalala, C., Seydel, K.B., Molyneux, M.E., Taylor, T.E., 2015. Quantitative assessment of multiorgan sequestration of parasites in fatal pediatric cerebral malaria. J. Infect. Dis. 8, 1317–1321. https://doi.org/10.1093/infdis/jiv205.

Mijiddorj, B., Kaneda, S., Sato, H., Kitahashi, Y., Javkhlantugs, N., Naito, A., Ueda, K., Kawamura, I., 2018. The role of d-allo-isoleucine in the deposition of the anti-Leishmania peptide bombinin H4 as revealed by 31P solid-state NMR, VCD spectroscopy, and MD simulation. Biochim. Biophys. Acta (BBA) Proteins Proteom. 1866, 789–798. https://doi.org/10.1016/J.BBAPAP.2018.01.005.

Mirzaei, F., Khanahmad, H., Namdar, F., Izadi, S., Hejazi, S.H., 2019. A novel strategy for enhance potentiation of meglumine anti-moniate against Leishmania major *in vitro*. Iran. J. Parasitol. 14, 542–551. https://doi.org/10.18502/ijpa.v14i4.2096.

Montazeri, M., Mehrzadi, S., Sharif, M., Sarvi, S., Tanzifi, A., Aghayan, S.A., Daryani, A., 2018. Drug resistance in toxoplasma gondii. Front. Microbiol. 9, 2587. https://doi.org/10.3389/FMICB.2018.02587/BIBTEX.

Monteiro, M.L., Lima, D.B., de Menezes, R.R.P.P.B., Sampaio, T.L., Silva, B.P., Serra Nunes, J.V., Cavalcanti, M.M., Morlighem, J.E., Martins, A.M.C., 2020. Antichagasic effect of hemocyanin derived from antimicrobial peptides of penaeus monodon shrimp. Exp. Parasitol. 215, 107930 https://doi.org/10.1016/J.EXPPARA.2020.107930.

Moraes, C.B., Witt, G., Kuzikov, M., Ellinger, B., Calogeropoulou, T., Prousis, K.C., Mangani, S., di Pisa, F., Landi, G., dello Iacono, L., Pozzi, C., Freitas-Junior, L.H., dos Santos Pascoalino, B., Bertolacini, C.P., Behrens, B., Keminer, O., Leu, J., Wolf, M., Reinshagen, J., Cordeiro-da-Silva, A., Santarem, N., Venturelli, A., Wrigley, S., Karunakaran, D., Kebede, B., Pöhner, I., Müller, W., Panecka-Hofman, J., Wade, R. C., Fenske, M., Clos, J., Alunda, J.M., Corral, M.J., Uliassi, E., Bolognesi, M.L., Linciano, P., Quotadamo, A., Ferrari, S., Santucci, M., Borsari, C., Costi, M.P., Gul, S., 2019. Accelerating drug discovery efforts for trypanosomatidic infections using an integrated transnational academic drug discovery platform. SLAS Discov. 24, 346–361. https://doi.org/10.1177/2472555218823171.

Moreira, C.K., Rodrigues, F.G., Ghosh, A., Varotti, F.de P., Miranda, A., Daffre, S., Jacobs-Lorena, M., Moreira, L.A., 2007. Effect of the antimicrobial peptide gomesin against different life stages of plasmodium spp. Exp. Parasitol. 116, 346. https://doi.org/10.1016/J.EXPPARA.2007.01.022.

