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ALCIDES JAIRON LACERDA CINTRA

PURIFICAÇÃO DE LECTINA DE FOLHAS DE *Bowdichia virgilioides*, INTERAÇÃO MOLECULAR E AVALIAÇÃO FITOQUÍMICA E BIOLÓGICA DO EXTRATO SALINO

RECIFE

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Tese apresentada ao Programa de Pós-Graduação em Bioquímica e Fisiologia da Universidade Federal de Pernambuco, como cumprimento parcial das exigências para a obtenção do título de Doutor em Bioquímica e Fisiologia.

Orientadora: Prof.ª Dr.ª Maria Tereza dos Santos Correia

Coorientadores: Prof. Dr. Jorge Luiz Neves e Prof. Dr. Cristian Torres León

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Aprovado em 29 de julho de 2021.

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RESUMO

As plantas são fontes naturais de muitas moléculas biologicamente ativas, como lectinas, que são proteínas ou glicoproteína capazes de reconhecer e se ligar a carboidratos ou glicoconjugados de maneira específica e reversível. E também é fonte de muitos outros metabólitos com alto potencial biotecnológico. Nesse sentido, o objetivo desta tese foi purificar lectina de folhas de *B. virgiliodes,* analisar sua interação molecular, e avaliar o extrato salino quanto a composição química e atividades biológicas. Para purificação da lectina foi preparado extrato salino das folhas, em seguida foi centrifugado, filtrado, e o sobrenadante foi dialisado e liofilizado (extrato salino). Posteriormente esse produto foi ressuspendido em água destilada e purificado em duas etapas de cromatografia de gel filtração, para obtenção da lectina. Após a purificação, foi realizado AH, inibição da AH por proteínas e carboidratos, e avaliação da interação lectina-proteína por espectroscopia de fluorescência intrínseca. Com o extrato salino foi analisado a composição química por LC-MS e avaliado sua ação antioxidante, antibacteriana, antifúngica, antibiofilme, citotóxica, hemolítica, anti-hemolítica, fotoprotetora, larvicida, e proteolítica. Por fim, foi quantificado fenóis, proteínas, carboidratos (totais e redutores) e ácido hexurônico. Os resultados apresentaram a purificação da lectina de folhas de *B. virgilioides*, BovLL. A qual apresentou interação com proteínas e carboidratos, em todos os ensaios avaliados. Nos ensaios com o extrato, foram identificados seis compostos majoritários e suas respectivas massas. O mesmo apresentou atividade antioxidante por DPPH, ABTS e FRAP. Atividade antibacteriana para Gram-positivas, *S. pyogenes*, *S. aureus* e *E. faecalis* (CMI e CMB: 250 e 500; 500 e 1000; e 1000 e 2000 µg / mL, respectivamente). Citotoxicidade em células cancerígenas (HT-29) ≥ 125 µg / mL e não para células normais (L929) até 500 µg / mL. Não apresentou toxicidade em larvas de *A. salina* e *A. aegypti* até 250 µg / mL e 1000 µg / mL, respectivamente. Não foi hemolítico até 2000 µg / mL, e foi anti-hemolítico até 1,95 µg / mL. O espectro de absorção revelou pico máximo em 280 nm (UVB) e FPS de 25,24 a 2000 µg / mL. Também apresentou 80% (U / mg) de atividade proteolítica. Finalmente foi quantificado o conteúdo de proteínas, carboidratos (totais e redutores), ácido hexurônico, e fenóis, respectivamente: 10 mg / mL, 62,85%, 17,0%, 19,18%, e 235,759 mg GAE / g. Portanto, foi purificada a primeira lectina de folhas de *B. virgilioides* (BovLL), com interações e biologia estudadas. E também foi obtido o extrato salino, com seis compostos químicos identificados e diferentes atividades biológicas testadas.

Palavras-Chave: *Bowdichia virgilioides*; Lectina; Purificação; Interação Molecular; Extrato; Fitoquímica; Atividades Biológicas.

ABSTRACT

Plants are natural sources of many biologically active molecules, such as lectins, which are proteins or glycoproteins capable of recognizing and binding carbohydrates or glycoconjugates in a specific and reversible manner. It is also a source of many other metabolites with high biotechnological potential. In this sense, the objective of this thesis was to purify lectin from leaves of *B. virgiliodes*, analyze its molecular interaction, and evaluate its saline extract, in terms of chemical composition and biological activities. For lectin purification, a saline extract of the leaves was prepared, then it was centrifuged, filtered, and the supernatant was dialyzed and lyophilized (saline extract). Subsequently, this product was resuspended in distilled water and purified in two steps of gel filtration chromatography, to obtain the lectin. After purification, HA, inhibition of HA by proteins and carbohydrates, and evaluation of the lectin-protein interaction by intrinsic fluorescence spectroscopy were performed. With the saline extract, the chemical composition was analyzed by LC-MS and its action was evaluated: antioxidant, antibacterial, antifungal, antibiofilm, cytotoxic, hemolytic, antihemolytic, photoprotective, larvicidal and proteolytic. Finally, phenols, proteins, carbohydrates (total and reducing agents) and hexuronic acid were quantified. The results showed the purification of lectin from leaves of *B. virgilioides*, BovLL. Which showed interaction with proteins and carbohydrates in all assays evaluated. In the tests with the extract, six major compounds and their respective masses were identified. It showed antioxidant activity by DPPH, ABTS and FRAP. Antibacterial activity for Gram-positive, *S. pyogenes, S. aureus* and *E. faecalis* (MIC and MBC: 250 and 500; 500 and 1000; and 1000 and 2000 µg / mL, respectively). Cytotoxicity in cancer cells (HT-29) ≥ 125 µg / mL and not for normal cells (L929) up to 500 µg / mL. It did not show toxicity in *A. salina* and *A. aegypti* larvae up to 250 µg / mL and 1000 µg / mL, respectively. It was non-hemolytic up to 2000 µg / mL, and anti-hemolytic up to 1.95 µg / mL. The absorption spectrum revealed maximum peak at 280 nm (UVB) and SPF of 25.24 to 2000 µg / mL. It also showed 80% (U / mg) of proteolytic activity. Finally, the content of proteins, carbohydrates (total and reducing), hexuronic acid, and phenols was quantified, respectively: 10 mg / mL, 62.85%, 17.0%, 19.18%, and 235.759 mg GAE / g. Therefore, the first lectin from leaves of *B. virgilioides* (BovLL) was purified, with interactions and biology studied. And the saline extract was also obtained, with six chemical compounds identified and different biological activities tested.

Key words: *Bowdichia virgilioides*; Lectin; Purification; Molecular Interaction; Extract; Phytochemistry; Biological Activities.

SUMÁRIO

1 INTRODUÇÃO

As plantas são fontes naturais de muitas moléculas biologicamente ativas, com capacidade antimicrobiana, anticancerígena, antioxidante, marcação celular e muitas outras. E por isso, são de grande interesse da indústria, do ponto de vista clínico, farmacêutico e biotecnológico (JUCÁ et al., 2020; SANTOS et al., 2020a; SOROKINA; STEINBECK, 2020). Suas diferentes partes (folhas, frutos, caules, raízes, troncos, etc) são muito estudadas por diferentes grupos de pesquisas em todo mundo (BENARBA; PANDIELLA, 2020; NEWMAN; CRAGG, 2020; SHOURIE, 2017). Entre os muitos tipos de moléculas estudadas no mundo, estão as lectinas, um grupo bastante específico de proteínas, com amplas aplicações e atividades biológicas (TSANEVA; VAN DAMME, 2020).

As lectinas são encontradas nas mais variadas fontes da natureza, por exemplo, são encontradas em: vírus (THOMPSON et al., 2020), fungos (SINGH; WALIA; KENNEDY, 2019), bactérias (SÝKOROVÁ et al., 2020; THUENAUER et al., 2020), protozoários (SINGH; WALIA; KANWAR, 2016), plantas (HIREMATH et al., 2020), algas marinhas (WU et al., 2016), animais, como, cobras (CEZARETTE; SARTIM; SAMPAIO, 2020), aves (ZHANG et al., 2017), crustáceos (ZHANG et al., 2018b), peixes (YANG et al., 2020), equinodermos (UNNO; GODA; HATAKEYAMA, 2014) e muitas outras fontes. Mas entre todas as fontes dessas moléculas, as lectinas de plantas são as mais estudadas. Tendo muitos estudos de purificação, caracterização e atividades biológicas, publicados periodicamente (TSANEVA; VAN DAMME, 2020). As partes das plantas estudadas também variam muito, desde folhas (PROCÓPIO et al., 2017; RADHA; URLA, 2019), sementes (CAVADA et al., 2020), troncos (ARAÚJO et al., 2012), raízes (SILVA et al., 2019a), caules (rizomas) (AHSANUL KABIR et al., 2019), floema dos frutos (NAREDDY; BOBBILI; SWAMY, 2017), sarcotesta dos frutos (SILVA et al., 2016a), e látex dos troncos (SIRITAPETAWEE et al., 2018), por exemplo.

Como as lectinas são moléculas amplamente estudadas, já possuem muitas atividades biológicas descritas, como: antiproliferativa (AHSANUL KABIR et al., 2019), antibacteriana (LIU; DANG, 2020), antifúngica (NOVA et al., 2020), mitogênica (SINGH; WALIA; KENNEDY, 2019), antibiofilme (MOURA et al., 2020), antioxidante (FREIRE et al., 2020), anti-inflamatória (LIU et al., 2019), leishmanicida (ARANDA-SOUZA et al., 2018), inseticida (OLIVEIRA et al., 2020b), nematicida (MEDEIROS et al., 2020), hipoglicemiante (FREIRE et al., 2020), antinociceptiva (PIRES et al., 2019), anti-hemolítica (RODRIGO et al., 2015), imunomoduladora (SANTOS et al., 2020a), antiviral (AGARWAL; TRIVEDI; MITRA, 2020),

antiulcerogênica (PINTO et al., 2019a), antilipidêmica (FREIRE et al., 2020), pró-inflamatória (CEZARETTE; SARTIM; SAMPAIO, 2020), anxiolítica (ARAÚJO et al., 2020), controle da resposta asmática (BORTOLOZZO et al., 2018) e anti-mutagênica (FRASSINETTI et al., 2015).

Além dessas atividades biológicas citadas anteriormente, também possuem muitos outros estudos com diferentes aplicações, como: produção de hidrogel contendo lectina, para tratamento de queimaduras (PEREIRA et al., 2019), desenvolvimento de sondas bifuncionais cliváveis para detecção de glicanos (MA et al., 2019), sinergismo com antibióticos para combater organismos resistentes (SANTOS et al., 2019b), detecção de patógenos bacterianos por citometria de fluxo, utilizando lectina como ligante específico aos microrganismos (HENDRICKSON et al., 2019), avaliação de perfis de glicose e manose em espécies de Cândida, usando pontos quânticos conjugados a lectina Cramoll (nanosondas fluorescentes) (OLIVEIRA et al., 2020c) e encapsulação em nanocarreadores para tratar células cancerígenas (WIJETUNGE et al., 2020). E também aplicada em métodos analíticos tradicionais, como: cromatografia de afinidade com lectina, histoquímica e citoquímica com lectina, blotting lectina, microarrays lectina, biossensores de lectina, microplaca à base de lectina (ELLSA) e ensaio de fluxo lateral com lectina (LFA) (HENDRICKSON; ZHERDEV, 2018; TSANEVA; VAN DAMME, 2020).

Além de lectinas, as plantas possuem estudos com muitos outros produtos delas obtidos, como: óleos (fixos e essenciais) e extratos (orgânicos, aquosos e salinos). O extrato salino, por exemplo, é muito utilizado em experimentos, devido sua riqueza de compostos, fácil obtenção e baixa toxicidade do solvente (CARVALHO et al., 2020; EBRAHIMI et al., 2020; MACÊDO et al., 2020; OLIVEIRA et al., 2020a; SOUZA et al., 2020). Recentemente muitos extratos salinos de plantas têm sido estudados, apresentando diferentes atividades biológicas, como, antioxidante de *Rosmarinus officinalis* (REZANEJAD et al., 2020), antibacteriana de *Monochoria vaginalis* (RAJAPAKSHA et al., 2020), antifúngica e inseticida de *Abarema cochliocarpos* (SILVA et al., 2020a) e antitumoral de *Schinus terebinthifolia* (RAMOS et al., 2019).

Entre as plantas com alto potencial biológico está a *Bowdichia virgilioides,* espécie pertencente à família Fabaceae, subfamília Faboideae e nativa do Brasil (ocorrendo em todos os seus estados e biomas). Popularmente é conhecida como sucupira, sucupira preta, sucupira-roxa, entre outros, a depender da localidade. *B. virgilioides* é resistente a solos de baixa fertilidade, apresenta grande porte, podendo chegar a 20 metros de altura, e sua madeira tem

grande valor econômico na construção civil. Na medicina popular, é muito usada para tratar tuberculose (SHARIFI-RAD et al., 2017), cicatrização de feridas, inflamação vaginal e do útero, dor de garganta (SOUZA et al., 2014), reumatismo (SARAIVA et al., 2015), dor de cabeça, aneurisma (GOMES et al., 2008), entre outras. Também possui muitos estudos científicos já descritos, por exemplo, antioxidante (FARIAS et al., 2013), antinociceptivo, anti-inflamatório (THOMAZZI et al., 2010), anti-hiperglicêmico (SILVA et al., 2015) e cicatrizante (BESERRA et al., 2018, 2019).

Tendo em vista a importância dos produtos naturais de plantas e a necessidade constante de descobrir novas moléculas com potencial biotecnológico, essa tese se propôs a purificar lectina de folhas de *B. virgilioides* e analisar sua interação molecular. Bem como avaliar a composição química e propriedades biológica do extrato salino de *B. virgilioides*.

1.1 OBJETIVOS

1.1.1 Objetivo geral

- Purificar lectina de folhas de *B. virgilioides*, analisar sua interação com moléculas e avaliar a composição química e propriedades biológicas do extrato salino.

1.1.2 Objetivos específicos

- Purificar lectina de folhas de *B. virgilioides*;
- Determinar sua atividade hemaglutinante (AH);
- Determinar a inibição da atividade hemaglutinante por carboidratos e proteínas;
- Avaliar a homogeneidade da amostra e estimar a massa relativa da lectina por eletroforese em condições desnaturantes (SDS-PAGE);
- Avaliar a interação da lectina BovLL com proteínas por espectroscopia de fluorescência intrínseca;
- Identificar e determinar a massa dos compostos majoritários do extrato de *B. virgilioides* por LC-MS;
- Realizar atividade antioxidante do extrato por DPPH, ABTS e FRAP;
- Determinar a atividade antibacteriana, antifúngica e antibiofilme do extrato;
- Realizar a citotoxicidade do extrato em linhagens de células normais e cancerígenas;
- Determinar a viabilidade de larvas de *A. salina* e *A. aegypti,* frente ao extrato;
- Avaliar a capacidade hemolítica e anti-hemolítica do extrato;
- Determinar o espectro de absorção ultravioleta e o potencial fotoprotetor do extrato;
- Avaliar a atividade proteolítica total do extrato;
- Avaliar a concentração de fenóis, proteínas, carboidratos (totais e redutores) e ácido hexurônico do extrato.

2 FUNDAMENTAÇÃO TEÓRICA

2.1 LECTINAS

A palavra “lectina” deriva do verbo latino “legere”, que significa selecionar, ler ou reunir. Boyd e Shapleigh em 1954, cunharam o termo “lectina” para descrever aglutininas derivadas de plantas, que eram específicas para grupos sanguíneos, após investigar a especificidade para eritrócitos específicos. As proteínas que apresentam atividade hemaglutinante são mencionadas como aglutininas, hemaglutininas, fito-hemaglutininas ou fito-glutininas (BOYD; SHAPLEIGH, 1954; GOLDSTEIN, 1980; ALLEN; BRILLIANTINE, 1969; LIS; SHARON, 1973).

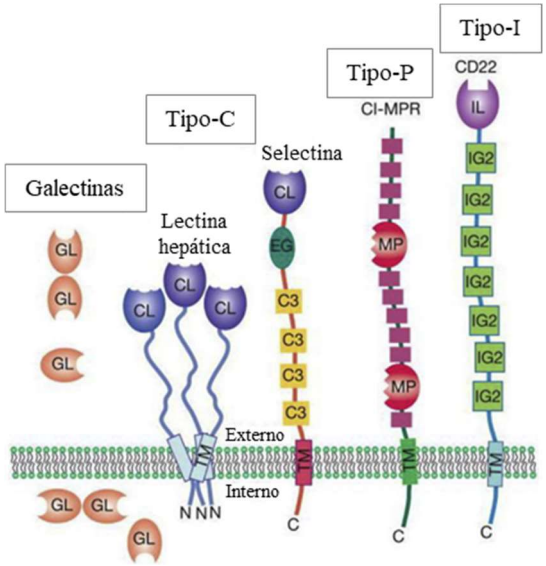
A definição de lectinas está em constante evolução, conforme as novas descobertas e estudos. Podem ser citadas, por exemplo, algumas definições, desde mais antigas até outras mais recentes: a) lectinas são proteínas ou glicoproteínas de origem não imune, associadas a carboidratos por pelo menos dois locais de ligação, aglutinando células de plantas e/ou animais, e precipitando polissacarídeos, glicoproteínas ou glicolipídios (GOLDSTEIN, 1980); b) lectinas são proteínas de ligação a carboidratos (não imunoglobulinas), que não exibem atividade enzimática em relação aos açúcares reconhecidos (LORIS, 2002); c) lectinas são proteínas com vários domínios de origem não imune, capazes de se ligar (reversível e não covalentemente) a açúcares específicos (isoladamente ou em estruturas maiores, tais como glicoproteínas e glicolipídeos), devido a uma cadeia polipeptídica específica dentro da sua estrutura, conhecida como: domínio de reconhecimento de carboidratos (DRC) (CAGLIARI; KREMER; PINTO, 2018); e mais recente, d) lectinas são proteínas com diversas estruturas moleculares que compartilham a capacidade de reconhecer e se ligar de forma específica e reversível às estruturas de carboidratos, sem alterar a porção do carboidrato (TSANEVA; VAN DAMME, 2020).

As fontes de lectinas são muito amplas, como citado na introdução, elas são encontradas em praticamente todos os organismos da natureza, desde: plantas (HIREMATH et al., 2020), bactérias (DIGGLE et al., 2006), fungos (SINGH; WALIA; KENNEDY, 2019), protozoários (SINGH; WALIA; KANWAR, 2016), algas (WU et al., 2016), animais, como, cobras (NUNES et al., 2011), crustáceos (ZHANG et al., 2018b), peixes (RUBEENA et al., 2019), e muitos outros.

Com relação a classificação das lectinas, no momento, não existe uma universalmente aceita (JUAN et al., 2017; TSANEVA; VAN DAMME, 2020). Mas elas podem ser agrupadas

de maneira mais geral com base na fonte de obtenção (planta, animais, etc) e de maneira mais específica, por exemplo, através de sua sequência e homologia estrutural (figura 1) em: a) Lectinas do tipo C, são as que requerem cálcio para reconhecimento. b) Lectinas do tipo S (galectinas), requerem ligações dissulfeto para estabilidade e reconhecimento. c) Lectinas do tipo P, reconhecem manose-6-fosfato. d) Lectinas do tipo I, são imunoglobulinas que podem distinguir carboidratos. e) E um subgrupo dessas moléculas foi designado como Siglecs, que se ligam especificamente aos ácidos siálicos.

Figura 1. Classificação de lectinas por sua sequência e homologia estrutural.



Fonte: (JUAN et al., 2017).

As lectinas possuem diversas atividades, como será visto mais à frente, mas a sua ação biológica mais característica, é a sua atividade hemaglutinante (AH), ou seja, sua capacidade de aglutinar eritrócitos. Essa atividade é muito usada em muitas etapas de estudos de purificação dessas macromoléculas, tanto na caracterização quanto no acompanhamento da purificação. Pois, a partir do momento em que se identifica e padroniza a AH da lectina em estudo, pode-se verificar a influência de diferentes agentes, como: moléculas, pHs, temperaturas e diferentes soluções iônicas, nessa atividade. Mas vale ressaltar que nem todas as lectinas são capazes dessa atividade, a exemplo, das lectinas que só possuem um único domínio de reconhecimento a carboidrato (DRC).

A partir da atividade hemaglutinante e da concentração (mg / mL) da lectina, pode-se obter índices importantes na purificação, como, atividade hemaglutinante específica (AHE), fator de purificação e rendimento (%). Cada proteína isolada, apresenta índices próprios de purificação (tabela 1). Por exemplo, a lectina PeRoL e EantH apresentam alto valor de AHE,

respectivamente 34.133 e 20.480 (SILVA et al., 2019a; SIRITAPETAWEE et al., 2018), quando comparado com a ApuL e EuniSL, respectivamente 182,8 e 85,3 (BRITO et al., 2017; OLIVEIRA et al., 2008). Com relação ao fator de purificação pode-se ver, por exemplo, que a MvFL 3.413 (PATRIOTA et al., 2017) e a PeRoL 22.021 (SILVA et al., 2019a) apresentaram valores muito mais altos, quando comparados a GDL 26,8 (AHSANUL KABIR et al., 2019) e GaBL 2,9 (COSTA et al., 2018). Esses e outros diferentes índices podem ser explicados por vários motivos, como: a estrutura da molécula, número e tipo de glicosilação, interações e ligações intramoleculares, do método de extração e purificação, do método de dosagem, e do sangue utilizado, por exemplo.

Tabela 1. Índices utilizados na purificação de lectinas.

Lectinas	mg / mL	AH	AHE	P	%	Referências
AFL	0,1	256	2.560	56	49	(CARNEIRO et al., 2019)
BlL	-	-	10.240	75	25	(NUNES et al., 2011)
CasuL	0,025	32	1.280	111,8	26,4	(PROCÓPIO et al., 2017)
cMoL	0,296	256	864	2,3	59	(SANTOS et al., 2009)
CrataBL	1,255	128	102	1,17	100	(ARAÚJO et al., 2012)
EantH	0,5	1.024	20.480	22,1	22,6	(SIRITAPETAWEE et al., 2018)
EuniSL	1,77	151	85,3	11,68	11	(OLIVEIRA et al., 2008)
PeRoL	0,12	4.096	34.133	22.021	-	(SILVA et al., 2019a)
GDL	-	-	1.716	26,8	84	(AHSANUL KABIR et al., 2019)
Penicillium proteolyticum lectin	1,52	256	168,42	52,30	84,21	(SINGH; WALIA; KENNEDY, 2019)
ApulSL	0,062	512	8.258	124,8	-	(CARVALHO et al., 2015)
ApuL	1,4	256	182,8	11,2	-	(BRITO et al., 2017)
GaBL	0,557	1.024	1.838	2,9	100	(COSTA et al., 2018)
MvFL	0,20	2.048	10.240	3.413	7,5	(PATRIOTA et al., 2017)

mg / mL: concentração de lectina em mg / mL. AH: atividade hemaglutinante. AHE: atividade hemaglutinante específica, que corresponde à razão entre HA e concentração de proteína (mg / mL). P: fator de purificação, foi calculado pela razão entre o AHE da etapa e a AHE do extrato, corresponde ao número de vezes que a pureza da

lectina foi aumentada. Rendimento (%): relação entre AH total da etapa e a AH total do extrato, corresponde ao percentual da AH total recuperada em cada etapa. Iso: Isoforma.

Além desses índices muito utilizados em estudos de purificação, outra atividade muito importante, derivada da atividade hemaglutinante, é a inibição da atividade hemaglutinante (IAH). Trata-se de uma atividade que revela quais os tipos de moléculas que interferem na AH da lectina em estudo, ou seja, moléculas que quando incubadas com a lectina, diminuem sua capacidade de aglutinar eritrócitos, normalmente as moléculas utilizadas são carboidratos e/ou proteínas. Uma vez determinado as moléculas específicas que afetam essa atividade (tabela 2), pode-se, por exemplo, melhor sugerir matrizes para utilização nas colunas de purificação, ou fornecer dados iniciais para outros estudos que visem investigar as interações moleculares entre a lectina e a molécula inibidora.

Por exemplo, em estudo realizado por Rubeena et al. (2019), foram avaliados 15 carboidratos (n-acetil-glicosamina, maltose, n-acetil-galactosamina, 2-desoxi-d-glicose, ramnose, manose, glicose, metil-α-d-galactopiranosídeo, sacarose, arabinose, xilose, galactose, ribose, lactose e rafinose) para inibição da AH da lectina de *Etroplus suratensis, Es-Lec*. Verificou-se que apenas glicose (0,06 mM), manose (0,06 mM), n-acetil-d-glicosamina (0,06 mM), maltose (0,24 mM) e 2-deoxi-d-glicose (0,24 mM) inibiram a AH da lectina. Sendo glicose, manose e n-acetil-glicosamina os principais inibidores, agindo mesmo em concentrações de 0,06 mM. Nesse estudo a *Es-Lec* foi purificada utilizando uma matriz de afinidade manose-Sepharose CL-4B, apresentando, assim, relação entre os melhores carboidratos da IAH e a matriz utilizada para isolamento (RUBEENA et al., 2019). Na tabela 2, abaixo, pode-se observar outras lectinas e respectivas moléculas (carboidratos e/ou proteínas) inibidoras de sua AH.

Tabela 2. Carboidratos e proteínas que inibem a AH de diferentes lectinas.

Lectinas	Carboidratos	Proteínas	Referências
SteLL	N-Acetil-D-Glicosamina	-	(GOMES et al., 2013)
BmoLL	D-Galactose D-Rafinose Metil-β-D-Galactopiranosídeo	-	(COELHO; SILVA, 2000)
CasuL	-	Ovalbumina Fetuína Seroalbumina	(PROCÓPIO et al., 2018)
cMoL	-	Azocaseína Asialofetuína	(SANTOS et al., 2009)
WSMoL	Frutose Glicose	Tiroglobulina	(SANTOS et al., 2005)

	Rafinose		
Cramoll 1	Metil-α-D-Manosídeo Trealose Metil-α-D-Glicopiranosídeo Manose Frutose Glicose Ramnose	-	(CORREIA; COELHO, 1995)
PgteL	-	Caseína Ovalbumina	(SILVA et al., 2016a)
PeRoL	Trealose Galactose Glicose Manose N-Acetil-D-Glicosamina	Tiroglobulina Fetuína	(SILVA et al., 2019a)
CrataBL	Galactose Glicose Frutose Manose N-Acetil-D-Glicosamina	Caseína Fetuína Ovalbumina	(ARAÚJO et al., 2012)
MvFL	-	Fetuína Ovalbumina	(PATRIOTA et al., 2017)
ConBr	Glicose Manose Frutose	-	(MOREIRA; GAVADA, 1984)
PpaL	Glicose Galactose Manose Metil-α-D-Manosídeo	-	(CAVADA et al., 2020)
Es-Lec	Glicose Manose N-Acetil-D-Glicosamina Maltose 2-Deoxi-D-Glicose	-	(RUBEENA et al., 2019)

-: não apresentou inibição ou não foi descrito/testado.

As estruturas já publicadas, dessas macromoléculas, apresentam muitas formas, e a maioria delas foram resolvidas por difração de raios X. De maneira geral, elas podem ser formadas por uma única cadeia polipeptídica ou mais de uma cadeia, formando assim monômeros, dímeros, trímeros, tetrâmero, etc. Alguns exemplos: a lectina Gal-10, formada por uma única cadeia polipeptídica (ITOH et al., 2020), a SPL-1, por duas cadeias (UNNO et al., 2019), a BC2L-C, por três (BERMEO; BERNARDI; VARROT, 2020), LecB, quatro (BAERISWYL et al., 2019), Clec4f, seis (OUYANG et al., 2020), e CEL-III, sete (UNNO; GODA; HATAKEYAMA, 2014). Essas cadeias polipeptídicas podem ser iguais ou diferentes,

quando iguais, adiciona-se o prefixo “homo”, quando diferentes “hetero” (exemplos: lectinas homodiméricas e lectinas heterodiméricas).

A maior ou menor complexidade dessas estruturas, desde o número de cadeias polipeptídicas mencionadas a cima, dobramentos dessas cadeias, pontes dissulfetos e outra modificações pós-traducionais, pode ser explicado pelo: organismo produtor e sua maquinaria celular, bem como a função que a mesma desempenha, no seu ambiente nativo. Podendo ser, por exemplo, um receptor celular de macrófagos, Clec2d (LAI; CRUZ; ROCK, 2020) ou uma lectina formadora de poro CEL-III, de pepino do mar *Cucumaria echinata* (UNNO; GODA; HATAKEYAMA, 2014). Para desvendar essas estruturas, são necessários estudos de extração, purificação e caracterizações, que forneçam subsídios para o melhor entendimento de sua conformação. Nos próximos parágrafos será abordado uma visão geral sobre purificação e caracterização de lectinas.

A purificação de uma lectina pode ser realizada de diferentes formas, isso depende de vários fatores, como equipamentos disponíveis, objetivo do estudo, experiência do pesquisador e outros fatores. É muito comum grupos de pesquisa realizarem as seguintes etapas para obtenção de lectinas: extração, fracionamento salino, e cromatografia em coluna. Mas também pode ser utilizado outras abordagens, como: micelas reversas, sistemas de duas fases, clonagem e expressão, e outras.

Para extração podem ser utilizados diferentes solventes em diferentes concentrações e pHs, como: 0,15 M de NaCl (PATRIOTA et al., 2017), 100 mM de Tris-HCl pH 7,6 contendo 150 mM de NaCl (SANTOS et al., 2019a), e 50 mM de Tris-HCl pH 8,0 contendo 150 mM de NaCl e 0,1 mM de PMSF (fluoreto de fenilmetilsulfonil) (CARNEIRO et al., 2019). Para o fracionamento (utilizando solução de saturação), podem ser usadas diferentes soluções, como, soluções salinas, alcoólicas, metanólicas, etc. Salinas, podem ser citadas, com sulfato de amônio, por exemplo: 70 % de saturação (CARNEIRO et al., 2019), 40 % de saturação (BRITO et al., 2017), ou utilizar duas etapas, como, 0-30 % e depois 30-60 % (PIRES et al., 2019), ou mais etapas, e até mesmo não utilizar nenhum tipo de fracionamento (AHSANUL KABIR et al., 2019).

Da mesma forma, para a cromatografia em coluna as matrizes utilizadas são muito variadas, podendo ser, de troca iônica (catiônica ou aniônica), afinidade, filtração em gel (exclusão molecular), interação hidrofóbica e adsorção, são algumas. Exemplos de diferentes matrizes: Sepharose (CARNEIRO et al., 2019), Quitina (SILVA et al., 2019a) Gel de Guar

(SANTOS et al., 2009), Mannose-Agarose, DEAE-Sephacel (SANTOS et al., 2019a), Sepharose 4B acoplada com Concanavalina A (DA SILVA et al., 2012), DEAE-Sepharose, Sephadex G-100 (SINGH; WALIA; KENNEDY, 2018), e Sepharose CL-4B acoplada com Mannose (RUBEENA et al., 2019). Também o número de cromatografias, ou etapas cromatográficas, podem ser variadas, onde pode ser observado estudos com uma única etapa cromatográfica (AHSANUL KABIR et al., 2019), com duas etapas (SIRITAPETAWEE et al., 2018), três etapas (PIRES et al., 2019), quatro (SILVA et al., 2012), ou até mais.

