UNIVERSIDADE FEDERAL DO TRIÂNGULO MINEIRO

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Orientadora: Profª Drª Renata Pereira Alves Balvedi

Coorientador: Wellington Francisco Rodrigues

Uberaba

2022

Catalogação na fonte: Biblioteca da Universidade Federal do Triângulo Mineiro

Barbosa, Yanne Oliveira
B213r Revisão sistemática das moléculas presentes na saliva de carrapatos com propriedades benéficas em humanos / Yanne Oliveira Barbosa. -- 2022.
54 f. : il., graf., tab.

Dissertação (Mestrado em Ciências Fisiológicas) -- Univer - sidade Federal do Triângulo Mineiro, Uberaba, MG, 2022
Orientadora: Profa. Profa. Dra. Renata Pereira Alves Balvedi

1. Carrapatos. 2. Vetores artrópodes. 3. Proteínas. 4. Pro- dutos biológicos. 5. Saliva. I. Balvedi, Renata Pereira. II. Universidade Federal do Triângulo Mineiro. III.Título.

CDU 595.42

Mirtes Soares - Bibliotecária CRB-6/2181

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Uberaba, 30 de novembro de 2021.

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2020 e no art. 34 da Portaria Reitoria/UFTM nº 87, de 17 de agosto de 2021.

Documento assinado eletronicamente por **Vanessa da Silva Ribeiro**, **Usuário Externo**, em 08/12/2021, às 10:36, conforme horário oficial de Brasília, com fundamento no § 3º do art. 4º do Decreto nº 10.543, de 13 de novembro de 2020 e no art. 34 da Portaria Reitoria/UFTM nº 87, de 17 de agosto de 2021.

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DEDICATÓRIA

À minha vozinha Dona Alda de Oliveira, que está com Deus;

Ao tio Vitor, que está com Deus;

À minha madrinha Maria Angélica;

À minha irmã Aldinha;

Ao meu pai Enismar;

À minha mãe Cláudia;

À tia Eliete;

À minha madrasta Gláucia;

À todos que sempre torceram por mim.

AGRADECIMENTOS

Agradeço à Deus por ter me permitido chegar até aqui, nesse momento que estamos vivendo tão desafiador. Agradeço à minha família que sempre me apoiou principalmente a minha madrinha Maria Angélica sempre ao meu lado me mostrando o caminho, ao meu pai Enismar que sempre entendeu minha distância e sempre foi paciente comigo e sempre tem as palavras certas, à minha irmã Alda Vitória que é o meu alicerce, à minha mãe.

Agradeço Beatriz (Bia), por toda ajuda;

À minha orientadora Drª Renata por tanta paciência, generosidade e ensinamentos;

Agradeço a vida;

E aos demais colegas que contribuíram para esse trabalho;

Agradeço ao universo;

À UFTM, PROPPG, PPGCF, PPGMEDTROP, aos órgãos de fomento às pesquisas. À Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), pelo apoio financeiro para a realização desta pesquisa. E, o presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) - pela concessão de bolsas aos demais pesquisadores envolvidos. À CNPq, por apoiar e financiar as pesquisas brasileiras. E assim, propiciar o crescimento do país e o avanço nas pesquisas.

EPÍGRAFE

"Então lembre-se de olhar para as estrelas, e não para seus pés. Tente achar um sentido para o que vê, e pergunte-se o porquê da existência do universo. Seja curioso. E não importa o quão difícil a vida seja, há sempre algo que você pode fazer e ter sucesso. Não desista. É um tempo maravilhoso para estar vivo."

Stephen Hawking.

RESUMO

Introdução: Apesar dos prejuízos diretos e indiretos aos seus hospedeiros, carrapatos são artrópodes hematófagos que apresentam, em sua saliva, moléculas bioativas com grande potencial farmacológico para modular atividades hemostáticas e imunomoduladoras em seres humanos. **Objetivo:** Sabendo disso, esse estudo buscou realizar a busca sistematizada por biomoléculas presentes na saliva de carrapatos de maneira a descrever aquelas que apresentam interesse farmacológico publicados nos últimos dez anos. **Metodologia:** A partir da pergunta estruturada: “Há biomoléculas em saliva de carrapatos que possuam aplicações farmacológicas em humanos?”, propomos desenvolver estudo secundário. As recomendações da ferramenta Prisma foram seguidas. Os descritores foram "Arthropod Proteins”, “Tick”, “Saliva”, “Biological Products” e os sinônimos foram Tick Proteins, Tick, Salivas, Biologic Drugs, Biologic Medicines, Biologic Pharmaceuticals, Biologic Products, Biological Drugs, Biological Medicines, Biological(s), Biologic(s), Biopharmaceuticals, Drugs, Biologic, Drugs, Natural Products, Pharmaceuticals, Products. Foram elegíveis estudos com caráter sistematizado com ou sem meta-análise e não houve restrição de idioma ou país para as seleções. Foram excluídos estudos que incluíram artrópodes diferente de carrapatos e estudos que o emprego da saliva não tivesse aplicações farmacológicas. As buscas foram realizadas no período de 01/01/2010 a 25/05/2021 nas bases de dados: MEDLINE®/PubMed®, Web of Science, LILACS, EMBASE, Cochrane e SCOPUS. Além disso, publicações, consulta com especialista e publicações não convencionais foram consideradas. A qualidade metodológica dos estudos elegíveis foi avaliada pelas ferramentas disponíveis no Joanna Briggs. Todas as etapas foram conduzidas por dois ou mais avaliadores de forma independente. Os dados gerados foram tabulados e sumarizados por meio de análise qualitativa narrativa. **Resultados:** A busca inicial resultou em 170 artigos e foram selecionados 19 artigos que atenderam os critérios de elegibilidade do estudo. Relatamos quantitativamente a prevalência de espécies, biomoléculas presentes e aplicação farmacológica. A saliva de hard ticks, predominantemente encontrados nas Américas, mostrou-se mais promissora quando utilizadas em estudos experimentais com células humanas. A elucidação das biomoléculas foi possível, sendo evasina e serpina as biomoléculas com seus potenciais farmacológicos de ação anti-inflamatórias mais evidentes. **Discussão:** Nos estudos selecionados nos últimos 10 anos identifica-se a limitação, pois encontramos apenas estudos experimentais, e nenhum estudo pré-clínico ou clínico, dificultando a qualificação metodológica; bem como em alguns estudos com a biomolécula Evasina e Serpina, há apontamentos da necessidade da elucidação, das biomoléculas em questão. **Conclusão:** Sintetizamos as evidências disponíveis de que as salivas dos carrapatos duros americanos são mais estudados para aplicações farmacológicas de ação anti-inflamatória e imunomoduladoras.

Palavras-chave: saliva; carrapato; potencial farmacológico; biomoléculas salivares.

ABSTRACT

Introduction: Despite the direct and indirect damage to their hosts, ticks are hematophagous arthropods that present, in their saliva, bioactive molecules with great pharmacological potential for modulating hemostatic and immunomodulatory activities in human beings. **Objective:** Knowing this, this study sought to carry out a systematic search for biomolecules present in tick saliva in order to describe those of pharmacological interest published in the last ten years. **Methodology:** Based on the structured question: “Are there biomolecules in tick saliva that have pharmacological applications in humans?”, we propose to develop a secondary study. The Prism tool recommendations were followed. The descriptors were "Arthropod Proteins", "Tick", "Saliva", "Biological Products" and the synonyms were Tick Proteins, Tick, Saliva, Biological Drugs, Biological Drugs, Biological Pharmaceuticals, Biological Products, Biological Drugs, Biological Drugs , Biological(s), Biological(s), Biopharmaceuticals, Pharmaceuticals, Biologicals, Pharmaceuticals, Natural Products, Pharmaceuticals, Products. Original primary studies were eligible, in addition to secondary studies with a systematic character with or without meta-analysis, and there was no restriction of language or country for the selections. Studies that included arthropods other than ticks and studies that the use of saliva had no pharmacological applications were excluded. ® / PubMed®, Web of Science, LILACS, EMBASE, Cochrane and SCOPUS. In addition, publications, expert consultation and unconventional publications were evaluated. The methodological quality of eligible studies is assessed by the tools available from Joanna Briggs. All steps were independently conducted by two or more evaluators. The data generated were tabulated and summarized through qualitative narrative analysis. **Results:** The initial search resulted in 170 articles and 19 articles that meet the study's eligibility criteria were selected. We quantitatively report the prevalence of species, biomolecules present and pharmacological application. A saliva from hard ticks, predominantly found in the Americas, shows more promise when used in experimental studies with human cells. The elucidation of the biomolecules was possible, being evasine and serpin as biomolecules with their pharmacological potentials of anti-inflammatory action more evident. **Discussion:** In the studies selected in the last 10 years, the limitation is identified, as we found only experimental studies, and no pre-clinical or clinical studies, making methodological qualification difficult; as well as in some studies with the Evasina and Serpina biomolecule, there are indications of the need for elucidation of the biomolecules in question. **Conclusion:** We summarize the available evidence that the saliva of American hard ticks are more studied for pharmacological applications with anti-inflammatory and immunomodulatory action.

Keywords: Arthropod proteins; Biological products; Saliva; Tick; Tick proteins.

