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

Biomoléculas presentes na saliva do carrapato com potencial farmacológico: uma revisão sistemática

Biomoléculas presentes en la saliva de garrapatas con potencial farmacológico: una revisión sistemática

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Yanne Oliveira Barbosa
ORCID: https://orcid.org/0000-0002-4862-4563
Federal University of Triangulo Mineiro, Brazil
E-mail: yanne.way@gmail.com
Beatriz Rodrigues Martins
ORCID: https://orcid.org/0000-0002-4626-8390
Federal University of Triangulo Mineiro, Brazil
E-mail: biaroma_95@hotmail.com
Cristhianne Molinero Andrade Ratkevicius
ORCID: https://orcid.org/0000-0003-3469-0022
Federal University of Triangulo Mineiro, Brazil
E-mail: cristhianne_m@hotmail.com
Wellington Francisco Rodrigues
ORCID: https://orcid.org/0000-0002-3426-2186
Federal University of Triangulo Mineiro, Brazil
E-mail: wellington.frodrigues60001@gmail.com
Carlo José Freire Oliveira
ORCID: https://orcid.org/0000-0003-2211-7333
Federal University of Triangulo Mineiro, Brazil
E-mail: carlo.oliveira@uftm.edu.br
Ana Paul aEspindula
ORCID: https://orcid.org/0000-0002-9282-4482
Federal University of Triangulo Mineiro, Brazil
E-mail: ana.espindula@uftm.edu.br
Renata Pereira Alves
ORCID: https://orcid.org/0000-0003-2411-614X
Federal University of Triangulo Mineiro, Brazil
E-mail: renata.pereira@uftm.edu.br

Abstract
Knowing that ticks have bioactive molecules in their saliva which modulate hemostatic and immunomodulatory activities in humans, we carried out a systematic search for biomolecules present in tick saliva with great pharmacological potential. We evaluated studies published in the last ten years. Following the recommendations of the Prisma tool, primary and secondary studies of a systematic nature were selected, with no language or country restriction. Studies that included arthropods other than ticks and studies in which the use of saliva had no pharmacological application were excluded. For searches, we used the following databases: MEDLINE®/PubMed®, Web of Science, LILACS, EMBASE, Cochrane and SCOPUS. The methodological quality was performed using the tools available in Joanna Briggs, always with two or more independent evaluators. The generated data were tabulated and summarized through qualitative narrative analysis. The methodology selected 19 articles that met the eligibility criteria. The saliva of hard ticks, found in the Americas, is more promising when used in experimental studies with human cells. The elucidation of the biomolecules was possible, with evasin and serpine being the biomolecules with the most evident pharmacological potential for anti-inflammatory action. In the selected studies, we found only experimental studies, with no pre-clinical or clinical studies, making methodological qualification difficult; in some studies, with the biomolecule Evasin and Serpin, the need for elucidation of these biomolecules in question was suggested. Thus, we found evidence that the saliva of American hard ticks is the most studied for pharmacological applications of anti-inflammatory and immunomodulatory action.
Keywords: Saliva; Tick; Pharmacological potential; Salivary biomolecules.

Resumo

Sabendo que os carrapatos possuem moléculas bioativas em sua saliva as quais modulam atividades hemostáticas e imunomoduladoras em humanos, realizamos uma busca sistemática de biomoléculas presentes na saliva do carrapato com grande potencial farmacológico. Avaliamos estudos publicados nos últimos dez anos. Seguindo as recomendações da ferramenta Prisma, foram selecionados estudos primários e secundários de caráter sistemático, não havendo restrição de idioma ou país. Foram excluídos estudos que incluíam artrópodes diferentes de carrapatos e estudos em que o uso de saliva não tinha aplicação farmacológica. Para as buscas, utilizamos as seguintes bases de dados: MEDLINE®/PubMed®, Web of Science, LILACS, EMBASE, Cochrane e SCOPUS. A qualidade metodológica foi realizada com as ferramentas disponíveis no Joanna Briggs, sempre com dois ou mais avaliadores independentes. Os dados gerados foram tabulados e resumidos por meio de análise narrativa qualitativa. A metodologia selecionou 19 artigos que atenderam aos critérios de elegibilidade. As salivas de carrapatos duros, encontrados nas Américas, são mais promissoras quando utilizadas em estudos experimentais com células humanas. A elucidação das biomoléculas foi possível, sendo a evasina e a serpina as biomoléculas com os potenciais farmacológicos mais evidentes para ação anti-inflamatória. Nos estudos selecionados encontramos apenas estudos experimentais, não havendo estudos pré-clínicos ou clínicos, dificultando a qualificação metodológica; em alguns estudos, com a biomolécula Evasin e Serpin, sugeriu-se a necessidade de elucidação dessas biomoléculas em questão. Assim, localizamos evidências que a saliva de carrapatos duros americanos é a mais estudada para aplicações farmacológicas de ação anti-inflamatória e imunomoduladora.