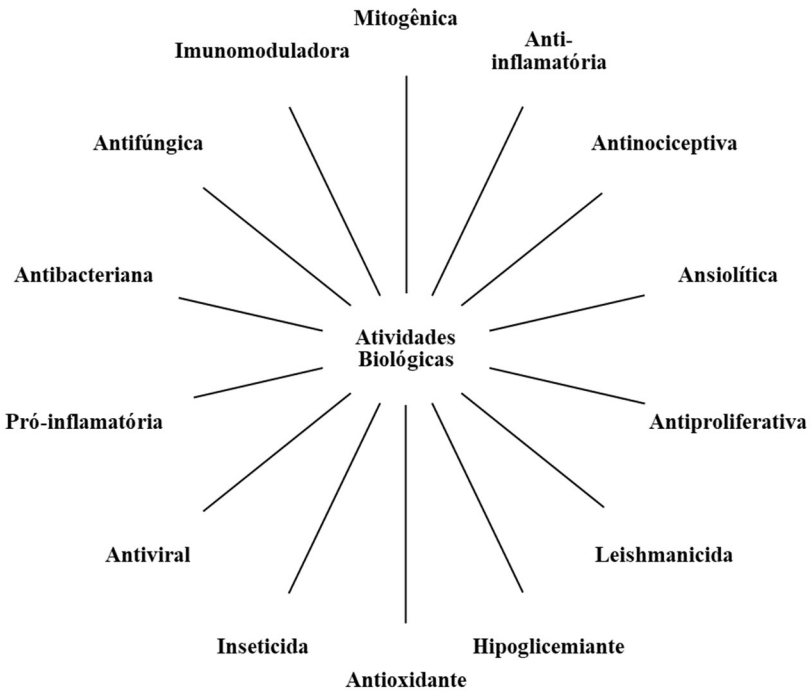
As soluções de equilíbrio, lavagem e eluição utilizadas nessas matrizes variam de acordo com o protocolo estabelecido, que leva em conta pontos como o tipo de matriz e o tipo de molécula que se pretende obter. Podendo ser soluções de água destilada, NaCl, ácido acético, tampões (contendo ou não um sal), utilização ou não de gradiente salino, etc. As dimensões das colunas (comprimento x diâmetro), como se pode ver a seguir, são também diversos: 30,0 x 1,5 cm (BRITO et al., 2017), 7,5 × 1,5 cm (SILVA et al., 2019a), 60 × 1,6 cm (COSTA et al., 2018) e 3,0 cm × 1,5 cm (SIRITAPETAWEE et al., 2018), mas normalmente as colunas de gel filtração são maiores, porque as dimensões são cruciais para a melhor resolução da separação molecular. Dependendo do tipo de coluna, elas podem ser produzidas (montadas) em laboratório ou compradas comercialmente de diferentes fabricantes.

Após a purificação ou concomitantemente, são utilizadas técnicas ou métodos para caracterização das lectinas em estudo. Para esse fim, podem ser utilizadas diferentes abordagens, a depender do objetivo. Podem ser citadas por exemplo: espectroscopia UV-Vis, eletroforeses em condições desnaturantes (SDS-PAGE) e nativas (acida ou básica), eletroforese bidimensional, cromatografia líquida de alta eficiência (CLAE), espectrometria de massas, espectroscopia de fluorescência, espectroscopia de dicroísmo circular, técnicas calorimétricas (DSC, DTG, ITG), difração por raios X (DRX), espectroscopia de infravermelho com transformada de Fourier (EITF) e espectroscopia de ressonância magnética nuclear (ERMN), são algumas muito utilizadas.

2.1.1 Atividades biológicas e aplicações

As lectinas como já citado, possuem muitas atividades biológicas descritas na literatura. E isso se deve, entre outros fatores, ao grande número de lectinas já purificadas e a sua ampla distribuição nos diferentes organismos da natureza. Na figura 2 são citadas algumas atividades já testadas.

Figura 2. Atividades biológicas de lectinas.



Fonte: Autor próprio.

As atividades biológicas já descritas com lectinas são muito variadas, como, antiviral (AGARWAL; TRIVEDI; MITRA, 2020), mitogênica (SINGH; THAKUR; KENNEDY, 2020), antiproliferativa (RAMOS et al., 2019), antioxidante (LACERDA et al., 2017), antinociceptiva (PIRES et al., 2019), antifúngica (NOVA et al., 2020), antibacteriana (RUBEENA et al., 2019), anti-inflamatória (CAMPOS et al., 2016), imunomoduladora (SANTOS et al., 2020a), inseticida (OLIVEIRA et al., 2020b) pró-inflamatória (LAI; CRUZ; ROCK, 2020), anxiolítica (ARAÚJO et al., 2020) gastroprotetora (PINTO et al., 2019a), anti-necrótica (OLIVEIRA et al., 2021), leishmanicida (ARANDA-SOUZA et al., 2018) e hipoglicemiante (LU et al., 2018), são exemplos (figura 2). Esses testes biológicos foram feitos a partir de trabalhos de purificação de lectinas de diferentes organismos e de diferentes partes dos organismos, através de abordagens e métodos particulares de cada estudo. Por exemplo: antibiofilme de Es-Lec (de soro de *Etroplus suratensis*, peixe) (RUBEENA et al., 2019), imunomoduladora de SteLL (de folhas de *Schinus terebinthifolia*, planta) (LIMA et al., 2019b), e pró-inflamatória de BlL (do veneno de *Bothrops leucurus*, cobra) (ARANDA-SOUZA et al., 2019).

Pode-se perceber também nos estudos, que diferentes lectinas podem apresentar atividades com efeitos inversos, como, anti-inflamatória de LAL (PIRES et al., 2016) e pró-inflamatória de PPL (WANG et al., 2019). O mesmo pode ser observado, com a rLSL que possui atividade hemolítica (TATENO; GOLDSTEIN, 2003), MpLeC que é anti-hemolítica

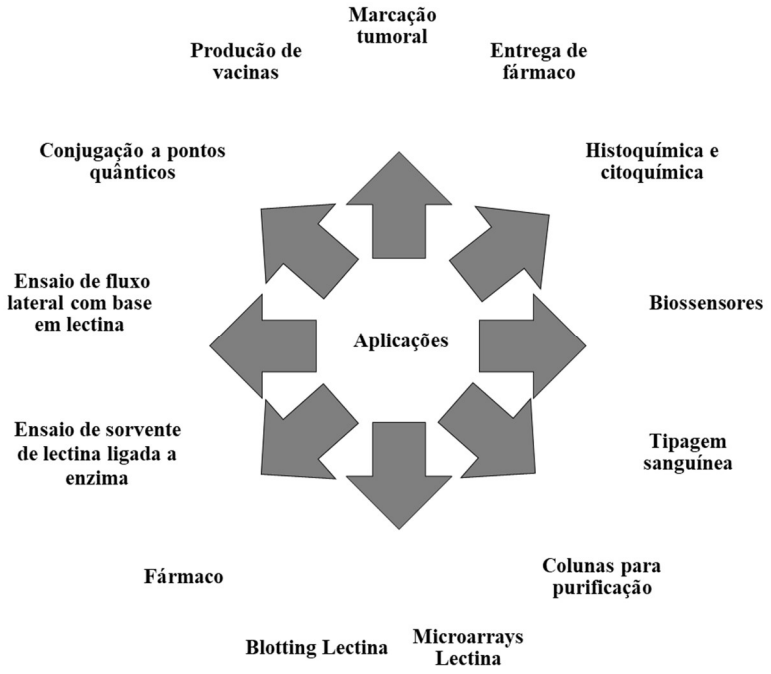
(PINTO et al., 2019a) e PeRoL que é não-hemolítica (SILVA et al., 2019a). Ou seja, dependendo: do tipo de estrutura, do seu enovelamento, fosforilação, do tipo de carboidrato que ela tem afinidade, da concentração testada, do pH e temperatura do meio que ela está inserida, e de muitos outros fatores, a lectina pode apresentar diferentes atividades e funções. E essas propriedades podem ter ou não, relação direta com a função que a lectina desempenha na sua fonte natural ou ambiente nativo. Pois a mesma pode ter sofrido alguma alteração no seu processo de obtenção, no ambiente de aplicação/teste, e outras, mesmo que mínima.

Por fim, vali salientar também, a versatilidade dessas moléculas, pois não é incomum que uma única lectina possa apresentar diferentes atividades, como no caso da CrataBL, que tem estudos: antitumoral, anti-inflamatório, antinociceptivo (ARAÚJO et al., 2011), inseticida (ARAÚJO et al., 2012), hipoglicemiante (ROCHA et al., 2013), citotoxicidade em células cancerígenas (FERREIRA et al., 2013), ação no bloqueio da coagulação e formação de trombo arterial (SALU et al., 2014), atenuação de alterações inflamatórias (OLIVA et al., 2015), controle de larvas de *Callosobruchus maculatus* (NUNES et al., 2015), desempenha papel no controle da resposta da asma, e mais. E de outro modo, muitas lectinas podem apresentar atividades em comum, como, atividade antibiofilme: apresentada por Es-Lec (RUBEENA et al., 2019), AFL (CARNEIRO et al., 2019), PgTeL (SILVA et al., 2019c) e ConA (JIN; LEE; HONG, 2019), por exemplo.

Além dessas muitas atividades biológicas, também existem muitos outros estudos visando diferentes aplicações (figura 3), como hidrogel contendo lectina (100 μg / mL), para tratamento de queimaduras de segundo grau (PEREIRA et al., 2019), formulação de andaime de membrana e hidrogel, contendo Frutalina, para cicatrização de feridas dérmicas (SOUSA et al., 2019), expressão heteróloga de lectinas, para facilitar produção em larga escala (LEYVA et al., 2019; LI et al., 2019; PINTO et al., 2019b; VAREJÃO et al., 2010), efeito da radiação na atividade de lectina (SANTOS et al., 2018b), padrão de ligação à lectina na placenta de mamíferos (DORSCH et al., 2019), funcionalização de nanotubos de dióxido de titânio (OLIVEIRA et al., 2018), sondas bifuncionais cliváveis para detecção de glicanos (MA et al., 2019), utilização conjunta de lectina e antibiótico para combater microrganismos multirresistentes (MDR) (SANTOS et al., 2019b), avaliação de afinidade a superfície celular, e do efeito sobre a atividade metabólica, de lectinas, em cianobactéria de água doce (TAKAARA et al., 2019), detecção de patógenos bacterianos por citometria de fluxo, utilizando lectina como ligante específico aos microrganismos (HENDRICKSON et al., 2019), estudos de hetero-multivalência (WORSTELL et al., 2018; WORSTELL; SINGLA; WU, 2019), pró-

fármaco de lectina-doxorrubicina fotoativável (DION et al., 2019), nanopartículas de sílica mesoporosa funcionalizadas com lectinas, tendo como alvo células cancerígenas (BHAT et al., 2018; CHEN et al., 2017; MARTÍNEZ-CARMONA et al., 2018), como agente de reconhecimento, para o enriquecimento de nanopartículas magnéticas em *S. aureus* (YANG et al., 2019), e para avaliar mecanismo de ação em *Aedes aegypti* (ALVES et al., 2019).

Figura 3. Aplicações de lectinas.



Fonte: Autor próprio.

Esses são alguns exemplos do grande número de aplicações, já estudadas, para esse amplo grupo de glicoproteínas. Nos próximos três parágrafos, serão citados alguns estudos recentes de lectinas, relacionadas com pontos quânticos, lipossomas e marcação de células cancerígenas, respectivamente, como forma de reforçar a sua importância, tendo em vista a novidades dos estudos.

Interação de lectinas com pontos quânticos (PQ) e lectinas conjugadas a PQ, têm sido muito aplicadas para marcação de superfícies, organismos, células, avaliar mecanismos de ação, ligações a organismos alvos, como em alguns exemplos citados a seguir: avaliação de perfis de glicose e manose em espécies de Cândida, usando pontos quânticos conjugados com a lectina Cramoll, como nanossondas fluorescentes (OLIVEIRA et al., 2020c); interações da lectina de ligação da manose com glóbulos vermelhos, empregando pontos quânticos catiônicos (LIMA et al., 2019a); avaliação do glicofenótipo em tecidos de câncer de mama com conjugados PQ-

Cramoll lectina (CARVALHO et al., 2019); e avaliação do mecanismo de ação em *A. aegypti*, baseado em PQ-lectina-marcação (ARRUDA et al., 2019).

Os estudos de lectinas relacionados a lipossomas são muito amplos, mas basicamente estão ligados ao direcionamento específico da formulação lipossomal revestida com lectinas e/ou encapsulação das lectinas bioativas, para melhorar a biodisponibilidade das mesmas, e assim, melhorar sua atividade. Exemplos: ligação e captação comparativas de lipossomas decorados com oligossacarídeos de manose por células que expressam o receptor de manose ou DC-SIGN (GAO et al., 2020a); nanocápsulas lipossômicas de Tarin, controlam o glioblastoma humano e a proliferação celular de adenocarcinoma mamário (CORRÊA et al., 2019); e lipossomas de aglutinina de germe de trigo com ciclodextrinas enxertadas na superfície como nanocarreadores bioadesivos de entrega dupla de drogas para tratar células orais (WIJETUNGE et al., 2020).

As lectinas, por possuírem capacidade de ligação e reconhecimento de carboidratos específicos, são muito exploradas para detecção e marcação de células cancerígenas. Como: lectina de *Sclerotium rolfsii* induz efeitos opostos em PBMCs normais e células leucêmicas Molt-4, ao reconhecer o antígeno TF e suas variantes como receptores (CHACHADI et al., 2020); nanopartículas de lectina para detecção de glicovariantes do antígeno 15-3 (CA15-3) associados ao câncer de mama no plasma humano (TERÄVÄ et al., 2019); lectinas na detecção de células cancerígenas utilizando o fenômeno de emissão induzida por agregação (AIE) (WANG; HANG; HUA, 2019); e detecção de anticorpos para o HCV E1E2 por captura de lectina (ELLSA) (MAJOR; LAW, 2019).

Por fim, é valido destacar que lectinas e kits baseados em lectinas (ou seja, ferramentas biotecnológicas derivadas de lectina) já são vendidos comercialmente, por exemplo (respectivamente, produto e empresa): lectina blotting kits L2650 (Sigma-Aldrich); colunas com lectinas para isolamento de glicoproteínas (Thermo Scientific e GE Healthcare Life Sciences); lectinas para histoquímica e citoquímica (Sigma-Aldrich, EY Laboratories, Roche, e Life Technologies); e lectina microarrays (Plexera e RayBiotech) (DAN; LIU; NG, 2016; HENDRICKSON; ZHERDEV, 2018).

2.1.2 Lectinas de folhas

Até o momento as lectinas foram tratadas de maneira geral, a partir deste item, serão abordadas apenas lectinas de folhas, devido a presente tese se dedicar a purificação desse grupo de lectinas. As lectinas de folhas são um subgrupo das lectinas de plantas, nesse sentido é

importante ressaltar brevemente a importância das plantas como grande fonte de produtos naturais, e com muitos estudos apresentando metabólitos primários e secundários com alto potencial biotecnológico. Isso se deve entre outros aspectos, a ampla distribuição da flora, e a sua infinita diversidade. Tendo hoje no mercado, inúmeros fármacos e produtos biotecnológicos extraídos e purificados de plantas. E as lectinas são um desses grupos de biomoléculas de alta relevância, por sua diversidade estrutural, química e biológica, o que permite muitas aplicações, como já citado.

O maior número de estudos de purificação de lectinas publicados te como fonte as plantas, sendo assim, é o grupo onde se encontra a maior diversidade de lectinas, atividades biológicas e aplicações. Esses estudos realizaram purificação e caracterização lectinas de diferentes partes das plantas, como folhas (HIREMATH et al., 2020; NOVA et al., 2020), sementes (CAVADA et al., 2020; NASCIMENTO et al., 2020), raízes (SILVA et al., 2019a), rizomas (AHSANUL KABIR et al., 2019), casca do tronco (COSTA et al., 2018), sarcotesta de sementes (SILVA et al., 2016a), exsudato do floema de frutos (NAREDDY; BOBBILI; SWAMY, 2017), bulbo (VAN DAMME, 2020) e látex do caule (SIRITAPETAWEE et al., 2018), por exemplo.

As lectinas de plantas possuem diferentes tipos de classificações, e de maneira mais geral podem ser classificadas pela fonte de obtenção (tecido ou parte da planta), como lectinas de folhas, sementes, etc. E mais especificamente nas seguintes classificações:, como, 1) pelo domínio de ligação a carboidratos (DRC) em: Merolectinas (domínio único de ligação a carboidratos); Hololectinas (pelo menos dois domínios de ligação a carboidratos, idênticos e similares); Superlectinas (possuem dois domínios de ligação a carboidratos não idênticos, que reconhecem açúcares estruturalmente diferentes); e Quimerolectinas (que possuem um domínio de ligação a carboidratos e outro domínio com atividade enzimática) (VAN DAMME et al., 1997; BARBIERI et al., 1993; COLLINGE et al., 1993). 2) Classificação das lectinas com base em proteínas relacionadas estrutural e evolutivamente: Lectinas da família do Amaranto; Lectinas de ligação à quitina; Lectinas do floema de Cucurbitaceae; Lectinas relacionadas a jacalina; Lectinas de leguminosas; Lectinas de monocotiledôneas ligadoras de manose; Lectinas inativadoras de ribossomos tipo 2 (RIP 2). E também 3) Baseado na afinidade de ligação à porção de carboidratos: afinidade por Glicose; Galactose e N-acetil-β-galactosamina; L-fucose; e Ácido siálico (MISHRA et al., 2019).

As lectinas de folhas, ou seja, lectinas que foram purificadas a partir de diferentes partes das folhas, também são muito estudadas. Delas já foram purificadas muitas dessas

macromoléculas, por exemplo: LCL, MvFL, MuLL, CasuL, ARLL, AAA, Aloctin 1, BmoLL e PptyLL (tabela 3). Vali salientar que as lectinas de sementes são também muito estudadas, sendo, por alguns autores, consideradas as mais estudadas, tendo sido purificadas lectinas muito conhecidas e bem caracterizadas, seja físico-química ou biologicamente, como, a ConA, ConBr e Cramoll. Mas as lectinas de folhas têm a vantagem de serem de fácil obtenção, estarem disponível em algumas espécies o ano todo e em grande quantidade, isso quando são comparadas com lectinas de frutos, sementes, e flores, por exemplo, que muitas vezes tem que se esperar determinada época do ano para realizar sua coleta.

Tabela 3. Lectinas de folhas de diferentes espécies de plantas.

Espécies	Lectinas	References
Agelanthus brunneus	Lectin de *Agelanthus brunneus*	(JOHNNY et al., 2016)
Agropyrum repens	ARLL	(CAMMUE; STINISSEN; PEUMANS, 1985)
Allium altaicum	AAA	(UPADHYAY et al., 2011)
Allium ascalonicum	AAL	(RAJA et al., 2016)
Allium sativum	ASAL	(SAHA et al., 2006)
Allium ursinum	AUAL	(SMEETS et al., 1997)
Aloe vera	Aloctin I	(AKEV et al., 2007)
Arisaema heterophyllum	AHA	(ZHAO et al., 2003)
Bauhinia monandra	BmoLL	(COELHO; SILVA, 2000)
Calliandra surinamensis	CasuL	(PROCÓPIO et al., 2017)
Chenopodium amaranticolor	Lectin de *Chenopodium amaranticolor*	(SUSEELAN; MITRA, 2001)
Chorchorus olitorius	JLL-1, JLL-2 e JLL-3	(KHAN et al., 2008)
Cycas annaikalensis	CAPL	(RADHA; URLA, 2019)
Cycas revoluta	CRLL	(SHIMOKAWA et al., 2016)
Dendrobium officinale	DOA2	(CHEN; SUN; TANG, 2005)
Dolichos lablab	DLL-II	(RAMESHWARAM; NADIMPALLI, 2008)
Dregea volubilis	DVLL	(PATIL; DESHPANDE, 2015)
Erythrina indica	EiLL	(KONOZY et al., 2002)
Euphorbia helioscopia	EHL	(RAFIQ et al., 2014)
Euphorbia tithymaloides	Lectin de *Euphorbia tithymaloides*	(JAWADE et al., 2016)
Glechoma hederacea	Gleheda	(WANG et al., 2003)
Griffonia simplicifolia	GS-II	(LAMB; SHIBATA; GOLDSTEIN, 1983)
Hydrangea macrophylla	HM30	(YANG; GONG, 2002)
Kalanchoe crenata	Lectin de *Kalanchoe crenata*	(ADENIKE; ERETAN, 2004)
Lantana camara	LCL	(HIREMATH et al., 2020)
Listera ovata	LOA	(VAN DAMME; ALLEN; PEUMANS, 1987)

Manihot esculenta	Lectin de *Manihot esculenta*	(SILVA et al., 2010b)
Microgramma vacciniifolia	MvFL	(PATRIOTA et al., 2017)
Moringa oleifera	SLL-1, SLL-2 e SLL-3	(KHATUN et al., 2009)
Morus alba	MLL	(DEEPA et al., 2012)
Morus rubra	MRL	(SURESHKUMAR; PRIYA, 2012)
Mussaenda alicia	Lectina de *Mussaenda alicia*	(NOVA et al., 2020)
Myracrodruon urundeuva	MuLL	(NAPOLEÃO et al., 2011)
Narcissus tazetta	Lectin de *Narcissus tazetta*	(OOI et al., 2000)
Neoregelia flandria	Lectin de *Neoregelia flandria*	(YAGI et al., 1996)
Nicotiana tabacum	Nictaba	(LANNOO et al., 2007)
Phaseolus vulgaris	GNLL	(KAMEMURA et al., 1996)
Phthirusa pyrifolia	PpyLL	(COSTA et al., 2010)
Sambucus ebulus	SELld	(ROJO et al., 2003)
Schinus terebinthifolius	SteLL	(GOMES et al., 2013)
Vicia unijuga	anti-N	(YANAGI et al., 1990)
Viscum album	ML I, ML II e ML III	(HINCHA; PFÜLLER; SCHMITT, 1997)
Withania somnifera	Lectin de *Withania somnifera*	(GHOSH, 2009)
Sophora japonica	LLI e LLII	(HANKINS; KINDINGER; SHANNON, 1987)
Iris hybrida	Lectina de *Iris hybrida*	(FERENS-SIECZKOWSKA; ORCZYK-PAWIŁOWICZ; MORAWIECKA, 1997)
Bauhinia variegata	BvaLL	(OLIVEIRA, 2006)
Indigofera suffruticosa	IsuLL	(PEREIRA, 2013)

Fonte: Autor próprio.

As famílias de plantas, fontes de lectinas de folhas, são as mais diversas, Fabaceae (PROCÓPIO et al., 2017), Anacardiaceae (NAPOLEÃO et al., 2011), Verbenaceae (HIREMATH et al., 2020), Polypodiaceae (PATRIOTA et al., 2017), Loranthaceae (JOHNNY et al., 2016), Amaryllidaceae (RAJA et al., 2016), Poaceae (CAMMUE; STINISSEN; PEUMANS, 1985), Xanthorrhoeaceae (AKEV et al., 2007), Araceae (ZHAO et al., 2003), Amaranthaceae (SUSEELAN; MITRA, 2001), Malvaceae (KHAN et al., 2008) Cycadaceae (RADHA; URLA, 2019) e Rubiaceae (NOVA et al., 2020), são exemplos. E essas famílias englobam, por sua vez, muitas espécies diferentes, das quais já foram isoladas muitas lectinas, como visto na tabela 3. O que resulta em uma enorme variedade de lectinas de folhas, seja no que diz respeito a fonte de obtenção, ou no que se refere as suas estruturas, tamanhos,

afinidades, domínios de ligação/reconhecimento a carboidratos (DRC), e outras características particulares.

Exemplificando alguma dessas particularidades pode-se observar as massas moleculares, e as melhores faixas de pHs e temperaturas de algumas lectinas, como: LCL que possui duas cadeias polipeptídicas de 14,49 kDa e 17,4 kDa, é termoestável em pH neutro até 80 °C e mostrou tolerância nos valores de faixa de pH de 4,3 a 10,0 (HIREMATH et al., 2020); MvFL tem 54 kDa, sua atividade hemaglutinante não foi alterada pelo aquecimento a 100 °C por 30 min, mas foi reduzido em pH alcalino (8,0 e 9,0) (PATRIOTA et al., 2017); CasuL tem 27, 13 e 10 kDa, mostrou valores mais altos de AH em pH 5,0, sendo também ativo em pH 7,0 (PROCÓPIO et al., 2017). Essas características conferem capacidades distintas as lectinas, explicando em parte muitas das diferentes atividades biológicas das mesmas, vista no próximo parágrafo.

Algumas atividades biológicas recentes de lectinas de folhas (lectina e atividade, respectivamente): Aloctin, anticancerígena (AKEV; CANDOKEN; KURUCA, 2020); LCL, mostrou forte ligação às células HT29 do adenocarcinoma do cólon humano, e possui potente atividade antibacteriana e antifúngica (HIREMATH et al., 2020); SteLL, imunomoduladora, alterando a liberação de citocinas pelos esplenócitos de camundongos (SANTOS et al., 2020a); CasuL, ação antimicrobiana, isoladamente e em combinação com antibióticos (PROCÓPIO et al., 2019); MuLL, termiticida (LIMA et al., 2018b); MLL, ação antimetastática (SARANYA et al., 2017); e BmoLL, anti-inflamatória e antinociceptiva (CAMPOS et al., 2016).

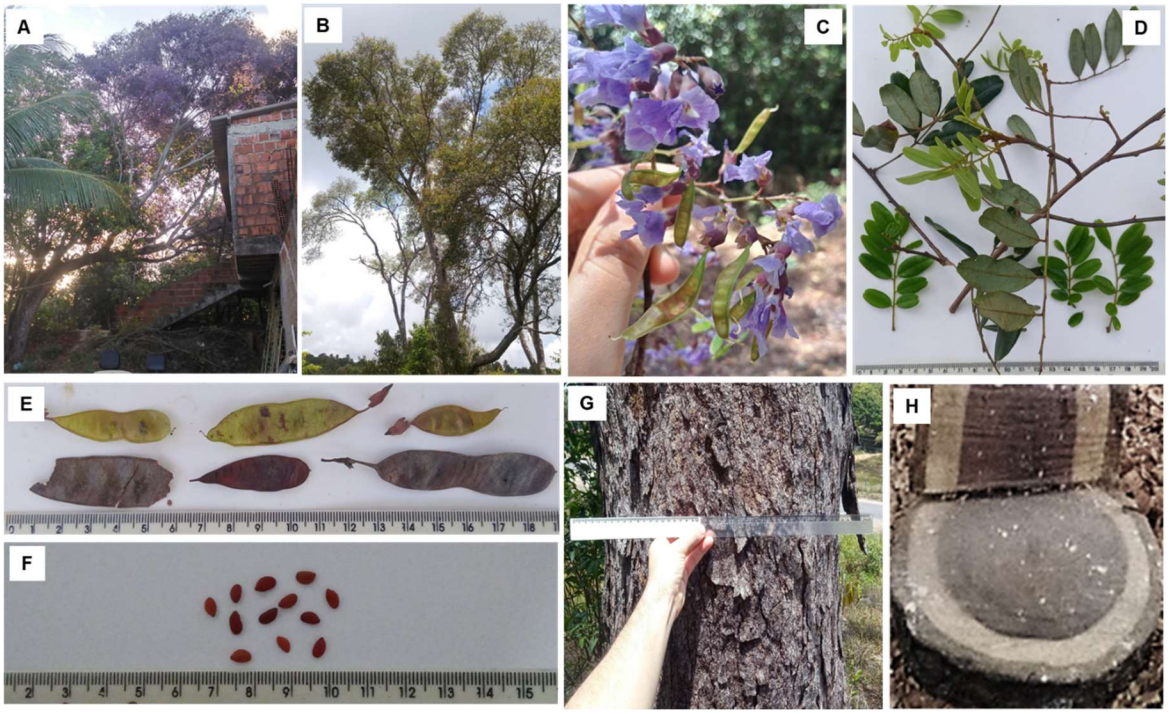
Por fim, vale destacar a identificação de 4 lectinas em folhas de *Viscum album* subsp. abietis, por análise proteômica: lectina específica de beta-galactosídeo 2 (ML-2), lectina específica de beta-galactosídeo 3 (ML-3), lectina específica de beta-galactosídeo 1 cadeia A isoforma 1 (ML-1) e lectina de ligação à quitina (cbML) (TSEKOURAS et al., 2020). Mostrando uma outra abordagem na pesquisa de lectinas, entre muitas possíveis, e também novas lectinas de folhas recentes descobertas.

Portanto, está claro que as lectinas são muito estudas e que as folhas de diferentes espécies são também fontes de muitas lectinas com alto potencial de aplicação. Essa tese apresenta no próximo item, a *Bowdichia virgilioides*, espécie fonte de compostos com alto potencial biotecnológico. E que já foi alvo de estudo de purificação de lectina da sua semente (LIMA, 2009).

2.2 BOWDICHIA VIRGILIOIDES

B. virgilioides (figura 4), popularmente chamada de sucupira-preta, e também conhecida por outros nomes, dependendo da região, como sucupira, sucupira-roxa, sucupira-mirin, sucupira-do-cerrado, sucupira-parda, sucupira-do-campo, coração-de-negro, paricarana e angelim-amargoso, e outros. Pertence à família Fabaceae (Leguminosae), subfamília Faboideae (Papilonoideae), é uma árvore de grande porte, com tronco de diâmetro de cerca de 60 centímetros e altura de aproximadamente 20 metros. Possui casca grossa e trincada, madeira ranchurada, pesada, cerne marrom escuro, e alta durabilidade. Floresce e frutifica anualmente entre, aproximadamente, setembro e dezembro, as pequenas flores com corola lilás, dão uma aparência ornamental e apícola (LORENZI, 2009; RIZZINI, 1990).

Figura 4. *B. virgilioides*. (A) Planta adulta com flores. (B) Planta adulta sem flores. (C) Inflorescências (flores e frutos). (D) Folhas. (E) Frutos verdes e maduros. (F) Sementes. (G) Tronco adulto, com diâmetro aproximado de 41cm. E (H) tronco em corte transversal no eixo vertical e horizontal, mostrando de dentro para fora respectivamente: o cerne, alburno e casca.



Fonte: Autor próprio.

Os frutos são samaróides, secos e indeiscentes. As sementes são ovóides, achatadas, têm hilo circular laranja, cercadas por arilo branco, rafe claramente visível, possuem cotilédones com reserva e eixo embrionário curto, com tamanho total e invaginado. A germinação é fanero-epígea-foliácea, unipolar, com eixo entre os cotilédones. As folhas compostas, cotiledonares, são verdes e com reservas, formato arredondado, pináculos e folhetos pubescentes (ALBUQUERQUE et al., 2015).

