UNIVERSIDADE FEDERAL DA PARAÍBA
CENTRO DE CIÊNCIAS EXATAS E DA NATUREZA
BACHARELADO EM CIÊNCIAS BIOLÓGICAS

EDUARDO AFONSO DA SILVA PEREIRA

LECTINAS E PEPTÍDEOS DERIVADOS COM POTENCIAL TERAPÊUTICO: AVALIAÇÃO DE RISCO DA LECTINA DE *ABELMOSCHUS ESCULENTUS* E PREDIÇÃO *IN SILICO* DE PEPTÍDEOS INIBIDORES DA ECA-I E DPP-IV

JOÃO PESSOA -PB
2021

EDUARDO AFONSO DA SILVA PEREIRA

LECTINAS E PEPTÍDEOS DERIVADOS COM POTENCIAL TERAPÊUTICO: AVALIAÇÃO DE RISCO DA LECTINA DE *ABELMOSCHUS ESCULENTUS* E PREDIÇÃO *IN SILICO* DE PEPTÍDEOS INIBIDORES DA ECA-I E DPP-IV

Monografia apresentada ao Curso de Ciências Biológicas (Trabalho Acadêmico de conclusão de Curso), como requisito parcial à obtenção do grau de Bacharel em Ciências Biológicas da Universidade Federal da Paraíba.

Orientador: Profº Dr. Davi Felipe Farias

JOÃO PESSOA
2021

Catalogação na publicação
Seção de Catalogação e Classificação

P4361 Pereira, Eduardo Afonso da Silva.
Lectinas e peptideos derivados com potencial terapêutico : avaliação de risco da lectina de abelmoschus esculentus e predição in silico de peptideos inibidores da ECA-I E DPP-IV / Eduardo Afonso da Silva Pereira. - João Pessoa, 2021.
191 f. : il.

Orientação: Davi Felipe Farias.
TCC (Graduação/Bacharelado em Ciências Biológicas) - UFPB/CCEN.

1. Proteínas - Alergenicidade. 2. Diabetes. 3. Hipertensão. 4. Proteínas terapêuticas. 5. In silico. I. Farias, Davi Felipe. II. Título.

UFPB/CCEN CDU 543.645.6(043.2)

Elaborado por Josélia Maria Oliveira da Silva - CRB-15/113

EDUARDO AFONSO DA SILVA PEREIRA

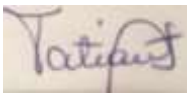
LECTINAS E PEPTÍDEOS DERIVADOS COM POTENCIAL TERAPÊUTICO: AVALIAÇÃO DE RISCO DA LECTINA DE *ABELMOSCHUS ESCULENTUS* E PREDIÇÃO *IN SILICO* DE PEPTÍDEOS INIBIDORES DA ECA-I E DPP-IV

Monografia apresentada ao Curso de Ciências Biológicas (Trabalho Acadêmico de conclusão de Curso), como requisito parcial à obtenção do grau de Bacharel em Ciências Biológicas da Universidade Federal da Paraíba.

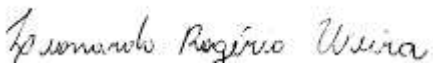
Data: 07 de julho de 2021
Resultado: Aprovado

BANCA EXAMINADORA:

Prof. Dr. Davi Felipe Farias
Universidade Federal da Paraíba (UFPB)
(Orientador)



Prof Drª Tatiane Santi-Gadelha
Universidade Federal da Paraíba (UFPB)
(Examinadora)



Me. Leonardo Rogério Vieira
Universidade Federal do Ceará (UFC)
(Examinador)

Prof. Dr. Augusto Cézar Vasconcelos de Freitas Júnior
Universidade Federal da Paraíba (UFPB)
(Suplente)

AGRADECIMENTOS

À Universidade Federal da Paraíba pelas bolsas de monitoria concedidas.

À Universidade Federal do Ceará pelas parcerias e colaborações feitas durante toda a graduação.

À Profª Dra. Tatiante Santi-Gadelha e à equipe do Laboratório de Bioquímica Genética e Radiológica (BIOGER) pela orientação e colaboração.

À equipe do Laboratório de Avaliação de Risco e Novas Tecnologias (LabRisco) pelos trabalhos e amizade construída.

Gostaria de agradecer, em especial, aos meus familiares e amigos da graduação e aos velhos amigos que me ajudaram durante toda a caminhada até aqui.

RESUMO

As lectinas compreendem uma classe heterogênea de proteínas que apresenta uma grande diversidade de propriedades terapêuticas. Neste contexto, a lectina isolada das sementes do quiabo (*Abelmoschus esculentus*), "*A. esculentus* lectin" (AEL), tem se destacado como um promissor agente terapêutico, tendo em vista suas propriedades anti-inflamatórias, anticâncer e antinociceptivas. Ainda no contexto das proteínas terapêuticas, peptídeos encriptados em sequências de proteínas bioativas têm-se mostrado como ferramentas promissoras para aplicação no tratamento de várias doenças, incluindo diabetes e hipertensão. Sendo assim, lectinas (incluindo a AEL) podem ser investigadas como fontes potenciais de peptídeos inibidores da enzima conversora de angiotensina-I (ECA-I) e da dipeptil-peptidase IV (DPP-IV). O objetivo geral deste estudo foi (*i*) realizar a avaliação de risco da lectina de *A. esculentus*, candidata a várias aplicações farmacológicas, bem como (*ii*) prospectar e caracterizar *in silico* peptídeos inibitórios da ECA-I e DPP-IV presentes em sequências primárias de aminoácidos de dez lectinas vegetais. Na Parte I (avaliação de risco da AEL) foram realizadas: (1) análises de bioinformática para a predição de potenciais efeitos adversos (alergenicidade, efeito anitnutricional e toxicidade) e susceptibilidade à digestão; (2) capacidade inibitória de proteases intestinais e susceptibilidade à digestão e ao tratamento térmico; e (3) teste de toxicidade aguda em embriões de peixe-zebra (*Danio rerio*). Já na Parte II (predição de peptídeos bioativos) foram realizadas as seguintes análises: (1) digestão sequencial *in silico* e predição da bioatividade; (2) predição de toxicidade e parâmetros bioquímicos; e (3) análise de "docking" molecular e interação atômica. Na Parte I, os resultados da avaliação de risco da AEL não mostraram identidade relevante (> 50%) com proteínas alergênicas, tóxicas e/ou antinutricionais. Contudo, a AEL apresentou identidade > 35% com inibidores de proteases alergênicos, utilizando o parâmetro janela de 80 aminoácidos. A AEL apresentou atividade inibitória de tripsina (90%) e quimotripsina (97%). Ainda, a AEL se mostrou resistente à pepsina, embora tenha sido completamente digerida com tripsina. A AEL não causou efeitos adversos aos embriões de peixe-zebra. Dessa forma, AEL mostrou-se uma molécula segura para aplicações terapêuticas. Quanto à Parte II, as lectinas apresentaram um grande número de potenciais peptídeos inibidores de ECA-I e DPP-IV. Nenhum dos peptídeos mais promissores apresentou toxicidade. Todos os peptídeos apresentaram baixa solubilidade. Os resultados do docking revelaram que todos os peptídeos são capazes de se ligar com alta especificidade às enzimas alvo. A energia de ligação entre os peptídeos MF, CF e FFL e ECA-I variou de -671,7 kJ/mol^{-1} a -518,4 kJ/ mol^{-1}, enquanto para os peptídeos WF, MF e GF e DPP-IV a energia de ligação variou de - 697,3 kJ/mol^{-1} a -415,7 kJ/mol^{-1}. Os principais tipos de interação encontrados foram interações hidrofóbicas e ligações de hidrogênio. A falta de interação dos peptídeos inibitórios com o sítio ativo das enzimas sugere uma inibição do tipo não competitivo. Estes peptídeos são candidatos promissores ao desenvolvimento de agentes anti-hipertensivos e antidiabéticos.

Palavras-chave: Alergenicidade, avaliação de segurança de uso, diabetes, hipertensão, predição *in silico*, proteínas terapêuticas.

ABSTRACT

Lectins comprise a heterogeneous class of proteins that have a wide range of therapeutic properties. In this context, the lectin isolated from okra (*Abelmoschus esculentus*) seeds, named "*A. esculentus* lectin" (AEL), has stood out as a promising therapeutic agent in view of its anti-inflammatory, anticancer and antinociceptive properties. Also in the context of therapeutic proteins, peptides encrypted in bioactive protein sequences have been shown to be promising tools for application in the treatment of various diseases, including diabetes and hypertension. Thus, lectins (including AEL) can be investigated as potential sources of inhibitory peptides of the angiotensin-I-converting enzyme (ACE-I) and dipeptidyl peptidase-IV (DPP-IV). The general aim of this study was (i) to carry out the risk assessment of the *A. esculentus* lectin that is a candidate for several pharmacological applications, as well as (ii) to prospect and characterize *in silico* ACE-I and DPP-IV inhibitory peptides present in primary amino acid sequences of ten plant lectins. In the Part I (AEL risk assessment) the following analyses were carried out: (1) bioinformatics analyses for the prediction of potential adverse effects (allergenicity, anitnutritional effect and toxicity) and susceptibility to digestion; (2) inhibitory capacity of intestinal proteases and susceptibility to digestion and heat treatment; and (3) acute toxicity testing on zebrafish (*Danio rerio*) embryos. In the Part II (prediction of bioactive peptides) the following analyses were performed: (1) sequential *in silico* digestion and prediction of bioactivity; (2) prediction of toxicity and biochemical parameters; and (3) molecular docking and atomic interaction analyses. In Part I, the results of the AEL risk assessment did not show relevant identity (> 50%) with allergenic, toxic and/or anti-nutritional proteins. However, AEL showed > 35% identity with allergenic protease inhibitors, using the 80 amino acid window parameter. AEL showed inhibitory activity against trypsin (90%) and chymotrypsin (97%). Furthermore, AEL was resistant to pepsin, although it was completely digested with trypsin. AEL did not cause adverse effects to zebrafish embryos. Thus, AEL proved to be a safe molecule for therapeutic applications. As for Part II, the lectins presented a large number of potential ACE-I and DPP-IV inhibitory peptides. None of the most promising peptides showed toxicity. All peptides had low solubility. The docking results revealed that all peptides are able to bind with high specificity to the target enzymes. The binding energy between peptides MF, CF and FFL and ACE-I ranged from -671.7 kJ/mol^{-1} to -518.4 kJ/mol^{-1}, while for peptides WF, MF and GF and DPP- IV the binding energy ranged from -697.3 kJ/mol^{-1} to -415.7 kJ/mol^{-1}. The main types of interaction found were hydrophobic interactions and hydrogen bonds. The lack of interaction of inhibitory peptides with the active site of the enzymes suggests a non-competitive type of inhibition. These peptides are promising candidates for the development of antihypertensive and antidiabetic agents.

Keywords: Allergenicity, diabetes, hypertension, *in silico* prediction, safe use assessment, therapeutic proteins.

LISTA DE ABREVIAÇÕES

A – Frequência de ocorrência de fragmentos bioativos em uma seqûencia de proteína

a – Número de fragmentos com uma dada atividade

AAL - *Andira anthelmia* lectin (AAL)

AE – Frequência de liberação de fragmentos com uma dada atividade por uma enzima específica

AEL - *Abelmoschus esculentus* lectin

ECA-I - Enzima conversora de angiotensina-I

AOL – "Allergen Online"

BIOPEP – "Database of bioactive peptides"

BP – "Bioactive Peptides"

BLAST – "Basic Local Alignment Search Tool"

BLOSUM – "BLOcks of Amino Acid SUbstitution Matrix"

BmoLL - *Bauhinia monandra* lectin

BtL - *Bryothamnion triquetrum* lectin

CDR – "Carbohydrate Recognition Domain"

CAT - Catalase

CEUA – "Committee on Ethical Use of Animals in Research"

CFAL - *Clitoria fairchildiana* lectin

ConA – ConcanavalinA (*Canavalia ensiformis*)

ConBr – ConcanavalinaBr (*Canavalia ensiformis*)

CRISPR – "Clustered Regularly Interspaced Short Palindromic Repeats"

D – Número de peptideos com a atividade específica liberada por uma enzima específica

Da – Dalton

DHt – Grau teórico de hidrólise

DLasil - *Dioclea lasiocarpa* lectin

DPP-IV – “Dipeptidyl peptidase-4”

Drfl - *Dioclea reflexa* lectin

EFSA - “European Food Safet Authority”

EROS – Espécies Reativas de Oxigênio

EUL - *Euonymus europaeus* lectin

ExPASy – “Expert Protein Analysis System”

FDA – “Food and Drug Administration”

FAO – “Food and Agriculture Organization”

FET – “Fish Embryo Acute Toxicity”

GIP – “Gastric inhibitory polypeptide”

GLP-1 – “Glucagon-like peptide-1”

GLUT-2 – Transportador de Glicose-2

GMO – “Genetically Modified Organism”

GPU - “Graphics Processing Unit”

GPX – Glutationa Peroxidase

GSH - Glutationa

HOSU – “History of Safe Use”

IgE – Imunoglobulina E

Ka – Constante de Afinidade

kDa – kilodaltons

LAL - *Lonchocarpus araripensis* lectin (LAL)

LCL - *Lantana câmara* lectin

N – “Number of aminoacids residues”

NCBI – “National Center for Biotechnology Information”

NO – “Nitric Oxide”

NR – “Non-Redundant”

OECD – “Organisation for Economic Co-operation and Development”

PBL - *Parkia biglobosa* lectin

PDB – “Protein Data Bank”

PGE2 – Prostaglandina 2

PPAR – “Peroxisome Proliferator-Activated Receptor”

QSAR – “Quantitative Structure–Activity Relationship”

RAS – “Renin–Angiotensin System”

RIP - Proteína inativadora de ribossomo

SBA – “Soybean agglutinin”

SDAP – “Structural Database of Allergenic Proteins”

SIF – “Simulated Intestinal Fluid”

SGF – “Simulated Gastric Fluid”

SNS – Sistema Nervoso Simpático

SOD – “Superoxide Dismutase”

SteLL - *Schinus terebinthifolia* lectin

SVM – “Support Vector Machine”

VEGF – “Vascular Endothelial Growth Factor”

W – Número de peptídeos com uma dada atividade liberados por uma enzima específica

WGA – “Wheat germ agglutinin’

WHO – “World Health Organization”

ZET – “Zebrafish Embryotoxicity Test”

LISTA DE FIGURAS

Sumário

1. INTRODUÇÃO

As lectinas compreendem uma classe heterogênea de proteínas que se ligam aos carboidratos e desempenham diferentes atividades biológicas, como por exemplo, melhorar a função renal (JIANDONG et al., 2019), possuem atividade antioxidante (SABITHA et al., 2012), atividade antifúngica (WU et al., 2016a) e efeito antitumoral (DE BRITO MARQUES RAMOS et al., 2019). No entanto, já está bem documentado que essas proteínas podem exercer atividades pró-inflamatórias, oxidativas e citotóxico para células epiteliais do intestino (MIYAKE; TANAKA; MCNEIL, 2007; WANG et al., 2019).

No campo medicinal, a lectina de *Abelmoschus esculentus* (AEL) tem-se destacado como um agente terapêutico promissor. Estudos pioneiros demonstraram que esta lectina apresenta atividade anti-inflamatória e antinociceptiva em camundongos e, de forma semelhante a outras lectinas vegetais, apresenta atividade hemaglutinante (DE SOUSA FERREIRA SOARES et al., 2012).

Em estudos recentes, foi possível verificar a atividade citotóxica seletiva dessa lectina contra uma linhagem de câncer de mama através de um mecanismo pró-apoptótico (MONTE et al., 2014). Ainda em relação ao seu potencial uso farmacológico, a AEL vem ganhando destaque como agente analgésico, antinociceptivo e anti-inflamatório no tratamento de desordens temporomandibulares, em parte devido à interação com receptores opióides e redução de citocinas pró-inflamatórias (ALVES et al., 2018; FREITAS et al., 2016).

No entanto, poucos estudos procuraram investigar possíveis efeitos indesejáveis dessa lectina, embora já tenha sido relatado que o extrato de *A. esculentus* apresentou alergenicidade moderada e atividade inibitória de tripsina (DATTA et al., 2019; MANDA; TADERA; AOYANA, 1992). Assim, é necessária uma análise mais consistente da segurança do uso desta proteína para fins terapêuticos.

Embora as proteínas sejam atraentes do ponto de vista médico, é necessário avaliar a segurança do uso por diferentes abordagens, pois é amplamente relatado que muitos alérgenos, toxinas e antinutrientes são de natureza protéica (DELANEY et al., 2008). As avaliações de risco compreendem estratégias diferentes para investigar potenciais efeitos adversos de uma molécula e garantir seu uso seguro com mínimo impacto negativo.

A segurança do uso de novas proteínas, seja na perspectiva dietética ou terapêutica, têm sido realizada principalmente com base nas diretrizes para avaliação de risco de alergenicidade para OGM (Organismos Geneticamente Modificados) propostas pela EFSA em 2010 (Painel

GMO da EFSA, 2010; VERHOECKX et al., 2016). Portanto, é importante que proteínas com grande potencial farmacológico e que não sejam originárias de OGM também sejam avaliadas quanto à segurança de uso.

Peptídeos bioativos são pequenas moléculas obtidas a partir da hidrólise de uma proteína parental que podem ser obtidas através de diferentes processos enzimáticos (SÁNCHEZ e VÁSQUEZ, 2017). Em meio a uma grande diversidade de peptídeos e diferentes propriedades exercidas por estes, muitos desses peptídeos têm se destacado na literatura devido ao seu potencial como agente terapêutico (MAESTRI et al., 2016).

Em razão da sua grande diversidade, as lectinas tem ganhado espaço na descoberta de novas agentes terapêuticos fato este que se relaciona com o seu amplo espectro de atividades farmacológicas (LARGADA-DIAZ et al. 2017).

Nesse cenário de atividades farmacológicas, os peptídeos anti-hipertensivos e anti-diabéticos merecem um destaque especial, devido a sua frequente presença em sequências peptídicas (BHAGYAWANT et al. 2019). O principai alvo molecular das doenças citadas como forma de tratamento é a inibição das enzimas ECA-I (enzima conversora de angiotensina-I) e DDP-IV (dipeptidil peptidase-IV), respectivamente (WU et al., 2009; OPARIL et al., 2018). Embora distintas do ponto de vista fisiológico e celular, ambas as enzimas compartilham uma inibição por pequenas sequências hidrofóbicas e de baixo peso molecular. Em muitos casos, a relação enzima-inibidor ocorre por interações não-covalentes (NONGONIERMA et al., 2014).

Os peptídeos bioativos apresentam resultados com grande potencial farmacológico, porém estudos sobre a segurança de uso desses peptídeos ainda continuam escassos (LIU et al., 2020). Durante sua produção por hidrólise, sequências tóxicas e/ou alergênicas podem surgir da sequência parental, resultando em efeitos colaterais não desejados (RUTHERFURD-MARKWICK, Kay J., 2012). Embora estudos *in silico* tenham aberto um caminho na investigação da segurança de uso, estudos *in vivo* precisam ser realizados para analisar os riscos associados ao seu uso e confirmar ou não os estudos *in vitro* (LIU et al., 2020).

Dado o exposto, o objetivo geral deste estudo foi (*i*) realizar a avaliação de risco da lectina de *A. esculentus* (AEL) que é candidata a várias aplicações farmacológicas, bem como (*ii*) prospectar e caracterizar *in silico* peptídeos inibitórios da ECA-I e DPP-IV nas sequências primárias de aminoácidos de dez lectinas vegetais. A fim de facilitar a compreensão da fundamentação teórica, esta será apresentada em três partes: Parte I – Introdução ao estudo das lectinas, Parte II - Avaliação de risco da lectina de *A. esculentus* e Parte II – Predição de peptídeos inibidores da ECA-I e DPP-IV em sequências de lectinas vegetais.

2. FUNDAMENTAÇÃO TEÓRICA

2.1 Parte I – Introdução ao estudo das lectinas

2.1.1 Histórico e classificação das lectinas

As lectinas compreendem um grupo de moléculas de origem não imune que se liga de forma específica e reversível a grupos de açúcares de outras moléculas e através dessa interação apresentam suas diferentes atividades biológicas (PEUMANS; VAN DAMME, 1995). Inicialmente essas proteínas foram denominadas como aglutininas devido a sua capacidade de aglutinar eritrócitos presentes no sangue humanos. Outros sinônimos para, as lectinas são hemaglutininas ou fitohemaglutininas (devido a sua presença em extratos vegetais) (SHARON; LIS, 2004).

O termo lectina seja amplamente empregado para proteínas de ligação a carboidratos, o termo hemaglutinina seria mais apropriado pois define a capacidade de aglutinar eritrócitos, apesar das lectinas aglutinarem outros grupos de células (VAN DAMME et al., 1998). No final do século XX, o termo lectina passou a ser empregado apenas para proteínas que possuem pelo menos um domínio não catalítico que se ligue reversivelmente a mono- e oligossacarídeos específicos (PEUMANS; VAN DAMME, 1995). Os estudos sobre esse grupo de proteínas, campo de estudo mais tarde denominado lectinologia, teve seu início com os experimentos de Silas Weir Mitchell em 1860, ao estudar a ação do veneno da *Crotalus durissus*, ele percebeu que o sangue se aglutinava rapidamente na presença do veneno (MITCHELL, 2011).

No entanto, as primeiras proteínas deste grupo, foram identificadas apenas no ano de 1888 pelo pesquisador Peter Hermann Stillmark, quando tentava isolar das sementes de *Ricinus communis* (conhecida como mamona) a lectina posteriormente chamada de ricina. Nos anos seguintes outras lectinas começaram a ser identificadas como a abrina de *Abrus precatorius* (SHARON; LIS, 2004).

A primeira cristalização de uma lectina foi liderada por James B. Sumner em 1938, que purificou a lectina concanavalina A de *Canavalia ensiformis* e demonstrou pela primeira vez a especificidade a açúcares das lectinas, tendo observado a inibição dessa lectina pela sacarose (SUMNER; GRALÉN; ERIKSSON-QUENSEL, 1938).

Outro passo importante na caracterização das lectinas, foi sua capacidade de aglutinar células do sistema sanguíneo (Sistema ABO), foi demonstrado que algumas dessas proteínas

possuem a habilidade de aglutinar tipos sanguíneos específicos, por exemplo, as lectinas de *Phaseolus limensis* e *Vicia cracca* são capazes de aglutinar células do tipo A mas não do tipo B ou O (SHARON; LIS, 2004). Diversos estudos foram realizados para tentar caracterizar lectinas de várias espécies de plantas, animais e fungos nos seus aspectos funcionais e estruturais (DE HOFF; BRILL; HIRSCH, 2009; DE FREITAS PIRES et al., 2019).

Nessa perspectiva, seguindo os avanços na Biologia Molecular, a busca pela estrutura tridimensional das proteínas levou o pesquisador Edelman em 1972 a identificar não apenas a sequência de aminoácidos primária da Concavalina A, mas junto com outros grupos de pesquisa conseguiram identificar sua estrutura tridimensional através de cristalografia por raio-X de alta resolução (EDELMAN et al., 1972; HARDMAN; AINSWORTH, 1972).

A capacidade de ligação à carboidratos das lectinas está intimamente relacionada ao seu enovelamento (NAGAE e YAMAGUCHI, 2019). Hoje, sabe-se que existe uma grande disponibilidade de informações sobre a biologia estrutural de diversas proteínas distribuídas em diversos grupos taxonômicos (VASTA e FENG, 2020). A compreensão das possíveis estruturas adotadas pelas lectinas nos ajudou a compreender sobre suas origens evolutivas e suas funções (VAN HOLLE; VAN DAMME, 2019). Muitas das classificações que foram adotadas na lectinologia usam como base características estruturais de sequências específicas nessas proteínas que se conservaram ao longo do tempo (SHARON; LIS, 2004).

A maioria das lectinas possuem um enovelamento comum durante seu processamento chamado de enovelamento de lectina de leguminosa. Esse padrão de enovelamento foi inicialmente observado na concanavalin (ConA). Tal descoberta deve-se aos estudos feitos por Edelman e colaboradores (EDELMAN et al., 1972). Um padrão comum de ser encontrado nas lectinas é o chamado C-lectina, embora seja mais comum de ser encontrado em animais (CHANDRA et al., 2001).

A capacidade de ligação das lectinas é conferida pelo domínio de reconhecimento de carboidratos (CDR). Esse domínio habilita essas proteínas às suas diferentes atividades biológicas, como defesa, infecções, regulação do metabolismo e vários outros processos fisiológicos (SHARON; LIS, 2004). Com base no tamanho da sequência que determina o CDR nas lectinas, pode-se dividir esse domínio em dois grupos. O primeiro compreende as lectinas que apresentam um CDR entre 120-150 resíduos formando apenas uma unidade única ou globular, como as lectinas de legumes, de moraceae, galectina, lectinas tipo-C e hemaglutinina do vírus influenza. O segundo grupo contém mais de um CDR por unidade única e apresentam

CDR entre 40 e 50 resíduos de aminoácidos de comprimento como as lectinas de bulbos e cereais (VIJAYAN e CHANDRA, 1999).

As lectinas podem se ligar a uma diversidade de carboidratos na forma de monossacarídeos ou polissacarídeos como glicose, galactose, manose, *N*-acetil-glicosamina, *N*-acetil-galactosamina, *L*-fucose entre outros. A especificidade gerada na síntese das lectinas parece ser diferente para diversos grupos de lectinas, levando a formação de diferentes CDR. A oligomerização parece ser um fator determinante na formação dessa especificidade e consequentemente na sua atividade biológica. Por exemplo, a lectina do alho apresenta-se como um dímero e não possui atividade anti-retroviral, embora lectinas com enovelamento similar não dímerícos são anti-retrovirais. Outro exemplo é a jacalina, que precisa passar por modificações pós-tranducionais para manter sua especificidade à galactose (SANKARANARAYANAN et al., 1996).

Inicialmente, as lectinas foram classificadas em 3 grupos com base no número de CDRs: merolectinas, hololectinas e quimerolectinas. Merolectinas são proteínas que possuem um domínio de ligação a carboidrato e não possuem atividade hemaglutinante. As hololectinas têm pelo menos 2 domínios de ligação a carboidratos e a maioria das lectinas estão classificadas neste grupo. Quimerolectinas têm um domínio de ligação a carboidratos e um domínio de atividade catalítica (PEUMANS; VAN DAMME, 1995). As superlectinas que possuem pelo menos dois domínios de ligação a açúcar, mas reconhecem açúcares diferentes foram posteriormente adicionados a essa classificação (VAN DAMME et al., 1998).

Classificações mais antigas costumavam dividir as lectinas de acordo com a especificidade à grupos específicos de açúcares. Embora pouco interessantes do ponto de vista evolutivo, constituíram uma importante fase do estudo das lectinas acerca da função biológica (VAN DAMME et al., 1998). Contudo, o avanço da Bioquímica e da Biologia Molecular permitiu grandes descobertas no campo da biologia estrutural e parte desse conhecimento vêm sendo utilizado para sistematizar melhores classificações para as lectinas baseado em regiões evolutivamente conservadas entre elas.

Com base nisso, foi possível estabelecer uma nova classificação para esse grupo com base em estruturas evolutivamente e estruturalmente relacionadas dos domínios de ligação à carboidrato. Estão inseridas nessa classificação: lectina de leguminosa, lectina ligadora de manose em monocotiledônea, lectina ligadora de quitina com domínio heveína, proteína inativadora de ribossomo (RIP) do tipo 2, Jacalina, lectina da família Amarantina e lectina do floema de Cucurbitaceae (VAN DAMME et al., 1998).

Estas classes são apresentadas a seguir:

- As lectinas de leguminosas são encontradas exclusivamente nas leguminosas, possuem um CDR contendo 25-30 kDa e sítio de ligação para cátions divalentes (Ca^{2+} e Mn^{2+}) (LAGARDA-DIAZ; GUZMAN-PARTIDA; VAZQUEZ-MORENO, 2017).
- As lectinas ligadoras de manose de monocotiledôneas, se ligam especificamente a manose e foram encontradas apenas nas famílias Alliaceae, Amaryllidaceae, Araceae, Bromeliaceae, Liliaceae e Orchidaceae (BARRE et al., 1996). Os protômeros que formam esse grupo podem ser divididos em dois: com apenas um domínio de 11 a 14 kDa ou com dois domínios de cerca de 30 kDa (VAN DAMME et al., 1998).
- As lectina ligadora de quitina com domínio heveína são caracterizadas por um CDR com especificidade para GlcNAc (N-acetil-glicosamina), o monômero que forma a quitina (ITAKURA et al., 2017). Existem lectinas ligadoras de quitina que não apresentam o domínio heveína. Heveína consiste em uma sequência de aproximadamente 43 aminoácidos encontrada em *Hevea brasiliensis* (VAN DAMME et al., 1998). Exemplos desta lectina podem ser encontradas nas famílias Gramineae, Solanaceae, Phytolaccaceae, Urticaceae, Veraceae e Viscaceae.
- As proteínas inativadoras de ribossomos (RIP) do tipo 2 são conhecidas por inativar através da catálise os ribossomos dos eucariotos, levando a uma deficiência na síntese proteica, o que caracteriza a sua potencial toxicidade. A ação sobre os ribossomos ocorre devido a presença da atividade N-glicosidase presente na cadeia A dessa proteína. A cadeia B possui a atividade de ligação a carboidrato, é encontrada nas famílias Euphorbiaceae, Leguminoseae, Viscaceae, Passifloraceae, Ranunculaceae, Lauraceae, Sambucaceae, Iridaceae e Cucurbitaceae (VAN DAMME et al., 1998).
- Lectinas relacionados à Jacalina são proteínas que apresentam um domínio relacionado a jacalina (lectina purificada de *Artocarpus heterophyllus*). Ocorre principalmente em plantas monocotiledôneas. Acredita-se que suas atividades estão relacionadas a resistência ao estresse biótico e abiótico, ferimentos e doenças causadas por insetos (SONG et al., 2014). Essa classificação possui duas subfamílias importantes, as lectinas da Moraceae e as lectinas de Convolvulaceae (VAN DAMME et al., 1998).
- As lectinas da família Amarantina são proteínas que apresentam especificidade para o carboidrato *N*-acetil-D-galactosamina. Amarantina é uma lectina purificada das

sementes de *Amaranthus caudatus*, da família Amaranthaceae, está relacionada com defesa a predadores nas plantas (VAN DAMME et al., 1998).

- As lectinas do floema Cucurbitaceae apresentam um sítio de ligação à quitina, *N*-acetilglicosamina especificamente, e não apresentam domínios heveína. São encontradas principalmente no floema das plantas da família Cucurbitaceae. Essas lectinas possuem subunidades não glicosiladas (VAN DAMME et al., 1998).
- Outras lectinas não entraram em nenhuma das categorias acima por falta de similaridade estrutural. Contudo, sabe-se que existem as lectinas purificadas de *Apiaceae, Araucariaceae, Celastraceae, Cucurbitaceae, Euphorbiaceae, Gramineae e Labiatae* (VAN DAMME et al., 1998).

No ano de 2008 foi proposta uma nova classificação para as lectinas vegetais com base nos domínios de ligação à carboidratos. Muitos anos se passaram desde a última tentativa de organizar as lectinas com base em estruturas evolutivamente conservadas. O avanço da pesquisa sobre lectinas levou à descoberta de 12 domínios diferentes de ligação a carboidratos, sendo a classificação atualmente mais utilizada para lectinas vegetais (VAN DAMME, 2014; VAN DAMME; LANNOO; PEUMANS, 2008). Estão inseridas nessa classificação: Aglutinina homóloga à *Agaricus bisporus,* Amarantina, homólogos da quitinase classe V com atividade da lectina, Família de cianovirina, Família de aglutinina de *Euonymus europaeus*, Família de aglutinina de *Galanthus nivalis*, Proteínas com domínios heveína, Jacalina, Proteínas com domínios de lectina de leguminosas, Domínio Lys/M, Família de aglutinina de *Nicotiana tabacum* (anteriormente lectinas do floema de *Cucurbitaceae*) e Família Ricina-B (VAN DAMME; LANNOO; PEUMANS, 2008).

Levando em consideração as atualizações mais recentes nos estudos das lectinas, algumas modificações podem ser observadas desde a última classificação por Van Damme em 2008. As pequenas modificações ocorridas são principalmente em relação a nomenclatura das famílias existentes e a exclusão da categoria Homólogos da quitinase classe V com atividade da lectina. A nova classificação inclui portanto apenas 11 famílias de lectinas: Aglutinina de *A. bisporus*, Amarantina, Cianovirina, Lectina relacionada à *Euonymus* (EUL), Lectina de *G. nivalis* (GNA), Hevein, Jacalina, Lectina de leguminosa, LysM: motivo de lisina, Aglutinina de *N. tabacum* (Nictaba) e Ricina-B (VAN HOLLE; VAN DAMME, 2019).

2.1.2 Atividades biológicas das lectinas de interesse terapêutico

O papel que as lectinas exercem nas plantas ainda é pouco compreendido. No entanto, diversos estudos vêm demonstrando que sua principal função nas plantas é auxiliar na defesa contra patógenos. Seu potencial de se ligar a carboidratos da superfície dos patógenos e inativá-los faz desse grupo de proteínas uma importante estratégia evolutiva de sobrevivência contra fitopatógenos (PEUMANS; VAN DAMME, 1995). Outro possível papel importante das lectinas, é que sua ligação a carboidratos de bactérias como as do gênero *Rhizobium* pode conferir a planta uma melhor adaptação ao processo de simbiose e oferta de nutrientes em leguminosas a partir da associação com as micorrizas (DE HOFF; BRILL; HIRSCH, 2009). Seu papel na defesa contra predadores ganha força ao reconhecer que a maior concentração de lectinas encontram-se normalmente em parte da planta que costumam ser mais susceptíveis à predação, embora as lectinas possam ser encontradas em praticamente todas as partes das plantas (PEUMANS; VAN DAMME, 1995).

As lectinas vegetais são conhecidas por exercerem um grande número de atividades biológicas nos organismos. Desde o século passado, várias lectinas foram purificadas de diversas espécies e o seu potencial farmacológico e medicinal vem se tornando cada vez mais relevante na área médica. Um grande número de atividades biológicas das lectinas vêm sendo descrito na literatura tais como atividades antioxidante, antimicrobiana, antifúngica, antiviral, anti-inflamatória, antinociceptiva e anticâncer (LAGARDA-DIAZ; GUZMAN-PARTIDA; VAZQUEZ-MORENO, 2017). As atividades anti-inflamatória, antinociceptiva e anticâncer e os efeitos tóxicos das lectinas serão descritos em maiores detalhes a seguir.

2.1.2.1 Atividade anti-inflamatória

As lectinas vegetais com propriedades anti-inflamatórias são amplamente descritas na literatura e vários são os mecanismos pela qual elas exercem essa atividade (BEZERRA, et al., 2014; ABREU et al., 2016). A lectina de *Lonchocarpus araripensis* (LAL), uma lectina não tóxica com especificidade para *N*-acetil-glicosamina mostrou ação anti-inflamatória, sendo capaz de inibir a formação de edemas e aumento da permeabilidade vascular, inibição da migração de neutrófilos e redução de mediadores inflamatórios tais como PGE2, óxido nítrico e TNF-α (PIRES et al., 2016).

Resultados similares foram encontrados para a lectina ligadora de manose (LCaL) purificada de *L. campestris.* A lectina demonstrou atividade antiedematogênica além de inibir o aumento da permeabilidade vascular e a migração leucocitária (DE FREITAS PIRES et al., 2019).

Outras lectinas como a BtL purificada de *Bryothamnion triquetrum* também apresentam atividade anti-inflamatória e antiedematogênica. Em estudos com camundongos, BtL se mostrou eficiente em reduzir o edema de pata induzida por carragenina, diminuindo a migração leucocitária e de neutrófilos além de reduzir os níveis de TNF-α, interleucina IL-1β e mieloperoxidase (FONTENELLE et al., 2018).

A lectina de *Schinus terebinthifolia* (SteLL), que é conhecida por sua atividade anticâncer e anti-microbiana vêm ganhando espaço como um promissor agente terapêutico no tratamento de inflamções. Não foi identificada toxicidade à esplenócitos de camundongos, apresentou atividade imunomoduladora auxiliando na expressão de citocinas pró-inflamatórias tais como (IL-17A, TNF-α, IFN-γ and IL-2) e aumento da citocina anti-inflamatória IL-4. Os níveis de óxido nítrico também reduziram, no entanto, parâmetros como Ca^{2+} citosólico e espécies reativas de oxigênios não mostraram alteração significativa (DOS SANTOS et al., 2020). Em modelos de peritonite, a lectina de *Parkia biglobosa* (PBL) apresentou um potente efeito anti-inflamatório evidenciado principalmente pela redução leucocitária nos sítios de inflamação e redução de citocinas pró-inflamatórias (SILVA et al., 2013).

Contudo, é válido ressaltar que alguns efeitos adversos podem ser percebidos após a exposição de algumas lectinas. Foi demonstrado por Pinto-Junior e colaboradores que a lectina Drfl purificada das sementes de *Dioclea reflexa* apesar de apresentar baixa toxicidade em exposição à náuplios de *Artemia* sp., possui efeito edematogênico significativo em camundongos (PINTO-JUNIOR et al., 2016). No entanto, cerca de 20% da sua atividade inflamatória foi reduzida quando a lectina foi conjugada com seu carboidrato de ligação. Efeitos edematogênicos induzidos por lectinas foram verificados com a ConV, uma lectina isolada das sementes de *C. virosa*, seu efeito foi sido inibido pela conjugação com glicose e representa um efeito de curta duração quando comparado com o efeito edematogênico da carragenina (OSTERNE et al., 2017).

2.1.2.2 Atividade antinociceptiva

O perfil inflamatório tem como uma de suas principais características a presença de dor, seja ela localizada ou sistêmica. No mecanismo de ação de medicamentos antinociceptivos, utilizados para minimizar a percepção da dor, é comum que ocorra interação do ligante à receptores opióides tais como delta, kappa e mi para a promoção de efeitos como a analgesia e termorregulação (MARTINS et al., 2012). A antinocicepção tem se tornado um alvo importante na caracterização de novas lectinas e seus resultados mostram um grande potencial

farmacológico para o tratamento de dores presentes em diversas doenças e condições metabólicas (ABREU et al., 2016; CAMPOS et al., 2016).

É descrito na literatura que existe uma grande diversidade de lectinas com atividade antinociceptiva e muitos dos seus mecanismos de ação já foram decifrados. Camundongos induzidos por ácido acético e formalina mostraram um resultado significativo para os parâmetros contorções e número de lambidas, respectivamente, quando foram previamente tratados com a lectina de *Clitoria fairchildiana* (CFAL) (LEITE et al., 2012). A redução no número de contorções e lambidas que o animal realiza durante a indução da nocicepção já foi relatada em diversos trabalhos, evidenciando um grande número de lectinas com essa atividade, por exemplo, a lectina das folhas de *Bauhinia monandra* (BmoLL) (CAMPOS et al., 2016), a lectina de *Solieria filiformis* (ABREU et al., 2016), a lectina de *Andira anthelmia* (AAL) (NASCIMENTO et al., 2016) e a lectina de *L. araripensis* (LAL) (AMORIM et al., 2016).

Em muitas respostas antinociceptivas parece haver uma interação entre as lectinas com receptores opióides, responsáveis pela sensação de dor. A lectina de *C. boliviana* (CboL) apresentou atividade antinociceptiva confirmada pelo número reduzido de contorções após tratamento com a proteína. É sugerido que seu mecanismo de ação envolva dois receptores opióides (FIGUEIREDO et al., 2009). Resultados semelhantes foram verificados para a lectina de *C. brasiliensis* (ConBr). Esta lectina possui efeito antinociceptivo dose-dependente para a redução de contorções e lambidas. A utilização de inibidores dos receptores opióides mostrou que apenas antagonistas do receptor mi não inibiram o efeito antinociceptivo, revelando uma relação entre a ConBr e os receptores kappa e delta na promoção da atividade antinociceptiva (DE FREITAS PIRES et al., 2013).

2.1.2.3 Atividade anticâncer

Sabendo que as células tumorais podem apresentar um padrão de glicosilação diferente nas suas proteínas de superfície, as lectinas têm se tornado uma interessante estratégia para entender os mecanismos de distribuição de metástase e progressão tumoral levando em consideração sua ligação altamente específica a estes carboidratos de superfície (LITYŃSKA et al., 2001). Nesse sentido, as lectinas vêm se destacando como uma abordagem promissora no tratamento, prognóstico e diagnóstico de diversos tipos de cânceres (YAU et al., 2015).

Alguns mecanismos básicos pelos quais as lectinas com propriedades antitumorais exercem sua atividade já são bem conhecidas, como a citotoxicidade, inibição do crescimento tumoral, ligação à membrana da célula cancerosa, alteração da sinalização das vias metabólicas (em especial, a apoptose), inativação dos ribossomos, autofagia e desregulação da atividade

telomerásica (DE MEJÍA; PRISECARU, 2005; JIANG et al., 2015). A lectina isolada de *Pisum sativum* L., por exemplo, apresenta atividade anticâncer em linhagens de câncer colorretal por inibir o crescimento e proliferação celular, bem como induz a apoptose mediada pela expressão de p21 e p53 (ISLAM et al., 2018). Outro estudo demonstrou que a lectina de *P. acutifolius* apresentou atividade semelhante na indução da apoptose em linhagens de células de câncer de cólon mediada por uma aumento na fosforilação da p53, maior atividade de caspase-3 e aumento de células em G0/G1 durante o ciclo celular (MORENO-CELIS et al., 2020).

Estudos *in vitr*o identificaram que a lectina de *Lantana camara* (LCL) possui atividade seletiva contra câncer de cólon, interagindo com os tumores na fase metastática e não se ligando às células do tecido normal. Além disso, o estudo também verificou que a LCL possui atividade inibitória no crescimento celular desse tipo de câncer, evidenciando o potencial antitumoral dessa lectina como agente terapêutico (HIREMATH et al., 2020). A DLasil, uma lectina purificada de *D. lasiocarpa*, apresenta atividade antiproliferativa contra diversos tipos de carcinomas humanos, incluindo câncer de ovário, pulmão, próstata e mama. A linhagem de células tumorais de câncer de ovário (A2780) foi mais suscetível à parada do ciclo celular em G2/M. Este resultado sugere que a DLasil seja capaz de reconhecer com maior especificidade carboidratos da superfície de linhagem A2780 (GONDIM et al., 2017).

2.1.2.4 Efeitos tóxicos

Vale ressaltar que algumas lectinas de origem vegetal também podem exercer efeitos adversos (WANG et al., 2019; WONG et al. 2020). As lectinas ConA (ConcanavalinA), LCA (*Lens culinaris* lectin), WGA (Wheat germ agglutinin), SBA (Soybean aglutinin), aglutinina do amendoim, aglutinina de *Ulex europaeus* e a jacalina são capazes de induzir a ativação de caspase-1, promovendo uma maior secreção de interleucina inflamatória IL-1B, resultando na formação de inflamassomas (NLRP3) e inflamação *in vivo*, especialmente Con A e WGA (GONG et al., 2017). As lectinas mais conhecidas por sua potente toxicidade são provavelmente a ricina e a abrina. A ricina é uma lectina extraída de *R. communis* que tem como principal mecanismo de ação a inativação enzimática dos ribossomos. A cadeia A desta lectina apresenta citotoxicidade para linfocitos e macrófagos (EL-NIKHELY et al., 2007). A abrina isolada de *A. precatorius* é outra lectina comumente usada como referência sobre o potencial toxicológico das lectinas, apresenta atividade inativadora de ribossomos assim como a ricina (WORBS et al., 2021), ambas inibem a síntese proteica, levando à morte celular (HEGDE; MAITI; PODDER, 1991).

2.2 Parte II – Avaliação de risco da lectina de *A. esculentus*

2.2.1 *A. esculentus*: aspectos botânicos, uso medicinal e propriedades terapêuticas

A. esculentus (ou *Hibiscus esculentus* em classificações mais antigas), conhecido popularmente como quiabo, é uma planta medicinal nativa do continente africano e faz parte da família Malvaceae (Figura 1). Acredita-se que tenha surgido no país que hoje fica a Etiópia (DAVIES et al., 1989; SINGH et al., 2014). De acordo com a etnobiologia, acredita-se que essa espécie tenha se estabelecido no Brasil durante o tráfico de escravos africanos com destino às colônias portuguesas na América por volta do século XVI (SILVA et al., 2016).