Murray, C.J.L., Ortblad, K.F., Guinovart, C., Lim, S.S., Wolock, T.M., Roberts, D.A., Dansereau, E.A., Graetz, N., Barber, R.M., Brown, J.C., Wang, H., Duber, H.C., Naghavi, M., Dicker, D., Dandona, L., Salomon, J.A., Heuton, K.R., Foreman, K., Phillips, D.E., Fleming, T.D., Flaxman, A.D., Phillips, B.K., Johnson, E.K., Coggeshall, M.S., Abd-Allah, F., Abera, S.F., Abraham, J.P., Abubakar, I., Abu-Raddad, L.J., Abu-Rmeileh, N.M., Achoki, T., Adeyemo, A.O., Adou, A.K., Adsuar, J. C., Agardh, E.E., Akena, D., al Kahbouri, M.J., Alasfoor, D., Albittar, M.I., Alcalá-Cerra, G., Alegretti, M.A., Alemu, Z.A., Alfonso-Cristancho, R., Alhabib, S., Ali, R., Alla, F., Allen, P.J., Alsharif, U., Alvarez, E., Alvis-Guzman, N., Amankwaa, A.A., Amare, A.T., Amini, H., Ammar, W., Anderson, B.O., Antonio, C.A.T., Anwari, P., Ärnlöv, J., Arsenijevic, V.S.A., Artaman, A., Asghar, R.J., Assadi, R., Atkins, L.S., Badawi, A., Balakrishnan, K., Banerjee, A., Basu, S., Beardsley, J., Bekele, T., Bell, M. L., Bernabe, E., Beyene, T.J., Bhala, N., Bhalla, A., Bhutta, Z.A., bin Abdulhak, A., Binagwaho, A., Blore, J.D., Basara, B.Bora, Bose, D., Brainin, M., Breitborde, N., Castañeda-Orjuela, C.A., Catalá-López, F., Chadha, V.K., Chang, J.C., Chiang, P.P.C., Chuang, T.W., Colomar, M., Cooper, L.T., Cooper, C., Courville, K.J., Cowie, B.C., Criqui, M.H., Dandona, R., Dayama, A., de Leo, D., Degenhardt, L., del Pozo-Cruz, B., Deribe, K., des Jarlais, D.C., Dessalegn, M., Dharmaratne, S.D., Dilmen, U., Ding, E. L., Driscoll, T.R., Durrani, A.M., Ellenbogen, R.G., Ermakov, S.P., Esteghamati, A., Faraon, E.J.A., Farzadfar, F., Fereshtehnejad, S.M., Fijabi, D.O., Forouzanfar, M.H., Paleo, U.F., Gaffikin, L., Gamkrelidze, A., Gankpé, F.G., Geleijnse, J.M., Gessner, B. D., Gibney, K.B., Ginawi, I.A.M., Glaser, E.L., Gona, P., Goto, A., Gouda, H.N., Gugnani, H.C., Gupta, R., Gupta, R., Hafezi-Nejad, N., Hamadeh, R.R., Hammami, M., Hankey, G.J., Harb, H.L., Haro, J.M., Havmoeller, R., Hay, S.I., Hedayati, M.T., Pi, I.B.Heredia, Hoek, H.W., Hornberger, J.C., Hosgood, H.D., Hotez, P.J., Hoy, D.G., Huang, J.J., Iburg, K.M., Idrisov, B.T., Innos, K., Jacobsen, K. H., Jeemon, P., Jensen, P.N., Jha, V., Jiang, G., Jonas, J.B., Juel, K., Kan, H., Kankindi, I., Karam, N.E., Karch, A., Karema, C.K., Kaul, A., Kawakami, N., Kazi, D. S., Kemp, A.H., Kengne, A.P., Keren, A., Kereselidze, M., Khader, Y.S., Khalifa, S.E.A. H., Khan, E.A., Khang, Y.H., Khonelidze, I., Kinfu, Y., Kinge, J.M., Knibbs, L., Kokubo, Y., Kosen, S., Defo, B.Kuate, Kulkarni, V.S., Kulkarni, C., Kumar, K., Kumar, R.B., Kumar, G.A., Kwan, G.F., Lai, T., Balaji, A.L., Lam, H., Lan, Q., Lansingh, V.C., Larson, H.J., Larsson, A., Lee, J.T., Leigh, J., Leinsalu, M., Leung, R., Li, Y., Li, Y., de