É uma planta nativa e não endêmica do Brasil, ou seja, não foi introduzida pelo homem e não é exclusiva de bioma brasileiro (ocorrendo também em outros países). No Brasil, sua distribuição geográfica é confirmada em todas as regiões (Norte, Nordeste, Centro-Oeste, Sudeste e Sul). Seus domínios fitogeográficos são: Amazônia, Caatinga, Cerrado, Mata Atlântica e Pantanal (JUCK et al., 2006; MACHADO et al., 2018; REFLORA, 2019).

Pesquisas etnofarmacológicas têm contribuído significativamente na pesquisa de plantas com potencial terapêutico (SARAIVA et al., 2015). E muitos estudos relatam o uso de *B. virgilioides* para vários fins medicinais, como para tuberculose (SHARIFI-RAD et al., 2017), inflamação do útero, cicatrização de feridas, inflamação vaginal, dor de garganta (SOUZA et al., 2014), tônico, diaforético, dermatoses (BRANDÃO et al., 2008), sífilis, gota, artrite (AQUINO; WALTER; RIBEIRO, 2007), purificadora do sangue, inchaço, vermes, dor abdominal (BOTINI et al., 2015), dor na coluna (dor no pescoço, dor nas costas, lombalgia com ciática) (RIBEIRO; MELO; BARROS, 2016), reumatismo (SARAIVA et al., 2015), dor de cabeça, aneurisma (GOMES et al., 2008), infecções diversas, amigdalites (CONCEIÇÃO et al., 2011), diabetes (MACEDO; FERREIRA, 2004), impotência sexual (afrodisíaco) e dor óssea (RIBEIRO et al., 2014a), são exemplos.

2.2.1 Atividades biológicas e compostos

B. virgilioides, como mostrado no item anterior, é amplamente utilizada na medicina popular para uma ampla gama de doenças. Em vista disso, muitos estudos se propuseram a testar suas atividades biológicas, como pode ser visto no próximo parágrafo. Pode-se observar nele, que a espécie *B. virgilioide*s possui atividades testadas a partir de distintas partes da planta e com diferentes tipos de amostras: extratos, frações, óleos e moléculas isoladas.

Alguns exemplos de atividades biológicas de diferentes partes de *B. virgilioides* são: cicatrização (com Lupeol, molécula purificada da casca do caule) (BESERRA et al., 2019), anti-hiperglicêmica (do extrato metanólico da casca da raiz) (SILVA et al., 2015), larvicida (a partir de fração ciclohexânica e etanólica do cerne do tronco) (BEZERRA-SILVA et al., 2015), antioxidante e antinociceptiva (do extrato etanólico 90%, e frações metanólica, hexânica, clorofórmica, acetato de etila e hidrometanólica da casca) (SHAN et al., 2016), antimicrobiana (do óleo da folha e da semente) (ALMEIDA et al., 2006)(LIMA et al., 2016), tripanocida (a partir de etanol 70% de folhas e cascas) (ORTIZ et al., 2010), hipoglicêmica (com benzofuranóides, da planta inteira) (LAKSHMI et al., 2016), antinociceptiva e anti-inflamatória (do extrato aquoso da folha e cerne da casca do tronco) (THOMAZZI et al., 2010), e inibição da inflamação alérgica (a partir do extrato aquoso da casca do caule) (JULIANE et al., 2016).

Estudos fitoquímicos anteriores com *B. virgilioides* descrevem moléculas de grande interesse químico por sua novidade estrutural, e do ponto de vista medicinal das várias atividades biológicas. Isso é muito positivo, sabendo que o interesse e a demanda por novos compostos de fontes naturais com função biológica, só crescem ano a ano, devido aos seus efeitos benéficos à saúde. No próximo parágrafo podem ser vistos alguns compostos identificados em *B. virgilioides*.

Como mencionado, muitos compostos já foram encontrados em *B. virgilioides*, e em diferentes partes, por exemplo: das folhas - triterpenos (Lupeol, Lupenona, β-sitosterol, Stigmasterol e β-amina) e ácidos graxos (não identificados) (TICONA et al., 2006); do cerne do tronco - isoflavonóides (Medicarpin e Maackiain) (BEZERRA-SILVA et al., 2015); das sementes - diterpenóides do tipo furanocassano (Sucupiraninas M-Q) (ENDO et al., 2019); da casca do tronco - fenóis, taninos, flavonóides, xantonas, catequinas, triterpenos pentacíclicos, esteróides livres, saponinas e alcalóides (SHAN et al., 2016); da casca e cerne do tronco (Ácido clorogênico, Ácido caféico, Rutina, Kaempferol e Quercetina) (LEITE et al., 2014); do fruto (Geraniol e Cariofileno) (ARRIAGA; GOMES; BRAZ-FILHO, 2000); da casca do caule (Ormosanina e Homoormosanina) (BRAVO et al., 2002); do caule (Lupeol, Lupenona, β-sitosterol, Stigmasterol, em mistura, e Siringaresinol, Bowdenol, Ácido trans-p-coumárico derivado éster, 8-metoxicoumestrol, 3,4-hidroxi-7-metoxi-isoflavona, 7,3'-di-hidroxi-4'-metoxi-isoflavona, 5,4'-di-hidroxi-7'-metoxi-isoflavona, isolados) (SILVA et al., 2019e).

2.2.2 Outros estudos

B. virgilioides é uma planta amplamente estudada, como já mostrado, com vários estudos de diferentes partes da planta e com diferentes focos. Além dos estudos etnofarmacológicos, atividades biológicas, identificação de compostos e isolamento de moléculas, já mostrados, também existem muitos estudos: ecológicos, agronômicos, morfológicos, entre outros. Mas vale ressaltar o grande número de pesquisas relacionadas às sementes, principalmente sobre germinação.

Por exemplo, estudos de florestamento, reflorestamento, conservação e paisagem (RANGEL; LEDO; ROCHA, 2009; SMIDERLE; SCHWENGBER, 2011), morfologia e comportamento das raízes (SALIS et al., 2014), morfologia de plântulas (RODRIGUES; TOZZI, 2007), anatomia da lâmina foliar (FERREIRA et al., 2015), aspectos morfológicos de frutos, sementes e mudas (ALBUQUERQUE et al., 2015), cultivo, desenvolvimento e biologia reprodutiva (DOMENE et al., 2014; SILVA; CHAVES; BRITO, 2011; VITAL et al., 2017),

estudos fenológicos (BULHÃO; FIGUEIREDO, 2002; LOCATELLI; MACHADO, 2004), e de composição florística e fitossociologia (BALDUINO et al., 2005; FELFILI et al., 1992, 2002).

E, como já mencionado, muitas pesquisas sobre sementes, especialmente sobre germinação, exemplos : como superar ou interromper a dormência (ANDRADE et al., 1997; SMIDERLE; SCHWENGBER, 2011; SMIDERLE; SOUSA, 2003), influência da amplitude da amostra nas medidas de germinação (RIBEIRO-OLIVEIRA; RANAL; DE SANTANA, 2013), caracterização física e avaliação da pré-embebição na germinação (GONÇALVES et al., 2008), alterações fisiológicas e bioquímicas durante a embebição (ALBUQUERQUE et al., 2009), germinação sob estresse hídrico (SILVA; AGUIAR; RODRIGUES, 2001), influência da cor do revestimento das sementes na germinação (SMIDERLE et al., 2015), validação de métodos de análise de germinação (CRUZ et al., 2012), estudos de tolerância à dessecação (MATHEUS et al., 2009), condutividade elétrica (DALANHOL et al., 2014), avaliação da qualidade das sementes por teste de raios-x (ALBUQUERQUE; GUIMARÃES, 2008), caracterização da qualidade por imagem (LIMA et al., 2018a), comportamento fisiológico sob diferentes temperaturas e condições de luz (ALBUQUERQUE; GUIMARÃES, 2007), e uso de biomassa de algas na peletização de sementes (MONTANHIM et al., 2014).

Os estudos mais recentes sobre *B. virgilioides*, são: a identificação de Sucupiraninas M-Q (cinco novos diterpenóides do tipo furanocassano) (ENDO et al., 2019), inibidores da catepsina V (SILVA et al., 2019e), atividade de folhas no amadurecimento de frutos (NASCIMENTO et al., 2019), atividade acaricida (NUMA et al., 2018), novo método proposto para discriminar espécies de madeira, entre elas *B. virgilioides*, combinando espectroscopia de refletância de infravermelho próximo (NIRS) e espectroscopia de ruptura induzida por laser (LIBS), e utilizando quimiometria para análise dos dados (LEANDRO; GONZAGA; LATORRACA, 2019), caracterização da qualidade das sementes por imagem (LIMA et al., 2018a), estudo de germinação de sementes (BARRETO et al., 2019; COÊLHO; PAULO; VIANA, 2019), conservação ambiental e ecológica (CONCEIÇÃO et al., 2019; NÓBREGA et al., 2020; OLIVEIRA et al., 2019a, 2019b; RICARDI-BRANCO; RIOS; PEREIRA, 2020), e avaliação do Lupeol na melhora da cicatrização de feridas em ratos hiperglicêmicos, com efeitos moduladores na inflamação, estresse oxidativo e angiogênese (BESERRA et al., 2019), além de seu efeito cicatrizante *in vitro* (BESERRA et al., 2018).

Portanto, foi observado nesta fundamentação teórica: a) a importância das lectinas, através de muitos estudos já realizados, de purificação, caracterização e atividades biológicas; b) bem como a importância da *B. virgilioides*, uma espécie nativa do Brasil, com alto potencial biotecnológico, grande variedade de moléculas já isoladas e muitas atividades biológicas já testadas. Tendo em vista todo o conteúdo abordado, essa tese se debruçou em purificar uma nova lectina de folhas de *B. virgilioides* e avaliar sua interação molecular. E também, realizar estudos com o extrato salino das folhas de *B. virgilioides*, com identificação dos compostos majoritários e avaliação do potencial biológico.

3 RESULTADOS

3.1 ARTIGO 1 - OVERVIEW ON PURIFICATION, BIOLOGICAL ACTIVITIES AND HETEROLOGOUS EXPRESSION OF LECTINS

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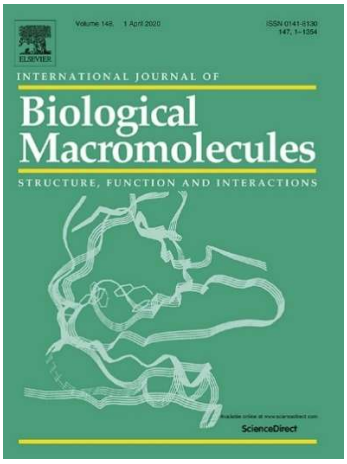

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Abstract

Lectins are proteins that recognize and bind carbohydrates and glycoconjugates in a specific and reversible way, and are found widely distributed in nature. Considering the importance and the large number of studies with lectins already published, this work proposed to carry out a review on purification, biological activities (highlighting healing) and heterologous expression of lectins. The methodology used was a broad bibliographic search in the main scientific research sites, using the terms: "lectin purification", "lectin characterization", "lectin biology activity", "lectin healing activity" and "heterologous expression of lectins". As well as the crossing of these terms. As a result of this review, it can be seen that lectins can be purified from various sources, such as microorganisms, plants and animals. That these purifications can be carried out in various ways, using different types of extractions, fractionations and chromatographs. As well as, using other methodological approaches, which include stages of biphasic systems, reverse micelles, and proteomics. It is still possible to have a broad view of a substantial number of their biological activities (such as antioxidant, antitumor, antiviral, and antibacterial, for example). In addition to these, the healing activity of lectins acting on the skin, liver, intestine, and eye was highlighted. Finally, heterologous expression studies were reviewed, showing the different models and respective lectins obtained.

Keywords: Lectins. Purification. Biological activities. Expression.

3.1.1 Introduction

Lectins are proteins or glycoproteins that have the ability to recognize and bind in a specific and reversible way to carbohydrates and glycoconjugates without changing the carbohydrate fraction (CAGLIARI; KREMER; PINTO, 2018; TSANEVA; VAN DAMME, 2020). They are widely distributed in nature, being found in the most varied sources, for example: plants (CAVADA et al., 2020), bacteria (SÝKOROVÁ et al., 2020; THUENAUER et al., 2020), fungi (SINGH; WALIA; KENNEDY, 2019), virus (THOMPSON et al., 2020), protozoa (SINGH; WALIA; KANWAR, 2016), seaweed (WU et al., 2016), animals, such as, snakes (CEZARETTE; SARTIM; SAMPAIO, 2020), chicken (ZHANG et al., 2017), crustaceans (ZHANG et al., 2018b), fish (YANG et al., 2020) and echinoderms (UNNO; GODA; HATAKEYAMA, 2014). Among these and other sources, plants are the most explored in the purification of lectins and can be isolated from different parts, such as leaves (HIREMATH et al., 2020), leaflets (RADHA; URLA, 2019), fronds (PATRIOTA et al., 2017), seeds (CAVADA et al., 2020), roots (SILVA et al., 2019a), rhizomes (AHSANUL KABIR et al., 2019), stem bark (trunk) (ARAÚJO et al., 2012), sarcotesta (SILVA et al., 2016a), phloem exudate from fruits (NAREDDY; BOBBILI; SWAMY, 2017), and latex stems (SIRITAPETAWEE et al., 2018).

Since these proteins have many purification studies, with lectins being discovered and published periodically, there are also many studies revealing their different biological activities, such as: insecticide (OLIVEIRA et al., 2020b), antiviral (AGARWAL; TRIVEDI; MITRA, 2020), antiulcer (PINTO et al., 2019a), immunomodulator (SANTOS et al., 2020a), anxiolytic (ARAÚJO et al., 2020), anticancer (PINTO et al., 2019b; RAMOS et al., 2019), antibacterial (LIU; DANG, 2020), antifungal (NOVA et al., 2020), antihyperglycemic, antioxidant (FREIRE et al., 2020), formation of amyloid-like aggregates (ARANDA-SOUZA et al., 2019), anthelmintic activity for gastrointestinal nematodes (MEDEIROS et al., 2018), anti-inflammation (LIU et al., 2019), proinflammatory (LAI; CRUZ; ROCK, 2020), cell signaling and cytokine production (PROCÓPIO et al., 2018), antibiofilm (JIN; LEE; HONG, 2019) and control of asthma response (BORTOLOZZO et al., 2018).

In addition to the purification of new lectins and studies of biological activities, there are also many studies of heterologous expression of lectins. Which are very important for making many applications of these macromolecules feasible, given that certain applications require large-scale production. For this, the studies have been dedicated to the use of different methodologies, using different expression systems, aiming to obtain the recombinant lectin as

close as possible to the native lectin, but at the same time taking into account some points such as cost, complexity and production time of each expression system (ABD WAHID et al., 2017; CARVALHO et al., 2008; CHEN et al., 2018; GAO et al., 2020b; MARTÍNEZ-ALARCÓN; BLANCO-LABRA; GARCÍA-GASCA, 2018; ZHANG et al., 2017).

Considering the importance and the large number of studies with lectins, this work proposed to carry out a review on purification, biological activities (highlighting healing) and heterologous expression of lectins.

3.1.2 Purification of lectins

Lectins can be obtained from different sources (table 4 and figure 5), and purified using different methodological approaches, such as shown in the figure 5. Among the different approaches used, one of the most traditional is the purification of lectins, in which the following steps are highlighted: 1) extraction, 2) fractionation, and 3) chromatography, respectively.

The extraction of lectins can be performed by various types of solvents whose concentration and pHs are also distinct (table 4). In addition, there are other variables in the purification process, such as extraction time, agitation speed (or rotation of the equipment used), temperature, filtration, and centrifugation, which can be seen in the following examples: the extraction of lectin LCL, for example, was made at 10% w/v in acetate buffer (0.05 M containing 0.1 M NaCl, pH 4.2), overnight at 4 °C, then filtered, and then centrifuged at 10,000 rpm for 20 min at 4 °C (HIREMATH et al., 2020); MvFL was extracted at 10% w/v in 0.15 M NaCl, for 16 h at 25 °C, filtered, and then centrifuged at 9,000 x g, for 15 min at 4 °C (PATRIOTA et al., 2017); and 10% w/v CasuL in 0.15 M NaCl, for 16 h, filtered, and then centrifuged at 12,000 x g for 15 min at 4 °C (PROCÓPIO et al., 2017).

Table 4. Extraction and chromatography data of lectins.

Lectin and its source	**Extraction (concentration/ solvent)**	**Fractionation**	**Matrix (diameter x length)**	**Equilibration solution/ Eluent solution**	**References**
LCL from *Lantana camara* leaves	10% w/v in 50 mM acetate buffer pH 4.2 containing 100 mM NaCl	50% saturation ammonium sulfate	1) CM-Cellulose (Carboxymethyl-Cellulose)	1) Acetate buffer 50 mM (pH 4.2) containing 100 mM NaCl / 50 mM acetate buffer (pH 4.2) containing 500 mM NaCl	(HIREMATH et al., 2020)
			2) Sepharose (Separation Pharmacia Agarose) 4B coupled to Mucin	2) Phosphate buffer saline (PBS) / 100 mM Glycine-HCl buffer (pH 2.0) containing 500 mM NaCl	
			3) Superdex G75 (connected to the purification system AKTA prime plus)	3) Tris buffer saline (TBS) (pH 7.5) / Tris buffer saline (TBS) (pH 7.5)	
AFL from Marine sponge *Aplysina fulva*	10% w/v in 50 mM Tris-HCl pH 8.0 containing 150 mM NaCl and 0.1 mM PMSF (phenylmethane sulfonyl fluoride)	70% saturation ammonium sulfate	1) Sepharose (1.0 x 10.0 cm)	1) 50 mM Tris-HCl buffer pH 8.0 containing 150 mM NaCl / 50 mM glycine buffer pH 2.6 containing 150 mM NaCl	(CARNEIRO et al., 2019)
ApuL from *Alpinia*	10% w/v in 0.15 M NaCl	40% saturation ammonium sulfate	1) Sephadex (Separation Pharmacia Dextran) G-75 (1.5 x 30 cm)	1) 0.15 M NaCl / 0.15 M NaCl	(BRITO et al., 2017)

purpurata bracts					
ApulSL from *Apuleia leiocarpa* seeds	10% w/v in 0.15 M NaCl	-	1) Chitin (1.5 x 7.5 cm)	1) 0.15 M NaCl / 1.0 M acetic acid	(CARVALHO et al., 2015)
CasuL from *Calliandra surinamensis* leaf pinnulae	10% w/v in 0.15 M NaCl	60% saturation ammonium sulfate	1) Sephadex G-75 (1.0 x 30.0 cm)	1) 0.15 M NaCl / 0.15 M NaCl	(PROCÓPIO et al., 2017)
cMoL from *Moringa oleifera* seeds	10% w/v in 0.15 M NaCl	0-60% ammonium sulfate	1) Guar gel (1.0 x 10.0 cm)	1) 0.15 M NaCl / 1.0 M NaCl	(SANTOS et al., 2009)
Cramoll (iso 1) from *Cratylia mollis* seeds	10% (w/v) / 0.15 M NaC1	0-40 and 40-60% saturation ammonium sulfate	1) Sephadex G-75 (70.0 x 1.9 cm) 2) CM-Celulose (31.0 x 1.5 cm) Peak II	1) 0.15 M NaCl / 0.3 M D-glicose in 0,15 M NaCl 2) 10 mM citrate-phosphate pH 5.5 / 0-0.4 M NaCl linear gradient	(CORREIA; COELHO, 1995)
Cramoll (iso 4) from *Cratylia mollis* seeds	10% (w/v) / 0.15 M NaC1	0-40 and 40-60% saturation ammonium sulfate	1) Sephadex G-75 (70.0 x 1.9 cm) 2) CM-Celulose (31.0 x 1.5 cm) Peak I	1) 0.15M NaCl / 0.15M NaC1 / 0.3 M D-glicose in NaCl 0.15 M 2) 10 mM citrate-phosphate pH 5.5/ 0-0.4 M NaC1 linear gradient	
Cramoll (iso 2) from *Cratylia mollis* seeds	10% (w/v) / 0.15 M NaCl	0-40% and 40-60% saturation ammonium sulfate	1) Sephadex G-75 (6.0 x 1.46 cm) (unadsorbed) 2) Bio-Gel P-200 (6.0x 1.46 cm)	1) 0.15 M NaCl / 0.15 M NaCl 2) 0.15 M NaCl / 0.15 M NaCl	(PAIVA; COELHO, 1992)

Cramoll (iso 3) from *Cratylia mollis* seeds	10% (w/v) / 0.15 M NaCl	0-40% saturation ammonium sulfate	1) CM-Cellulose (31.0 x 1.44 cm) 2) Sephadex G-75 (unadsorbed)	1) 10 mM citrate-phosphate pH 5.5 containing 0.15 M NaCl / 0-0.4 M NaCl linear gradient 2) 0.15 M NaC1 / 0.15 M NaC1	
CrataBL from *Crataeva tapia* bark	10% w/v in 0.15 M NaCl	30-60% saturation ammonium sulfate	1) CM-Cellulose (1.6 x 5.2 cm)	1) 0.01 M citrate-phosphate buffer pH 5.5 / 0.5 M NaCl	(ARAÚJO et al., 2012)
EantH from *Euphorbia antiquorum* L. latex	The latex was extracted, centrifuged and remained with the supernatant fraction	The supernatant fraction was fractionated with absolute ethanol and stayed with the precipitated fraction	1) HiTrap SP FF (Sepharose Fast Flow) 2) HiTrap Phenyl FF (high sub) (1.5 x 3.0 cm)	1) 25 mM sodium-acetate buffer pH 4.5 / 0.0-0.5 M NaCl linear gradient in 25 mM sodium-acetate buffer pH 4.5 2) 25 mM phosphate buffer pH 7.0 in 2.0 M $(NH_4)_2SO_4$ / 0.0-2.0 M $(NH_4)_2SO_4$ linear gradient in 25 mM phosphate buffer pH 7.0	(SIRITAPET AWEE et al., 2018)
Es-Lec from pearl spot (*Etroplus suratensis*) fish	The blood was centrifuged and the serum was fractionated	*	1) Sepharose CL-4B coupled mannose	1) 10 mM Tris–HCl buffer pH 7.4 containing 150 mM NaCl and 10 mM $CaCl_2$ / 10 mM Tris-HCl buffer pH 8.0 containing 140 mM NaCl and 3 mM EDTA	(RUBEENA et al., 2019)
GaBL from Genipa americana bark	20% w/v in 0.05 M Tris-HCl pH 4.0	20% saturation ammonium sulfate	1) Sephacryl (Separation Pharmacia Allyl Dextran/Bisacrylamide) S-	1) 0.05 M Tris-HCl buffer pH 8.0 / 0.05 M Tris-HCl buffer pH 8.0	(COSTA et al., 2018)

			100 Hiprep (in AKTA) (1.6 x 60 cm)		
GDL from *Geodorum densiflorum* rhizome	10% w/v in 10 mM Tris-HCl pH 8.2	-	1) QA Cellulose (2.5 x 20 cm)	1) 10 mM Tris-HCl buffer pH 8.2 / 0.0-0.5 M NaCl linear gradient in 10 mM Tris-HCl buffer pH 8.2 (Fraction not connected HA)	(AHSANUL KABIR et al., 2019)
LCaL from *Lonchocarpus campestris* seeds	10% w/v in 50 mM Tris-HCl	30-60% saturation ammonium sulfate	1) DEAE (Diethylaminoethyl)-Sephacel (Separation Pharmacia Cellulose) (10 mL)	1) 50 mM Tris-HCl buffer pH 7.6 containing 0.15 M NaCl / 0.25-1.0 M NaCl linear gradient in 50 mM Tris-HCl buffer pH 7.6	(PIRES et al., 2019)
			2) HiTrap DEAE FF (5 mL) in HPLC/FPLC (High Performance Liquid Chromatography/Fast Protein Liquid Chromatography)	2) 50 mM Tris-HCl buffer pH 7.0 / 0.0-1.0 M NaCl linear gradient in 50 mM Tris-HCl buffer pH 7.0	
			3) TSKgel silica (0.75 x 60 cm)	3) 50 mM Tris-HCl buffer pH 7.6 / 50 mM Tris-HCl buffer pH 7.6	
MaL from *Machaerium acutifolium* seeds	10% w/v in 100 mM Tris-HCl pH 7.6 containing 150 mM NaCl	0-60% saturation ammonium sulfate	1) Mannose-agarose (1.0 x 3.5 cm)	1) 100 mM Tris-HCl buffer pH 7.6 containing 150 mM NaCl / 100 mM glycine buffer pH 2.6	(SANTOS et al., 2019a)
			2) DEAE-Sephacel (2.0 x 5.0 cm)	2) 25 mM Tris–HCl buffer pH 8.0 / 0.0-1.0 M NaCl linear gradient in 25 mM Tris-HCl buffer pH 8.0	
MvFL from *Microgramma*	10% w/v in 0.15 M NaCl	-	1) Sephadex G-75 (1.5 x 30 cm)	1) distilled water / distilled water	(PATRIOTA et al., 2017)

vacciniifolia fronds			2) DEAE-Sephadex A25 (2.0 x 3.0 cm)	2) 0.1 M Tris-HCl buffer pH 8.0 / 0.1 M Tris-HCl buffer pH 8.0 containing 1.0 M NaCl	
MuLL from *Myracrodruon urundeuva* leaves	10% p/v in NaCl 0.15 M	60-80% saturation ammonium sulphate	1) Chitin (7.5 × 1.5 cm)	1) 0.15 M NaCl / Acetic Acid 1.0 M	(NAPOLEÃO et al., 2011)
OniL from Nile tilapia (*Oreochromis niloticus*) serum	The blood was centrifuged and the serum was fractionated	20-40% saturation ammonium sulphate	1) Sepharose 4B coupled Concanavalin A	1) Tris-buffered saline (TBS) pH 8.0 containing 10 mM $CaCl_2$ and 10 mM $MnCl_2$ / TBS containing 200 mM methyl-α-D-mannopyranoside	(DA SILVA et al., 2012)
Penicillium duclauxii lectin	The fungal extract (in PBS containing 1 mM PMSF) was centrifuged and remained with the supernatant fraction	-	1) DEAE-Sepharose (2.0 x 7.0 cm) 2) Sephadex G-100 (1.0 x 40.0 cm)	1) 0.01 M PBS pH 6.0 / 0.01 M PBS pH 6.0 containing 0.25 M NaCl 2) 0.1 M PBS pH 7.2 / 0.1 M PBS pH 7.2	(SINGH; WALIA; KENNEDY, 2018)
Penicillium proteolyticum lectin	The fungal extract (in PBS supplemented with 1 mM benzamidine) was centrifuged and remained with the	-	1) DEAE-Sepharose (2.0 x 7.0 cm) 2) Sephadex G-100 (1.0 x 40 cm)	1) 0.01 M PBS pH 5.8 / 0.01 M PBS pH 5.8 containing 0.5 M NaCl 2) 0.1 M PBS pH 7.2 / 0.1 M PBS pH 7.2	(SINGH; WALIA; KENNEDY, 2019)

	supernatant fraction				
PeRoL from *Portulaca elatior* root	10% w/v in 0.15 M NaCl	-	1) Chitin (1.5 x 7.5 cm)	1) 0.15 M NaCl / 1.0 M acetic acid	(SILVA et al., 2019a)
PgTeL from *Punica granatum* sarcotesta	90% v/v, 90 mL de juice de sarcotesta of *P. granatum* in 10 mL 0.15 M NaCl	30% saturation ammonium sulphate	1) Chitin (1.5 x 7.5 cm)	1) 0.15 M NaCl / 1.0 M acetic acid	(SILVA et al., 2016a)
PLUN from seeds of *Phaseolus lunatus* L. var. cascavel	10% w/v in 0.5 M NaCl	*	1) Sephadex G-100 (280 mL)	1) 0.15 M NaCl / 0.15 M NaCl	(LACERDA et al., 2017)

w/v: weight/volume. v/v: volume/volume. -: no fractionation was performed. *: Not informed.

Various types of fractionations can also be performed and in different concentrations, as well as, may or not use fractionation in the purification process. For example, absolute alcohol can be used to fractionate the latex supernatant, after centrifugation (SIRITAPETAWEE et al., 2018), or use of ammonium sulphate to fractionate the product from saline extraction (PROCÓPIO et al., 2017), or even, do not use this step (AHSANUL KABIR et al., 2019). In addition to these details, such as the type and concentration of substance used for fractionation and, this procedure has other variables such as the number of fractionation steps, agitation speed or rotation of the equipment used, time, temperature, centrifugation, and fraction selection (supernatant or precipitate).

After the extraction and fractionation, the chromatographic steps are proceeded, and in them, some main characteristics can be observed, such as matrices, column length and diameter, and different solutions for equilibration, washing and elution. The matrices used in columns for chromatography of lectins are many, such as: (i) anionic exchange chromatography = DEAE-Sepharose, DEAE-Sephacel, QA Cellulose, HiTrap Q-Sepharose HP, HiTrap DEAE-Sepharose FF; (ii) cation exchange chromatography = CM-Celulose, HiTrap SP HP, HiTrap SP FF; (iii) hydrophobic interaction chromatography = Sepharose, HiTrap Phenyl FF (high sub); (iv) molecular exclusion chromatography = Sephadex, Superdex, Sephacryl S-100 Hiprep, Bio-Gel P-200, TSKgel silica; (v) affinity chromatography = Guar gel, Chitin, Sepharose CL-4B coupled mannose, Lactamyl-Seralose 4B, Lactamyl-Agarose, Lactose-Agarose, among others. In addition, some columns can work with more than one function, which can be molecular exclusion and affinity (Sephadex G75) and also anion exchange with molecular exclusion (DEAE-Sephadex A25), for example (table 4).

The length and diameter of these columns are also very different, depending on the protocol established in the study, for example: DEAE-Sephacel (0.2 x 5.0 cm) (SANTOS et al., 2019a) and DEAE-Sephacel (0.75 x 75 cm) (CAVADA et al., 2020). The equilibrium, washing and elution solutions used in these matrices also vary widely, according to the established purification protocol, which takes into account points such as the type of matrix and the type of molecule that is to be obtained. It can be solutions of distilled water, NaCl, acetic acid, buffers (whether or not containing a salt, or other substance), whether or not to use a salt gradient (table 4).

In the establishment of purification protocols, different methodological approaches can be adopted to obtain isolated lectins. After selecting the source of protein extraction, be it

vegetable, fungus, animal or other, different steps can be performed according to the objective, planning, technical knowledge, reagents and equipment available. It can be seen in figure 5 that the purification processes of the lectins can be grouped into four groups: (i) those that used only extraction, fractionation and chromatography; (ii) using heterologous expression; (iii) through the aqueous two-phase systems (ATPS); and (iv) employing reverse micelles.