Palavras-chave: Saliva; Carrapato; Potencial farmacológico; Biomoléculas salivares.

Resumen

Sabiendo que las garrapatas tienen moléculas bioactivas en su saliva que modulan las actividades hemostáticas e inmunomoduladoras en humanos, llevamos a cabo una búsqueda sistemática de biomoléculas presentes en la saliva de las garrapatas con gran potencial farmacológico. Los estudios publicados en los últimos diez años. Siguiendo las recomendaciones de la herramienta Prisma, se seleccionaron estudios primarios y secundarios de carácter sistemático, sin restricción de idioma o país. Se excluyeron los estudios que incluyeron artrópodos distintos a las garrapatas y los estudios en los que el uso de saliva no tuvo aplicación farmacológica. Para las búsquedas utilizamos las bases de datos: MEDLINE®/PubMed®, Web of Science, LILACS, EMBASE, Cochrane y SCOPUS. La calidad metodológica se realizó utilizando las herramientas disponibles en Joanna Briggs. Los datos generados fueron tabulados y resumidos mediante análisis narrativo cualitativo. La metodología seleccionó 19 artículos que cumplían con los criterios de elegibilidad. La saliva de las garrapatas duras, es más prometedora cuando se usa en estudios experimentales con células humanas. La elucidación de las biomoléculas fue posible, siendo la evasina y la serpina las biomoléculas con el potencial farmacológico más evidente para la acción antiinflamatoria. En los estudios seleccionados, encontramos solo estudios experimentales, sin estudios preclínicos ni clínicos, lo que dificulta la calificación metodológica; en algunos estudios, con las biomoléculas Evasin y Serpin, se sugirió la necesidad de dilucidar estas biomoléculas en cuestión. Así, encontramos evidencia de que la saliva de la garrapata dura americana es la más estudiada para aplicaciones farmacológicas de acción antiinflamatoria e inmunomoduladora.

Palabras clave: Saliva, Garrapata; Potencial farmacológico; Biomoléculas salivales.

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. Methodology

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) (Haddaway & 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 2023). 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 06/02/2023, 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 02/06/2023.

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/alternative terms were selected come from DeCS. 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 – 2023.

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.) 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.).

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.