Figura 1: Classificação taxonômica de *A. esculentus.*

Fonte: Adaptado de Islam, 2019.

A família malvaceae se caracteriza por apresentar um hábito arbóreo, arbustivo, lianas ou ervas. As folhas geralmente são alternadas, espiraladas ou dísticas. Apresentam ainda venação palmada ou peninérvea e presença de estípula. Possuem inflorescência indeterminadas ou mistas. As flores são bissexuais ou unisexuais, geralmente radiais, normalmente associada com brácteas que podem formar um epicálice. Suas flores apresentam diferentes morfologias, atraindo polinizadores como abelhas, morcegos, moscas, vespas, formigas, mariposas e aves. As sépalas costumam aparecer em um total de 5 nessa família, mas podem estar ausentes. Estames podem ser 5 ou numerosos. Os grãos de pólen são geralmente tricolporados, triporados ou poliporados, as vezes espinhosos. Apresentam dois carpelos ou mais e são conatos. Possuem ovário súpero e placentação geralmente axial. Os nectários são compostos por pêlos glandulares multicelulares densamente agrupados. O fruto é normalmente uma cápsula, noz, agregado de

folículo, baga ou drupa. As sementes podem apresentar pêlos ou serem ariladas, mas em poucos casos podem ser aladas. O endosperma está presente nas malvaceae (JUDD et al., 2009).

O quiabeiro apresenta distribuição principalmente em regiões tropicais e subtropicais (Figura 2). Apresenta caule ereto semi lenhoso, é uma planta anual e podem chegar até 2,5 metros de altura. As folhas são pecioladas e podem chegar até 20 e 30 cm de comprimento. A corola apresenta cor amarela, sendo a base um tom de púrpura. Seu fruto é do tipo cápsula e de acordo com o tipo de cultivar esse fruto pode apresentar colorações como verde, violeta, vermelho, embora a cor verde seja predominante. O tamanho do fruto pode chegar até 25 cm. A cápsula é responsável por manter as sementes em proteção. Suas sementes apresentam um boa fonte de proteínas, minerais além de elementos como o ferro, potássio, cálcio e magnésio (GURGEL; MITIDIERI, 1956).

Nas sementes está presente grande parte dos compostos bioativos que exercem suas atividades antioxidantes e antimicrobianas (PETROPOULOS et al., 2017). No Brasil, *A. esculentus* está presente principalmente na região nordeste devido as características morfo-climáticas da região, mas sua ocorrência também é relatada no sudeste do país (REFLORA, 2020).

Figura 2: Representa a planta de *A. esculentus*. A) Hábito arbustivo da planta. B) Características da flor e do fruto. C) Secção transversal do fruto, evidenciando as sementes.

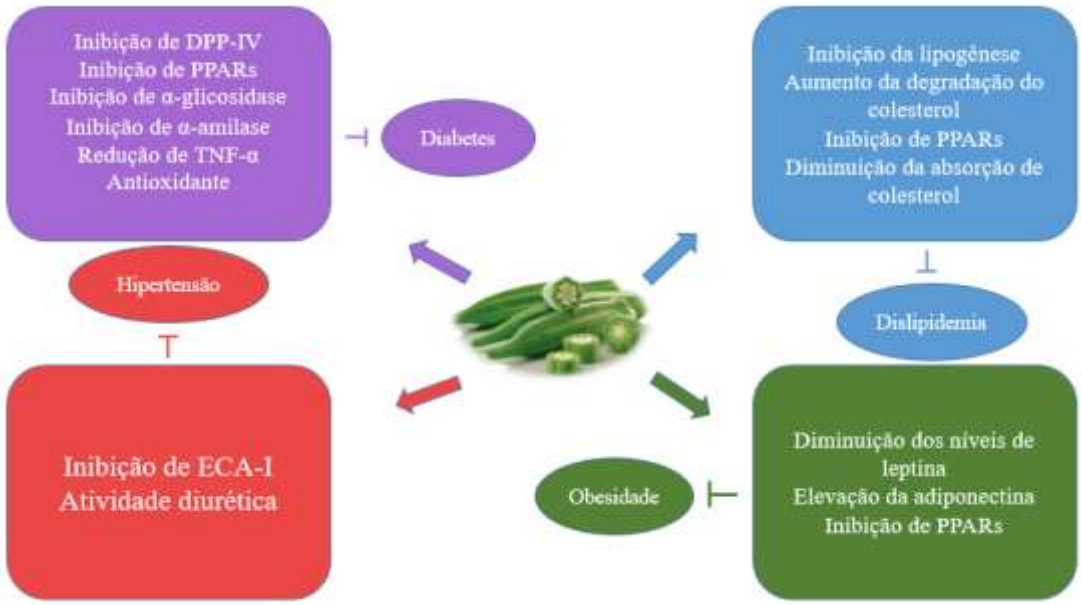
A) B) C)

Fonte: Google imagens.

No campo medicinal, essa planta vêm se destacando devido as suas propriedades anti-inflamatória, auxilia no bom funcionamento intestinal, tratamento de úlceras, doenças do aparelho urinário, dentre outros (JAIN et al., 2012). A figura 3 representa as principais atividades do quiabo. A composição diversa de proteínas, carboidratos e compostos fenólicos

de *A. esculentus* faz dela uma espécie com grande potencial farmacológico no tratamento contra várias doenças seja através de extratos ou compostos isolados, especialmente no tratamento do Diabetes Mellitus tipo 2 (PETROPOULOS et al., 2017).

Figura 3: Principais propriedades terapêuticas de *A. esculentus.*



Fonte: Adaptado de Esmaeilzadeh et al., 2020.

As propriedades antidiabéticas desta planta vem ganhando destaque no tratamento do diabetes tipo 2, pois ela apresenta efeitos hipoglicêmicos, reduzindo os níveis de glicose no sangue, entre outros benefícios (KHOSROZADEH; HEYDARI; ABOOTALEBI, 2016; HUANG et al., 2017; PENG et al., 2019; DURAZZO et al., 2019). Outro efeito bem estudado é a sua capacidade inibitória de enzimas importantes no contexto patológico da doença, como a alfa-amilase e a alfa-glucosidase (SABITHA; PANNEERSELVAM; RAMACHANDRAN, 2012). Vários polissacarídeos estão sendo descobertos por serem responsáveis pelas atividades antidiabéticas que são encontradas no quiabo, entre eles a ramnogalacturonana (LIAO et al., 2019; LIU et al., 2018b).

Muitos medicamentos utilizados no tratamento da diabetes atuam inibindo receptores específicos, tais como o PPARs (Receptores Ativados por Proliferadores de Peroxissoma). Extratos em pó do quiabo já demonstraram resultados positivos sobre a ação do genes PPARs, onde sua desregulação foi associada a uma melhora dos níveis de glicose no sangue (MAJD et al., 2018). Os flavonóides presentes no quiabo, em especial a quercetina e seu derivados são responsáveis em partes por sua atividade anti-hiperglicêmica e antioxidante, através da redução

dos níveis de malondialdeído, um subproduto da peroxidação lipídica (DAMAYANTHI; RIMBAWAN; HANDHARYANI, 2018). O quiabo também foi capaz de reduzir o estresse oxidativo em ratos diabéticos através do aumento da atividade das enzimas glutationa peroxidase (GPX) e catalase (CAT) (MAJD et al., 2019).

Os efeitos gastroprotetores do quiabo têm sido evidenciados principalmente devido a sua complexa composição de polissacarídeos. A fração obtida dos frutos do quiabo, apresentando vários carboidratos na sua composição, foi capaz de inibir a bactéria *Helicobacter pylori* devido às suas propriedades anti-adesivas, reduzindo a ligação da bactéria à mucosa gástrica do hospedeiro (LENGSFELD et al., 2004). Outro estudo demonstrou que o extrato do quiabo é responsável por reduzir os danos gástricos induzidos pelo álcool, além de evitar a formação de úlceras e melhorar o perfil antioxidante através da redução de malondialdeído e aumento do antioxidante GSH (Glutationa peroxidase), neste trabalho, foi visto também uma menor infiltração celular, redução de edema e hemorragia após o tratamento com o extrato do quiabo (ORTAÇ et al., 2018).

O quiabo é bastante conhecido na medicina tradicional devido às suas atividades antioxidantes. Um estudo feito com a mucilagem das vagens do quiabo foi possível identificar que existe uma quantidade desejável de antioxidantes naturais nessa planta (GEMEDE et al., 2018). Essa mesma capacidade antioxidante tem sido reportada em estudos que utilizaram o extrato metanólico e aquoso das sementes do quiabo.

No entanto, os resultados *in vitro* mostram que para esses extratos, a atividade antioxidante existe, porém é menor do que a normalmente relatada para outros tipos de extrato (DOREDDULA et al., 2014).

A composição variada de polifenóis e polissacarídeos de *A. esculentus* é responsável, em parte, por sua atividade antioxidante e estudos com esses compostos têm mostrado reduções nos níveis de malondialdeído, bem como aumento na expressão de genes antioxidantes como glutationa (GSH), GPX e superóxido dismutase (SOD) (XIA et al., 2015). Em adição a estes resultados, sabe-se que a atividade antioxidante do quiabo varia de acordo com os diferentes estágios de maturação da planta, já é conhecido que os compostos fenólicos apresentam diferenças em sua concentração de acordo com o amadurecimento (SHEN et al., 2019).

Algumas desordens no metabolismo apresentam vários marcadores bioquímicos que podem ser utilizados como parâmetro de avaliação. Entre eles podemos destacar o aumento na formação espécies reativas de oxigênio, responsável por causar danos a biomoléculas como proteínas, lipídeos e DNA. Um perfil oxidativo elevado está normalmente associado a fenótipos

negativos e respostas a este estado se traduzem em alterações na expressão de proteínas antioxidantes, tais como SOD, CAT e GSH. O quiabo, pode ser utilizado como uma boa estratégia para minimizar essa ação oxidante, que desempenha uma papel importante na patogênese e progressão de várias doenças de distribuição mundial como o diabetes e a obesidade (SABITHA et al., 2012).

A. esculentus vêm ganhando espaço como coadjuvante na luta contra o câncer. Vários estudos vêm relatando sua eficácia contra linhagens de células tumorais específicas e, até o momento, os resultados têm se mostrado bastante promissores. Um estudo recente identificou que utilização do extrato das sementes do quiabo promoveu a inibição da migração e proliferação celular, inibição de VEGF (Fator de Crescimento Endotelial Vascular), bem como aumento do número de células apoptóticas nas linhagens tumorais MCF-7, HeLa e HepG2. Ainda, o estudo indicou que a linhagem MCF-7 foi mais responsiva aos efeitos citotóxicos do extrato das sementes do quiabo (CHAEMSAWANG et al., 2019).

Estudos *in vitro* demonstraram que o polissacarídeo ramnogalacturonana obtido de *A. esculentus* possui eficiente atividade antiproliferativa e pró-apoptótica em linhagens de melanoma (B16F10). Outros efeitos desse polissacarídeo são a inibição do ciclo celular e indução da apoptose, além da redução da expressão de caderinas, moléculas importantes na progressão de diversos tipos de câncer (VAYSSADE et al., 2010).

Outros benefícios de *A. esculentus* vem sendo evidenciados na literatura, seu extrato etanólico e a presença de polissacarídeos foi eficaz em aumentar a glicose hepática, além de reduzir os níveis de ácido lático no sangue, propiciando dessa forma um efeito antifadiga (LI et al., 2016b; XIA et al., 2015). A síndrome metabólica é uma desordem caracterizada por uma série de fatores como a resistência insulínica, dislipidemia, pressão sanguínea alta, obesidade e hipertriglicemia. O quiabo vem ganhando destaque no combate a essa síndrome por apresentar atividade antidiabética, antiobesidade e antihipertensiva (ESMAEILZADEH; RAZAVI; HOSSEINZADEH, 2020).

O quiabo também apresenta atividade imunomoduladora devido aos seus polissacarídeos, aumentando a atividade fagocítica, a proliferação de células do sistema imune como células T, células B e macrófagos, aumento da produção de óxido nítrico (NO) além de modular a expressão de citocinas importantes na defesa contra patógenos, em especial *Staphylococcus aureus* (CHEN et al., 2016; WAHYUNINGSIH et al., 2018). Foi verificado também que alguns flavonoides presentes no quiabo mostraram atividade protetora contra radiação UV-B através do seu potencial antioxidante, reduzindo o dano oxidativo ao DNA além

de uma diminuição nos níveis de espécies reativas de oxigênio (EROS) mediada pela via Nrf2 (PATWARDHAN; BHATT, 2016).

2.2.1.1 Lectina de *A. esculentus (AEL)*

As sementes do quiabo apresentam um grande quantidade de proteínas com diversas atividades biológicas (PETROPOULOS et al., 2017). Nesse contexto, a lectina de *A. esculentus* (AEL) purificada das sementes do quiabo por Soares e colaboradores demonstrou várias atividades biológicas promissoras (DE SOUSA FERREIRA SOARES et al., 2012). Os autores identificaram que a AEL possui atividade anti-inflamatória e antinociceptiva e sua atividade hemaglutinante é diferente de outras lectinas, sendo metalo-independente. Sua atividade de hemaglutinação é inibida por lactose, frutose e manose. O perfil eletroforético da AEL identificou duas bandas de proteínas de 15,0 kDa e 21,0 kDa. Os resultados da espectrometria de massas revelaram um monômero de 10,29 kDa e um dímero de 20,58 kDa (DE SOUSA FERREIRA SOARES et al., 2012).

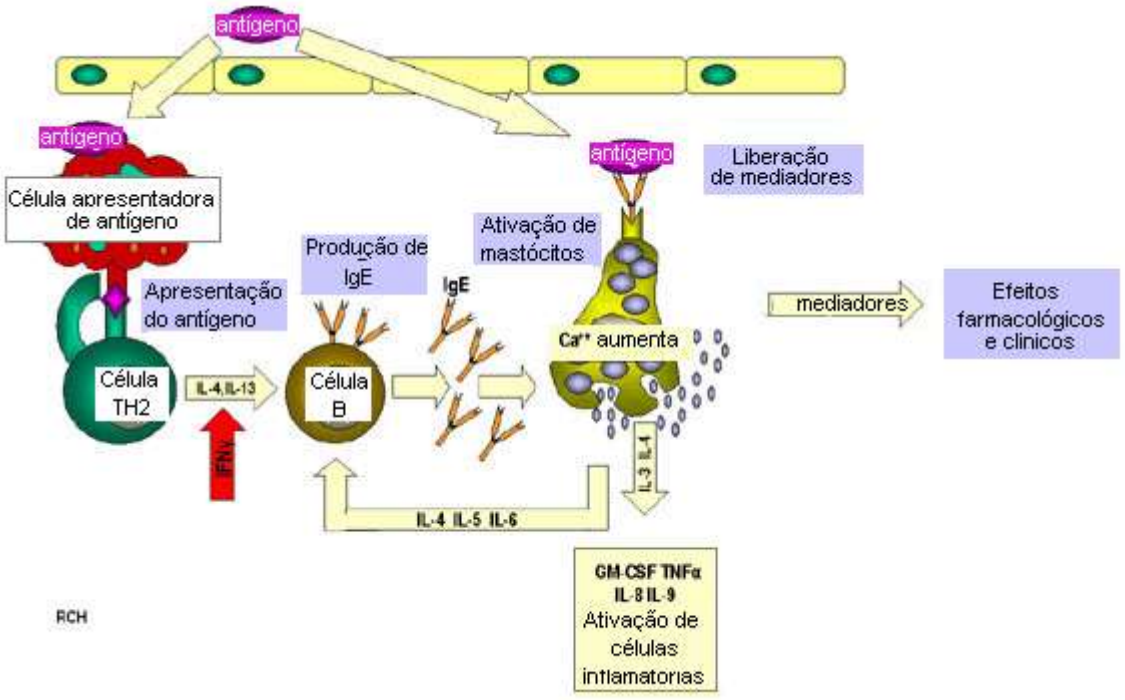
Outra atividade relevante da AEL que já foi investigada é a sua atividade fungicida, principalmente contra *Candida parapsilosis*. Acredita-se que sua atividade inibitória se deva à capacidade de ligação aos carboidratos da parede celular dos fungos, causando assim sua inibição (KLAFKE et al., 2013). A AEL mostrou atividade citotóxica seletiva para a linhagem celular de câncer de mama MCF7, promovendo alterações na permeabilidade da membrana celular e na expressão de genes relacionados à apoptose, como caspase-3, Bax, p21 e Bcl-2. Assim como ocorre com outros efeitos mediados por lectina, presume-se que ocorra uma interação entre a lectina e os açúcares da superfície celular da linhagem de células MCF7, uma vez que as células saudáveis não são afetadas (MONTE et al., 2014).

A AEL também possui grande potencial farmacológico no tratamento de dores na articulação temporomandibular através de seus efeitos anti-inflamatórios e antinociceptivos, reduzindo os níveis de TNF-α e IL-1β e a ativação dos receptores kappa e delta opióides (FREITAS et al., 2016). Além disso, esta lectina apresenta um efeito gastroprotetor em modelos induzidos pelo etanol, provavelmente devido à sua atividade antioxidante e à ativação de receptores opióides (A. RIBEIRO et al., 2016).

2.2.2 Alergias

A hipersensibilidade pode ser caracterizada como uma resposta imune a antígenos que leva a reações sintomáticas após sua exposição (MURPHY; WEAVER, 2016). As hipersensibilidades são classificadas em quatro tipos de acordo com o tipo de resposta imune e o mecanismo efetor: as hipersensibilidade do tipo 1, 2, 3 e 4 (ABUL K; ANDREW H. LICHTMAN MD; SHIV PILLAI MBBS, 2015). As alergias propriamente ditas constituem o grupo da hipersensibilidade 1 e seu mecanismo de ação é ilustrado na Figura 4.

Figura 4: Mecanismos básicos no processo de hipersensibilidade do tipo 1.

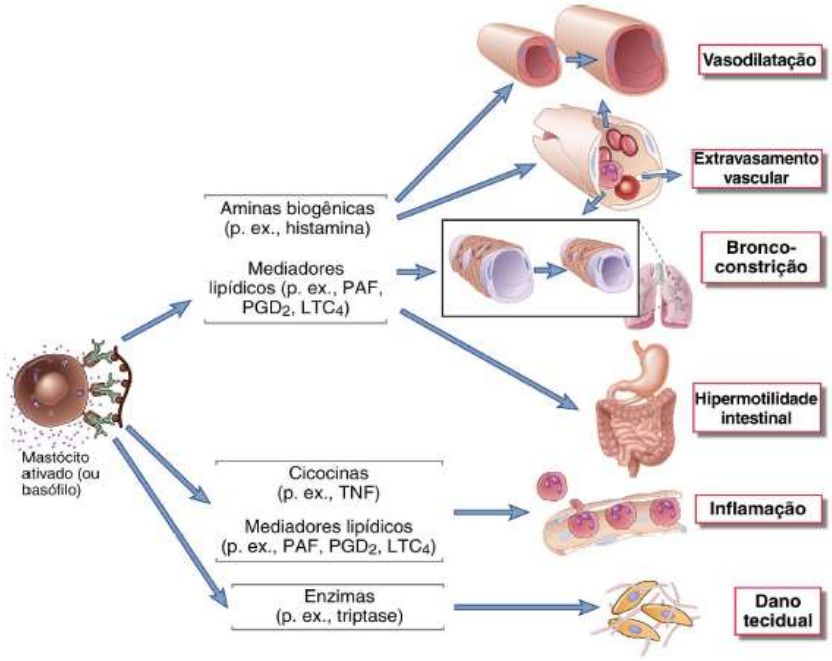


Fonte: Google imagens.

Uma característica marcante nas alergias é a capacidade que anticorpos IgE têm de serem produzidos e estarem aptos a se ligarem a determinados antígenos. Seu surgimento está normalmente associado com ativação de células T auxiliares (T *helper*), as quais são capazes de produzir as interleucinas IL-4, IL-5 e IL-13, levando a produção de anticorpos IgE. Em seguida, ocorre a ligação do anticorpo a receptores Fc de mastócitos (chamada fase de sensibilização) e ativação de mastócitos através da reexposição ao antígeno (degranulação), resultando na liberação de mediadores que culminará em uma reação alérgica de caráter inflamatório. Mastócitos ativados secretam uma variedade de mediadores que são responsáveis

pelas manifestações das reações alérgicas (BERIN, 2015; SABBAN; YE; HELM, 2014). (Figura 5).

Figura 5: Principais efeitos clínicos observados após a ligação do IgE aos receptores Fc dos mastócitos.



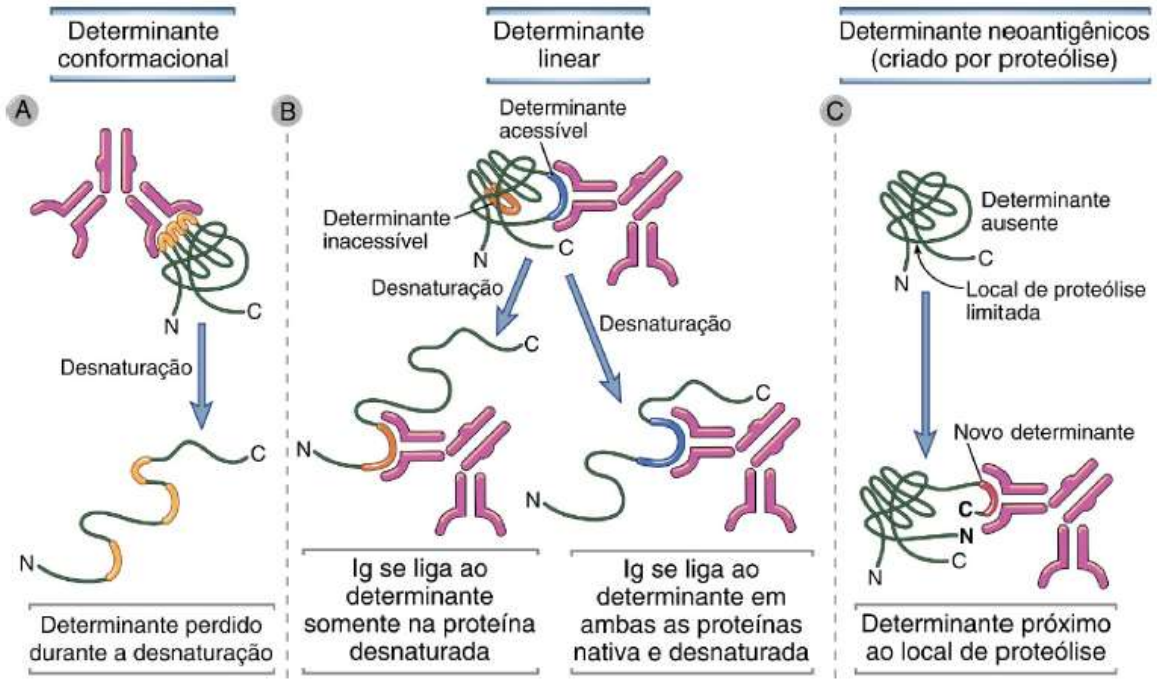
Fonte: Abul K et al., 2015.

Anticorpos ou imunoglobulinas, são uma família de glicoproteínas estruturalmente relacionadas e produzidas na forma ligada à membrana ou secretadas pelos linfócitos B (HNASKO, 2015). Os anticorpos apresentam diferentes isoformas responsáveis por funções específicas e são nomeadas como IgA, IgD, IgE, IgG e IgM (MA; O'KENNEDY, 2015). Todas as moléculas de anticorpo compartilham as mesmas características estruturais básicas, mas apresentam marcante variabilidade nas regiões onde os antígenos se ligam (SCHROEDER; CAVACINI, 2010). Um antígeno é qualquer substância que pode ser especificamente ligada por uma molécula de anticorpo ou receptor de célula T (HNASKO, 2015).

Moléculas que estimulam as respostas imunes são chamadas de imunógenos (CRUMPTON, 1974; VAN REGENMORTEL, 2001). Macromoléculas, tais como proteínas, polissacarídeos e ácidos nucleicos, normalmente são muito maiores do que a região de ligação do antígeno de uma molécula de anticorpo (ABUL K; ANDREW H. LICHTMAN MD; SHIV PILLAI MBBS, 2015). Dessa maneira, qualquer anticorpo se liga a somente uma porção da macromolécula, que é chamada de determinante ou um epítopo (Figura 6). Quando um anticorpo se liga a uma proteína, os locais de ligação resultantes no anticorpo e na proteína são

chamados o paratopo e epítopo, respectivamente (EL-MANZALAWY; DOBBS; HONAVAR, 2017). Os epítopos podem ser classificados em lineares (contínuos) ou conformacionais (descontínuos) (VAN REGENMORTEL, 2001). Os epítopos lineares/contínuos são definidos pela sequência de aminoácidos residente na sequência primária da proteína onde os anticorpos podem se ligar. Os epítopos conformacionais/descontínuos se caracterizam por sequências peptídicas agrupadas na estrutura tridimensional que reagem aos anticorpos. Se os determinantes lineares surgem na superfície externa ou em uma região de conformação estendida na proteína dobrada nativa, eles podem ser acessíveis aos anticorpos. Os determinantes lineares podem estar inacessíveis na conformação nativa e aparecem somente quando a proteína é desnaturada (EL-MANZALAWY; DOBBS; HONAVAR, 2017; NIELSEN; MARCATILI, 2015). A predição de epitopos alergênicos leva em consideração características bioquímicas da proteína como flexibilidade, antigenicidade, hidrofilicidade, voltas beta e acessibilidade (KOZLOVA et al., 2015).

Figura 6: Características dos determinantes antigênicos encontrados em proteínas. A) Os determinantes conformacionais estão expostos apenas na conformação nativa das proteínas. B) Os determinantes lineares podem surgir após a desnaturação da proteína, expondo a sequência de ligação. C) Os determinantes neoantigênicos podem ser formados devido às modificações que ocorrem posterior a síntese das proteínas.



Fonte: ABBAS et al, 2008.

O local de ligação do antígeno de um anticorpo pode acomodar um determinante linear composto por cerca de seis aminoácidos (FAO / WHO, 2001). As proteínas podem estar sujeitas

a modificações como glicosilação, fosforilação, ubiquitinação, acetilação e proteólise (WALSH; JEFFERIS, 2006). Essas modificações, por alteração na estrutura da proteína, podem produzir novos epítopos. Tais epítopos são chamados de determinantes neoantigênicos e eles também podem ser reconhecidos por anticorpos específicos (ISLAM et al., 2017).

O reconhecimento do antígeno pelo anticorpo envolve princípios termodinâmicos como por exemplo, a Constante de afinidade (Ka) (HNASKO, 2015). Vários tipos de interações não covalentes podem contribuir para a ligação do anticorpo ao antígeno, incluindo forças eletrostáticas, ligações de hidrogênio, forças de Van der Waals e interações hidrofóbicas (BRADEN; POLJAK, 1995).

Os indivíduos atópicos produzem altos níveis de IgE em resposta a alérgenos ambientais, enquanto os indivíduos normais geralmente produzem outros isotipos de Ig, como IgM e IgG, e apenas pequenas quantidades de IgE (JOHANSSON, 2016). A quantidade de IgE sintetizada depende da propensão de um indivíduo a gerar células T auxiliares específicas de alérgenos que produzem IL-4 e IL-13, porque estas citocinas estimulam a mudança da classe dos anticorpos de células B para IgE. O anticorpo IgE é responsável pela sensibilização dos mastócitos e fornece o reconhecimento de antígenos para as reações de hipersensibilidade imediata (LEHRER; HORNER; REESE, 1996).

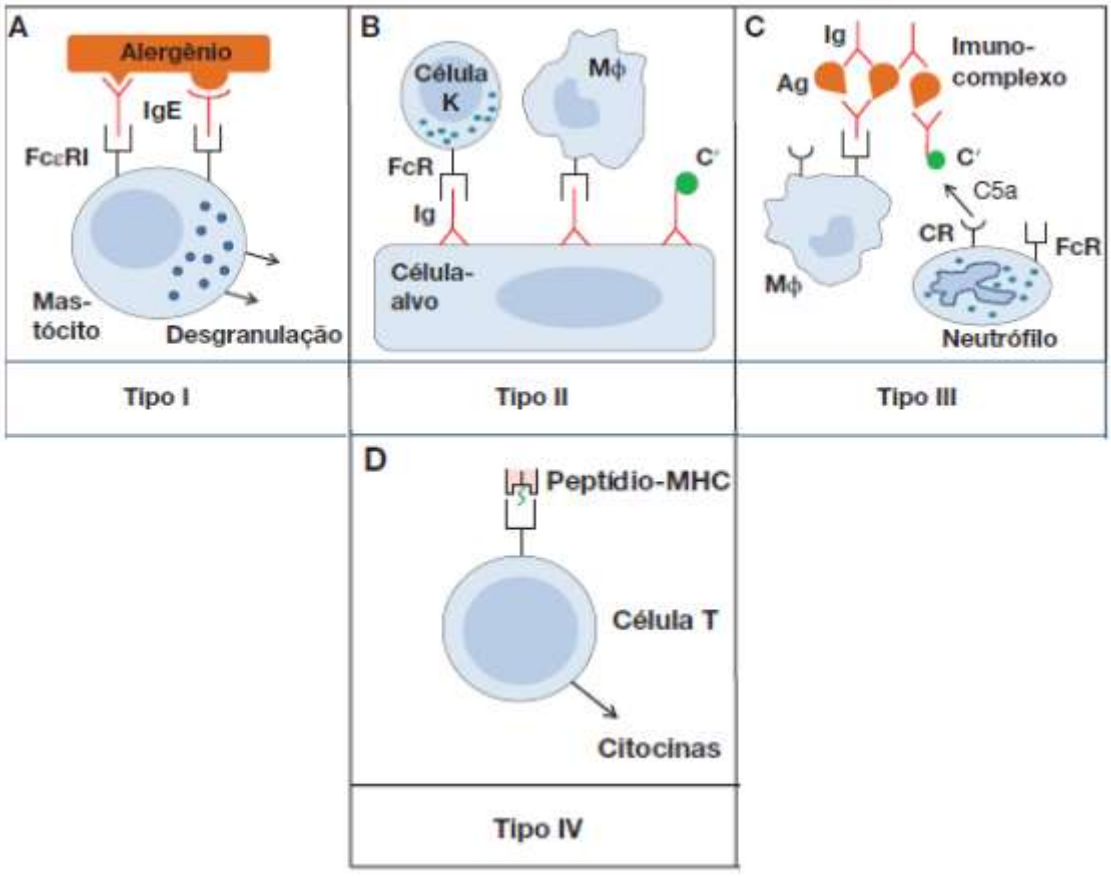
Alérgenos típicos incluem proteínas no pólen, ácaros domésticos, pêlos de animais, alimentos e produtos químicos como o antibiótico penicilina (IVANCIUC et al., 2009). Algumas características são típicas de alérgenos como médio peso molecular (pelo menos 3,5 kDa), a estabilidade e a glicosilação (FAO / WHO, 2001).

Em um indivíduo alérgico a um antígeno particular, uma grande parte da IgE ligada ao FcεRI na superfície dos mastócitos é específica para este antígeno (RIOS; KALESNIKOFF, 2015). A exposição ao antígeno irá cruzar moléculas de IgE suficientes para desencadear a ativação dos mastócitos. A ativação dos mastócitos resulta em três tipos de resposta biológica: a secreção do conteúdo dos grânulos pré-formados por exocitose (degranulação), a síntese e secreção dos mediadores lipídicos e a síntese e secreção de citocinas (HAYGLASS, 1995).

Outras reações de hipersensibilidades ocorrem nos organismos, mas estas não são consideradas alergias propriamente ditas, contudo, são responsáveis por alterações imunológicas importantes (ABUL K; ANDREW H. LICHTMAN MD; SHIV PILLAI MBBS, 2015). A hipersensibilidade do tipo 2 é mediada por anticorpos IgG e IgM e atuam por meio da ativação do sistema complemento, recrutando células inflamatórias e interferindo nas funções celulares normais. Esses anticorpos são específicos para antígenos de determinadas células e

podem ser encontradas de forma livre na circulação. A hipersensibilidade do tipo 3 é caracterizada principalmente pela formação de imunocomplexos na circulação (complexo anticorpo-antígeno), que se depositam nos vasos e provocam lesões. A hipersensibilidade do tipo 4 é causada pela ativação das células T auxiliares CD4+ T, que pode induzir um processo inflamatório ou matar suas células-alvo através da secreção de citocinas e produção de anticorpos que danificam os tecidos (ABUL K; ANDREW H. LICHTMAN MD; SHIV PILLAI MBBS, 2015; DELVES et al., 2017; JUSTIZ VAILLANT; ZITO, 2018). Um esquema demonstrando os principais mecanismos de hipersensibilidade é mostrado na Figura 7.

Figura 7: Reações imunológicas de hipersensibilidade. A) Hipersensibilidade do tipo 1. B) Hipersensibilidade do tipo 2. C) Hipersensibilidade do tipo 3. D) Hipersensibilidade do tipo 4.



Fonte: Delves et al, 2017.

2.2.3 Avaliação de risco de novas proteínas

A segurança de uso de novas proteínas, seja ela com perspectiva alimentar ou terapêutica, tem sido realizada principalmente com base nas orientações de avaliação de risco

de alergenicidade para OGMs (Organismos Geneticamente Modificados) proposta pela EFSA em 2010 (“Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed”, 2010; VERHOECKX et al., 2016). Novas atualizações desta diretriz foram feitas em 2017, no entanto nenhuma abordagem específica para proteínas terapêuticas foi mencionada. A avaliação de risco de novas proteínas continua utilizando as abordagens “peso de evidência” e “abordagem caso a caso” para avaliar a segurança dessas proteínas quanto ao seu potencial alergênico e toxicológico (VERHOECKX et al., 2016, 2019).

As abordagens que são realizadas para a segurança dos OGM podem ser adaptadas para garantir o uso seguro de novas proteínas com atividade terapêutica na perspectiva de novas formas de tratamento para muitas doenças. Entre essas abordagens, podemos citar:

1. **Elaboração de um Histórico de Uso Seguro**: Uma ampla busca sobre as utilizações da proteína de interesse bem como do organismo da qual ela foi extraída. Tem como objetivo identificar os efeitos terapêuticos, tóxicos, antinutricionais e alergênicos sobre o uso do organismo/proteína com base na literatura (CONSTABLE etal., 2007).
2. **Busca por sequências homólogas a alérgenos**: As ferramentas de bioinformática vêm se tornando uma abordagem de grande peso na tomada de decisões sobre a potencial alergenicidade de proteínas de origem animal e vegetal. Os bancos de dados de alérgenos são utilizados para comparar a sequência primária da proteína de interesse com proteínas reconhecidamente alergênicas. Os padrões de homologia (35% de identidade para uma janela de 80 aminoácidos) entre as sequências ainda continuam sendo os mesmo que os descritos pela FAO/WHO, 2001. Os principais bancos utilizados são o AllergenOnline, Allermatch, SDAP e AllerCatPro (IVANCIUC et al., 2003; FIERS et al., 2004; GOODMAN et al., 2016; MAURER-STROH et al., 2019).
3. **Capacidade de ligação de IgE a epitopos alergênicos**: A ligação de IgE representa um dos principais indícios da alergenicidade de uma proteína. Diversas ferramentas de bioinformática vêm sendo utilizadas para fazer a predição dos sítios de ligação da IgE em epitopos alergênicos da sequência alvo. O percentual de identidade com epítopos alergênicos de outras proteínas é um importante indicativo da capacidade de ligação da IgE com a proteína alvo (FAO/WHO, 2001).
4. **Estabilidade de proteínas e resistência às proteases intestinais**: Testes *in vitro* ainda permanecem como boas estratégias para validar a resistência de vários alérgenos de natureza proteica. Esse teste simula de forma *in vitro* a digestão gástrica e intestinal

como naturalmente ocorreria. Proteínas que são resistentes às principais proteases intestinais tais como pepsina, tripsina e pancreatina (mistura de proteases produzidas no pâncreas) são consideradas potenciais alérgenos (VERHOECKX et al., 2016).

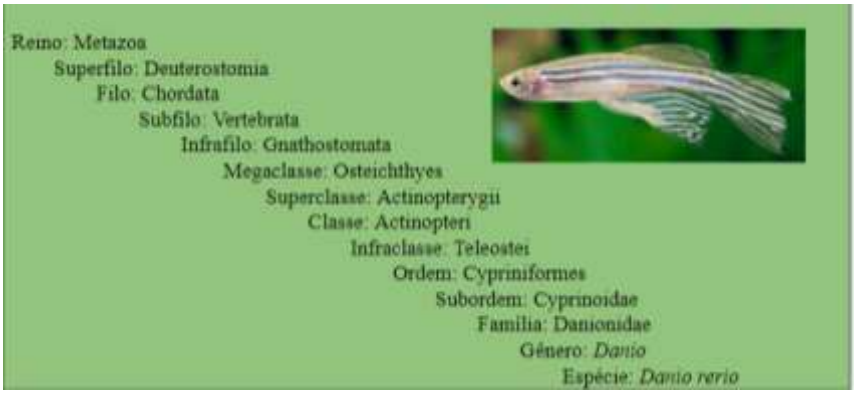
5. **Avaliação de toxicidade aguda:** A utilização de modelos animais é um importante método para avaliar os efeitos tóxicos da exposição de substâncias que apresentem algum risco em sua utilização. Os testes podem ser feitos seguindo as diretrizes da OECD ("Organisation for Economic Co-operation and Development") e buscam identificar a toxicidade e as concentrações seguras da substância no contexto farmacológico e terapêutico (BARRÉ-SINOUSSI e MONTAGUTELLI, 2015).

Nesse sentido, fica claro a necessidade de realização das avaliações de risco para novas proteínas terapêuticas baseadas em estratégias holísticas. A segurança dessas proteínas deve levar em consideração abordagens *in silico*, *in vitro* e *in vivo* para compreender a natureza da proteína e os efeitos sistêmicos que ela pode exercer numa possível reação com os sistemas biológicos (FASOLIN et al., 2019).

2.2.4 *Danio rerio*

O peixe-zebra (*D. rerio*), também conhecido como zebrafish ou peixe paulistinha, é um peixe teleósteo da família Danionidae (STOUT et al., 2016). Em classificações mais antigas, *D. rerio* era classificado como Cyprinidae. No entanto, estudos genômicos auxiliaram na modificação da filogenia do grupo dos teleósteos incluindo este peixe como pertencente à família Danionidae (BETANCUR et al., 2017; MCCLUSKEY; BRAASCH, 2019). Uma classificação filogenética recente é mostrada na Figura 8.

Figura 8: Atualizações taxonômicas de *Danio rerio*. A figura mostra uma classificação filogenética recente da espécie após modificações taxonômicas ocorridas no grupo dos teleósteos nos últimos anos.



Fonte: Adaptado de McCluskey and Braasch, 2019.

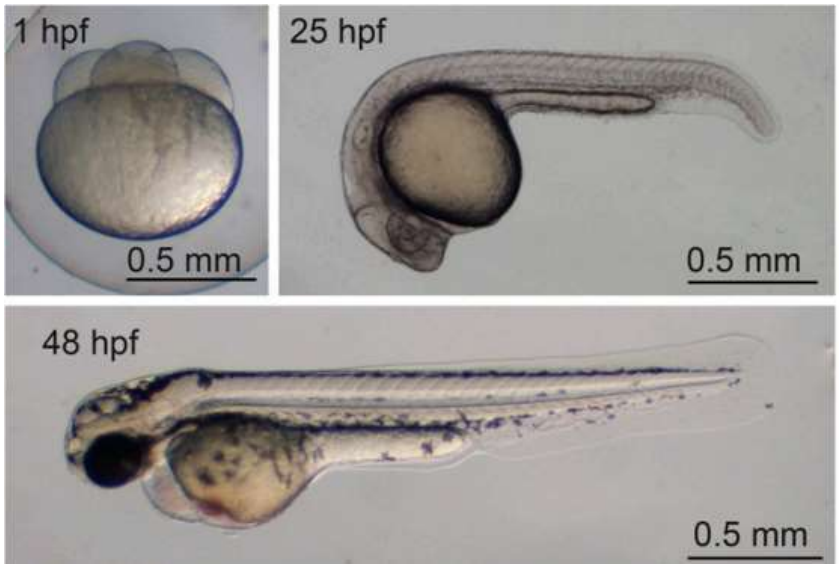
D. rerio é um peixe tropical de origem asiática e é encontrado principalmente em rios, pequenos riachos e lagos rasos, especialmente àqueles encontrados próximos às plantações de arroz (ENGESZER et al., 2007). Os animais adultos podem chegar a 4-5 cm de comprimento, apresentando dimorfismo sexual visível (Figura 9). As fêmeas são maiores que os machos, apresentando um abdômen mais proeminente devido a postura de ovos, enquanto os machos são menores e mais longilíneos, com formato semelhante a um torpedo (HOLTZMAN et al., 2016; LAALE, 1977). Os animais adultos apresentam um padrão de listras ao longo do corpo que é característico da espécie.

Figura 9: Visão lateral de animais adultos de *Danio rerio*. O dimorfismo sexual presente nos peixes adultos da espécie é marcado pelo formato longilíneo em forma de torpedo dos machos, enquanto as fêmeas são caracterizadas por um abdômen proeminente. O padrão de listras horizontais ao longo do corpo dos animais é característico da espécie.

Fonte: Holtzman et al, 2016.

São animais que na natureza formam agregados sociais para proteção contra predadores, forrageamento e acasalamento (PARICHY, 2015). *D. rerio* apresenta fecundação externa. A reprodução dessa espécie ocorre o ano todo, garantindo que uma grande quantidade de animais seja gerada o ano inteiro. A espécie apresenta ovo do tipo telolécito, ou seja, a maior parte do citoplasma é composto por vitelo, garantindo a nutrição do embrião nos estágios iniciais do seu desenvolvimento. Os embriões apresentam um padrão de clivagem meroblástica e discoidal (GILBERT; BARRESI, 2017; LAALE, 1977). Umas de suas características mais marcantes como modelo experimental é a velocidade do seu desenvolvimento, como mostrado no Figura 10. Após a fertilização os embriões passam por diversos estágios do desenvolvimento como o período de zigoto (1 célula), clivagem (2 até 64 células), blástula (128 células até 30% do processo de epibolia), gástrula (50% de epibolia até a formação do broto), segmentação (1 até 26 somitos), faríngula (estágio 5 dos primórdios da linha lateral até o surgimento das nadadeiras peitorais rudimentares), incubação (continuação da morfogênese) e o desenvolvimento das larvas (GILBERT; BARRESI, 2017; KIMMEL et al., 1995).

Figura 10: Estágio embrionários de *Danio rerio*. Em 1 hpf (horas pós fertilização) o embrião encontra-se no estágio de 4 células, concentradas no polo animal. Em 25 hpf as principais estruturas e modificações corporais já ocorreram, como a formação da cabeça, desprendimento da cauda, formação dos somitos, surgimento dos otólitos. Em 48 hpf o embrião apresenta coluna reta e a maior parte dos seus órgãos estão desenvolvidos.



Fonte: Holtzman *et al*. (2016).

2.2.4.1 Peixe-zebra (D. rerio) como sistema modelo

Os roedores (por exemplo, ratos e camundongos) têm sido classicamente utilizados na pesquisa devido a sua proximidade filogenética com os seres humanos e suas semelhanças fisiológicas (VANDAMME, 2014). Outros sistemas modelo tais como a *Drosophila melanogaster* (SANTALLA; PORTIANSKY; FERRERO, 2016), *Caenorhabditis elegans* (HUNT, 2017) e *Artemia* spp. (OS, 2019) ainda são bastante utilizados devido a traços fenotípicos facilmente distinguíveis e amplo conhecimento sobre o desenvolvimento desses organismos. Nessa perspectiva, *D. rerio* tem emergido como excelente sistema modelo de vertebrados devido às características do seu desenvolvimento, comportamento e morfologia, permitindo análises genéticas, bioquímicas e um bom poder de visualização celular devido a sua transparência nos estágios iniciais do desenvolvimento (PARICHY, 2015).