LISTA DE ILUSTRAÇÕES

SUMÁRIO

1 INTRODUÇÃO

Os carrapatos estão classificados na classe Arachnida, dentro da Ordem Acari, Subordem Ixodida possuem (955 espécies), são constituídos por três famílias: Nuttalliellidae que possui uma espécie, Argasidae é composta por 218 espécies e Ixodidae é a espécie em maior número com aproximadamente 736 espécies (Dantas-Torres, Filipe *et al*., 2019), vivem como ectoparasitas de vertebrados, apresentam ampla distribuição pelo mundo e são reconhecidos como importantes transmissores de doenças. Em alguns dos seus estágios de desenvolvimento os carrapatos, necessitam de sangue e se alimentam de mamíferos, pássaros, répteis e anfíbios. Dessa forma são denominados hematófagos obrigatórios, necessitam ingerir sangue para seu desenvolvimento. O ciclo de vida e o desenvolvimento populacional dos carrapatos estão relacionados à interação com seus hospedeiros, pois o que vai determinar sua sobrevivência é o desempenho ao longo da alimentação, e também sua capacidade de mudar para o próximo estádio, seu desenvolvimento, e sua reprodução.

No Brasil, diversas espécies endêmicas de carrapatos foram descritas, estão distribuídas entre vários gêneros diferentes, tais como: Argas; Antricola; Carios; Ornithodoros; Amblyomma; Dermacentor; Haemaphysalis; Ixodes e Rhipicephalus (Dantas-Torres *et al*., 2009).

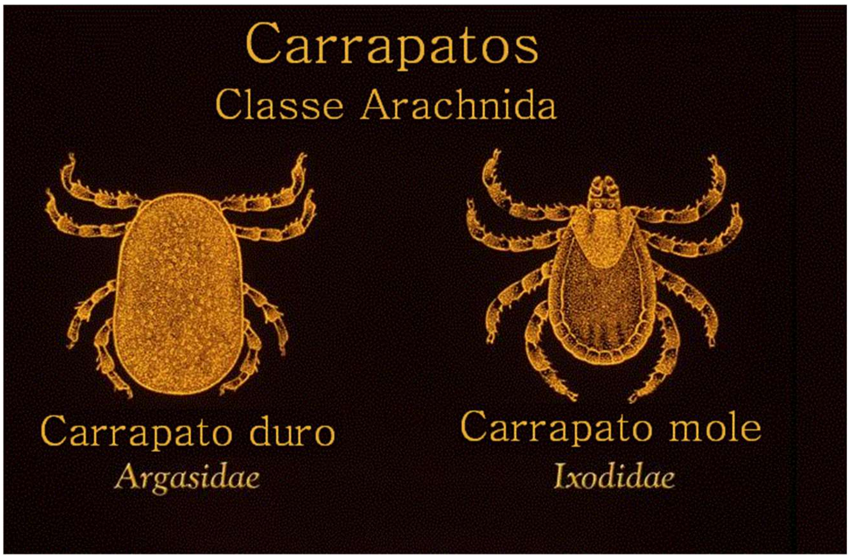
O longo período em que carrapatos da família Ixodidae permanecem fixados na pele do hospedeiro ao se alimentar permitiu que desenvolvesse diversos mecanismos reparatórios como: hemostasia, controle da inflamação e da resposta imune (Abreu, J, 2018), essas respostas dificultam a aquisição de sangue após a fixação no hospedeiro.

Os carrapatos desenvolveram uma série de mecanismos para obter com sucesso sangue de seu hospedeiro, possuem em sua saliva diversas moléculas capazes de induzir modificações fisiológicas no local da lesão tecidual no hospedeiro e concluir a ingestão do sangue. Após selecionar o local de fixação, o processo de penetração na pele do hospedeiro e sua permanecia no local leva um tempo. O carrapato corta a pele com suas quelíceras, em seguida, insere o seu hipostômio em forma espatulada, coberto por pequenos dentes, que será o canal para deposição de saliva (Figura 2). Os carrapatos ixodídeos ficam por longos períodos fixados, esse contato e a grande exposição a repetidas picadas, faz com que sejam desenvolvidas diversas respostas reparatórias sistêmicas e locais como hemostasia, respostas inflamatórias e respostas imunes (Francischetii I *et al.*, 2008).

Devem enfrentar o sistema imunológico do hospedeiro, composto por muitas células e moléculas de sinalização, principalmente citocinas e fatores de crescimento. O hospedeiro vertebrado possui mecanismos que deveriam ser eficientes contra esses artrópodes, como imunidade inata, adquirida e o sistema hemostático. Porém, a saliva desses carrapatos possui algumas ações farmacológicas, ordenadas por componentes proteicos e outras moléculas como, por exemplo, os lipídios, que desencadeiam ações vasodilatadoras, agregação plaquetária/coagulação sanguínea inibida, atividade anti-inflamatória, imunossupressora e altera a barreira endotelial refletindo na sinalização de inflamação (Tirloni L *et al.,* 2016; Francischetti I *et al*., 2008; Maritz-Olivier C *et al.,* 2007).

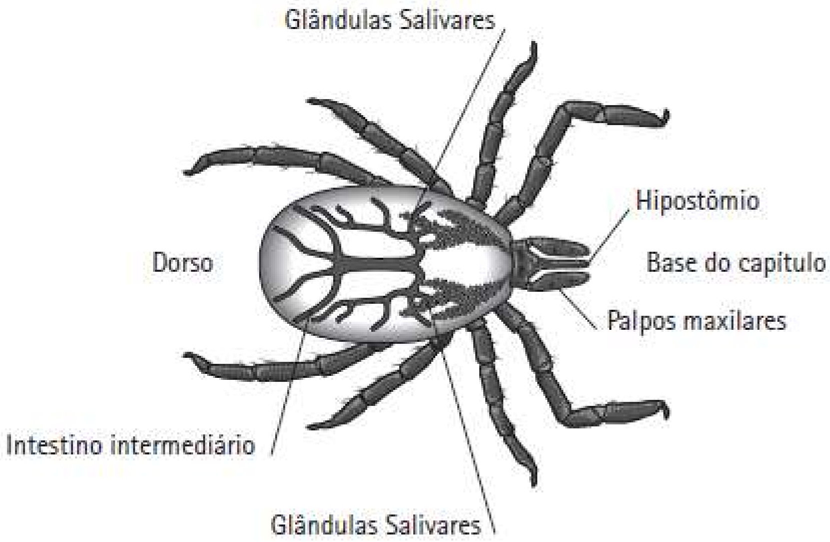
Os conhecimentos sobre os mecanismos utilizados por carrapatos para burlar os sistemas humanos, nos permite identificar quais moléculas em sua saliva, pode ser utilizados como estratégia para a busca por novos compostos farmacológicos a partir da exploração de substâncias ativas. Nesse sentido, evidências mostram que a saliva de carrapatos apresenta potente atividade imunomoduladoras e anti-hemostáticos, e outros ainda não explorados.

Figura 1: Diferenças de carrapatos duro e mole



Fonte: microbiologybook.org

Figura 2: Morfologia do carrapato

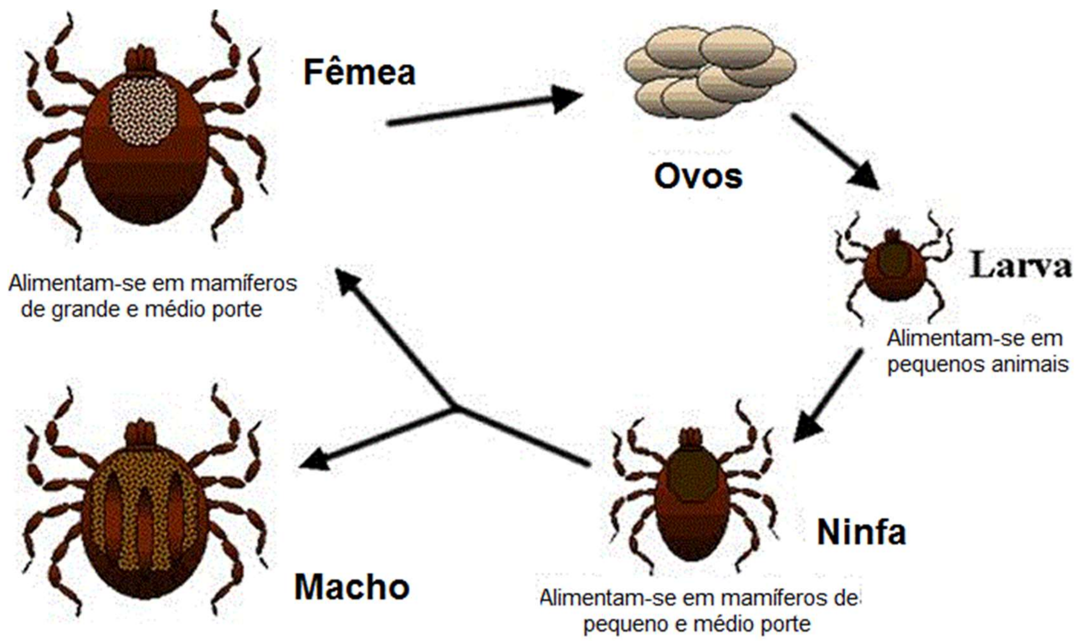


Fonte: Vestsmart

2 CICLO DE VIDA

Os carrapatos possuem um ciclo de vida que incluí a fase de ovo, larva, ninfa e adulto. Após o ingerir o sangue do hospedeiro a fêmea teleógina ingurgitada (alimentada) se desprende do mesmo, depositando milhares de ovos, no ambiente e logo em seguida morre dando inicio a um novo ciclo. Desses ovos, que dependem das condições ambientais para eclodir dão origem as larvas (fase de muda), estas após a maturação, sobem em capins, folhas baixas até entrarem em contato com possível hospedeiro e se fixa a ele. No hospedeiro as larvas realizam o repasto sanguíneo e ficam complemente ingurgitadas, as larvas se desprendem dando origem a fase de ecdise e assim surgem as ninfas que assim como as larvas também ficam à espera de um hospedeiro se alimentam e assim realizam novamente a fase de ecdise para o período adulto onde irão se fixar novamente no hospedeiro para se alimentar e assim fazer o depósito de ovos (Rodrigues *et al.*, 2015) (Figura 3).