In the first group (i), it can be seen, for example, that CasuL, Cramoll 1,4 and BmoLL, were extracted, fractionated, and underwent a single chromatography step. CasuL and Cramoll 1,4 were extracted with 0.15 M NaCl, and BmoLL with citrate-phosphate buffered saline. The saline fractionation with ammonium sulfate was performed in a single step for Casul and BmoLL (both 0-60%), and in two stages for Cramoll 1.4 (0-40 and 40-60%). For chromatography, Sephadex G-75 was used for CasuL and Cramoll 1,4; while Guar gel was used for BmoLL (COELHO; SILVA, 2000; CORREIA; COELHO, 1995; PROCÓPIO et al., 2017).

LCaL and BfL were obtained using the same approach (extraction, fractionation and chromatography). LCaL used three chromatographic steps (DEAE-Sephacel, HiTrap DEAE FF, and TSKgel silica), and BfL four (DEAE-Sephadex, Sepharose-4B, Chitin and Superdex 75). For extraction, LCaL used Tris-HCl buffer, and BfL was with PBS (phosphate buffered saline). For saline fractionation, precipitation with ammonium sulfate in two stages was used, for both lectins, LCaL (0-30 and 30-60%) and BfL (0-40 and 40-80%) (PIRES et al., 2019; SILVA et al., 2012).

EantH was purified without the protein extraction step, because the starting material was the plant's latex, so it has already been followed by fractionation with organic solvent (absolute ethanol). Then it went through two chromatography steps (HiTrap SP FF and HiTrap Phenyl FF). Likewise, Es-Lec did not go through a protein extraction stage, the material collected was blood, which was centrifuged and fractionated serum, then a chromatography stage was performed (Sepharose CL-4B coupled mannose) (RUBEENA et al., 2019; SIRITAPETAWEE et al., 2018).

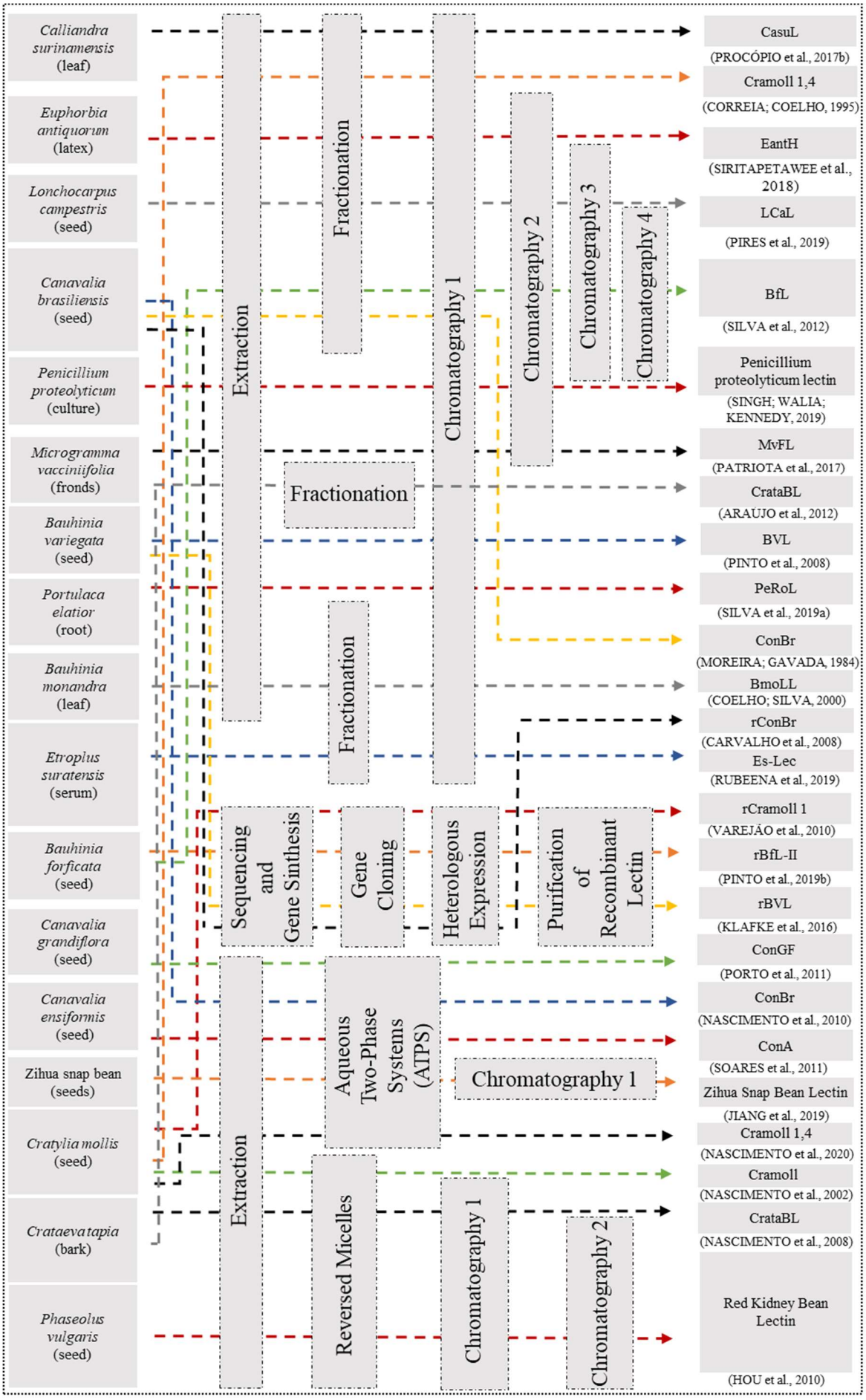

Calliandra surinamensis (leaf)
Euphorbia antiquorum (latex)
Lonchocarpus campestris (seed)
Canavalia brasiliensis (seed)
Penicillium proteolyticum (culture)
Microgramma vacciniifolia (fronds)
Bauhinia variegata (seed)
Portulaca elatior (root)
Bauhinia monandra (leaf)
Etroplus suratensis (serum)
Bauhinia forficata (seed)
Canavalia grandiflora (seed)
Canavalia ensiformis (seed)
Zihua snap bean (seeds)
Cratylia mollis (seed)
Crataeva tapia (bark)
Phaseolus vulgaris (seed)
Extraction
Fractionation
Chromatography 1
Chromatography 2
Chromatography 3
Chromatography 4
Fractionation
Fractionation
Sequencing and Gene Sinthesis
Gene Cloning
Heterologous Expression
Purification of Recombinant Lectin
Extraction
Aqueous Two-Phase Systems (ATPS)
Chromatography 1
Reversed Micelles
Chromatography 1
Chromatography 2
CasuL
(PROCÓPIO et al., 2017b)
Cramoll 1,4
(CORREIA; COELHO, 1995)
EantH
(SIRITAPETAWEE et al., 2018)
LCaL
(PIRES et al., 2019)
BfL
(SILVA et al., 2012)
Penicillium proteolyticum lectin
(SINGH; WALIA; KENNEDY, 2019)
MvFL
(PATRIOTA et al., 2017)
CrataBL
(ARAUJO et al., 2012)
BVL
(PINTO et al., 2008)
PeRoL
(SILVA et al., 2019a)
ConBr
(MOREIRA; GAVADA, 1984)
BmoLL
(COELHO; SILVA, 2000)
rConBr
(CARVALHO et al., 2008)
Es-Lec
(RUBEENA et al., 2019)
rCramoll 1
(VAREJÃO et al., 2010)
rBfL-II
(PINTO et al., 2019b)
rBVL
(KLAFKE et al., 2016)
ConGF
(PORTO et al., 2011)
ConBr
(NASCIMENTO et al., 2010)
ConA
(SOARES et al., 2011)
Zihua Snap Bean Lectin
(JIANG et al., 2019)
Cramoll 1,4
(NASCIMENTO et al., 2020)
Cramoll
(NASCIMENTO et al., 2002)
CrataBL
(NASCIMENTO et al., 2008)
Red Kidney Bean Lectin
(HOU et al., 2010)

Figure 5. Scheme showing an overview of different approaches in the purification of lectins highlighting some steps. It is organized as follows: on the left side, the and its respective source; in the center, the different stages used by each study (through which arrows of different colors pass), and on the right side the different lectins obtained and their respective references. Extraction is the step that uses some solvent (for example, aqueous solution, saline, or buffer) to extract the lectin from the source. The fractionation corresponds to the use of a substance (such as salt and alcohol) for precipitation or selective separation of proteins.

The studies with MvFL, BVL and PeRoL did not use the fractionation step, followed by the direct extraction for the chromatographic step. MvFL and PeRoL were extracted with 0.15 M NaCl and BVL with Tris-HCl saline buffer. To obtain PeRoL and BVL, a single chromatographic step was used (Chitin column and Lactose-Agarose column, respectively). In turn, to obtain MvFL, two chromatographic steps were used (Sephadex G-75 and DEAE-Sephadex A25) (PATRIOTA et al., 2017; PINTO et al., 2008; SILVA et al., 2019a).

Some recombinant lectins were obtained by heterologous expression using gene sequencing/synthesis, cloning, expression, and purification. rCramoll 1 and rBfL-II were expressed in *Escherichia coli*, and rBVL and rConBr in *Pichia pastoris*. After expression: rCramoll 1 was purified by chromatography on Sephadex G-75; rBfL-II was purified by nickel affinity chromatography on a HiTrap chelating column; rBVL was using α-Lactose-Agarose column chromatography; and rConBr did not go through a chromatographic step, it was only identified in the cell extract by western blot and electrophoresis (CARVALHO et al., 2008; KLAFKE et al., 2016; PINTO et al., 2019b; VAREJÃO et al., 2010).

Other lectins have been supported by the ATPS process, such as ConA, Cramoll 1,4, ConGF and ConBr, which were obtained through saline extraction (0.15 M NaCl, 10% w/v) and a second stage based on aqueous two-phase system. ConBr and Cramoll were obtained using a two-phase system PEG (polyethyleneglycol)/phosphate buffer; and ConA and ConGF using PEG/citrate buffer (NASCIMENTO et al., 2020, 2010; PORTO et al., 2011; SOARES et al., 2011). It is noteworthy that purification by aqueous two-phase system, is the approach that uses fewer steps, considering the scheme of figure 5, only extraction and ATPS.

Using the step of reversed micelles, can be mentioned the purification of Cramoll, CrataBL and the lectin from red kidney bean; these three lectins were obtained using, at first, saline extraction in NaCl 0.15 M 10% w/v, and then the reverse micellar system consisting of anionic surfactant dioctyl sulfosuccinate sodium salt (DOSS or AOT), in isooctane. Then, to obtain CrataBL and lectin from red kidney bean, one and two chromatography steps were performed, respectively. No chromatographic step was performed (or was not mentioned in the article) for Cramoll (HOU et al., 2010; NASCIMENTO et al., 2002, 2008).

As seen in figure 5, the same lectin can be purified in different ways, like the Cramoll that was obtained by extraction, fractionation and chromatography (CORREIA; COELHO, 1995), heterologous expression (VAREJÃO et al., 2010), two-phase system (NASCIMENTO et al., 2020), and reverse micelles (NASCIMENTO et al., 2002). CrataBL by extraction, fractionation and chromatography (ARAÚJO et al., 2012), and reverse micelles (NASCIMENTO et al., 2008). ConBr obtained by extraction, fractionation and chromatography (MOREIRA; GAVADA, 1984), heterologous expression (CARVALHO et al., 2008), and two-phase system (NASCIMENTO et al., 2010). BfL purified by extraction, fractionation and chromatography (SILVA et al., 2012) and heterologous expression (PINTO et al., 2019b). BVL acquired by extraction and chromatography (without fractionation) (PINTO et al., 2008) and also by heterologous expression (KLAFKE et al., 2016).

In addition (figure 6), it is worth highlighting the proteomic analysis, which can be included as an important step, within the purification cycle, helping to identify lectins, by comparing primary sequences deposited in databases. For example, its use to identify four lectins in the leaf genome of *Viscum album* subsp. abietis, which have been identified as beta-galactoside specific lectin 2 (ML-2), beta-galactoside-specific lectin 3 (ML-3), beta-galactoside-specific lectin 1 (chain A isoform 1) (ML-1) and chitin-binding lectin (cbML). Basically, the methodology consisted of extracting proteins from the leaves, centrifuging, trypsinization of the sample, and later application in high performance liquid chromatography equipment coupled to a *in tandem* mass spectrometer (HPLC-MS/MS), finally, a proteomic analysis was performed to identify the lectins, mentioned above (TSEKOURAS et al., 2020).

In another study including a proteomic analysis stage, *Bothrops jararacussu* venom was centrifuged and the supernatant applied to a chromatographic column of molecular exclusion, the fractions collected from the column were evaluated for antimicrobial activity, and two fractions, with better activities, were analyzed by electrophoresis, presenting a band of 15 KDa, in both fractions. Then, the most intense (most concentrated) electrophoresis band was selected, corresponding to only one of the fractions. This was trypsinized, and then applied to high performance liquid chromatography coupled to an in-tandem mass spectrometer (HPLC-MS/MS). Finally, sequencing and proteomic analysis was performed to identify the peptides present in the sample. A type C lectin was then identified, with a 94% correlation. Because these lectins have galactose binding properties, a galactose affinity matrix was selected, and the sample was then chromatographed to obtain pure lectin, a type C lectin (KLEIN et al., 2015).

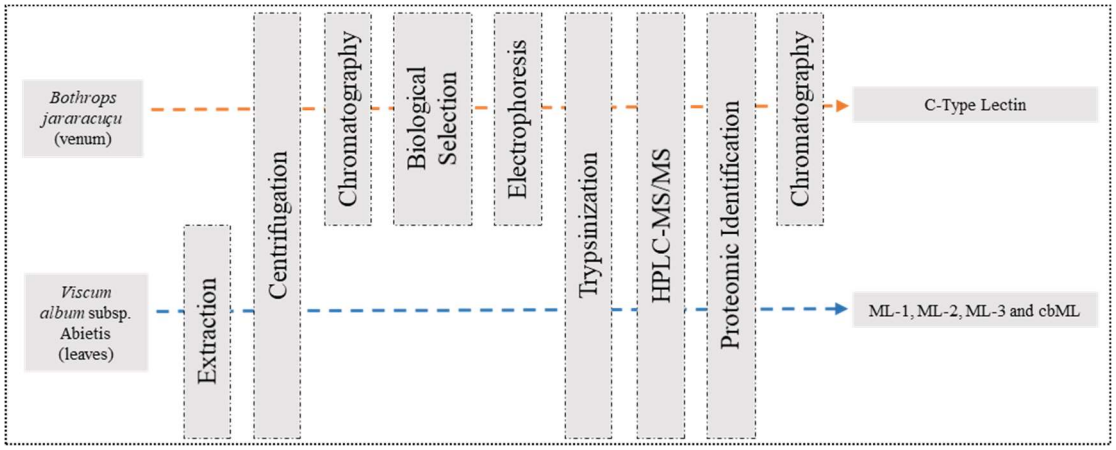


Figure 6. Scheme showing two studies that used proteomics to identify lectins.

In addition to the proteomic analysis stage carried out in these two studies, it is worth noting other points: 1) in the first article, fewer stages are performed and the lectins are not purified, only identified in the proteome. In addition, in this study, the sample taken for HPLC-MS/MS did not come from electrophoresis, but from trypsinization of the supernatant of the extract centrifugate (TSEKOURAS et al., 2020). 2) In the second article, many more steps were taken, and the lectin was purified at the end of the process. And the sample taken for HPLC-MS/MS came from electrophoresis. And the most interesting detail in this second study, is that a biological screening of the chromatographed fractions was carried out, and from there, a fraction was selected to continue the purification (KLEIN et al., 2015).

Also, to the purification approaches and methodological data dealt with so far, it is also worth mentioning in this review other techniques used both during the purification process and after obtaining pure lectin. These techniques include chromatography, electrophoresis, X-ray diffraction, circular dichroism, nuclear magnetic resonance, electron microscopy, calorimetry, flow cytometry, and others, these techniques can provide structural data of lectins, their biological effect on other organisms, mechanism of action, type of interaction between lectin and another molecule or conjugate, as well as characterization of formulations containing lectin, for example (table 5). In this sense, the table below was constructed, with a variety of techniques and methods used with lectins, focusing exclusively on what was studied from lectin with the respective technique, and not on the principle or other methodological information that the technique can provide. It is also worth noting that some techniques can be used for many purposes, depending on the nature of the study, what is intended, the degree of knowledge of the professionals involved, among other variables.

Their most common classification for these proteins is based on their source. However, it is also possible to classify them through their structural homology in: C-type lectins

- are those that require calcium for recognition; S-type lectins or galectins - were a group that need of free thiols (-SH) for stability; P-type lectins - which recognize mannose-6-phosphate; I-type lectins - are immunoglobulins that can distinguish carbohydrates; finally, a subgroup, of these molecules, which specifically binds to sialic acids, which has been designated as Siglecs. At the moment, there is no single universally accepted classification for lectins (JUAN et al., 2017). It is also worth mentioning that there are other classifications, such as plant lectins, which are the most studied, classified according with source, CRD, based on its structural homology and by the affinity of binding to the carbohydrate portion (MISHRA et al., 2019).

Finally, it is important to mention that lectins have different activities as will be seen later in this article, but their most characteristic biological action, is its ability to agglutinate, be it erythrocytes, bacteria, viruses, fungi, or other structures, but the hemagglutinating activity (HA), that is, its ability to agglutinate erythrocytes, is the best known. This activity is widely used in many stages of purification studies of these macromolecules, both in the characterization and in the monitoring of purification. Because, from the moment the HA of the lectin under study is identified and standardized, one can verify the influence of different factors in this activity, such as: pHs, temperatures, ions, carbohydrates, proteins, glycolipids, and others. That is, it is possible to map and obtain some information about these proteins, through HA. However, it is worth mentioning that not all lectins are capable of this activity, for example, lectins that only have a single CRD (BARRE et al., 2019; CAGLIARI; KREMER; PINTO, 2018).

Table 5. Techniques/methods used in studies involving lectins.

Characterization*	Grouping*	Techniques/Methods	Lectins	Studied	References
Structural and Biological	Microscopy	Atomic Force Microscope (AFM)	Cramoll	Characterization of Cramoll immobilization on nanostructured surfaces of titanium dioxide.	(OLIVEIRA et al., 2018)
Structural and Biological	Microscopy	Confocal Laser Scanning Microscopy (CLSM) or Laser Confocal Scanning Microscopy (LCSM)	PgTeL	Analysis of the effect caused by PgTeL on the cell wall of fungi.	(SILVA et al., 2018)
Structural and Biological	Microscopy	Confocal Laser Scanning Microscopy (CLSM) or Laser Confocal Scanning Microscopy (LCSM	WSMoL	Analyzed the effect of WSMoL on biofilm formation and integrity of the cell membrane of *S. marcescens* and Bacillus sp.	(MOURA et al., 2017)
Structural and Biological	Microscopy	Confocal Laser Scanning Microscopy (CLSM) or Laser Confocal Scanning Microscopy (LCSM	Es-Lec	Visualization of inhibition of the biofilm of Gram-negative bacteria *A. hydrophila* and *V. parahaemolyticus* by Es-lec, in different concentrations, using acridine orange staining.	(RUBEENA et al., 2019)
Structural and Biological	Microscopy	Confocal Raman Microscopy (CRM)	ConA	Detection of lectin-ligand binding in Nanopore-Supported Phospholipid Bilayers.	(BRYCE; KITT; HARRIS, 2018)
Structural and Biological	Microscopy	Fluorescence Microscopy (FM)	AAL	Verification of cell membrane marking by AAL and evaluation of nanocarrier delivery.	(BHAT et al., 2018)
Structural and Biological	Microscopy	Fluorescence Microscopy (FM)	Cramoll	The adhesion of nanotubes functionalized with Cramoll on the surface of cells similar to osteoblasts was visualized.	(OLIVEIRA et al., 2018)
Structural and Biological	Microscopy	Fluorescence Microscopy (FM)	FRIL	It was used to verify the effect of FRIL on the production of SARS-CoV-2 Spike proteins (S) and	(LIU et al., 2020)

				Nucleocapsid proteins (N), by means of immunofluorescence microscopy tracking.	
Structural and Biological	Microscopy	High-Speed Atomic Force Microscopy (HS-AFM)	HA0	Record of HA0 conformational changes, changing the conditions from neutral to acidic, on a millisecond scale.	(LIM et al., 2020)
Structural and Biological	Microscopy	Optical Microscopy (OM) or Light Microscope (LM)	Frutalin	Histological analysis of the healing of dermal wounds with hydrogels and membrane scaffolds, containing Frutalin in different concentrations.	(SOUSA et al., 2019)
Structural and Biological	Microscopy	Optical Microscopy (OM) or Light Microscope (LM	MBL	Analysis of the interaction profile between red blood cells and MBL.	(LIMA et al., 2019a)
Structural and Biological	Microscopy	Optical Microscopy (OM) or Light Microscope (LM	rPcLec6	The effect of bacteria agglutination caused by rPcLec6 was observed.	(ZHANG et al., 2018b)
Structural and Biological	Microscopy	Optical Microscopy (OM) or Light Microscope (LM	FRIL	Visualization of the cytopathic effect in cells infected with SARS-CoV-2 and treated with FRIL in different concentrations.	(LIU et al., 2020)
Structural and Biological	Microscopy	Optical Microscopy (OM) or Light Microscope (LM	Es-Lec	Visualization of aggregation of erythrocytes, fungi and bacteria. And also visualization of antibiofilm and antiviral (anticytopathic) effect.	(RUBEENA et al., 2019)
Structural and Biological	Microscopy	Scanning Electron Microscope (SEM)	PgTeL	Investigation of the effects of PgTeL on the ultrastructure of *C. krusei* and *C. albicans*.	(SILVA et al., 2018)
Structural and Biological	Microscopy	Scanning Electron Microscope (SEM)	MBL	Evaluation of morphological changes in red blood cells, due to the binding of MBL lectin.	(LIMA et al., 2019a)
Structural and Biological	Microscopy	Scanning Electron Microscope (SEM)	Frutalin	Evaluation of galactomannan-based membrane scaffolds images,	(SOUSA et al., 2019)

				containing different concentrations of Frutalin.	
Structural and Biological	Microscopy	Transmission Electron Cryomicroscopy (CryoTEM) ou Electron Cryomicroscopy (CryoEM)	DNGR-1	Visualization of the native structure of the F-actin complex linked to the DNGR-1 lectin, in a frozen hydrated state. And acquisition of three-dimensional helical image of lectin.	(HANČ et al., 2015)
Structural and Biological	Microscopy	Transmission Electron Cryomicroscopy (CryoTEM) ou Electron Cryomicroscopy (CryoEM)	HE	Evaluation of the native structure of Hemagglutinin Esterase. And acquisition of three-dimensional image of lectin.	(HURDISS et al., 2020)
Structural and Biological	Microscopy	Transmission Electron Microscopy (TEM)	UEA1	The stability of the dispersion of nanoparticles in murine mucus and in PBS (TEM liquid state) was evaluated.	(CHEN et al., 2017)
Structural and Biological	Microscopy	Transmission Electron Microscopy (TEM)	BlL	The effect of BlL on macrophage morphology and viability was evaluated. The presence of fibrils, similar to amyloid, was found in macrophages treated with BlL.	(ARANDA-SOUZA et al., 2019)
Structural and Biological	Microscopy	Transmission Electron Microscopy (TEM)	FRIL	Obtaining a structure of electron microscopy of negative staining of the FRIL tetramer. And visualization of viral aggregation caused by FRIL.	(LIU et al., 2020)
Structural	Calorimetry	Derived Thermogravimetry (DTG)	ConA	Evaluation of P1 nuclease immobilization in microcarriers, induced by ConA. The decomposition patterns of the different microcarriers are shown in the DTG curves.	(HUANG et al., 2018)

Structural	Calorimetry	Differential Scanning Calorimetry (DSC)	CPL	The thermal unfolding of CPL lectin was evaluated, varying pH and temperature.	(NAREDDY; SWAMY, 2018)
Structural	Calorimetry	Differential Scanning Calorimetry (DSC)	rBanLec	Evaluation of the thermal stability of lectin using thermodynamic parameters: maximum transition temperature (T_m), calorimetric enthalpy (H^{cal}) and van´t Hoff´s enthalpy (H^{vH}).	(DIMITRIJEVIC et al., 2010)
Structural	Calorimetry	Thermogravimetric Analysis (TGA)	ConA	Evaluation of P1 nuclease immobilization in microcarriers, induced by ConA.	(HUANG et al., 2018)
Structural	Calorimetry	Thermogravimetric Analysis (TGA)	UEA1	Used to assess the degree of nanoparticulate-lectin conjugation.	(CHEN et al., 2017)
Structural	Calorimetry	Thermogravimetric Analysis (TGA)	AAL	Used to determine the organic content of each component of the developed nanocarrier system.	(BHAT et al., 2018)
Structural	Calorimetry	Isothermal Titration Calorimetry (ITC)	CPL	Evaluation of CPL binding to chitooligosaccharides (chitotetraose and chitohexaose).	(NAREDDY; BOBBILI; SWAMY, 2017)
Structural	Calorimetry	Isothermal Titration Calorimetry (ITC)	rWGA	Evaluation of binding of rWGA and its 4 isostructural domains to chitooligosaccharides (chitotriosis).	(LEYVA et al., 2019)
Structural	Edman Degradation	Edman Degradation	BfL	Degradation of lectin, generating peptides for sequencing and obtaining native primary structure.	(SILVA et al., 2012)
Structural	Edman Degradation	Edman Degradation	CrataBL	Degradation of lectin, generating peptides for sequencing and obtaining native primary structure.	(FERREIRA et al., 2013)
Structural	Edman Degradation	Edman Degradation	CrataBL	CrataBL degradation for N-terminal residue sequencing.	(ARAÚJO et al., 2012)

Structural	Spectroscopy	Circular Dichroism Spectroscopy (CD)	BfL	Obtaining the percentage of secondary structures of lectin.	(SILVA et al., 2012)
Structural	Spectroscopy	Circular Dichroism Spectroscopy (CD)	CPL	Evaluation of the effect of different temperatures and pHs on the secondary structures of lectin.	(NAREDDY; BOBBILI; SWAMY, 2017)
Structural	Spectroscopy	Dynamic Light Scattering (DLS) or Photon Correlation Spectroscopy (PCS)	BVL	Verified the effect of BVL on the size and polydispersity of free and containing liposomal vesicles was evaluated.	(SANTOS et al., 2018a)
Structural	Spectroscopy	Dynamic Light Scattering (DLS) or Photon Correlation Spectroscopy (PCS)	UEA1	Verified the size and polydispersity of nanoparticles with and without UEA1 lectin.	(CHEN et al., 2017)
Structural	Spectroscopy	Dynamic Light Scattering (DLS) or Photon Correlation Spectroscopy (PCS)	AAL	DLS was used to evaluate the hydrodynamic diameter of the nanoparticles containing and without the presence of AAL.	(BHAT et al., 2018)
Structural	Spectroscopy	Dynamic Light Scattering (DLS) or Photon Correlation Spectroscopy (PCS)	FRIL	Analysis of virus particle aggregation under increasing concentrations of FRIL.	(LIU et al., 2020)
Structural	Spectroscopy	Electron Paramagnetic Resonance Spectroscopy (EPR Spectroscopy)	ConBr ConM DLasiL DSclerL	It was used to confirm the presence of Manganese in lectins.	(GONDIM et al., 2017)
Structural	Spectroscopy	Fluorescence Spectroscopy (FS)	MvFL	The effects of pH and temperature on the conformation of MvFL were evaluated by analysis of intrinsic and extrinsic fluorescence.	(PATRIOTA et al., 2017)
Structural	Spectroscopy	Fluorescence Spectroscopy (FS)	CPL	The chemical unfolding of CPL was evaluated using guanidine chloride,	(NAREDDY; SWAMY, 2018)

				by intrinsic fluorescence spectroscopy.	
Structural	Spectroscopy	Fluorescence Spectroscopy (FS)	PgTeL	Evaluation of the effects of urea, pH and temperature on PgTeL conformation, by extrinsic fluorometric analysis.	(SILVA et al., 2018)
Structural	Spectroscopy	Fluorescence Spectroscopy (FS)	EgviL	Evaluation of EgviL-Carbohydrate interactions (Glucose and Galactose) by fluorescence extinction. It allowed to elucidate the thermodynamic parameters involved in the protein-ligand interaction.	(GOMES et al., 2020)
Structural	Spectroscopy	Fourier-Transform Infrared Spectroscopy (FTIR)	Zihua Snap Bean Lectin	FTIR was used to study changes in the secondary structure of the Zihua Snap Bean Lectin during heat treatment.	(JIANG et al., 2019)
Structural	Spectroscopy	Fourier-Transform Infrared Spectroscopy (FTIR)	ConA	Obtaining the FTIR spectra of different samples, during the process of modification and immobilization of nuclease P1 in granulo-porous carrier microparticles, induced by ConA.	(HUANG et al., 2018)
Structural	Spectroscopy	Fourier-Transform Infrared Spectroscopy (FTIR)	Cramoll	Characterization of nanotubular arrangements of titanium dioxide before and after functionalization with Cramoll.	(OLIVEIRA et al., 2018)
Structural	Spectroscopy	Fourier-Transform Infrared Spectroscopy (FTIR)	Frutalin	Characterization of galactomannan-based membrane scaffolds formulations, containing different concentrations of Frutalin.	(SOUSA et al., 2019)
Structural	Spectroscopy	Horizontal Attenuated Total Reflectance-Fourier	BVL	Phosphatidylcholine (liposomal) interferograms without and with	(SANTOS et al., 2018a)