Os primeiros estudos feitos com peixe-zebra datam de 1930 como destaca Laale (1977). No entanto, o trabalho publicado em 1981 por Streininger e colaboradores é um dos grandes marcos na pesquisa e divulgação de estudos utilizando o peixe-zebra como modelo experimental. O grupo se dedicou a estudar a genética e mutagênese de clones de peixe-zebra (STREISINGER et al., 1981). Embora conhecido há bastante tempo, sua utilização na ciência teve um crescimento exponencial frente à outros modelos devido a sua versatilidade de trabalho e a decifração do seu genoma, que impulsionou os campos da genômica e biologia do desenvolvimento dos vertebrados (STRÄHLE et al., 2012).

Várias características fizeram de *D. rerio* um animal popular entre os cientistas, entre eles sua facilidade de criação em biotérios, menores custos de manutenção e manejo, fácil reprodução, tempo de geração curto, alta fecundidade, transparência óptica do embrião, rápido desenvolvimento e disponibilidade de recursos genômicos (LAALE, 1977; NASIADKA; CLARK, 2012).

No campo da toxicologia clínica e ambiental, *D. rerio* vem se destacando como um relevante modelo para avaliação da toxicidade de diversos herbicidas e pesticidas, tais como o Glifosato e o 2,4-D, bem como de formulações contendo estes princípios ativos (GAAIED et al., 2020; GONÇALVES et al., 2020; LANZARIN et al., 2020). O desenvolvimento das estratégias ômicas na ciência vem ganhando destaque nas pesquisas da Biologia Molecular devido ao grande impacto dos resultados gerados. Essas técnicas permitem analisar de forma holística os processos biológicos e *D. rerio* vem servindo como alvo dessas abordagens devido as suas grandes utilizações na pesquisa como mencionado (VIEIRA et al., 2020, 2021). Estudos

com peixe-zebra tem evidenciado alterações no perfil epigenômico após a exposição de partículas de arsênico inorgânicos, com importantes mudanças pós-transgeracionais revelando alteração no perfil de metilação das histonas e na expressão de fatores de transcrição neuronais. O estudo ressalta o peixe-zebra como um bom modelo para avaliação da expressão gênica com foco em modificações químicas de caráter reversível no DNA (VALLES et al., 2020).

De acordo com Williams et al. (2014), áreas como a toxicogenômica e a toxicoepigenômica têm sido amplamente beneficiadas com o crescimento de *D. rerio* no estudo da toxicologia, especialmente devido à conservação no perfil de transcrição e vias metabólicas encontradas neste teleósteo, possibilitando a extrapolação de resultados para outros organismos (WILLIAMS; MIRBAHAI; CHIPMAN, 2014). Foi demonstrado também que *D. rerio* é um bom modelo experimental para avaliação do consumo dietético de ácidos graxos ômega-6 e ômega-3 no desenvolvimento de obesidade induzida, na perspectiva de avaliar o impacto do consumo desses lipídeos sobre parâmetros no sucesso reprodutivos entre machos e fêmeas bem como a viabilidade dos embriões (FOWLER et al., 2020).

A similaridade funcional e organização morfológica do pâncreas de *D. rerio* com o pâncreas de mamíferos têm proporcionado o estudo das relações hormonais e genéticas relacionados ao Diabetes Mellitus, usando principalmente linhagens transgênicas desse organismo (KIMMEL; MEYER, 2016). Já é conhecido que no desenvolvimento de humanos e peixe-zebra, as células que expressam insulina surgem antes do aparecimento das células capazes de produzir glucagon, enquanto nos roedores essa ordem é inversa. Assim, existe uma maior similaridade entre humanos e peixes, no que diz respeito à formação de algumas regiões do pâncreas, do que de humanos e camundongos (PRINCE; ANDERSON; DALGIN, 2017).

A técnica CRISPR (“Clustered Regularly Interspaced Short Palindromic Repeats”) vem revolucionando a área da genética e da genômica. Aliado a isso, vários estudos vêm demonstrando a utilização de *D. rerio* para edições genéticas devido às vantagens conferidas pelo rápido desenvolvimento e similaridades genéticas com humanos. Sua utilização vem sendo feita principalmente para a geração de linhagens “knock-out” e “knock-in” para genes específicos (LI et al., 2016a).

No campo da oncologia, *D. rerio* tem sido eficiente em mimetizar neuroblastomas devido a indução de genes característicos das células deste tumor. Além disso, esse modelo vem auxiliando na compreensão dos mecanismos de progressão da doença, assim como na elaboração de novas drogas e formas de tratamento (ZHU; THOMAS LOOK, 2016).

Outro aspecto importante da utilização desse modelo na oncologia diz respeito a sua similaridade de telômeros, estruturas que estão associadas à senescência celular e também alguns tipos de cânceres. Tanto o comprimento dessas estruturas quanto a regulação da sua atividade na célula parecem ocorrer de forma similar àquela de humanos (BARRIUSO; NAGARAJU; HURLSTONE, 2015).

Outros estudos ressaltam *D. rerio* como adequado para o estudo de condições neurológicas como as convulsões epiléticas, tendo em vista que este organismo apresentou alterações na expressão gênica e proteica similares àquelas encontradas em modelos de roedores classicamente utilizadas nos ensaios pré-clínicos (PAUDEL et al., 2020). Ainda, como sistema modelo *D. rerio* tem se mostrado uma valiosa estratégia para estudar a patofisiologia e gênese de várias doenças como: doenças renais (MORALES; WINGERT, 2017), doenças hematopoiéticas (GORE et al., 2018), desordens cerebrais (KALUEFF; STEWART; GERLAI, 2014), doenças no fígado (GOESSLING; SADLER, 2015), doenças humanas raras (ADAMSON; SHERIDAN; GRIERSON, 2018) e doenças cardiovasculares e metabólicas (GUT et al., 2017). Além disso, esse modelo tem sido útil na área da neurofarmacologia (DE ABREU et al., 2019) em estudos toxinológicos (VARGAS; SARMIENTO; VÁSQUEZ, 2015), compreensão da evolução do sistema imunológico de vertebrados (GARCÍA-MORENO et al., 2019), regeneração de órgãos (SHI et al., 2015) e entendimento do comportamento social (GENG; PETERSON, 2019).

2.3 Parte III – Predição de peptídeos inibidores da ECA-I e DPP-IV em sequências de lectinas vegetais

2.3.1 Proteínas e peptídeos terapêuticos

Proteínas e peptídeos terapêuticos são moléculas de natureza proteica que apresentam atividade biológica relevante no contexto das patologias e bem-estar metabólico. As várias funções que as proteínas exercem fazem delas grandes alvos da pesquisa moderna a fim de buscar novas atividades e potenciais aplicações (SÁNCHEZ-RIVERA et al., 2014; HAYES, 2018).

Nessa perspectiva, as proteínas de origem vegetal vem se destacando como interessantes recursos para a prospecção e identificação de novas proteínas, especialmente com potencial farmacológico (LEAL et al., 2016). As diversas aplicações das proteínas terapêuticas vegetais vêm sendo evidenciadas devido às suas propriedades anti-inflamatórias, antioxidantes, antitumoral, antiparasitária, antidiabéticas (MAESTRI; MARMIROLI; MARMIROLI, 2016).

A principal justificativa para o uso de proteínas e peptídeos como forma de tratamento resulta da sua alta especificidade, alta atividade, baixa toxicidade e o mínimo de interações inespecíficas quando administrada (RENUKUNTLA et al., 2013).

Vale ressaltar que além dos polímeros de proteínas bioativos encontradas na maioria das plantas, diversos peptídeos com relevante significado biológico são produzidos a partir da quebra dessas moléculas (HAJFATHALIAN et al., 2018). Durante a ingestão, as proteínas são quebradas enzimaticamente em tamanhos menores para que possam ser absorvidas pelo organismo, normalmente na forma de aminoácidos. No entanto, é esperado que alguns peptídeos não sejam digeridos e dessa forma serão absorvidos pelo organismo em sua forma peptídica contendo números diferentes de resíduos de aminoácidos (TOLDRÁ et al., 2018). Outro processo comum na produção desses peptídeos endogenamente é a fermentação, este processo é mediado por bactérias da flora intestinal que secretam proteases para promover a hidrólise das proteínas (HAJFATHALIAN et al., 2018).

A utilização de fármacos de natureza proteica possui diferentes formas de administração, garantindo a manutenção da estrutura primária da proteína e consequentemente sua função (JITENDRA et al., 2011). Vale ressaltar ainda, que a forma de administração das proteínas terapêuticas deve ser levada em consideração, pois sabe-se que a via oral irá degradar as proteínas em diferentes estágios (RENUKUNTLA et al., 2013). Outra forma de utilização das proteínas terapêuticas que vem se destacando é a formulação em cápsula. Este método objetiva realizar a entrega da proteína apenas em compartimentos ideais, como o intestino, preservando a atividade e o potencial terapêutico da proteína testada (KWON; DANIELL, 2016). Uma forma recentemente utilizada para efetuar a entrega de proteínas e peptídeos com atividade terapêutica é a bioencapsulação. Devido a composição da parede celular das plantas (carboidratos como a lignina e a celulose), cápsulas com essa estrutura vem sendo feitas para realizar a entrega dessas drogas no epitélio intestinal, pois sabe-se que haverá normalmente um resistência às proteases humanas, que não possuem especificidade para clivar a ligação desses açúcares da parede celular vegetal (XIAO et al., 2016).

Centenas de proteínas e peptídeos com perfil terapêutico já tiveram sua bioatividade validada e a expectativa é que esse número aumente nos próximos anos (SÁNCHEZ; VÁZQUEZ, 2017). Este cenário tem sido favorecido pelo avanço das ferramentas *in silico* e desenvolvimento de novas metodologias para prever a função de proteínas e peptídeos (MINKIEWICZ et al., 2019). Metodologias computacionais na caracterização de novas moléculas podem reduzir tempo e dinheiro gasto nos estágios inicias da pesquisa, além de gerar

dados robustos para as próximas fases da pesquisa (KRALJEVIC et al., 2004) Por exemplo, a base de dados BIOPEP (http://www.uwm.edu.pl/biochemia/index.php/pl/biopep) é utilizada para deposição e digestão de sequências peptídicas, obtenção do grau teórico de hidrólise, entre outras; ToxinPred (http://crdd.osdd.net/raghava/toxinpred/) é usado para analisar o potencial tóxico de sequências de peptídeos; e o PeptideRanker (http://distilldeep.ucd.ie/PeptideRanker/) usado para avaliar o potencial bioativo das sequências de interesse. Neste sentido, peptídeos derivados de plantas tê-se mostrado como alternativas terapêutica e nutracêutica promissoras no tratamento de algumas doenças, tendo em vista as diferentes atividades já descritas na literatura (GUANG; PHILLIPS, 2009; MAESTRI; MARMIROLI; MARMIROLI, 2016). Dentre estas atividades, as propriedades anti-hipertensiva e antibiabética dos peptídeos serão apresentadas em maiores detalhes a seguir.

2.3.1.1 Peptídeos Anti-hipertensivos

Hipertensão é uma doença caracteriza pelo controle inadequado da pressão sanguínea, com indivíduos apresentando valores de pressão arterial >140/90 mmHg. Os principais componentes que atuam na regulação da pressão sanguínea são: sistema nervoso simpático (SNS), regulação por hormônios, sistema renina-angiotensina-aldosterona, além do controle mecânico promovido pelo coração (O'SHEA; GRIFFIN; FITZGIBBON, 2017). Devido a essas elavadas taxas, muito indivíduos se tornam mais suscetíveis ao risco de desenvolver doenças cardiovasculares (ALUKO, 2015; POULTER; PRABHAKARAN; CAULFIELD, 2015). A hipertensão é uma das doenças crônicas que mais afetam as pessoas em todo o mundo (WHO, 2020). O estilo de vida é um dos principais fatores relacionados ao início da doença, bem como sua progressão e complicações (CHEUNG; LI, 2012). Além disso, características genéticas podem estar envolvidas na etiologia dessa desordem. Estudos têm mostrado que as variantes genéticas são fundamentais para prever a suscetibilidade de desenvolver tais doenças (ONG et al., 2008).

A pressão sanguínea é controlada principalmente pelo sistema renina-angiotensina-aldosterona (RAA). Quando a pressão sanguínea está baixa, o rim expressa a proteína renina na sua forma inativa chamada prorenina que será posteriormente clivada para tornar-se biologicamente ativa (PATEL et al., 2017). A renina por sua vez desempenha um papel importante no início do sistema RAA pois é responsável pela conversão do angiotensinogênio para angiotensina I. Em seguida, a angiotensina I será clivada pela ECA-I formando como produto a angiotensina-II (MIRABITO COLAFELLA; BOVÉE; DANSER, 2019). Esta, por sua vez, atua como um potente vasoconstritor e retentor de sal. A produção de angiotensina-II

leva à vasoconstrição de veias e artérias, elevando dessa forma a pressão sanguínea (NATESH et al., 2003). Um dos eventos finais desse sistema é a expressão de aldosterona, que funcionára regulando a concentração dos íons sódio e potássio (AMES; ATKINS; PITT, 2019). Diferentes inibidores para as enzimas desse sistema têm sido identificados, e estes atuam impedindo principalmente a formação de angiotensina-II (HE; LIU; MA, 2013).

ECA-I é uma dipeptidil carboxidase do grupo das metaloproteinases de zinco que participa do sistema RAA e tem sido alvo farmacológico para o tratamento da hipertensão há muitas décadas (VUKIC et al., 2017). ECA-I atua no sistema RAA transformando a angiotensina-I (decapeptídeo) em angiotensina-II (octapeptídeo) através da remoção dos resíduos His-Leu na porção C-terminal (FOUNTAIN; LAPPIN, 2018). Além disso, ECA-I também é responsável pela degradação de bradicinina, uma vasodilator que desempenha papéis importantes na inflamação (PIRAHANCHI; SHARMA, 2019). Estruturalmente, a ECA-I tem três bolsões de sítio ativo bem caracterizado: S1 (ALA_{354}, GLU_{384} and TYR_{523}), S2 (GLN_{281}, HIS_{353}, LYS_{511}, HIS_{513} and $TYR5_{20}$) and S1'(GLU_{162}) (WU et al., 2015).

Peptídeos inibidores de ECA-I foram identificados em uma ampla gama de espécies de plantas (DASKAYA-DIKMEN et al., 2017). Os inibidores de ECA-I de origem natural ainda são uma alternativa de baixo custo com menos efeitos colaterais quando comparados aos anti-hipertensivos mais comumente usados, tais como captopril e lisinopril (ERDMANN; CHEUNG; SCHRÖDER, 2008; FITZGERALD; MEISEL, 2000).

Entre as principais características compartilhadas pelos peptídeos inibidores da ECA-I estão a sequência, composição de aminoácidos e tamanho da cadeia (DASKAYA-DIKMEN et al., 2017). Os peptídeos inibidores de ECA-I geralmente têm entre 2 e 12 resíduos de aminoácidos e peso molecular inferior a 3 kDa (LÓPEZ-FANDIÑO; OTTE; VAN CAMP, 2006). Peptídeos com potencial inibitório de ECA-I de tamanhos maiores podem ter sua atividade reduzida devido à dificuldade encontrada para se ligar ao sítio ativo da enzima (NATESH et al., 2003). Embora os tripeptídeos geralmente tenham maiores atividades inibitórias da ECA-I, os dipeptídeos podem ter sua atividade aumentada (ou diminuída) se houver a adição de um aminoácido específico à sua porção N-terminal, transformando-se em tripeptídeos inibidores (LI et al., 2004).

Os peptídeos inibidores de ECA-I apresentam forte hidrofobicidade em suas cadeias e esta característica tem sido fortemente associada ao seu mecanismo inibitório, principalmente quando os aminoácidos Trp, Phe, Glx ou Pro estão presentes na porção C-terminal (CHEUNG et al., 2009). A presença de aminoácidos aromáticos (como triptofano, tirosina e fenilalanina)

e cadeias laterais ramificadas na porção COOH-terminal dos peptídeos foram relatadas como características importantes encontradas em peptídeos inibidores de ECA-I, enquanto a porção NH_2-terminal geralmente tem aminoácidos ramificados (GUANG; PHILLIPS, 2009; LI et al., 2004).

Os peptídeos inibidores da ECA-I também apresentam efeito imunomodulador quando os peptídeos possuem arginina na porção C-terminal, podendo se ligar a receptores de células T e modular a expressão de linfocinas (MEISEL, 1997; SANTIAGO-LÓPEZ et al., 2016). Sequências ricas em peptídeos hidrofílicos reduzem o acesso dessas sequências ao sítio ativo da enzima e consequentemente reduzem a atividade inibitória (LI et al., 2004). O tamanho desses peptídeos tem uma forte influência na forma como eles são entregues às células porque a taxa de absorção de pequenos peptídeos (di e tripeptídeos) é maior do que a taxa de absorção de aminoácidos livres (WEBB, 1990). Embora não seja comum, tem sido sugerido que mesmo pequenas quantidades de peptídeos bioativos na dieta podem ter um efeito biológico (ROBERTS et al., 1999). Outro mecanismo de inibição proposto para ECA-I é a quelação do átomo de zinco presente no sítio ativo (SUETSUNA, 1998). A quelação de átomos de zinco mediada por peptídeo ocorre principalmente quando a carga do peptídeo é negativa devido à presença de aminoácidos como Asp e Glu, criando interações iônicas com o metal necessário para a atividade catalítica da enzima (ALUKO, 2015).

Para exemplificar os peptídeos anti-hipertensivos, temos o peptídeo Leu-Leu obtido a partir do hidrolisado de proteína de soro de leite que é capaz de se ligar aos aminoácidos que compõem o sítio ativo da ECA-I e estabilizar sua ligação por meio do hidrogênio. Essa interação resultou em uma alta interação peptídeo-enzima, evidenciada por uma baixa energia de ligação (PAN et al., 2012). Peptídeos anti-hipertensivos das proteínas do mel mostraram uma potente inibição da ECA-I através da interação por ligações de hidrogênio com o bolsão S1 e S2 da enzima (TAHIR et al., 2020). Como inibidores não competitivos de ECA-I, Ala-Val-Lys-Val-Leu, Tyr-Leu-Val-Arg e Thr-Leu-Val-Gly-Arg de *Corylus heterophylla* (avelã) têm a capacidade de se ligar a locais diferentes do local ao qual o substrato enzimático natural se liga e, assim, inibir a formação do complexo enzima-substrato (LIU et al., 2018a). Análises *in silico* revelaram que a digestão da enzima RuBisCO gera vários peptídeos com bioatividade relevante, principalmente antioxidantes e inibidores de ECA-I e DPP-IV (UDENIGWE; GONG; WU, 2013).

O peptídeo Thr-Asn-Leu-Asp-Trp-Glx das sementes de *Ginkgo biloba* também foi capaz de inibir a ECA-I ao se ligar ao átomo de zinco, criando ligações de hidrogênio e forças

de Van der Waals com aminoácidos presentes nos bolsões S1 e S2. Por outro lado, o peptídeo Arg-Ala-Asp-Phe-Tyr de *G. biloba* embora apresente os mesmos tipos de interação entre os aminoácidos, apenas o bolsão S2 ativo está envolvido na inibição (MA et al., 2019). A atividade anti-hipertensiva de peptídeos obtidos de *Gracilariopsis lemaneiformis* (Rhodophyta), tem mostrado que ambas as sequências atuam por inibição não competitiva, formando ligações de hidrogênio principalmente com resíduos de aminoácidos presentes nos bolsões S1 e S2 (DENG et al., 2018).

2.3.1.2 Peptídeos Antidiabéticos

Diabetes tipo 2 é uma desordem metabólica multifatorial caracterizada pelo aumento dos níveis glicêmicos de forma crônica em seus portadores, sendo em muitos casos acompanhado da deficiência na secreção da insulina (GUTHRIE; GUTHRIE, 2004). De acordo com dados da Organização Mundial da Saúde (OMS), estima-se que existam cerca de 422 milhões de pessoas vivendo com diabetes mellitus no mundo (WHO, 2020). Embora amplamente distribuída no mundo, atualmente China, Índia e Estados Unidos são os países com o maior número de pacientes diabéticos. Além disso, o número crescento de novos casos ao longo dos anos alcançou em 2015 o número de diabéticos previsto para o ano de 2030. Grande parte dessa mudança deve-se a fatores como estilo de vida sedentário, hábitos alimentares não saudáveis, histórico familiar, status sócio-econômico, idade e fatores genéticos (GUTHRIE; GUTHRIE, 2004; HURTADO; VELLA, 2019; ZHENG; LEY; HU, 2018).

A obesidade tem se destacado com um dos principais fatores de risco para o surgimento da diabetes e um dos seus efeitos no metabolismo corresponde à diminuição na eficiência do transporte de glicose e alterações na sensibilidade da insulina sobre seus receptores, provocando nos tecidos resistência a esse hormônio (MALONE; HANSEN, 2019). A resistência insulínica é um evento comum na progressão dessa doença e, em muitos casos, o acúmulo de tecido adiposo pode interferir no metabolismo e secreção de substâncias que regulam as funções da célula (AL-GOBLAN; AL-ALFI; KHAN, 2014; RODRIGUES et al., 2019). As complicações que caracterizam a diabetes e podem acompanhar a evolução dessa doença incluem a cetose diabética, retinopatia, neuropatia e nefropatia diabética, assim como outras complicações micro e macrovasculares que são na maior parte decorrentes de eventos hiperglicêmicos não controlados (FITE; LAKE; HANFORE, 2019; FOWLER, 2008; WU, 2015).

Biomarcados não genéticos têm sido utilizados há bastante tempo para a avaliação da diabetes, como, por exemplo, a avaliação de HbA1c e hormônios endócrinos envolvidos na diabetes. No entanto, os biomarcadores não-genéticos como micro-RNAs, embora não

apresentem resultados sólidos na literatura, podem ser no futuro uma alternativa mais personalizada de avaliação da doença (LAAKSO, 2019). A diabetes mellitus do tipo 2 pode ser dividida em cinco estágios: estágio 1 - pré-diabetes; estágio 2 - diabetes sem complicações; estágio 3 - diabetes com complicações moderadas; estágio 4 - diabetes com deficiência absoluta de insulina e estágio 5 - diabetes com complicações sérias (WU, 2015).

Além do hormônio insulina, que desempenha um papel fundamental na patofisiologia da diabetes, outros hormônios de grande relevância no contexto da diabetes são as incretinas (DEACON; AHRÉN, 2011). Estes hormônios são produzidos no trato gastrointestinal e estima-se que as incretinas estejam envolvidos na homeostase da glicose, respondendo a quase 50% da liberação de insulina (TSENG; ZHANG; WOLFE, 1999). As duas principais incretinas GIP ("Glucose-dependent insulinotropic polypeptide") e GLP-1 ("Glucagon-like peptide-1") apresentam níveis sanguíneos baixos em indivíduos em jejum e níveis aumentados quando ocorre a ingestão de alimentos que elevem os níveis glicêmicos (NAUCK; MEIER, 2018).

Estima-se que que GIP e GLP-1 apresentem uma meia-vida de 7 e 2 minutos na corrente sanguínea, embora não seja conhecido se o tempo de vida plasmática influencie na sua atividade insulinotrópica (SHUBROOK et al., 2011). O hormônio GIP é produzido pelas células-K na porção proximal do intestino, especialmente no duodeno. O hormônio GLP-1 é produzido pelas células-L na porção mais distal do intestino, como o íleo (DEACON; AHRÉN, 2011). GIP e GLP-1 estimulam a secreção de insulina principalmente através de receptores localizados nas células β-pancreáticas, além disso, o GLP-1 tem sido implicado na proliferação e diferenciação de células β pancreáticas (BUTEAU et al., 1999).

Os receptores das incretinas são receptores acoplados à proteína G e a ligação desses hormônios desencadeia uma cascata de sinalização que resulta em níveis elevados de cálcio intracelular e cAMP. Essa cascata libera insulina pré-formada em pequenos grânulos nas células β-pancreáticas através de exocitose (HOLST; GROMADA, 2004; POWER et al., 2014). As incretinas são hormônios importantes no contexto da diabetes, tem sido sugerido que pacientes com grau avançado da doença expressam e secretam GLP-1 em menor quantidade que indivíduos saudáveis (NAUCK; MEIER, 2018).

A inibição da enzima DPP-IV como estratégia para manter os níveis de insulina em níveis adequados continua sendo o assunto de pesquisas recentes no campo da farmacologia (GAO et al., 2015). As incretinas GIP e GLP-1 são os principais alvos da enzima dipeptidil peptidase-IV (DPP4). Essa enzima é uma serino-peptidase que regula a concentração de incretina no corpo auxiliando na homeostase da glicose sanguínea, principalmente pela

regulação de insulina e glucagon (MCINTOSH et al., 2005). DPP-IV atua enzimaticamente clivando aminoácidos que estejam após resíduos de alanina na posição 2 de uma sequência, principalmente na porção N-terminal (PATIL et al., 2015). Devido aos efeitos fisiológicos envolvendo essa enzima, a inibição de DPP-IV tem sido implicada como uma boa estratégia para manter os níveis insulínicos elevados e continua sendo alvo de importantes estudos na descoberta de novos fármacos antidiabéticos (LU et al., 2019).

Nesse contexto, os peptídeos inibidores da DPP-IV surgiram nesse cenário como uma alternativa ao uso dos antidiabéticos tradicionais. Considerando que a elevação de cAMP atua em diferentes mecanismos na célula, é possível que outras vias de sinalização como a MAP quinase e as vias da fosfatidilinositol 3-quinase / proteína quinase B também estejam envolvidas na ativação dos receptores de incretinas (HOLST; GROMADA, 2004). Também é sugerido que a via da fosfatidilinositol 3-quinase ativada pela incretina é responsável pela proliferação de células beta no pâncreas devido ao aumento dos níveis de PDX-1, um importante fator de transcrição envolvido no desenvolvimento do pâncreas, e na expressão de genes como transportadores de insulina, glucosinase e GLUT-2 (BUTEAU et al., 1999). O GLP-1 tem sido implicado na redução da apoptose em células pancreáticas, evidenciado pela baixa condensação nuclear, superexpressão do gene antiapoptótico Bcl-2 e subexpressão da caspase-3 (FARILLA et al., 2003).

Os inibidores DPP-IV são principalmente inibidores competitivos, bloqueando a ligação da enzima e seu substrato natural (PATIL et al., 2015). No entanto, foi observado que os inibidores da DPP-IV de longa ação podem atuar como inibidores não competitivos, ligando-se a sítios secundários e modificando a ação da enzima (NONGONIERMA; FITZGERALD, 2013; POWER et al., 2014).

Diferentes fontes nutricionais já apresentam potencial inibitório para esta enzima como alternativa terapêutica no tratamento do Diabetes (PATIL et al., 2015; POWER et al., 2014). Embora várias frações de hidrolisados de proteína de soro de leite com cerca de 3-10 kDa possam inibir DPP-IV, os peptídeos que mostraram as atividades inibitórias mais altas têm um peso molecular inferior a 3 kDa (KONRAD et al., 2014).

Estudos *in vivo* demonstraram que o hidrolisado de proteína zeína de milho, contendo peptídeos inibidores de DPP-IV, foi capaz de aumentar a concentração de insulina no sangue em até 6,3 vezes em comparação com os ratos do grupo controle (MOCHIDA; HIRA; HARA, 2010). Embora menos comum, DPP-IV pode ser inibida por aminoácidos livres, com exceção da leucina, metionina e triptofano (POWER et al., 2014). Porém, estudos realizados com a

proteína zeína do milho, mostraram que os aminoácidos livres obtidos a partir dos hidrolisados são menos eficazes na promoção da secreção de GLP-1 pela inibição da DPP-IV (MOCHIDA; HIRA; HARA, 2010). *Phalaris canariensis* (capim-canário), que pertencente à família das gramíneas, apresentou peptídeos inibitórios para ambas as enzimas, DPP-IV e ECA-I, encriptados em proteínas de armazenamento (ESTRADA-SALAS et al., 2014). Vários peptídeos inibidores de DPP-IV de diferentes fontes alimentares, como ovos de galinha, carne bovina, soja, cevada, dentre outros, já foram preditos usando abordagens computacionais (LACROIX; LI-CHAN, 2012).

Em hidrolisados preparados a partir de sementes de *A. hipocondriacus* L. observou-se que os peptídeos inibidores de DPP-IV apresentam tamanhos menores que 10 kDa. Além disso, esses peptídeos interagem com DPP-IV principalmente por meio de ligações de hidrogênio e interações hidrofóbicas (VELARDE-SALCEDO et al., 2013). Como visto para os peptídeos obtidos a partir do hidrolisado de suco de cozimento de atum, a maioria dos peptídeos com atividade inibitória da DPP-IV tem aminoácidos hidrofóbicos, como Val, Leu, Ile, Trp, Phe e Cys (HUANG et al., 2012).

Vários outros peptídeos inibidores já foram identificados em *A. hypochondriacus*, porém os inibidores DPP-IV e ECA-I têm a maior frequência de ocorrência (SILVA-SÁNCHEZ et al., 2008). Peptídeos inibidores DPP-IV com cerca de 300 Da foram encontrados nas sementes de *Oryza sativa* (arroz), com Ile-Pro / Met-Pro e Pro-Ile / Gly-Pro mostrando a maior e menor atividade inibitória DPP-IV, respectivamente (HATANAKA et al., 2012). Outros aminoácidos hidrofóbicos e hidrofílicos também podem estar presentes, embora o papel dos aminoácidos hidrofílicos ainda não seja bem compreendido (NONGONIERMA; FITZGERALD, 2019). O GLP-1 e o GIP são hormônios com atividade insulinotrópica, que perdem essa atividade após a inibição pela DPP-IV. Desta forma, a inibição da DPP-IV leva a uma maior estimulação da insulina enquanto os níveis de glucagon são reduzidos, promovendo um balanço positivo na homeostase da glicose em pacientes diabéticos (POWER et al., 2014).

Um heptapeptídeo interagindo com os aminoácidos da enzima DPP-IV foi identificado no colostro de búfala e o aminoácido ARG125 parece exercer uma grande influência nesta ligação, uma vez que a Diprotina (A), um conhecido inibidor da DPP-IV, também requer esta interação em sua inibição (ASHOK; BRIJESHA; APARNA, 2019). O peptídeo TTAGLLE isolado de feijão caupi (*Vigna unguiculata*) foi o único entre várias sequências peptídicas capaz de interagir com o sítio ativo e os bolsões S2/S3 por meio principalmente de interações eletrostáticas, embora não tenha apresentado os melhores valores de energia de ligação livre

(DE SOUZA ROCHA et al., 2014). Como esperado para a maioria dos peptídeos inibidores da DPP-IV, os peptídeos extraídos do amaranto (*A. hypochondriacus* L.) interagem com o sítio ativo da enzima por meio de interações hidrofóbicas e ligações de hidrogênio. No entanto, em uma proporção muito pequena, alguns resíduos de aminoácidos positivos, como histidina e arginina, também foram capazes de interagir com a enzima (VELARDE-SALCEDO et al., 2013). Dentre vários peptídeos obtidos a partir da hidrólise da napina, principal a proteína da colza (*Brassica napus*), observou-se que o número de ligações de hidrogênio e as interações totais entre os aminoácidos do complexo peptídeo-enzima não tiveram relação com a intensidade de inibição enzimática e os diferentes peptídeos obtidos conseguiram interagir nos bolsões S1, S2 e S3 da enzima DPP-IV (XU et al., 2019).

3. REFERÊNCIAS

A. RIBEIRO, K. et al. ?lpha-2 Adrenergic and Opioids Receptors Participation in Mice Gastroprotection of Abelmoschus esculentus Lectin. **Current Pharmaceutical Design**, v. 22, n. 30, 2016.

ABREU, T. M. et al. Antinociceptive and Anti-inflammatory Activities of the Lectin from Marine Red Alga Solieria filiformis. **Planta Medica**, 2016.

ABUL K, A. M.; ANDREW H. LICHTMAN MD, P.; SHIV PILLAI MBBS, P. **ABBAS Celular and Molecular Immunology** . [s.l: s.n.]. v. 9

ADAMSON, K. I.; SHERIDAN, E.; GRIERSON, A. J. Use of zebrafish models to investigate rare human disease. **Journal of Medical Genetics**, v. 55, n. 10, 2018.

AL-GOBLAN, A. S.; AL-ALFI, M. A.; KHAN, M. Z. Mechanism linking diabetes mellitus and obesity. **Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy**, v. 7, p. 587–591, 2014.

ALUKO, R. E. **Structure and function of plant protein-derived antihypertensive peptidesCurrent Opinion in Food Science**, 2015.

ALVES, S. M. et al. The efficacy of a lectin from Abelmoschus Esculentus depends on central opioid receptor activation to reduce temporomandibular joint hypernociception in rats. **Biomedicine and Pharmacotherapy**, v. 101, 2018.

AMES, M. K.; ATKINS, C. E.; PITT, B. **The renin-angiotensin-aldosterone system and its suppressionJournal of Veterinary Internal Medicine**, 2019.

AMORIM, R. M. F. et al. The leguminous lectin of Lonchocarpus araripensis promotes antinociception via mechanisms that include neuronal inhibition of Na+ currents. **Inflammation Research**, v. 65, n. 9, 2016.

ASHOK, A.; BRIJESHA, N.; APARNA, H. S. Discovery, synthesis, and in vitro evaluation of a novel bioactive peptide for ACE and DPP-IV inhibitory activity. **European Journal of Medicinal Chemistry**, v. 180, 2019.

BARRE, A. et al. Structure-function relationship of monocot mannose-binding lectins. **Plant Physiology**, v. 112, n. 4, 1996.

BARRÉ-SINOUSSI, Françoise; MONTAGUTELLI, Xavier. Animal models are essential to biological research: issues and perspectives. **Future science OA**, v. 1, n. 4, 2015.

BARRIUSO, J.; NAGARAJU, R.; HURLSTONE, A. **Zebrafish in oncologyAging**, 2015.

BERIN, M. C. Pathogenesis of IgE-mediated food allergy. **Clinical and Experimental Allergy**, v. 45, n. 10, 2015.

BETANCUR, R. R. et al. **Phylogenetic classification of bony fishesBMC Evolutionary Biology**, 2017.

BEZERRA, Gustavo Arruda et al. Structural studies of an anti-inflammatory lectin from Canavalia boliviana seeds in complex with dimannosides. **PloS one**, v. 9, n. 5, p. e97015, 2014.

BHAGYAWANT, Sameer S. et al. Chickpea (Cicer arietinum L.) Lectin Exhibit Inhibition of ACE-I, α-amylase and α-glucosidase Activity. **Protein and peptide letters**, v. 26, n. 7, p. 494-501, 2019.

BRADEN, B. C.; POLJAK, R. J. Structural features of the reactions between antibodies and protein antigens. **The FASEB Journal**, v. 9, n. 1, 1995.

BUTEAU, J. et al. Glucagon-like peptide-1 promotes DNA synthesis, activates phosphatidylinositol 3-kinase and increases transcription factor pancreatic and duodenal homeobox gene 1 (PDX-1) DNA binding activity in beta (INS-1)- cells. **Diabetologia**, v. 42, n. 7, 1999.

CAMPOS, J. K. L. et al. Anti-inflammatory and antinociceptive activities of Bauhinia monandra leaf lectin. **Biochimie Open**, v. 2, 2016.

CERRATO, A. et al. Identification and antimicrobial activity of medium-sized and short peptides from yellowfin tuna (Thunnus albacares) simulated gastrointestinal digestion. **Foods**, v. 9, n. 9, 2020.

CHAEMSAWANG, W. et al. The Effect of Okra (Abelmoschus esculentus (L.) Moench) Seed

Extract on Human Cancer Cell Lines Delivered in Its Native Form and Loaded in Polymeric Micelles. **International Journal of Biomaterials**, v. 2019, 2019.

CHANDRA, N. R. et al. Structural similarity and functional diversity in proteins containing the legume lectin fold. **Protein Engineering**, v. 14, n. 11, 2001.

CHEN, H. et al. In Vitro and in Vivo Immunomodulatory Activity of Okra (Abelmoschus esculentus L.) Polysaccharides. **Journal of Medicinal Food**, v. 19, n. 3, 2016.

CHEUNG, B. M. Y.; LI, C. **Diabetes and hypertension: Is there a common metabolic pathway?Current Atherosclerosis Reports**, 2012.

CHEUNG, I. W. Y. et al. Angiotensim-I converting enzyme inhibitory activity of hydrolysates from oat (Avena sativa) proteins by in silico and in vitro analyses. **Journal of Agricultural and Food Chemistry**, 2009.

CONSTABLE, A. et al. History of safe use as applied to the safety assessment of novel foods and foods derived from genetically modified organisms. **Food and Chemical Toxicology**, v. 45, n. 12, p. 2513-2525, 2007.

COVÉS-DATSON, E. M. et al. Inhibition of Ebola Virus by a Molecularly Engineered Banana Lectin. **PLoS Neglected Tropical Diseases**, v. 13, n. 7, 2019.

CRUMPTON, M. J. Protein Antigens: The Molecular Bases of Antigenicity and Immunogenicity. In: **The Antigens**. [s.l: s.n.].

DAMAYANTHI, E.; RIMBAWAN, R.; HANDHARYANI, E. Potential of okra (Abelmoschus esculentus L.) extract to reduce blood glucose and malondialdehyde (MDA) liver in streptozotocin-induced diabetic rats. **Jurnal Gizi dan Pangan**, v. 13, n. 1, 2018.

DASKAYA-DIKMEN, C. et al. Angiotensin-I-converting enzyme (ACE)-inhibitory peptides from plants. **Nutrients**, v. 9, n. 4, p. 1–19, 2017.

DATTA, D. et al. Macromolecular properties and partial amino acid sequence of a Kunitz-type protease inhibitor from okra (Abelmoschus esculentus) seeds. **Journal of Biosciences**, v. 44, n. 2, 2019.

DAVIES, M. S. et al. The Use of Plant Genetic Resources. **The Journal of Ecology**, v. 77, n.

4, 1989.

DE ABREU, M. S. et al. **Neuropharmacology, pharmacogenetics and pharmacogenomics of aggression: The zebrafish modelPharmacological Research**, 2019.

DE BRITO MARQUES RAMOS, D. et al. Evaluation of antitumor activity and toxicity of Schinus terebinthifolia leaf extract and lectin (SteLL) in sarcoma 180-bearing mice. **Journal of Ethnopharmacology**, v. 233, 2019.

DE CAMPOS ZANI, S. C.; WU, J.; CHAN, C. B. **Egg and soy-derived peptides and hydrolysates: A review of their physiological actions against diabetes and obesityNutrients**, 2018.

DE FREITAS PIRES, A. et al. Opioid-like antinociceptive effects of oral administration of a lectin purified from the seeds of Canavalia brasiliensis. **Fundamental and Clinical Pharmacology**, v. 27, n. 2, 2013.

DE FREITAS PIRES, A. et al. Lectin purified from Lonchocarpus campestris seeds inhibits inflammatory nociception. **International Journal of Biological Macromolecules**, v. 125, 2019.

DE HOFF, P. L.; BRILL, L. M.; HIRSCH, A. M. **Plant lectins: The ties that bind in root symbiosis and plant defenseMolecular Genetics and Genomics**, 2009.

DE MEJÍA, E. G.; PRISECARU, V. I. Lectins as bioactive plant proteins: A potential in cancer treatment. **Critical Reviews in Food Science and Nutrition**, v. 45, n. 6, 2005.

DE SOUSA FERREIRA SOARES, G. et al. Purification and biological activities of abelmoschus esculentus seed lectin. **Protein Journal**, v. 31, n. 8, 2012.

DE SOUZA ROCHA, T. et al. Impact of germination and enzymatic hydrolysis of cowpea bean (Vigna unguiculata) on the generation of peptides capable of inhibiting dipeptidyl peptidase IV. **Food Research International**, v. 64, 2014.

DEACON, C. F.; AHRÉN, B. **Physiology of incretins in health and diseaseReview of Diabetic Studies**, 2011.

DELANEY, B. et al. Evaluation of protein safety in the context of agricultural biotechnology.

Food and Chemical Toxicology, v. 46, n. SUPPL. 2, 2008.

DELVES, P. J. et al. **Roitt's Essential Immunology 13th edition**. [s.l: s.n.].

DENG, Z. et al. Antihypertensive effects of two novel angiotensin i-converting enzyme (ace) inhibitory peptides from gracilariopsis lemaneiformis (Rhodophyta) in spontaneously hypertensive rats (SHRs). **Marine Drugs**, v. 16, n. 9, 2018.

DOREDDULA, S. K. et al. Phytochemical analysis, antioxidant, antistress, and nootropic activities of aqueous and methanolic seed extracts of ladies finger (Abelmoschus esculentus L.) in Mice. **Scientific World Journal**, v. 2014, 2014.

DOS SANTOS, A. J. C. A. et al. Schinus terebinthifolia leaf lectin (SteLL) is an immunomodulatory agent by altering cytokine release by mice splenocytes. **3 Biotech**, v. 10, n. 4, 2020.

DURAZZO, A. et al. **Abelmoschus esculentus (L.): Bioactive components' beneficial properties-focused on antidiabetic role-for sustainable health applicationsMolecules**, 2019.

E LACERDA, R. R. et al. Lectin from seeds of a Brazilian lima bean variety (Phaseolus lunatus L. var. cascavel) presents antioxidant, antitumour and gastroprotective activities. **International Journal of Biological Macromolecules**, v. 95, 2017.

EDELMAN, G. M. et al. The covalent and three-dimensional structure of concanavalin A. **Proceedings of the National Academy of Sciences of the United States of America**, v. 69, n. 9, 1972.

EL-MANZALAWY, Y.; DOBBS, D.; HONAVAR, V. G. In silico prediction of linear B-cell epitopes on proteins. In: **Methods in Molecular Biology**. [s.l: s.n.]. v. 1484.

EL-NIKHELY, N. et al. Ricin A chain from Ricinus sanguineus: DNA sequence, structure and toxicity. **Protein Journal**, v. 26, n. 7, 2007.

ENGESZER, R. E. et al. **Zebrafish in the wild: A review of natural history and new notes from the fieldZebrafish**, 2007.

ERDMANN, K.; CHEUNG, B. W. Y.; SCHRÖDER, H. **The possible roles of food-derived**

bioactive peptides in reducing the risk of cardiovascular diseaseJournal of Nutritional Biochemistry, 2008.

ESMAEILZADEH, D.; RAZAVI, B. M.; HOSSEINZADEH, H. **Effect of Abelmoschus esculentus (okra) on metabolic syndrome: A reviewPhytotherapy Research**, 2020.

ESTRADA-SALAS, P. A. et al. Characterization of antidiabetic and antihypertensive properties of canary seed (Phalaris canariensis L.) peptides. **Journal of Agricultural and Food Chemistry**, v. 62, n. 2, 2014.

FARILLA, L. et al. Glucagon-Like Peptide 1 Inhibits Cell Apoptosis and Improves Glucose Responsiveness of Freshly Isolated Human Islets. **Endocrinology**, v. 144, n. 12, 2003.

FASOLIN, Luiz H. et al. Emergent food proteins–Towards sustainability, health and innovation. **Food research international**, v. 125, p. 108586, 2019.

FIERS, Mark WEJ et al. Allermatch™, a webtool for the prediction of potential allergenicity according to current FAO/WHO Codex alimentarius guidelines. **BMC bioinformatics**, v. 5, n. 1, p. 1-6, 2004.

FIGUEIREDO, J. G. et al. Antinociceptive activity and toxicology of the lectin from Canavalia boliviana seeds in mice. **Naunyn-Schmiedeberg's Archives of Pharmacology**, v. 380, n. 5, 2009.

FITE, R. O.; LAKE, E. A.; HANFORE, L. K. **Diabetic retinopathy in Ethiopia: A systematic review and meta-analysisDiabetes and Metabolic Syndrome: Clinical Research and Reviews**, 2019.

FITZGERALD, R. J.; MEISEL, H. **Milk protein-derived peptide inhibitors of angiotensin-I-converting enzymeBritish Journal of Nutrition**, 2000.

FONTENELLE, T. P. C. et al. Lectin obtained from the red seaweed Bryothamnion triquetrum: Secondary structure and anti-inflammatory activity in mice. **International Journal of Biological Macromolecules**, v. 112, 2018.

FOUNTAIN, J. H.; LAPPIN, S. L. **Physiology, Renin Angiotensin System**. [s.l: s.n.].

FOWLER, L. A. et al. Both dietary ratio of n-6 to n-3 fatty acids and total dietary lipid are positively associated with adiposity and reproductive health in zebrafish. **Current Developments in Nutrition**, v. 4, n. 4, 2020.

FOWLER, M. J. **Microvascular and macrovascular complications of diabetesClinical Diabetes**, 2008.