Figura 3. Ciclo biológico de grande parte dos carrapatos.



Fonte: Extinset, 2021

3 HEMATOFAGIA E A SALIVA DE CARRAPATOS

A hematofagia é uma das fases mais complicadas para o carrapato, eles precisam localizar a fonte alimentar e penetrar suas peças bucais na pele para obter o sangue sem que o hospedeiro o note. Os carrapatos principalmente os ixodídeos ficam muito tempo em contato com seus hospedeiros, com isso faz com que sejam desenvolvidas diversas respostas reparatórias sistêmicas e locais como hemostasia, respostas inflamatórias e respostas imunes (Valenzuela, 2005). Essas respostas podem dificultar o processo de aquisição de sangue, assim, a glândula salivar do carrapato produz a saliva, um fluido composto por várias moléculas farmacologicamente ativas que quando injetadas no hospedeiro, produzem modificações fisiológicas no local da picada que vão desencadear no hospedeiro diversas reações e garantir que o artrópode consiga ingerir sangue (Francischetti *et al*., 2009).

Inicialmente as secreções salivares inibe a coagulação do sangue, dilatam os capilares da pele suprime as respostas inflamatórias do hospedeiro, estimula o sistema imunológico do hospedeiro e interage com os componentes do sistema imunológico. Os carrapatos ixodídeos possuem uma alimentação lenta isso devido à necessidade de produzir uma nova cutícula para acomodar o volume cada vez maior de sangue. Ao se alimentar a fêmea totalmente alimentada fertilizada pode pesar de 100 a 120 vezes seu peso original. A produção de anticorpos, o sistema complemento, linfócitos T e células apresentadoras de antígenos são respostas do hospedeiro à essa alimentação dos carrapatos, no entanto muitos hospedeiros desenvolvem uma resposta imunológica a exposições repetidas a carrapatos. O processo de alimentação de carrapatos de corpo mole é relativamente rápido em comparação com carrapatos de corpo duro. Os carrapatos de corpo mole começam a se alimentar de sangue quase imediatamente após a fixação, não secretam cimento, não formam uma nova cutícula (Anderson & Magnarelli, 2008).

3.1 AÇÕES FARMACOLÓGICAS

As principais ações farmacológicas identificadas são modulação de resposta anti-inflamatória e anticoagulante, inicialmente, para obter grande fluxo sanguíneo no local de fixação, os carrapatos secretam na saliva compostos lipídicos que são capazes de provocar a dilatação dos vasos sanguíneos. A capacidade da saliva de carrapatos de inibir a agregação plaquetária decorre da presença de moléculas específicas que bloqueiam a interação entre plaquetas, inibindo assim o processo de agregação, ou da presença de proteínas com atividade enzimática que destroem ou inibem os agonistas que ativam a agregação. As salivas de carrapatos possuem em uma composição moléculas com atividade anticoagulante inibindo moléculas de trombina ou de fator Xa, os inibidores de coagulação se alternam em mecanismo de ação, variando desde pequenos peptídeos a moléculas maiores, como proteínas pertencentes à família Kunitz e à família das serpinas (MARITZ-OLIVIER *et al*., 2007).

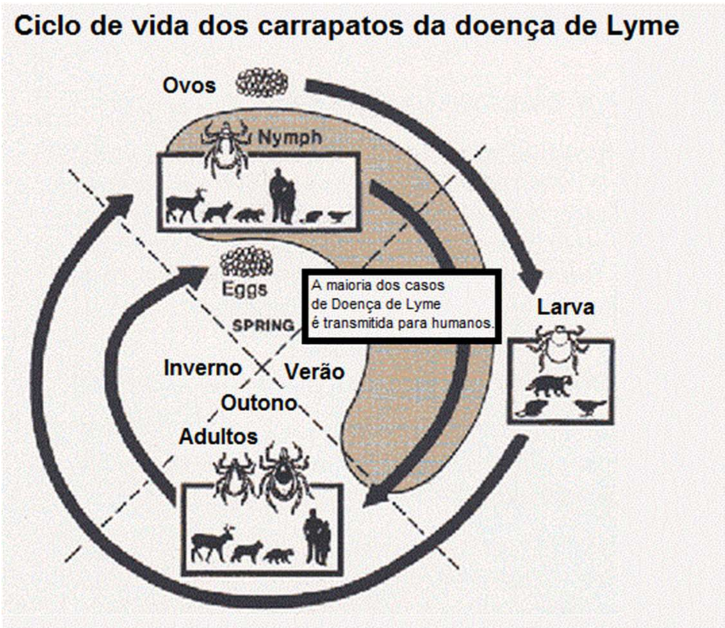
A inflamação ocorre como resposta em decorrência de uma injúria local, envolvendo células de defesa, quimiocinas, enzimas, mediadores inflamatórios lipídicos e citocinas. (FRANCISCHETTI *et al*., 2009).

3.2 PROBLEMAS PARA SERES HUMANOS

Os carrapatos além de possuir em sua saliva benefícios, os malefícios estão presentes também para seres humanos. Sabe-se que em sua saliva é possível a transmissão de patógenos, apesar de as picadas da maioria dos carrapatos não apresentarem consequências, elas podem trazer uma quantidade de agentes causadores de doença humana como vírus, bactérias e até protozoários.

Uma das doenças mais conhecidas é a doença de Lyme, é causada pela bactéria *Borrelia burgdorferi* (figura 4) que infecta mamíferos nos Estados Unidos, são transmitidos para humanos pelos carrapatos *Ixodídeos* de pernas pretas (carrapatos do veado).

Figura 4: Transmissão da Doença de Lyme



Fonte: microbiologybook.org

Há outras doenças bacterianas como a febre maculosa da montanha rochosa, atingem crianças abaixo de 15 anos. É causada pela bactéria *Ricketsia rickettsii*, é transmitida pelo carrapato marrom do cão e o carrapato da madeira da Montanha Rochosa (os dois são espécies *Dermacentor* dos Estados Unidos). A tularemia é uma doença também transmitida pelas duas espécies de Dermacentor, é causada pela bactéria *Francisella tularensis*.

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APÊNDICE A – Artigo submetido

Biomolecules with pharmacological potential present in tick saliva: a systematic review

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All authors read and approved the final manuscript.

Note: Supplementary data associated with this article

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Abstract

Ticks have bioactive molecules in their saliva with great pharmacological potential to modulate hemostatic and immunomodulatory activities in humans. A systematic search was carried out for biomolecules present in tick saliva and show those of pharmacological interest published in the last ten years. A secondary study was developed following the Prisma tool recommendations. Original primary studies were used, in addition to secondary studies with a systematic character with or without meta-analysis, and there was no language or country restriction for these choices. Studies that included arthropods other than ticks and studies that the use of saliva had no pharmacological application were excluded. Searches were performed in the following databases: MEDLINE®/PubMed®, Web of Science, LILACS, EMBASE, Cochrane and SCOPUS. In addition, specialist orientations and unconventional publications were considered. The methodological quality was performed using the tools available at Joanna Briggs. All steps were conducted by two or more independent evaluators. The data generated was charted and summarized through qualitative narrative analysis. The initial search resulted in 170 articles and 19 articles that met the study's eligibility criteria were selected. We quantitatively report the prevalence of species, biomolecules present and pharmacological application. The saliva of hard ticks, found in the Americas, are more promising when used in experimental studies with human cells. The elucidation of the biomolecules was possible, with evasin and serpin being the biomolecules with the most evident pharmacological potentials for anti-inflammatory action. In the selected studies we found only experimental studies, and no pre-clinical or clinical studies, making methodological qualification difficult; in some studies, with the Evasin and Serpin biomolecule, it suggested the need for elucidation of these biomolecules in question. We summarized the available evidence that the saliva of American hard ticks is the most

studied for pharmacological applications of anti-inflammatory and immunomodulating action.

Keywords: saliva, tick, pharmacological potential, salivary biomolecules.

1. Introduction

Ticks are distributed in the Arachnida class, Acari order, of the suborder Ixodida and 955 species have already been listed. The tick species are distributed into three families: Nuttalliellidae, which has only one species, Argasidae (soft tick) which is composed of 218 species and Ixodidae (hard tick) with approximately 736 species (Dantas et al; 2019), they are obligatory hematophagous arthropods that feed repeatedly by minutes, hours, days, or weeks on their hosts (Francischetti et al; 2009). Due to this, to successfully obtain blood from their host, these invertebrate ectoparasites have developed a series of mechanisms that bypass vertebrate defenses. Among these mechanisms, we can highlight the production of saliva, which is a secretion rich in components that favor the success of blood acquisition and the perpetuation of its host's tick. In the vertebrate host, the insertion of the oral tract triggers the recruitment of defense cells and the production of chemokines, lipid inflammatory mediators and cytokines (Francischetti et al; 2009). The presence of secreted saliva, exactly where the tick's mouthparts are fixed, is the main reason for its permanence, since it is where the cells and molecules of the host act precisely (Tatchell; 1967).