Lima, G.M.F., Lin, H.H., Lipshultz, S.E., Liu, S., Liu, Y., Lloyd, B.K., Lotufo, P.A., Machado, V.M.P., Maclachlan, J.H., Magis-Rodriguez, C., Majdan, M., Mapoma, C.C., Marcenes, W., Marzan, M.B., Masci, J.R., Mashal, M.T., Mason-Jones, A.J., Mayosi, B.M., Mazorodze, T.T., McKay, A.C., Meaney, P.A., Mehndiratta, M.M., Mejia-Rodriguez, F., Melaku, Y.A., Memish, Z.A., Mendoza, W., Miller, T.R., Mills, E.J., Mohammad, K.A., Mokdad, A.H., Mola, G.L., Monasta, L., Montico, M., Moore, A.R., Mori, R., Moturi, W.N., Mukaigawara, M., Murthy, K.S., Naheed, A., Naidoo, K.S., Naldi, L., Nangia, V., Narayan, K.M.V., Nash, D., Nejjari, C., Nelson, R.G., Neupane, S.P., Newton, C.R., Ng, M., Nisar, M.I., Nolte, S., Norheim, O.F., Nowaseb, V., Nyakarahuka, L., Oh, I.H., Ohkubo, T., Olusanya, B.O., Omer, S.B., Opio, J.N., Orisakwe, O.E., Pandian, J.D., Papachristou, C., Caicedo, A.J. P., Patten, S.B., Paul, V.K., Pavlin, B.I., Pearce, N., Pereira, D.M., Pervaiz, A., Pesudovs, K., Petzold, M., Pourmalek, F., Qato, D., Quezada, A.D., Quistberg, D.A., Rafay, A., Rahimi, K., Rahimi-Movaghar, V., Rahman, S.U., Raju, M., Rana, S.M., Razavi, H., Reilly, R.Q., Remuzzi, G., Richardus, J.H., Ronfani, L., Roy, N., Sabin, N., Saeedi, M.Y., Sahraian, M.A., Samonte, G.M.J., Sawhney, M., Schneider, I.J.C., Schwebel, D.C., Seedat, S., Sepanlou, S.G., Servan-Mori, E.E., Sheikhbahaei, S., Shibuya, K., Shin, H.H., Shiue, I., Shivakoti, R., Sigfusdottir, I.D., Silberberg, D.H., Silva, A.P., Simard, E.P., Singh, J.A., Skirbekk, V., Sliwa, K., Soneji, S., Soshnikov, S. S., Sreeramareddy, C.T., Stathopoulou, V.K., Stroumpoulis, K., Swaminathan, S., Sykes, B.L., Tabb, K.M., Talongwa, R.T., Tenkorang, E.Y., Terkawi, A.S., Thomson, A. J., Thorne-Lyman, A.L., Towbin, J.A., Traebert, J., Tran, B.X., Dimbuene, Z.Tsala, Tsilimbaris, M., Uchendu, U.S., Ukwaja, K.N., Uzun, S.B., Vallely, A.J., Vasankari, T. J., Venketasubramanian, N., Violante, F.S., Vlassov, V.V., Vollset, S.E., Waller, S., Wallin, M.T., Wang, L., Wang, X.R., Wang, Y., Weichenthal, S., Weiderpass, E., Weintraub, R.G., Westerman, R., White, R.A., Wilkinson, J.D., Williams, T.N., Woldeyohannes, S.M., Wong, J.Q., Xu, G., Yang, Y.C., Yano, Y., Yentur, G.K., Yip, P., Yonemoto, N., Yoon, S.J., Younis, M., Yu, C., Jin, K.Y., el Sayed Zaki, M., Zhao, Y., Zheng, Y., Zhou, M., Zhu, J., Zou, X.N., Lopez, A.D., Vos, T., 2014. Global, regional, and national incidence and mortality for HIV, tuberculosis, and malaria during 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet N. Am. Ed. 384, 1005–1070. https://doi.org/10.1016/S0140-6736(14)60844-8.