FREITAS, R. S. et al. Lectin from Abelmoschus esculentus reduces zymosan-induced temporomandibular joint inflammatory hypernociception in rats via heme oxygenase-1 pathway integrity and tnf-α and il-1β suppression. **International Immunopharmacology**, v. 38, 2016.

GAAIED, S. et al. 2,4-Dichlorophenoxyacetic acid herbicide effects on zebrafish larvae: development, neurotransmission and behavior as sensitive endpoints. **Environmental Science and Pollution Research**, v. 27, n. 4, 2020.

GAITONDE, P.; BALU-IYER, S. V. In vitro immunogenicity risk assessment of therapeutic proteins in preclinical setting. **Methods in molecular biology (Clifton, N.J.)**, v. 716, 2011.

GAO, Y. et al. **Recent progress in natural products as DPP-4 inhibitorsFuture Medicinal Chemistry**, 2015.

GARCÍA-MORENO, D. et al. The zebrafish: A research model to understand the evolution of vertebrate immunity. **Fish and Shellfish Immunology**, v. 90, 2019.

GAUTAM, A. K. et al. Characterization of chickpea (Cicer arietinum L.) lectin for biological activity. **Physiology and Molecular Biology of Plants**, v. 24, n. 3, 2018.

GEMEDE, H. F. et al. Indigenous Ethiopian okra (Abelmoschus esculentus) mucilage: A novel ingredient with functional and antioxidant properties. **Food Science and Nutrition**, v. 6, n. 3, 2018.

GENG, Y.; PETERSON, R. T. **The zebrafish subcortical social brain as a model for studying social behavior disordersDMM Disease Models and Mechanisms**, 2019.

GILBERT, S. F.; BARRESI, M. J. F. DEVELOPMENTAL BIOLOGY, 11TH EDITION 2016. **American Journal of Medical Genetics Part A**, v. 173, n. 5, 2017.

GOESSLING, W.; SADLER, K. C. **Zebrafish: An Important Tool for Liver Disease ResearchGastroenterology**, 2015.

GONDIM, A. C. S. et al. The potent anti-cancer activity of Dioclea lasiocarpa lectin. **Journal of Inorganic Biochemistry**, v. 175, 2017.

GONG, T. et al. Plant Lectins Activate the NLRP3 Inflammasome To Promote Inflammatory Disorders. **The Journal of Immunology**, v. 198, n. 5, 2017.

GONZÁLEZ-MONTOYA, M. et al. Bioactive peptides from germinated soybean with anti-diabetic potential by inhibition of dipeptidyl peptidase-IV, α-amylase, and α-glucosidase enzymes. **International Journal of Molecular Sciences**, v. 19, n. 10, 2018.

GOODMAN, Richard E. et al. AllergenOnline: a peer-reviewed, curated allergen database to assess novel food proteins for potential cross-reactivity. **Molecular nutrition & food research**, v. 60, n. 5, p. 1183-1198, 2016.

GORE, A. V. et al. The zebrafish: A fintastic model for hematopoietic development and disease. **Wiley Interdisciplinary Reviews: Developmental Biology**, v. 7, n. 3, 2018.

GUANG, C.; PHILLIPS, R. D. Plant food-derived angiotensin i converting enzyme inhibitory peptides. **Journal of Agricultural and Food Chemistry**, 2009.

GUO, H. et al. Novel dipeptidyl peptidase-IV and angiotensin-I-converting enzyme inhibitory peptides released from quinoa protein by in silico proteolysis. **Food Science and Nutrition**, 2020.

GURGEL, J. T. A.; MITIDIERI, J. Estudos sôbre o quiabeiro II: efeitos da autofecundação e do cruzamento. **Anais da Escola Superior de Agricultura Luiz de Queiroz**, v. 12–13, n. 0, 1956.

GURUNG, M. et al. **Role of gut microbiota in type 2 diabetes pathophysiologyEBioMedicine**, 2020.

GUT, P. et al. Little fish, big data: Zebrafish as a model for cardiovascular and metabolic disease. **Physiological Reviews**, v. 97, n. 3, 2017.

GUTHRIE, R. A.; GUTHRIE, D. W. **Pathophysiology of Diabetes MellitusCritical Care**

Nursing Quarterly, 2004.

HAJFATHALIAN, M. et al. **Peptides: Production, bioactivity, functionality, and applicationsCritical Reviews in Food Science and Nutrition**, 2018.

HARDMAN, K. D.; AINSWORTH, C. F. Structure of Concanavalin a at 2.4-å Resolution. **Biochemistry**, v. 11, n. 26, 1972.

HATANAKA, T. et al. Production of dipeptidyl peptidase IV inhibitory peptides from defatted rice bran. **Food Chemistry**, v. 134, n. 2, 2012.

HAYES, M. **Food proteins and bioactive peptides: New and novel sources, characterisation strategies and applicationsFoods**, 2018.

HAYGLASS, K. T. Allergy: who, why and what to do about it? **Immunology Today**, v. 16, n. 11, 1995.

HE, H. L.; LIU, D.; MA, C. B. **Review on the Angiotensin-I-Converting Enzyme (ACE) Inhibitor Peptides from Marine ProteinsApplied Biochemistry and Biotechnology**, 2013.

HEGDE, R.; MAITI, T. K.; PODDER, S. K. Purification and characterization of three toxins and two agglutinins from Abrus precatorius seed by using lactamyl-Sepharose affinity chromatography. **Analytical Biochemistry**, v. 194, n. 1, 1991.

HIREMATH, K. Y. et al. A lectin with anti-microbial and anti proliferative activities from Lantana camara, a medicinal plant. **Protein Expression and Purification**, v. 170, 2020.

HNASKO, R. M. The biochemical properties of antibodies and their fragments. **Methods in Molecular Biology**, v. 1318, 2015.

HOLST, J. J.; GROMADA, J. **Role of incretin hormones in the regulation of insulin secretion in diabetic and nondiabetic humansAmerican Journal of Physiology - Endocrinology and Metabolism**, 2004.

HOLTZMAN, N. G. et al. Learning to fish with genetics: A primer on the vertebrate model Danio rerio. **Genetics**, v. 203, n. 3, 2016.

HUANG, C. N. et al. The nutraceutical benefits of subfractions of Abelmoschus esculentus in

treating type 2 diabetes mellitus. **PLoS ONE**, v. 12, n. 12, 2017.

HUANG, S. L. et al. Dipeptidyl-peptidase IV inhibitory activity of peptides derived from tuna cooking juice hydrolysates. **Peptides**, v. 35, n. 1, 2012.

HUNT, P. R. **The C. elegans model in toxicity testingJournal of Applied Toxicology**, 2017.

HURTADO, M. D.; VELLA, A. **What is type 2 diabetes?Medicine (United Kingdom)**, 2019.

ISLAM, F. et al. Pea lectin inhibits cell growth by inducing apoptosis in SW480 and SW48 cell lines. **International Journal of Biological Macromolecules**, v. 117, 2018.

ISLAM, S. et al. Neo-epitopes generated on hydroxyl radical modified glycatedigg have role in immunopathology of diabetes type 2. **PLoS ONE**, v. 12, n. 1, 2017.

ITAKURA, Y. et al. Sugar-binding profiles of chitin-binding lectins from the hevein family: A comprehensive study. **International Journal of Molecular Sciences**, v. 18, n. 6, 2017.

IVANCIUC, O. et al. Structural analysis of linear and conformational epitopes of allergens. **Regulatory Toxicology and Pharmacology**, v. 54, n. 3 SUPPL., 2009.

IVANCIUC, Ovidiu; SCHEIN, Catherine H.; BRAUN, Werner. SDAP: database and computational tools for allergenic proteins. **Nucleic acids research**, v. 31, n. 1, p. 359-362, 2003.

JAHANBANI, R. et al. Antioxidant and Anticancer Activities of Walnut (Juglans regia L.) Protein Hydrolysates Using Different Proteases. **Plant Foods for Human Nutrition**, v. 71, n. 4, 2016.

JAIN, N. et al. a Review on : Abelmoschus Esculentus 1 1 1 1. v. 462036, n. June, p. 87, 2012.

JIANDONG, L. et al. Trichosanthes kirilowii lectin ameliorates streptozocin-induced kidney injury via modulation of the balance between M1/M2 phenotype macrophage. **Biomedicine and Pharmacotherapy**, v. 109, 2019.

JIANG, Q. L. et al. Plant lectins, from ancient sugar-binding proteins to emerging anti-cancer drugs in apoptosis and autophagy. **Cell Proliferation**, v. 48, n. 1, 2015.

JITENDRA et al. **Noninvasive routes of proteins and peptides drug deliveryIndian Journal of Pharmaceutical Sciences**, 2011.

JOHANSSON, S. G. O. The discovery of IgE. **Journal of Allergy and Clinical Immunology**, v. 137, n. 6, 2016.

JUDD, W. S. et al. **Sistemática Vegetal - Um Enfoque FilogenéticoTaxon**, 2009.

JUSTIZ VAILLANT, A. A.; ZITO, P. M. **Hypersensitivity Reactions, Immediate**. [s.l: s.n.].

KALUEFF, A. V.; STEWART, A. M.; GERLAI, R. **Zebrafish as an emerging model for studying complex brain disordersTrends in Pharmacological Sciences**, 2014.

KAUR, R. et al. Glycan-dependent chikungunya viral infection divulged by antiviral activity of NAG specific chi-like lectin. **Virology**, v. 526, 2019.

KEYAERTS, E. et al. Plant lectins are potent inhibitors of coronaviruses by interfering with two targets in the viral replication cycle. **Antiviral Research**, v. 75, n. 3, 2007.

KHOSROZADEH, M.; HEYDARI, N.; ABOOTALEBI, M. The Effect of Abelmoschus Esculentus on Blood Levels of Glucose in Diabetes Mellitus. **Iranian journal of medical sciences**, v. 41, n. 3 Suppl, 2016.

KIMMEL, C. B. et al. Stages of embryonic development of the zebrafish. **Developmental Dynamics**, v. 203, n. 3, 1995.

KIMMEL, R. A.; MEYER, D. Zebrafish pancreas as a model for development and disease. **Methods in Cell Biology**, v. 134, 2016.

KIRSHNER, S. L. **Mechanisms underlying the immunogenicity of therapeutic proteins: Risk assessment and management strategiesJournal of Interferon and Cytokine Research**, 2014.

KLAFKE, G. B. et al. Assessment of Plant Lectin Antifungal Potential Against Yeasts of Major Importance in Medical Mycology. **Mycopathologia**, v. 175, n. 1–2, 2013.

KOHARUDIN, L. M.; GRONENBORN, A. M. **Antiviral lectins as potential HIV microbicidesCurrent Opinion in Virology**, 2014.

KONRAD, B. et al. The evaluation of dipeptidyl peptidase (DPP)-IV, α-glucosidase and angiotensin converting enzyme (ACE) inhibitory activities of whey proteins hydrolyzed with serine protease isolated from asian pumpkin (Cucurbita ficifolia). **International Journal of Peptide Research and Therapeutics**, v. 20, n. 4, 2014.

KOZLOVA, E. E. G. et al. Classification epitopes in groups based on their protein family. **BMC Bioinformatics**, v. 16, n. 19, 2015.

KRALJEVIC, Sandra; STAMBROOK, Peter J.; PAVELIC, Kresimir. Accelerating drug discovery: Although the evolution of '-omics' methodologies is still in its infancy, both the pharmaceutical industry and patients could benefit from their implementation in the drug development process. **EMBO reports**, v. 5, n. 9, p. 837-842, 2004.

KWON, K. C.; DANIELL, H. **Oral Delivery of Protein Drugs Bioencapsulated in Plant CellsMolecular Therapy**, 2016.

LAAKSO, M. **Biomarkers for type 2 diabetesMolecular Metabolism**, 2019.

LAALE, H. W. The biology and use of zebrafish, Brachydanio rerio in fisheries research. A literature review. **Journal of Fish Biology**, v. 10, n. 2, 1977.

LACROIX, I. M. E.; LI-CHAN, E. C. Y. Evaluation of the potential of dietary proteins as precursors of dipeptidyl peptidase (DPP)-IV inhibitors by an in silico approach. **Journal of Functional Foods**, v. 4, n. 2, 2012.

LAGARDA-DIAZ, I.; GUZMAN-PARTIDA, A. M.; VAZQUEZ-MORENO, L. **Legume lectins: Proteins with diverse applicationsInternational Journal of Molecular Sciences**, 2017.

LANZARIN, G. A. B. et al. Behavioural toxicity of environmental relevant concentrations of a glyphosate commercial formulation - RoundUp® UltraMax - During zebrafish embryogenesis. **Chemosphere**, v. 253, 2020.

LEAL, L. S. S. et al. The anti-inflammatory and antinociceptive effects of proteins extracted from Acacia farnesiana seeds. **Revista Brasileira de Plantas Medicinais**, v. 18, n. 1, 2016.

LEHRER, S. B.; HORNER, W. E.; REESE, G. Why are Some Proteins Allergenic? Implications for Biotechnology. **Critical Reviews in Food Science and Nutrition**, v. 36, n. 6, 1996.

LEITE, J. F. M. et al. Antinociceptive and anti-inflammatory effects of a lectin-like substance from Clitoria fairchildiana R. Howard seeds. **Molecules**, v. 17, n. 3, 2012.

LENGSFELD, C. et al. Glycosylated Compounds from Okra Inhibit Adhesion of Helicobacter pylori to Human Gastric Mucosa. **Journal of Agricultural and Food Chemistry**, v. 52, n. 6, 2004.

LI, G. H. et al. Angiotensin I-converting enzyme inhibitory peptides derived from food proteins and their physiological and pharmacological effects. **Nutrition Research**, 2004.

LI, M. et al. **Zebrafish Genome Engineering Using the CRISPR–Cas9 SystemTrends in Genetics**, 2016a.

LI, Y. X. et al. Antifatigue effects of ethanol extracts and polysaccharides isolated from Abelmoschus esculentus. **Pharmacognosy Magazine**, v. 12, n. 47, 2016b.

LIAO, Z. et al. Polysaccharide from Okra (Abelmoschus esculentus (L.) Moench) Improves Antioxidant Capacity via PI3K/AKT Pathways and Nrf2 Translocation in a Type 2 Diabetes Model. **Molecules**, v. 24, n. 10, 2019.

LITYŃSKA, A. et al. Comparison of the lectin-binding pattern in different human melanoma cell lines. **Melanoma Research**, v. 11, n. 3, 2001.

LIU, C. et al. Exploration of the molecular interactions between angiotensin-I-converting enzyme (ACE) and the inhibitory peptides derived from hazelnut (Corylus heterophylla Fisch.). **Food Chemistry**, v. 245, 2018a.

LIU, J. et al. Structure characterisation of polysaccharides in vegetable “okra” and evaluation of hypoglycemic activity. **Food Chemistry**, v. 242, 2018b.

LIU, Ling et al. Safety considerations on food protein-derived bioactive peptides. **Trends in Food Science & Technology**, v. 96, p. 199-207, 2020.

LÓPEZ-FANDIÑO, R.; OTTE, J.; VAN CAMP, J. **Physiological, chemical and technological**

aspects of milk-protein-derived peptides with antihypertensive and ACE-inhibitory activityInternational Dairy Journal, 2006.

LU, Y. et al. A novel dipeptidyl peptidase IV inhibitory tea peptide improves pancreatic β-cell function and reduces α-cell proliferation in streptozotocin-induced diabetic mice. **International Journal of Molecular Sciences**, 2019.

LUNA VITAL, D. A. et al. Peptides in common bean fractions inhibit human colorectal cancer cells. **Food Chemistry**, v. 157, 2014.

MA, F. F. et al. Three novel ACE inhibitory peptides isolated from ginkgo biloba seeds: Purification, inhibitory kinetic and mechanism. **Frontiers in Pharmacology**, v. 9, n. JAN, 2019.

MA, H.; O'KENNEDY, R. The structure of natural and recombinant antibodies. In: **Methods in Molecular Biology**. [s.l: s.n.]. v. 1348.

MAESTRI, E.; MARMIROLI, M.; MARMIROLI, N. **Bioactive peptides in plant-derived foodstuffsJournal of Proteomics**, 2016.

MAJD, N. E. et al. Okra (Abelmoscus esculentus) Improved Islets Structure, and Down-Regulated PPARs Gene Expression in Pancreas of High-Fat Diet and Streptozotocin-Induced Diabetic Rats. **Cell Journal**, v. 20, n. 1, 2018.

MAJD, N. E. et al. Effect of Abelmoschus esculentus powder on ovarian histology, expression of apoptotic genes and oxidative stress in diabetic rats fed with high fat diet. **Iranian Journal of Pharmaceutical Research**, v. 18, n. 1, 2019.

MALONE, J. I.; HANSEN, B. C. **Does obesity cause type 2 diabetes mellitus (T2DM)? Or is it the opposite?Pediatric Diabetes**, 2019.

MANDA, F.; TADERA, K.; AOYANA, K. Skin lesions due to okra (Hibiscus esculentus L): proteolytic activity and allergenicity of okra. **Contact Dermatitis**, v. 26, n. 2, 1992.

MARTÍNEZ LEO, E. E.; ACEVEDO FERNÁNDEZ, J. J.; SEGURA CAMPOS, M. R. **Biopeptides with antioxidant and anti-inflammatory potential in the prevention and treatment of diabesity diseaseBiomedicine and Pharmacotherapy**, 2016.

MARTINS, R. T. et al. Receptores opioides até o contexto atual. **Revista Dor**, v. 13, n. 1, 2012.

MAURER-STROH, Sebastian et al. AllerCatPro—prediction of protein allergenicity potential from the protein sequence. **Bioinformatics**, v. 35, n. 17, p. 3020-3027, 2019.

MAZALOVSKA, M.; KOUOKAM, J. C. **Lectins as promising therapeutics for the prevention and treatment of HIV and other potential coinfectionsBioMed Research International**, 2018.

MAZORRA-MANZANO, M. A.; RAMÍREZ-SUAREZ, J. C.; YADA, R. Y. **Plant proteases for bioactive peptides release: A reviewCritical Reviews in Food Science and Nutrition**, 2018.

MCCLUSKEY, B. M.; BRAASCH, I. Zebrafish phylogeny and taxonomy. In: **The Zebrafish in Biomedical Research: Biology, Husbandry, Diseases, and Research Applications**. [s.l: s.n.].

MCINTOSH, C. H. S. et al. **Dipeptidyl peptidase IV inhibitors: How do they work as new antidiabetic agents?Regulatory Peptides**, 2005.

MEISEL, H. Biochemical properties of bioactive peptides derived from milk proteins: Potential nutraceuticals for food and pharmaceutical applications. **Livestock Production Science**, 1997.

MINKIEWICZ, Piotr; IWANIAK, Anna; DAREWICZ, Małgorzata. BIOPEP-UWM database of bioactive peptides: Current opportunities. **International journal of molecular sciences**, v. 20, n. 23, p. 5978, 2019.

MIRABITO COLAFELLA, K. M.; BOVÉE, D. M.; DANSER, A. H. J. **The renin-angiotensin-aldosterone system and its therapeutic targetsExperimental Eye Research**, 2019.

MITCHELL, C. A.; RAMESSAR, K.; O'KEEFE, B. R. **Antiviral lectins: Selective inhibitors of viral entryAntiviral Research**, 2017.

MITCHELL, S. W. **Researches upon the venom of the rattlesnake : with an investigation of the anatomy and physiology of the organs concerned / By S. Weir Mitchell.** [s.l: s.n.].

MIYAKE, K.; TANAKA, T.; MCNEIL, P. L. Lectin-based food poisoning: A new mechanism of protein toxicity. **PLoS ONE**, v. 2, n. 8, 2007.

MOCHIDA, T.; HIRA, T.; HARA, H. The corn protein, zein hydrolysate, administered into the ileum attenuates hyperglycemia via its dual action on glucagon-like peptide-1 secretion and dipeptidyl peptidase-IV activity in rats. **Endocrinology**, v. 151, n. 7, 2010.

MONTE, L. G. et al. Lectin of Abelmoschus esculentus (okra) promotes selective antitumor effects in human breast cancer cells. **Biotechnology Letters**, v. 36, n. 3, 2014.

MORALES, E. E.; WINGERT, R. A. Zebrafish as a model of kidney disease. In: **Results and Problems in Cell Differentiation**. [s.l: s.n.]. v. 60.

MORENO-CELIS, U. et al. Tepary Bean (Phaseolus acutifolius) lectins induce apoptosis and cell arrest in G0/G1 by P53(Ser46) phosphorylation in colon cancer cells. **Molecules**, v. 25, n. 5, 2020.

MURPHY, K.; WEAVER, C. **Janeway's Immunobiology**. [s.l: s.n.].

NAGAE, Masamichi; YAMAGUCHI, Yoshiki. Structural Aspects of Carbohydrate Recognition Mechanisms of C-Type Lectins. **C-Type Lectins in Immune Homeostasis**, p. 147-176, 2019.

NASCIMENTO, K. S. et al. Purification of a thermostable antinociceptive lectin isolated from Andira anthelmia. **Journal of Molecular Recognition**, v. 29, n. 6, 2016.

NASIADKA, A.; CLARK, M. D. Zebrafish breeding in the laboratory environment. **ILAR Journal**, v. 53, n. 2, 2012.

NATESH, R. et al. Crystal structure of the human angiotensin-converting enzyme-lisinopril complex. **Nature**, 2003.

NAUCK, M. A.; MEIER, J. J. **Incretin hormones: Their role in health and diseaseDiabetes, Obesity and Metabolism**, 2018.

NG, T. B. et al. Purification and Characterization of a Lectin from Green Split Peas (Pisum sativum). **Applied Biochemistry and Biotechnology**, v. 177, n. 6, 2015.

NIELSEN, M.; MARCATILI, P. Prediction of antibody epitopes. In: **Methods in Molecular Biology**. [s.l: s.n.]. v. 1348.

NONGONIERMA, A. B.; FITZGERALD, R. J. Inhibition of dipeptidyl peptidase IV (DPP-IV) by proline containing casein-derived peptides. **Journal of Functional Foods**, v. 5, n. 4, 2013.

NONGONIERMA, Alice B. et al. In silico approaches to predict the potential of milk protein-derived peptides as dipeptidyl peptidase IV (DPP-IV) inhibitors. **Peptides**, v. 57, p. 43-51, 2014.

NONGONIERMA, A. B.; FITZGERALD, R. J. **Features of dipeptidyl peptidase IV (DPP-IV) inhibitory peptides from dietary proteinsJournal of Food Biochemistry**, 2019.

NWACHUKWU, I. D.; ALUKO, R. E. **Structural and functional properties of food protein-derived antioxidant peptidesJournal of Food Biochemistry**, 2019.

O'SHEA, P. M.; GRIFFIN, T. P.; FITZGIBBON, M. **Hypertension: The role of biochemistry in the diagnosis and managementClinica Chimica Acta**, 2017.

ONG, K. L. et al. Association of a polymorphism in the lipin 1 gene with systolic blood pressure in men. **American Journal of Hypertension**, 2008.

OPARIL, Suzanne et al. Hypertension. **Nat. Rev. Dis. Primers**, v. 4, 2018.

ORTAÇ, D. et al. In vivo anti-ulcerogenic effect of okra (Abelmoschus esculentus) on ethanol-induced acute gastric mucosal lesions. **Pharmaceutical Biology**, v. 56, n. 1, 2018.

OS, A. Assessment of Cytotoxic Activity of Calotropis Procera's Extracts Using Artemia Salina Assay. **Biomedical Journal of Scientific & Technical Research**, v. 18, n. 5, 2019.

OSTERNE, V. J. S. et al. Structural characterization of a lectin from Canavalia virosa seeds with inflammatory and cytotoxic activities. **International Journal of Biological Macromolecules**, v. 94, 2017.

PAN, D. et al. Studies on purification and the molecular mechanism of a novel ACE inhibitory peptide from whey protein hydrolysate. **Food Chemistry**, v. 130, n. 1, 2012.

PAN, W. L.; NG, T. B. A dimeric Phaseolus coccineus lectin with anti-oxidative, anti-

proliferative and cytokine-inducing activities. **International Journal of Biological Macromolecules**, v. 81, 2015.

PARICHY, D. M. **Advancing biology through a deeper understanding of zebrafish ecology and evolutioneLife**, 2015.

PATEL, S. et al. Renin-angiotensin-aldosterone (RAAS): The ubiquitous system for homeostasis and pathologies. **Biomedicine and Pharmacotherapy**, v. 94, 2017.

PATIL, P. et al. **Food protein-derived bioactive peptides in management of type 2 diabetesEuropean Journal of Nutrition**, 2015.

PATWARDHAN, J.; BHATT, P. Flavonoids derived from Abelmoschus esculentus attenuates UV-B induced cell damage in human dermal fibroblasts through Nrf2-ARE pathway. **Pharmacognosy Magazine**, v. 0, n. 0, 2016.

PAUDEL, Y. N. et al. Pilocarpine induced behavioral and biochemical alterations in chronic seizure-like condition in adult Zebrafish. **International Journal of Molecular Sciences**, v. 21, n. 7, 2020.

PENG, C. H. et al. Abelmoschus esculentus subfractions improved nephropathy with regulating dipeptidyl peptidase-4 and type 1 glucagon-like peptide receptor in type 2 diabetic rats. **Journal of Food and Drug Analysis**, v. 27, n. 1, 2019.

PETROPOULOS, S. et al. The chemical composition, nutritional value and antimicrobial properties of: Abelmoschus esculentus seeds. **Food and Function**, v. 8, n. 12, 2017.

PEUMANS, W. J.; STINISSEN, H. M.; CARLIER, A. R. Lectin synthesis in developing and germinating wheat and rye embryos. **Planta**, v. 156, n. 1, 1982.

PEUMANS, W. J.; VAN DAMME, E. J. M. **The role of lectins in plant defenceThe Histochemical Journal**, 1995.

PINTO-JUNIOR, V. R. et al. Purification and molecular characterization of a novel mannose-specific lectin from Dioclea reflexa hook seeds with inflammatory activity. **Journal of Molecular Recognition**, v. 29, n. 4, 2016.

PIRAHANCHI, Y.; SHARMA, S. **Physiology, Bradykinin**. [s.l: s.n.].

PIRES, A. F. et al. A novel N-acetyl-glucosamine lectin of Lonchocarpus araripensis attenuates acute cellular inflammation in mice. **Inflammation Research**, v. 65, n. 1, 2016.

POULTER, N. R.; PRABHAKARAN, D.; CAULFIELD, M. **Hypertension**. The Lancet. **Anais**...2015

POWER, O. et al. Food protein hydrolysates as a source of dipeptidyl peptidase IV inhibitory peptides for the management of type 2 diabetes. **Proceedings of the Nutrition Society**, v. 73, n. 1, 2014.

PRINCE, V. E.; ANDERSON, R. M.; DALGIN, G. Zebrafish Pancreas Development and Regeneration: Fishing for Diabetes Therapies. In: **Current Topics in Developmental Biology**. [s.l: s.n.]. v. 124.

RAVESCHOT, C. et al. **Production of bioactive peptides by lactobacillus species: From gene to applicationFrontiers in Microbiology**, 2018.

REDDY, N.; YANG, Y. **Potential of plant proteins for medical applicationsTrends in Biotechnology**, 2011.

RENUKUNTLA, J. et al. **Approaches for enhancing oral bioavailability of peptides and proteinsInternational Journal of Pharmaceutics**, 2013.

RIOS, E. J.; KALESNIKOFF, J. FcεRI expression and dynamics on mast cells. **Methods in Molecular Biology**, v. 1220, 2015.

ROBERTS, P. R. et al. Effect of chain length on absorption of biologically active peptides from the gastrointestinal tract. **Digestion**, 1999.

RODRIGUES, K. F. et al. Haptoglobin levels are influenced by Hp1–Hp2 polymorphism, obesity, inflammation, and hypertension in type 2 diabetes mellitus. **Endocrinologia, Diabetes y Nutricion**, v. 66, n. 2, 2019.

RUTHERFURD-MARKWICK, Kay J. Food proteins as a source of bioactive peptides with diverse functions. **British Journal of Nutrition**, v. 108, n. S2, p. S149-S157, 2012.

SABBAN, S.; YE, H.; HELM, B. Development of an in vitro model system for studying the interaction of equus caballus ige with its high-affinity receptor FcεRI. **Journal of Visualized**

Experiments, n. 93, 2014.

SABITHA, V. et al. Investigation of in vivo antioxidant property of Abelmoschus esculentus (L) moench. fruit seed and peel powders in streptozotocin-induced diabetic rats. **Journal of Ayurveda and Integrative Medicine**, v. 3, n. 4, 2012.

SABITHA, V.; PANNEERSELVAM, K.; RAMACHANDRAN, S. In vitro α-glucosidase and α-amylase enzyme inhibitory effects in aqueous extracts of Abelmoscus esculentus (L.) Moench. **Asian Pacific Journal of Tropical Biomedicine**, v. 2, n. 1 SUPPL., 2012.

SALAS, C. E. et al. Biologically active and antimicrobial peptides from plants. **BioMed Research International**, 2015.

SÁNCHEZ-RIVERA, L. et al. **Peptidomics for discovery, bioavailability and monitoring of dairy bioactive peptidesFood Research International**, 2014.

SÁNCHEZ, A.; VÁZQUEZ, A. **Bioactive peptides: A reviewFood Quality and Safety**, 2017.

SANKARANARAYANAN, R. et al. A novel mode of carbohydrate recognition in jacalin, a Moraceae plant lectin with a β-prism fold. **Nature Structural Biology**, v. 3, n. 7, 1996.

SANTALLA, M.; PORTIANSKY, E. L.; FERRERO, P. V. Drosophila melanogaster, an emerging animal model for the study of human cardiac diseases. **Revista Argentina de Cardiologia**, v. 84, n. 5, 2016.

SANTIAGO-LÓPEZ, L. et al. **Food-derived immunomodulatory peptidesJournal of the science of food and agriculture**, 2016.

SATO, Y. et al. High mannose-specific lectin (KAA-2) from the red alga Kappaphycus alvarezii potently inhibits influenza virus infection in a strain-independent manner. **Biochemical and Biophysical Research Communications**, v. 405, n. 2, 2011.

SCHROEDER, H. W.; CAVACINI, L. Structure and function of immunoglobulins. **Journal of Allergy and Clinical Immunology**, v. 125, n. 2 SUPPL. 2, 2010.

Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. **EFSA Journal**, v. 8, n. 7, 2010.

SHARON, N.; LIS, H. **History of lectins: From hemagglutinins to biological recognition moleculesGlycobiology**, 2004.

SHEN, D. D. et al. Physicochemical properties, phenolic profiles, antioxidant capacities, and inhibitory effects on digestive enzymes of okra (Abelmoschus esculentus) fruit at different maturation stages. **Journal of Food Science and Technology**, v. 56, n. 3, 2019.

SHI, W. C. et al. **Using zebrafish as the model organism to understand organ regenerationScience China Life Sciences**, 2015.

SHUBROOK, J. et al. **Saxagliptin: A selective DPP-4 inhibitor for the treatment of type 2 diabetes mellitusClinical Medicine Insights: Endocrinology and Diabetes**, 2011.

SILVA-SÁNCHEZ, C. et al. Bioactive peptides in amaranth (Amaranthus hypochondriacus) seed. **Journal of Agricultural and Food Chemistry**, v. 56, n. 4, 2008.

SILVA, J. G. DA et al. Plantas medicinais trazidas da África para o Brasil: africanizando o Brasil. **Anais do Congresso Nordestino de Biólogos - Congrebio 2016**, v. 6, 2016.

SILVA, H. C. et al. Purification and primary structure of a mannose/glucose-binding lectin from Parkia biglobosa Jacq. seeds with antinociceptive and anti-inflammatory properties. **Journal of Molecular Recognition**, v. 26, n. 10, 2013.

SINGH, P. et al. AN OVERVIEW ON OKRA (Abelmoschus esculentus) AND IT'S IMPORTANCE AS A NUTRITIVE VEGETABLE IN THE WORLD. **International Journal of Pharmacy and Biological Science**, v. 4, n. 2, 2014.

SONG, M. et al. Association of jacalin-related lectins with wheat responses to stresses revealed by transcriptional profiling. **Plant Molecular Biology**, v. 84, n. 1–2, 2014.

STOUT, C. C. et al. Resolving Cypriniformes relationships using an anchored enrichment approach. **BMC Evolutionary Biology**, v. 16, n. 1, 2016.

STRÄHLE, U. et al. Zebrafish embryos as an alternative to animal experiments-A commentary on the definition of the onset of protected life stages in animal welfare regulations. **Reproductive Toxicology**, v. 33, n. 2, 2012.

STREISINGER, G. et al. Production of clones of homozygous diploid zebra fish (Brachydanio

rerio). **Nature**, v. 291, n. 5813, 1981.

SUETSUNA, K. Isolation and characterization of angiotensin I-converting enzyme inhibitor dipeptides derived from Allium sativum L (garlic). **Journal of Nutritional Biochemistry**, 1998.

SUMNER, J. B.; GRALÉN, N.; ERIKSSON-QUENSEL, I. B. The molecular weights of urease, canavalin, concanavalin A and concanavalin B. **Science**, v. 87, n. 2261, 1938.

TAHIR, R. A. et al. In Silico identification of angiotensin-converting enzyme inhibitory peptides from MRJP1. **PLoS ONE**, v. 15, n. 2, 2020.

TOLDRÁ, F. et al. Generation of bioactive peptides during food processing. **Food Chemistry**, 2018.

TRAN, N.; PHAM, B.; LE, L. **Bioactive compounds in anti-diabetic plants: From herbal medicine to modern drug discoveryBiology**, 2020.

TSENG, C. C.; ZHANG, X. Y.; WOLFE, M. M. Effect of GIP and GLP-1 antagonists on insulin release in the rat. **American Journal of Physiology - Endocrinology and Metabolism**, v. 276, n. 6 39-6, 1999.

UDENIGWE, C. C.; GONG, M.; WU, S. In silico analysis of the large and small subunits of cereal RuBisCO as precursors of cryptic bioactive peptides. **Process Biochemistry**, v. 48, n. 11, 2013.

VALLES, S. et al. Exposure to low doses of inorganic arsenic induces transgenerational changes on behavioral and epigenetic markers in zebrafish (Danio rerio). **Toxicology and Applied Pharmacology**, v. 396, 2020.

VAN DAMME, E. J. M. et al. **Plant lectins: A composite of several distinct families of structurally and evolutionary related proteins with diverse biological rolesCritical Reviews in Plant Sciences**, 1998.

VAN DAMME, E. J. M. **History of plant lectin researchMethods in Molecular Biology**, 2014.

VAN DAMME, E. J. M.; LANNOO, N.; PEUMANS, W. J. Chapter 3 Plant Lectins. In:

Advances in Botanical Research. [s.l: s.n.]. v. 48.

VAN HOLLE, S.; VAN DAMME, E. J. M. Messages from the past: New insights in plant lectin evolution. **Frontiers in Plant Science**, 2019.

VAN REGENMORTEL, M. H. V. **Antigenicity and immunogenicity of synthetic peptides**. Biologicals. **Anais**...2001

VANDAMME, T. **Use of rodents as models of human diseasesJournal of Pharmacy and Bioallied Sciences**, 2014.

VARGAS, R. A.; SARMIENTO, K.; VÁSQUEZ, I. C. **Zebrafish (Danio rerio): A Potential Model for Toxinological StudiesZebrafish**, 2015.

VASTA, Gerardo R.; FENG, Chiguang. F-Type Lectins: Structure, Function, and Evolution. In: **Lectin Purification and Analysis**. Humana, New York, NY, 2020. p. 225-239.

VAYSSADE, M. et al. Antiproliferative and proapoptotic actions of okra pectin on B16F10 melanoma cells. **Phytotherapy Research**, v. 24, n. 7, 2010.

VELARDE-SALCEDO, A. J. et al. In vitro inhibition of dipeptidyl peptidase IV by peptides derived from the hydrolysis of amaranth (Amaranthus hypochondriacus L.) proteins. **Food Chemistry**, v. 136, n. 2, 2013.

VERHOECKX, K. et al. **Allergenicity assessment strategy for novel food proteins and protein sourcesRegulatory Toxicology and Pharmacology**, 2016.

VERHOECKX, K. et al. The relevance of a digestibility evaluation in the allergenicity risk assessment of novel proteins. Opinion of a joint initiative of COST action ImpARAS and COST action INFOGEST. **Food and Chemical Toxicology**, v. 129, 2019.

VUKIC, V. R. et al. In silico identification of milk antihypertensive di- and tripeptides involved in angiotensin I–converting enzyme inhibitory activity. **Nutrition Research**, 2017.

WAHYUNINGSIH, S. P. A. et al. Crude polysaccharides from okra pods (abelmoschus esculentus) grown in Indonesia enhance the immune response due to bacterial infection. **Advances in Pharmacological Sciences**, v. 2018, 2018.

WALSH, G.; JEFFERIS, R. **Post-translational modifications in the context of therapeutic proteinsNature Biotechnology**, 2006.

WANG, K. et al. Differential apoptotic and mitogenic effects of lectins in Zebrafish. **Frontiers in Endocrinology**, v. 10, n. JUN, 2019.

WEBB, K. E. **Intestinal absorption of protein hydrolysis products: a review.Journal of animal science**, 1990.

WILLIAMS, T. D.; MIRBAHAI, L.; CHIPMAN, J. K. The toxicological application of transcriptomics and epigenomics in zebrafish and other teleosts. **Briefings in Functional Genomics**, v. 13, n. 2, 2014.

WONG, K. L. et al. **Bioactive proteins and peptides isolated from Chinese medicines with pharmaceutical potentialChinese Medicine (United Kingdom)**, 2014.

WONG, Jack Ho et al. New ribosome-inactivating proteins and other proteins with protein synthesis–inhibiting activities. **Applied microbiology and biotechnology**, v. 104, n. 10, p. 4211-4226, 2020.

WORBS, Sylvia et al. Differentiation, Quantification and Identification of Abrin and Abrus precatorius Agglutinin. **Toxins**, v. 13, n. 4, p. 284, 2021.

WU, Jing et al. Dipeptidyl peptidase IV (DPP IV): a novel emerging target for the treatment of type 2 diabetes. **Journal of Nanjing Medical University**, v. 23, n. 4, p. 228-235, 2009.

WU, J. et al. Lunatin, a novel lectin with antifungal and antiproliferative bioactivities from Phaseolus lunatus billb. **International Journal of Biological Macromolecules**, v. 89, 2016a.

WU, M. et al. A novel thyroglobulin-binding lectin from the brown alga Hizikia fusiformis and its antioxidant activities. **Food Chemistry**, v. 201, 2016b.

WU, Q. et al. A novel angiotensin-I converting enzyme (ACE) inhibitory peptide from gastrointestinal protease hydrolysate of silkworm pupa (Bombyx mori) protein: Biochemical characterization and molecular docking study. **Peptides**, v. 68, 2015.

WU, S. L. Staging of type 2 diabetes mellitus. **Genetics and Molecular Research**, v. 14, n. 1,

2015.

XIA, F. et al. Antioxidant and anti-fatigue constituents of Okra. **Nutrients**, v. 7, n. 10, 2015.

XIAO, Y. et al. Low cost delivery of proteins bioencapsulated in plant cells to human non-immune or immune modulatory cells. **Biomaterials**, v. 80, 2016.

XU, F. et al. Identification and Quantification of DPP-IV-Inhibitory Peptides from Hydrolyzed-Rapeseed-Protein-Derived Napin with Analysis of the Interactions between Key Residues and Protein Domains. **Journal of Agricultural and Food Chemistry**, v. 67, n. 13, 2019.

YAU, T. et al. **Lectins with potential for anti-cancer therapyMolecules**, 2015.

ZHENG, Y.; LEY, S. H.; HU, F. B. **Global aetiology and epidemiology of type 2 diabetes mellitus and its complicationsNature Reviews Endocrinology**, 2018.

ZHOU, R. et al. GalNAc-Specific Soybean Lectin Inhibits HIV Infection of Macrophages through Induction of Antiviral Factors. **Journal of Virology**, v. 92, n. 6, 2017.

ZHU, S.; THOMAS LOOK, A. Neuroblastoma and its zebrafish model. In: **Advances in Experimental Medicine and Biology**. [s.l: s.n.]. v. 916.

4. ARTIGO CIENTÍFICO 1

Risk assessment of the *Abelmoschus esculentus* (okra) lectin, a candidate protein for pharmaceutical applications

Eduardo Afonso Silva Pereira[a], Louise Mangueira Lima[a], Íris Flávia Sousa Gonçalves[a], Terezinha Souza[a], José Thalles Jocelino Gomes Lacerda[c], Joanderson Pereira Cândido Silva[c], Leonardo Rogério Vieira[a], Carlos Alberto Almeida Gadelha[d], Tatiane Santi-Gadelha[c] and Davi Farias[a,*]

[a]Laboratory for Risk Assessment of Novel Technologies (LabRisk), Department of Molecular Biology, Federal University of Paraiba, 58051-900, João Pessoa, Brazil.
[c]Laboratory of Genetics and Radiobiological Biochemistry, Department of Molecular Biology, Federal University of Paraiba, 58051-900, João Pessoa, Brazil.
[d]Laboratory of Structural Proteomics, Department of Molecular Biology, Federal University of Paraiba, 58051-900, João Pessoa, Brazil.

*Corresponding author:
Prof. Davi Farias, Ph.D.
E-mail: davi@dbm.ufpb.br
Fone/Fax: +55 83 3216 7633
Orcid: 0000-0001-5438-1919

List of abbreviations:

AEL: *Abelmoschus esculentus* lectin; AOL – Allergen Online; BLAST - Basic Local Alignment Search Tool; BLOSUM - BLOcks of Amino Acid SUbstitution Matrix; CEUA - Committee on Ethical Use of Animals in Research; CDR - Carbohydrate Recognition Domain; Da – Dalton; EFSA - European Food Safet Authority; ExPASy - Expert Protein Analysis System; FAO - Food and Agriculture Organization; FET - Fish Embryo Acute Toxicity; GMO – Genetically Modified Organism; HOSU - History of Safe Use; NCBI – National Center for Biotechnology Information; NR – Non-Redundant; OECD - Organisation for Economic Co-operation and Development; SDAP - Structural Database of Allergenic Proteins; SGF - Simulated Gastric

Fluid; SIF – Simulated Intestinal Fluid; WHO - World Health Organization; ZET – Zebrafish Embryotoxicity Test.

Highlights:

- The okra lectin (AEL) presents anticancer and anti-inflammatory properties.

- AEL showed relevant identity with antinutritional and allergenic proteins.

- AEL showed *in vitro* inhibitory activity of intestinal proteases.

- AEL was susceptible to *in vitro* simulated gastrointestinal digestion.

- AEL did not cause mortality or morphological changes in zebrafish embryos.

Abstract

The *Abelmoschus esculentus* lectin (AEL) has stood out as a promising therapeutic agent in view of its anti-inflammatory, anticancer and antinociceptive properties. Thus, this study aimed to carried out an early risk assessment to gather information on the safety of AEL for pharmaceutical applications. The history of safe use showed no hazard reports for the *A. esculentus* species and AEL, while for the lectin class its toxic and/or anti-nutritional effects are described. No relevant identity of AEL full-length amino acids sequence with allergenic, toxic and/or antinutritional proteins was observed in NCBI databases. Likewise, no identity > 50% in allergen databases was found. However, the AEL sequence showed similarity with allergens (mainly serine protease inhibitors) when comparisons were made using 80 amino acids sliding window parameter (identity > 35%). AEL showed *in vitro* inhibitory activity of trypsin and chymotrypsin (90% and 97% inhibition, respectively) which were resistant to heat treatment. In contrast, the lectin had its digestion completed in the first minutes of the intestinal phase of *in vitro* sequential digestion. AEL did not cause acute adverse effects to zebrafish embryos. Taken together, the results indicate that AEL is potentially safe for further exploration in therapeutic applications.

Keywords: allergenicity, *in vitro* digestibility, plant lectin, protease inhibitor, embryotoxicity.