Just as researchers and pharmaceutical companies seek to discover synthetic or plant-derived bioactive molecules, they also seek to find molecules derived from vertebrate and invertebrate animals. In the case of hematophagous arthropods, such as ticks, mosquitoes, sandflies and triatomines, it is known that they are capable of producing and secreting potent bioactive molecules. Among these, there`s no doubt that ticks are the species with the best-known molecules and with a greater number of activities that have already been previously determined. However, there still hasn`t been any careful evaluation of how many molecules that have already been identified to show a potential effect on the biology of human cells and molecules. For this reason, our proposal is to identify the main biomolecules existing in the saliva of ticks studied in the last decade in order to evaluate their interaction routes and potential pharmacological actions in human cells and molecules.

2. Materials and methods

This is a secondary study developed through a systematic review, following the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 (Haddaway and McGuinness; 2020).

The study used a structure question with the aid of the acronym POT (P = population, O = outcome, T = type of study), being: P = Biomolecules with pharmacological activity present in tick saliva; O = type of activity; T = primary and secondary studies (only systematic review with or without meta-analysis), assisting in the stages of development of the methodological protocol.

2.1 Eligibility criteria

The criteria for inclusions were: types of original primary studies, in addition to secondary studies with a systematic character with or without meta-analysis, which covered the period of the last decade (2010 to 2021). For the present study, there were no language or country restrictions for the selections.

On the other hand, studies that were duplicated or that included only arthropods other than ticks, studies in which the use of tick saliva did not have pharmacological applications and, finally, *in silico* studies (bioinformatics) were excluded.

2.2 Information sources

The searches took place from 01/01/2010 to 05/25/2021, defining that for conventional publications the following 05 databases would be used: Medical Literature Analysis and

Retrieval System Online (MEDLINE/PubMed), Web of Science, Latin American and Caribbean Health Sciences Literature (LILACS), Excerpta Medica Abstract Journal (EMBASE), Cochrane Library (Cochrane) and SciVerse Scopus.

For evaluation and the search in gray literature, unconventional publications, the following were investigated: Theses and dissertations cataloged by the Coordination for the Improvement of Higher Education Personnel (CAPES) and a detailed search on the topics indicated and suggested by the Gray's Matter manual (Grey Matters; 2020). In the theses and dissertations cataloged in the 04 most referenced universities in the study of ticks: Federal University of Triângulo Mineiro (UFTM), Federal University of Uberlândia (UFU), University of São Paulo (USP) and Osvaldo Cruz Foundation (FIOCRUZ). In addition, a consultation with an expert (CJFO) was carried out to evaluate the results, and to adjust the search strategy. A consultation was also carried out with the librarian on 08/26/2021, to confirm the searches and results, we closed the date of analysis of the articles 2010 on 05/25/2021.

2.3 Search strategy

To ensure accuracy in the search, descriptors and synonyms were established after searching the Medical Subject Headings (MeSH®).

Based on the structure question "Are there biomolecules in tick saliva that have pharmacological applications in humans?" descriptors and synonyms were selected. The descriptors were "Arthropod Proteins", "Tick", "Saliva", "Biological Products" and the synonyms were Tick Proteins, Tick, Salivas, Biologic Drugs, Biologic Medicines, Biologic Pharmaceuticals, Biologic Products, Biological Drugs, Biological Medicines, Biological (s), Biologic(s), Biopharmaceuticals, Drugs, Biologic, Drugs, Natural Products, Pharmaceuticals, Products.

The search strategy was adapted for each type of base evaluated. The Boolean operators "or" and "and" were used to guarantee the proper associations. The search key was generated automatically and below is an example used in the Medline/Pubmed database: "Ticks"[Mesh] OR Ticks OR Tick OR Ixodida OR Ixodidas AND "Saliva"[Mesh] OR Saliva OR salivas AND "Arthropod Proteins"[Mesh] OR (Arthropod Proteins) OR (Tick Proteins) AND "Biological Products"[Mesh] OR (Biological Products) OR (Products, Biological) OR (Biological Product) OR (Product, Biological) OR (Biological Product) OR (Product, Biologic) OR (Biologic Products) OR Biopharmaceutical OR Biopharmaceutical OR Biological OR Biological OR (Biological Drug) OR (Drug, Biological) OR (Biologic Drugs) OR (Drugs, Biologic) OR (Biological Medicine) OR (Medicine, Biological) OR (Biological Medicines) OR (Medicines, Biological) OR Biologicals OR (Biologic Medicines) OR (Medicines, Biologic) OR (Biologic Pharmaceuticals) OR (Pharmaceuticals, Biologic) OR Biologics OR (Biologic Drug) OR (Drug, Biologic) OR (Biological Drugs) OR (Drugs, Biological) OR (Natural Products) OR (Natural Product) OR (Product, Natural) filter=years.2010 – 2021 (Supplementary material A).

2.4 Study selection

The study selections were carried out independently by two researchers (Y.O.B. and C.M.A.R) and the disagreements were resolved by consensus and, when necessary, a third evaluator with broad experience for decision-making (R.P.A.B) was included. The kappa coefficient for agreements was used to determine possible significant variations between the evaluators in different stages.

Articles were first selected based on their titles and abstracts, and those that were duplicates were excluded. Then, the complete essays were independently evaluated by the evaluators, and those that met the eligibility criteria were selected for this study.

2.5 Extraction of the data

Data from the selected studies were entered into a previously standardized Excel spreadsheet (Microsoft®), following the selection of the independent evaluators (Y.O.B. and C.M.A.R) and checked, when necessary, by a third evaluator (R.P.A.B.) (Supplementary material B).

The analyzed data to be extracted from the eligible studies were study type, tick taxonomy, the place of origin of the tick, techniques used, anatomical parts that were extracted from the tick, molecules involved with pharmacological activity, promoted action and the methodology used.

2.6 Methodological quality assessment

Parameters linked to the methodological quality of the selected studies were carefully evaluated for all the selected studies. The recommendations from the Joanna Briggs; 2020 tools and the Checklist for Analytical Cross Sectional Studies form were followed (Supplementary material C).

For each study, a percentage of achievement was assigned in the topics that were suggested by the tool that was used, so that studies that met all quality topics were assigned 100% achievement, and reductions were associated with absences in the description and/or non-clear descriptions.

2.7 Data analysis

The data was charted in Microsoft® Excel and for the analysis and visual display of the data, the “Prism” program from Graphpad version 8.0 was also used. A qualitative narrative synthesis was carried out with the exposure of absolute (number) and relative (percentage) frequencies. Associations were assessed using the Chi-square test. For the temporal correlation of the frequencies of scientific production, the Person correlation test was used, after the notice of normality by the D'Agostino & Pearson test. The significance level used for all assessments was of 5% (Arango; 2001).

2.8 Records

The results pointed out in this systematic review are recorded at https://osf.io/yjuar/

3. Results

3.1 Study Selection

The databases initially presented 170 articles and no records. With individual analysis, we observed that there were 26 duplicated articles. Among the 144, all titles and abstracts were read and the inclusion and exclusion criteria was applied. 125 articles were excluded for not being related to the topic, and none of them could have been used.

After completely reading the 45 studies, 26 were excluded for the following reasons: narrative reviews (n = 10), (n = 5) articles were analyzed exclusively by bioinformatics and did not relate to the topic (n = 11).

Finally, the systematic search resulted in the selection of 19 articles according to the criteria established in the methodology and presented in detail in Figure 1 – flowchart.

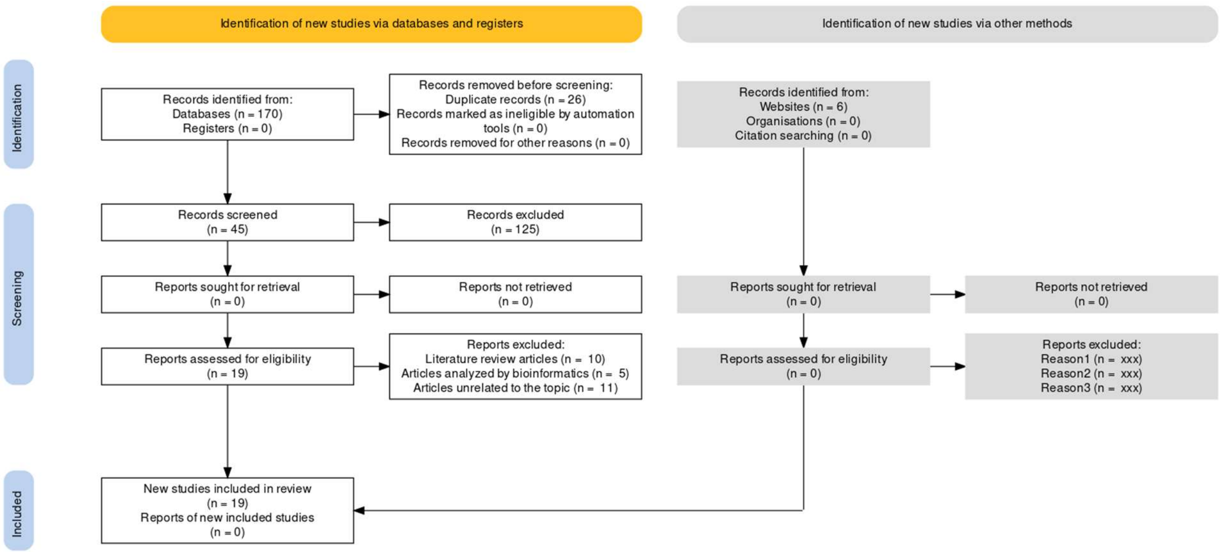

Fig. 1. Flowchart belonging to the identification of the studies collected in database/records (in yellow) and Identification of studies collected by other methodologies (in gray).