		Transform Infrared Spectroscopy (HATR-FTIR)		BVL were evaluated. Showing the alteration of some chemical groups, the result of interaction.	
Structural	Spectroscopy	Nuclear Magnetic Resonance Spectroscopy (NMRS)	rWGA	Evaluation of the type of binding of rWGA with chitotriosis.	(LEYVA et al., 2019)
Structural	Spectroscopy	Nuclear Magnetic Resonance Spectroscopy (NMRS)	PhoSL	Obtaining three-dimensional native trimeric structure and fucose binding mechanism.	(YAMASAKI; YAMASAKI; TATENO, 2018)
Structural	Spectroscopy	Nuclear Magnetic Resonance Spectroscopy (NMRS)	PSA	Structural assessment of Glycan-PSA interactions.	(DIERCKS et al., 2018)
Structural	Spectroscopy	Nuclear Magnetic Resonance Spectroscopy (NMRS)	EgviL	Structural evaluation of EgviL-Carbohydrate (Galactose) interactions, to identify the region of EgviL that interacts with the monosaccharide galactose.	(GOMES et al., 2020)
Structural	Spectroscopy	Nuclear Magnetic Resonance Spectroscopy (NMRS)	BVL	Evaluation of the interaction of BVL and phosphatidylcholine (liposomal).	(SANTOS et al., 2018a)
Structural	Spectroscopy	Raman Spectroscopy (RS)	Lens culinaris lectin	Structural evaluation and identification of chemical groups of Lens culinaris lectin using different excitation wavelengths. The presence of a phosphate ion strongly linked to lectin was verified.	(RYGULA et al., 2013)
Structural	Spectroscopy	Surface Plasmon Resonance (SPR)	Lens culinaris lectin	Detection of mannose-lectin interaction in graphene-on-gold interface modified with mannose.	(PENEZIC et al., 2014)
Structural	Spectroscopy	Surface Plasmon Resonance (SPR)	ML-I	It was used to establish an innovative, simple and rapid assay	(TAO et al., 2009)

				for detecting ML-1 in buffer, serum and injectable drugs.	
Structural	Spectroscopy	Surface Plasmon Resonance (SPR)	GRFT	It was used to assess the binding of GRFT to glycoproteins on viral surfaces.	(LEVENDOSKY et al., 2015)
Structural	Spectroscopy	UV–Vis Spectroscopy	BVL	It was used to better understand qualitatively, the interactions between BVL and phosphatidylcholine (liposomal). Showing that the electronic properties of BVL do not seem to be affected by the presence of phosphatidylcholine (liposomal).	(SANTOS et al., 2018a)
Structural	Spectroscopy	UV–Vis Spectroscopy	EgviL	Evaluation of EgviL-Carbohydrate interaction (Glucose and Galactose). Showing that there were no conformational changes in the structure of EgviL due to changes in the molar absorptivity coefficient.	(GOMES et al., 2020)
Structural	Mass Spectrometry	Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)	ConBr ConM DLasiL DSclerL	It was used to determine the metal content (concentration) of the lectins.	(GONDIM et al., 2017)
Structural	Mass Spectrometry	Electrospray Ionization Mass Spectrometry (ESI-MS)	BfL	Used to determine the native molecular mass, and to determine the primary sequence of BfL. And also, to obtain the molecular weights of the glycosylated and deglycosylated peptides.	(SILVA et al., 2012)
Structural	Mass Spectrometry	Electrospray Ionization Mass Spectrometry (ESI-MS)	PpaL	To determine the isotopic mean molecular weight of PpaL. And for	(CAVADA et al., 2020)

				later determination of the primary and secondary protein sequence.	
Structural	Mass Spectrometry	Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry (MALDI-MS)	CPL	Determination of the molecular weight of native CPL and peptides, for further sequencing and obtaining the primary structure.	(NAREDDY; BOBBILI; SWAMY, 2017)
Structural	Mass Spectrometry	Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry (MALDI-MS)	CrataBL	Determination of the molecular mass of glycosylated CrataBL and the mass of its peptides, for further sequencing and obtaining primary structure.	(FERREIRA et al., 2013)
Structural	X-ray	X-ray Diffraction (XRD) ou X-ray Cristalography (XRC)	CrataBL	Obtaining crystalline structure and prediction of its quaternary three-dimensional structure. Featuring a dimer (two distinct monomers) of CrataBL per asymmetric unit.	(FERREIRA et al., 2013)
Structural	X-ray	X-ray Diffraction (XRD) ou X-ray Cristalography (XRC)	POL	Obtaining three-dimensional crystalline structure and binding properties with melibiose ligand.	(PERDUCA et al., 2020)
Structural	X-ray	X-ray Diffraction (XRD) ou X-ray Cristalography (XRC)	rFTP	Obtaining crystalline structure and prediction of its quaternary three-dimensional structure. One tetramer (four monomers) was found per asymmetric unit.	(SOUSA et al., 2017)
Structural	X-ray	Small-Angle X-ray Scattering (SAXS)	CEL-III	Evaluation of structural parameters of monomeric and oligomeric CEL-III: Radius of rotation (Rg), Intensity of direct scattering normalized in relation to protein concentration (J(0)/C), Molecular mass and Maximum particle size (Dmax).	(UNNO; GODA; HATAKEYAMA, 2014)

Structural	X-ray	Small-Angle X-ray Scattering (SAXS)	BVL	The thinning of the lipid bilayer of BVL-containing liposomes in the outer and inner lining was revealed.	(SANTOS et al., 2018a)
Structural	X-ray	X-ray photoelectron spectroscopy (XPS)	ConA	Evaluation of the ConA-carbohydrate bond in microcarriers, by XPS chemical bond analysis.	(HUANG et al., 2018)
Purification and Homogeneity	Chromatography	High Performance Liquid Chromatography (HPLC)	CrataBL	Purity evaluation and verification of native homodimeric CrataBL, for later use in XDR (Crystallization).	(FERREIRA et al., 2013)
Purification and Homogeneity	Chromatography	High Performance Liquid Chromatography (HPLC)	BfL	BfL homogeneity/purity was analyzed.	(SILVA et al., 2012)
Purification and Homogeneity	Chromatography	Ultra-Performance/Pressure Liquid Chromatography (UPLC)	PpaL	Used in the second stage of PpaL purification. And later to obtain its relative molecular mass.	(CAVADA et al., 2020)
Purification and Homogeneity	Chromatography	Fast Protein Liquid Chromatography (FPLC)	cMoL	Evaluation of purity and determination of relative molecular mass of native lectin cMoL.	(SANTOS et al., 2009)
Purification and Homogeneity	Chromatography	Fast Protein Liquid Chromatography (FPLC)	CrataBL	Evaluation of purity and determination of relative molecular mass of native lectin CrataBL.	(ARAÚJO et al., 2012)
Purification and Homogeneity	Chromatography	Fast Protein Liquid Chromatography (FPLC)	PeRoL	Evaluation of purity and determination of relative molecular mass of native lectin PeRoL.	(SILVA et al., 2019a)
Purification and Homogeneity	Electric-separation	Isoelectric focusing	PeRoL	Obtaining the isoelectric point of PeRoL.	(SILVA et al., 2019a)
Purification and Homogeneity	Electric-separation	Isoelectric focusing	CasuL	Obtaining the isoelectric point of CasuL.	(PROCÓPIO et al., 2017)
Purification and Homogeneity	Electric-separation	Electrophoresis 2D	ApulSL	Obtaining the isoelectric point and detecting isoforms.	(CARVALHO et al., 2015)
Purification and Homogeneity	Electric-separation	Electrophoresis 2D	EgviL	Obtaining the isoelectric point and not presenting isoforms.	(GOMES et al., 2020)

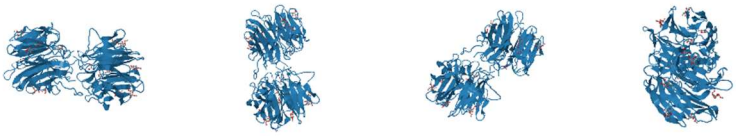
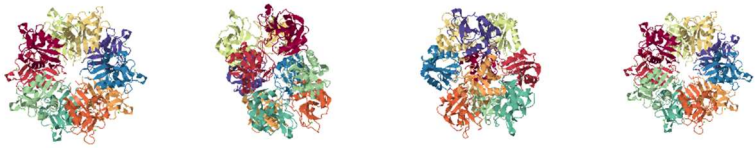
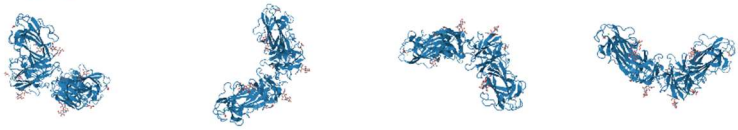
Purification and Homogeneity	Electric-separation	Electrophoresis Denaturing and Native	ApuL	Evaluation of sample homogeneity, verification of polypeptide subunits and determination of relative molecular mass.	(BRITO et al., 2017)
Purification and Homogeneity	Electric-separation	Electrophoresis Denaturing and Native	CrataBL	Evaluation of sample homogeneity, verification of polypeptide subunits and determination of relative molecular mass.	(ARAÚJO et al., 2012)
Purification and Homogeneity	Electric-separation	Electrophoresis Denaturing and Native	PeRoL	Evaluation of sample homogeneity, verification of polypeptide subunits and determination of relative molecular mass.	(SILVA et al., 2019a)
Purification and Homogeneity	Electric-separation	Electrophoretic light scattering (ELS) ou Zeta Potential	UEA1	Measurement of the variation in the surface electrical charge of nanoparticles with and without UEA1.	(CHEN et al., 2017)
Purification and Homogeneity	Electric-separation	Electrophoretic light scattering (ELS) ou Zeta Potential	BVL	Measurement of the variation in the superficial electrical charge of liposomes with and without BVL.	(SANTOS et al., 2018a)
Biological	Cytometry	Flow Cytometry (FCM)	PgTeL	Used to check the viability of bacterial cells treated with PgTeL.	(SILVA et al., 2019c)
Biological	Cytometry	Flow Cytometry (FCM)	WGA	Microorganism detection by flow cytometry, based on the interaction between lectin-*Escherichia coli* and *Staphylococcus aureus*.	(HENDRICKSON et al., 2019)
Biological	Immunology	Enzyme-Linked Immunosorbent Assay (ELISA)	FRIL	ELISA was used to determine the affinity of FRIL binding to the recombinant Spike protein of SARS-CoV-2, produced with predominantly complex type glycans and produced with mannose-rich glycans.	(LIU et al., 2020)

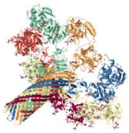
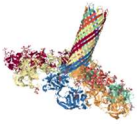
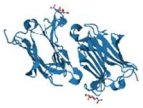
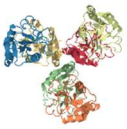
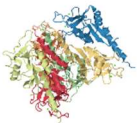
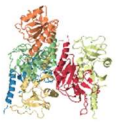
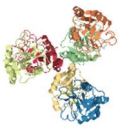
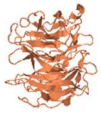
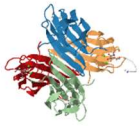
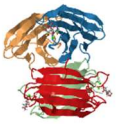
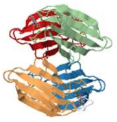
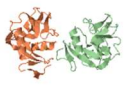
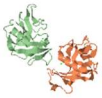
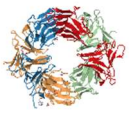
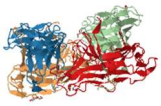
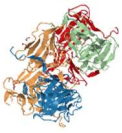
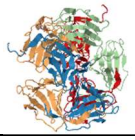
Biological	Immunology	Enzyme-Linked Immunosorbent Assay (ELISA)	rPcLec6	Performed to detect the binding of rPcLec6 to polysaccharides (LPS, LTA and peptidoglycan).	(ZHANG et al., 2018b)
Biological	Immunology	Reverse Transcription Polymerase Chain Reaction quantitative real time (qRT-PCR)	rPcLec6	It was used to detect the level of transcriptional expression of PcLec6.	(ZHANG et al., 2018b)
Biological	Immunology	Western Blot, Western Blotting or Protein Immunoblotting	rPcLec6	Immunodetection of rPcLec6 linked to Gram-positive and negative bacteria.	(ZHANG et al., 2018b)
Biological	Immunology	Western Blot, Western Blotting or Protein Immunoblotting	rConBr	Immunodetection of heterologous expression of rConBr.	(CARVALHO et al., 2008)
Biological	Immunology	Western Blot, Western Blotting or Protein Immunoblotting	FRIL	Immunoblotting showed the type of glycoprotein that FRIL had the most binding affinity.	(LIU et al., 2020)

*: The techniques can be classified in different categories and even be in more than one category, everything will depend on the parameters adopted for this classification, as well as the focus that is intended to be adopted in the presentation of the same. In this study, the main objective is not in the classification, but in showing the most varied techniques used in the characterization of lectins and what was obtained in the study. Thus, it can reinforce the use of this technique in some more well-known characterizations and also open the horizon for their use for some uses, perhaps, not very conventional or classically known.

It is important to note that the structure of proteins is something very complex, because they are macromolecules, and therefore, have many physical-chemical and biological specificities. One of these specificities concerns its molecular dynamics. Although these structures, as seen in table 6, are represented in a static way, in nature these macromolecules are in constant dynamics between their structural and conformational levels, both to perform their functions, as well as to adapt to different conditions of pH and ionic strength of the medium. For example, constant transitions (dynamic equilibrium) dimer-monomer or dimer-tetramer by lectins ins under different pH conditions (VAREJÃO et al., 2010; ZHANG et al., 2017).

Table 6. Three-dimensional structures of different lectins, by different angles.

Lectins	**Structures**	**References**
RSLex		(GUAGNINI et al., 2020)
AAL		(HOUSER et al., 2017)
BJcul		(SARTIM et al., 2017)
POL		(PERDUCA et al., 2020)
Omentin -1		(MCMAHON et al., 2020)
BfL		(LUBKOWSKI et al., 2017)
BC2L-C		(BERMEO; BERNARDI; VARROT, 2020)
VML		(SOUSA et al., 2016)

CEL-III					(UNNO; GODA; HATAKEYAMA, 2014)
Gal-10					(ITOH et al., 2020)
Clec4f					(OUYANG et al., 2020)
PLL3					(FALTINEK et al., 2019)
LecB					(BAERISWYL et al., 2019)
SPL-1					(UNNO et al., 2019)
Tarin					(PEREIRA et al., 2017)

In general, these macromolecules can be formed by a single polypeptide chain (monomer) or more than one chain, thus forming dimers, trimers, tetramers among another. Thus, some examples included Gal-10 lectin formed by a single polypeptide chain (ITOH et al., 2020), SPL-1 by two chains (UNNO et al., 2019), BC2L-C for three (BERMEO; BERNARDI; VARROT, 2020), LecB is a tetramer (BAERISWYL et al., 2019), Clec4f id formed for six (OUYANG et al., 2020), and CEL-III for seven (UNNO; GODA; HATAKEYAMA, 2014). These polypeptide chains can be the same or different, when equal, the prefix “homo” is added, when different “hetero” (example: homodimers and heterodimers).

The complexity of these structures, such as the number of polypeptide chains as well as its folding, the presence of disulfide bridges and other post-translational modifications, can be explained by: lectin-producing organism and its cellular machinery and also for the role that lectin plays in its native environment. It could be, for example, a cellular receptor of mouse macrophages, such as Clec2d lectin, capable of immune detection of cell death (LAI; CRUZ; ROCK, 2020), mouse Kupffer cell receptor like Clec4f lectin (OUYANG et al., 2020), or a

pore-forming lectin as CEL-III lectin, purified from the body fluid of sea cucumber *Cucumaria echinata*, which have hemolytic capacity (UNNO; GODA; HATAKEYAMA, 2014).

3.1.3 Biological activities

In addition to the purification and characterization process, it is important to visualize an application for lectins. And one of its many application possibilities, is as a pharmacological agent (COELHO et al., 2017; JUAN et al., 2017), for this, many studies test its potential through different biological activities (table 7). And these studies are very relevant not only because of the results presented, but also because many of the lectins tested are from natural sources, which also has beneficial repercussions for the preservation of ecosystems. In addition, many synthetic chemicals have problems such as: resistance of target organisms and high collateral toxicity (DUTTA et al., 2019; KUMAR; JAITAK, 2019; PÉREZ; CARRETERO; CONTRERAS, 2019).

The biological activities already described with lectins are very varied, depending on the focus of the study. Some studies, such as evaluation of the antibacterial, antioxidant, anticancer, anti-inflammatory capacity, among others, can be seen in table 7. These biological tests were made from lectins from different organisms and from different parts of these organisms. For example: antibiofilm of Es-Lec (from *Etroplus suratensis* serum, fish) (RUBEENA et al., 2019), immunomodulator of SteLL (from *Schinus terebinthifolia* leaves, plant) (LIMA et al., 2019b), and proinflammatory of BlL (from *Bothrops leucurus* venom, snake) (ARANDA-SOUZA et al., 2019).

Table 7. Different lectin biological activities.

Activities	Lectins	References
Antibacterial	CiMBL	(LIU; DANG, 2020)
Antibacterial	Es-Lec	(RUBEENA et al., 2019)
Antibacterial	CasuL	(PROCÓPIO et al., 2019)
Antibiofilm	Es-Lec	(RUBEENA et al., 2019)
Antibiofilm	AFL	(CARNEIRO et al., 2019)
Antibiofilm	ConA	(JIN; LEE; HONG, 2019)
Antibiofilm	MuBL, MuHL and MuLL	(MOURA et al., 2020)
Antidepressant	ConBr	(BARAUNA et al., 2006)
Antifugal	PeRoL	(SILVA et al., 2019a)
Antifugal	ApuL	(FERREIRA et al., 2018)
Antifugal	Lectin de *Mussaenda alicia*	(NOVA et al., 2020)
Anti-inflammatory	OppCTL	(LIU et al., 2019)

Anti-inflammatory	ConA and ConBr	(OLIVEIRA et al., 2021)
Anti-inflammatory	LAL	(PIRES et al., 2016)
Antilipidemic	BSL	(FREIRE et al., 2020)
Antimutagenic	Lys LJ	(FRASSINETTI et al., 2015)
Anti-necrotic	ConA and ConBr	(OLIVEIRA et al., 2021)
Antinociceptive	LCaL	(PIRES et al., 2019)
Antinociceptive	MaL	(SANTOS et al., 2019a)
Antinociceptive	BmoLL	(CAMPOS et al., 2016)
Antinutritional	MvRL	(ALBUQUERQUE et al., 2020)
Antioxidant	BSL	(FREIRE et al., 2020)
Antioxidant	PLUN	(LACERDA et al., 2017)
Antioxidant	HFL	(WU et al., 2016)
Antiproliferative	GDL	(AHSANUL KABIR et al., 2019)
Antiproliferative	BfL-II	(PINTO et al., 2019b)
Antiproliferative	SteLL	(RAMOS et al., 2019)
Antiulcer or Gastroprotective	MpLec	(PINTO et al., 2019a)
Antiulcer or Gastroprotective	PLUN	(LACERDA et al., 2017)
Antiviral	FRIL	(LIU et al., 2020)
Antiviral	*Es-Lec*	(RUBEENA et al., 2019)
Antiviral	Siglec-1	(UCHIL et al., 2019)
Antiviral	BanLec	(BATCHA; WADHWANI; SUBRAMANIAM, 2020)
Antiviral	rCV-N	(AGARWAL; TRIVEDI; MITRA, 2020)
Anxiolytic	DAL	(ARAÚJO et al., 2020)
Asthma Response Control	CrataBL	(BORTOLOZZO et al., 2018)
Erythrocytic (Antihemolytic)	MpLeC	(RODRIGO et al., 2015)
Erythrocytic (Hemolytic)	CEL-III	(UNNO; GODA; HATAKEYAMA, 2014)
Erythrocytic (Hemolytic)	Clec2d	(LAI; CRUZ; ROCK, 2020)
Erythrocytic (No Hemolytic)	PeRoL	(SILVA et al., 2019a)
Formation of amyloid-like aggregates	B1L	(ARANDA-SOUZA et al., 2019)
Healing	Cramoll	(ANDRADE et al., 2021)
Healing	Frutalin	(SOUSA et al., 2019)
Healing	Cramoll	(ALBUQUERQUE et al., 2017)
Hypoglycemic or Anti-hyperglycemic	BSL	(FREIRE et al., 2020)
Hypoglycemic or Anti-hyperglycemic	TKL	(LU et al., 2018)
Immunomodulator	CFL and ConBr	(BATISTA et al., 2017)
Immunomodulator	Tarin	(CORRÊA et al., 2019)
Immunomodulator	SteLL	(SANTOS et al., 2020a)

Insecticide	Lectin from *Solanum integrifolium*	(CHEN et al., 2018)
Insecticide	PPA	(RAHIMI et al., 2018)
Insecticide	WSMoL	(OLIVEIRA et al., 2020b)
Insecticide	MuBL and MuHL	(ALVES et al., 2019)
Mitogenic	CAL	(GAUTAM et al., 2018)
Mitogenic	*Penicillium duclauxii* lectin	(SINGH; WALIA; KENNEDY, 2018)
Mitogenic	*Penicillium proteolyticum* lectin	(SINGH; WALIA; KENNEDY, 2019)
Mitogenic	RSB	(UNE; NONAKA; AKIYAMA, 2018)
Parasiticide	BlL	(ARANDA-SOUZA et al., 2018)
Parasiticide	WSMoL	(MEDEIROS et al., 2020)
Proinflammatory	BjcuL	(CEZARETTE; SARTIM; SAMPAIO, 2020)
Proinflammatory	Clec2d	(LAI; CRUZ; ROCK, 2020)
Proinflammatory	PPL	(WANG et al., 2019)

It can also be seen in the table 7, that different lectins can present activities with inverse effects, such as anti-inflammatory by ALL (PIRES et al., 2016) and pro-inflammatory by PPL (WANG et al., 2019). It can also be seen that the rLSL has hemolytic activity (TATENO; GOLDSTEIN, 2003), MpLeC is antihemolytic (PINTO et al., 2019a), and PeRoL is non-hemolytic (SILVA et al., 2019a). They can present different activities as seen, and it depends: of type of structure, its folding, phosphorylation, type of carbohydrate it has affinity, tested concentration, pH, temperature and the medium it is inserted in, for example. In addition, many of these activities may be directly related to the role that lectin plays in its natural source.

It is also worth noting that a single lectin can have different activities, such as CrataBL, which has antitumor, anti-inflammatory, antinociceptive (ARAÚJO et al., 2011), insecticide (ARAÚJO et al., 2012), hypoglycemic (ROCHA et al., 2013), cancer cell cytotoxicity (FERREIRA et al., 2013), action in blocking coagulation and formation of arterial thrombus (SALU et al., 2014), attenuates inflammatory changes (OLIVA et al., 2015), larvae control of *Callosobruchus maculatus* (NUNES et al., 2015), plays a role in controlling the asthma response, and more. In another way, many lectins may have activities in common, such as antibiofilm activity: presented by Es-Lec (RUBEENA et al., 2019), AFL (CARNEIRO et al., 2019), PgTeL (SILVA et al., 2019c) and ConA (JIN; LEE; HONG, 2019), for example. Next, the healing activity will be highlighted, in order to exemplify the activity of lectins in more

detail and also because there is no presentation of healing activity in the literature with this type of approach: lectins involved in cutaneous, hepatic, intestinal and ocular healing.

3.1.3.1 Healing activity

3.1.3.1.1 Cutaneous

The main role of the skin is to serve as a protective barrier against the environment. Loss of skin integrity, due to damage or disease, can lead to severe disability or even death (KONDO; ISHIDA, 2010), and its restoration consists of a dynamic and well-organized biological process (SANTORO; GAUDINO, 2005) that covers a varied number of cellular and biochemical components used in the recovery of tissue morphology and function (KAPOOR et al., 2006).

Skin lesions are the result of disruption of tissue integrity (DAVIS; PEREZ, 2009). Tissue damage initiates a cascade of reactions, including inflammation, tissue formation and remodeling (granulation tissue), leading to partial or total reorganization of the affected area (CLARK, 1998; SCHREML et al., 2010). If a wound is a disruption of the anatomical and physiological continuity of an organ or tissue, a scar is an attempt by the body to restore integrity (MORGAN, C. J.; PLEDGER, 1992). Thus, the development of engineering based on natural sources for the healing of wounds or skin ulcers is of great interest to researchers and other parts of the field of biomedical research (DAVIS; PEREZ, 2009), in particular, the search for agents capable of improving tissue repair and reducing healing time (SCHULTZ et al., 2005; SÜNTAR et al., 2010).

Nowadays, lectins have been extensively studied for the development of new technologies. This group of proteins, so structurally diverse, has several biomedical and biotechnological applications (LAM; NG, 2011), capable of activating immune cells, neutrophils, macrophages, and mast cells (MORENO et al., 2003; PANUNTO-CASTELO et al., 2001), thus demonstrating a potential to accelerate wound healing and epithelial tissue regeneration (SILVA et al., 2004).

BVL, a lectin from *Bauhinia variegata* (a species of orchid), has at least two isoforms (BVL-1 and BVL-2). Both isoforms are galactose ligands with a molecular mass of 32 kDa and structurally similar to others found in Caesalpinoideae species (PINTO et al., 2008). This lectin and its recombinant isoform 1 (rBVL-1), have the ability to stimulate the mitogenic activity of resident cells, transforming them into potent chemotactic agents for the recruitment of neutrophils, through the release of cytokines, as observed in studies on other lectins of

binding to galactose, such as those isolated from *Vatairea macrocarpa* and *Artocarpus integrifolia* (ALENCAR et al., 2007; CHAHUD et al., 2009).

In a study by Neto et al. (2011), tests were carried out to analyze the ability to optimize the healing process of the two isoforms of Bauhinia variegata lectin, with better results for rBVL-1, where on the seventh day of treatment there was already the presence of regenerated skin, demonstrating better results in repair, compared to BVL-1. This difference in wound healing observed between animals treated with BVL and rBVL-1 was probably due to structural differences in the domains of these lectins. The rBVL-1 lectin showed an excellent ability to stimulate the healing process through the action of immune cells, increasing the synthesis of collagen from fibroblasts and angiogenesis, presenting a greater capacity for contraction of the lesion.

Still, in studies applied to healing, in Parkia pendula lectin, in tests with immunocompetent and immunocompromised animals, it was observed that on the 12th day of treatment, the immunocompromised animals, in addition to having their lesions closed first than their respective control groups, showed excellent repair in relation to some parameters, such as collagen deposition, for example. The control group showed significant collagen deposition and reepithelialization. The histological analysis of the control group showed that there was a matrix poor in collagen fibers at the wound site that conferred fragility of the local tension and inefficient tissue repair. The results demonstrate that the lectin from *P. pendula* seeds induces the repair of skin wounds in normal and immunosuppressed organisms, thus suggesting a potential biopharmaceutical for action in the healing process of skin wounds (table 8) (CORIOLANO et al., 2014).

Another source of studies has been the application of lectins against Leishmania sp. Cutaneous leishmaniasis is one of the most common infections caused by protozoan parasites of the genus Leishmania. Patients with cutaneous leishmaniasis can manifest one or more altered skin lesions that are compared to other dermatological diseases (VAN KESTEREN et al., 2020). Afonso-Cardoso et al. (2007), demonstrated immunization by the lectin ScLL, from *Synadenium carinatum*, compared to the use of an SLA antigen, where immunization with SLA associated with ScLL conferred partial protections for the animals. And the protection afforded by lectin alone at a concentration of 100 µg/animal, was greater compared to SLA+ScLL. Despite the fact that the group immunized with SLA associated with ScLL (100 µg/animal) gave early protection to the animals in the sixth week after infection. BALB/c mice immunized with ScLL only (10, 50 or 100 µg/animal) were partially protected at the end of 10 weeks, when compared to groups immunized with SLA and control only. It was also observed that the

parasitic load of animals immunized with SLA+ScLL or ScLL alone decreased, showing a significant reduction when compared to groups immunized only with SLA and control.

Studies carried out by Panunto-Castelo et al. (2001), evaluated the resistance of animals to Leishmania after treatment with the lectin KM+, from *Artocarpus integrifolia*, where it was observed that KM+ induces macrophages to produce IL-12p40, an important cytokine, known to lead the immune response to a Th1 pattern, thus bringing resistance to *Leishmania major* infection. A significant reduction in the number of parasites in the animals' paws was observed, thus leading to a decrease in paw thickness.

Frutalin is a galactose-binding lectin, purified from *Artocarpus incisa* seeds (MOREIRA et al., 1998). This lectin has several biological activities, such as cytotoxicity to tumor cells, mitogenic activity in human lymphocytes and chemotaxis to neutrophils (BRANDO-LIMA et al., 2005, 2006; OLIVEIRA et al., 2011). Frutalin was evaluated for action on a fibroblast culture and was subsequently incorporated into a galactomannan-based hydrogel to treat skin lesions induced in mice, where neutrophil chemotaxis was evidenced (SOUSA et al., 2019).

When adding Frutalin to the fibroblast cultures, significantly increased levels of MyD88, a fundamental protein for the activation of immune cells through TLR receptors in innate immunity, were found (AKIRA; TAKEDA, 2004). Frutalin also activated the TLR4 receptor, a key participant in the detection of pathogens, activation of neutrophils and regulation of the immune system (GARCÍA-CULEBRAS et al., 2019), fact that explains chemotaxis to neutrophils. Increased IL-6 levels by 5 times with the addition of Frutalin for 24 hours, which in addition to stimulating angiogenesis, is also associated with cell proliferation and cell migration (KUMARI et al., 2016; TAKEUCHI; AKIRA, 2010). Histological analysis was marked by an intense inflammatory infiltrate on days 3 and 7, while from the 11th day onwards there was proliferation of fibroblasts and the beginning of total reepithelization of the previously ulcerated area. The healing process was marked by intense angiogenesis (OLIVEIRA et al., 2011).

In continuity with skin lesions, Brustein et al. (2012), were able to evaluate the antimicrobial and healing activity of EmaL by performing treatment on induced skin lesions. EmaL lectin is extracted from the seeds of *Eugenia malaccensis*, a medicinal plant popularly known as red jambo and commonly consumed as food, by the Brazilian population (OLIVEIRA et al., 2006).

EmaL showed excellent antimicrobial activity against Streptococcus sp., *Pseudomonas aeruginosa* and *Staphylococcus aureus*, the latter being, a pathogen commonly associated with skin infections (GOMPELMAN et al., 2018). When the biopsy was performed, the group treated with EmaL presented well-organized transition areas, showing more extensive reepithelialization towards the center of the lesion, however, difficult to visualize; in addition, the granulation tissue was fibrous with some small vessels. The control group, on the other hand, only presented granulation tissue with fibrovascular and vascular characteristics (BRUSTEIN et al., 2012).

Cramoll is a lectin with a specific binding site for glucose and mannose (PATRICIO et al., 2011), and it has four isoforms: Cramoll 1, Cramoll 2, Cramoll 3 and Cramoll 4 (CORREIA; COELHO, 1995; PAIVA; COELHO, 1992). It comes from the seeds of *Cratylia mollis*, a plant native to northeastern Brazil (MELO et al., 2010). Several beneficial functions of this lectin have already been demonstrated, such as: immunomodulatory capacity, proliferative in lymphocytes, antitumor and tissue repair (ANDRADE et al., 2004; BELTRÃO et al., 1998; MACIEL et al., 2004).

In a study using Cramoll 1,4 for topical treatment of second-degree burns, induced in rats, it was observed that the group treated with lectin had a better response to treatment than the control group, with the process of tissue repair accelerated and getting better response in the inflammatory process. The treated mice showed an increase in the number of fibroblasts, the presence of organized collagen in the center of the lesion and a shorter reepithelization time compared to the control group. This occurs due to the tissue repair activity that Cramoll 1,4 has: immunomodulatory action and proliferative activity in lymphocytes, which assist in the recruitment of fibroblasts and the consequent acceleration of tissue repair (PEREIRA et al., 2012).

In continuity with the lectin Cramoll 1,4, there was an acceleration of reepithelialization when treating lesions induced in healthy and immunosuppressed mice for 12 days. Lesions in healthy mice treated with Cramoll 1,4 showed the highest prevalence of granulation tissue and the highest collagen deposition. All mice had complete lesion closure at the end of 12 days, however, 90% of lesions were closed on the 10th day in the healthy Cramoll 1,4 group. The immunosuppressed mice treated with Cramoll 1,4 showed inferior results compared to the healthy group, since the models of this group were induced to immunosuppression before starting the treatment (MELO et al., 2011). According to Li et al. (2018), this leads to a decrease in leukocyte migration and, consequently, a delay in the healing

process, since lymphocytes and macrophages are responsible for stimulating the proliferation of fibroblasts and angiogenesis, intrinsic factors for repair, tissue repair.