1. Introduction

Lectins comprise a heterogeneous class of proteins that selectively bind to carbohydrates and are present in a wide variety of plant foods, especially seeds and tubers like cereals, potatoes and beans (Hamid and Masood, 2009; Miyake et al., 2007). Research focused on lectin-based drug development has been given much attention due to the large number of their biological activities, including the improvement of renal function (Jiandong et al., 2019), antioxidant activity (Sabitha et al., 2012), antifungal (Wu et al., 2016) and anti-tumor effects (de Brito Marques Ramos et al., 2019). These molecules are also known to cause acute toxic effects when lectin-containing undercooked vegetables are ingested, which is accompanied by nausea, vomiting and diarrhea (Vasconcelos and Oliveira, 2004; Miyake et al., 2007). Despite being one of the most studied classes of proteins, the mechanism behind the toxicity of lectins remains poorly understood (Miyake et al., 2007). However, the broad profile of biological activities carried out by lectins sheds light on many medical fields as they are considered a promising therapeutic protein class.

In this context, the lectin from *Abelmoschus esculentus* (AEL) seeds has been highlighted as a promising candidate for medical and pharmaceutical applications. Pioneering studies have shown that this lectin presents anti-inflammatory and antinociceptive activity in mice, and equally to other plant lectins, presents hemagglutinating activity (De Sousa Ferreira Soares et al., 2012). In a recent study, it was reported the selective activity of AEL against the MCF-7 breast cancer cell line through a proapoptotic mechanism (Monte et al., 2014). Also regarding its potential pharmacological use, this lectin has been gaining prominence as an analgesic, antinociceptive and anti-inflammatory agent for treatment of temporomandibular disorders, in part due to its interaction with opioid receptors and reduction of pro-inflammatory cytokines (Alves et al., 2018; Freitas et al., 2016). Nevertheless, few studies have sought to assess potential undesirable effects of this lectin, since it has already been reported that the

extract of *A. esculentus* has moderate allergenicity (Manda et al., 1992) and trypsin inhibitory activity (Datta et al., 2019).

The safety of use of novel proteins, whether with a dietary or therapeutic perspective, has been carried out mainly based on the guidelines for risk assessment of allergenicity for GMOs (Genetically Modified Organisms) proposed by EFSA in 2010 (EFSA GMO Panel, 2010; Verhoeckx et al., 2019). In that context, the use of holistic approaches in the evaluation of new molecules has been of great value in generating more robust results about their safety (Crump et al., 2010). Results provided in these analysis when taken together provide a solid base of information to be used to reduce the time and costs with pre-clinical trials, also because they are limiting factors for the characterization of a new therapeutic agent (Parng et al., 2002; Kraljevic et al. 2004). At the same time, such approaches are also capable of improving *in vivo* assays, reducing the number of animals in experimental tests (Franceschi et al., 2017). Therefore, it is important that candidate proteins for drug development, and not just those originated from GMOs, are also assessed to their safety. A recently published study demonstrated the safety of the protein miraculin, commonly used as a food additive, by adopting an *in silico* and *in vitro* approach to evaluate its allergenic and toxicological potential based on the FAO/WHO and Codex Alimentarius guidelines (Tafazoli et al., 2019).

It is well known that some proteins can provoke an immune response due to cross-reactivity with endogenous proteins (Kirshner, 2014). The use of these proteins as a form of treatment deserves attention regarding the safety of use and the potential unintended effects that may arise. Much attention has been paid to multidisciplinary risk assessments especially as they have become an important tool to validate the safety of these proteins for this purpose, especially related to immunogenicity (Gaitonde and Balu-Iyer, 2011). Although proteins are attractive from the medical point of view, it is necessary to assess their risks of use through

different approaches, as it is widely reported that many allergens, toxins and antinutrients are of protein nature (Delaney et al., 2008; Farias et al., 2015).

Here, we aim to assess the safety of the lectin from *A. esculentus* seeds through assessments *in silico*, *in vitro* and *in vivo*. First, we have initially carried out a literature review to construct its history of safe use (HOSU) followed by *in silico* analysis to search similarities of the full-length amino acids sequence of AEL with those of proteins described as allergenic, toxic and/or antinutritional. *In vitro* tests were performed to evaluate the protease inhibitory activity of AEL, as well as its susceptibility to digestion and thermal stability. Finally, this lectin was assessed for its acute toxic effects by using the zebrafish embryotoxicity test (ZET).

2. Materials and Methods

2.1 *A. esculentus* (AEL) sample

A lyophilized sample of 50 mg of *A. esculentus* lectin was kindly provided by Professor Tatiane Santi-Gadelha, coordinator of the Laboratory of Biochemistry, Genetics and Radiobiology, Federal University of Paraiba (João Pessoa, Brazil), and kept refrigerated until the moment of the analysis. The isolation of AEL followed the methodology pioneering described by De Sousa Ferreira Soares et al. (2012). The AEL sample provided was >99% of purity (SDS-PAGE analysis).

2.2 History of safe use

A literature review was carried out regarding the HOSU of the *A. esculentus* species and lectins with emphasis on AEL. To guide this search, the following parameters were followed: (1) exposure to humans or animals and their respective results; (2) advantages or disadvantages associated with the use and consumption; (3) biological activities reported. For this, the PubMed database (pubmed.ncbi.nlm.nih.gov/) was accessed. To broaden this research, the

synonymy most used for the species (*Hibiscus esculentus*) was also used. The search was carried out using keywords in different combinations: *Hibiscus esculentus; Hibiscus esculentus* and allergy*; Hibiscus esculentus* and use*; Abelmoschus esculentus; Abelmoschus esculentus* and intake; *Abelmoschus esculentus* and lectin; *Abelmoschus esculentus* and toxicity; *Abelmoschus esculentus* and use; *Abelmoschus esculentus* and allergenicity; *Abelmoschus esculentus* and AEL. The search was conducted between September and November 2020.

2.3 Bioinformatics analysis

The primary amino acids sequence of AEL was provided by Prof. Tatiane Santi Gadelha from the Federal University of Paraiba, João Pessoa, Brazil (Figure 1).

An *in silico* search was carried out to assess the degree of similarity of the full-length amino acids sequence of AEL with those of proteins known to be allergenic, toxic and/or antinutritional for humans. The AEL sequence in FASTA format (Pearson; Lipman, 1988) was run against proteins deposited in the NR and Model Organisms (https://www.ncbi.nlm.nih.gov/) reference databases. The algorithm used was BLASTP+ and the scoring matrix was BLOSUM62. The alignments were carefully checked for specific details [E-value <0.01, shared identity >50%, alignment size and gap frequency (gap) <6%] to indicate the relevance of any similarity found as suggested by Sá et al. (2020). Then, the results of the alignment considered relevant were searched in the literature to find reports of adverse effects (toxicity, antinutritional and allergenicity).

Another *in silico* search was carried out to specifically assess the degree of similarity of the AEL amino acids sequence with allergenic proteins, using the criteria established by FAO/WHO (2001). The lectin sequence was compared to the allergen sequences deposited in the following databases: (1) Structural Database of Allergenic Proteins (SDAP), from the University of Texas (fermi.utmb.edu/SDAP/); (2) AllergenOnline (AOL) from the University

of Nebraska-Lincoln (allergenonline.com/) and (3) Allermatch from the Wageningen University and Research Centre (allermatch.org/). The complete sequence of AEL in FASTA format was subjected to comparisons, using as filter (cutoff) E-value < 0.1 and alignments with identity > 50% being considered relevant (Aalberse and Van Ree, 2000). AEL sequence comparisons were also performed against allergenic proteins using the parameter of 80 amino acids sliding window for detection of identity > 35%, and contiguous sequences of eight, seven or six amino acids showing 100% identity. All comparisons were run during the period from October to November 2020.

2.4 Mode of action and specificity

According to the indications of Delaney et al. (2008), a search was conducted in order to summarize the data available in the literature on the mode of action and specificity of lectins with focus on *A. esculentus* lectin. To this end, the PubMed database (ncbi.nlm.nih.gov/pubmed/) was accessed, using combinations of keywords as follows: (1) mode of action and lectins; (2) mode of action and lectins and specificity; (3) mode of action and *Abelmoschus esculentus* lectin; (4) mode of action and *Abelmoschus esculentus* lectin and specificity. The search was carried out in the period from October to November 2020.

2.5 Inhibitory activity of intestinal proteases

Evaluation of intestinal protease inhibitory activity by AEL was performed following the protocol described by Erlanger et al. (1961). Initially trypsin (0.1 mg/mL) and chymotrypsin (0.2 mg/mL) solutions were prepared using 1 mM HCl, and a 2% azocasein solution using 50 mM Tris-HCl buffer, pH 7.5, for the construction of an enzyme curve with different enzyme volumes in order to establish the most appropriate volume to be used in the evaluation of the inhibitory activity. Volumes of 10 and 15 µL for trypsin and chymotrypsin, respectively, were

established. The trypsin and chymotrypsin enzymes were incubated with 50 mM Tris-HCl buffer with 20 mM CaCl2, pH 7.5, at a constant temperature of 37 °C. To the tests and blanks were added 100 µL of AEL at a concentration of 1 mg/mL followed by an incubation for 15 min at 37 °C. At the end of this period, the 2% azocasein substrate was added to the standards and tests. Again, an incubation of this mixture was done for 15 min. Trichloroacetic acid 20% was added to stop the reaction. Finally, 2% azocasein solution was added to the blanks and the inhibitory activity promoted by AEL was measured spectrophotometrically at the wavelength of 440 nm. Inhibitory activity was calculated following the formula: Inhibitory Activity (%) = (Test Abs - White Abs) x 100 / Standard Abs. Assays were performed in quadruplicate for each test sample.

2.6 *In silico* prediction of susceptibility to digestion

An *in silico* prediction of AEL susceptibility to digestion was carried out to evaluate its resistance to major gastrointestinal proteases. Using the Peptide Mass tool from the ExPASy Bioinformatics Resource Portal (web.expasy.org/peptide_mass/), the complete AEL amino acids sequence was firstly submitted to pepsin proteolysis (pH 1.3). Then each peptide sequence obtained was subjected to a new trypsin proteolysis. The number and mass (Da) of the peptides generated were registered in each stage.

2.7 Sequential *in vitro* digestion

The AEL susceptibility to sequential *in vitro* digestion followed the protocol described by Farias et al. (2015), with slight modifications. Initially, pepsin (P7012, Sigma) and AEL were prepared separately in the concentrations of 1.0 and 2.5 mg/mL, respectively, using as a solvent an acidic solution (NaCl 34 mM, HCl 0,7% pH 1,0-2,0). Then, 250 µL of pepsin solution were mixed with AEL solution, in the proportion of 1:10 (v/v), and incubated at 37 °C

in a water bath with continuous stirring (80 rpm). The mixture remained incubated for 2 h at 37 ºC under stirring, and aliquots of 100 μL were collected at different times of incubation (1, 5, 15, 30, 60 and 120 min), and then transferred to appropriate stopping buffer (2% SDS, 10% Glycerol, 6% β-mercaptoethanol, 0.01% bromophenol blue, 200 mM DTT and 500 mM Tris-HCl pH 2.0) in the ratio of 1: 1 (v/v) followed by heating at 100 ºC for 5 min and then kept frozen until electrophoresis analysis. Then, the pH of the remaining solution was adjusted to 8.0 with 1 M Tris-HCl, and an alkaline solution of 50 mM potassium phosphate, pH 8.0 and 1 mg/mL trypsin (T4799, Sigma) in the proportion of 1:1 was added. The mixture was incubated and aliquots were collected as described, and then they were transferred to stopping buffer (3% SDS, 17% glycerol, 8.5% β-mercaptoethanol, 0.01% bromophenol blue, 170 mM DTT, 6 mM PMSF, 200 mM Tris-HCl pH 7.2). Aliquots were subjected to heating and finally were kept frozen until electrophoresis analysis. The digestibility of AEL was monitored by Tricine-SDS-PAGE (15%) (Schägger and von Jagow, 1987), stained using Coomassie Blue R-250 following the Blue silver method (Candiano et al., 2004).

2.8 Stability of the protease inhibitory activity to thermal treatment

AEL samples at a concentration of 1.0 mg/mL were incubated in a water bath at approximately 100 °C for 10, 30 and 60 min. After the different times of incubation, the samples were rapidly cooled down and kept frozen until the time of analysis. The stability of trypsin and chymotrypsin inhibitory activities of AEL heat-treated samples was assessed as described earlier (2.5). Inhibition of the inhibitory activity was calculated as follows: Inhibition (%) = 100 - Inhibitory Activity (%). Assays were performed in quadruplicate for each test sample.

2.9 Toxicity assay

2.9.1 Zebrafish embryos

The zebrafish (*Danio rerio*) embryos were provided by the zebrafish facility established at the Department of Molecular Biology, Federal University of Paraiba (João Pessoa, Brazil). Zebrafish adults (wild type strain) were kept at 26 ± 1 ºC under a 14:10 h (light:dark) photoperiod. The water quality was maintained by activated-carbon filtration, conductivity at 750 ± 50 µS, and dissolved oxygen above 95% saturation. Fishes were fed daily with commercial food (Tetra ColorBits, Germany) and *Artemia* sp. nauplii, being also monitored for abnormal behavior or disease development.

To obtain embryos, an egg trap was placed overnight in a tank containing male and female specimens (1:1 ratio) one day prior to testing. One hour after the beginning of the light cycle, eggs were collected with a Pasteur pipette and rinsed with E3 medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$ and 0.33 mM $MgSO_4$) for subsequent selection of embryos by using an inverted light microscope (100x magnification). Viable fertilized eggs were selected for embryotoxicity analysis. All experiments were conducted with zebrafish in this study were approved by the Committee on Ethical Use of Animals in Research (CEUA) of Federal University of Paraiba, Brazil, certified by number 6743030518.

2.9.2 Acute toxicity test using zebrafish embryos

The Fish Embryo Acute Toxicity (FET) test was conducted with AEL according to OECD's guideline number 236 (OECD, 2013), with modifications. Zebrafish embryos with up to 3 hpf (hours post fertilization) of age were exposed to 0.1 mg/mL of AEL (concentration that corresponds to the IC_{50} against the cancer cell line MCF7 and also that of greater solubility) solubilized in E3 medium containing 0.1 M of sodium phosphate buffer, pH 7.4. For that, it was prepared a 24-well plate containing 20 fertilized eggs (1 embryo per well in 2.0 mL of solution) exposed to the test sample and 4 embryos were exposed only to E3 medium (internal controls). Additional plates containing embryos exposed only to E3 medium (negative control) or E3

medium containing 0.1 M of sodium phosphate buffer pH 7.4 (solvent control) were also assayed. The exposure was performed for 96 h, and the embryos were analyzed every 24 h for the apical endpoints: egg coagulation; lack of somite formation; lack of detachment of the tail-bud from the yolk sac and lack of heartbeat. In the presence of any of these endpoints the embryo/larva was considered dead. The exposures were under static conditions (without renovation of test sample or controls). Observations were performed in a stereomicroscope (80x magnification) and photographed (Televal 31, Zeiss). After 96 h, surviving larvae were euthanized with eugenol and properly discarded.

The % mortality was calculated using Abbott's formula after correction for control: % mortality = (% test mortality - % test control) / 100 - % control mortality (Abbott, 1925).

3. Results and Discussion

The okra lectin, AEL, has been considered a candidate protein for application in the pharmaceutical industry as a potential therapeutic agent in the breast cancer treatment and also as an anti-inflammatory and antinociceptive agent (Monte et al., 2014; Freitas et al., 2016). However, very little is known about the presence of possible undesirable properties of this protein (*e.g*. allergenicity and toxicity), which could hamper further steps on the research and drug development. Thus, in this study, the AEL was analyzed holistically from a series of *in silico*, *in vitro* and *in vivo* analysis to gather information that attested to the safety of this molecule that may also serve as experimental basis for guidance further studies.

3.1 History of safe use

The AEL risk assessment began with search in the scientific literature to determine the HOSU of *A. esculentus* species and plant lectins with focus on AEL. The safe use of okra is consolidated due to its ancient consumption as food (Huang et al., 2017; Liao et al., 2019;

Sabitha et al., 2011). In addition, different extracts and substances isolated from various parts of the plant have numerous beneficial (pharmacological or medicinal properties) (Doreddula et al., 2014; Erfani Majd et al., 2018; Luo et al., 2018) (Table 1). We found only two reports of moderate hypersensitivity of okra extracts (Manda et al., 1992; Ueda et al 1993). According to Manda and colleagues, the hypersensitivity is possibly triggered by a non-IgE-mediated process, supporting the low presence of IgE-binding epitopes following the AEL (Manda et al., 1992). On the other hand, a moderate IgE-mediated allergenicity was observed by Ueda and colleagues (Ueda et al 1993). Regarding AEL, few reports were found and these refer to the presence of beneficial pharmacological activities to human health (Monte et al., 2014; Ribeiro et al., 2016; Freitas et al., 2016; Alves et al., 2018). However, it is reported that most lectins display toxic and/or antinutritional effects, which can be deflated with domestic heat treatment before consumption (Manda et al., 1992; Vasconcelos and Oliveira, 2004). Thermal or hydrolytic processing could not be a viable alternative to curb undesirable effects of lectins since it could modify their three-dimensional structure impairing the biological activities of interest.

3.2 Bioinformatics analysis

Another important aspect in the safety assessment of a protein is to compare its primary amino acids sequence with that of allergenic, toxic and antinutritional proteins, in order to detect the degree of identity between them (Codex Alimentarius, 2009; Madduri et al., 2012). Thus, the AEL sequence was compared to protein sequences deposited in large public databases. Here, the sequences deposited in NCBI's nr and Model Organisms databases were used. No identity of AEL with allergenic, toxic and/or antinutritional proteins was observed according to the parameters used (Table S1). AEL showed identity mostly with proteins from species of the genus *Durio, Theobroma* and *Gossypium*, but none of them have reports of toxic, antinutritional

or allergenic properties. The comparison of the AEL sequence with other proteins in the databases identified similarity with several hypothetical and putative proteins, but because they are predicted sequences the confidence to assess their potential allergen risk may be limited. Although some proteins of the *Theobroma cacao* species are known to have allergenic potential, there are few studies that confirm the allergenicity of this fruit (Menezes et al., 2012).

The allergenic potential of a protein can also be evaluated by comparing its primary sequence of amino acids with proteins deposited in specific allergen databases (FAO/WHO, 2001; Ladics, 2008; Codex Alimentarius, 2009; Ladics et al., 2011). The allergenicity prediction of AEL in the AOL, SDAP and Allermatch databases showed no identity > 50% with any allergenic protein using full FASTA comparisons (Table 2). According to Aalberse and Van Ree (2000), complete sequence alignments with identity > 50% may indicate that protein can trigger allergenic processes or cause cross-reactions with IgE antibodies. On the other hand, the AEL sequence presented similarity with allergenic proteins in the three databases accessed when comparisons were made using an 80 amino acids window and identity > 35% (Tables 2 and 3). All protease inhibitors showed similarity to *Solanum tuberosum*. In the AOL database, only one protease inhibitor was found. In the SDAP, inhibitors of cysteine and aspartic proteases were found, while in Allermatch, inhibitors of cysteine and serine proteases were identified (Table 3). According to the search parameter of six and seven contiguous amino acids in the SDAP database, four and one sequence of allergenic proteins identical to those of the AEL were found, respectively (Table S2). In the Allermatch database, two and one sequence of allergenic proteins were found in the search for six and seven contiguous amino acids. Regarding the search for sequences of eight contiguous amino acids, searches in the AOL, SDAP and Allermatch databases did not find any allergenic proteins (Table 2). However, searches for matches of short amino acid sequences (≤ 8 amino acids) to detect proteins as

potential cross-reactive allergens has been taken as a product of chance which add little value to the allergenicity prediction due to the high rate of false positives (Silvanovich et al., 2006).

The AOL, Allermatch and SDAP databases are versatile tools capable of quickly indicate the potential of a protein to be allergenic or not. These tools have been applied, for example, to investigate the allergenicity of proteins used in the development of transgenic cultures such as an osmotine from *Nicotiana tabacum*, where similarities greater than 35% with potential allergens have been identified (Sharma et al., 2011). It is possible to note that the search for IgE-binding sites in protein sequences in databases for allergens has long been used in earlier studies, and robust results have been generated from these analysis (Ivanciuc et al., 2003; Menezes et al., 2012; Wang et al., 2020). In the golden era of bioinformatics, new approaches based on high-throughput technologies have been used as alternative tools to identify allergens. It is noteworthy that, although omics approaches present a new evaluation parameter for new allergens (a field called allergomics), traditional methodologies continue to be widely used in research to detect and validate potential allergens (Wang et al., 2020).

Although the databases for allergens are based mainly on percentages of identity with other proteins deposited in banks, providing good results on the allergenicity of the proteins of interest, it is suggested that in *vitro* tests should be carried out to validate the results *in silico* in addition to certifying their biological mechanisms.

3.3 Mode of action and specificity

Lectins comprise a group of molecules that have been extensively studied since the last century, and are characterized by being able to bind to groups of sugars from other molecules. This binding ability, conferred by the carbohydrate recognition domain (CDR), gives these proteins their ability to participate into different biological events such as defense, infections, metabolism regulation and several other physiological processes (Sharon and Lis, 2004). It is

well known that lectins can fold in different conformations and to these changes are credited the ability of lectins to bind to carbohydrates and perform their great diversity of biological properties (Vijayan and Chandra, 1999).

This remarkable capacity for specific binding to sugars seems to be the initial molecular event for the development of the pharmacological activities presented by AEL, as well as responsible for its specificity for certain biological targets. AEL has been investigated for its fungicidal activity, especially against *Candida parapsilosis*. It is believed that its inhibitory activity is due to the capacity of binding to carbohydrates of the fungal cell wall, causing its inhibition (Klafke et al., 2013). This lectin also showed selective cytotoxicity against the breast cancer cell line (MCF7), causing changes in the cell membrane permeability and changes in the expression of apoptosis-related genes such as caspase-3, Bax, p21 and Bcl-2. As with other lectin-mediated effects, an interaction between AEL lectin and surface sugars of the MCF7 strain is assumed to occur because healthy cells are not affected (Monte et al., 2014).

AEL also has anti-inflammatory and antinociceptive effects, reducing the levels of TNF-α and IL-1β and activation of κ and **δ** opioid receptors (Freitas et al., 2016; Alves et al., 2018). Furthermore, this lectin has a gastroprotective effect in models induced by ethanol, probably due to its antioxidant activity and its activation of opioid receptors (Ribeiro et al., 2016).

Despite all the potential of AEL as a therapeutic agent, more studies are needed to deepen the knowledge about the mode of action, specificity of this lectin and its three-dimensional structures.

3.4 Susceptibility to digestion

In order to obtain prior information on the susceptibility of AEL to digestion, we performed an *in silico* prediction of susceptibility to pepsin digestion followed by trypsin to simulate the normal digestive process flow. The peptides generated in digestion were divided

into three groups (less than 6 amino acids, between 6 and 10 amino acids and above 10 amino acids) (Figure 2A). AEL presented several cleavage points for pepsin, producing a large number of peptides with <10 amino acids, 11 of the 28 peptides formed (Figures 2A and 2B). When these peptides were subjected to trypsin digestion, new peptides were formed, with a large increase in the number of peptides with <10 amino acids (30 of 46 formed). These results preliminarily point out that AEL can be completely digested.

To validate the predictions obtained *in silico*, we conducted *in vitro* tests to assess the digestibility of AEL samples using the sequential digestion assay to monitor the susceptibility of this lectin to common proteases of the human gastrointestinal tract. As shown in Figure 2C, after 2 h of pepsin digestion, one AEL band of approximately 14 kDa remained visible on the electrophoresis gel. However, this gastric-resistant peptide could be fully digested in just 5 min of incubation with trypsin in the intestinal phase. Likewise, no peptide derived from AEL digestion with molecular mass ≥ 3.5 kDa can be seen minutes after the begin of the intestinal phase. This data is relevant because it is known that this is the minimum size that a peptide must have to trigger an IgE-mediated immune response (FAO/WHO, 2001).

Acidity and temperature treatments are known to be indicators of allergenicity, as these conditions can alter the conformation of protein epitopes, important regions for IgE binding (Taylor and Lehrer, 1996). For example, *in vitro* evaluations showed that rice chitinase was detected in SDS-PAGE even after 60 min incubated with pepsin (Mishra et al., 2015). A similar result was found for other rice allergenic proteins, showing that although digestibility varied in simulated gastric fluid (SGF) according to pH, solubility and heat treatment conditions, all proteins were digested in simulated intestinal fluid (SIF) (Lang et al., 2015). Another example is *Phaseolus vulgaris* lectin, which was resistant to proteolysis by pepsin in the gastric phase, but in SIF the lectin bands were almost imperceptible. In addition, the allergenicity of this lectin

could be reduced when incubated at low pH, reducing the ability to bind to IgE (Zhao et al., 2019).

Here, a clear difference between *in silico* and *in vitro* results were observed. Similar divergences have been seen in other studies, showing different results obtained by *in vitro* and *in silico* tests. In most cases, this can occur due to the perfect conditions for *in silico* assays, reducing factors that decreased the optimal enzymatic catalysis usually seen in bench tests (Chatterjee et al., 2015). In other studies, *in silico* digestion was successfully used to confirm the non-allergenicity of novel proteins such as in the risk assessment study of a chimeric Cry protein which showed its safety for development of transgenic crops (Rathinam et al., 2017).

3.4 Trypsin and chymotrypsin inhibitory activities and thermal stability

The bioinformatics analysis showed that AEL has significant similarity with serine protease inhibitors. Protease inhibitors are considered classic antinutritional factors and when ingested or administered in their native form, they can cause great nutritional damage (Clemente and Del Carmen Arques, 2014). In this sense, AEL was analyzed for its ability to inhibit the activity of major serine proteases, such as trypsin and chymotrypsin.

AEL showed inhibitory activity of trypsin and chymotrypsin (90 and 97% inhibition, respectively) (Figure 3). To assess the susceptibility of these activities to heat treatment, a sample of AEL was heat treated by incubation for 10, 30 and 60 min at ~ 100 °C. As can be seen in Figure 3, the trypsin inhibitory activity was quite stable to heat treatment. Even after 1 h incubation, the lectin still succeeded to inhibit trypsin proteolytic activity by approximately 50%. Regarding chymotrypsin inhibitory activity, AEL was even more resistant. After 1 h incubation, lectin was still able to inhibit 84.4% of chymotrypsin proteolytic activity in relation to the standard. Like AEL, the Kunitz-type protease inhibitor AKPI2, purified from *Apios americana*, exhibited inhibitory activity against trypsin and chymotrypsin, although for AKPI2

the heat treatment time between 37 and 100 °C does not seem to influence the decrease of its inhibitory activity (Liu et al., 2019).

At first glance, the use of AEL orally as a therapeutic strategy may represent a considerable risk given its inhibition of intestinal proteases and resistance to treatment. However, this result is mitigated by the fact that this lectin is digested as previously shown.

3.7 Toxicity assay

Historically, most studies have used rodents as model organisms to investigate the acute toxicity of candidate molecules for agricultural and biomedical applications (Farias et al., 2015). Recently, zebrafish has stood out as an important tool for assessing the risks of therapeutic drugs due to its biological attributes (Parng et al., 2002). Among the advantages of using zebrafish embryos for toxicological studies, it is possible to highlight its rapid development, low production cost, transparent embryos and great biochemical and genetic similarity with vertebrate organisms including humans (Gonçalves et al., 2020). Fertilized zebrafish embryos were exposed to 0.1 mg/mL of AEL. This lectin did not cause mortality or morphological changes, as well as no delay in embryo/larvae developments were registered (Figure 4). In the last years, several studies have been carried out in order to understand the toxicological potential that lectins have on living beings due to their presence in a large number of plants, many of them used for consumption. Similar to the results found here, the PNA lectin (Peanut agglutinin) did not show morphological or developmental changes in zebrafish embryos at even higher higher concentrations than the one used in this study, i.e., 500 ug/mL, although it was able to stimulate cell proliferation in cell culture. On the other hand, Soybean agglutinin (SBA) and Wheat germ agglutinin (WGA) when evaluated in the same concentrations as PNA promoted pericardial edema and induction of systemic apoptosis in the body and in regions of the tail (Wang et al., 2019).

Therefore, this result contributes to the use of the acute toxicity test in fish embryos as an alternative to the use of rodents in the risk assessment of candidate proteins in preliminary risk assessments, in line with the 3R principle.

4. Conclusions

The early risk assessment of the *A. esculentus* lectin, AEL, gathered relevant information on the safety of this candidate molecule for pharmaceutical applications. The HOSU did not present convincing hazard reports, while the bioinformatics analysis showed similarity with antinutritional and allergenic proteins, especially serine protease inhibitors. AEL inhibited intestinal proteases (trypsin and chymotrypsin), even after heat treatment. However, the protein had its digestion completed in the first minutes of the intestinal phase of *in vitro* sequential digestion (pepsin followed by trypsin). In addition, the lectin did not cause acute adverse effects to zebrafish embryos in the therapeutic concentration tested. If taken together, the results indicate that AEL is potentially safety to be used for therapeutic applications.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We thank to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (Brazil) and Universidade Federal da Paraíba (João Pessoa, Brazil) for providing scholarships to the students involved for the development of the experimental activities.

REFERENCES

A. Ribeiro, K., V. Chaves, H., Mateus Pereira Filho, S., Ribeiro Pinto, I., Andressa Martins Monteiro, D., Oliveira Matos, S., Santi-Gadelha, T., Alberto de Almeida Gadelha, C., Thalles Jocelino Gomes de Lacerda, J., M. V. Aguiar, L., M. A. Pereira, K., M. B. Benevides, N., de Paulo T. Pinto, V., Cristino Filho, G., M. Bezerra, M., A. R. Silva, A., 2016. ?lpha-2 Adrenergic and Opioids Receptors Participation in Mice Gastroprotection of Abelmoschus esculentus Lectin. Curr. Pharm. Des. 22, 4736–4742. https://doi.org/10.2174/1381612822666160201152438.

Aalberse, R.C., Van Ree, R., 2000. Monoclonal antibody assays for allergens: Pick your antibodies with care! J. Allergy Clin. Immunol. 106, 625–626. https://doi.org/10.1067/mai.2000.110226

Abbott, W.S., 1925. Method of Computing the Effectiveness of an Insecticide | Journal of Economic Entomology | Oxford Academic. J. Econ. Entomol. 265–267.

Akbari, F., Shahinfard, N., Mirhoseini, M., Shirzad, H., Heidarian, E., Hajian, S., & Rafieian-Kopaei, M. (2016). Impacts of Hibiscus esculentus extract on glucose and lipid profile of diabetic rats. *Journal of nephropharmacology*, *5*(2), 80.

Alqasoumi, S. I. (2012). 'Okra'Hibiscus esculentus L.: A study of its hepatoprotective activity. *Saudi Pharmaceutical Journal*, *20*(2), 135-141.

Alves, S.M., Freitas, R.S., do Val, D.R., Vieira, L.V., de Assis, E.L., Gomes, F.I.F., Gadelha, C.A. de A., Gadelha, T.S., de Lacerda, J.T.J.G., Clemente-Napimoga, J.T., Pinto, V. de P.T., Cristino Filho, G., Bezerra, M.M., Chaves, H.V., 2018. The efficacy of a lectin from Abelmoschus Esculentus depends on central opioid receptor activation to reduce temporomandibular joint hypernociception in rats. Biomed. Pharmacother. 101, 478–484. https://doi.org/10.1016/j.biopha.2018.02.117.

Atawodi, S. E., Atawodi, J. C., Idakwo, G. A., Pfundstein, B., Haubner, R., Wurtele, G., ... &

Owen, R. W. (2009). Polyphenol composition and antioxidant potential of Hibiscus esculentus L. fruit cultivated in Nigeria. *Journal of medicinal food*, *12*(6), 1316-1320.

Baktavachalam, G.B., Delaney, B., Fisher, T.L., Ladics, G.S., Layton, R.J., Locke, M.E. h., Schmidt, J., Anderson, J.A., Weber, N.N., Herman, R.A., Evans, S.L., 2015. Transgenic maize event TC1507: Global status of food, feed, and environmental safety. GM Crops Food. https://doi.org/10.1080/21645698.2015.1054093.

Candiano, G., Bruschi, M., Musante, L., Santucci, L., Ghiggeri, G.M., Carnemolla, B., Orecchia, P., Zardi, L., Righetti, P.G., 2004. Blue silver: A very sensitive colloidal Coomassie G-250 staining for proteome analysis. Electrophoresis 25, 1327–1333. https://doi.org/10.1002/elps.200305844.

Chatterjee, A., Kanawjia, S.K., Khetra, Y., Saini, P., 2015. Discordance between in silico & in vitro analyses of ACE inhibitory & antioxidative peptides from mixed milk tryptic whey protein hydrolysate. J. Food Sci. Technol. 52, 5621–5630. https://doi.org/10.1007/s13197-014-1669-z.

Clemente, A., Del Carmen Arques, M., 2014. Bowman-Birk inhibitors from legumes as colorectal chemopreventive agents. World J. Gastroenterol. 20, 10305–10315. https://doi.org/10.3748/wjg.v20.i30.10305

Crump, Kenny S., Chao Chen, and Thomas A. Louis. "The future use of in vitro data in risk assessment to set human exposure standards: challenging problems and familiar solutions." *Environmental health perspectives* 118.10 (2010): 1350-1354. https://doi.org/10.1289/ehp.1001931

CODEX ALIMENTARIUS, 2009. Foods derived from modern biotechnology Second edition.

Datta, D., Pohlentz, G., Mondal, S., Divya, M.B., Guruprasad, L., Mormann, M., Swamy, M.J., 2019. Macromolecular properties and partial amino acid sequence of a Kunitz-type

protease inhibitor from okra (Abelmoschus esculentus) seeds. J. Biosci. 44, 1–11. https://doi.org/10.1007/s12038-019-9859-5

de Brito Marques Ramos, D., de Moura Fontes Araújo, M.T., de Lima Araújo, T.C., dos Santos Neto, O.G., e Silva, M.G., Silva, Y.A., Lira Torres, D.J., de Siqueira Patriota, L.L., de Melo, C.M.L., de Lorena, V.M.B., Guedes Paiva, P.M., Mendes, R.L., Napoleão, T.H., 2019. Evaluation of antitumor activity and toxicity of Schinus terebinthifolia leaf extract and lectin (SteLL) in sarcoma 180-bearing mice. J. Ethnopharmacol. 233, 148–157. https://doi.org/10.1016/j.jep.2019.01.011

De Sousa Ferreira Soares, G., Assreuy, A.M.S., De Almeida Gadelha, C.A., De Morais Gomes, V., Delatorre, P., Da Conceicao Simoes, R., Cavada, B.S., Leite, J.F., Nagano, C.S., Pinto, N.V., De Luna Freire Pessoa, H., Santi-Gadelha, T., 2012. Purification and biological activities of abelmoschus esculentus seed lectin. Protein J. 31, 674–680. https://doi.org/10.1007/s10930-012-9447-0

Delaney, B., Astwood, J.D., Cunny, H., Conn, R.E., Herouet-Guicheney, C., MacIntosh, S., Meyer, L.S., Privalle, L., Gao, Y., Mattsson, J., Levine, M., 2008. Evaluation of protein safety in the context of agricultural biotechnology. Food Chem. Toxicol. 46. https://doi.org/10.1016/j.fct.2008.01.045

Doreddula, S.K., Bonam, S.R., Gaddam, D.P., Desu, B.S.R., Ramarao, N., Pandy, V., 2014. Phytochemical analysis, antioxidant, antistress, and nootropic activities of aqueous and methanolic seed extracts of ladies finger (Abelmoschus esculentus L.) in Mice. Sci. World J. 2014. https://doi.org/10.1155/2014/519848

Ebrahimzadeh, M. A., Nabavi, S. M., & Nabavi, S. F. (2013). Antidepressant activity of Hibiscus esculentus L. *European review for medical and pharmacological sciences*, *17*(19), 2609-2612.

Erfani Majd, N., Tabandeh, M.R., Shahriari, A., Soleimani, Z., 2018. Okra (Abelmoscus esculentus) Improved Islets Structure, and Down-Regulated PPARs Gene Expression in Pancreas of High-Fat Diet and Streptozotocin-Induced Diabetic Rats. Cell J. 20, 31–40. https://doi.org/10.22074/cellj.2018.4819

Erlanger, B.F., Kokowsky, N., Cohen, W., 1961. The preparation and properties of two new chromogenic substrates of trypsin. Arch. Biochem. Biophys. 95, 271–278. https://doi.org/10.1016/0003-9861(61)90145-X

FAO/WHO, n.d. Evaluation of Allergenicity of Genetically Modified Foods Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology.

Farias, D.F., Viana, M.P., Oliveira, G.R., Santos, V.O., Pinto, C.E.M., Viana, D.A., Vasconcelos, I.M., Grossi-de-Sa, M.F., Carvalho, A.F.U., 2015. Food safety assessment of Cry8Ka5 mutant protein using Cry1Ac as a control Bt protein. Food Chem. Toxicol. 81, 81–91. https://doi.org/10.1016/J.FCT.2015.04.008.

Franceschi, N., Paraskevopoulos, K., Waigmann, E., & Ramon, M. (2017). Predictive protein toxicity and its use in risk assessment. *Trends in Biotechnology*, *35*(6), 483-486. https://doi.org/10.1016/j.tibtech.2017.03.010

Freitas, R.S., do Val, D.R., Fernandes, M.E.F., Gomes, F.I.F., de Lacerda, J.T.J.G., SantiGadelha, T., de Almeida Gadelha, C.A., de Paulo Teixeira Pinto, V., Cristino-Filho, G., Pereira, K.M.A., de Castro Brito, G.A., Bezerra, M.M., Chaves, H.V., 2016. Lectin from Abelmoschus esculentus reduces zymosan-induced temporomandibular joint inflammatory hypernociception in rats via heme oxygenase-1 pathway integrity and tnf-α and il-1β suppression. Int. Immunopharmacol. 38, 313–323. https://doi.org/10.1016/j.intimp.2016.06.012

Gaitonde, P., Balu-Iyer, S. V., 2011. In vitro immunogenicity risk assessment of therapeutic proteins in preclinical setting. Methods Mol. Biol. 716, 267–280.

https://doi.org/10.1007/978-1-61779-012-6_16

Gonçalves, Í.F.S., Souza, T.M., Vieira, L.R., Marchi, F.C., Nascimento, A.P., Farias, D.F., 2020. Toxicity testing of pesticides in zebrafish—a systematic review on chemicals and associated toxicological endpoints. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-020-07902-5

Hamid, R., Masood, A., 2009. Dietary Lectins as Disease Causing Toxicants. Pakistan J. Nutr. 8, 293–303. https://doi.org/10.3923/pjn.2009.293.303

Huang, C.N., Wang, C.J., Lin, C.L., Lin, H.T., Peng, C.H., 2017. The nutraceutical benefits of subfractions of Abelmoschus esculentus in treating type 2 diabetes mellitus. PLoS One 12, 2–13. https://doi.org/10.1371/journal.pone.0189065

Ivanciuc, O., Mathura, V., Midoro-Horiuti, T., Braun, W., Goldblum, R.M., Schein, C.H., 2003. Detecting potential IgE-reactive sites on food proteins using a sequence and structure database, SDAP-food. J. Agric. Food Chem. 51, 4830–4837. https://doi.org/10.1021/jf034218r

Jiandong, L., Yang, Y., Peng, J., Xiang, M., Wang, D., Xiong, G., Li, S., 2019. Trichosanthes kirilowii lectin ameliorates streptozocin-induced kidney injury via modulation of the balance between M1/M2 phenotype macrophage. Biomed. Pharmacother. 109, 93–102. https://doi.org/10.1016/j.biopha.2018.10.060

Kirshner, S.L., 2014. Mechanisms underlying the immunogenicity of therapeutic proteins: Risk assessment and management strategies. J. Interf. Cytokine Res. https://doi.org/10.1089/jir.2013.0011

Klafke, G.B., Moreira, G.M.S.G., Monte, L.G., Pereira, J.L., Brandolt, T.M., Xavier, M.O., Santi-Gadelha, T., Dellagostin, O.A., da Silva Pinto, L., 2013. Assessment of Plant Lectin Antifungal Potential Against Yeasts of Major Importance in Medical Mycology. Mycopathologia 175, 147–151. https://doi.org/10.1007/s11046-012-9596-x

Kraljevic, Sandra, Peter J. Stambrook, and Kresimir Pavelic. "Accelerating drug discovery: Although the evolution of '-omics' methodologies is still in its infancy, both the pharmaceutical industry and patients could benefit from their implementation in the drug development process." *EMBO reports* 5.9 (2004): 837-842. https://doi.org/10.1038/sj.embor.7400236

Ladics, G.S., 2008. Current codex guidelines for assessment of potential protein allergenicity. Food Chem. Toxicol. 46, S20–S23. https://doi.org/10.1016/j.fct.2008.07.021

Ladics, G.S., Cressman, R.F., Herouet-Guicheney, C., Herman, R.A., Privalle, L., Song, P., Ward, J.M., McClain, S., 2011. Bioinformatics and the allergy assessment of agricultural biotechnology products: Industry practices and recommendations. Regul. Toxicol. Pharmacol. 60, 46–53. https://doi.org/10.1016/j.yrtph.2011.02.004

Lang, G.H., Kagiya, Y., Kitta, K., 2015. Multiplex comparison of the digestibility of allergenic and non-allergenic proteins in rice grains by in vitro digestion. Food Chem. 168, 606–614. https://doi.org/10.1016/j.foodchem.2014.07.089

Liao, Z., Zhang, J., Liu, B., Yan, T., Xu, F., Xiao, F., Wu, B., Bi, K., Jia, Y., 2019. Polysaccharide from Okra (Abelmoschus esculentus (L.) Moench) Improves Antioxidant Capacity via PI3K/AKT Pathways and Nrf2 Translocation in a Type 2 Diabetes Model. Molecules 24. https://doi.org/10.3390/molecules24101906

Liu, X., Li, X., Peng, M., Wang, X., Du, X., Meng, L., Zhai, J., Liu, J., Yu, H., Zhang, Q., 2019. A novel C-type lectin from spotted knifejaw, Oplegnathus punctatus possesses antibacterial and anti-inflammatory activity. Fish Shellfish Immunol. 92, 11–20. https://doi.org/10.1016/j.fsi.2019.05.054

Luo, Y., Cui, H.X., Jia, A., Jia, S.S., Yuan, K., 2018. The Protective Effect of the Total Flavonoids of Abelmoschus esculentus L. Flowers on Transient Cerebral Ischemia-Reperfusion Injury Is due to Activation of the Nrf2-ARE Pathway. Oxid. Med. Cell.

Longev. 2018. https://doi.org/10.1155/2018/8987173

Madduri, K.M., Schafer, B.W., Hasler, J.M., Lin, G., Foster, M.L., Embrey, S.K., Sastry-Dent, L., Song, P., Larrinua, I.M., Gachotte, D.J., Herman, R.A., 2012. Preliminary safety assessment of a membrane-bound delta 9 desaturase candidate protein for transgenic oilseed crops. Food Chem. Toxicol. 50, 3776–3784. https://doi.org/10.1016/j.fct.2012.07.013

Manda, F., Tadera, K., Aoyana, K., 1992. Skin lesions due to okra (Hibiscus esculentus L): proteolytic activity and allergenicity of okra. Contact Dermatitis 26, 95–100. https://doi.org/10.1111/j.1600-0536.1992.tb00891.x

Menezes, S.P., dos Santos, J.L., Cardoso, T.H.S., Pirovani, C.P., Micheli, F., Noronha, F.S.M., Alves, A.C., Faria, A.M.C., da Silva Gesteira, A., 2012. Evaluation of the Allergenicity Potential of TcPR-10 Protein from Theobroma cacao. PLoS One 7, e37969. https://doi.org/10.1371/journal.pone.0037969

Matsushita, T., Aoyama, K., Manda, F., Ueda, A., Yoshida, M., & Okamura, J. (1989). Occupational dermatoses in farmers growing okra (Hibiscus esculentus L.). *Contact dermatitis*, *21*(5), 321-325.