3.2 Characteristics of the studies

After selecting the eligible studies, data such as: author and year, title, database, journal of publication, type of study and methodological quality were set in place in Table 1.

Among the 05 databases evaluated, it was possible to identify that those eligible studies are in 02: Medline/Pubmed (n = 15 - 79%) and Web of Science (n = 4 - 21%).

In the evaluated articles, no preclinical or clinical studies were found. On the other hand, 79% of the studies used 2 or more study designs and 21% only used one. Assessing the study models separately these strategies were identified: in vitro (n = 16, 43%), in vivo (n = 13, 35%) and *in silico* (n = 8, 22%).

All selected studies were evaluated for methodological quality. The data showed a variation between “moderate” (minimum value found = 62.50% of achievement) to “high” (100% of achievement). In the assessment of methodological quality, there was an average and standard deviation of quality achievement of 95 ± 10.18 (%). Of the 19 selected studies, 13 (68.42%) had the maximum achievement (100%) and only one study had the lowest achievement (62.5%).

Table 1. Characteristic of studies and methodological quality. *PM: Pubmed/ WS: Web of Science

Author / Year	Study	Data base	Journal/periodical	Type of study	Methodological quality
28 (2010)	A new Factor Xa inhibitor from *Amblyomma cajennense* with a unique domain composition.	PM	Elsevier - Archives of Biochemistry and Biophysics	In vitro, in vivo and in silico experimental study	93,75%
18 (2011)	Ixodid tick salivary gland products target host wound healing growth factors	PM	Elsevier - International Journal for Parasitology	In vitro, in vivo and in silico experimental study	62,50%
24 (2011)	The action of *Amblyomma cajennense* tick saliva in compounds of the hemostatic system and cytotoxicity in tumor cell lines	PM	Elvesier - Biomedicine e Pharmacotherapy	In vitro experimental study	100%
43 (2011)	Deconstructing tick saliva: non-protein molecules with potent immunomodulatory properties	WS	Jounal of Biological Chemisty	In vivo and in vitro experimental study	100%
12 (2013)	Evasin-4, a tick-derived chemokine-binding protein with broad selectivity can be modified for use in preclinical disease models	PM	The Febs Journal	In vivo and in vitro experimental study	90%
39 (2013)	Characterization of Ixophilin, A Thrombin Inhibitor from the Gut of *Ixodes scapularis*	PM	Plos One	In vivo and in vitro experimental study	100%
33 (2014)	Antihistamine response: a dynamically refined function at the host-tick interface	PM	Parasites and Vectors	In silico observational study	75%
41 (2014)	Longistatin in tick saliva blocks advanced glycation end-product receptor activation	PM	JCI - The Journal of Clinical Investigation	In vivo and in vitro experimental study	100%
20 (2015)	Effective inhibition of thrombin by *Rhipicephalus microplus* serpin-15 (RmS-15) obtained in the yeast *Pichia pastoris*	WS	Ticks and Tick-borne Diseases	In vivo experimental study	87,50%
21 (2015)	*Rhipicephalus microplus* serine protease inhibitor family: annotation, expression and functional characterisation assessment	WS	Parasites and vectors	In vivo and in vitro experimental study	100%
16 (2017)	Yeast surface display identifies a family of evasins from ticks with novel polyvalent CC chemokine-binding activities	PM	Nature – scientific reports	In vivo and in silico experimental study	100%
34 (2017)	Avathrin: a novel thrombin inhibitor derived from a multicopy precursor in the salivary glands of the ixodid tick, *Amblyomma variegatum*	PM	The Faseb	In vitro, in vivo and in silico experimental study	100%
40 (2018)	Ixonnexin from Tick Saliva Promotes Fibrinolysis by Interacting with Plasminogen and Tissue-Type Plasminogen Activator, and Prevents Arterial Thrombosis	PM	Nature Scientific Reports	In vivo and in vitro experimental study	100%
23 (2019)	Antitumoral effects of *Amblyomma sculptum Berlese* saliva in neuroblastoma cell lines involve cytoskeletal deconstruction and cell cycle arrest	PM	Brazilian Journal of Veterinary Parasitology	In vitro experimental study	100%
30 (2019)	The immunosuppressive functions of two novel tick serpins, HlSerpin-a and HlSerpin-b, from *Haemaphysalis longicornis*	PM	Immunology	In vitro, in vivo and in silico experimental study	100%
36 (2019)	Immunosuppressive effects of sialostatin L1 and L2 isolated from the taiga tick *Ixodes persulcatus Schulze*	PM	Elsevier - Ticks and Tick-borne Diseases	In vivo and in vitro experimental study	100%
37 (2019)	The structure and function of Iristatin, a novel immunosuppressive tick salivary cystatin	PM	Cellular and Molecular Life Sciences	In vivo, in vitro and in silico experimental study	100%
42 (2019)	Rapid assembly and profiling of an anticoagulant sulfoprotein library	WS	PNAS - Proceedings of the National Academy of Sciences of the United States of America	In vitro and in silico experimental study	93,75%
17 (2020)	Semisynthesis of an evasin from tick saliva reveals a critical role of tyrosine sulfation for chemokine binding and inhibition	PM	PNAS - Proceedings of the National Academy of Sciences of the United States of America	In vitro and in silico experimental study	100%

Figure 2 shows the distribution of studies that evaluated bioactive molecules for pharmacological application between the periods of 2010 to 2021, and it was observed that annually it had an average percentage of 8.33 with a frequency of 1.33 studies. With these findings, it was not possible to identify a significant time correlation in the frequency of studies with the theme (p>0.05).

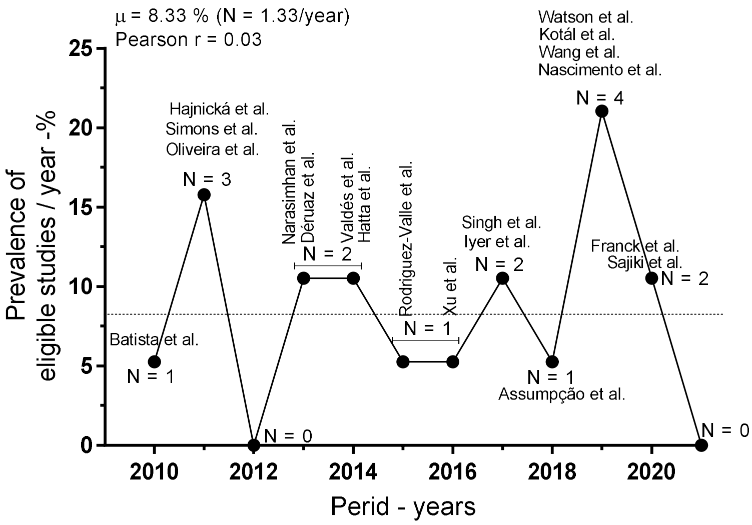

Fig. 2. Time correlation of studies that evaluated the pharmacological action of biomolecules extracted from tick saliva.

3.3 Report biasing

It was possible to qualify the distribution of tick genera and species evaluated in experimental studies for the application of saliva as a therapeutic form, as shown in Table2. The selected articles allowed the extraction of the following data: there are three genera of ticks, all of the Ixodidae family (hard tick) whose saliva are promising in pharmacological actions, since they contain bioactive compounds. The prevalence of the genera and respective species with the highest prevalence can be observed: *Amblyoma sp* in 31.03% (*Amblyomma cajennense* 13.79% cited), *Rhipicephalus sp* 27.59% (*Rhipicephalus sanguineus* 10.34% cited) and *Ixodes sp* 20, 69% (*Ixodes scapularis* 10.34% cited), *Haemaphysalis sp* 10.34% (Haemaphysalis longicornis 10.34%), *Dermacentor sp 6.90% (Dermacentor andersoni* and Dermacentorreticulatuse 3.45% each) and *Hyalomma sp.*3.45% (*Hyalomma marginatum rufipes* with 3.45%).

Genus	Prevalence % (n)	Species	Prevalence % (n)	Region	Study
Amblyomma sp.	31.03 (9)	*Amblyomma cajennense*	13.79 (4)	Brazil and Mexico	Batista et al., 2010, Simons et al., 2011, Franck et al.,2020, Singh et al.,2017
		Amblyomma maculatum	3.45 (1)	Brazil and Mexico	Singh et al.,2017
		Amblyomma parvum	3.45 (1)	Brazil and Mexico	Singh et al.,2017
		Amblyomma sculptum	3.45 (1)	Brazil	Nascimento et al.,2019
		Amblyomma variegatum	6.90 (2)	Africa and Slovakia	Hajnická et al.,2011, Iyer et al., 2017
Dermacentor sp	6.90 (2)	*Dermacentor andersoni*	3.45 (1)	United States	Watson et al.,2019
		Dermacentor reticulatus	3.45 (1)	Western Asia and Europe	Hajnická et al.,2011
Haemaphysalis sp.	10.34 (3)	*Haemaphysalis longicornis*	10.34 (3)	Australia and Asia	Wang et al.,2016, Anisuzzaman et al.,2014, Watson et al.,2019
Hyalomma sp.	3.45 (1)	*Hyalomma marginatum rufipes*	3.45 (1)	Africa, Asia and Europe	Watson et al.,2019
Ixades sp.	20.69 (6)	*Ixodes persulcatus*	3.45 (1)	Europe, China and Japan	Sajiki et al.,2020
		Ixodes ricinus	6.90 (2)	Europe and Asia	Hajnická et al.,2011, Kotál et al.,2019,
		Ixodes scapularis	10.34 (3)	United States, Canada and Slovakia	Hajnická et al.,2011, Narasimhan et al.,2013, Assumpção et al 2018
Rhipicephalus sp.	27.59 (8)	*Rhipicephalus (Boophilus) microplus*	6.90 (2)	Australia, South Africa and South America	Tao et al.,2016, Rodriguez-Valle et al.,2015
		Rhipicephalus appendiculatus	6.90 (2)	Africa	Hajnická et al.,2011, Valdés 2014
		Rhipicephalus pulchellus	3.45 (1)	Africa	Singh et al.,2014
		Rhipicephalus sanguineus	10.34 (3)	Brazil	Déruaz et al.,2013, Singh et al.,2017, Oliveira et al.,2011
Total	100 (29)		100 (29)		

Table 2 - Prevalence of species and regions of ticks selected in the selected articles.