Naoyoshi, S., Haruhisa, S., Atsushi, S., Ikuo, I., Yoshitaka, O., Humio, O., 1990. Biological activity of obiopeptide-1, a synthetic peptide derived from the native immune-regulator obioactin. Nihon Juigaku Zasshi 907–914. https://doi.org/10.1292/jvms1939.52.907.

Oliveira, M., Georgina Gomes-Alves, A., Sousa, C., Mirta Marani, M., Pl acido, A., Vale, N., Delerue-Matos, C., Gameiro, P., Kückelhaus, S.A., Tomas, A.M., Roberto A Leite, J.S., Eaton, P., 2016. Ocellatin-PT antimicrobial peptides: high-resolution microscopy studies in antileishmania models and interactions with mimetic membrane systems Antimicrobial peptides, atomic force microscopy, scanning electron microscopy, Leishmania infantum, lipid membranes, surface plasmon resonance. Biopolymers. https://doi.org/10.1002/bip.22925.

Oréfice, F., Cunha Filho, R., Lúcia Barboza, A., Oréfice, J.L., Calucci, D., 2010. Toxoplasmose ocular adquirida toxoplasmose ocular pós-natal acquired ocular toxoplasmosis post-birth ocular toxoplasmosis ARTIGO DE REVISÃO. Rev. Bras. Oftalmol. 69, 184–207.

Pagheh, A.S., Daryani, A., Alizadeh, P., Hassannia, H., Rodrigues Oliveira, S.M., Kazemi, T., Rezaei, F., Pereira, M.de L., Ahmadpour, E., 2021. Protective effect of a DNA vaccine cocktail encoding ROP13 and GRA14 with Alum nano-adjuvant against Toxoplasma gondii infection in mice. Int. J. Biochem. Cell Biol. 132, 105920 https://doi.org/10.1016/J.BIOCEL.2021.105920.

Paik, D., Pramanik, P.K., Chakraborti, T., 2020. Curative efficacy of purified serine protease inhibitor PTF3 from potato tuber in experimental visceral Leishmaniasis. Int. Immunopharmacol. 85 https://doi.org/10.1016/J.INTIMP.2020.106623.

Patiño-Márquez, I.A., Patiño-González, E., Hernández-Villa, L., Ortíz-Reyes, B., Manrique-Moreno, M., 2018a. Identification and evaluation of Galleria mellonella peptides with antileishmanial activity. Anal. Biochem. 546, 35–42. https://doi.org/10.1016/J.AB.2018.01.029.

Parthasarathy, A., Kalesh, K., 2020. Defeating the trypanosomatid trio: proteomics of the protozoan parasites causing neglected tropical diseases. RSC Med. Chem. 11, 625–645. https://doi.org/10.1039/D0MD00122H.

Pedron, C.N., Freire, K.A., Torres, M.D.T., Lima, D.B., Monteiro, M.L., de Menezes, R.R.P. P.B., Martins, A.M.C., Oliveira, V.X., 2020. Arg-substituted VmCT1 analogs reveals promising candidate for the development of new antichagasic agent. Parasitology 147, 1810–1818. https://doi.org/10.1017/S0031182020001882.

Pérez-Cordero, J.J., Lozano, J.M., Cortés, J., Delgado, G., 2011. Leishmanicidal activity of synthetic antimicrobial peptides in an infection model with human dendritic cells. Peptides 32, 683–690. https://doi.org/10.1016/J.PEPTIDES.2011.01.011 (N.Y.).