Mishra, A., Gaur, S.N., Lavasa, S., Arora, N., 2015. In vitro assessment of allergenicity features and localization of probable IgE binding regions. Food Chem. Toxicol. 84, 181–187. https://doi.org/10.1016/j.fct.2015.08.018

Miyake, K., Tanaka, T., McNeil, P.L., 2007. Lectin-Based Food Poisoning: A New Mechanism of Protein Toxicity. PLoS One 2, e687. https://doi.org/10.1371/journal.pone.0000687

Monte, L.G., Santi-Gadelha, T., Reis, L.B., Braganhol, E., Prietsch, R.F., Dellagostin, O.A., e Lacerda, R.R., Gadelha, C.A.A., Conceição, F.R., Pinto, L.S., 2014. Lectin of Abelmoschus esculentus (okra) promotes selective antitumor effects in human breast

cancer cells. Biotechnol. Lett. 36, 461–469. https://doi.org/10.1007/s10529-013-1382-4

OECD, 2013. Test No. 236: Fish Embryo Acute Toxicity (FET) Test. OECD Guidel. Test. Chem. Sect. 2, OECD Publ. 1–22. https://doi.org/10.1787/9789264203709-en

Parng, C., Seng, W. L., Semino, C., & McGrath, P. (2002). Zebrafish: a preclinical model for drug screening. *Assay and drug development technologies*, *1*(1), 41-48

Rathinam, M., Singh, S., Pattanayak, D., Sreevathsa, R., 2017. Comprehensive in silico allergenicity assessment of novel protein engineered chimeric Cry proteins for safe deployment in crops. BMC Biotechnol. 17, 64. https://doi.org/10.1186/s12896-017-0384-z

Sá, C.A., Vieira, L.R., Pereira Almeida Filho, L.C., Real-Guerra, R., Lopes, F.C., Souza, T.M., Vasconcelos, I.M., Staniscuaski, F., Carlini, C.R., Urano Carvalho, A.F., Farias, D.F., 2020. Risk assessment of the antifungal and insecticidal peptide Jaburetox and its parental protein the Jack bean (Canavalia ensiformis) urease. Food Chem. Toxicol. 136, 110977. https://doi.org/10.1016/j.fct.2019.110977

Sabitha, V., Ramachandran, S., Naveen, K.R., Panneerselvam, K., 2012. Investigation of in vivo antioxidant property of Abelmoschus esculentus (L) moench. fruit seed and peel powders in streptozotocin-induced diabetic rats. J. Ayurveda Integr. Med. 3, 188–193. https://doi.org/10.4103/0975-9476.104432

Sabitha, V., Ramachandran, S., Naveen, K.R., Panneerselvam, K., 2011. Antidiabetic and antihyperlipidemic potential of Abelmoschus esculentus (L.) Moench. in streptozotocin-induced diabetic rats. J. Pharm. Bioallied Sci. 3, 397–402. https://doi.org/10.4103/0975-7406.84447

Schägger, H., von Jagow, G., 1987. Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. Anal.

Biochem. 166, 368–379. https://doi.org/10.1016/0003-2697(87)90587-2

Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed, 2010. . EFSA J. 8, 1700. https://doi.org/10.2903/j.efsa.2010.1700

Sharma, P., Singh, A.K., Singh, B.P., Gaur, S.N., Arora, N., 2011. Allergenicity assessment of osmotin, a pathogenesis-related protein, used for transgenic crops. J. Agric. Food Chem. 59, 9990–9995. https://doi.org/10.1021/jf202265d

Sharon, N., Lis, H., 2004. History of lectins: From hemagglutinins to biological recognition molecules. Glycobiology. https://doi.org/10.1093/glycob/cwh122

Tafazoli, S., Vo, T.D., Roberts, A., Rodriguez, C., Viñas, R., Madonna, M.E., Chiang, Y.H., Noronha, J.W., Holguin, J.C., Ryder, J.A., Perlstein, A., 2019. Safety assessment of miraculin using in silico and in vitro digestibility analyses. Food Chem. Toxicol. 133, 110762. https://doi.org/10.1016/j.fct.2019.110762

Taylor, S.L., Lehrer, S.B., 1996. Principles and characteristics of food allergens. Crit. Rev. Food Sci. Nutr. 36. https://doi.org/10.1080/10408399609527761

Ueda, A., Manda, F. U. M. I., Aoyama, K. O. H. J. I., Ueda, T. A. D. A. K. O., Obama, K., Li, Q. I. N. G., & Tochigi, T. (1993). Immediate-type allergy related to okra (Hibiscus esculentus Linn) picking and packing. *Environmental research*, *62*(2), 189-199.

Vasconcelos, I.M., Oliveira, J.T.A., 2004. Antinutritional properties of plant lectins. Toxicon. https://doi.org/10.1016/j.toxicon.2004.05.005

Verhoeckx, K., Bøgh, K.L., Dupont, D., Egger, L., Gadermaier, G., Larré, C., Mackie, A., Menard, O., Adel-Patient, K., Picariello, G., Portmann, R., Smit, J., Turner, P., Untersmayr, E., Epstein, M.M., 2019. The relevance of a digestibility evaluation in the allergenicity risk assessment of novel proteins. Opinion of a joint initiative of COST action ImpARAS and COST action INFOGEST. Food Chem. Toxicol. 129, 405–423. https://doi.org/10.1016/j.fct.2019.04.052

Vijayan, M., Chandra, N., 1999. Lectins. Curr. Opin. Struct. Biol. https://doi.org/10.1016/S0959-440X(99)00034-2

Wang, C., Wang, Y., Liu, G., Fu, L., 2020. Food allergomics based on high-throughput and bioinformatics technologies. Food Res. Int. https://doi.org/10.1016/j.foodres.2019.108942

Wang, K., Liu, C., Hou, Y., Zhou, H., Wang, X., Mai, K., He, G., 2019. Differential apoptotic and mitogenic effects of lectins in Zebrafish. Front. Endocrinol. (Lausanne). 10. https://doi.org/10.3389/fendo.2019.00356

Wu, J., Wang, J., Wang, S., Rao, P., 2016. Lunatin, a novel lectin with antifungal and antiproliferative bioactivities from Phaseolus lunatus billb. Int. J. Biol. Macromol. 89, 717–724. https://doi.org/10.1016/j.ijbiomac.2016.04.092

Zhao, J., He, S., Tang, M., Sun, X., Zhang, Z., Ye, Y., Cao, X., Sun, H., 2019. Low-pH induced structural changes, allergenicity and in vitro digestibility of lectin from black turtle bean (Phaseolus vulgaris L.). Food Chem. 283, 183–190. https://doi.org/10.1016/j.foodchem.2018.12.134

Figure captions

Figure 1. Primary amino acid sequence of *Abelmoschus esculentus* lectin (AEL).

Figure 2. Susceptibility to digestion of *Abelmoschus esculentus* lectin (AEL). (A) Number of predicted peptide fragments generated at each stage of *in silico* sequential digestion (pepsin followed by trypsin) by using the PeptideCutter program. (B) AEL cleavage sites simulating a sequential digestion: Pn 1.3 - Pepsin cleavage sites at pH 1.3 and Tryps - trypsin cleavage sites. (C) Tricine-SDS-PAGE (15%) of AEL (1 mg/mL) after increasing periods of incubation with pepsin (gastric phase of digestion) followed by trypsin (intestinal phase of digestion). M = Marker: beta-galactosidase (116.0 kDa), phosphorylase B (97.0 kDa), bovine serum albumin (66.0 kDa), glutamic dehydrogenase (55.0 kDa),ovalbumin (45.0 kDa), glyceraldehyde-3-phosphate dehydrogenase (36.0 kDa), carbonic anhydrase (29.0kDa), trypsinogen (24.0 kDa), trypsin inhibitor (20.1 kDa) and alpha-lactalbumin (14.2 kDa).

Figure 3. *A. esculentus* lectin (AEL) inhibitory activity (1 mg/mL) after different heating times (0, 10, 30 and 60 min at ~ 100 °C) on trypsin (0.1 mg/mL) (A) and quimotrypsin (0.2 mg/mL) (B) proteolytic activities.

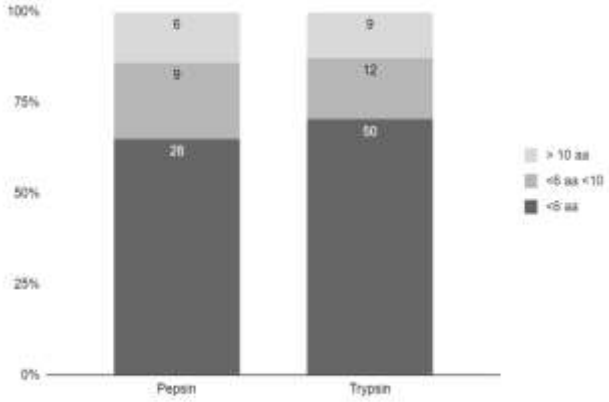
Figure 4. 96 hpf-old zebrafish (*D. rerio*) larva after acute exposure to AEL. A) Control larvae kept only in embryo medium. B) Larvae exposed to AEL 0.1 mg/mL. No morphological or developmental changes in the embryos at the tested concentration were observed.

Figure 1.

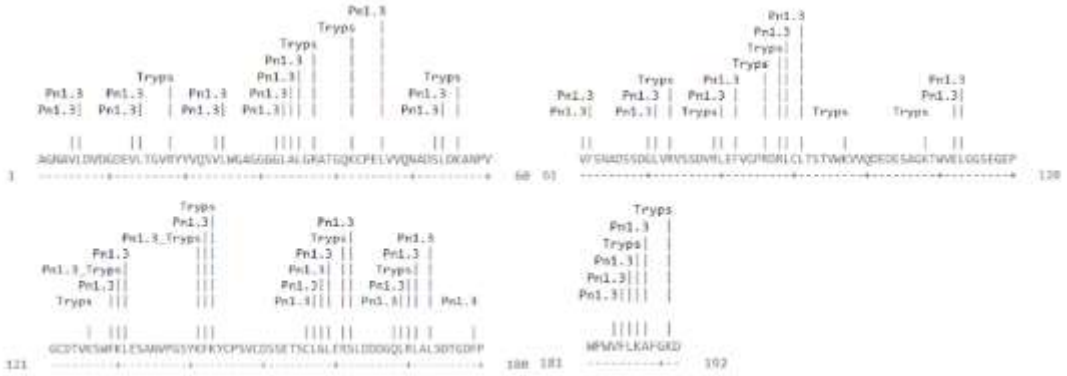
>
AGNAVLDVDGDEVLTGVRYYVQSVLWGAGGGGLALGRATGQKCPELVVQNADSLDKANP
VVFSNADSSDGLVRVSSDVRLEFVGPRDRLCLTSTVWKVVQDEDESAGKTWVELGGSEGEP
GCDTVKSWFKLESANVPGSYKFKYCPSVCDSSETSCLGLERSLDDDGQLRLALSDTGDFPWP
WVFLKAFGKD

Figure 2.

A)


100%
75%
50%
25%
0%
6
9
20
9
12
50
> 10 aa
<6 aa <10
<6 aa
Pepsin
Trypsin

B)



C)

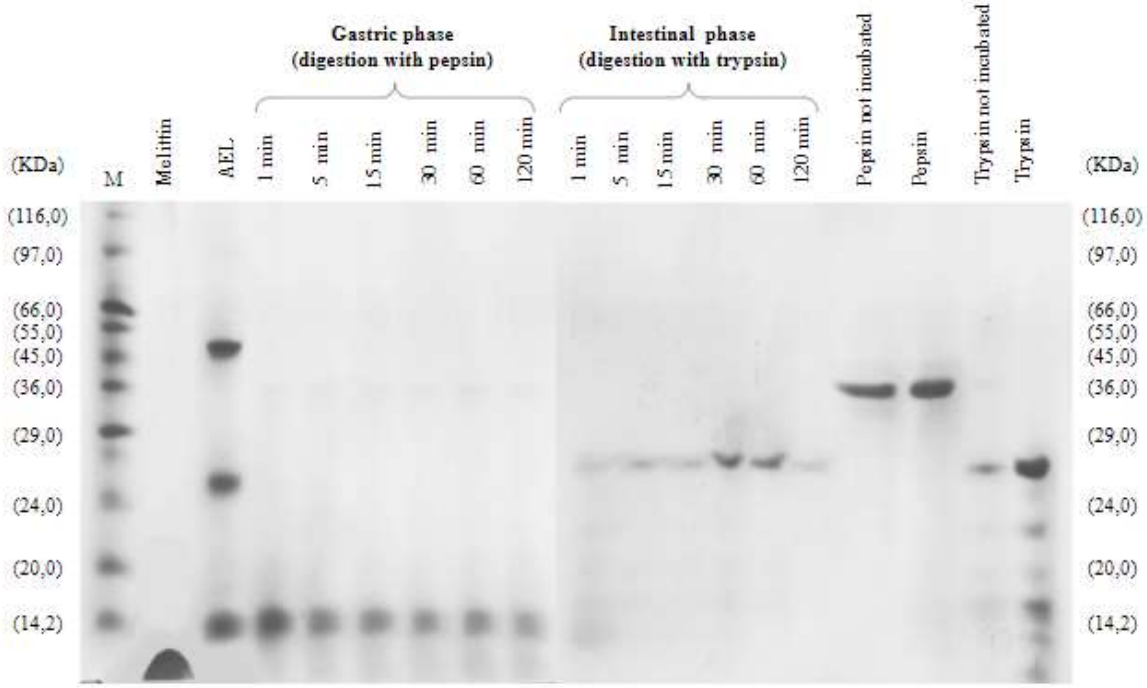

Gastric phase
(digestion with pepsin)
Intestinal phase
(digestion with trypsin)
(KDa)
M
Melittin
AEL
1 min
5 min
15 min
30 min
60 min
120 min
1 min
5 min
15 min
30 min
60 min
120 min
Pepsin not incubated
Pepsin
Trypsin not incubated
Trypsin
(KDa)
(116,0)
(97,0)
(66,0)
(55,0)
(45,0)
(36,0)
(29,0)
(24,0)
(20,0)
(14,2)

Figure 3.

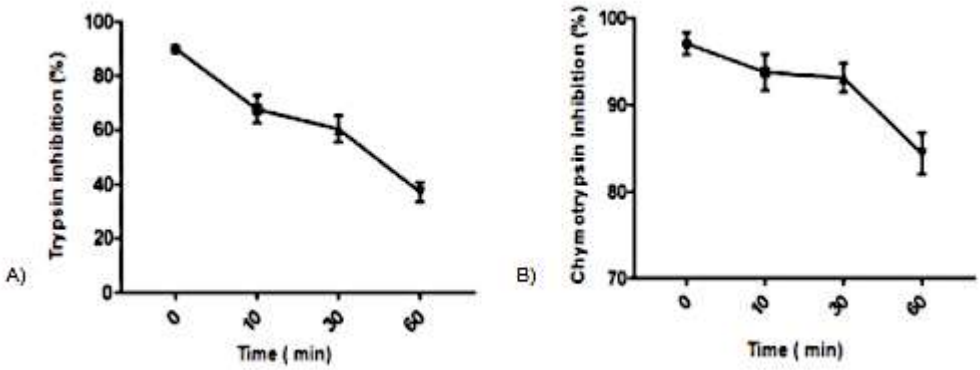

A)
Trypsin inhibition (%)
100
80
60
40
20
0
0
10
30
60
Time (min)
B)
Chymotrypsin inhibition (%)
100
90
80
70
0
10
30
60
Time (min)

Figure 4.

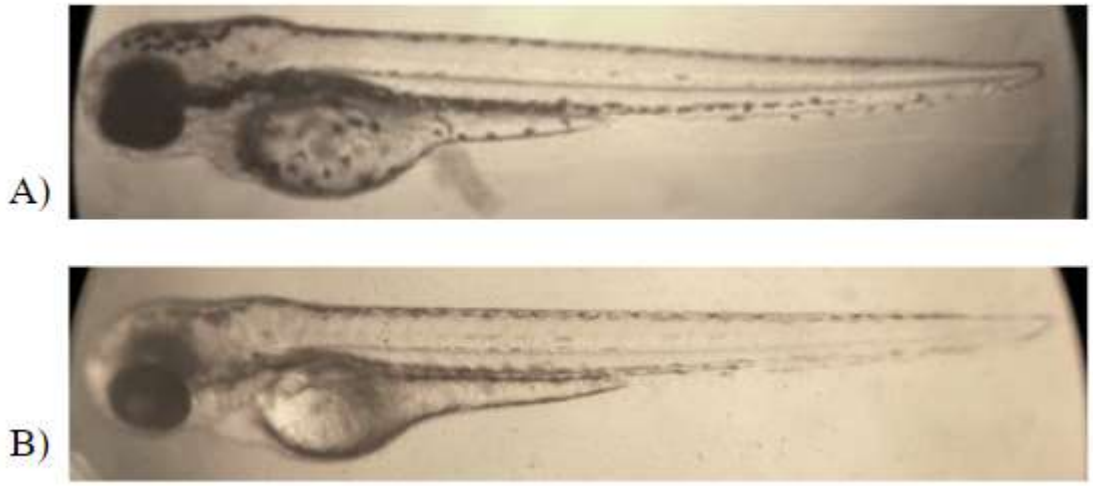


Table contents

Table 1 - Sequences of 6 and 7 contiguous amino acids of allergenic proteins identified (100% identity) in lectin of *Abelmoschus esculentus* (AEL) in allergen databases.

Sequence	Number of access	Description/ Species	Database
DSSDGLV	Q96385	Pectate lyase -*Chamaecyparis obtusa*	SDAP
AGNAVL	17291858	Aldehyde dehydrogenase -*Harmonia axyridis*	SDAP
DSSDGL	Q96385	Aldehyde dehydrogenase - *Chamaecyparis obtusa*	SDAP
SSDGLV	BAA05543	Pectate lyase -*Cryptomeria japonica*	SDAP

DSSDGLV	Q96385	Pectate lyase- *Chamaecyparis obtusa*	Allermatch
SSDGLV	Q96385	Pectate lyase - *Chamaecyparis obtusa*	Allermatch

* Not provided by the database.

Source: Direct Search 2020.

Table 2 – Similarity search of the complete primary amino acids sequence of the *Abelmoschus esculentus* lectin (AEL) with toxic, antinutritional and allergenic proteins deposited in the NCBI general databases*

Database	**Access number**	**Protein matched**	**Identity**	**E-value**	**Gap**	**Adverse effects***
NR†	EOY21251.1	Seed protein *Theobroma cacao*	61%	2e-55	3%	_**
NR	OMO94886.1	Proteinase inhibitor I3, Kunitz legume *Corchorus olitorius*	57%	2.9	2%	–

NR	AAL85648.1	Trypsin inhibitor, partial - *Theobroma obovatum*	66%	2e-51	3%	–
NR	KJB73820.1	Hypothetical protein - *Gossypium raimondii*	54%	1e-46	3%	–
NR	XP_022738218.1	Seed protein-like *Durio zibethinus*	58%	4e-55	3%	–
NR†	EOY21247.1	Seed protein *Theobroma cacao*	61%	4-e55	3%	_
NR	XP_017985239.1	Seed protein-like *Theobroma cacao*	60%	2e-54	3%	_
NR	AAV41233.1	Putative trypsin inhibitor - *Theobroma bicolor*	63%	2e-66	2%	_

NR	XP_007036743.1	Seed protein - *Theobroma cacao*	62%	3e-58	2%	_
NR	prf||1802409A	Albumin *Theobroma cacao*	62%	4e-58	2%	_
NR	AAV41231.1	Putative trypsin inhibitor *Theobroma cacao*	56%	2e-54	2%	_
NR	XP_016733195.1	Predicted: seed protein-like, partial *Gossypium hirsutum*	58%	5e-54	1%	_
NR	OMO94897.1	Proteinase inhibitor I3, Kunitz legume *Corchorus olitorius*	57%	3e-53	2%	_
NR	XP_022734232.1	Seed protein-like *Durio zibethinus*	56%	1e-52	3%	_
			58%	6e-52	3%	_

NR	XP_022734258.1	Seed protein-like *Durio zibethinus*				
NR	AAL85648.1	Trypsin inhibitor, partial *Theobroma obovatum*	66%	2e-51	3%	_
NR	AAL85644.1	Trypsin inhibitor, partial *Theobroma subincanum*	65%	3e-51	3%	_
NR	OMO67988.1	Proteinase inhibitor I3, Kunitz legume *Corchorus olitorius*	57%	1e-50	2%	_
NR	XP_022733892.1	Seed protein-like *Durio zibethinus*	57%	2e-50	3%	_
NR	AAL85643.1	Trypsin inhibitor, partial *Theobroma angustifolium*	63%	5e-50	2%	-

NR	XP_022734216.1	Seed protein-like *Durio zibethinus*	55%	1e-49	3%	_
NR	XP_022734369.1	Seed protein-like *Durio zibethinus*	55%	2e-49	3%	_
NR	XP_022734070.1	Seed protein-like *Durio zibethinus*	55%	2e-49	3%	_
NR	AAL85653.1	Trypsin inhibitor, partial - *Theobroma simiarum*	64%	3e-49	1%	_
NR	AAL85657.1	Trypsin inhibitor, partial, *Theobroma sylvestre*	66%	3e-49	1%	_
NR	XP_022733898.1	Seed protein-like *Durio zibethinus*	56%	4e-49	3%	_

NR	AAL85650.1	Trypsin inhibitor, partial - *Theobroma mammosum*	63%	4e-49	1%	–
NR	AAL85651.1	Trypsin inhibitor, partial - *Theobroma mammosum*	62%	4e-49	1%	–
NR	AAL85652.1	Trypsin inhibitor, partial - *Theobroma mammosum*	62%	5e-49	1%	–
NR	AAL85640.1	Trypsin inhibitor, partial - *Herrania albiflora*	64%	5e-49	1%	–
NR	AAL85659.1	Trypsin inhibitor, partial - *Theobroma speciosum*	65%	1e-48	1%	–

NR	AAL85637.1	Trypsin inhibitor, partial - *Herrania mariae*	61%	1e-48	1%	_
NR	AAL85655.1	Trypsin inhibitor, partial - *Theobroma bicolor*	61%	2e-48	1%	_
NR	AAL85660.1	Trypsin inhibitor, partial - *Theobroma sylvestre*	63%	1e-47	1%	_
NR	AAL85642.1	Trypsin inhibitor, partial - *Theobroma speciosum*	63%	6e-47	1%	_
NR	KJB73820.1	Hypothetical protein -*Gossypium raimondii*	54%	1e-46	3%	_

NR	AAL85645.1	Trypsin inhibitor, partial - *Theobroma grandiflorum*	64%	1e-46	3%	_
NR	AAL85654.1	Trypsin inhibitor, partial -*Theobroma cacao*	64%	3e-46	1%	_
NR	AAL85656.1	Trypsin inhibitor, partial -*Theobroma microcarpum*	61%	3e-46	1%	_
NR	XP_016726747.1	Predicted: seed protein-like - *Gossypium hirsutum*	56%	5e-46	3%	_
NR	TYG51640.1	Hypothetical protein -*Gossypium darwinii*	56%	1e-45	3%	_
NR	EOY21249.1		53%	5e-45	3%	_

		Seed protein, putative - *Theobroma cacao*				
NR	XP_016724672.1	Predicted: seed protein-like - *Gossypium hirsutum*	54%	5e-45	3%	_
NR	KAB2063681.1	Hypothetical protein -*Gossypium barbadense*	54%	7e-45	4%	_
NR	XP_012456563.1	Predicted:seed protein-like - *Gossypium raimondii*	55%	9e-45	3%	_
NR	XP_016678869.1	Predicted: seed protein-like - *Gossypium hirsutum*	54%	2e-44	4%	_
NR	XP_017985238.1		53%	7e-44	3%	_

		Predicted: seed protein-like - Theobroma cacao				
NR	KAB2010646.1		54%	8e-44	4%	_
NR	XP_017648613.1	Hypothetical protein -*Gossypium barbadense*	54%	1e-43	4%	_
NR	XP_016726748.1	Predicted: seed protein-like - *Gossypium arboreum*	51%	1e-43	3%	_
NR	KAB2010641.1	Predicted: seed protein-like - *Gossypium hirsutum*	54%	6e-43	3%	_
NR	TYI62648.1	Hypothetical protein -*Gossypium barbadense*	54%	6e-43	3%	_

NR	KAB2010645.1	Hypothetical protein -*Gossypium mustelinum*	52%	1e-42	3%	_
NR	TYG51636.1	Hypothetical protein -*Gossypium barbadense*	51%	1e-42	3%	_
NR	XP_016726751.1	Hypothetical protein -*Gossypium darwinii*	54%	2e-42	4%	_
NR	AAV41232.1	Predicted: seed protein-like - *Gossypium hirsutum*	52%	2e-42	2%	_
NR	XP_012456564.1	Putative trypsin inhibitor - *Theobroma grandiflorum*	53%	3e-42	3%	_
		Predicted: seed protein-like -				

NR	XP_012457838.1	*Gossypium raimondii*	53%	8e-42	3%	_
NR	XP_016726750.1	Predicted: seed protein-like - *Gossypium raimondii*	53%	9e-42	3%	_
NR	KAB2010648.1	Predicted: seed protein-like - *Gossypium hirsutum*	54%	9e-42	3%	_
NR	TYI62654.1	Hypothetical protein -*Gossypium barbadense*	53%	2e-41	3%	_
NR	TYG51634.1	Hypothetical protein -*Gossypium mustelinum*	52%	7e-41	4%	_
NR	TYH51456.1	Hypothetical protein -*Gossypium darwinii*	52%	7e-41	4%	_

NR	KAB2010643.1	Hypothetical protein -*Gossypium tomentosum*	52%	7e-41	4%	_
NR	XP_016726764.1	Hypothetical protein - Gossypium barbadense Predicted: seed protein-like - *Gossypium hirsutum*	52%	7e-41	4%	_
NR	TYI62650.1	Hypothetical protein -*Gossypium mustelinum*	52%	8e-41	4%	_
NR	KAA3481788.1	Seed protein-like - *Gossypium australe*	52%	2e-40	4%	_

NR	XP_017618846.1	Predicted: seed protein-like - *Gossypium arboreum*	51%	4e-40	1%	_
NR	PPD74598.1	Hypothetical protein -*Gossypium barbadense*	50%	2e-39	3%	_
NR	XP_012457615.1	Predicted: seed protein-like - *Gossypium raimondii*	51%	4e-39	4%	_
NR	KAB2010647.1	Hypothetical protein -*Gossypium barbadense*	52%	7e-39	3%	_
NR	XP_016678874.1	Predicted: seed protein-like - *Gossypium hirsutum*	51%	1e-38	2%	_
NR	KAB2063685.1		52%	1e-38	2%	_

NR	OMO68115.1	Hypothetical protein -*Gossypium barbadense*	56%	4e-38	1%	_
NR	TYJ16232.1	Proteinase inhibitor I3, Kunitz legume -*Corchorus capsularis*	51%	1e-37	2%	_
NR	PPR93593.1	Hypothetical protein -*Gossypium mustelinum*	52%	2e-37	2%	_
NR	TYH51460.1	Hypothetical protein -*Gossypium barbadense*	52%	2e-37	3%	_
NR	AAL85661.1	Hypothetical protein -*Gossypium tomentosum*	55%	6e-37	1%	_
		Trypsin inhibitor, partial -*Theobroma bicolor*				

*Results with E-value < 0.01, shared identity > 50%, alignment size and gap frequency <6% were considered relevant; †NCBI database (http://www.ncbi.nlm.nih.gov/).

** No similarity was found with toxic, anti-nutritional and / or allergenic proteins deposited in the NCBI general databases. No published articles were found reporting adverse effects.

Table 3 - Identified proteins (>35% identity) for *Abelmoschus esculentus* lectin (AEL) in two allergen databases using as a search parameter a sliding window of 80 amino acids.

Database	Number access	Description/Species	Identity (%)	E-value
AllergenOnline	gi|994779|gid|195	Proteinase inhibitor – *Solanum tuberosum*	36.00	1.7e-016
SDAP	O24383	Cysteine protease inhibitor – *Solanum tuberosum*	35.00	-*
SDAP	CAA45723	Aspartic protease inhibitor; PIG; PIGEN1; STPIB; pF4 - *Solanum tuberosum*	35.00	-*
Allermatch	P30941	Serine protease inhibitor 7 - *Solanum tuberosum*	36.00	-*
Allermatch	P20347	Cysteine protease inhibitor 1- *Solanum tuberosum*	35.00	-*

*Not provided by the database.

Supplementary tables

Table S1 - Literature search on the history of safe use of okra (*Abelmoschus esculentus*) and its lectin (AEL).

Sample	Form of Use	Positive Aspects	References	Negative Aspects	References
Abelmoschus esculentus	Extract (powder)	Antidiabetic, antihyperglycemic and antihyperlipidemic	Sabitha et al. (2011)	-	-
	Extract (powder)	Antihyperglycemic activity and effect hypolipidemic	Erfani et al. 2018	-	-
	Extract (purified)	Anti-fatigue activity	Gao et al. (2018)	-	-
	Extract (powder)	Antidiabetic, antihyperglycemic,	Huang et al. (2017)	-	

	reduced insulin resistance and plasma fatty acid concentration			-
Extract (powder)	Antioxidant and anti-apoptotic activity in the nervous system, neuroprotective effect	Luo et al. (2018)	-	-
Extract (powder)	Immunomodulatory and immunostimulatory activity, Increase phagocytic activity and splenocyte proliferation during induced immune response	Wahyuningsih et al. (2018)	Immunosuppressive activity	Wahyuningsih et al. (2018)
Extract (phenolic)	Hypoglycemic, antiapoptotic, antioxidant, anti-glycotoxicity, anti-lipid peroxidation	Erfani Majd et al. (2019)	-	-

Extract (aqueous)	Hypoglicemic activity, oxidative stress modulation and reduction of pathological processes of diabetes	Liao et al. (2019)	-	-
Extract (alcoholic and aqueous)	Antioxidant activity, reduction of lipid peroxidation, reductions of renal fibrosis and fat deposition	Peng et al. (2019).	-	-
Extract (aqueous and methanolic)	Antioxidant, anti-stress and nootropic activity	Doredulla et al. (2014)	-	-
Extract (powder)	Antioxidant properties in diabetic conditions	Sabitha et al. (2012)	-	-

Extract (ethanolic)	Reversal of cognitive deficits and toothed gyrus cell proliferations	Tongiaroenbuan-gam et al. (2011)	-	-
Extract (ethanolic and polysaccharide)	Anti-fatigue effect	Li et al. (2016)	-	-
Extract (powder)	Potassium-rich and may contributed to maintaining blood pressure and the protective role of the heart	Hailu et al. (2016)	-	-
Extract (aqueous)	Immunomodulatory activity	Chen et al. (2016)	-	-

Dry okra pruning accesses	Relieves problems related to malnutrition in countries	Gemede et al. (2016)	-	-
Extract (alcoholic)	Gastroprotective effect, reducing gastrointestinal ulcers, inflammation, bleeding, lipid peroxidation and increasing antioxidant defense	Ortaç et al (2018)	-	-
Extract (powder)	-	-	Moderate allergenicity and development of skin lesions	Manda et al. (1992)
Purified protein	Antidiabetic	Akbari et al (2016)	-	-
Extract (powder)	Antidepressant		-	-

		Ebrahimzadeh et al (2013)			
Extract (methanolic)	Antioxidant and hepatoprotective	Alqasoumi et al (2012)	-	-	
Extract (alcoholic)	Antioxidant	Atawodi et al (2009)	-	-	
Extract (methanolic)	-	-	Dermatitis	Matsushita et al (1989)	
Extract (powder)	-	-	Moderate allergenicity	Ueda et al (1993)	

Lectin from *Abelmoschus esculentus*	Purified protein	Antinociceptive and anti-inflammatory	Freitas et al.(2016)	-	-
	Purified protein	Antioxidant activity	Ribeiro et al. (2016)	-	-
	Purified protein	Displays anti-inflammatory, antinociceptive and hemagglutination activity	Soares et al. (2012)	-	-
	Purified protein	Antinociceptive and anti-inflammatory activity	Alves et al. (2018)	-	-

		-	-
Anticancer (breast cancer) and anti-apoptotic	Monte et al.,(2014).		

Supplementary tables

Table S2 - Similarity of the full-length primary amino acids sequence of *Abelmoschus esculentus* lectin (AEL) with allergenic proteins deposited in allergen databases.

Databases	Parameters used		
	CS[†]	**80 aa's**[‡]	**6/7/8 aa's**[¶]
AllergenOnline	0	1	[¥]/[¥]/0
SDAP	0	2	4/1/0
Allermatch	0	2	2/1/0

CS: Complete sequence of amino acids (aa's).

[†]To be considered relevant the identity must be greater than 50% (AALBERSE, 2000).

[‡]To be considered relevant the identity must be greater than 35% (CODEX ALIMENTARIUS, 2009).

[¶]To be considered relevant the identity must be 100% (CODEX ALIMENTARIUS, 2009).

[¥] In this database it is not possible to do search for 6 and 7 contiguous amino acids.

*Number of similar sequences found according to the specificities for each parameter analyzed

Source: Direct Search 2020.

5. ARTIGO CIENTÍFICO 2

Prediction of dipeptidyl peptidase-IV and angiotensin-I-converting enzyme inhibitory peptides from plant lectins using *in silico* approaches

Running head: Prediction of bioactive peptides from lectins

Eduardo Afonso da Silva Pereira[a], Tatiane Santi-Gadelha[b] and Davi Farias[a,*]

[a]Laboratory for Risk Assessment of Novel Technologies (LabRisk), Department of Molecular Biology, Federal University of Paraiba, 58051-900, João Pessoa, Brazil.

[b]Laboratory of Genetics and Radiobiological Biochemistry, Department of Molecular Biology, Federal University of Paraiba, 58051-900, João Pessoa, Brazil.

*Corresponding author:

Prof. Davi Farias, Ph.D.

E-mail: davi@dbm.ufpb.br

Phone: +55 83 3216 7633

Orcid: 0000-0001-5438-1919

List of abbreviations:

AE - Frequency of release of fragments with a given activity by selected enzymes; ACE-I - Angiotensin-I-converting *enzyme;* A – Frequency of biactive fragments occurrence in a protein sequence; a - Number of fragments with a given activity; 2; BIOPEP - Database of bioactive peptides; BP – Bioactive peptides; D – Number of peptides with a given activity

released by a given enzyme; DHt – Theoretical degree of hydrolysis; DPP-IV - Dipeptidyl peptidase-4; FDA – Food and Drug Administration; GIP - Gastric inhibitory polypeptide; GLP-1 - *Glucagon-like peptide-1*; GPU- Graphics Processing Unit; N - Number of aminoacids residues; NCBI – National Center for Biotechnology Information; PDB – Protein Data Bank; QSAR - Quantitative Structure–Activity Relationship; *RAS* - Renin–Angiotensin System; SVM – Support Vector Machine; W – Number of peptides with a given activity released by a given enzyme.

Highlights:

- Lectins are good sources of antihypertensive and anti-diabetic peptides.
- Dipeptides and tripeptides comprise the majority of ACE-I and DPP-IV inhibitors found.
- ACE-I and DPP-IV inhibitors are mainly composed of hydrophobic amino acids.
- ACE-I and DPP-IV inhibitory peptides are non-competitive inhibitors.

Abstract

Plant lectins are recognized for their several biological activities which make them a promising platform for identification of bioactive peptides (BP). This work aimed to predict through *in silico* approaches the presence of dipeptidyl peptidase-IV (DPP-IV) and angiotensin-I-converting enzyme (ACE-I) inhibitory peptides in sequences of plant lectins, as well as to verify their binding capacity by molecular docking. *In silico* tools were used to identify and characterize peptides that potentially have inhibitory activity of the ACE-I and DPP-IV enzymes, as well as to assess their toxicity. Plant lectins possess a large number potential inhibitory peptides of ACE-I and DPP-IV. None of the most promising peptides presented toxicity according to ToxinPred, and all peptides showed low solubility according to PepCalc. The results of docking revealed that all peptides are capable of binding with high specificity to the target enzymes. The binding energy between MF, CF and FFL peptides and ACE-I ranged from -671.7 kJ/mol^{-1} to -518.4 kJ/mol^{-1}, while for WF, MF and GF peptides and DPP-IV the binding energy varied from -697.3 kJ/mol^{-1} to -415.7 kJ/mol^{-1}. The main types of interactions found were hydrophobic interactions and hydrogen bonds. The lack of interaction of the inhibitory peptides with the active site of the enzymes suggests an inhibition of the non-competitive type. Although these BP are promising inhibitors, *in vivo* studies must be performed to confirm their biological activity, and also to assess their toxicity and safety as new therapeutic tools.

Keywords: Antidiabetics, antihypertensives, bioactive peptides, hydrophobic interactions, non-competitive inhibition

1. Introduction

Bioactive peptides (BP) are organic molecules of protein nature that are encrypted in a parental protein and released mainly by enzymatic processes (Sánchez & Vázquez, 2017). BP usually have between 2-20 amino acids and a molecular weight of less than 6,000 Da (Sarmadi & Ismail, 2010). BP have gained ground as important molecules used in the treatment of different diseases, whether they are of natural or synthetic origin (Fosgerau & Hoffmann, 2015).

Plants have long been recognized as an excellent source of therapeutic proteins (Tran et al., 2020). In addition to some classes of plant proteins, many peptides generated from the digestion of these molecules have been considered potent therapeutic agents (Maestri et al., 2016). In this sense, plant lectins have figured out as a class of great interest due to their diversity of pharmacological activities (Lagarda-Diaz et al., 2017), specially for the treatment of diseases that currently occupy a large space on the world stage such as diabetes and hypertension (Bhagyawant et al., 2019). The remaining question is whether these lectins would be able to show any biological activity when present only as small peptides from their native sequences.

According to the World Health Organization (WHO) the prevalence of diabetes mellitus and hypertension affects about 422 million and 1.3 billion people worldwide, respectively (WHO, 2020). In a global context, both diseases are considered public health problems since they can in the long term lead their patients to debilitating conditions (Al-Lawati, 2017; Laurent et al., 2012). More recently, a new concern has emerged since diabetes and previous vascular disease are risk factors for the severity and mortality of COVID-19 (Valencia et al., 2020). The great spread of diabetes and hypertension in the world has stimulated the discovery of new molecules capable of treating them by different mechanisms (Miller et al., 2014). Amidst the traditional and widely commercialized drugs for the treatment of these

diseases, BP are emerging as promising approaches to the control of diabetes and hypertension (Guo et al., 2020).

According to the Food and Drug Administration (FDA), there are currently approximately twelve classes of drugs used to treat diabetes (Gourgari et al., 2017). Taking into account the different mechanisms of action and possible combinations of these drugs, inhibitors of the dypeptydil peptidase-IV enzyme (DPP-IV) are usually one of the main choices in the treatment of diabetes (Pratley, 2008; J. Wu et al., 2009). DPP-IV is a serine aminopeptidase that acts on the body by degrading a class of molecules called incretins. GLP-1 and GPI are gut hormones and the main incretins that act as primary regulators of glucose homeostasis, regulating the secretion of insulin and glucagon (Patil et al., 2015; Power et al., 2014). Furthermore GLP-1 has been implicated in the proliferation and differentiation of pancreatic β-cell (Holst & Gromada, 2004). Inhibition of the DPP-IV enzyme as a strategy to maintain insulin levels at adequate levels remains the subject of recent research in the field of pharmacology (Lu et al., 2019).

Among the different classes of antihypertensive drugs, the inhibitory substances of the angiotensin-I converting enzyme (ACE-I) is one of the first-line drugs for the treatment of high blood pressure (Oparil et al., 2018). ACE-I is a dipeptidyl carboxidase from the group of zinc metalloproteinases and participates in the renin-angiotensin-aldosterone system (RAS), transforming angiotensin-I (Asp-Arg-Val-Try-Ile-His-Pro-Phe-His-Leu) in angiotensin-II (Arg-Val-Try-Ile-His-Pro-Phe) by removing the dipeptide His-Leu in the terminal portion. Angiotensin II acts as a potent vasoconstrictor and salt retainer (Vukic et al., 2017). ACE-I inhibitors by preventing the formation of angiotensin-II lead to dilation of the arteries, reducing blood pressure (Ribeiro-Oliveira et al., 2008). Besides that, ACE-I inhibitors are known to raise levels of bradykinin, an important molecule that helps lower blood pressure (Sharma, 2010).

In the past few years, great efforts have been made to characterize BP from natural sources with relevant pharmacological and nutraceutical properties (but with reduced side effects) by using *in vitro* and *in silico* methods (Liu et al., 2019). The reduced cost to identify, isolate and characterize peptides and proteins with potent pharmacological activity using computational approaches represents an additional advantage to the traditional methodologies used in the pharmaceutical industry. An increasing advance has been seen in the use of *in silico* tools to predict the function of proteins and peptides. For example, the BIOPEP database (http://www.uwm.edu.pl/biochemia/index.php/pl/biopep) is used for depositing and digesting peptide sequences, obtaining the theoretical degree of hydrolysis, among others; ToxinPred (http://crdd.osdd.net/raghava/toxinpred/) is used to analyze the toxic potential of peptide sequences; and the PeptideRanker (http://distilldeep.ucd.ie/PeptideRanker/) used to evaluate the bioactive potential of the sequences of interest.

Considering that lectins could be a source of peptides with biological activities of pharmacological and nutraceutical interests, this work aimed to identify BP from lectins of different plant origin, mainly legumes. For that, different *in silico* approaches were used to generate and identify the peptides that potentially have inhibitory activity of the ACE-I and DPP-IV enzymes, as well as to assess their toxicity.

2. Material and Methods

2.1 Lectin amino acids sequences

The justification for choosing lectins for study was based on previous reports of pharmacological activities in the literature and availability of primary amino acids sequences. Thus, the *in silico* analyses were performed with the following plant lectins: AEL - *Abelmoschus esculentus* (de Lacerda et al., 2017), ConA - *Canavalia ensiformes* (Acession

number P02866-CONA_CANEN), ConBr - *C. brasiliensis* (P55915), ConMa - *C. rosea* (P81364), MusaLec - *Musa acuminata* (Q8L5H4), Pea Lec - *Pisum sativum* (P02867), Soybn Lec - *Glycine max* (P05046), Lencu Lec - *Lens culinaris* (P02870), PHA-E - *Phaseolus vulgaris* (Q8RW23), Favin - *Vicia faba* (P02871), Allce Lec - *Allium cepa* (C0HJM8), Allsa Lec - *Allium sativum* (P83886) and Coles Lec from *Colocasia esculenta* (A5HMM7). These sequences were obtained from the NCBI database (https://www.ncbi.nlm.nih.gov/) with the exception of that of AEL. The peptide sequence in .pdb format was obtained from the PeptideDB database (http://www4g.biotec.or.th/FeptideDB/index.php). To simulate the gastrointestinal digestion *in silico*, the pepsin (EC 3.4.23.1) and trypsin (EC 3.4.21.4) enzymes were used.

2.2 Bioactivity search of plant lectin peptides

The activity profile of the BP encrypted in each protein sequence was analyzed individually at BIOPEP (http://www.uwm.edu.pl/biochemia/index.php/pl/biopep). Proteins were analyzed for the frequency of bioactive peptides which can occur in their sequences (A): $A = a / N$, where (a) is the number of fragments with a given activity and (N) is the number of amino acids residues. The individual values of (A) for each biological activity were used to generate the data plot on the HeatMap. The data were generated with the RStudio program. The profile of biological activity in the heat map varied between 0 and 12 for each activity with 0 being a low activity potential and 12 a high profile of biological activity. In total, 18 different biological activities performed by the selected lectins were counted. The values of (A) added together resulted in the variable $\sum A$ - total frequency of occurrence of all bioactive peptides. The values of $\sum A$ were also used to identify the total number of biological activities that the different lectins have in their sequences.

2.3 Evaluation of the frequency of release of ACE-I and DPP-IV inhibitory peptides from plant lectins

Enzymatic simulation and prediction of bioactive peptides were carried out at BIOPEP. For that, it was used pepsin alone or pepsin followed by trypsin to simulate the gastrointestinal digestion. The sequences were assessed for their frequency of release of fragments with a given activity by the selected enzymes (AE) using the formula: AE = d / N, where (d) is the number of peptides with a given activity released by a given enzyme and (N) is the number of amino acid residues in protein. In addition, the relative frequency of release of fragments with a given activity by selected enzymes (W) was evaluated applying the formula: W = AE / A. Finally, the theoretical degree of hydrolysis (DHt) was analyzed according to the the formula: DHt = d / D × 100%, where (d) is the number of hydrolyzed peptide bonds in a protein / peptide chain and (D) is the total number of peptide bonds in a protein / peptide chain (Minkiewicz et al, 2019).