It can also be observed that studies involving species with a prevalence higher than 10% converge to the statement that used ticks from the regions of Brazil, Mexico, United States, Canada, Slovakia, Asia, Africa and Australia.

To better assess these regional data, Table 3 shows the continental prevalence of ticks, whose saliva has possible pharmacological usage. It is interesting to note the predominance and frequency of ticks found in America, followed closely tied by Europe and Asia; followed by Africa and with lower prevalence in Oceania.

Genus	Continent					Total	P-value
	America	Europe	Asia	Africa	Oceania		
Amblyomma sp.	4 (66.67)	1 (16.67)	0 (0.00)	1 (16.66)	0 (0.00)	6 (100)	<0.0001
Dermacentor sp.	1 (33.33)	1 (33.33)	1 (33.34)	0 (0.00)	0 (0.00)	3 (100)	
Haemaphysalis sp.	0 (0.00)	0 (0.00)	1 (50.00)	0 (0.00)	1 (50.00)	2 (100)	
Hyalomma sp.	0 (0.00)	1 (33.33)	1 (33.33)	1 (33.34)	0 (0.00)	3 (100)	
Ixades sp.	2 (25.00)	3 (37.50)	3 (37.50)	0 (0.00)	0 (0.00)	8 (100)	
Rhipicephalus sp.	2 (33.33)	0 (0.00)	0 (0.00)	3 (50.00)	1 (16.67)	6 (100)	
Total	9 (158.33)	6 (120.83)	6 (154.17)	5 (100.00)	2 (66.67)	28	

Table 3. Continental prevalence that assesses possible pharmacological applications for tick saliva.

Corresponding to the characteristics of the samples used through Table 4. We can stratify that 78.95% of the studies used human material, 68.42% used animals (non-humans) and 47.37% used unicellular organisms and other types of cells. It is noteworthy that the interaction of human and non-human materials (a+b) occupied third place in this prevalence, 57.89%, followed by the interaction of human materials and unicellular organisms (a+c) and non-human materials and unicellular organisms (b+c) with 31.58%.

Sample characteristic	Events (N)	Prevalence - % (N = 19)
Human - (a)	15	78.95
Non-human animal - (b)	13	68.42
Unicellular organism - (c)	9	47.37
"In silico" - (d)	4	21.05
(a+b)	11	57.89
(a+c)	6	31.58
(a+d)	2	10.53
(b+c)	6	31.58
(b+d)	2	10.53
(c+d)	3	15.79
(a+b+c)	4	21.05
(a+b+d)	2	10.53
(a+c+d)	2	10.53
(b+c+d)	2	10.53
(a+b+c+d)	2	10.53

Table 4. Stratification of the characteristics of the samples used.

Table 5 summarizes the molecules shown in the studies as present in the saliva of ticks capable of interacting and acting on various molecules in the human body. Molecules listed in the studies include enzymes, cytokines, components of the complement system, antibodies, cell signaling molecules, and immune cell receptors.

In selected studies and charted data, we can observe the prevalence of biomolecules and their respective pharmacological actions: Evasine (21.05%) that binds to host chemokines as an anti-inflammatory strategy; Serpin (15.79%) that acts as a protease inhibitor, also relating them to anti-inflammatory action. Assessing gross saliva (10.53%), the studies point to a possible antitumor and anticoagulant pharmacological action. The other biomolecules presented at lower frequencies (5.26%) had other specific actions such as: Amblyomin-X indicating anticoagulant and protease inhibitor action; Lipocalin with antihistamine action; Avatrin with anti-hemostatic activity; Ixophyllin: anticoagulant/thrombin inhibitor; Sialostatin with immunosuppressive and anti-inflammatory action; Iristatin: immunosuppressive; Ixonexin: possible anticoagulant; Longistatin: suppressor of adhesion molecule expression, cytokine secretion, prevention of NF-κB translocation, and reduction of cellular oxidative stress; Sulfoproteins: anticoagulant; Ado and PGE2 with important immunomodulating and inflammatory

responses modulating action.

Biomolecule	**Pharmacological Action**	**Prevalence %**
Evasin	Blocking / chemokine function, Anti-inflammatory (inhibition of chemokine), Action on inflammatory diseases.	21.050%
Serpin	Action on inflammatory diseases, Protease inhibition	15.790%
Crude saliva	Antitumor action, Cytotoxic effects; Induction of cell death in cancer cell lines; Coagulation systems;Fibrinolysis and action on platelet aggregation; Factor Xa and inhibition of thrombin.	10.530%
Amblyomin-X	Anti-coagulant; Protease inhibitor.	5.263%
Lipocalin	Antihistamine	5.263%
Avatrina	Anticoagulant (thrombin inhibitor); Anti-hemostatic compounds	5.263%
Sialostatin	Immunosuppressant; Anti-inflammatory	5.263%
Ixophylline	Anticoagulant action (thrombin inhibitor)	5.263%
Ixonnexin	Anticoagulant	5.263%
Iristatin	Immunosuppressant	5.263%
Longistatin	Suppression of the expression of adhesion molecules Cytokine secretion; Prevention of NF-κB translocation; Reduction of cellular oxidative stress.	5.263%
Sulfoproteins	Anticoagulant	5.263%
Ado e PGE2	Modulation of immune and inflammatory responses	5.263%
Total		100%

Table 5. Prevalence of biomolecules

Table 6 shows the distribution focused on the potential pharmacological activities of tick saliva in different types of pathologies (target diseases). You can observe the prevalence of 42.11% on the selected studies, identifying molecules with anticoagulant activity (thrombin blockers). This pharmacological action is important in pathologies such as thrombosis, thromboembolism and stroke. Bioactive molecules with anti-inflammatory action were identified in the same percentage, which can help in therapies for pro-inflammatory pathologies such as arthritis. Anti-tumor and immunosuppressive pharmacological actions prevailed in 10.53% of the articles and in 5.26% anti-platelet aggregation, antihistamine and protease inhibitor actions. In combined/accumulated evaluation, we observed a greater tendency of studies to point out the use of tick saliva in pharmacological actions in inflammatory diseases (63.16%), followed by hemostatic activity (47.37%).

Pharmacological action	**Target diseases**	**Prevalence - %**
Anticoagulant (blocks thrombin)	Thrombosis, thromboembolism, Brain stroke	42.11
Platelet anti-aggregation	Essential thrombocythemia, Polycythemia Vera	5.26
Antihistamine	Hypersensitivity (Allergies, Asthma)	5.26
Anti-inflammatory	Pro-inflammatory diseases (Arthritis, etc.)	42.11
Antitumor activity	Cancer	10.53
Imunossupressors	Autoimmune diseases	10.53
Protease inhibition	Acute pancreatitis	5.26
		Accumulated prevalence - %
Hemostatic activity	Thrombosis, thromboembolism, Brain stroke Essential thrombocythemia, Polycythemia Vera	47.37
Inflammatory diseases	Hypersensitivity (Allergies, Asthma) Pro-inflammatory diseases (Arthritis, etc.) Autoimmune diseases, Acute pancreatitis	63.16

Table 6. Potential pharmacological activities for tick saliva in different types of pathologies.

4. Discussion

Pubmed is a search engine with open access to the MEDLINE database that contains publications of research articles in biomedicine. MEDLINE has around 4,800 magazines published in the United States and in more than 70 countries around the world and has been active from 1966 to the present day. Web of Science is a website that provides subscription-based access to 6 online databases that provide comprehensive information and citations for many different academic disciplines. LILACS is a health-specific database that provides articles published in 26 countries in Latin America and the Caribbean, important in our region. COCHRANE is a collection of databases focused on the health area with high quality evidence and also has a specific database for systematic reviews. EMBASE a database for pharmacological and biomedical articles covers a range of international publications. SCOPUS is a database where you can find a vast literature of publications such as articles, books, scientific journals and events in the most diverse areas such as: health, arts, medicine and others.

Of these, at least five are mandatory for the study to be carried out in a systematic review and this has assigned six bases to compose the search strategies. For this reason, the journals found in these databases were chosen because they relate to the theme, research profile and methodology determined in this systematic review. The systematic review, by limiting the investigation time in the last 10 years, allowed us to identify possible pharmacological applications with potential in humans. We observed a lack of scientific records on preclinical and clinical studies as shown in table 7.

Finding only experimental studies (*in vitro, in vivo, in silico) made* the methodological classification difficult and there is still a lack of notes that need to be investigated to demonstrate the real action in human beings.