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/

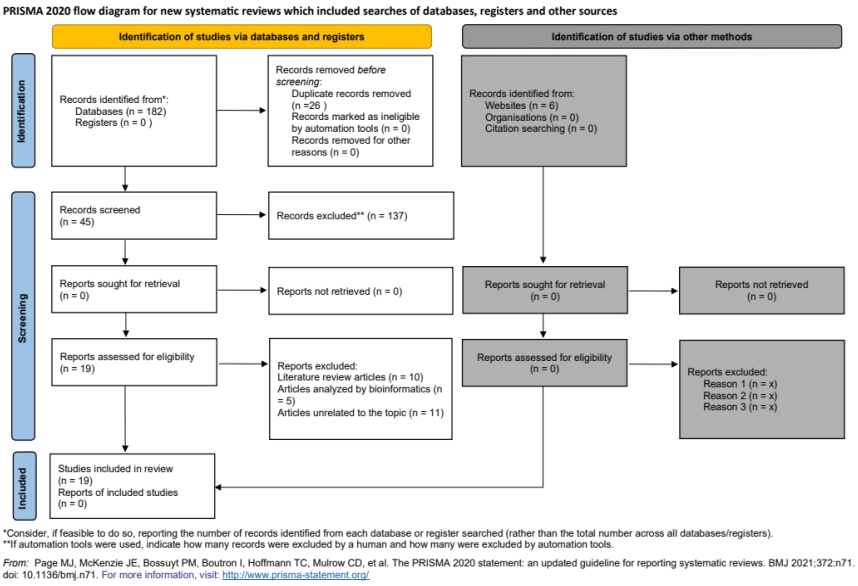
3. Results

3.1 Study Selection

The databases initially presented 182 articles and no records. With individual analysis, we observed that there were 26 duplicated articles. Among the 166, all titles and abstracts were read and the inclusion and exclusion criteria was applied. 137 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.

Figure 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).



Source: Authors.

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.

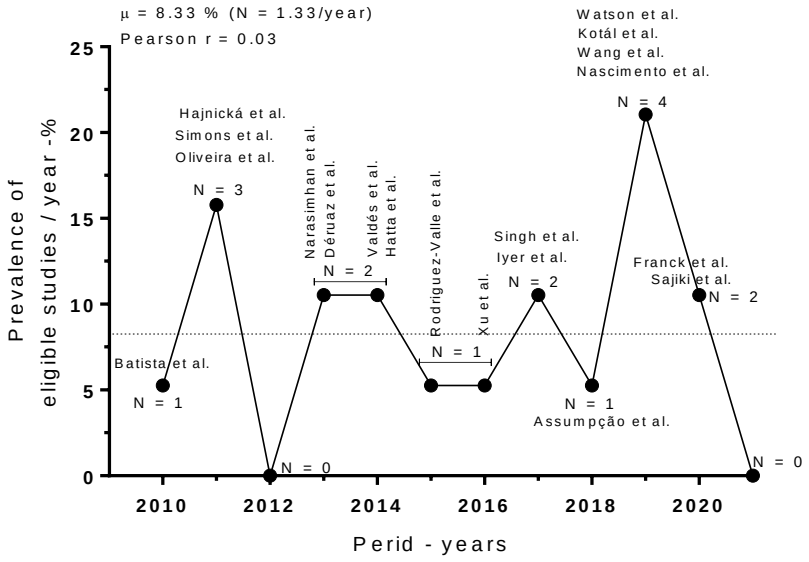
Author / Year	Study	Data base	Journal/periodical	Type of study	Methodological quality
28 (2010)	A new Factor Xa inhibitor from *Amblyomma cajennense* with a unique domain composition.	PM	Elsevier - Archives of Biochemistry and Biophysics	In vitro, in vivo and in silico experimental study	93,75%
18 (2011)	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%

Source: Authors.

Figure 2 shows the distribution of studies that evaluated bioactive molecules for pharmacological application between the periods of 2010 to 2023, 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 ($p>0.05$).

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



Source: Authors.

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 Table 2.

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%).

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

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 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)		

Source: Authors.

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.

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

Genus	Continent					Total
	America	Europe	Asia	Africa	Oceania	
Amblyomma sp.	4 (66.67)	1 (16.67)	0 (0.00)	1 (16.66)	0 (0.00)	6 (100)
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

P-value <0.0001. Source: Authors.

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%.

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

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)/(b+c)	6/6	31.58/31.58
(a+d)	2	10.53
(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

Source: Authors.

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 response modulation action.

Table 5 - Prevalence of biomolecules.

Biomolecule	Pharmacological Action	Prevalence %
Evasin	Anti-inflammatory (inhibition of chemokine), Action on inflammatory diseases (ID).	21.050%
Serpin	Action on ID, 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 oxidative stress.	5.263%
Sulfoproteins	Anticoagulant	5.263%
Ado e PGE2	Modulation - immune and inflammatory responses	5.263%
Total		100%

Source: Authors.

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%).

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

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 Hypersensitivity (Allergies, Asthma)	47.37
Inflammatory diseases	Pro-inflammatory diseases (Arthritis, etc.) Autoimmune diseases, Acute pancreatitis	63.16

Source: Authors.

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.

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

Biomolecule	Possible human applications	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	

Source: Authors.

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, for futures research, 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.

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PROPPG/UFTM, PPGCF, PPGCS, PPGMTI.

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