2.4 Ranking and toxicity prediction of ACE-I and DPP-IV inhibitory peptides

In order to rank the biologically active peptides found, the PeptideRanker Score (http://distilldeep.ucd.ie/PeptideRanker/) was used to select the top-three peptides that obtained the highest scores among the DPP-IV and ACE-I inhibitory peptides. The results were represented between 0 and 1, with 0 being a low bioactive potential and 1 a high bioactivity potential. After selection, each peptide was evaluated *in silico* for toxicity in the ToxinPred database (http://crdd.osdd.net/raghava/toxinpred/). The prediction method for the toxicity analysis was based on support vector machine (SVM). The E-value cut-off for motif-bases method was 10. For further analysis of the peptides such as solubility, molar extinction coefficient and molecular weight, the PepCalc bank was used (http://pepcalc.com/).

2.5 Analysis of molecular docking

The peptides used in docking were chosen according to their bioactivity profile provided by the PeptideRanker Score and inhibitory activity (A) provided by BIOPEP. The sequences of the DPP-IV and ACE-I proteins to be used in the docking were obtained in .pdb format in the PBD Database (https://www.rcsb.org/). Docking analysis of DPP-IV and ACE-I and the inhibitory peptides were performed using the ClusPro 2.0 database (https://cluspro.bu.edu). The GPU option was chosen to work with the docking models. The best models were chosen based on the balanced scoring scheme and the lowest binding energy values. The molds with the lowest binding-free energies were chosen as the most representative of enzyme inhibition. The Ligplot software was used to analyze in 2D figures the hydrogen bonds and hydrophobic interactions between ligand-enzyme. PIC server (http://pic.mbu.iisc.ernet.in/) was used to verify and quantify the interaction between the amino acids involved in enzyme inhibition. The chosen interaction cut-off value followed the recommendations of the software. PyMOL software was used to visualize the result of molecular docking in 3D.

3. Results and Discussion

3.1 Evaluation of the biological activity profile in plant lectins

Amid a wide variety of physiological effects, plant-based proteins such as lectins have gained attention as promising alternatives in the treatment of diabetes and hypertension diseases. In this context, we aimed to identify peptides with high biological activity present in plant lectin sequences with inhibitory activity against the ACE-I and DPP-IV enzymes.

According to the BIOPEP database, a total of 18 biological activities were found in the sequences of the selected lectins (Figure 1). Among them are: ACE-I inhibitor, activating ubiquitin-mediated proteolysis, α-glucosidase inhibitor, antiamnestic peptide, anticancer

peptide, antioxidant peptide, antithrombotic peptide, bacterial permease ligand, CaMPDE inhibitor, DPP-III inhibitor, DPP-IV inhibitor, hypolipidemic, HMGG-CoA reductase inhibitor, immunomodulating peptide, neuropeptide, renin inhibitor, and regulating and stimulating.

3.2 Frequency of release of ACE-I and DPP-IV inhibitory peptides from plant lectins

The total frequency of occurrence of all bioactive peptides (∑A) showed that the lectins with the highest biological activity values were AEL (∑A 1,3481), Coles Lec (∑A 1,225), Favin (∑A 1.2666), PHA-E (∑A 1.2091), Soybn Lec (∑A 1.1769) and Musa Lec (∑A 1.1794) (Table 1). However, in number of biological activities observed, the main lectins were Soybn lec, PHA-E, AEL and Musa lec presenting 16 activities, Allce lec with 15 activities and Allsa lec, and Favin with 14 biological activities (Table 1). Although other biological activities have been presented, only two activities have been chosen for a more detailed analysis of the sequences responsible for such activities. Based on the frequency of the occurrence of peptides with given activity (A), only ACE-I and DPP-IV inhibitory activities were present in all lectins analyzed according to the heatmap (Figure 1). The lectins with the highest values of (A) for ACE-I inhibition were Musa Lec (A 0.4279), Coles Lec (A 0.3979) and AEL (A 0.3937). For the inhibition of DDP-IV, the lectins with the highest values were Favin (A 0.6421), AEL (A 0.6199) and PHA-E (A 0.6184) (Table 1).

All BP encrypted in the lectin sequences which had between 2 and 5 amino acids and associated with ACE-I and DDP-IV inhibition were systematically annotated (Supplementary Table 1). The lectins presented a high amount of ACE-I and DPP-IV inhibitory peptides when analyzed individually and also in the total sum of inhibitory peptides (Supplementary Table 2). Among all lectins studied, Coles Lec presented the greatest number of ACE-I inhibitory peptides, accounting for a total of 110 peptides. As to

the inhibition of DDP-IV, 197 inhibitory peptides were found in the Soybn Lec sequence (Figure 2 and Supplementary Table 1).

The Theoretical Degree of Hydrolysis (DHt) in an enzymatic reaction is defined as the total number of cleaved peptide bonds and it shows the enzyme's effectiveness in producing peptides (Iwaniak et al., 2020; Rutherfurd, 2010). Our results showed that the DHt using pepsin ranged from 6.1111% (Allsa Lec) to 23.7288% (Pea Lec and Lencu Lec), while for the combination of pepsin and trypsin ranged from 15.0000% (Allsa Lec) to 30.5085% (PHA-E) (Table 2). For all lectin sequences, the DHt values were higher when there was a combination of pepsin and trypsin. DHt values vary according to the combination of enzyme and the sequence of amino acids. Moreover, the release of peptides can occur at different DHt values (Iwaniak et al., 2020). A release of antioxidant peptides of plant origin revealed a DHt that varied between 27% and 66% when the enzymes bromelain, ficin and pepsin were used (Szerszunowicz & Kłobukowski, 2020). Plant proteases usually have higher DHt values because they have more recognition sites than the proteases pepsin, trypsin and chymotrypsin and therefore also generate a greater number of peptides (Ji et al., 2019) and higher DHt values (Barbana & Boye, 2010). In order to simulate the enzymatic digestion that occurs in animals, plant proteases were not included in our analyses. However, the DHt results of the pepsin and trypsin proteases are promising and capable of generating a large number of BP as demonstrated here.

The maximum and minimum values of (AE) for ACE-I inhibitory activity of the peptides obtained were 0.0500 and 0.0055 (Lencu lec and Allsa lec peptides, respectively). For DPP-IV inhibitory activity, the highest and lowest values of (AE) were 0.0667 and 0.0166 (Favin and Allsa lec peptides, respectively). In turn, the maximum and minimum values found of (W) were the same for all analyzed lectins (Table 2). Futhermore, it was possible to observe that the values of (AE) for the same lectin were generally higher for the inhibitory activity

of DPP-IV when compared with the data of ACE-I. Despite the use of pepsin and trypsin for all lectin hydrolysis simulations, the different DHt, AE and W values existed due to the different amino acids compositions and the different cleavage sites of each lectin (Table 2). The results obtained showed that the release of BP is greater in the intestinal phase (after pepsin digestion) than in the gastric phase (where only pepsin is present). Analyses of wheat peptides showed a similar result, by identifying a higher number and release of peptides in the intestinal phase due to the presence of trypsin and chymotrypsin (Perçin & Karakaya, 2020).

ACE-I inhibitory peptides have been identified in a wide range of plant species (Silva do Nascimento et al., 2021). ACE-I inhibitors of natural origin are still a low-cost alternative with fewer side effects when compared to the most commonly used antihypertensive drugs, namely captopril and lisinopril (Aluko, 2015; Segura Campos et al., 2013). ACE-I inhibitors still remain the main therapeutic strategy to regulate blood pressure in hypertensive patients. Different natural sources of peptides have been identified, such as in hydrolysates of oat (I. W. Y. Cheung et al., 2009), wheat germ (Matsui et al., 2000), quinoa (Guo et al., 2020), chia (Segura Campos et al., 2013) and *Zizyphus jujuba* (Memarpoor-Yazdi et al., 2020).

In general, ACE-I inhibitory peptides have between 2-12 amino acids and those of long size can be prevented from binding to the enzyme active site (Daskaya-Dikmen et al., 2017; Natesh et al., 2003). The presence of aromatic amino acids (such as tryptophan, tyrosine and phenylalanine) and branched side chains in the COOH-terminal portion of the peptides have been reported as important characteristics found in ACE-inhibiting peptides, while the NH2-terminal portion usually has aliphatic and branched amino acids (Rezaei et al., 2019; Wilson et al., 2011).

It was seen that in the hydrolysates of chickpea and yellow pea proteins, ACE-I inhibitory peptides had molecular weight below 4 kDa and the profile of inhibitory peptides

changed according to the enzymes used in the digestion (Barbana & Boye, 2010). In peptides derived from garlic, all dipeptides characterized presented the aromatic amino acids tyrosine or phenylalanine as C-terminal residues, being Phe-Tyr the most potent among them (Suetsuna, 1998). Futhermore, the presence of aliphatic amino acids in the NH2-terminal portion is observed in potent ACE-I inhibiting dipeptides, such as Val-Trp (H.-S. Cheung et al., 1980).

In order to have any *in vivo* effect, the peptides must be absorbed by the cells of the intestine and reach the bloodstream without being degraded by plasma peptidases (Li et al., 2004). Small peptides, as those presented in this study, are easily absorbed by the transcellular pathway through the intestinal cells, while oligopeptides usually follow the paracellular route (Shen & Matsui, 2017). Due to the high concentration of peptides observed in the inhibition of ACE-I, it has been proposed that another mechanism of action in the inhibition of ACE-I is the chelation of zinc molecules in the active site of this protein, which acts as an important cofactor in the activity of ACE-I (Suetsuna, 1998).

Many inhibitors have been discovered for DPP-IV in order to improve the effectiveness of drugs currently available on the market. Thus, incretin-based therapies appear to be a viable and promising alternative in the treatment of the disease (Nauck et al., 2009). The main substrates of the DPP-IV serine dipeptidase are the gut hormones, namely GLP-1 and GIP and have a plasma half-life of approximately 2 and 7 minutes, respectively (Shubrook et al., 2011). GLP-1 and GIP act on receptors coupled to G protein. The binding of these hormones to their receptors initiates a signaling cascade raising levels of cyclic AMP and intracellular calcium. Then, the calcium concentration leads to the release of insulin contained in granules by exocytosis (Power et al., 2014).

It has been suggested that roughly half of the insulin released after a meal is mediated by the action of the GLP-1 and GIP incretins (Tseng et al., 1999). DPP-IV cleaves dipeptides

that are just after proline or alanine residues at position 2 of the inhibitory sequence in the N-terminal portion (Patil et al., 2015). The discovery of new DPP-IV inhibitors has been driven not only by its physiological effects, but also by safety and tolerability as an oral hypoglycemic agent. Inhibition of DPP-IV prolongs the half-life of GLP-1 and GIP incretins, reducing glucagon secretion and consequently increasing insulin levels in the bloodstream (Patil et al., 2015)**.** DPP-IV inhibitory peptides usually have a sequence of up to 7 amino acids and hydrophobic characteristics (Huang et al., 2012). Although large peptides are less able to inhibit DDP-IV, it has been seen that the sequence and amino acids composition of the peptides are more important than their size (Huang et al., 2012; Nongonierma & FitzGerald, 2019). Among the DPP-IV inhibitory peptides, it is common to find the amino acids tryptophan, threonine and methionine in the N-terminal portion and alanine, leucine and histidine in the C-terminal portion, although potent inhibitors have proline or alanine in the N-terminal portion (Power et al., 2014). The presence of proline in the inhibitory sequence despite being an important feature in the inhibition of DPP-IV, for some sequences not to exert the same pattern of inhibition, since sequences showing proline were unable to inhibit the enzyme (Nongonierma & FitzGerald, 2013).

Because the inhibition of DDP-IV requires a high concentration of peptides, it is noteworthy that in order to have a significant and long-lasting physiological effect in diabetic patients, foods containing these peptides should preferably be consumed daily. It has been reported that the MF dipeptide has inhibitory activity and ACE-I, however it was seen in our work that this same sequence also has DPP-IV inhibitory activity (Yu et al., 2019).

3.3 Top-three ACE-I and DPP-IV inhibitory peptides and toxicity

The six peptides chosen (three for each inhibitory activity) were defined as the most promising peptides in the context of enzyme inhibition and they were used in the further

molecular docking analyses to verify their inhibition mechanism (Table 4). The peptides that obtained the highest inhibitory activity for ACE-I were the peptides MF (from ConA, ConBr and ConMa), CF (Coles lec) and FFL (SBA). The peptides that obtained the best values for DPP-IV inhibitory activity were WF (from AEL), MF (ConA, ConBr and ConMa) and GF (ConMa, MusaLec, PEA, SBA, LCA, PHA-E, Favin).

Since toxicity is an important assessment in the development of new peptides, as well as biochemical properties (e.g. solubility), the toxicity of all peptides was analyzed using the ToxinPred and PepCalc softwares (Gupta et al., 2013). The result of the analyses showed that the selected peptides were non-toxic according to the parameters used in the software, and all of them showed low solubility due to its composition of hydrophobic amino acids (Table 3). As expected the peptides also had low molecular mass due to the size of their sequences. According to our knowledge, no lethality has been associated to the use of DPP-IV inhibitors. However it is possible that non-lethal effects observed in studies with mice such as alopecia, bloody diarrhea, thrombocytopenia and splenomegaly were due to DDP8/9 inhibition (Lankas et al., 2005).

3.4 Analysis of molecular docking

Among the thermodynamic properties that can be evaluated computationally is the free binding energy, which will indicate the binding between the substrate-ligand complex. In this context, bioinformatics tools were used to perform a protein-ligand docking in order to understand the inhibitory activity of the peptides from lectins. The lowest binding energy represents a strong interaction between peptide and enzyme. The three-dimensional structure of the ACE-I and DPP-IV enzymes was obtained from the PDB Databank using the codes 1O8A and 1NU6, respectively. Molecular docking analyses using ClusPro2 revealed that all peptides had very low peptide-protein binding energies, revealing a high level of interaction

between peptides and enzymes. The binding energy values of the inhibitory peptides for ACE-I ranged from -671.7 kJ/mol^{-1} to -518.4 kJ/mol^{-1}, and for DPP-IV from -697.3 kJ/mol^{-1} to -415.7 kJ/mol^{-1} (Table 4).

The binding energy between the MF peptide and ACE-I was -536.7 kJ/mol^{-1} (Table 4). The main types of interaction between the enzyme-inhibitor complex were hydrophobic interactions and hydrogen bonds. The residues responsible for hydrophobic interactions were TYR_{146} (PHE_2); LEU_{161} (PHE_2), TRP_{185} (PHE_2); TRP_{279} (PHE_2); VAL_{350} (MET_1), PHE_{512} (PHE_2); GLU_{349} (MET_1); VAL_{151} (PHE_2). Hydrogen bonds were found mainly between residues CYS352 (MET_1); ALA_{149} (MET_1); GLU_{162} (MET_1) (Table 4 and Figure 3A). The MF peptide is able to interact with the ACE-I S1' pocket and with amino acids neighboring the S2 pocket - LYS_{511}, HIS_{513}. A non-competitive inhibition is suggested for the MF dipeptide due to the lack of interaction between the main amino acids that make up the ACE-I active site.

The CF inhibitory sequence for the ACE-I enzyme had a binding energy of -518.4 kJ/mol^{-1} (Table 4). The CF peptide does not interact with amino acids present in the active site. A single interaction was observed between the CF cysteine and the GLU_{162} residue from the S1'pocket, through hydrogen bonds. Thus, CF is expected to be a non-competitive inhibitor of this enzyme, due to the low interaction with the active site of the enzyme. The interaction between the CF-ACE-I complex occurs through hydrogen bonds and hydrophobic interactions, the latter occurring mainly by aromatic chain amino acids. Hydrophobic interactions occurred between amino acids TYR_{146}, LEU_{161}, TRP_{279}, PHE_{512}, HIS_{353} and SER_{147} with the phenylalanine residue of the dipeptide. Hydrogen bonds occurred between the residues of GLU_{162}, ALA_{149} and CYS_{352} with the CYS residue from CF (Table 4 and Figure 3B).

The FFL tripeptide obtained the lowest binding energy among ACE-I inhibitory peptides, with an energy of -671.7 kJ/mol^{-1} (Table 4). As expected for ACE-I inhibiting peptides, the peptides showed mainly hydrophobic interactions between the peptides and the enzyme. However, no interactions of the dipeptide amino acids with any amino acid that makes up the active site of the enzyme were observed, indicating a possible non-competitive inhibition by the FFL sequence. The interactions occurred between residues of TYR_{51} (PHE_1); TYR_{62} (LEU_3); ILE_{88} (PHE_2); ILE_{88} (LEU_3); ALA_{89} (PHE_2); VAL_{119} (PHE_1), LEU_{122} (PHE_2); TYR_{360} (LEU_3); GLU_{123} (PHE_2). Hydrogen bonds were present between VAL_{119} (PHE_1); THR_{92} (PHE_1); ARG_{124} (LEU_3) (Table 4 and Figure 3C). Two Cation-Pi interactions were also observed between residues LYS_{118} (PHE_1) and ARG_{124} (PHE_2).

ACE-I has three pockets of active sites, being them: S1 (ALA_{354}, GLU_{384} and TYR_{523}), S2 (GLN_{281}, HIS_{353}, LYS_{511}, HIS_{513} and $TYR5_{20}$) and S1'(GLU_{162}) (Q. Wu et al., 2015). As noted in the results, all peptides showed hydrophobic peptides (Phe, Trp, Met and Gly). Inhibition of ACE-I can occur through competitive, non-competitive or mixed inhibition (Tahir et al., 2020), however, small peptides usually act through competitive inhibition.

The inhibitory activity of ACE-I is strongly influenced by the composition of amino acids present in the C-terminal portion and amino acids with hydrophobic characteristics, namely aromatic amino acids and branched side-chains (Fitzgerald & Meisel, 2000). For inhibitory peptides of small size, the composition of amino acids seems to be a more important characteristic for the inhibition of this enzyme than the N-terminal portion. Besides that, long peptide sequences less effectively inhibit ACE-I (Pripp et al., 2004). The chebulin peptide (Asp–Glu–Asn–Ser–Lys–Phe) obtained from *Terminalia chebula* is capable of inhibiting ACE-I by non-competitive inhibition. The presence of phenylalanine in the C-terminal portion of this hexapeptide is related to the formation of hydrogen bonds and hydrophobic interactions (Sornwatana et al., 2015). The EWL tripeptide obtained from

ovotransferrin proteolysis showed a potent inhibition of ACE-I through molecular docking, and the main interactions between ligand-enzyme were pi interaction, attractive charge and conventional hydrogen bond (Yu et al., 2019).

The WG and PRY peptides derived from patatin (potato) are able to inhibit ACE-I in a mixed and competitive way, respectively, mainly for being able to form hydrogen bonds and stabilizing the enzyme-peptide complex. Furthermore, the WG peptide was able to interact with the zinc atom (Zn) in the enzyme, compromising the activity of ACE-I (Fu et al., 2017). The amino acid isoleucine provides more flexibility to the IPP tripeptide (-19.02 kJ/mol^{-1}) and greater inhibitory capacity compared to the VPP tripeptide (-18.69 kJ/mol^{-1}) in terms of enzyme inhibition, which explains the different binding energy values of these peptides , both obtained from casein (Pina & Roque, 2009).

The result of molecular docking with the DPP-IV inhibitory peptides indicated that the binding energy found for the WF peptide was -697.3 kJ/mol^{-1}, the lowest binding energy among all analyzed sequences (Table 4). The interaction between the WF peptide and DPP-IV was due to hydrophobic interactions and hydrogen bonds. Ionic and Cation-Pi interactions were observed in the protein structure, but these bonds were not involved in the inhibition of DPP-IV. The WF peptide was able to interact through hydrophobic interactions with the $LEU_{236\text{-}A}$ (PHE_2) residues; $TYR_{238\text{-}A}$ (PHE_2); $TYR_{241\text{-}A}$ (TRP_1); $TYR_{241\text{-}B}$ (TRP_1/PHE_2); $PHE_{713\text{-}A}$ (TRP_1/PHE_2); $ALA_{717\text{-}B}$ (TRP_1); $TRP_{734\text{-}A}$ (TRP_1). In that case, both chains of the DPP-IV protein were involved in inhibition. Hydrogen bonds were also observed between residues GLN_{714} (PHE_2) and SER_{239} (TRP_1) (Table 4 and Figure 4A). The mode of inhibition was thought to be non-competitive due to WF bonds in several other amino acids that do not make up the DPP-IV active site.

The binding energy found for the inhibition of DPP-IV by the MF peptide was -540.6 kJ/mol^{-1} (Table 4). A non-competitive mode of inhibition has been thought due to low

interaction with amino acids residues from the DPP-IV active site or any of the enzyme pockets. The residues that interacted through hydrogen bonds were $ILE_{236\text{-}A}$ (PHE_2); $TYR_{238\text{-}A}$ (PHE); $TYR_{241\text{-}A}$ (PHE_2/MET_1); $TYR_{241\text{-}B}$ (PHE_2); $PHE_{713\text{-}A}$ (PHE_2/MET_1); $PHE_{713\text{-}B}$ (MET_1); $ALA_{717\text{-}B}$ (MET_1); $TRP_{734\text{-}A}$ (MET_1); THR $_{706\text{-}A/B}$ (MET_1). The hydrogen bonds between the peptide and DPP-IV occurred mainly between $SER_{239\text{-}A}$ (PHE_2); $TYR_{241\text{-}A}$ (PHE_2) (Table 4 and Figure 4B). Ionic and Cation-Pi interactions were also observed, but not involving the MF peptide.

For the GF peptide, a binding energy of -415.7 kJ/mol^{-1} was found (Table 4). As seen for the other DPP-IV inhibitors, ionic and Cation-Pi interactions were observed in some residues, but were not present in the interaction of the peptides and the enzyme. Hydrophobic interactions were also observed between various residues, being them: $TYR_{238\text{-}A}$ (PHE_2); $TYR_{241\text{-}B}$ (PHE_2); $HIS_{712\text{-}A}$ (PHE_2); $GLU_{237\text{-}A}$ (PHE_2); $THR_{706\text{-}A}$ (PHE_2); $PHE_{713\text{-}A}$ (GLY_1). Among the hydrogen bonds, interactions were found between $TYR_{241\text{-}B}$ (GLY_1); $TYR_{241\text{-}A}$ (PHE_2); $SER_{239\text{-}A}$ (PHE_2) (Table 4 nad Figure 4C). No interactions of the amino acids of the inhibitory peptide with amino acids residues from the DPP-IV active site or the S1, S2 or S3 pockets were observed, indicating a possible non-competitive inhibition model.

In relation to DPP-IV, its catalytic site consists of the amino acids SER_{630}, ASP_{708}, and HIS_{740} located between the two domains of the protein. It is still observed the presence of a S1 pocket, formed by the amino acids VAL_{711}, VAL_{656}, TYR_{662}, TYR_{666}, TRP_{659}, and TYR_{631} (Thoma et al., 2003), S2 pocket formed by the amino acids ARP_{125}, GLU_{205}, and GLU_{206}, PHE_{357}, TYR_{547}, PRO_{550}, TYR_{631}, and TYR_{666} (Aertgeerts, 2004) and S3 pocket SER_{209}, TYR_{547}, ARG_{358}, and PHE_{357} (de Souza Rocha et al., 2014; Xu et al., 2019). Since the active site is located in a small pocket, it is more likely that small side chain amino acids like proline, alanine and glycine were able to interact with the amino acids in that pocket (Rasmussen et al., 2003).

It is common to notice in DPP-IV inhibitory peptides the presence of hydrophobic amino acids or aromatic chain in the N-terminal portion such as Ala, Val, Ile, Leu, Met, Phe, Tyr or Trp (Nongonierma et al., 2014). In addition, the presence of tryptophan as seen in our work is an important feature that appears in most non-competitive inhibitors. In fact, the presence of hydrophobic amino acids in the N-terminal portion of the inhibitory peptide appears to be more important than the total hydrophobicity of the peptide in terms of enzyme inhibition.(Nongonierma et al., 2014).

Although less common, peptides from soy were able to perform ionic interactions during the inhibition of DPP-IV through the amino acid ARG_{358}. This residue is also involved in the inhibition of DPP-IV by the oral antidiabetic omarigliptin (Lammi et al., 2016). The smallest peptides inhibiting DPP-IV of *P. vulgaris* have the ability to bind to S1 and S2 pocket through the HIS_{740} and ARG_{125} residues, respectively. On the other hand, pocket S3 had no relation to the inhibitory activity of the analyzed sequences (Oseguera-Toledo et al., 2015).

4. Conclusions

Our results indicate that plant lectins of different origins, especially from legume species, are good sources of antihypertensive and antidiabetic peptides. The peptides found, mainly di and tripeptides, are hydrophobic in nature and interact with the ACE-I and DPP-IV enzymes through hydrophobic interactions and hydrogen bonds. The non-competitive inhibition mode was thought to be the most representative since no interaction with residues of the active enzyme site was observed. Since DPP-IV and ACE-I inhibitors are widely used in the pharmaceutical industry, the results achieved in this work may help in the development of new drugs based on proteins from natural sources as an adjunct in the management of diseases of wide occurrence such as diabetes and hypertension. However, *in vivo* studies

must be performed to confirm their biological activity, as well as to assess the toxicity and safety os these peptides as new therapeutic tools.

Acknowledgements

The authors acknowledge the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq-Brazil) by Scientific undergraduate scholarship of Mr. Eduardo Pereira

Conflict of Interest Statement

The authors declare no conflict of interest.

Ethical Statements

This article contains only *in silico* results. Thus, no study with humans and animals has been carried out that would require approval from the Ethics Committee.

References

Aertgeerts, K. (2004). Crystal structure of human dipeptidyl peptidase IV in complex with a decapeptide reveals details on substrate specificity and tetrahedral intermediate formation. *Protein Science*, *13*(2). https://doi.org/10.1110/ps.03460604

Al-Lawati, J. A. (2017). Diabetes mellitus: A local and global public health emergency! In *Oman Medical Journal*. https://doi.org/10.5001/omj.2017.34

Aluko, R. E. (2015). Structure and function of plant protein-derived antihypertensive peptides. In *Current Opinion in Food Science*. https://doi.org/10.1016/j.cofs.2015.05.002

Barbana, C., & Boye, J. I. (2010). Angiotensin I-converting enzyme inhibitory activity of

chickpea and pea protein hydrolysates. *Food Research International*, *43*(6). https://doi.org/10.1016/j.foodres.2010.05.003

Bhagyawant, S. S., Narvekar, D. T., Gupta, N., Bhadkaria, A., Gautam, A. K., & Srivastava, N. (2019). Chickpea (Cicer arietinum L.) Lectin Exhibit Inhibition of ACE-I, α-amylase and α-glucosidase Activity. *Protein & Peptide Letters*. https://doi.org/10.2174/0929866526666190327130037

Cheung, H.-S., Wang, F.-L., Ondetti, M. A., Sabo, E. F., & Cushman, D. W. (1980). Binding of peptide substrates and inhibitors of angiotensin-converting enzyme. *The Journal of Biological Chemistry*.

Cheung, I. W. Y., Nakayama, S., Hsu, M. N. K., Samaranayaka, A. G. P., & Li-Chan, E. C. Y. (2009). Angiotensim-I converting enzyme inhibitory activity of hydrolysates from oat (Avena sativa) proteins by in silico and in vitro analyses. *Journal of Agricultural and Food Chemistry*. https://doi.org/10.1021/jf9018245

Daskaya-Dikmen, C., Yucetepe, A., Karbancioglu-Guler, F., Daskaya, H., & Ozcelik, B. (2017). Angiotensin-I-converting enzyme (ACE)-inhibitory peptides from plants. *Nutrients*, *9*(4), 1–19. https://doi.org/10.3390/nu9040316

de Lacerda, J. T. J. G., e Lacerda, R. R., Assunção, N. A., Tashima, A. K., Juliano, M. A., dos Santos, G. A., dos Santos de Souza, M., de Luna Batista, J., Rossi, C. E., de Almeida Gadelha, C. A., & Santi-Gadelha, T. (2017). New insights into lectin from Abelmoschus esculentus seeds as a Kunitz-type inhibitor and its toxic effects on Ceratitis capitata and root-knot nematodes Meloidogyne spp. *Process Biochemistry*, *63*. https://doi.org/10.1016/j.procbio.2017.09.003

de Souza Rocha, T., Hernandez, L. M. R., Chang, Y. K., & de Mejía, E. G. (2014). Impact of germination and enzymatic hydrolysis of cowpea bean (Vigna unguiculata) on the generation of peptides capable of inhibiting dipeptidyl peptidase IV. *Food Research International*, *64*. https://doi.org/10.1016/j.foodres.2014.08.016

Fitzgerald, R. J., & Meisel, H. (2000). Milk protein-derived peptide inhibitors of angiotensin-I-converting enzyme. In *British Journal of Nutrition*. https://doi.org/10.1017/s0007114500002221

Fosgerau, K., & Hoffmann, T. (2015). Peptide therapeutics: Current status and future directions. In *Drug Discovery Today*. https://doi.org/10.1016/j.drudis.2014.10.003

Fu, Y., Alashi, A. M., Young, J. F., Therkildsen, M., & Aluko, R. E. (2017). Enzyme inhibition kinetics and molecular interactions of patatin peptides with angiotensin I-converting enzyme and renin. *International Journal of Biological Macromolecules*, *101*. https://doi.org/10.1016/j.ijbiomac.2017.03.054

Gourgari, E., Wilhelm, E. E., Hassanzadeh, H., Aroda, V. R., & Shoulson, I. (2017). A comprehensive review of the FDA-approved labels of diabetes drugs: Indications, safety, and emerging cardiovascular safety data. *Journal of Diabetes and Its Complications*. https://doi.org/10.1016/j.jdiacomp.2017.08.005

Guo, H., Richel, A., Hao, Y., Fan, X., Everaert, N., Yang, X., & Ren, G. (2020). Novel dipeptidyl peptidase-IV and angiotensin-I-converting enzyme inhibitory peptides released from quinoa protein by in silico proteolysis. *Food Science and Nutrition*. https://doi.org/10.1002/fsn3.1423

Gupta, S., Kapoor, P., Chaudhary, K., Gautam, A., Kumar, R., & Raghava, G. P. S. (2013). In Silico Approach for Predicting Toxicity of Peptides and Proteins. *PLoS ONE*, *8*(9). https://doi.org/10.1371/journal.pone.0073957

Holst, J. J., & Gromada, J. (2004). Role of incretin hormones in the regulation of insulin secretion in diabetic and nondiabetic humans. In *American Journal of Physiology - Endocrinology and Metabolism* (Vol. 287, Issues 2 50-2). https://doi.org/10.1152/ajpendo.00545.2003

Huang, S. L., Jao, C. L., Ho, K. P., & Hsu, K. C. (2012). Dipeptidyl-peptidase IV inhibitory activity of peptides derived from tuna cooking juice hydrolysates. *Peptides*,

35(1). https://doi.org/10.1016/j.peptides.2012.03.006

Iwaniak, A., Minkiewicz, P., Pliszka, M., Mogut, D., & Darewicz, M. (2020). Characteristics of Biopeptides Released in Silico from Collagens Using Quantitative Parameters. *Foods*. https://doi.org/10.3390/foods9070965

Ji, D., Udenigwe, C. C., & Agyei, D. (2019). Antioxidant peptides encrypted in flaxseed proteome: An in silico assessment. *Food Science and Human Wellness*. https://doi.org/10.1016/j.fshw.2019.08.002

Lagarda-Diaz, I., Guzman-Partida, A. M., & Vazquez-Moreno, L. (2017). Legume lectins: Proteins with diverse applications. In *International Journal of Molecular Sciences*. https://doi.org/10.3390/ijms18061242

Lammi, C., Zanoni, C., Arnoldi, A., & Vistoli, G. (2016). Peptides Derived from Soy and Lupin Protein as Dipeptidyl-Peptidase IV Inhibitors: In Vitro Biochemical Screening and in Silico Molecular Modeling Study. *Journal of Agricultural and Food Chemistry*, *64*(51). https://doi.org/10.1021/acs.jafc.6b04041

Lankas, G. R., Leiting, B., Roy, R. S., Eiermann, G. J., Beconi, M. G., Biftu, T., Chan, C. C., Edmondson, S., Feeney, W. P., He, H., Ippolito, D. E., Kim, D., Lyons, K. A., Ok, H. O., Patel, R. A., Petrov, A. N., Pryor, K. A., Qian, X., Reigle, L., … Thornberry, N. A. (2005). Dipeptidyl peptidase IV inhibition for the treatment of type 2 diabetes: Potential importance of selectivity over dipeptidyl peptidases 8 and 9. *Diabetes*, *54*(10). https://doi.org/10.2337/diabetes.54.10.2988

Laurent, S., Schlaich, M., & Esler, M. (2012). New drugs, procedures, and devices for hypertension. *The Lancet*. https://doi.org/10.1016/S0140-6736(12)60825-3

Li, G. H., Le, G. W., Shi, Y. H., & Shrestha, S. (2004). Angiotensin I-converting enzyme inhibitory peptides derived from food proteins and their physiological and pharmacological effects. *Nutrition Research*. https://doi.org/10.1016/j.nutres.2003.10.014

Liu, R., Cheng, J., & Wu, H. (2019). Discovery of food-derived dipeptidyl peptidase IV inhibitory peptides: A review. In *International Journal of Molecular Sciences*. https://doi.org/10.3390/ijms20030463

Lu, Y., Lu, P., Wang, Y., Fang, X., Wu, J., & Wang, X. (2019). A novel dipeptidyl peptidase IV inhibitory tea peptide improves pancreatic β-cell function and reduces α-cell proliferation in streptozotocin-induced diabetic mice. *International Journal of Molecular Sciences*. https://doi.org/10.3390/ijms20020322

Matsui, T., Li, C. H., Tanaka, T., Maki, T., Osajima, Y., & Matsumoto, K. (2000). Depressor effect of wheat germ hydrolysate and its novel angiotensin I- converting enzyme inhibitory peptide, Ile-Val-Tyr, and the metabolism in rat and human plasma. *Biological and Pharmaceutical Bulletin*. https://doi.org/10.1248/bpb.23.427

Memarpoor-Yazdi, M., Zare-Zardini, H., Mogharrab, N., & Navapour, L. (2020). Purification, Characterization and Mechanistic Evaluation of Angiotensin Converting Enzyme Inhibitory Peptides Derived from Zizyphus Jujuba Fruit. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-60972-w

Miller, B. R., Nguyen, H., Hu, C. J. H., Lin, C., & Nguyen, Q. T. (2014). New and emerging drugs and targets for type 2 diabetes: Reviewing the evidence. In *American Health and Drug Benefits*.

Natesh, R., Schwager, S. L. U., Sturrock, E. D., & Acharya, K. R. (2003). Crystal structure of the human angiotensin-converting enzyme-lisinopril complex. *Nature*. https://doi.org/10.1038/nature01370

Nauck, M. A., Vilsbøll, T., Gallwitz, B., Garber, A., & Madsbad, S. (2009). Incretin-based therapies: viewpoints on the way to consensus. In *Diabetes care: Vol. 32 Suppl 2*. https://doi.org/10.2337/dc09-s315

Nongonierma, A. B., & FitzGerald, R. J. (2013). Inhibition of dipeptidyl peptidase IV (DPP-IV) by proline containing casein-derived peptides. *Journal of Functional*

Foods, *5*(4). https://doi.org/10.1016/j.jff.2013.09.012

Nongonierma, A. B., & FitzGerald, R. J. (2019). Features of dipeptidyl peptidase IV (DPP-IV) inhibitory peptides from dietary proteins. In *Journal of Food Biochemistry* (Vol. 43, Issue 1). https://doi.org/10.1111/jfbc.12451

Nongonierma, A. B., Mooney, C., Shields, D. C., & Fitzgerald, R. J. (2014). In silico approaches to predict the potential of milk protein-derived peptides as dipeptidyl peptidase IV (DPP-IV) inhibitors. *Peptides*, *57*. https://doi.org/10.1016/j.peptides.2014.04.018

Oparil, S., Acelajado, M. C., Bakris, G. L., Berlowitz, D. R., Cífková, R., Dominiczak, A. F., Grassi, G., Jordan, J., Poulter, N. R., Rodgers, A., & Whelton, P. K. (2018). Hypertension. In *Nature Reviews Disease Primers*. https://doi.org/10.1038/nrdp.2018.14

Oseguera-Toledo, M. E., Gonzalez de Mejia, E., & Amaya-Llano, S. L. (2015). Hard-to-cook bean (Phaseolus vulgaris L.) proteins hydrolyzed by alcalase and bromelain produced bioactive peptide fractions that inhibit targets of type-2 diabetes and oxidative stress. *Food Research International*, *76*. https://doi.org/10.1016/j.foodres.2015.07.046

Patil, P., Mandal, S., Tomar, S. K., & Anand, S. (2015). Food protein-derived bioactive peptides in management of type 2 diabetes. In *European Journal of Nutrition* (Vol. 54, Issue 6). https://doi.org/10.1007/s00394-015-0974-2

Perçin, P. S., & Karakaya, S. (2020). Evaluation of Protein Profiles, Bioactivity, Allergenicity and Toxicity of Peptides Generated After in silico Digestion of Common Wheat and Einkorn Wheat. *Turkish Journal of Agriculture - Food Science and Technology*. https://doi.org/10.24925/turjaf.v8i4.901-911.3072

Pina, A. S., & Roque, A. C. A. (2009). Studies on the molecular recognition between bioactive peptides and angiotensin-converting enzyme. *Journal of Molecular*

Recognition, *22*(2). https://doi.org/10.1002/jmr.905

Power, O., Nongonierma, A. B., Jakeman, P., & Fitzgerald, R. J. (2014). Food protein hydrolysates as a source of dipeptidyl peptidase IV inhibitory peptides for the management of type 2 diabetes. *Proceedings of the Nutrition Society*, *73*(1). https://doi.org/10.1017/S0029665113003601

Pratley, R. E. (2008). Overview of glucagon-like peptide-1 analogs and dipeptidyl peptidase-4 inhibitors for type 2 diabetes. In *MedGenMed Medscape General Medicine*.

Pripp, A. H., Isaksson, T., Stepaniak, L., & Sørhaug, T. (2004). Quantitative structure-activity relationship modelling of ACE-inhibitory peptides derived from milk proteins. *European Food Research and Technology*, *219*(6). https://doi.org/10.1007/s00217-004-1004-4

Rasmussen, H. B., Branner, S., Wiberg, F. C., & Wagtmann, N. (2003). Crystal structure of human dipeptidyl peptidase IV/CD26 in complex with a substrate analog. *Nature Structural Biology*, *10*(1). https://doi.org/10.1038/nsb882

Rezaei, A., Amirdivani, S., Khosrowshahi Asl, A., Malekinejad, H., Zomorodi, S., & Hosseinmardi, F. (2019). Inhibition of the Angiotensin I Converting Enzyme (ACE) and proteolysis of non-fat probiotic yogurt. *Brazilian Journal of Food Technology*, *22*. https://doi.org/10.1590/1981-6723.23418

Ribeiro-Oliveira, A., Nogueira, A. I., Pereira, R. M., Vilas Boas, W. W., Souza dos Santos, R. A., & Simões e Silva, A. C. (2008). The renin-angiotensin system and diabetes: An update. In *Vascular Health and Risk Management*. https://doi.org/10.2147/vhrm.s1905

Rutherfurd, S. M. (2010). Methodology for determining degree of hydrolysis of proteins in hydrolysates: A Review. In *Journal of AOAC International*. https://doi.org/10.1093/jaoac/93.5.1515

Sánchez, A., & Vázquez, A. (2017). Bioactive peptides: A review. In *Food Quality and Safety*. https://doi.org/10.1093/fqsafe/fyx006

Sarmadi, B. H., & Ismail, A. (2010). Antioxidative peptides from food proteins: A review. In *Peptides*. https://doi.org/10.1016/j.peptides.2010.06.020

Segura Campos, M. R., Peralta González, F., Chel Guerrero, L., & Betancur Ancona, D. (2013). Angiotensin I-converting enzyme inhibitory peptides of chia (Salvia hispanica) produced by enzymatic hydrolysis. *International Journal of Food Science, 2013*. https://doi.org/10.1155/2013/158482

Sharma, J. N. (2010). Activation of the bradykinin system by angiotensin-converting enzyme inhibitors. In *European Journal of Inflammation*. https://doi.org/10.1177/1721727X1000800201

Shen, W., & Matsui, T. (2017). Current knowledge of intestinal absorption of bioactive peptides. In *Food and Function*. https://doi.org/10.1039/C7FO01185G

Shubrook, J., Colucci, R., Guo, A., & Schwartz, F. (2011). Saxagliptin: A selective DPP-4 inhibitor for the treatment of type 2 diabetes mellitus. In *Clinical Medicine Insights: Endocrinology and Diabetes* (Vol. 4). https://doi.org/10.4137/CMED.S5114

Silva do Nascimento, E., Anaya, K., de Oliveira, J. M. C., de Lacerda, J. T. J. G., Miller, M. E., Dias, M., Mendes, M. A., de Azevedo Lima Pallone, J., Weis Arns, C., Juliano, M. A., Santi Gadelha, T., Bertoldo Pacheco, M. T., & de Almeida Gadelha, C. A. (2021). Identification of bioactive peptides released from in vitro gastrointestinal digestion of yam proteins (Dioscorea cayennensis). *Food Research International, 143*. https://doi.org/10.1016/j.foodres.2021.110286

Sornwatana, T., Bangphoomi, K., Roytrakul, S., Wetprasit, N., Choowongkomon, K., & Ratanapo, S. (2015). Chebulin: Terminalia chebula Retz. fruit-derived peptide with angiotensin-I-converting enzyme inhibitory activity. *Biotechnology and Applied*

Biochemistry, *62*(6). https://doi.org/10.1002/bab.1321

Suetsuna, K. (1998). Isolation and characterization of angiotensin I-converting enzyme inhibitor dipeptides derived from Allium sativum L (garlic). *Journal of Nutritional Biochemistry*. https://doi.org/10.1016/S0955-2863(98)00036-9

Szerszunowicz, I., & Kłobukowski, J. (2020). Characteristics of potential protein nutraceuticals of plant origin with antioxidant activity. *Molecules*. https://doi.org/10.3390/molecules25071621

Tahir, R. A., Bashir, A., Yousaf, M. N., Ahmed, A., Dali, Y., Khan, S., & Sehgal, S. A. (2020). In Silico identification of angiotensin-converting enzyme inhibitory peptides from MRJP1. *PLoS ONE*, *15*(2). https://doi.org/10.1371/journal.pone.0228265

Thoma, R., Löffler, B., Stihle, M., Huber, W., Ruf, A., & Hennig, M. (2003). Structural basis of proline-specific exopeptidase activity as observed in human dipeptidyl peptidase-IV. *Structure*, *11*(8). https://doi.org/10.1016/S0969-2126(03)00160-6

Tran, N., Pham, B., & Le, L. (2020). Bioactive compounds in anti-diabetic plants: From herbal medicine to modern drug discovery. In *Biology*. https://doi.org/10.3390/biology9090252

Tseng, C. C., Zhang, X. Y., & Wolfe, M. M. (1999). Effect of GIP and GLP-1 antagonists on insulin release in the rat. *American Journal of Physiology - Endocrinology and Metabolism*, *276*(6 39-6). https://doi.org/10.1152/ajpendo.1999.276.6.e1049

Valencia, I., Peiró, C., Lorenzo, Ó., Sánchez-Ferrer, C. F., Eckel, J., & Romacho, T. (2020). DPP4 and ACE2 in Diabetes and COVID-19: Therapeutic Targets for Cardiovascular Complications? In *Frontiers in Pharmacology* (Vol. 11). https://doi.org/10.3389/fphar.2020.01161

Vukic, V. R., Vukic, D. V., Milanovic, S. D., Ilicic, M. D., Kanuric, K. G., & Johnson, M. S. (2017). In silico identification of milk antihypertensive di- and tripeptides involved

in angiotensin I–converting enzyme inhibitory activity. *Nutrition Research*. https://doi.org/10.1016/j.nutres.2017.07.009

Wilson, J., Hayes, M., & Carney, B. (2011). Angiotensin-I-converting enzyme and prolyl endopeptidase inhibitory peptides from natural sources with a focus on marine processing by-products. In *Food Chemistry*. https://doi.org/10.1016/j.foodchem.2011.04.081

Wu, J., Chen, Y., Shi, X., & Gu, W. (2009). Dipeptidyl peptidase IV(DPP IV): a novel emerging target for the treatment of type 2 diabetes. In *Journal of Nanjing Medical University*. https://doi.org/10.1016/S1007-4376(09)60061-7

Wu, Q., Jia, J., Yan, H., Du, J., & Gui, Z. (2015). A novel angiotensin-I converting enzyme (ACE) inhibitory peptide from gastrointestinal protease hydrolysate of silkworm pupa (Bombyx mori) protein: Biochemical characterization and molecular docking study. *Peptides*, *68*. https://doi.org/10.1016/j.peptides.2014.07.026

Xu, F., Yao, Y., Xu, X., Wang, M., Pan, M., Ji, S., Wu, J., Jiang, D., Ju, X., & Wang, L. (2019). Identification and Quantification of DPP-IV-Inhibitory Peptides from Hydrolyzed-Rapeseed-Protein-Derived Napin with Analysis of the Interactions between Key Residues and Protein Domains. *Journal of Agricultural and Food Chemistry*, *67*(13). https://doi.org/10.1021/acs.jafc.9b01069

Yu, Z., Chen, Y., Zhao, W., Zheng, F., Ding, L., & Liu, J. (2019). Novel ACE inhibitory tripeptides from ovotransferrin using bioinformatics and peptidomics approaches. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-019-53964-y

WHO. (2021). Global status report on noncommunicable diseases 2021. World Health Organization, WHO Press.