Biomolecule	Possible human applications	Tests: In vivo	In vitro	Clinic	In Silico	Reference*	Reference literature
Evasin	Wound healing, inflammatory diseases	Yes	Yes	No	No	Déruaz et al.,2013, Singh et al., 2017, Frank et al.,2020, Hajnická et al., 2020	Chmelař et al., 2019, Bonvin, et al; 2016, Déruaz et al; 2019, Frauenschuh et al., 2007, Vieira et al., 2009)
Serpin	Coagulation diseases, digestive system, pancreatic insufficiency and Alleviate Joint Swelling and Inflammatory Response in Arthritis Models	Yes	Yes	No	Yes	Tao et al.,2016, Rodriguez-Valle et al.,2015, Wang et al.,2020	Chmelař et al., 2019
Crude saliva	Neuroblastoma cell lines, effect on blood clotting, fibrinolysis and platelet aggregation, Cell death induction in cancer cell lines	No	Yes	No	No	Nascimento et al., 2019, Simons et al 2011	-
Amblyomin-X	Coagulation diseases	Yes	Yes	No	Yes	Batista et al 2010	(Chmelař et al., 2019, Branco et al.,2016, Decrem et al., 2009, Corral et al.,2016, Chudzinski-Tavassi et al 2010)
Lipocalin	Allergy and asthma	No	No	No	Yes	Valdés., 2014	Wang et al., 2016, Paesen et al.,2000, Sangamnatdej et al., 2002
Avatrina	Thrombosis	Yes	Yes	No	Yes	Iyer et al., 2017	Kotsyfakis et al., 2006
Sialostatin	Immunopathies and inflammatory diseases	Yes	Yes	No	No	Sajiki et al., 2020	-
Ixophylline	Coagulation diseases	Yes	Yes	No	No	Narasimhan et al.,2013	Kotsyfakis et al., 2006, Aounallah et al., 2020
Ixonnexin	Thrombosis	Yes	Yes	No	No	Assumpção et al., 2018	-
Iristatin	Immunopathies and inflammatory diseases	Yes	Yes	No	Yes	Kotál et al., 2018	Narasimhan et al., 2013
Longistatin	Inflammatory diseases	Yes	Yes	No	No	Anisuzzaman et al., 2014	-
Sulfoproteins	Coagulation diseases	No	Yes	No	Yes	Watson et al., 2019	-
Ado e PGE2	Immunopathies and inflammatory diseases	Yes	Yes	No	No	Oliveira et al 2011	-
Total						19	

Table 7. Pharmacological applications in humans; *Articles selected in this systematic review.

Evaluations of this same search in periods prior to 2010 will bring other results and discussions since 2000 new experimental techniques have been developed since then, as well as bioinformatics. However, to evaluate the scientific evidence that is closer to the discoveries and potential applicability, this temporal limitation was preferably chosen. Since the results of the last ten years did not show clinical studies, our review points to results with potential signaling of possible studies more focused on these biomolecules for the next ten years.

The ticks listed are all from the Ixodidae (hard tick) family. Ticks successfully perform the blood meal, through the bite on the host's skin, spend days and even weeks to complete their feeding. For this to be successful, through their saliva, they trigger a series of immunomodulatory and homeostatic responses in the host, in addition to presenting major interferences in wound healing and suppressing the inflammatory response, and due to this their saliva are studied in search for possible actions and interesting pharmacological contributions (Nutall 2019; Bowman et al., 2008).

Considering the question on this systematic review and the data found, we converged information on some molecules present in tick saliva (regardless of genus and species) that are known for their specific pharmacological actions and functions in human target molecules and cells. We confirm the quantified results, highlighting the molecules that have been listed, favoring a greater elucidation of this pharmacological role and using as the base structure the associations found in the results with other scientific literature.

As evaluated, molecules have applications defined by their actions. Initial tests will guide the isolated or combined effects of these biomolecules so that in the next 10 years they can scientifically act in human therapeutics.

Evasin and Serpin prevailed in the results and the need for better elucidation of studies with crude or isolated saliva are still made noticed, since in this analysis there are interesting results with a promising percentage in antitumor action, as well as anticoagulant and anti-inflammatory actions.

5. Conclusion

It is observed that tick saliva is a promising universe to be explored. The prevalence of studies is not correlated with the greater effectiveness of the pharmacological action, but with the great number of studies identified in the systematic review. We summarized the available evidence that the saliva of American hard ticks is the one with the most studies for pharmacological applications referring to anti-inflammatory and immunomodulatory action.

Financing

PROPPG/UFTM, PPGCF, PPGCS, PPGMTI.

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APÊNDICE B – Dados Suplementares

Estratégias de Busca

Material suplementar A

STUDENT: Yanne Barbosa

COURSE Mestrado em Ciências Fisiológicas

ADVISOR: Profa. Renata Alves

THEME: Biomolecules with pharmacological potential present in tick saliva: a systematic review

DESCRIPTORS: Arthropod protein; tick; saliva; biological products.

DATE: 25/08/2021

LIBRARY: Sônia Maria Rezende Paolinelli – CRB-6/1191

SEARCH STRATEGY - LILACS

	mh:Carrapatos OR carrapatos OR Ixodida OR Ticks OR Ixodida Ixodidas OR Tick OR Garrapatas OR Ixodida OR mh:B01.050.500.131.166.132.832$
AND	**mh:Saliva** OR Saliva OR Salivas OR mh:A12.200.666$
AND	**mh:"Proteína de artrópodes"** OR (Proteína de artrópodes) OR (Arthropod Proteins) OR (Tick Proteins) OR (Proteínas de Artrópodos) OR mh:D12.776.093$
AND	**Mh:"Produtos biológicos"** OR (Produtos biológicos) OR Biofármaco Biofármacos OR (Droga Biológica) OR (Drogas Biológicas) OR (Fármaco Biológico) OR (Fármacos Biológicos) OR (Medicamento Biológico) OR (Medicamentos Biológicos) OR (Produto Biológico) OR (Produto Natural) OR (Produtos Biofarmacêuticos) OR (Produtos Naturais) OR (Remédio Biológica) OR (Remédios Biológicos) OR (Substâncias Biológicas) OR (Biological Products) OR Biologic (Biologic Drug) OR (Biologic Drugs) OR (Biologic Medicines) OR (Biologic Pharmaceuticals) OR (Biologic Product) OR (Biologic Products) OR Biological OR (Biological Drug) OR (Biological Drugs) OR (Biological Medicine) OR (Biological Medicines) OR (Biological Product) OR Biologicals OR Biologics OR Biopharmaceutical OR Biopharmaceuticals OR (Drug, Biologic) OR (Drug, Biological) OR (Drugs, Biologic) OR (Drugs, Biological) OR (Medicine, Biological) OR (Medicines, Biologic) OR (Medicines, Biological) OR (Natural Product) OR (Natural Products) OR (Pharmaceuticals, Biologic) OR (Product,

	Biologic) OR (Product, Biological) OR (Product, Natural) OR (Products, Biological) OR (Productos Biológicos) OR Biofarmacéuticos OR (Drogas Biológicas) OR (Fármaco Biológico) OR (Fármacos Biológicos) OR (Medicamento Biológico) OR (Medicamentos Biológicos) OR (Producto Biofarmacéutico) OR (Producto Biológico) OR (Producto Natural) OR (Productos Biofarmacéuticos) OR (Productos Naturales) OR (Sustancias Biológicas) OR mh:D20.215$ OR mh:VS2.002.001.010$