Another study also showed the healing activity of Cramoll 1,4 incorporated in different concentrations in a film based on the galactomannan polysaccharide and applied to lesions induced in rats (ALBUQUERQUE et al., 2017). Galactomannan is a vegetable polysaccharide formed by a main chain of D-Mannose with side groups of D-Galactose, existing in the endosperm of *Gleditsia sinensis* (SIQUEIRA et al., 2015; ZHAO et al., 2019b).

The study revealed that rats treated with films containing Cramoll 1,4 lectin showed a significant increase in the percentage of wound contraction, greater collagen deposition and greater degree of healing compared to control rats. Cramoll 1,4 immobilized on galactomannan films presented itself as a promising dressing, maintaining the biological activities of lectin, including mitogenic pattern, immunomodulation and healing agent (ALBUQUERQUE et al., 2017).

Table 8. Lectins with healing action on different types of injuries.

Lectin	**Plant**	**Specificity**	**Type of Injury**	**Animal Model Used**	**Infection**	**Reference**
Artin M	*Artocarpus heterophyllus*	Mannose	Cutaneous	Camundongo	*Leishmania major*	(PANUNTO-CASTELO et al., 2001; SOUZA et al., 2013)
			Hepatic	Camundongo	*Candida albicans*	(LOYOLA et al., 2012)
ConA	*Canavalia ensiformis*	Mannose	Hepatic	Camundongo	*Klebsiella pneumoniae*	(KUO et al., 2007)
			Intestinal	Rat	-	(YASUOKA et al., 2003)
Cramoll 1,4	*Cratylia mollis*	Glucose / Mannose	Cutaneous	Rat	-	(ALBUQUERQUE et al., 2017; PEREIRA et al., 2012)
			Cutaneous	Camundongo	-	(MELO et al., 2011)
Cramoll	*Cratylia mollis*	Glucose / Mannose	Cutaneous	Camundongo	-	(ANDRADE et al., 2021)
EmaL	*Eugenia malaccensis*	Glucose	Cutaneous	Camundongo	-	(BRUSTEIN et al., 2012)
Frutalin	*Artocarpus incisa*	Galactose	Cutaneous	Camundongo	-	(SOUSA et al., 2019)
KM+	*Artocarpus integrifolia*	Mannose	Ocular	Rabbit	-	(CHAHUD et al., 2009)

BVL	*Bauhinia variegata*	Galactose	Cutaneous	Camundongo	-	(NETO et al., 2011)
PHA	*Phaseolus vulgaris*	Galactose	Intestinal	Rat	-	(YASUOKA et al., 2003)
PpaL	*Parkia pendula*	Glucose / Mannose	Cutaneous	Camundongo	-	(CORIOLANO et al., 2014)
SBA	*Glycine max*	Galactose	Ocular	Rat	-	(GORDON, 2011)
ScLL	*Synadenium carinatum*	Galactose	Cutaneous	Camundongo	*Leishmania major*	(AFONSO-CARDOSO et al., 2007)

- Not identified

3.1.3.1.2 Hepatic

Concanavalin A (ConA) is a vegetable lectin from the seeds of *Canavalia ensiformis*, commonly known as hog bean, and which has affinity for mannose binding sites (ZHAO et al., 2017). In a study that evaluated the healing of a liver abscess induced by *Klebsiella pneumoniae* via a gastroesophageal tube in a murine model of infection, it was noted that the administration of ConA - 2h before infection or at intervals after injury induction - significantly inhibited the bacterial growth log (KUO et al., 2007). Although it has already been described that ConA can be used as an experimental model for inducing hepatitis (GRECO et al., 2016; LEE et al., 2016; LI et al., 2016) and has the ability to recruit and activate neutrophils, through the massive expression of β2-integrin, which are directly responsible for the intense inflammatory process through the release of oxidative radicals (BONDER et al., 2004).

In addition, it was shown that in mice treated with ConA (using non-hepatotoxic doses), on the third day, the liver abscess was full of activated macrophages and infiltrating neutrophils in interaction, which demonstrates that lectin effectively showed an immunomodulatory effect by increasing interactions between phagocytic cells in the liver, leading to digestion and degradation of apoptotic neutrophils by resident Kupffer cells, thus blocking the release of toxic metabolites and harmful tissue enzymes, conditions that promoted and intensified the tissue repair process (KUO et al., 2007).

Also related to liver damage, according to tests carried out by Loyola et al. (2012), the lectin from the seed of *Artocarpus heterophyllus*, known as ArtinM, also managed to significantly attenuate the phagocytic activity of hepatic and peritoneal macrophages in mice infected by *Candida albicans*, through interaction with Mannose and Dectin-1 Receptors. *C. albicans* is an important human pathogen and the target of several studies, mainly due to its high capacity to cause infections and injuries in various tissues of the body, such as the liver (GULATI; NOBILE, 2016), and its recognition by the immune system occurs even through

type C Lectin Receptors (CLRs) (WANG, 2015). The study reported that animals pretreated with lectin performed better against yeast cells with dose-dependent effect between 48 and 72 hours after administration (LOYOLA et al., 2012), suggesting that this compound is a relevant accelerator of the healing process by removing inflammatory and infectious agents in the liver.

3.1.3.1.3 Intestinal

Another animal clinical trial demonstrated that ConA and PHA, (galactose ligand lectin from *Phaseolus vulgaris*, red beans), have a healing effect on intestinal lesions induced by indomethacin. It was noted that the two groups of rats with a diet supplemented by each of these lectins demonstrated minor macroscopic inflammation compared to animals that received only a control diet (NAGAE et al., 2014; WOLTERS-EISFELD; SCHUMACHER, 2017; YASUOKA et al., 2003).

Collections every 2 hours showed that the rats that were supplemented with lectins demonstrated better healing results, with emphasis on the animals of the PHA group, that there was rapid formation of granulation tissue, where the ulcer was completely covered with epithelial tissue. Both lectins also managed to decrease the rates of MPO in the intestinal mucosa, an enzyme found in leukocytes and that can be used to quantify inflammation. Therefore, both ConA and PHA were able to significantly reduce the area of the lesions, promoted the epithelial healing of the small intestine and, still, preserved the integrity of the animals' mucosa (HARTMAN; FORD, 2018; UL-AIN BALOCH; N IBEABUCHI, 2018; YASUOKA et al., 2003).

3.1.3.1.4 Ocular

A model of eye injury induced in male albino rabbits proved that the lectin KM+, found in seeds of *Artocarpus integrifolia*, managed to promote and accelerate the corneal epithelial healing process (CHAHUD et al., 2009), proving a previous study that had already elucidated its molecular mechanisms of immunomodulation (PEREIRA-DA-SILVA et al., 2006). From the scraping of the superficial ocular tissue, the animals were divided into groups, in the group treated with lectin, doses were applied directly to the eyes of the animals, at 2 hours intervals, for 12 hours. Through optical microscopy, it was found that there was a significant increase in the influx of neutrophils in the frontal ocular stroma of the group treated in the first 12 hours after the injury (when compared to the group with only buffer solution) (CHAHUD et al., 2009), crucial period of the initial corneal repair phase as neutrophils are closely related to the secretion of substances involved in the healing process (LATEEF et al., 2019; REINKE; SORG, 2012).

It was also observed that these migratory neutrophils did not accumulate for longer than necessary in the injured region, since the exacerbated release of pro-inflammatory compounds could impair the healing process (KIM et al., 2012). The study also reported that 24 hours after the injury, more epithelial cells were detected with antibodies to PCNA (nuclear antigen of proliferating cells), p63, VEGF (vascular endothelial growth factor), c-Met and Laminin. Thus, during the same evaluation period, 70% of the animals treated with KM + had their lesions healed, against only 30% of the control group (CHAHUD et al., 2009).

Another curative activity of lectins in eye cells has shown that SBA lectin, extracted from soy (*Glycine max*), has the ability to induce endothelial proliferation in the cornea of rats by increasing the rates of surface galactose binding of tissue to SBA during the initial phase of the healing process, when a fully structured endothelial monolayer does not yet exist, where intense recruitment of adjacent cells occurs to repair the injured region. The study shows that healthy eye tissues were not able to bind as effectively to SBA, but when corneal injury occurred, these rates of attachment increased considerably. Thus, it was described that during the initial repair phase, specific protein markers slightly identified transitory bonds to lectin throughout the injured surface, indicating a probable complementary action of recognition and adjacent adhesion. And as the compromised tissue regenerated, these markers were directed to the cell periphery, as a way to boost the migration of cells to the regions in the process of repair, until the complete restoration of the junctional complex. The more structured the endothelial cell monolayer was, the more the animal eye tissue links to SBA gradually disappeared (GORDON, 2011; UD-DIN; BAYAT, 2017; YANNAS; TZERANIS; SO, 2017).

3.1.4 Expression of recombinant lectins

The purpose of staggered production of lectins by conventional methods (of collection, extraction and purification from the biological source) are not the most suitable since these processes demand more time and a large amount of raw material (ANDERSEN; KRUMMEN, 2002; OLIVEIRA; TEIXEIRA; DOMINGUES, 2013; SCHMIDT, 2004). In this sense, the heterologous expression of bioactive lectins is of great value once it can facilitate large-scale production, obtain higher yields, reduce costs and time, avoid contamination problems, separate isoforms (producing a single product, with well-defined characteristics), in addition to being able to study the function of sequences by mutagenesis directed (MARTÍNEZ-ALARCÓN; BLANCO-LABRA; GARCÍA-GASCA, 2018). The production of recombinant lectins, such as mentioned, in figure 5, consists of the synthesis and cloning of the gene of

interest, heterologous expression of lectin, and its purification (GOLOTIN et al., 2019; LEYVA et al., 2019; TATENO; GOLDSTEIN, 2003).

Table 9. Recombinant lectins obtained from sequences of native lectins of various species, produced in organisms/cells of different groups of nature.

Species of Native Lectin	Group	Organism/Cell	Recombinant Lectins	References
Aleuria aurantia	Bacteria	*E. coli*	rAAL	(ROMANO et al., 2011)
Artocarpus incisa	Bacteria	*E. coli*	rFTP	(SOUSA et al., 2017)
Bauhinia forficata	Bacteria	*E. coli*	rBfL-II	(PINTO et al., 2019b)
Bauhinia variegata	Fungus	*P. pastoris*	rBVL-Ip	(KLAFKE et al., 2016)
Bauhinia variegata	Bacteria	*E. coli*	rBVL-Ie	(KLAFKE et al., 2013)
Canavalia brasiliensis	Bacteria	*E. coli*	rConBr	(NOGUEIRA et al., 2005)
Canavalia brasiliensis	Fungus	*P. pastoris*	rConBr	(CARVALHO et al., 2008)
Chicken liver tissue	Human	HeLa Cells	RcMBL	(ZHANG et al., 2017)
Cratylia mollis	Bacteria	*E. coli*	rCramoll 1	(VAREJÃO et al., 2010)
Cycas annaikalensis,	Bacteria	*E. coli*	CAPL	(RADHA; URLA, 2019)
Gallus Gallus	Human	HeLa R19 Cells	RcMBL	(ZHANG et al., 2017)
Glycine max	Bacteria and Monkey	*E. coli* and BS-C-1 cells	SBA	(ADAR et al., 1997)
Helix pomatia	Bacteria	*E. coli*	HPA	(MARKIV et al., 2011)
Homo sapiens	Hamster	Chinese hamster ovary cells	MBL	(OHTANI et al., 1999)
Laetiporus sulphureus	Bacteria	*E. coli*	rLSL	(TATENO; GOLDSTEIN, 2003)
Lignosus rhinocerus	Bacteria	*E. coli*	rRhinocelectin	(CHEONG et al., 2019)
Litopenaeus vannamei	Bacteria	*E. coli*	rLvLTLC1	(TIAN et al., 2018)
Moringa oleifera	Fungus	*P. pastoris*	MoL	(ABD WAHID et al., 2017)
Musa acuminata	Bacteria	*E. coli*	rBanLec	(GAVROVIC-JANKULOVIC et al., 2008)

Mytilus trossulus	Bacteria	*E. coli*	r-MTL	(GOLOTIN et al., 2019)
Nostoc ellipsosporum	Bacteria	*E. coli*	rCV-N	(AGARWAL; TRIVEDI; MITRA, 2020)
Oplegnathus punctatus	Bacteria	*E. coli*	rOppCTL	(LIU et al., 2019)
Penaeus japonicus	Bacteria	*E. coli*	rPjLec2	(GAO et al., 2020b)
Portunus trituberculatus	Bacteria	*E. coli*	rPtCTL4	(ZHANG et al., 2018a)
Procambarus clarkii	Bacteria	*E. coli*	rPcLec6	(ZHANG et al., 2018b)
Rimicaris exoculata	Bacteria	*E. coli*	rReCTL-1 rReCTL-2	(WANG et al., 2020)
Ruditapes philippinarum	Bacteria	*E. coli*	rRpCTL	(LI et al., 2019)
Solanum integrifolium	Human	293-F cell	rCBL	(CHEN et al., 2018)
Triticum vulgaris	Bacteria	*E. coli*	rWGA	(LEYVA et al., 2019)
Urtica doica	Plant	*Nicotiana tabacum* cell	UDA	(DOES et al., 1999)

E. coli = Escherichia coli and *P. pastoris = Pichia pastoris*.

There are recombinant lectins expressed in a diversity of cells, both in eukaryotes and prokaryotes, for example, *Escherichia coli* (bacterium), *Pichia pastoris* (fungus), BS-C-1 cells (primate), *Nicotiana tabacum* (plant), Chinese hamster ovary cells (rodent), 293-F Cell and HeLa R19 Cells (human), as can be seen in table 9. The sources of recombinant lectins also vary a lot, such as plants (*Artocarpus incisa*, *Bauhinia forficata, Bauhinia variegata, Cratylia mollis, Solanum integrifolium, Triticum vulgaris*), fungi *(Aleuria aurantia, Laetiporus sulphureus, Lignosus rhinocerus*), crustaceans (*Portunus trituberculatus, Penaeus japonicus, Litopenaeus vannamei, Procambarus clarkii*), mollusks (*Ruditapes philippinarum, Mytilus trossulus*), and fish (*Oplegnathus punctatus*).

One of the main questions in relation to the choice of model for the expression of recombinant lectin is the machinery that each cell has for the production of the protein. Prokaryotic organisms, such as *E. coli*, do not have the appropriate machinery composed of intracellular organelles capable of carrying out post-translational modifications, such as complex folding, formation of disulfide bonds, phosphorylation, glycosylation, and proteolytic processing, while eukaryotes like fungi, plants and animals have (CEREGHINO et al., 2002; DEMAIN; VAISHNAV, 2009). However, other issues are also very important when choosing the expression model, such as complexity of the system, ease of manipulation, knowledge of

its physiology and its genome, duplication rate and costs (BANEYX; MUJACIC, 2004; DEMAIN; VAISHNAV, 2009; ROMANOS; SCORER; CLARE, 1992; SØRENSEN; MORTENSEN, 2005). Usually, these factors mentioned are more advantageous in simpler organisms, such as bacteria and fungi, however, with the advances in genetic engineering and molecular biology techniques, the use of more complex models and with more adequate machinery (animal models, for example), become increasingly attractive as these variables are overcome (ALMO; LOVE, 2014; ANDERSEN; KRUMMEN, 2002; ROSANO; CECCARELLI, 2014).

Finally, it is worth mentioning that many of these lectins produced by heterologous expression have many activities already tested, for example, rRpCTL antimicrobial activity against bacteria (LI et al., 2019), antiproliferative activity of rBfL-II in human breast and colorectal cancer cells (MCF-7 and HT-29, respectively) (PINTO et al., 2019b), antibacterial and antiviral activities of rPcLec6 (ZHANG et al., 2018b), antibacterial and anti-inflammatory activity of rOppCTL (LIU et al., 2019), link with the innate immune response, of rPtCTL4 (ZHANG et al., 2018a) and having potential for modulation of the immune response. Increases T lymphocyte proliferation and secretion of interferon-gamma by mouse splenocytes of rBanLec (DIMITRIJEVIC et al., 2010, 2012; GAVROVIC-JANKULOVIC et al., 2008).

3.1.5 Conclusion

Therefore, this study demonstrates that lectins can be purified in different organisms and ways. A substantial number of biological activities were also presented, highlighting healing activity (cutaneous, hepatic, intestinal and ocular). Finally, some models of heterologous expression and the respective obtained lectins were presented.

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3.2 ARTIGO 2 - PURIFICATION OF *BOWDICHIA VIRGILIOIDES* LEAVES LECTIN AND ANALYSIS OF ITS MOLECULAR INTERACTION

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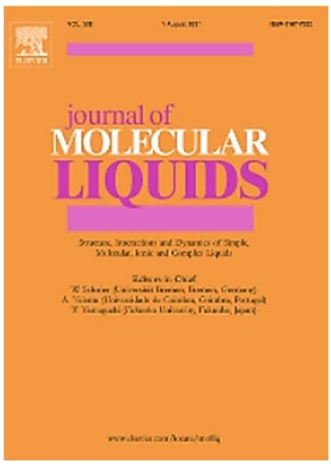

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Abstract

Lectins are proteins or glycoproteins, which can bind in a specific and reversible way to carbohydrates. They are found in various organisms such as viruses, bacteria, fungi, plants, animals, etc. They have many biological activities already studied as well as many applications. This work aims to purify the lectin from the leaves of *B. virgilioides* (BovLL), and to analyze the lectin-molecule interaction. For this, the extraction was carried out in 0.15 M NaCl (16 h stirring at 25°C); gel filtration chromatography (Sephadex® G75 and G50); hemagglutinating activity (HA); inhibition of HA; SDS-PAGE and fluorescence spectroscopy analysis. BovLL has a SHA of 1,481, the hemagglutinating activity of lectin was altered by azocasein, seroalbumin, ovalbumin, fetuin, casein, glucose, galactose, fructose and mannose. And unmodified by ConA, CrataBL, lactose and N-acetylglucosamine. SDS-PAGE under non-reducing and reducing conditions revealed a single band, 18 kDa and 9 kDa (respectively). In the fluorescence assay, BovLL showed maximum signal intensity at a wavelength of 320 nm (intensity 3.99823 a.u.). And its fluorescence intensity was changed after interaction with azocasein and albumin proteins. Therefore, this study purified the first *B. virgilioides* leaves lectin, and revealed its first molecular interaction data and biological properties.

Keywords: BovLL. Lectins. *B. virgilioides.* Purification. Molecular Interaction.

3.2.1 Introduction

Lectins are proteins with diverse molecular structures that share the ability to recognize and bind specifically and reversibly to carbohydrate structures without changing the carbohydrate moiety (TSANEVA; VAN DAMME, 2020). They are a group of proteins widely distributed in nature, with studies reported in the most different organisms, such as plants (AHSANUL KABIR et al., 2019; PIRES et al., 2019; SILVA et al., 2019a), bacteria (ALYOUSEF; ALQASIM; ALOAHD, 2018; DIGGLE et al., 2006), fungi (SINGH; WALIA; KENNEDY, 2019), virus (LIM et al., 2020), crustaceans (ZHANG et al., 2018b), fish (RUBEENA et al., 2019), mollusks (GOLOTIN et al., 2019; LI et al., 2019), snakes (NUNES et al., 2011), and others.

Lectins have many biological activities already studied, as an antioxidant (WU et al., 2016), antibacterial (SILVA et al., 2019b), antifungal (SILVA et al., 2019a), anti-inflammatory (PIRES et al., 2016), antibiofilm (CARNEIRO et al., 2019), antihemolytic (RODRIGO et al., 2015), antinociceptive (PIRES et al., 2019), hypoglycemic (ROCHA et al., 2013), pro-inflammatory (ARANDA-SOUZA et al., 2019), nematicidal (MEDEIROS et al., 2018), antiproliferative (RAMOS et al., 2019), antiviral (RUBEENA et al., 2019), gastroprotective (LACERDA et al., 2017), immunomodulator (LIMA et al., 2019b), insecticide (RAHIMI et al., 2018), mitogenic (SINGH; WALIA; KENNEDY, 2019), leishmanicidal (ARANDA-SOUZA et al., 2018), antimutagenic (FRASSINETTI et al., 2015), etc.

Other studies addressed different applications of lectins, some examples are: cancer marker (WANG; HANG; HUA, 2019), probes for detecting glycans (MA et al., 2019), hydrogel containing lectin to treat burns (PEREIRA et al., 2019) and for dermal wound healing (SOUSA et al., 2019), coating and encapsulation in liposomes, for greater bioavailability and specificity of the formulation (CORRÊA et al., 2019; WIJETUNGE et al., 2020), heterologous expression, to obtain on a large scale (GAO et al., 2020b; WANG et al., 2020), conjunction with quantum dots, for marking and visualizing different structures (OLIVEIRA et al., 2020c), functionalization of titanium dioxide nanotubes (OLIVEIRA et al., 2018), and lectin-functionalized mesoporous silica nanoparticles (BHAT et al., 2018).

In plants, lectins are widely studied, having isolated lectins and characterized from different parts of plants, such as root (SILVA et al., 2019a), inflorescence (BRITO et al., 2017), leaves (PROCÓPIO et al., 2017), sarcotesta (SILVA et al., 2016a), seeds (PIRES et al., 2019), rhizome (AHSANUL KABIR et al., 2019), bark (COSTA et al., 2018), bast (ARAÚJO et al.,

2012), fruit (NAREDDY; BOBBILI; SWAMY, 2017) and latex (SIRITAPETAWEE et al., 2018), are examples. As will be discussed in the present study and as already mentioned, other lectins have already been isolated from plant leaves, such as: CasuL of *Calliandra surinamensis* (PROCÓPIO et al., 2017), SteLL from *Schinus terebinthifolius* (GOMES et al., 2013), AAA from *Allium altaicum* (UPADHYAY et al., 2011), BmoLL from *Bauhinia monandra* (COELHO; SILVA, 2000), and many others. The advantages of studies with leaves are mainly in the low damage caused in the harvest and in the high bioavailability, because in many plants the leaves occur in great quantity and in practically all the months of the year, as in the case of *Bowdichia virgilioides*.

B. virgilioides is a plant native to Brazil. Its phytogeographic domains are: Amazon, Caatinga, Cerrado, Atlantic Forest and Pantanal. Its most well-known popular name is black sucupira. It belongs to the Fabaceae family, subfamily Faboideae. It is a large tree that can grow in low fertility soils (JUCK et al., 2006; MACHADO et al., 2018; REFLORA, 2019; SMIDERLE; SOUSA, 2003). It has many activities, from different parts of the plant, already studied, such as, for example, antimicrobial (LIMA et al., 2016), anti-inflammatory (BARROS et al., 2010; JULIANE et al., 2016; THOMAZZI et al., 2010), and anxiolytic-like effect (MACHADO et al., 2018; VIEIRA et al., 2013). Also, already isolated molecules, such as lupeol (BESERRA et al., 2018) and cathepsin V inhibitor (SILVA et al., 2019e). Thus, this study proposes the purification of lectin from leaves of *B. virgilioides* and analysis of the interaction with proteins and carbohydrates.

3.2.2 Materials and methods

3.2.2.1 Plant material and extraction

B. virgilioides leaves were collected in the Aldeia-Beberibe Environmental Protection Area (APA Aldeia-Beberibe), km19 of road Aldeia, Camaragibe, Pernambuco, Brazil. Coordinates origin: [*latitude*: -7.904639 *longitude*: -35.062278 WGS84]. A kind of confirmation is deposited in Professor Vasconcelos Sobrinho Herbarium (PEUFR), Voucher number 54010. The leaves were separated and dried at 28°C for 15 days. The dried material was milled and the resulting powder (10 g) suspended in 0.15 M sodium chloride (NaCl, 100 mL) for 16 h at 28°C under constant stirring. The suspension was passed through filter paper and gauze and the filtrate centrifuged (10 min, 3000 x *g*) to yield the extract. It was then dialyzed in distilled water (two 2 h changes) and finally lyophilized.

3.2.2.2 Purification of lectin BovLL

The extract (0.5 mL, 5 mg / mL) was applied in Sephadex® G75 column (35 x 1.1 cm) previously equilibrated with distilled water. After the total application of the sample, the column was eluted with distilled water, NaCl 0.15 M, NaCl 1 M and Glucose 0.5 M. The samples were collected in fractions of 2 mL and the absorbance at 280 nm was measured. Then they were gathered in groups of three, to perform the hemagglutinating activity (HA). The fractions with the highest HA were combined, and concentrated in lyophilizer, and later applied in Sephadex® G50 column (35 x 1.1 cm) (0.5 mL, 5 mg / mL), previously equilibrated with distilled water. After the total application of the sample, the column was eluted with distilled water. Again, the samples were collected in fractions of 2 mL and the absorbance was measured at 280 nm. Then they were gathered in groups of three to perform HA. The fractions with the best HA were combined, which were lyophilized, corresponding to BovLL.

3.2.2.3 Protein concentration assay

Protein determination (LOWRY et al., 1951), was performed using bovine serum albumin (BSA) as the standard (31.25 – 500 µg / mL).

3.2.2.4 Haemagglutinating activity (HA) assay

The haemagglutinating activity assay was performed in 96-well microtiter plates using a suspension of rabbit erythrocytes (2.5% v/v) previously treated with glutaraldehyde (BING; WEYAND; STAVITSKY, 1967). Briefly, the sample (50 µL) was serially two-fold diluted in 0.15 M NaCl in a plate row and then 50 µL of the erythrocyte suspension was added to each well. The number of haemagglutinating activity units (HAU) corresponded to the reciprocal of the highest dilution of the sample that promoted agglutination of erythrocytes. The specific HA was defined as the ratio between the HAU and the protein concentration (mg / mL). Collection of erythrocytes was approved by Ethics Committee on Animal Experimentation of the Universidade Federal de Pernambuco (process 23076.033782/2015-70).

3.2.2.5 Polyacrylamide gel electrophoresis (SDS-PAGE)

The BovLL lectin (50 µg) was submitted to SDS-PAGE at 12% (w/v) under non-reducing and reducing conditions (reducing agent β-mercaptoethanol) (LAEMMLI, 1970). BovLL polypeptide bands and molecular mass markers ((Amersham ECL Rainbow Marker – Full Range (12 000 – 225,000 Da)) were stained in 0.02% (w/v) Comassie Brilliant Blue R-250 in 10% acetic acid (v/v) and 40% methanol (v/v).

3.2.2.6 Evaluation of the effect of proteins and carbohydrates on lectin HA

The HA inhibition assay was used to evaluate the lectin molecule-binding specificity. The assay was similar to the HA assay except for: 1) the use of carbohydrate or protein solution in place of 0.15 M NaCl for serial dilution, 2) twice the concentration of carbohydrate or protein in the second well, and 3) incubation step of 15 min at 28°C was included before the addition of erythrocytes. The effects of proteins (1,000 µg / mL) azocasein, casein, fetuin, bovine seroalbumin, chicken ovalbumin, ConA and CrataBL, as well as the carbohydrates (0.2 M) glucose, fructose, mannose, galactose, lactose and N-acetyl-glucosamine were evaluated.

3.2.2.7 Fluorescence spectroscopy measurements

The fluorescence emission spectra at steady state were performed from titration of molecules (0 – 0.3 µM) in a solution of BovLL at a fixed concentration of 0.3 µM, at temperatures of 298.15, 303.15 and 308.15K. The excitation wavelength was set to 280 nm and the emission spectrum was recorded between 290 nm and 400 nm. The excitation and emission slits' width were 5 nm with a scan rate of 60 $nm.min^{-1}$. The spectra obtained here were corrected concerning the internal filter effect in a method previously described by Menezes et al. (2020).

3.2.2.8 Statistical analysis

The results of the present study were analyzed statistically with the program GraphPad Prism® 7.00. Using ANOVA (One-Way) followed by Tukey test. Values were considered significant when $p < 0.05$. For the tests that did not have to evaluate the relationship between groups, the results were expressed only in Mean ± Standard Deviation (SD).

3.2.3 Results and discussion

3.2.3.1 Extraction and purification

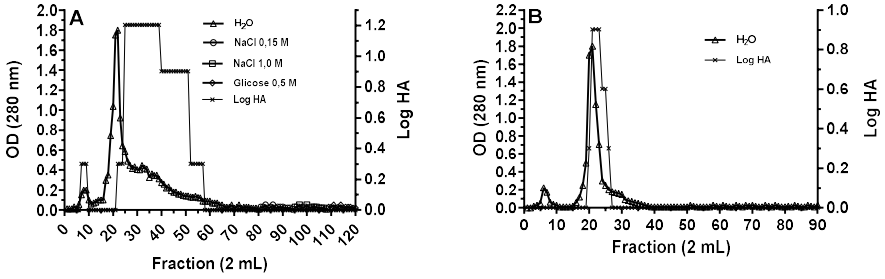


Figure 7. Purification of Bowdichia virgilioides leaf lectin (BovLL). Gel filtration chromatograms, using Sephadex® matrix G75 (A) and G50 (B). Evidencing the elution profile of BovLL, as well as the graph of the Log HA. OD: Optical Density.

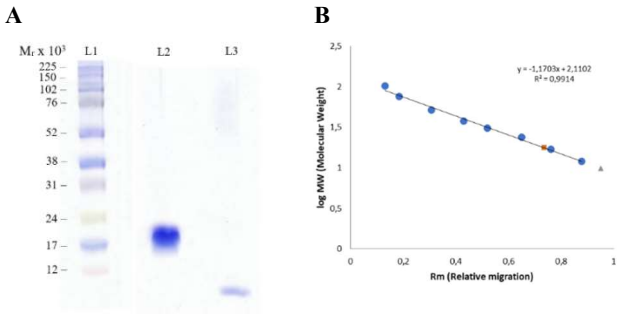


Figure 8. SDS-PAGE of BovLL. A. (L1) Molecular mass marker (12 – 225 kDa). (L2) BovLL. (L3) BovLL + β-mercaptoethanol. B. Molecular mass estimation of the BovLL by SDS–PAGE in 12% gel. The molecular weight size markers (●), BovLL (■) and BovLL + β-mercaptoethanol (▲).

Table 10. Summary of purification of lectin (BovLL) from *B. virgilioides*.

Sample	Protein Concentration	Hemagglutinating Activity (HA)		
		HU	SHA	Purification Factor
Extract	10	2,048	204.8	1.0
G75	0.132	64	484.84	2.37
G50 BovLL	0.086	32	1,481	7.23

Protein Concentration (mg/mL): is the amount of protein in mg contained in each mL of solution. SHA (HU/mg.mL^{-1}): Specific Hemagglutinating Activity, which corresponds to the ratio between HA and Protein Concentration. Purification Factor (fold): indicates how many times the purity has been improved, it is calculated by the ratio between the SHA in the stage and the SHA of the extract. HU: hemagglutinating units.