Pitale, D.M., Kaur, G., Baghel, M., Kaur, K.J., Shaha, C., 2020. Halictine-2 antimicrobial peptide shows promising antiparasitic activity against Leishmania spp. Exp. Parasitol. 218, 107987 https://doi.org/10.1016/J.EXPPARA.2020.107987.
Pinto, E.G., Pimenta, D.C., Antoniazzi, M.M., Jared, C., Tempone, A.G., 2013. Antimicrobial peptides isolated from Phyllomedusa nordestina (Amphibia) alter the permeability of plasma membrane of Leishmania and Trypanosoma cruzi. Exp. Parasitol. 135, 655–660. https://doi.org/10.1016/J.EXPPARA.2013.09.016.
Possani, L.D., Zurita, M., Delepierre, M., Herna Â Ndez, F.H., Rodri, M.H., Guez, Â., Possani, L.D., Zurita, M., Delepierre, M., Hernaâ Ndez, F.H., Rodrõâ, M.H., 1998. From Noxiustoxin to Shiva-3, a peptide toxic to the sporogonic development of Plasmodium berghei. Toxicon 36, 1683–1692. https://doi.org/10.1016/S0041-0101(98)00161-5.
Prado, A.A.F., Ferreira de Almeida, G., Silva Gontijo, L., Torres, M.L.M., 2011. Toxoplasmose: o que o profissional da saúde deve saber. Encicl. Biosf. 7, 1–30. https://www.conhecer.org.br/enciclop/2011a/agrarias/toxoplasmose.pdf (accessed April 21, 2022).
Pretzel, J., Mohring, F., Rahlfs, S., Becker, K., 2013. Antiparasitic peptides. Yellow Biotechnology I: Insect Biotechnologie in Drug Discovery and Preclinical Research. SpringerNature, pp. 157–192.
Raja, Z., AndreA, S., Abbassi, F., Humblot, V., Lequin, O., Bouceba, T., Correia, I., Casale, S., Foulon, T., Sereno, D., Oury, B., Ladram, A., 2017. Insight into the mechanism of action of temporin-SHa, a new broad-spectrum antiparasitic and antibacterial agent. PLoS One 12, e0174024. https://doi.org/10.1371/JOURNAL.PONE.0174024.
Raja, Z., André, S., Piesse, C., Sereno, D., Nicolas, P., Foulon, T., Oury, B., Ladram, A., 2013. Structure, antimicrobial activities and mode of interaction with membranes of bovel phylloseptins from the painted-belly leaf frog, phyllomedusa sauvagii. PLoS One 8, e70782. https://doi.org/10.1371/JOURNAL.PONE.0070782.
Radzishevsky, I.S., Rotem, S., Zaknoon, F., Gaidukov, L., Dagan, A., Mor, A., 2005. Effects of acyl versus aminoacyl conjugation on the properties of antimicrobial peptides. Antimicrob. Agents Chemother. 49, 2412–2420. https://doi.org/10.1128/AAC.49.6.2412-2420.2005.
Rangel, M., dos Santos Cabrera, M.P., Kazuma, K., Ando, K., Wang, X., Kato, M., ichi Nihei, K., Hirata, I.Y., Cross, T.J., Garcia, A.N., Faquim-Mauro, E.L., Franzolin, M.R., Fuchino, H., Mori-Yasumoto, K., Sekita, S., Kadowaki, M., Satake, M., Konno, K., 2011. Chemical and biological characterization of four new linear cationic α-helical peptides from the venoms of two solitary eumenine wasps. Toxicon 57, 1081–1092. https://doi.org/10.1016/J.TOXICON.2011.04.014.
Saha, B., Pai, K., Sundar, S., Bhattacharyya, M., Bodhale, N.P., 2021. The drug resistance mechanisms in Leishmania donovani are independent of immunosuppression. Cytokine 145, 1043–4666. https://doi.org/10.1016/J.CYTO.2020.155300.
Sardar, A.H., Das, S., Agnihorti, S., Kumar, M., Ghosh, A.K., Abhishek, K., Kumar, A., Purkait, B., Ansari, M.Y., Das, P., 2013. Spinigerin induces apoptotic like cell death in a caspase independent manner in Leishmania donovani. Exp. Parasitol. 135, 715–725. https://doi.org/10.1016/J.EXPPARA.2013.10.011.
Savoia, D., Guerrini, R., Marzola, E., Salvadori, S., 2008. Synthesis and antimicrobial activity of dermaseptin S1 analogues. Bioorg. Med. Chem. 16, 8205–8209. https://doi.org/10.1016/J.BMC.2008.07.032.
Shibeshi, M.A., Kifle, Z.D., Atnafie, S.A., 2020. Antimalarial drug resistance and novel targets for antimalarial drug discovery. Infect. Drug Resist. 13, 4047. https://doi.org/10.2147/IDR.S279433.