RESULT LILACS = 1 article

https://pesquisa.bvsalud.org/portal/?u_filter%5B%5D=fulltext&u_filter%5B%5D=db&u_filter%5B%5D=mj_cluster&u_filter%5B%5D=type_of_study&u_filter%5B%5D=la&u_filter%5B%5D=limit&u_filter%5B%5D=pais_assunto&u_filter%5B%5D=year_cluster&u_filter%5B%5D=type&fb=&output=&lang=pt&from=1&sort=&format=&count=&page=1&skfp=&index=&q=%28mh%3ACarrapatos+OR+carrapatos+OR+Ixodida+OR+Ticks+OR+Ixodida+Ixodidas+OR+Tick+OR+Garrapatas+OR+Ixodida+OR+mh%3AB01.050.500.131.166.132.832%24+%29+AND+%28mh%3ASaliva+OR+Saliva+OR+Salivas+OR+mh%3AA12.200.666%24%29+AND+%28mh%3A%22Prote%C3%ADna+de+artr%C3%B3podes%22+OR+%28Prote%C3%ADna+de+artr%C3%B3podes%29+OR+%28Arthropod+Proteins%29+OR+%28Tick+Proteins%29+OR+%28Prote%C3%ADnas+de+Artr%C3%B3podos%29+OR+mh%3AD12.776.093%24++%29+AND+%28Mh%3A%22Produtos+biol%C3%B3gicos%22+OR+%28Produtos+biol%C3%B3gicos%29+OR+Biof%C3%A1rmaco+Biof%C3%A1rmacos+OR+%28Droga+Biol%C3%B3gica%29+OR+%28Drogas+Biol%C3%B3gicas%29+OR+%28F%C3%A1rmaco+Biol%C3%B3gico%29+OR+%28F%C3%A1rmacos+Biol%C3%B3gicos%29+OR+%28Medicamento+Biol%C3%B3gico%29+OR+%28Medicamentos+Biol%C3%B3gicos%29+OR+%28Produto+Biol%C3%B3gico%29+OR+%28Produto+Natural%29+OR+%28Produtos+Biofarmac%C3%AAuticos%29+OR+%28Produtos+Naturais%29+OR+%28Rem%C3%A9dio+Biol%C3%B3gica%29+OR+%28Rem%C3%A9dios+Biol%C3%B3gicos%29+OR+%28Subst%C3%A2ncias+Biol%C3%B3gicas%29+OR+%28Biological+Products%29+OR+Biologic+%28Biologic+Drug%29+OR+%28Biologic+Drugs%29+OR+%28Biologic+Medicines%29+OR+%28Biologic+Pharmaceuticals%29+OR+%28Biologic+Product%29+OR+%28Biologic+Products%29+OR+Biological+OR+%28Biological+Drug%29+OR+%28Biological+Drugs%29+OR+%28Biological+Medicine%29+OR+%28Biological+Medicines%29+OR+%28Biological+Product%29+OR+Biologicals+OR+Biologics+OR+Biopharmaceutical+OR+Biopharmaceuticals+OR+%28Drug%2C+Biologic%29+OR+%28Drug%2C+Biological%29+OR+%28Drugs%2C+Biologic%29+OR+%28Drugs%2C+Biological%29+OR+%28Medicine%2C+Biological%29+OR+%28Medicines%2C+Biologic%29+OR+%28Medicines%2C+Biological%29+OR+%28Natural+Product%29+OR+%28Natural+Products%29+OR+%28Pharmaceuticals%2C+Biologic%29+OR+%28Product%2C+Biologic%29+OR+%28Product%2C+Biological%29+OR+%28Product%2C+Natural%29+OR+%28Products%2C+Biological%29+OR+%28Productos+Biol%C3%B3gicos%29+OR+Biofarmac%C3%A9uticos+OR+%28Drogas+Biol%C3%B3gicas%29+OR+%28F%C3%A1rmaco+Biol%C3%B3gico%29+OR+%28F%C3%A1rmacos+Biol%C3%B3gicos%29+OR+%28Medicamento+Biol%C3%B3gico%29+OR+%28Medicamentos+Biol%C3%B3gicos%29+OR+%28Producto+Biofar

mac%C3%A9utico%29+OR+%28Producto+Biol%C3%B3gico%29+OR+%28Producto+Natural%29+OR+%28Productos+Biofarmac%C3%A9uticos%29+OR+%28Productos+Naturales%29+OR+%28Sustancias+Biol%C3%B3gicas%29+OR+mh%3AD20.215%24+OR+mh%3AVS2.002.001.010%24%29&where=&range_year_start=&range_year_end=&filter%5Bdb%5D%5B%5D=LILACS&range_year_start=&range_year_end=

SEARCH STRATEGY - PUBMED

	"Ticks"[Mesh] OR Ticks OR Tick OR Ixodida OR Ixodidas
AND	"Saliva"[Mesh] OR Saliva OR salivas
AND	"Arthropod Proteins"[Mesh] OR (Arthropod Proteins) OR (Tick Proteins)
AND	"Biological Products"[Mesh] OR (Biological Products) OR (Products, Biological) OR (Biological Product) OR (Product, Biological) OR (Biologic Product) OR (Product, Biologic) OR (Biologic Products) OR Biopharmaceuticals OR Biopharmaceutical OR Biological OR Biologic OR (Biological Drug) OR (Drug, Biological) OR (Biologic Drugs) OR (Drugs, Biologic) OR (Biological Medicine) OR (Medicine, Biological) OR (Biological Medicines) OR (Medicines, Biological) OR Biologicals OR (Biologic Medicines) OR (Medicines, Biologic) OR (Biologic Pharmaceuticals) OR (Pharmaceuticals, Biologic) OR Biologics OR (Biologic Drug) OR (Drug, Biologic) OR (Biological Drugs) OR (Drugs, Biological) OR (Natural Products) OR (Natural Product) OR (Product, Natural)

RESULT: 121

LINK:
https://pubmed.ncbi.nlm.nih.gov/?term=%28%28%28%22Ticks%22%5BMesh%5D+OR+Ticks+OR+Tick+OR+Ixodida+OR+Ixodidas%29+AND+%28%22Saliva%22%5BMesh%5D+OR+Saliva+OR+salivas%29%29+AND+%28%22Arthropod+Proteins%22%5BMesh%5D+OR+%28Arthropod+Proteins%29+OR+%28Tick+Proteins%29%29%29+AND+%28%22Biological+Products%22%5BMesh%5D+OR+%28Biological+Products%29+OR+%28Products%2C+Biological%29+OR+%28Biological+Product%29+OR+%28Product%2C+Biological%29+OR+%28Biologic+Product%29+OR+%28Product%2C+Biologic%29+OR+%28Biologic+Products%29+OR+Biopharmaceuticals+OR+Biopharmaceutical+OR+Biological+OR+Biologic+OR+%28Biological+Drug%29+OR+%28Drug%2C+Biological%29+OR+%28Biologic+Drugs%29+OR+%28Drugs%2C+Biologic%29+OR+%28Biological+Medicine%29+OR+%28Medicine%2C+Biological%29+OR+%28Biological+Medicines%29+OR+%28Medicines%2C+Biological%29+OR+Biologicals+OR+%28Biologic+Medicines%29+OR+%28Medicines%2C+Biologic%29+OR+%28Biologic+Pharmaceuticals%29+OR+%28Pharmaceuticals%2C+Biologic%29+OR+Biologics+OR+%28Biologic+Drug%29+OR+%28Drug%2C+Biologic%29+OR+%28Biological+Drugs%29+OR+%28Drugs%2C+Biological%29+OR+%28Natural+Products%29+OR+%28Natural+Product%29+OR+%28Product%2C+Natural%29%29&filter=years.2010-2021

SEARCH STRATEGY - COCHRANE

	MeSH descriptor: [Ticks] explode all trees
AND	MeSH descriptor: [Saliva] explode all trees
AND	MeSH descriptor: [Arthropod Proteins] explode all trees
AND	MeSH descriptor: [Biological Products] explode all trees

RESULT = 0 artigos

SEARCH STRATEGY - EMBASE

	'tick'/exp OR ticks OR Ixodida OR Ixodoidea
AND	'saliva'/exp OR spittle
AND	'arthropod protein'/exp OR "arthropod proteins"
AND	'biological product'/exp OR biologic OR "biologic agent" OR "biologic agents" OR "biologic product" OR "biologic products" OR biological OR "biological agent" OR "biological agents" OR "biological products" OR biologicals OR biologics

RESULT =0 artigos

SEARCH STRATEGY – WEB OF SCIENCE, SCOPUS.

	Ticks OR Tick
AND	Saliva OR salivas
AND	"Arthropod Proteins" OR "Tick Proteins"
AND	**SCOPUS** "Biological Products" OR "Products, Biological" OR "Biological Product" OR "Product, Biological" OR "Biologic Product" OR "Product, Biologic" OR "Biologic Products" OR Biopharmaceuticals OR Biopharmaceutical OR Biological OR Biologic OR "Biological Drug" OR "Drug, Biological" OR "Biologic Drugs" OR "Drugs, Biologic" OR "Biological Medicine" OR "Medicine, Biological" OR "Biological Medicines" OR "Medicines, Biological" OR Biologicals OR "Biologic Medicines" OR "Medicines, Biologic" OR "Biologic Pharmaceuticals" OR "Pharmaceuticals, Biologic" OR Biologics OR "Biologic Drug" OR "Drug, Biologic" OR "Biological Drugs" OR "Drugs, Biological" OR "Natural Products" OR "Natural Product" OR "Product, Natural" **WEB OF SCIENCE** ALL=((Biological Products OR Biologic Drugs OR Biologic Medicines OR Biologic Pharmaceuticals OR Biologic Products OR Biological Drugs OR Biological Medicines OR Biologicals OR Biologics OR Biopharmaceuticals OR Drugs, Biologic OR Drugs, Biological OR Medicines, Biologic OR Medicines, Biological OR Natural Products OR Pharmaceuticals, Biologic OR Products, Biological) AND (Saliva OR Salivas) AND (Ticks OR Tick) AND (Arthropod Proteins OR Tick Proteins))

	Results: 48

LINK WEB OF SCIENCE: https://www.webofscience.com/wos/woscc/summary/731fd9e0-2ab6-4a48-8aaa-bfa86b7d69ea-062ab9dd/relevance/1

Material suplementar B

Seleção final dos artigos

https://docs.google.com/spreadsheets/d/1OakNLKZgEzEZpWvL6Ev4fxnqHts-m0ls/edit?usp=sharing&ouid=113357696022573599687&rtpof=true&sd=true

Material suplementar C

Avalição da qualidade metodológica

https://docs.google.com/spreadsheets/d/1JPZ5uHiXNclUQaX25v-JhlD0rIMbSNNCVy2_sHvWGwQ/edit?usp=sharing

APÊNDICE C – Lista de Coautores

CRediT author statement

“Biomoléculas com potencial farmacológico presente em saliva de carrapatos: uma revisão sistemática”

"Biomolecules with pharmacological potential present in tick saliva: a systematic review"

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Escrita – manuscrito original: Yanne Oliveira Barbosa; Wellington Francisco Rodrigues, Carlo José Freire de Oliveira, Renata Pereira Alves-Balvedi;

Escrita - Revisão & Edição: Yanne Oliveira Barbosa; Wellington Francisco Rodrigues, Carlo José Freire de Oliveira, Renata Pereira Alves-Balvedi;