BovLL was purified in two stages of filtration gel chromatography (Sephadex® G75 and G50) (table 10 and figure 7). It had a SHA of 1,481 HU / mg.mL^{-1} and a Purification Factor of 7.23 indicating an increase in SHA, that is, a ratio of activity to lectin concentration, 7.23 times of BovLL for the initial sample (extract), not chromatographed. Other plant lectins, recently purified by filtration gel, showed the following values of SHA and Purification Factor, respectively: Apul 182.8 HU / mg.mL^{-1} and 11.2 fold (BRITO et al., 2017) and CasuL 1,280 HU / mg.mL^{-1} and 111.8 fold (PROCÓPIO et al., 2018).

The electrophoretic profile (figure 8 A), under non-reducing conditions revealed the presence of a single protein band with a relative mass of 18 kDa. While under reducing conditions, with β-mercaptoethanol (reducing agent of disulfide bonds, which occur between thiol groups of cysteine amino acids), the presence of a single protein band with a relative mass of 9 kDa. These data preliminarily reveal the homogeneity of the sample.

3.2.3.2 Effect of proteins and carbohydrates on BovLL HA

The effect of proteins and carbohydrates was evaluated by inhibiting the hemagglutinating activity of lectin. The hemagglutinating activity of BovLL (512 HU) was reduced by: azocasein (0 HU), seroalbumin (2 HU), ovoalbumin (32 HU), fetuin (64 HU),

casein (64 HU), glucose 256 (HU) and galactose 256 (HU). Stimulated by fructose and mannose (8,192 HU). And it was not altered by ConA, CrataBL, lactose and N-acetylglucosamine. These data suggest that the tested proteins possibly succeeded in destabilizing the interaction of BovLL with erythrocytes or promoted changes in lectin that prevented the binding with erythrocytes, allowing their precipitation. And that the types of carbohydrates and their concentrations tested, were not able to interfere in the stability of the lectin-erythrocyte interaction, not affecting, respectively, the hemagglutinating activity of lectin. Other lectins also showed inhibition with proteins, such as the PeRoL (fetuin, ovalbumin, casein and thyroglobulin) (SILVA et al., 2019a), CasuL (ovalbumin, fetuin and seroalbumin) (PROCÓPIO et al., 2018), MvFL (fetuin and ovalbumin) (PATRIOTA et al., 2017), PgteL (casein and ovalbumin) (SILVA et al., 2016a) and cMoL (azocasein and asialofetuin) (SANTOS et al., 2009).

3.2.3.3 Fluorescence spectroscopy measurements

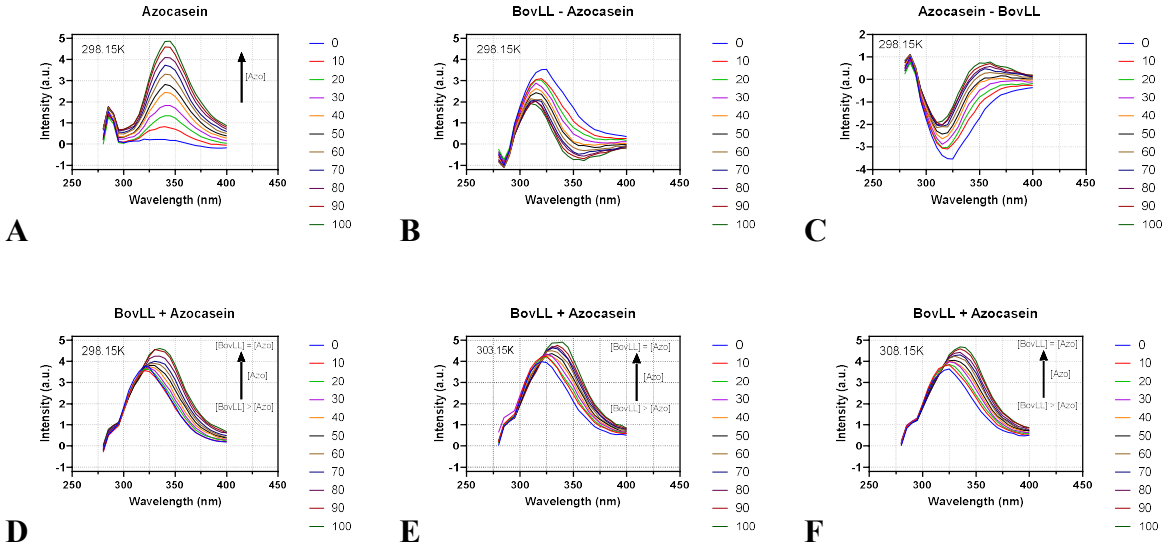
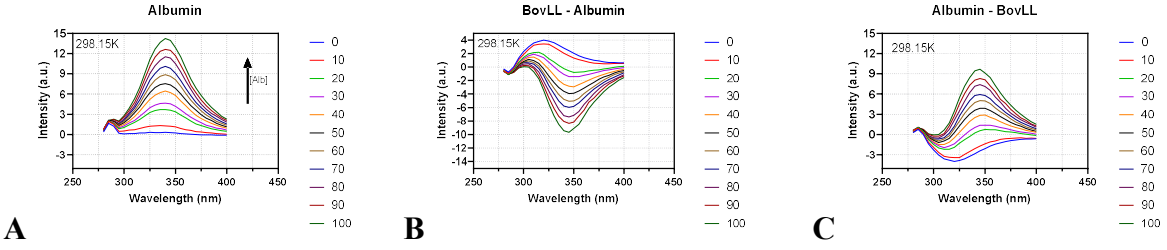


Figure 9. Intrinsic fluorescence spectrum of azocasein 298.15K (A), BovLL – azocasein 298.15K (B), azocasein – BovLL 298.15K (C), BovLL + azocasein 298.15K (D), BovLL + azocasein 303.15K (E), and BovLL + azocasein 308.15K (F). Axis (y) = intrinsic fluorescence intensity and Axis (x) = wavelength. a.u. (arbitrary unit). nm (nanometer).



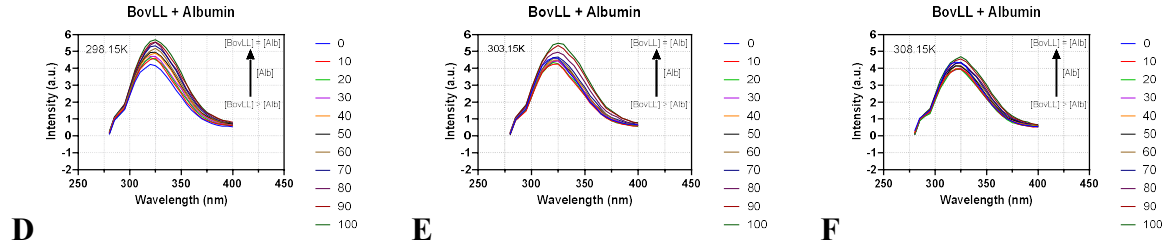


Figure 10. Intrinsic fluorescence spectrum of albumin 298.15K (A), BovLL – albumin 298.15K (B), albumin – BovLL 298.15K (C), BovLL + albumin 298.15K (D), BovLL + albumin 303.15K (E), and BovLL + albumin 308.15K (F). Axis (y) = intrinsic fluorescence intensity and Axis (x) = wavelength. a.u. (arbitrary unit). nm (nanometer).

BovLL showed maximum fluorescence intensity at a wavelength of 320 nm (intensity 3.99823 a.u.), at a concentration of 100 µg / mL at 25ºC. The intrinsic fluorescence of a protein basically corresponds to the aromatic amino acids tryptophan, tyrosine and phenylalanine, through its ability to absorb and emit fluorescence at different wavelengths (excitation (λ_{ex}) and emission (λ_{em}) wavelengths). But usually, the intrinsic fluorescence obtained in the studies, is a result of the excitation and emission of tryptophan (Trp) (which is usually excited at 280nm and emits between 300 and 400 nm), as in the spectra above. Although Trp generally occurs, in a lower concentration, in proteins, when compared to phenylalanine and tyrosine, it stands out, among other reasons, for the greater amount of energy (Q) that it can absorb and emit in a period of time (BRADER, 2020), consequently, the Trp residue is the most common fluorophore used to study protein aggregation, unfolding and dynamics by intrinsic fluorescence (KHAN et al., 2017). The amino acid Trp tends to be found in the hydrophobic nuclei of proteins, so when there is a change in the conformation of the protein by factors such as pH, temperature and ligand, for example, this native spectrum can be changed and measured, revealing important information about the stability of the protein (ZHAO et al., 2019a). In addition, knowing information about the location of these amino acids in the protein, it is possible to infer other more specific information regarding the conformational structure, such as, in which part of the molecule changes are taking place. In general, fluorescence spectroscopy may reflect changes in the tertiary structure of proteins (JIANG et al., 2019).

Other lectins also had their intrinsic fluorescence explored in their studies, such as: MvFL (PATRIOTA et al., 2017), Zihua Snap Bean Lectin (JIANG et al., 2019), Lectin from Black Turtle Bean (ZHAO et al., 2019a), ConA (KHAN et al., 2017) and Dectin-1 (DULAL et al., 2018), as will be seen next. In MvFL, the effect of temperature and pH on its conformation was evaluated. The MvFL samples were submitted to different pH and temperature conditions, and did not show significant changes in the maximum emission wavelength (about 336 nm), proving to be very stable (PATRIOTA et al., 2017).

With Zihua Snap Bean Lectin, the maximum λ_{em} was kept between 328–330 nm, when the heating time was short or the heating temperature was low. The fluorescence intensity increased when the heating time was long or the heating temperature was high, which may be related to thermal polymerization of multimers, according to the authors. And there was a redshift at maximum λ_{em}, representing a change in the microenvironment of the Trp residue (JIANG et al., 2019). Lectin from Black Turtle Bean, at 200 μg / mL, had a maximum λ_{em} at 328 nm, at pH 7.2, and showed structural changes induced by low pH (1.0 - 3.5) as a function of time. The fluorescence intensity was significantly decreased in the range of 0 - 8 h, and from 8 - 24 h there was no change in intensity, indicating that a steady state of the lectin tertiary structure was formed after 8 h. The authors also argue that there was an evident shift towards blue at maximum emission, with a considerable decrease in the fluorescence intensity of the treated lectin in the range 0-8 h, indicating that the amino acids on the protein's surface may gradually move towards non-polar environments, and the decrease in fluorescence intensity can be attributed to the deprotonation of neighboring basic amino acids (ZHAO et al., 2019a).

Other studies have evaluated the effect of the interaction of lectin with other molecules, by intrinsic fluorescence spectroscopy, such as: ConA with the anionic surfactant Gemini and Dectin-1 with the polysaccharide β-glucan Laminarina (DULAL et al., 2018; KHAN et al., 2017). ConA (anionic), when incubated with low concentrations of Gemini (cationic surfactant), the maximum λ_{em} was shifted to a lower wavelength and the fluorescence intensity decreased. As the Gemini concentration increased, the maximum λ_{em} was not changed, but the fluorescence intensity was increased (KHAN et al., 2017). With the lectin Dectin-1 it was revealed that it binds Laminarin cooperatively with a Hill coefficient of ~ 3, it was observed that Laminarin promotes cooperative oligomerization of Dectin-1 (DULAL et al., 2018).

3.2.4 Conclusion

Therefore, this study purified, by gel filtration chromatography, the first lectin from leaves of *B. virgilioides*, BovLL. Which presented mass of 18 kDa (non-reduced) and 9 kDa (reduced), and showed interaction with proteins and carbohydrates. This work, thus reveals a new molecule from native flora with high potential for new applications.

Acknowledgment

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3.3 ARTIGO 3 - NATURAL PRODUCT FROM *BOWDICHIA VIRGILIOIDES* LEAVES: CHEMICAL COMPOSITION AND PHARMACOLOGICAL POTENTIAL

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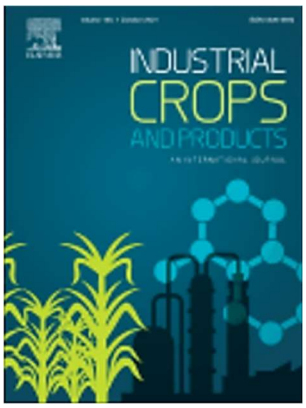

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Abstract

Although different types of products extracted from *Bowdichia virgilioides* are reported, no investigation has been carried out with leaves saline extract. Thus, this work prepared, identified (chemical composition) and evaluated the pharmacological potential of this natural product. The Liquid Chromatography-Mass Spectrometry (LC-MS) results revealed six main compounds. And its pharmacological potential showed different activities, such as, antioxidant by DPPH, ABTS and FRAP. Antibacterial in *Streptococcus pyogenes*, *Staphylococcus aureus,* and *Enterococcus faecalis* (MIC and MBC µg / mL, respectively): 250 and 500; 500 and 1000; and 1000 and 2000. Cytotoxicity in adenocarcinoma (≥ 125 µg / mL) and not in normal lymphocytes (up to 500 µg / mL). Non-hemolytic (up to 2000 µg / mL) and anti-hemolytic (up to 1.95 µg / mL). It showed photoprotection between 2000 and 62.5 µg / mL. Total proteolytic activity of 80% (U / mg). And it was not larvicidal for *Artemia salina* (up to 250 µg / mL) and *Aedes aegypti* (up to 1000 µg / mL). Finally, the content of phenols, proteins, carbohydrates (total and reducing) and hexuronic acid was determined (235.759 mg GAE / g, 10.0 mg / mL, 62.85%, 17.0%, and 19.18% respectively). All results expose the saline extract from *B. virgilioides* leaves as a promising new natural product for different biological applications.

Keywords: Natural product. *B. virgilioides.* Leaves. Saline extract. Chemical composition. Pharmacological potential.

3.3.1 Introduction

Bowdichia virgilioides Kunth belongs to the Fabaceae family, subfamily Faboideae, genus Bowdichia (ARRIAGA; GOMES; BRAZ-FILHO, 2000; REFLORA, 2019; THEPLANTLIST, 2019). It is a large tree, which reaches about 20 meters high and its wood is widely used in construction. Its most common popular name is black sucupira (BARROS et al., 2010; MACHADO et al., 2018; SILVA et al., 2010a; SMIDERLE; SOUSA, 2003). Its flowers are lilac, and its fruits are produced annually between september and december, depending on the region of occurrence. Although it is native to Brazil, its geographical distribution is confirmed in all regions (JUCK et al., 2006; MACHADO et al., 2018; REFLORA, 2019).

It is used in folk medicine as an anti-inflammatory (to combat uterus and vaginal inflammation), in wound healing, for headache, backache, sore throat, diabetes, rheumatism, aneurysm, and various infections (CONCEIÇÃO et al., 2011; GOMES et al., 2008; SARAIVA et al., 2015; SOUZA et al., 2014). Furthermore, *B. virgilioides* is indicated for bioprospecting investigations, according to its relative importance (IR) (determined to indicate species with bioeconomic potential) (SOUZA et al., 2014).

Also, *B. virgilioides* has many pharmacological properties that have already been tested, such as: anti-inflammatory (BARROS et al., 2010; JULIANE et al., 2016; THOMAZZI et al., 2010), anxiolytic (MACHADO et al., 2018; VIEIRA et al., 2013), acaricide (NUMA et al., 2018), antinociceptive (SILVA et al., 2010a; THOMAZZI et al., 2010), healing, hypoglycemic (MACHADO et al., 2018; SOUZA et al., 2009), antimicrobial (AGRA et al., 2013; ALMEIDA et al., 2006), antioxidant (FARIAS et al., 2013), leishmanicide (RIBEIRO et al., 2014b), termiticide (SANTANA et al., 2010), as a larvicidal agent against the *Aedes aegypti* (BEZERRA-SILVA et al., 2015), and good effect on fruit ripening (NASCIMENTO et al., 2019).

Some molecules with biological properties have already been obtained from *B. virgilioide*s, such as Bowdenol (2,3-Dihydrobenzofuran) (MELO et al., 2001), Bowdichine (diaza-adamantane alkaloid) (BARBOSA-FILHO et al., 2004), Lupeol (BESERRA et al., 2018), Odoratin (7-O-b-D-glucopyranoside) (VELOZO et al., 1999a), Sucupiranins M-Q, five new furanocassane-type diterpenoids (ENDO et al., 2019), two isoflavonoids (JUCK et al., 2006), and cathepsin V inhibitor (SILVA et al., 2019e). Nevertheless, the literature does not have reports involving saline extract of the *B. virgilioides* leaves.

Therefore, to provide the first biological data for this extract, this study prepared, identified (chemical composition) and evaluated its pharmacological potential. This was done basically through LC-MS and different biological activities (antioxidant, antibacterial, antifungal, antibiofilm, cytotoxic, hemolytic, anti-hemolytic, photoprotective, larvicidal and proteolytic).

3.3.2 Materials and methods

3.3.2.1 Plant material and extraction

B. virgilioides leaves were collected in the Aldeia-Beberibe Environmental Protection Area (APA Aldeia-Beberibe), km 19 of road Aldeia, Camaragibe, Pernambuco, Brazil. Coordinates origin: [*latitude*: -7.904639 *longitude*: -35.062278 WGS84]. A kind of confirmation is deposited in Professor Vasconcelos Sobrinho Herbarium (PEUFR), Voucher number 54010. The leaves were separated and dried at 28°C for 15 days. Next, the dried material was milled, and the resulting powder (10 g) was suspended in 0.15 M sodium chloride (NaCl, 100 mL) for 16 h at 28°C under constant stirring. Next, the suspension was passed through filter paper, and the filtrate was centrifuged (10 min, 3000 x *g*) to yield the extract. It was then dialyzed against distilled water (two 2h changes) and finally lyophilized.

3.3.2.2 Chemicals

DPPH (1,10-diphenyl-2-20-picrylhydrazyl), ABTS (2,20-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylicacid), Folin-Ciocalteu reagent (sodium 3,4-dioxo-3,4-dihydronaphthalene-1-sulfonate), Gallic acid (3,4,5-trihydroxybenzoic acid) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Solvents for HPLC analyses were HPLC grade. All other chemicals used were used for analytical grade purposes.

3.3.2.3 Analytical RP-HPLC/ESI-MS

The analyses by Reverse Phase-High Performance Liquid Chromatography were performed on a Varian HPLC system, including an autosampler (Varian Pro Star 410, USA), a ternary pump (Varian Pro Star 230I, USA) and a PDA detector (Varian Pro Star 330, USA). A liquid chromatographic ion trap mass spectrometer (Varian 500-MS IT Mass Spectrometer, USA) equipped with an electrospray ion source also was used. 5 μL samples were injected into a Denali C18 column (150 mm × 2.1 mm, 3 μm, Grace, USA). The thermostat temperature was maintained at 30°C. The eluents were as follows: formic acid (0.2%, v / v; A solvent) and acetonitrile (B solvent). The gradient was characterized by using initial, 3% B with a

retention time of 0–5 min, 9% B linear with a retention time of 5–15 min, 16% B linear with a retention time of 15–45 min, 50% B linear. The column was then washed and reconditioned. The elution was monitored at 245, 280, 320, and 550 nm while the flow rate was kept at 0.2 mL / min. A total effluent (0.2 mL / min) was injected into the mass spectrometer source without splitting. All MS experiments were carried out in negative mode $[M-H]^-$. Nitrogen and helium were used as nebulizing and damping gas, respectively. The ion source ran by setting the temperature of 350°C and a spray and capillary voltage of 5.0 V and 90.0 V. For the data recording and processing, the MS Workstation software (Version 6.9) was used. Samples were firstly analyzed in full scan mode acquired in the 50–2000 m/z range (TORRES-LEÓN et al., 2017a).

3.3.2.4 Determination of antioxidant capacity

3.3.2.4.1 DPPH• radical scavenging activity

The reduction of DPPH radical (2,2-Diphenyl-1-picrylhydrazyl) was made following the methodology reported by Blois (1958), with some modifications. A solution o DPPH at 60 μM was prepared, 193 μL of DPPH solution was mixed with 7 μL of sample in each microplate. After 30 min of reaction under dark conditions, absorbance was measured at 517 nm, using a spectrophotometer microplate reader (Epoch, BioTek Instruments, Inc.; Winooski, VT, USA) controlled with the Gen5 Data Analysis software interface. Trolox was dissolved in ethanol and then diluted to the appropriate concentration to establish the standard calibration curve.

3.3.2.4.2 ABTS•$^+$ radical cation-scavenging activity assay

This analysis was performed according to Re et al. (1999), 1 mL of the ethanolic solution of ABTS radical (2′,2-Azino-bis (3-etilbenzotiazolina-6-sulfonate)) was mixed with 10 μL of extract in a quartz cell. A spectrophotometer (Genesys 20, USA) has been applied to read the absorbance at 734 nm. Trolox was diluted to the appropriate concentration for the establishment of the standard calibration curve.

3.3.2.4.3 Ferric reducing antioxidant power (FRAP) assay

FRAP (Ferric reducing the ability of plasma) assay was performed as follows. An aliquot of 10 μL was mixed with 290 μL of FRAP reagent in a 96-well microplate. The reaction mixture was incubated for 15 min (at 37°C). The absorbance was evaluated at 593 nm against a blank of distilled water (Epoch, BioTek Instruments, Inc.; Winooski, VT, USA). Trolox was dissolved in ethanol and then diluted to the appropriate concentration to establish the standard calibration curve (BENZIE; STRAIN, 1996).

3.3.2.5 Antibacterial, antifungal and antibiofilm

3.3.2.5.1 Antibacterial

The bacterial isolates were obtained from the Collection of Microorganisms at UFPEDA of the Federal University of Pernambuco (UFPE): UFPEDA 07 *Streptococcus pyogenes* (+), UFPEDA 02 *Staphylococcus aureus* (+), UFPEDA 09 *Enterococcus faecalis* (+), UFPEDA 224 *Escherichia coli* (-), UFPEDA 396 *Klebsiella pneumoniae* (-), UFPEDA 767 *Proteus mirabilis* (-) and UFPEDA 416 *Pseudomonas aeruginosa* (-). The Minimum Inhibitory Concentration (MIC) determination of the *B. virgilioides* saline extract was performed in triplicate (in the range of 4,000 µg – 125 µg), according to the broth microdilution technique and the Clinical and Laboratory Standards Institute – (CLSI) recommendations. *In vitro* quantitative basic activity was accessed by measuring the optical density (OD) on a microplate reader (FLx800™ Multi-Detection) at 600 nm wavelength. In addition, Minimum Bactericidal Concentration (MBC) of the extract was performed by the Kirby-Bauer method (disk diffusion) (CLSI, 2020).

3.3.2.5.2 Antifungal

The antifungal sensitivity test followed the conditions described in document M38-A2 (CLSI, 2008). The culture medium used was RPMI 1640 (Sigma-Aldrich, USA) with L-glutamine, without sodium bicarbonate (pH 7.0 ± 0.1), and with morpholino propane sulfonic acid (MOPS; 0.165 mol / L; Sigma-Aldrich). The culture medium was sterilized on 0.22 µm membranes (Millipore, Darmstadt, Germany). Four yeast strains (*Candida albicans* 02, 04, 146 and 5879) from the UFPE Antibiotics department were used. The microorganisms were grown on Saboraud Dextrose Agar (SDA) at 35°C for 48 hours. The standard antifungal agent used was Fluconazol (Sigma-Aldrich®) (FCZ), at concentrations from 0.125 to 64 µg / mL, in water.

3.3.2.5.3 Antibiofilm

The antibiofilm test was carried out according to the method described by Trentin et al. (2011), with some adaptations. In microtiter plates, were distributed in each well 80 µL of bacterial suspension, 80 µL of saline extract (concentration of 4 mg, 2 mg, 1 mg and 0.5 mg) and 40 µL of BHI medium (Sigma-Aldrich, Co., USES). After incubation (37ºC / 24 h), or containing two wells, it was removed from the were plates washed three times with sterile saline solution. In a sequence, the were plates submerged in heat (60ºC / 1 h) to fix the added bacteria. Bacteria in biofilm formed with a 0.4% crystal violet solution for 15 min at room temperature. After this period, or ruddy biofilm was solubilized with absolute ethanol P.A. 99.8% (Neon,

São Paulo, BR) and absorbance measured at 570 nm in a microplate reader (FLx800™ Multi-Detection).

3.3.2.6 Cell line culture and cytotoxicity in HT-29 and L929

The HT-29 (human colon adenocarcinoma) and L929 (normal mouse fibroblast) cell lines are obtained from the Cell Culture Laboratory (localized at the Antibiotics Department - Federal University of Pernambuco). In brief, HT-29 and L929 strains were maintained in DMEM medium (HiMedia Laboratories Pvt. Ltd.) and with 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin and streptomycin) supplement. Cultivated in cell culture flasks under the following conditions 37°C, 5% CO_2 and humidified atmosphere. The viability of the cells was evaluated by the trypan blue exclusion method. Only cell samples in which the viability was higher than 98% were used in the experiments.

The cytotoxicity of extract in HT-29 and L929 cells was determined using the MTT assay (MOSMANN, 1983). HT-29 (10^5 cells / mL) and L929 (5×10^3 cells / mL) cells were plated in 96-well plates and incubated for 24 h. The extract dissolved in sterile distilled water was added to the wells at a 2000 dilution at 62.5 μg / mL and incubated for 72 h. Solution of cells only with medium and the solvent of the extract (sterile distilled water) was used as a negative control. After the incubation period, cells were reincubated with MTT (5 mg / mL) for 2 h, after which the supernatant was aspirated and the formazan crystals dissolved in DMSO. Then absorbance was measured by a plate spectrophotometer (ELISA) (Epoch - BioTek Instruments, USA) at a wavelength of 560 nm.

3.3.2.7 Lysis and protection in erythrocytes

3.3.2.7.1 Erythrocyte preparation

The erythrocytes were isolated by centrifugation (1032 x *g*, 10 min at 28°C) and followed by the plasma and buffy coat (leuco-platelet layer) removal, the erythrocytes were washed and centrifugation four times with 0.9% saline (physiological serum) (1032 x *g*, 10 min at 28°C) and then resuspended using the same buffer, ending with a suspension of erythrocytes at 2% (YUAN et al., 2005).

3.3.2.7.2 Hemolytic and antihemolytic assay

In the hemolytic assay each 2 mL Eppendorf tube received 1.1 mL 2% erythrocyte suspension and 0.4 mL extract (0.25 - 2000 μg / mL). Controls were 0.4 mL of 0.9% saline (physiological serum) (negative control) and 0.4 mL of saponin at a final concentration of 0.00125% (to 1.5 mL) (positive control). They were then gently homogenized. After incubation

for 2, 24 and 48 h (36.5°C), the cells were centrifuged (400 x g, 5 min at 28°C), and the supernatant absorbance was measured at 540 nm. For the antihemolytic assay, each 2 mL Eppendorf tube received 1.1 mL 2% erythrocyte suspension and 0.4 mL extract (0.25 - 2000 µg / mL). Controls were 0.4 mL of 0.9% saline (physiological serum) (negative control) and 0.4 mL of vitamin C (2 mg / mL). They were then gently homogenized and incubated for 4 h (36.5°C). Then 0.3 mL of saponin was added to a final concentration of 0.00125% (to 1.8 mL) in all tubes. Subsequently, they were again gently homogenized and incubated for 2 h, 24 h and 48 h (36.5°C). Soon after, the cells were centrifuged (400 x g, 5 min at 28°C), and the supernatant absorbance was measured at 540 nm. According to Oliveira et al. (2012) and Naim et al. (1976), with modifications.

3.3.2.8 Determination of the maximum absorption wavelength and *in vitro* sun protection factor (SPF)

The dried extract was diluted in distilled water (final concentrations: 0.0625, 0.125, 0.250, 0.500, 1000, and 2000 µg / mL) to determine the maximum absorption wavelength (λmax). Subsequently, spectrophotometric scanning was performed at wavelengths between 200 - 400 nm, with intervals of 5 nm. The readings were performed using a 1 cm quartz cell, and distilled water was used as blank (JUNIOR et al., 2013; VIOLANTE et al., 2009). Finally, calculation of SPF was obtained according to the equation developed by Mansur et al. (1986):

$$\mathbf{SPFspectrophotometric} = \mathbf{CF} \times \sum_{\mathbf{290}}^{\mathbf{320}} \mathbf{EE}\ (\boldsymbol{\lambda}) \times \mathbf{I}\ (\boldsymbol{\lambda}) \times \mathbf{Abs}\ (\boldsymbol{\lambda})$$

Where: EE (λ) – erythemal effect spectrum; I (λ) – solar intensity spectrum; Abs (λ) – absorbance of sunscreen product; CF – correction factor (= 10). The values of EE x I are constants. They were determined by Sayre et al. (1979) and are showed in table 11.

3.3.2.9 Protease activity assay

Protease activity was evaluated as described by Azeez et al. (2007), with minor modifications. The sample (100 µL), 0.6% (w / v) azocasein (50 µL) and 0.1% (v / v) Triton X-100 (100 µL) were added to 300 µL of 0.1 M sodium phosphate pH 7.5 and this mixture was incubated at 37°C for 3 h. The reaction was stopped by adding 200 µL - 10% (w / v) trichloroacetic acid (TCA), and the assay was incubated at 4°C for 30 min. Then, centrifugation (9,000 x *g*, 10 min) was performed, and the absorbance of the supernatant at 366 nm was read. Negative controls were carried out and differed from the test assays regarding the moment of TCA addition, which occurred before incubation at 37°C for 3 h, in order to prevent protease

action. One unit (U) of protease activity corresponded to the amount of enzyme able to generate peptides in a concentration enough to increase the absorbance by 0.01 compared to the control. The ratio between the enzyme activity (U) and the quantity of proteins (mg) used in the test was calculated by specific protease activity.

3.3.2.10 Toxicity in Artemia salina and Aedes aegypti larvae

A. salina eggs (25 mg) were hatched in natural seawater at 25 – 30 C. The pH was adjusted to 8.0 – 9.0 using Na_2CO_3 to prevent larval death as a result of decreased pH during incubation (LEWAN; ANDERSSON; MORALES, 1992). The eggs were placed in one of two compartments of a container separated by a boundary plate. The compartment with the eggs was covered to maintain a dark ambiance. The other compartment was illuminated to attract the phototropic newly-hatched larvae through perforations on the boundary plate. After 24 h, the brine shrimps that had moved to the illuminated compartment were collected using a Pasteur pipette and used in the lethality assay. The *B. virgilioides* leaves extract was evaluated for its effect on the survival of *A. salina* larvae according to the procedure described by Meyer et al. (1982). The assay was performed in 15 mL falcon tubes containing groups of 12 – 15 larvae exposed to leaves extract (31.25, 125, 500, and 2000 µg / mL) diluted in natural seawater to a final volume of 5 mL. After 48 h, the survival rate (%) was recorded. In the negative control, larvae were incubated in seawater.

The *A. aegypti* colony used (Rockefeller strain) is maintained at the Laboratório de Ecologia Química of the Universidade Federal de Pernambuco at 27 ± 1°C, 78 ± 2% relative humidity, and 14:10 (light/dark) photoperiod. The larvicidal activity was evaluated using an adaptation of the World Health Organization (1981) method, previously described by Coelho et al. (2009).