Shirian, S., Oryan, A., Hatam, G.R., Daneshbod, Y., 2013. Three Leishmania/L. species–L. infantum, L. major, L. tropica–as causative agents of mucosal Leishmaniasis in Iran. Pathog. Glob. Health 107, 267–272. https://doi.org/10.1179/2047773213Y.0000000098.
Silva, T., María, Abengózar, A.N., Fernández-Reyes, M., Andreu, D., Nazmi, K., Bolscher, J.G.M., Bastos, M., Rivas, L., 2012. Enhanced leishmanicidal activity of cryptopeptide chimeras from the active N1 domain of bovine lactoferrin. Amino Acids 43, 2265–2277. https://doi.org/10.1007/s00726-012-1304-0.
Silva, P.I., Daffre, S., Bulet, P., 2000. Isolation and characterization of gomesin, an 18-residue cysteine-rich defense peptide from the spider Acanthoscurria gomesiana hemocytes with sequence similarities to horseshoe crab antimicrobial peptides of the tachyplesin family. J. Biol. Chem. 275, 33464–33470. https://doi.org/10.1074/JBC.M001491200.
Souza, G.S., do Nascimento, V.V., de Carvalho, L.P., de Melo, E.J.T., Fernandes, K.V., Machado, O.L.T., Retamal, C.A., Gomes, V.M., Carvalho, A.de O., 2013. Activity of recombinant and natural defensins from Vigna unguiculata seeds against Leishmania amazonensis. Exp. Parasitol. 135, 116–125. https://doi.org/10.1016/J.EXPPARA.2013.06.005.
Souza, G.S., P. de Carvalho, L., de Melo, E.J.T., Vieira Da Silva, F.C., Machado, O.L.T., Gomes, V.M., Carvalho, A.D.O., 2019. A synthetic peptide derived of the β 2-β 3 loop of the plant defensin from Vigna unguiculata seeds induces Leishmania amazonensis apoptosis-like cell death. Amino Acids 51, 1633–1648. https://doi.org/10.1007/s00726-019-02800-8.
Souza, A.L.A., Faria, R.X., Calabrese, K.S., Hardoim, D.J., Taniwaki, N., Alves, L.A., de Simone, S.G., 2016. Temporizin and temporizin-1 peptides as novel candidates for eliminating trypanosoma cruzi. PLoS One 11, e0157673. https://doi.org/10.1371/JOURNAL.PONE.0157673.
Soravia, E., Martini, G., Zasloff, M., 1988. Antimicrobial properties of peptides from Xenopus granular gland secretions. FEBS Lett. 228, 337–340.
Steiner, H., Hultmark, D., Engström, Å., Bennich, H., Boman, H.G., 1981. Sequence and specificity of two antibacterial proteins involved in insect immunity. Nature 292, 246–248. https://doi.org/10.1038/292246a0, 1981 292:5820.
Tanaka, T., Morshedur, R., Battur, B., Boldbaatar, D., Liao, M., Umemiya-Shirafuji, R., Xuan, X., Fujisaki, K., 2010. Parasiticidal activity of human α-defensin-5 against Toxoplasma gondii. *In Vitro* Cell. Dev. Biol. Anim. 46, 560–565. https://doi.org/10.1007/s11626-009-9271-9.
Tanaka, T., Maeda, H., Matsuo, T., Boldbattar, D., Umemiya-Shirafuji, R., Kume, A., Suzuki, H., Xuan, X., Tsuji, N., Fujisaki, K., 2012. Parasiticidal activity of Haemaphysalis longicornis longicin P4 peptide against Toxoplasma gondii. Peptides 34, 242–250. https://doi.org/10.1016/J.PEPTIDES.2011.07.027 (N.Y.).
Tang, Y., Hou, S., Li, X., Wu, M., Ma, B., Wang, Z., Jiang, J., Deng, M., Duan, Z., Tang, X., Liu, Y., Wang, W., Han, X., Jiang, L., 2019. Antiparasitic effect on Toxoplasma gondii induced by a spider peptide lycosin-I. Exp. Parasitol. 198, 17–25. https://doi.org/10.1016/J.EXPPARA.2019.01.009.
Tam, J.P., Wang, S., Wong, K.H., Tan, W.L., 2015. Antimicrobial Peptides from Plants. Pharmaceuticals 8, 711–757. https://doi.org/10.3390/PH8040711, 2015, Vol. 8, Pages 711-757.
Téné, N., Bonnafé, E., Berger, F., Rifflet, A., Guilhaudis, L., Ségalas-Milazzo, I., Pipy, B., Coste, A., Leprince, J., Treilhou, M., 2016. Biochemical and biophysical combined study of bicarinalin, an ant venom antimicrobial peptide. Peptides 79, 103–113. https://doi.org/10.1016/J.PEPTIDES.2016.04.001 (N.Y.).
Torrent, Marc, Pulido, David, Rivas, Luis, Andreu, David, 2012. Antimicrobial peptide action on parasites | Bentham Science. Curr. Drug Targets. 1138–1147. https://doi.org/10.2174/138945012802002393.
do Nascimento, V.V., De O. Mello, E., Carvalho, L.P., de Melo, E.J.T., De O Carvalho, A., Fernandes, K.V.S., Gomes, V.M., 2015. PvD1 defensin, a plant antimicrobial peptide with inhibitory activity against Leishmania amazonensis. Biosci. Rep. 248. https://doi.org/10.1042/BSR20150060.
van Hofsten P., Faye I., Kockum K., Lee J.-Y., Xanthopoulos K.G., Boman I.A., Boman H. G., Engstromt A., Andreut D., Merrifieldt R.B., Molecular cloning, cDNA sequencing, and chemical synthesis of cecropin B from Hyalophora cecropia (insect immunity/solid-phase peptide synthesis/COOH-terminal amide/cecropin B precursor/antibacterial peptides), 1985. https://www.pnas.org.
Wang, Z.H., Zhang, W., Zhang, X.Z., Yao, X.R., Huang, W., Jia, H., Liu, X.L., Hou, S.H., Wang, X.J., 2021. Development of a real-time recombinase-aided amplification (RT-RAA) molecular diagnosis assay for sensitive and rapid detection of Toxoplasma gondii. Vet. Parasitol. 298, 109489 https://doi.org/10.1016/J.VETPAR.2021.109489.
Wheeler, R.J., Gluenz, E., Gull, K., 2011. The cell cycle of Leishmania: morphogenetic events and their implications for parasite biology. Mol. Microbiol. 79, 647. https://doi.org/10.1111/J.1365-2958.2010.07479.X.
Wichroski, M.J., Melton, J.A., Donahue, C.G., Tweten, R.K., Ward, G.E., 2002. Clostridium septicum alpha-toxin is active against the parasitic protozoan Toxoplasma gondii and targets members of the SAG family of glycosylphosphatidylinositol-anchored surface proteins. Infect. Immun. 70, 4353–4361. https://doi.org/10.1128/IAI.70.8.4353-4361.2002.
World Health Organization, Leishmaniose, (2022). https://www.who.int/news-room/fact-sheets/detail/leishmaniasis (accessed June 10, 2022).
Yang, Z., Zheng, J., Chan, C.F., Wong, I.L.K., Heater, B.S., Chow, L.M.C., Lee, M.M.M., Chan, M.K., 2019. Targeted delivery of antimicrobial peptide by Cry protein crystal to treat intramacrophage infection. Biomaterials 217. https://doi.org/10.1016/J.BIOMATERIALS.2019.119286.
Zahedifard, F., Lee, H., No, J.H., Salimi, M., Seyed, N., Asoodeh, A., Rafati, S., 2020. Comparative study of different forms of Jellein antimicrobial peptide on Leishmania parasite. Exp. Parasitol. 209 https://doi.org/10.1016/J.EXPPARA.2019.107823.
Zahedifard, F., Lee, H., No, J.H., Salimi, M., Seyed, N., Asoodeh, A., Rafati, S., 2019. Anti-leishmanial activity of Brevinin 2R and its Lauric acid conjugate type against L. major: *in vitro* mechanism of actions and *in vivo* treatment potentials. PLoS Negl. Trop. Dis. 13, e0007217 https://doi.org/10.1371/JOURNAL.PNTD.0007217.
Zampa, M.F., Araújo, I.M.S., Costa, V., Nery Costa, C.H., Santos, J.R., Zucolotto, V., Eiras, C., Leite, J.R.S.A., 2009. Leishmanicidal activity and immobilization of dermaseptin 01 antimicrobial peptides in ultrathin films for nanomedicine applications. Nanomed. Nanotechnol. Biol. Med. 5, 352–358. https://doi.org/10.1016/J.NANO.2008.11.001.
Zasloff, M., 1987. Magainins, a class of antimicrobial peptides from Xenopus skin: isolation, characterization of two active forms, and partial cDNA sequence of a precursor. Proc. Natl. Acad. Sci. USA 84, 5449. https://doi.org/10.1073/PNAS.84.15.5449.
Zhang, Y., Lai, B.S., Juhas, M., Zhang, Y., 2019. Toxoplasma gondii secretory proteins and their role in invasion and pathogenesis. Microbiol. Res. 227 https://doi.org/10.1016/J.MICRES.2019.06.003.