3.3.2.11 Contents

3.3.2.11.1 Total polyphenols

The hydrolyzable polyphenols content was evaluated with the Folin–Ciocalteu method (SINGLETON; ROSSI, 1965; VENTURA et al., 2008), the measurements were expressed as milligrams (mg) of gallic acid equivalents (GAE) per gram (g) of dry plant material (mg GAE / g) according to a gallic acid (GA) standard curve.

3.3.2.11.2 Toral proteins

Protein determination was conducted as described by Lowry et al. (1951), using bovine serum albumin (BSA) as the standard (31.25 – 500 µg / mL).

3.3.2.11.3 Total carbohydrates

The total carbohydrate content was evaluated by the phenol-sulfuric acid method, which is based on the carbonization of sugars and their subsequent complexation, based on the principles described by Dubois et al. (1956). It was used to make the standard curve to D-galactose. The readings were performed on a spectrophotometer (Biospectro-SP 22) at 490 nm.

3.3.2.11.4 Reducing sugars

The determination of reducing sugars was performed based on the DNS method (3,5-dinitrosalicylic acid) proposed by Miller (1959). The curve was made using glucose as standard and the readings performed in ELISA microplate reader (Synergy ™ HTX) at 490 nm.

3.3.2.11.5 Hexuronic acid

The content of hexuronic acid was determined according to the method of Dische (1947), where specific sugars are combined with carbazole, determining the characteristic color of the reaction. The lactan glucurone acid was used as standard and the readings carried out in a spectrophotometer (Biospectro-SP 22) at 525 nm.

3.3.2.12 Statistical analysis

The results were examined statistically with the program GraphPad Prism® 7.00. Using ANOVA (One-Way) followed by Tukey test (values were considered significant if $p < 0.05$). For the tests that did not have to evaluate the relationship between groups, the results were expressed only in Mean ± Standard Deviation (SD).

3.3.3 Results and discussion

3.3.3.1 Analytical LC-MS

This study is one of the few where *B. virgilioides* compounds are studied. LC-MS studies are not found in the literature. The results of this work are the basis for future studies. In the present study, 6 molecules were identified in the *B. virgilioides* extract (table 12 and figure 11). Peak 1 was identified as Ethyl digallate. This plant metabolite has been previously reported in *Galla chinensis* (TIAN et al., 2009). Peak 2 and 6 were identified as Procyanidin and Quercetin 3-O-glucuronide, respectively. These molecules belong to the Flavanols and Flavonoid families. These molecules have been previously reported in fruit extracts (ZHU et al., 2019). Peak 3 was identified as Tri (epi) cate-chin, this compound has been previously reported in the guava fruit. Rojas-Garbanzo et al. (2017), reported a molecule a $[M–H]^-$ ion at

m/z 865 built from three (epi) catechin units. Peaks 4 and 5 were identified as Procyanidin trimer C1 and Sinensetin, these molecules were reported from the database of the food research department of the Autonomous University of Coahuila (DIA/UADEC).

Previous phytochemical studies with *B. virgilioides* describe molecules of great chemical interest for their structural novelty and the pharmacological point of view of the various biological activities. As phenols, tannins, flavonoids, xanthones, catechins, pentacyclic triterpenes, free steroid, saponins, and alkaloids present in the bark extract (hydroethanol, hexane, chloroform, ethyl acetate and hydromethanol) (SHAN et al., 2016). 3,6-Dimethoxy-6′′,6′′-dimethylchromene-(7,8,2′′,3)-flavone, 3,5,6-trimethoxyfuran-(7,8,2′′,3")-flavone in roots, geraniol and caryophyllene in fruits (ARRIAGA; GOMES; BRAZ-FILHO, 2000). Chlorogenic acid, caffeic acid, rutin, kaempferol and quercetin in bark ethanolic extract and stem heartwood extract (LEITE et al., 2014). Lupeol, lupenone, β-sitosterol, stigmasterol and β-amyrine, as main triterpenes components along with unidentified fatty acids, present in dichloromethane extract of leaves (TICONA et al., 2006). Phenolic compounds, including flavonoids in hydroethanolic extract (SANTOS et al., 2014). Alkaloids (ormosanine and bowdichine), flavonoids (licoagroside F, liquiritigenin diglucoside, afrormosin malonylglucoside, afrormosin, and licoisoflavone B), xanthone (trihydroxyxanthone), phenylpropanoid (licoagrone), diterpene (sucutinirane C and sucutinirane A), and amide (lotusine B) present in ethanolic extract (NUMA et al., 2018). Finally, isoflavone derivatives were also identified (odoratin, afromosin, wistin, cladrastin, and cladrastin 7-O-β-D-glucoside) in *B. virgilioides* (VELOZO et al., 1999b).

3.3.3.2 Antioxidant capacity determination

The extract was tested in three antioxidant (figure 12) activities widely used for *in vitro* testing: DPPH, ABTS, and FRAP. With significant activity ≥ 62.5 µg / mL (DPPH) (Fig. 12 A) and ≥ 125 µg / mL (ABTS and FRAP). At a concentration of 2000 µg / mL practically 100 % antioxidant activity was achieved in the three tests. It is worth mentioning in this antioxidant activity, the concentration of 250 µg / mL. Because at this concentration, the extract also showed antibacterial, anticancer, antihemolytic, and photoprotective activity, in addition, it was not toxic to fibroblasts, erythrocytes, and *A. salina*, as will be seen in next topics in this article.

In comparison, can be mentioned some other plant products tested, such as: aqueous extract from *Myrciaria floribunda* (West ex Willd.) presented IC_{50} of 350.41 (µg / mL) (DPPH) and 793.44 (µM TE / g) (ABTS) (SANTOS et al., 2020b). Aqueous extract from *Arbutus unedo*

L. presented IC_{50} (µg / mL) of 7.956 ± 0.278 (DPPH) and 156.55 ± 17.40 (FRAP) (MOUALEK et al., 2016). Essential oil from *Hymenaea cangaceira* R.B.Pinto, Mansano & A.M.G.Azevedo with IC_{50} (µg / mL) of 127.51 ± 0.01 (DPPH) and 257.03 ± 0.04 (ABTS) (VERAS et al., 2020). Also, alcoholic extract from *Mentha pulegium* L. 0.88 ± 0.02 (mg AAE / mL) (DPPH), 10.29 ± 0.63 (µmol Fe^{2+} / mL) (FRAP) (ROCHA et al., 2019).

Other plant species also had the antioxidant potential of their tested extract, such as *Falcaria vulgari*s Bernh (GOORANI et al., 2019), *Vernonia amygdalina* Delile (TUNASAMY; SURYADEVARA; ATHIMOOLAM, 2019), *Centaurea cyanus* L. (ESCHER et al., 2018), and *Graptopetalum paraguayense* (N.E.Br.) E.Walther (AI et al., 2017), are some examples.

3.3.3.3 Antibacterial, antifungal and antibiofilm

Antimicrobial substances are considered bacteriostatic agents when the ratio MBC/MIC > 4 and bactericidal agents when the ratio MBC/MIC ≤ 4 (OLIVEIRA et al., 2012). Thus, the extract was a bactericidal agent for the Gram-positive pathogens tested (table 13). Initial results indicate bacterial activity against Gram-positive strains, with no action for Gram-negative strains. This activity should be directly linked to the bacterial coating structure of these groups. Because Gram-positive bacteria have a thick layer of peptidoglycan, and since this extract is very rich in protein, there may be some interaction between these components. However, this type of interaction is not possible with Gram-negative due to their second protective layer (KASHEF; HUANG; HAMBLIN, 2017).

The extract can be designed for dermal applications since the extract was not toxic to fibroblasts at 500 µg / mL (figure 14 B), and at the same time, it was toxic to *S. aureus* at 500 µg / mL and *S. pyogenes* at 250 µg / mL (table 13). It is also possible to think of synergism with other substances for applications against a wider spectrum of microorganisms.

3.3.3.4 Cytotoxicity in HT-29 and L292

The extract exhibited a significant cell viability decrease from 125 up to 2000 µg / mL for human colon epithelial cell adenocarcinoma (figure 14 A). However, for normal fibroblasts of mouse adipose tissue only presented toxicity over 1000 µg / mL (figure 14 B). These results preliminarily demonstrate the selectivity of *B. virgilioides* extract for higher toxicity in tumor cells, which may be linked to the expression of different molecules on the membrane surface of these cells.

Other leaf extracts also showed significant toxicity to human colon adenocarcinoma cells (AI et al., 2017; PINTAĆ et al., 2019; ROCHA et al., 2019). As well as other leaf extracts did not show toxicity to fibroblasts at concentrations up to 500 µg / mL, similar to this study (BOLLA et al., 2019; SADOWSKA et al., 2017).

3.3.3.5 Lysis and protection erythrocyte determination

In the hemolytic assay, no percentage of erythrocyte lysis was verified even at 2000 µg / mL, during 2, 24, and 48 h (figure 15 A). The hemolytic activity of extracts is due to the presence of saponins that have lytic action on the membranes of erythrocytes, altering their permeability and causing the destruction of these cells (SIMÕES et al., 2001). The presence of hemolysins and lytic enzymes may also be linked to extract hemolysis (KONOZY et al., 2002). Therefore, it is suggested that the saline extract of *B. virgilioides* does not present these components in significant amounts to lyse erythrocytes at the concentrations tested. This result is interesting for future *in vivo* applications. Aqueous extract from *Moringa oleifera* Lam. seeds (ARAÚJO et al., 2013) and hydroalcoholic extract from *Buchenavia tetraphylla* (Aubl.) R.A. Howard leaves (OLIVEIRA et al., 2012) also did not show hemolytic activity even at concentrations of 2000 µg / mL, as in this study. In turn, the saline extract of rhizome from *Microgramma vacciniifolia* (Langsd. & Fisch.) Copel. showed hemolytic activity of 1000 µg / mL (SILVA et al., 2020b).

In the anti-hemolytic assay, the extract showed protection against erythrocytes, even at concentrations of 1.95 µg / mL, during 2, 24, and 48 h (figure 15 B). This result demonstrates that the extract interacted with the erythrocyte membrane, so that saponin could not oxidize the membrane lipids and lyse the erythrocytes. Other studies with plant extracts have jointly evaluated anti-hemolytic and antioxidant activity, where correlations between antioxidant activity and erythrocyte lysis protection effect were observed (CHAKRABORTY; SHAH, 2011; ESCHER et al., 2018; LINS et al., 2018; MOUALEK et al., 2016).

3.3.3.6 Maximum absorption wavelength and sun protection factor (SPF) determination

The analysis of the absorption spectrum of the extract in different concentrations (2000 to 62.5 µg / mL), between the electromagnetic wavelengths of 200 to 400 nm, corresponding to the ultraviolet rays A, B, and C (UVA, UVB, and UVC) (figure 16 A), the absorption spectrum showed a maximum peak in 280 nm (UVB) (figure 16 B). Additionally, the *in vitro* sun protection factor of the extract was also evaluated (280 - 320 nm, SPF - UVB), showing the

significant capacity of protection of the extract against solar radiation even at very low concentrations of 62.5 µg / mL, and maximum SPF of 25.24 at 2000 µg / mL (figure 16 C).

These results are very promising, as the extract showed antioxidant activity at the same concentrations. Many cosmetic product formulas use photoprotective components that can fight free radicals. For instance, *Camellia sinensis* (L.) Kuntze leaves extract are presented in the internationally commercialized sunscreen composition (NEOSTRATA, 2019). In addition, many studies have jointly performed antioxidant and photoprotective activities of plant extracts (AQUINO et al., 2002; BAZYLKO; BORZYM; PARZONKO, 2015; BRAVO et al., 2017; NUNES et al., 2018; REIS MANSUR et al., 2016; SILVA et al., 2016b; WRÓBLEWSKA et al., 2019).

3.3.3.7 Proteolytic activity

The evaluation of the total proteolytic activity of *B. virgilioides* saline extract was 80.57% enzyme units by milligram of protein (U / mg). The presence of protease activity reveals the total enzymatic capacity of the extract by breaking the substrate used in the test (azocasein). In these tests, the enzymatic capacity of *B. virgilioides* extract was revealed, opening the possibility, for example, for purification of new enzymes. Other plants, such as *Microgramma vacciniifolia* (Langsd. & Fisch.) Copel., *Crataeva Tapia* L., and *Moringa oleifera* Lam., also demonstrated the presence of enzymatic activities (NUNES et al., 2015; PATRIOTA et al., 2017; ROCHA-FILHO et al., 2015).

3.3.3.8 Toxicity in *A. saline* and *A. aegypti* larvae

To obtain the first toxicity data on living organisms, toxicity was performed on *A. salina* and *A. aegypti*. The *A. salina* assay demonstrated toxicity ≥ 500 µg / mL (figure 17). This result points to the non-toxicity of the extract, according to the WHO, as these concentrations are already very high for *A. salina*, a very sensitive filtering aquatic organism (WHO, 1965, 2012). *Moringa oleifera* Lam. extract showed toxicity very close to the present study (ROCHA-FILHO et al., 2015). In the assay with *A. aegypti* larvae, no significant mortality was observed (even at 1000 µg/mL). Therefore, this extract is not indicated to control this vector at the concentrations tested. However, synergism with other chemical or natural products can be a good alternative. Other plant extracts have already presented positive results for this test, such as the extracts of *Dracaena loureiri* Gagnep and *Moringa oleifera* Lam. (SILVA et al., 2019d; THONGWAT et al., 2017).

3.3.3.9 Content of phenols, proteins, total and reducing carbohydrates and hexuronic acid

The *B. virgilioides* extract showed a total phenol content of 235.759 ± 14.476 (mg GAE / g) at 2,000 µg / mL (figure 18). In comparison, some other plant products can be mentioned, such as: aqueous extract from *Myrciaria floribunda* (West ex Willd.), which presented 62.28 (mg GAE / g) (SANTOS et al., 2020b). The aqueous extract from *Arbutus unedo* L. with 207.84 ± 15.03 (mg GAE / g) (MOUALEK et al., 2016). And the alcoholic extract from *Mentha pulegium* L. 23.55 ± 1.12 (mg GAE / g) (ROCHA et al., 2019). It is also important to mention, that the antioxidant activities of extracts may be related to the presence of phenolic compounds, as many studies reports that phenolic compounds play an important role in human health due to their antioxidant activity (MOUALEK et al., 2016). The graphs in figures 12 and 18 show a clear relationship of direct proportionality between the content of total phenols present in the different concentrations of the extract and the antioxidant activities tested. In addition, the protein content of the extract was 10 mg / mL. And the percentage of carbohydrates (total and reducing) and hexuronic acid was 62.85, 17.0, and 19.18%, respectively.

Figure captions

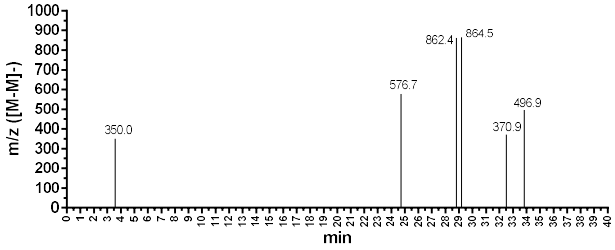

Figure 11. LC-MS spectrum. Showing the retention time and molecular mass of the main compounds identified.

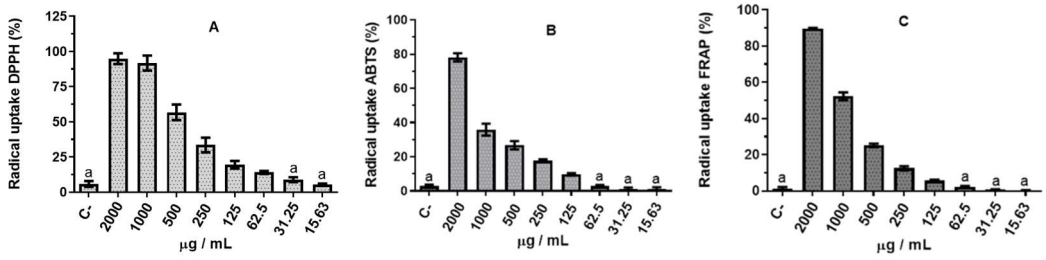

Figure 12. Antioxidant capacity of *B. virgilioides* extract. (A) Uptake of DPPH radical (%). (B) Uptake of ABTS•+ radical (%). (C) FRAP - Reducing power of the ferric ion. DPPH, ABTS, and FRAP in milligrams (mg) of trolox equivalent (TE) per gram (g) of dry plant material (mg TE / g). Mean ± SD (Standard deviation).

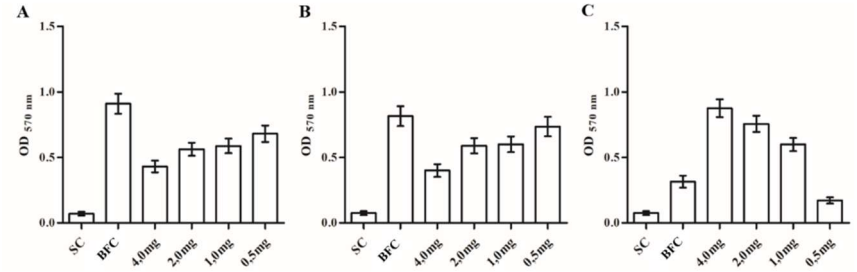

Figure 13. Gram-positive bacteria antibiofilm stained with violet crystal. The values of bacteria in biofilms were expressed as the average optical density (OD) at 570 nm compared to the Biofilm Formation Control (BFC). Sterility control (SC) was used only for assay control. *A: Enterococcus faecalis, B: Staphylococcus aureus, C: Streptococcus pyogenes.*

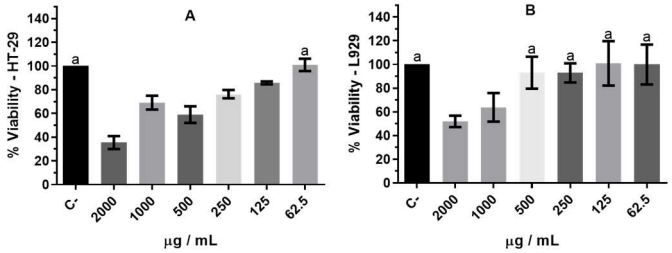

Figure 14. *B. virgilioides* extract cytotoxicity. (A) Percentage of HT-29 cell viability by MTT assay (HT-29 - Human colon epithelial cell adenocarcinoma). (B) Percentage of L292 cell viability by MTT assay (L292 - Normal fibroblast of mouse adipose tissue). $p < 0.05$. Mean ± SD (Standard deviation).

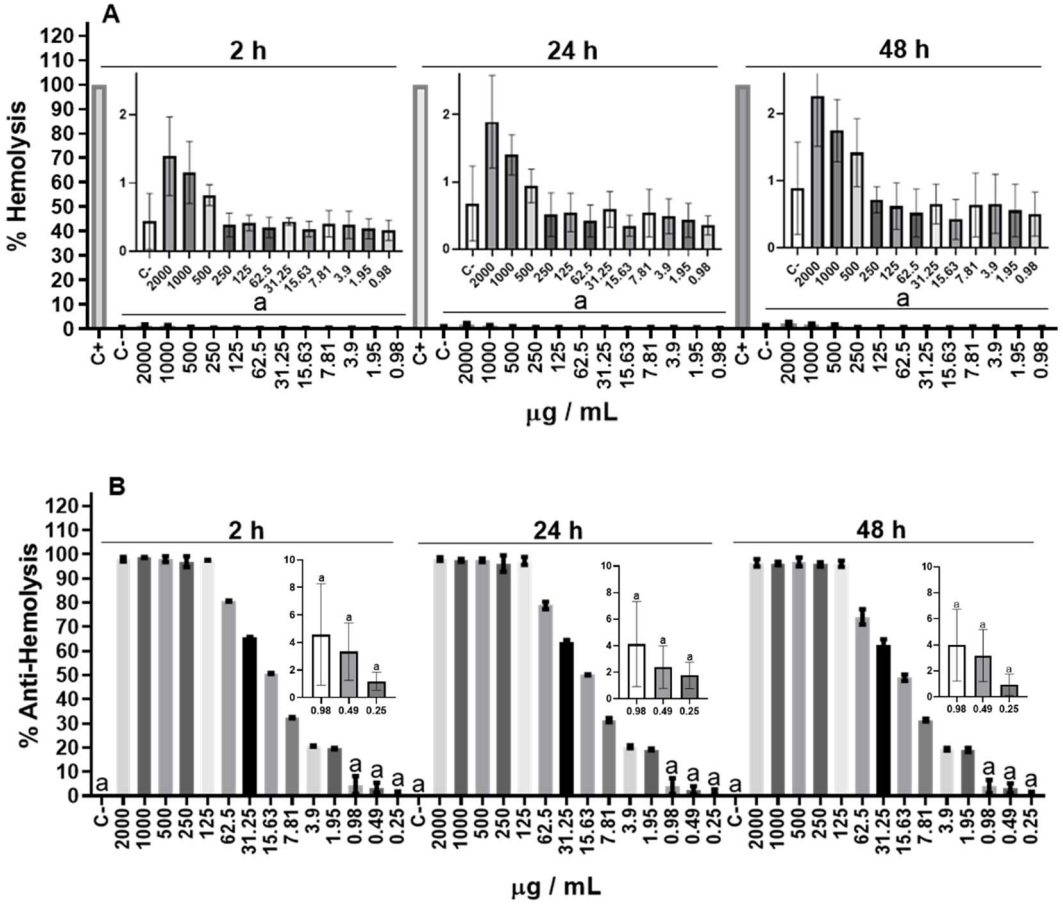

Figure 15. Hemolysis (A) and Anti-hemolysis (B) percentage of *B. virgilioides* extract. $p < 0.05$. Mean ± SD (Standard deviation).

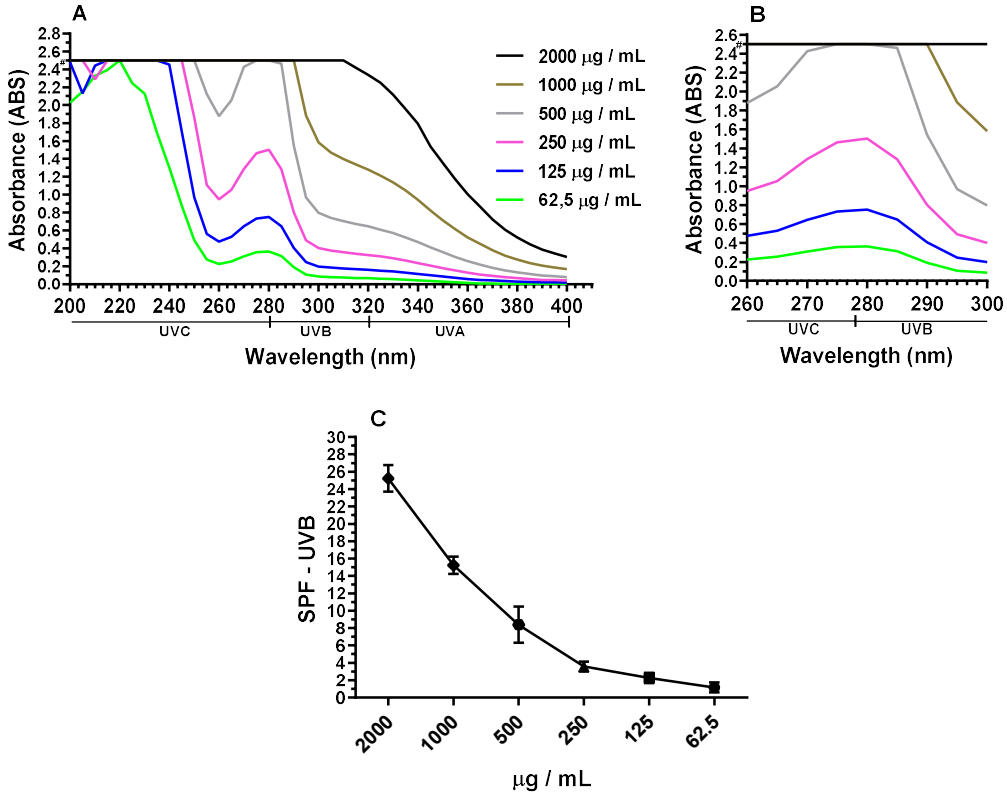

Figure 16. UV absorption spectrum and SPF of *B. virgilioides* extract. (A) The absorption spectrum in the region of ultraviolet (UVA, UVB and UVC). # 2.500 = maximum absorbance measured by equipment used. (B) The maximum absorption wavelength region is magnified in A. (C) *In vitro* Sun Protection Factor between 280 - 320 nm (SPF - UVB). p <0.05. Mean ± SD (Standard deviation).

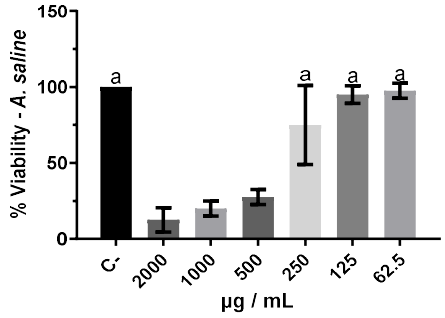

Figure 17. Larvicidal assay of *B. virglioides* extract. Evaluation of toxicity in *A. salina* larvae. p < 0.05. Mean ± SD (Standard deviation).

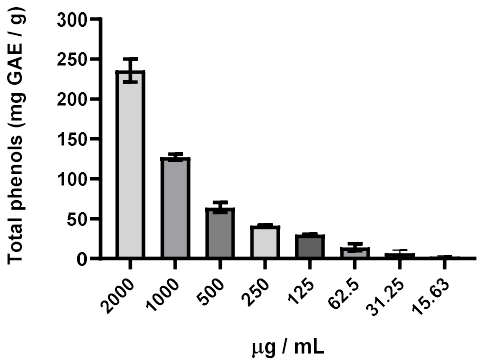

Figure 18. Total phenols of *B. virgilioides* extract. milligrams (mg) of gallic acid equivalents (GAE) per gram (g) of dry plant material (mg GAE / g). Mean ± SD (Standard deviation).

Tables

Table 11. Normalized product function used in the calculation of SPF.

Wavelength (nm)	EE x I (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1.0000

EE – erythemal effect spectrum; I – Solar intensity spectrum.

Table 12. Major compounds identified in *B. virgilioides* by LC-MS.

Peak	Retention time (min)	$[M-H]^-$	Compound name	Family
1	3.57	350	Ethyl digallate	Gallotannin
2	24.73	576.7	Procyanidin	Flavanols
3	28.81	862.4	Tri(epi)cate-chin	Proanthocyanidin
4	29.2	864.5	Procyanidin trimer C1	Proanthocyanidin trimers
5	32.52	370.9	Sinensetin	Methoxyflavones
6	33.82	496.9	Quercetin 3-O-glucuronide	Flavonoid

Table 13. Antibacterial activity of *B. virgilioides* extract.

Species	MIC (µg / mL)	MBC (µg / mL)	MBC/MIC
Streptococcus pyogenes (+)	250	500	2
Staphylococcus aureus (+)	500	1000	2
Enterococcus faecalis (+)	1000	2000	2
Escherichia coli (-)	-	-	-
Klebsiella pneumoniae (-)	-	-	-
Proteus mirabilis (-)	-	-	-
Pseudomonas aeruginosa (-)	-	-	-

Antimicrobial activity of *B. virgilioides* extract against Gram-positive (+) and Gram-negative (-) bacteria. Minimum Inhibitory Concentration (MIC); Minimum Bactericidal Concentration (MBC); No activity (-).

Table 14. Antifungal activity of *B. virgilioides* extract.

Species	MIC (µg / mL)	MFC (µg /mL)	MFC/MIC
Candida. Albicans (02)	1024	-	-
Candida. Albicans (04)	1024	-	-
Candida. Albicans (146)	1024	-	-
Candida. Albicans (5879)	1024	-	-

-: The Minimum Fungicidal Concentration (MFC) of the extract was not performed, as the MIC was not found. MIC of the positive control (Fluconazole): *Candida albicans* 32 µg / mL, *Candida albicans* 64 µg / mL, *Candida albicans* 4 µg / mL and *Candida albicans* 2 µg / mL.

3.3.4 Conclusions

The saline extract of *B. virgilioides* leaves was easily obtained and has a high pharmacological potential, with different possible applications, as activities were detected: antioxidant, antibacterial, cytotoxic, anti-hemolytic, photoprotective and proteolytic. The chemical composition by LC-MS detected six main compounds and their respective masses, and further studies are needed to reinforce the identification, as well as for structural clarification, and isolation. In addition, these are the first results with the saline extract of *B. virgilioides* leaves, which can be guiding for further studies.

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4 CONCLUSÕES

- Foi purificado a primeira lectina de folhas de *B. virgilioides*, denominada BovLL, com AHE de 1481;
- A atividade hemaglutinante da lectina, foi reduzida por azocaseína, seroalbumina, ovalbumina, fetuína, caseína, glicose e galactose. Estimulada por frutose e manose. E não alterada por ConA, CrataBL, lactose e N-acetilglicosamina;
- Eletroforese SDS-PAGE revelou uma única banda de 18 kDa (não redutora) e 9 kDa (redutora);
- BovLL mostrou fluorescência intrínseca, com intensidade de sinal máxima em um comprimento de onda de 320 nm (intensidade 3,99823 a.u.). E sua intensidade de fluorescência foi alterada após interação com proteínas azocaseína e albumina;
- No extrato, foram identificados seis compostos majoritários e suas respectivas massas;
- O extrato apresentou atividade antioxidante significativa a partir de 125 µg / mL, por DPPH, ABTS e FRAP;
- Atividade bacteriana para Gram-positivas, *S. pyogenes*, *S. aureus* e *E. faecalis* (CMI e CMB: 250 e 500; 500 e 1000; e 1000 e 2000 µg / mL, respectivamente) e não para Gram-negativas, *E. coli, K. pneumoniae, P. mirabilis* e *P. aeruginosa;*
- O extrato não apresentou atividade antifúngica para quatro cepas da espécie *Cândida albicans* até 1024 µg / mL;
- Atividade antibiofilme para *Enterococcus faecalis, Staphylococcus aureus* e *Streptococcus pyogenes.*
- Citotoxicidade em células cancerígenas (HT-29) ≥ 125 µg / mL e não citotóxico para células normais (L929) até 500 µg / mL;
- Não apresentou toxicidade em *A. salina* até 250 µg / mL e *A. aegypti* até 1000 µg / mL;
- Não foi hemolítico até 2000 µg / mL e foi anti-hemolítico até 1,95 µg / mL;
- O espectro de absorção revelou pico máximo em 280 nm (UVB) e FPS de 25,24 a 2000 µg / mL;
- Apresentou 80% (U / mg) de atividade proteolítica total;
- E por fim, foi quantificado o conteúdo de proteínas, carboidratos (totais e redutores), ácido hexurônico, e fenóis, respectivamente: 10 mg / mL, 62,85%, 17,0%, 19,18%, e 235,759 mg GAE / g.

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