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CERTIFICADO

Certificamos que o trabalho

Sensibilidade de isolados de Candida albicans frente ao fragmento peptídico Ctn [15-34]

cujos autores: **Gabriela Silva Cruz, Antônia Dalila Oliveira Alves, Erika Helena Salles de Brito, Gandhi Rádis Baptista**, foi submetido na modalidade **E-pôster**, na **57ª Edição do Congresso da Sociedade Brasileira de Medicina Tropical – MEDTROP 2022**, realizado no período de **13 a 16 de novembro de 2022**, no Hangar Centro de Convenções e Feiras da Amazônia na cidade de Belém, PARÁ.

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Sensibilidade de isolados de *Candida albicans* frente ao fragmento peptídico Ctn [15-34]

Introdução

A Crotalicidina (Ctn) um peptídeo alfa-helicoidal linear com 34 resíduos de aminoácidos, que pertence ao grupo dasvipericidinas e à família das catelicidinas (peptídeos antimicrobianos de vertebrados), foi caracterizada a partir da glândula de veneno da Crotalus durissus terrificus, cascavel da América do Sul, e sua atividade antiproliferativa foi demonstrada *in vitro* contra bactérias de fungos. A fração C-terminal com 20 resíduos, Ctn [15-34], mantém a atividade antimicrobiana.

Objetivo(s)

Determinar a sensibilidade de cepas de *Candida albicans* frente ao fragmento peptídico Ctn [15-34].

Material e Métodos

Para avaliar a atividade antifúngica foi empregado o teste de sensibilidade por microdiluição em caldo, de acordo com as normas do documento M27-A3 do CLSI (Clinical and Laboratory Standards Institute). O peptídeo previamente sintetizado e liofilizado foi reconstituído e diluído seriadamente em placas de poliestireno de 96 poços fundo U. Dessa forma, as concentrações finais obtidas variaram de 0,0195 μM a 40 μM. Em seguida, o inóculo fúngico foi adicionado aos poços da placa. Para os testes de sensibilidade foram utilizadas cepas de *Candida albicans*, de origem a partir de isolados clínicos ou da microbiota oral de indivíduos saudáveis e uma cepa ATCC 90028 (Candida albicans resistente ao fluconazol), totalizando 17 cepas. Esses isolados foram obtidos durante a realização de um estudo aprovado pelo Comitê de Ética em Pesquisa da UNILAB conforme protocolo CAAE: 59953716.5.0000.5576, número do parecer:1.937.092.

Resultados e Conclusão

A Concentração Inibitória Mínima (CIM) do fragmento peptídico variou entre 5 μM e 20 μM. Dentre os 17 isolados, para cinco desses, o valor de CIM foi de 5 μM; para seis cepas, incluindo a ATCC 90028, o valor de CIM foi de 10 μM; e para o restante, a CIM foi igual a 20 μM. Em ensaios realizados previamente com células eucarióticas (células renais humanas), o Ctn [15-34] demonstrou citotoxicidade apenas em concentrações mais elevadas (IC50 ≥ 50μM). Conclui-se que os isolados de Candida albicans selecionados para o teste foram sensíveis diante de baixas concentrações do fragmento peptídeo Ctn [15-34].