Figure captions

Figure 1 - Heatmap of the frequency of occurrence of peptides with given activity in plant lectins. The frequencies were obtained from the BIOPEP database. For each lectin, the highest frequency with a given activity obtains a maximal green color while the lowest frequency obtains a minimum red color.

Figure 2 - ACE-I and DPP-IV inhibitory peptides found in the lectin sequences. A) Unique peptides with antihypertensive and anti-diabetic activity. B) Total amount of ACE-I and DPP-IV inhibitory peptides found in lectins. C) Quantity of ACE-I and DPP-IV inhibitory peptides per protein sequence.

Figure 3 – Three-dimensional structure of ACE-I and its inhibitory peptides. The left side is showing the 3D structure of ACE-I complexed with its inhibitor. On the right side there is a 2D diagram showing the hydrophobic interactions (red) and the hydrogen bonds (green) existing between the inhibitory sequence and the enzyme. A) Non-competitive inhibition by the MF peptide. B) Non-competitive inhibition by the CF peptide. Non-competitive inhibition. C) Non-competitive inhibition by the FFL peptide. The 3D and 2D images were analyzed using PyMOL and LigPlot, respectively.

Figure 4 – Three-dimensional structure of DPP-IV and its inhibitory peptides. The left side is showing the 3D structure of DPP-IV complexed with its inhibitor. On the right side there is a 2D diagram showing the hydrophobic interactions (red) and the hydrogen bonds (green) existing between the inhibitory sequence and the enzyme. A) Non-competitive inhibition by the WF peptide. B) Non-competitive inhibition by the MF peptide. Non-competitive

inhibition. C) Non-competitive inhibition by the GF peptide. The 3D and 2D images were analyzed using PyMOL and LigPlot, respectively.

Figure 1.

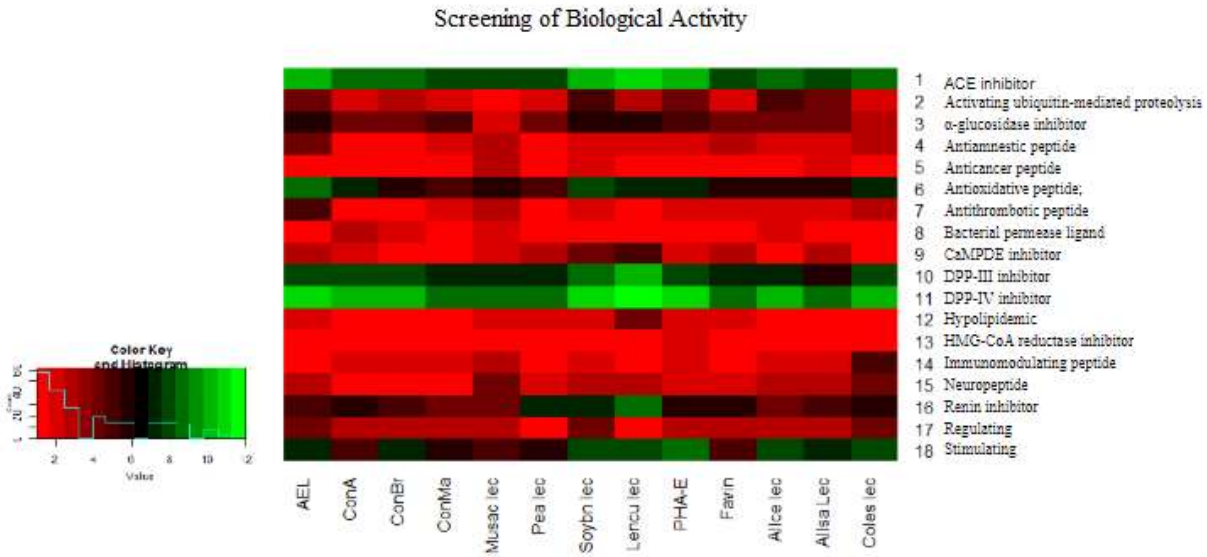


Figure 2.

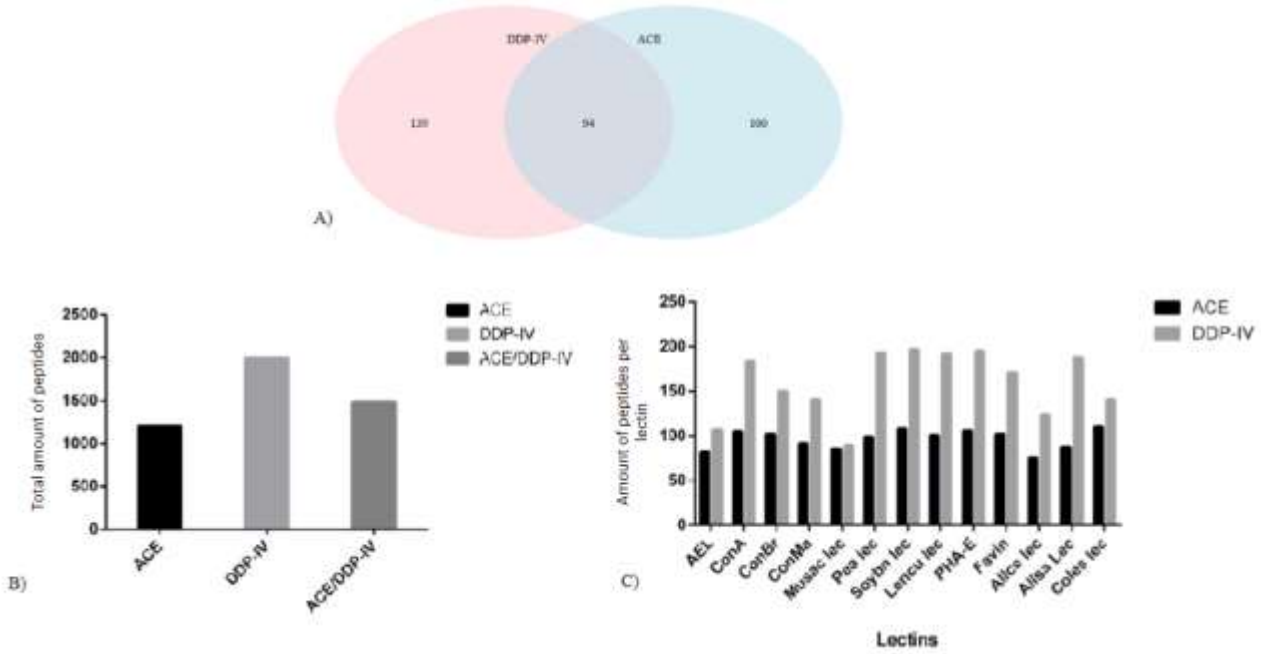


Figure 3.

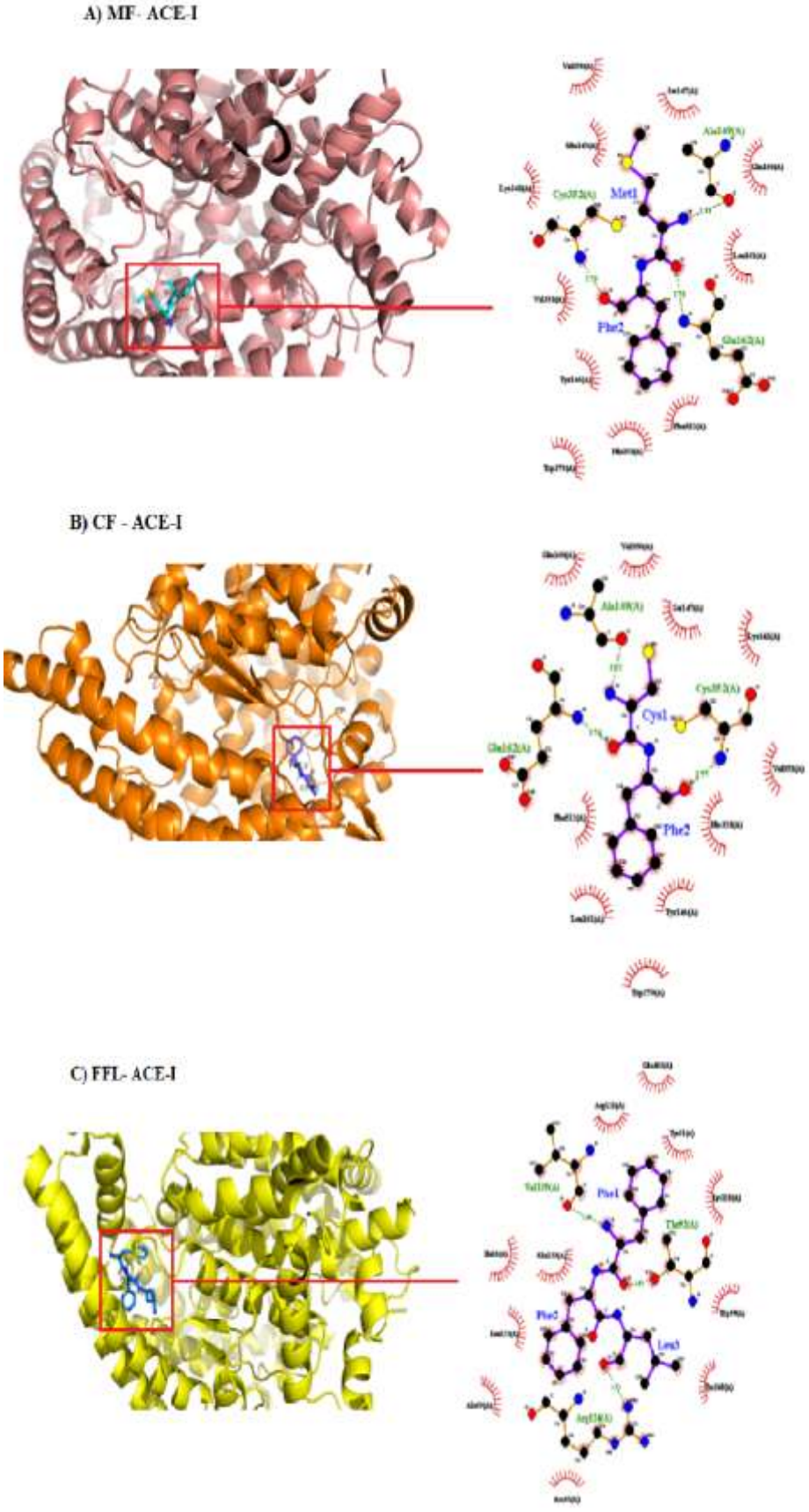

A) MF- ACE-I
Met1
Phe2
B) CF - ACE-I
Cys1
Phe2
C) FFL- ACE-I
Phe1
Phe2
Lys3

Figure 4.

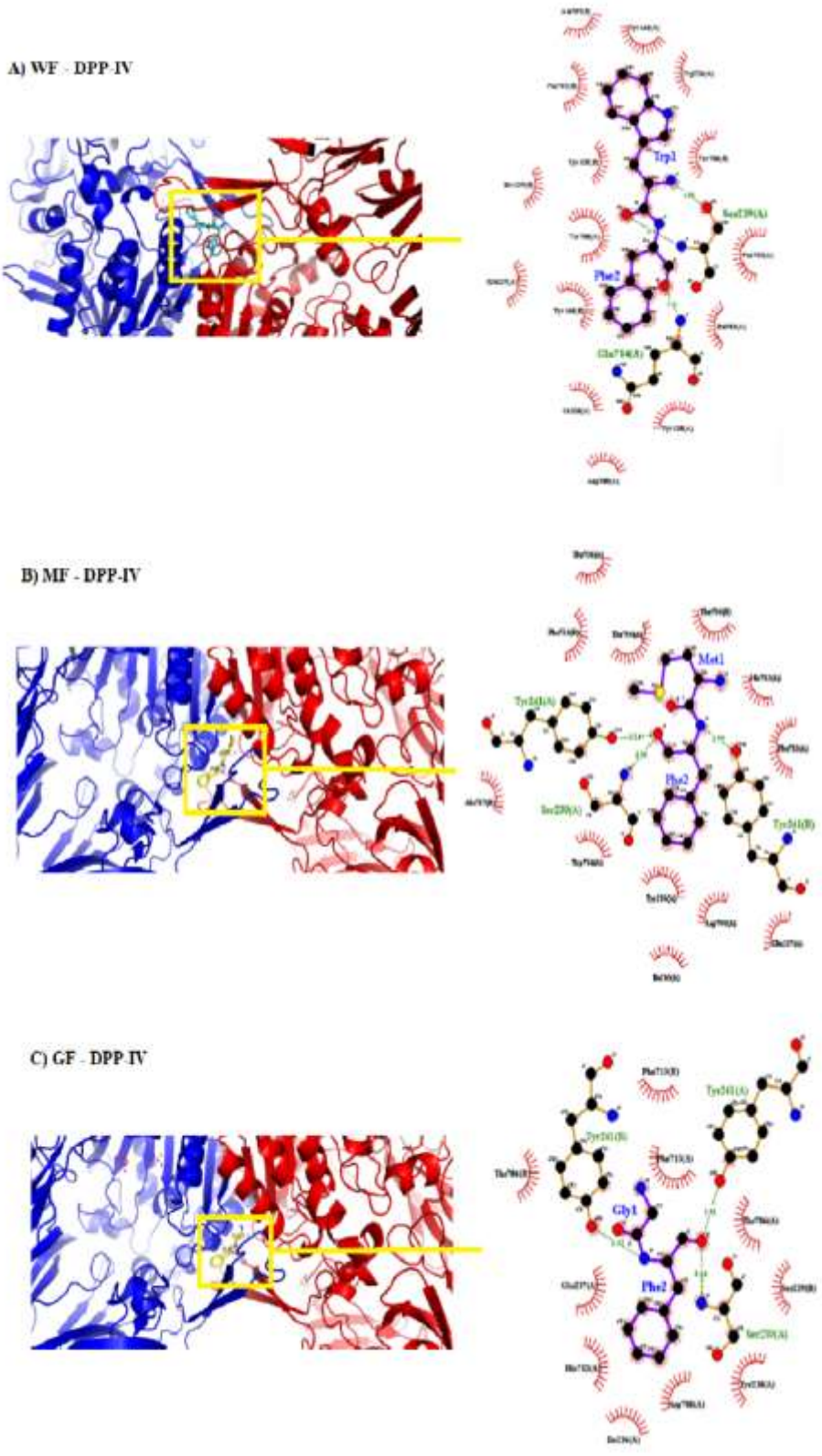


Table 1. The frequency of occurrence of peptides with a given activity (A) obtained from *in silico* digestion of plant lectins. The frequencies were obtained from the BIOPEP database.

Source	Protein	Number of activities	∑A*	A (ACE-I inhibitor)	A (DPP-IV inhibitor)
Abelmoschus esculentus	AEL	16	1.3481	0.3937	0.6199
Canavalia ensiformis	ConA	12	1.0838	0.3177	0.5691
Canavalia brasiliensis	ConBr	11	1.0893	0.3450	0.5463
Canavalia rosea	ConMa	12	1.0198	0.3191	0.5164
Musa acuminata	Musac lec	16	1.1794	0.4279	0.4925
Pisum sativum	Pea lec	11	1.1161	0.3065	0.6012
Glycine max	Soybn lec	16	1.1769	0.3217	0.5971
Lens culinaris	Lencu lec	12	1.1203	0.3102	0.5994
Phaseolus vulgaris	PHA-E	16	1.2091	0.3343	0.6184
Vicia faba	Favin	14	1.2666	0.3789	0.6421
Allium cepa	Allce lec	14	1.1974	0.3509	0.6096
Allium sativum	Allsa Lec	15	1.1246	0.3434	0.5547
Colocasia esculenta	Coles lec	13	1.225	0.3979	0.5329

*Total frequency of occurrence of all bioactive peptides.

Table 2. Prediction of the efficiency of release of bioactive peptides from plant lectins during *in silico* sequential digestion using pepsin and trypsin. The rate of release of the peptides was analyzed using the BIOPEP database.

Protein	Enzime	DHt* (%)	ACE inhibitor AE**	ACE inhibitor W#	DPP-IV inhibitor AE	DPP-IV inhibitor W
AEL	Pepsin	11.8182	0.0090	0.0229	0.0136	0.0219
	Pepsin/Trypsin	22.2727	0.0136	0.0345	0.0226	0.0365
ConA	Pepsin	16.2011	0.0278	0.0770	0.0333	0.0555
	Pepsin/Trypsin	21.7877	0.0278	0.0770	0.0500	0.0833
ConBr	Pepsin	12.2881	0.0211	0.0490	0.0253	0.0400
	Pepsin/Trypsin	20.3390	0.0211	0.0490	0.0464	0.0733
ConMa	Pepsin	11.4894	0.0127	0.0326	0.0169	0.0285
	Pepsin/Trypsin	17.8723	0.0169	0.0434	0.0339	0.0571
Musac lec	Pepsin	11.4286	0.0071	0.0118	0.0213	0.0337
	Pepsin/Trypsin	19.2857	0.0213	0.0353	0.0284	0.0450
Pea lec	Pepsin	23.7288	0.0167	0.0417	-	-
	Pepsin/Trypsin	28.8136	0.0333	0.0833	0.0167	0.0244
Soybn lec	Pepsin	16.1972	0.0140	0.0369	0.0386	0.0558

	Pepsin/Trypsin	23.5915	0.0246	0.0649	0.0456	0.0660
Lencu lec	Pepsin	23.7288	0.0333	0.0908	0.0167	0.0271
	Pepsin/Trypsin	30.5085	0.0500	0.1364	0.0333	0.0540
PHA-E	Pepsin	17.5182	0.0218	0.0555	0.0436	0.0615
	Pepsin/Trypsin	24.4526	0.0327	0.0833	0.0473	0.0667
Favin	Pepsin	13.5593	0.0167	0.0385	0.0167	0.0213
	Pepsin/Trypsin	25.4237	0.0333	0.0769	0.0667	0.0852
Allce lec	Pepsin	6.7485	-	-	0.0122	0.0163
	Pepsin/Trypsin	15.3374	0.0122	0.0267	0.0183	0.0244
Allsa Lec	Pepsin	6.1111	-	-	0.0110	0.0156
	Pepsin/Trypsin	15.0000	0.0055	0.0112	0.0166	0.0235
Coles lec	Pepsin	18.8341	0.0179	0.0361	0.0223	0.0359
	Pepsin/Trypsin	28.2511	0.0089	0.0180	0.0179	0.0288

*DHt – Theoretical degree of hydrolysis

**AE - Frequency of release of fragments with a given activity by selected enzymes

#W - Relative frequency of release of fragments with a given activity by selected enzymes

.

Table 3. Prediction of the chemical characteristics of peptides obtained from sequential *in silico* digestion of plant lectins that showed the greatest potential for bioactivity. The peptides were selected based on the score obtained in the PeptideRanker and analyzed for their toxicity in ToxinPred and solubility and molecular weight in PepCalc.

Peptide	Activity	Source	PeptideRanker Score*	Toxicity**	Solubility#	Molecular Weight (g/mol)[3]
MF	ACE inhibition	ConA, ConBr and ConMa	0.996643	Non-toxin	Poor solubility	296.39
CF	ACE inhibition	Coles	0.99641	Non-toxin	Poor solubility	268.33
FFL	ACE inhibition	SBA	0.995536	Non-toxin	Poor solubility	425.52
WF	DDP-IV inhibition	AEL	0.998831	Non-toxin	Poor solubility	351.4
MF	DDP-IV inhibition	ConA, ConBr and ConMa	0.996643	Non-toxin	Poor solubility	296.39
GF	DDP-IV inhibition	ConMa, MusaLec, PEA, SBA, LCA, PHA-E, Favin	0.994712	Non-toxin	Poor solubility	222.24

*Analyzed in the PeptideRanker;

**Analyzed in the ToxinPred;

#Analyzed in the PepCalc.

Table 4. Molecular interaction between ACE-I and DPP-IV inhibitory peptides. Docking was performed using the ClusPro server 2.0 and the interactions between amino acids were analyzed using LigPlot and PIC server software. The structures were visualized using the PyMOL program.

Activity	Peptide	Representative	Weighted Score* (kJ/mol-1)	Hydrofobic interations**	Hydrogen bonds#
ACE inhibition	MF	Lowest Energy	-536.7	$TYR1_{46}$ (PHE_2); LEU_{161} (PHE_2), TRP_{185} (PHE_2); $TRP2_{79}$ (PHE_2); VAL_{350}(MET_1), PHE_{512} (PHE_2); GLU_{349} (MET_1), VAL_{151} (PHE_2)	CYS_{352} (MET_1); ALA_{149} (MET_1); GLU_{162}(MET_1)
ACE inhibition	CF	Lowest Energy	-518.4	TYR_{146} (PHE_2); LEU_{161} (PHE_2); TRP_{279} (PHE_2); PHE_{512} (PHE_2), HIS_{353} (PHE_2); SER_{147} (PHE_2)	GLU_{162} (CYS_1); ALA_{149} (CYS_1); CYS_{352} (CYS_1)
ACE inhibition	FFL	Lowest Energy	-671.7	TYR_{51} (PHE_1); TYR_{62} (LEU_3); ILE_{88} (PHE_2); ILE_{88} (LEU_3); ALA_{89} (PHE_2); VAL_{119} (PHE_1); TYR_{360} (LEU_3), LEU_{122}(PHE_2), GLU_{123} (PHE_2);	VAL_{119} (PHE_1); THR_{92} (PHE_1); ARG_{124} (LEU_3)
DDP-IV inhibition	WF	Lowest Energy	-697.3	$ILE_{236\text{-}A}$† (PHE_2); $TYR_{238\text{-}A}$ (PHE_2); $TYR_{241\text{-}A}$ (TRP_1); $TYR_{241\text{-}B}$‡ (TRP_1/PHE_2); $PHE_{713\text{-}A}$ (TRP_1/ PHE_2); $ALA_{717\text{-}B}$ (TRP_1); $TRP_{734\text{-}A}$ (TRP_1)	GLN_{714} (PHE_2). SER_{239} (TRP_1)
DDP-IV inhibition	MF	Lowest Energy	-540.6	$ILE_{236\text{-}A}$ (PHE_2); $TYR_{238\text{-}A}$ (PHE_2); $TYR_{241\text{-}A}$ (PHE_2/MET_1); $TYR_{241\text{-}B}$ (PHE_2); $PHE_{713\text{-}A}$ (PHE_2/MET_1); $PHE_{713\text{-}B}$ (MET_1); $ALA_{717\text{-}B}$ (MET_1); $TRP_{734\text{-}A}$ (MET_1); $THR_{706\text{-}A/B}$ (MET_1)	$SER_{239\text{-}A}$ (PHE_2); $TYR_{241\text{-}A}$ (PHE_2)

DDP-IV inhibition	GF	Lowest Energy	-415.7	$TYR_{238\text{-}A}$ (PHE_2); $TYR_{241\text{-}B}$ (PHE_2); $HIS_{712\text{-}A}$ (PHE_2). $GLU_{237\text{-}A}$ (PHE_2); $THR_{706\text{-}A}$ (PHE_2); $PHE_{713\text{-}A}$ (GLY_1)	$TYR_{241\text{-}B}$ (GLY_1); $TYR_{241\text{-}A}$ (PHE_2); $SER_{239\text{-}A}$ (PHE_2);

*Calculated by the ClusPro server 2.0;

**Analyzed in the LigPot and PIC server;

†Chain A;

‡Chain B.

Supplementary Tables

Supplementary Table 1. Prediction of ACE-I and DPP-IV inhibitory peptides released from plant lectins after *in silico* sequential digestion using pepsin and trypsin.

	ACE inhibitor	DPP-IV inhibitor
Pepsin and Trypsin AEL	87 peptides	137 peptides
	RL[a](3[b]), IR(2) MF(1), KW(1), VW(1), RF(1), GY(1), LF(1), GP(1), AW(1), GEP(1), VK(1), AF(1), LA(2), VP(1), RA(1), AA(1), FR(1), IF(1), IG(1), GA(1), GL(1), AG(3), GR(2), KG(1), FG(1), GV(4), GQ(2), GK(1), GT(1), GE(1), GG(3), SG(1), LG(2), GD(1), TG(2), NG(1), PG(1), VR(1), GYK(1), DG(3), NY(1), SY(1), KF(1), YK(1), RR(1), KA(2), LLF(1), EI(1), IE(2), LQ(1), EW(1), EK(1), FVP(1), AV(1), TP(1), YV(1), WA(1), WM(1), ST(3), AVV(1), VVR(1), VVL(1), EF(1), YY(1), FF(1)	GP(1), VA(1), KA(2), LA(2), FA(1), VP(1), LL(2), VV(4), TP(1), SP(1), GA(1), RA(1), WA(1), EP(1), TA(2), WV(1), EK(1), AL(2), GL(1), VR(1), WRL(1), AA(1), WR(1), WM(1), WW(1), WF(1), AW(1), AD(1), AF(1), AG(3), AS(1), AT(2), AV(1), DN(4), DR(1), EI(1), EW(1), FR(1), GE(1), GG(3), GV(4), GY(1), HS(1), IR(2), KF(1), KG(1), KH(1), KI(1), KK(1), KS(1), KT(2), KW(1), MF(1), MK(1), NA(3), NE(1), NG(1), NT(1), NY(1), PG(1), PI(1), PN(1), PS(1), PV(2), QI(1), QS(1), QT(1), QV(1), QY(1), RH(1), RL(3), RR(1), SI(1), SK(3), SV(1), SW(1), SY(1), TD(3), TG(2), TI(1), TL(2), TS(2), TT(2), TV(1), VI(1), VK(1), VL(4), VN(2), VQ(2), VS(1), VT(1), VW(1), YD(1), YF(1), YK(1), YV(1), YY(1), FF(1)
Pepsin and Trypsin ConA	105 peptides	183 peptides

	RL(1), IR(1), LY(1), YL(1), MF(2), KW(1), FP(1), LF(3), FY(1), FNQ(1), AY(1), YP(3), IW(1), IA(1), PSY(1), IA(1), IP(2), AF(1), AP(1), KR(1), RA(1), YA(1), AA(1), IF(1), VG(3), IG(2), GI(2), GL(3), GR(2), DA(2), GS(3), GK(1), GT(1), GQ(2), SG(1), LG(1), GD(2), TG(3), EA(1), NG(1), VR(2), VAV(1), SHP(1), QK(1), DG(2), SY(3) SF(2), KL(1) YK(1), NK(1), EI(1), VE(1), LQ(1), AH(1), PQ(1), EW(1), KE(1), HP(1), PH(1), TF(3), AI(1), LSW(1), AV(3), GLY(1), GKV(1), DF(1), IL(2), ST(4), YN(2), AVV(2), IIY(1)	VA(2), MA(1), AP(1), PA(1), LP(2), LL(1), VV(4), IP(2), SP(2), FP(1), HP(1), YP(3), IA(1),RA(1), TA(2), FL(3), WV(1), AL(2), SL(1), GL(1), VR(2), AA(1), WN(1), WS(1), WE(1), VGL(1), AD(3), AF(1), AH(1), AS(2), AT(4), AV(3), AY(1), DN(1), DP(1), DQ(1), EI(1), ES(1), ET(2), EW(1), FN(2), GI(2), HE(1), HF(1), HI(3), II(1), IL(2), IR(1), IW(1), KE(1), KK(2), KR(1), KS(4), KT(1), KV(2), KW(1), LH(1), LI(2), LM(1), LT(1), MF(2), MQ(1), MV(1), NA(4), NE(1), NG(2), NL(1), NM(1), NQ(1), NT(2), NV(2), PH(1), PI(1), PN(2), PQ(1), PS(2), PV(1), QF(1), QG(2), QN(1), RL(2), RN(1), SF(1), SH(1), SI(1), SK(4), SV(3), SW(1), SY(3), TD(2), TF(3), TG(3), TH(1), TI(3), TM(1), TN(2), TR(1), TS(1), TT(2), TV(2), TY(1), VD(2), VE(1), VG(3), VH(1), VI(1), VL(1), VN(1), VS(4), YA(1), YD(1), YK(1), YN(2)
Pepsin and Trypsin ConBr	102 peptides	150 peptides
	VLP(1), RL(3), LY(1), IY(1), MF(1), KW(1), FP(1), LF(2), FY(1), FNQ(1), YP(3), IW(1), PSY(1), IA(1), IP(1), AF(1), AP(1), KR(1), RA(1), YA(1), VG(4), IG(2), GI(2), GL(3), GR(2), DA(2), GS(3), GK(2), GT(2), QG(2), SG(1), LG(1), GD(3), TG(3), EG(1), EA(1), NG(3), VR(2), VAV(1), SHP(1), QK(1), DG(1), SY(3), SF(2), KL(1), YK(1), VE(1), TE(1), LQ(1), AH(1), PQ(1), EW(1), KE(1), HP(1), PH(1), TF(2), LSW(1), AV(3), GLY(1), GKV(1), IL(2), ST(3), YN(1), AVV(2), LR(1), IIY(1),	VA(2), AP(1), PA(1), LP(1), LL(1), VV(2), IP(1), SP(2), FP(1), HP(1), YP(3), IA(1), RA(1), TA(2), FL(1), WV(1), AL(2), GL(3), VR(2), WN(1), WS(1), WE(1), VGL(1), AD(2), AF(1), AH(1), AS(2), AT(3), AV(3), DN(1), DP(1), DQ(1), EG(1), ES(1), ET(2), EW(1), FN(1), GI(2), HE(1), HF(1), HI(3), II(1), IL(2), IW(1), KE(1), KK(1), KR(1), KS(3), KT(1), KV(1), KW(1), LH(1), LI(2), LT(1), MF(1), MQ(1), NA(1), NG(3), NL(1), NM(1), NQ(1), NT(2), NV(1), PH(1), PN(2), PQ(1), PS(2), PV(1), QF(1), QG(2), QN(1), RL(3), SF(2), SH(1), SI(1), SK(3), SV(3), SW(1), SY(3), TD(1), TE(1), TF(2), TG(3), TH(1), TI(2), TN(2), TR(1), TS(1), TT(1),

		TV(1), TY(1), VD(1), VE(1), VG(4), VH(1), VL(1), VS(3), YA(1), YD(1), YK(1), YN(1),
Pepsin and Trypsin ConMA	91 peptides	140 peptides
	VLP(1), RL(2), LY(1), MF(1), KW(1), FP(1), LF(2), FY(1), FNQ(1), YP(3), GP(1), IW(1), PSY(1), IA(1), IP(1), AF(1), AP(1), KR(1), RA(1), YA(1), GF(1), VG(5), GI(1), GL(3), AG(1), GR(2), DA(3), GS(2), GK(2), GT(2), QG(1), SG(1), LG(1), GD(2), TG(2), NG(3), VR(2), VAV(1), SHP(1), QK(1), DG(2), SY(4), SF(1), KL(1), YK(1), VE(1), LQ(1), AH(1), EW(1), KE(1), HP(1), PH(1), TF(1), LSW(1), AV(2), GLY(1), GKV(1), IL(2), ST(4), YN(1), AVV(1)	GP(1), VA(2), AP(1), PA(1), LP(1), LL(1), VV(1), IP(1), SP(1), FP(1), HP(1), YP(3), IA(1), RA(1), TA(2), WV(1), AL(2), GL(3), VR(2), WN(1), WS(1), WE(1), VGL(1), AD(2), AF(1), AG(1), AH(1), AS(1), AT(4), AV(2), DN(1), DP(1), DQ(1), ES(1), ET(2), EW(1), FN(1), GF(1), GI(1), HE(1), HF(1), HI(2), IL(2), IW(1), KE(1), KR(1), KS(2), KV(1), KW(1), LH(1), LI(2), MF(1), MQ(1), NA(1), NG(3), NL(1), NM(1), NQ(1), NT(2), NV(1), PH(1), PN(2), PS(3), PV(1), QF(1), QG(1), QN(1), QS(1), RL(2), SF(1), SH(1), SI(1), SK(1), SV(2), SW(1), SY(4), TD(2), TF(1), TG(3), TH(1), TI(2), TK(1), TN(2), TR(1), TS(1), TT(1), TV(3), TY(1), VD(1), VE(1), VG(5), VH(1), VL(1), VS(3), YA(1), YD(1), YK(1), YN(1)
Pepsin and Trypsin MusaLec	85 peptides	89 peptides
	GPA(1), VY(1), YG(2), AY(1), GP(2), AW(1), VK(1), IA(1), AF(1), AA(1), GF(2), IF(1), VG(3), IG(1), GM(1), GA(2), AG(1), GR(1), KG(1), FG(4), DA(2), GS(2), GV(2), MG(1), GK(3), GT(2), WG(1), HG(1), GE(2), GG(6), SG(3), LG(1), GD(2), TG(1), EG(1), NG(2), MKG(1), NY(1), SY(1), KL(1), NK(1), EY(1), EI(1), TE(1), LQ(1), PH(1), TF(1), AI(2), AV(1),	GP(2), PA(1), LP(1), VV(4), TP(2), GPA(1), GA(2), IA(1), EP(1), SL(1), AA(1), AW(1), WG(1), AF(1), AG(1), AV(1), AY(1), EG(1), EI(1), ET(1), EY(1), GE(2), GF(2), GG(6), GV(2), HE(1), HF(1), II(1), KG(1), KI(2), KK(1), KS(1), KT(1), KV(1), LV(1), MG(1), MK(1), MN(1), NG(2), NT(1), NY(1), PF(2), PH(1), PI(1), QE(1), RG(1), RH(1), RI(1),

	GVY(1), TP(2), DF(1), DM(1), YH(1), MGP(1), RG(1), ST(1), FGG(1)	SV(1), SY(1), TE(1), TF(1), TG(1), TN(1), TR(1), TY(1), VD(2), VG(3), VK(1), VL(1), VT(1), VY(1), YG(2), YH(1), YL(2), YR(1), YY(1)
Pepsin and Trypsin PEA	99 peptides	193 peptides
	LY(1), VF(1), GGY(1), GY(2), LF(1), FY(2), YP(1), AW(1), IW(1), VK(1), IA(1), AF(1), AP(2), VP(1), RA(1), YA(2), AA(4), GF(2), IF(2), VG(1), IG(2), GI(1), GA(1), GR(1), DA(1), GV(1), GT(1), GE(1), GG(2), QG(1), SG(1), LG(1), GD(1), TG(3), EA(1), NG(1), VR(1), VAV(1), DG(2), NGF(1), SY(2), SF(4), KF(1), KL(2), KA(1), EY(2), KP(1), IAP(1), EV(1), VE(1), TE(1), LQ(2), TQ(2), AH(1), PQ(1), EW(1), EK(1), KE(1), TF(3), AI(1), LSW(1), AV(2), ASL(1), LEE(1), IFL(1), FYN(1), FQ(1), IL(2), TLS(1), ST(1), YN(2), LGV(1)	VA(3), MA(1), KA(1), AP(2), VP(1), LL(1), VV(3), SP(2), KP(1), YP(1), GA(1), IA(2), RA(1), FL(2), WV(1), EK(1), AL(1), SL(4), VR(1), AA(4), WK(1), WS(1), AW(1), YT(2), AD(2), AE(2), AF(1), AH(1), AS(1), AT(2), AV(2), DP(1), DQ(1), DR(2), ET(2), EV(1), EW(1), EY(2), FN(2), FQ(1), GE(1), GF(2), GG(2), GI(1), GV(1), GY(2), HE(1), HI(2), HS(1), IH(1), IL(2), IN(1), IW(1), KE(1), KF(1), KS(2), KT(1), KV(1), LI(2), LT(5), MI(1), NA(3), NF(1), NG(1), NL(1), NR(1), NT(2), NV(5), PI(1), PN(2), PQ(1), PS(1), PV(1), QA(1), QG(1), QN(2), QQ(1), QT(3), RH(1), RI(1), SF(4), SI(2), SK(1), SV(1), SW(2), SY(2), TE(1), TF(3), TG(3), TI(1), TK(5), TL(2), TN(1), TQ(2), TS(4), TT(5), TV(3), TY(1), VD(1), VE(1), VF(1), VG(1), VI(2), VK(1), VL(2), VN(3), VS(2), VT(2), WD(2), YA(2), YD(1), YL(1), YN(2), YS(1)
Pepsin and Trypsin Soybn Lec	108 peptides	197 peptides
	RL(1), IR(1), LVYP(1), SLVYP(1), LY(1), LVL(2), VY(1), VYP(1), GY(1), LF(1), FY(1), YP(1), VPK(1), PL(1), IW(1), FFL(1), LAP(1), IP(1), AF(1), AP(2), LA(4), KR(1), VP(1),	PP(1), VA(4), MA(1), KA(1), LA(4), FA(2), AP(2), LP(2), VP(1), LL(2), VV(4), HA(2), IP(1), TP(2), KP(2), YP(1), RA(1), NP(1), QP(1), FL(1), WV(1), AL(1), SL(6),

	RA(1), YA(1), AA(2), GF(1), FR(1), IG(2), GI(1), GL(3), AG(1), GR(1), DA(2), GS(1), GK(1), GT(1), GE(1), QG(1), SG(2), LG(2), GD(2), TG(2), EA(1), NG(1), PG(1), VR(1), VAV(1), DG(1), NF(1), SF(5), KF(1), KL(2), NK(3), KA(1), KP(2), VE(1), LQ(2), LN(1), TQ(1), PP(1), PQ(1), EW(1), KE(1), PH(2), TF(2), AI(2), LVY(1), LSW(1), FVP(1), AV(1), ASL(1), FNE(1), DGL(1), FNF(1), TP(2), IL(2), ST(2), QP(1)	GL(3), VR(1), AA(2), PL(1), WN(1), WS(1), AD(1), AE(1), AF(1), AG(1), AS(6), AT(2), AV(1), DP(2), DQ(1), ES(2), ET(2), EW(1), FN(2), FR(1), GE(1), GF(1), GI(1), GY(1), HD(1), HE(1), HI(2), IH(1), IL(2), IN(1), IR(1), IW(1), KE(1), KF(1), KR(1), KT(3), KV(3), LH(1), LI(1), LN(1), LT(4), LV(4), MI(1), NE(2), NF(1), NG(1), NL(1), NM(1), NN(1), NV(2), PG(1), PH(2), PI(2), PK(2), PN(2), PQ(1), PS(2), QG(1), QL(1), QN(1), QT(1), QV(1), RI(1), RL(1), RN(1), SF(5), SH(1), SI(2), SK(2), SV(1), SW(4), TF(2), TG(2), TH(1), TK(2), TL(2), TQ(1), TS(8), TT(1), TV(1), TY(1), VD(2), VE(1), VL(5), VN(1), VS(2), VT(1), VY(1), WD(3), YA(1), YD(1), YL(1), YS(1)
Pepsin and Trypsin LCA	101 peptides	191 peptides
	LY(1), VF(1), GGY(1), GY(2), FY(2), YP(1), PL(1), AW(1), IW(1), VK(1), IA(2), AF(1), AP(2), VP(2), RA(2), AA(4), GF(1), IF(3), VG(1), IG(2), GI(1), GA(1), GR(1), KG(1), DA(2), GV(1), GK(1), GT(1), GE(1), GG(3), QG(1), LG(1), GD(1), TG(3), NG(1), VR(1), VAV(1), QK(1), DG(1), NF(1), SY(2), SF(4), KF(1), KL(1), NK(1), KA(1), EY(1), KP(1), IAP(1), EV(2), VE(1), TE(1), LQ(2), LN(1), TQ(1), AH(1), PQ(1), EW(1), KE(2), TF(3), AV(2), ASL(1), LEE(1), IFL(1), FYN(1), TP(1), FQ(1), IL(1), ST(3), YN(2), LGV(1).	VA(3), MA(1), KA(1), FA(1), AP(2), VP(2), LL(1), VV(3), VPL(1), TP(1), SP(1), KP(1), YP(1), GA(1), IA(2), RA(2), FL(1), WV(1), AL(1), SL(2), VR(1), AA(4), PL(1), WN(1), WS(1), AW(1), YT(2), AD(2), AE(1), AF(1), AH(1), AS(1), AT(2), AV(2), DP(1), DQ(1), DR(1), ET(1), EV(2), EW(1), EY(1),FN(2), FQ(1), GE(1), GF(1), GG(3), GI(1), GV(1), GY(2), HE(1), HI(2), HS(2), IH(1), IL(1), IW(1), KE(2), KF(1), KG(1), KS(3), KT(1), KV(1), LI(2), LN(1), LT(5), MI(1), NA(2), NE(1), NF(1), NG(1), NL(2), NV(5), PI(1), PN(1), PQ(1), PS(2), PV(1), QA(1), QG(1), QN(1), QT(3), RH(1), RI(1), SF(4), SI(3), SK(2), SV(1), SW(2), SY(1), TE(1), TF(3), TG(3), TI(1), TK(5), TL(3), TN(1), TQ(1), TS(5), TT(4), TV(3), TY(1), VD(1), VE(1), VF(1), VG(1),

		VH(1), VI(2), VK(1), VL(1), VN(3), VT(3), WD(2), YD(1), YL(2), YN(2), YS(1)
Pepsin and Trypsin PHA-E	106 peptides	195 peptides
	VPL(1), RL(1), LVYP(1), SLVYP(1), GPA(1), LY(1), LVL(1), RF(1), VY(1), VYP(1), PR(1), LF(2), FY(1), YP(1), LLP(1), GP(1), IW(1), GEP(1), VK(1), AF(2), AP(1), LA(3), VP(1), RA(1), YA(1), GF(1), VG(2), IG(1), GI(1), GA(1), GL(4), GR(1), KG(4), DA(1), GS(1), QG(1), GT(1), GE(2), GG(1), SG(1), LG(2), TG(2), EG(1), NG(1), VAV(1), YKY(1), DG(2), NY(1), NF(1), SF(6), KY(1), KL(3), YK(1), KP(1), FAL(1), EV(2), LQ(1), LN(2), PT(1), AH(1), EW(1), TF(1), LVY(1), LSW(1), AV(2), ASL(1), FNE(1), DGL(1), DF(1), FQ(1), IL(3), SGP(1), TLS(1), ST(1), YN(1), LR(1), TLS(1), TTW(1), VHW(1)	GP(1), VA(3), MA(1), LA(3), FA(3), AP(1), PA(1), LP(2), VP(1), LL(5), KP(1), YP(1), GPA(1), GA(1), RA(1), EP(2), TA(1), FL(1), WV(1), AL(2), SL(4), GL(4), WS(1), AD(1), AE(1), AF(2), AH(1), AS(5), AT(3), AV(2), DN(2), DP(1), EG(1), ET(3), EV(1), EW(1), FN(3), FQ(1), GE(2), GF(1), GG(1), GI(2), HI(1), HT(1), HW(1), IL(3), IQ(1), IW(1), KG(4), KS(2), KT(2), KY(1), LI(2), LN(2), LT(2), LV(3), NA(2), ND(2), NE(1), NF(1), NG(1), NL(2), NN(2), NQ(1), NT(1), NV(3), NY(1), PI(1), PK(2), PN(1), PS(1), PT(1), PV(1), QI(2), QL(1), RH(1), RL(1), SF(6), SI(2), SK(3), SV(1), SW(1), TF(1), TG(2), TK(2), TL(2), TN(3), TS(4), TT(5), TV(3), TW(1), TY(2), VD(1), VE(2), VG(2), VH(1), VI(1), VK(1), VL(4), VN(2), VS(2), VY(1), WD(3), YA(1), YD(2), YK(1), YN(1), YS(1)
Pepsin and Trypsin Favin	102 peptides	171 peptides
	LY(2), VF(1), GGY(2), GY(4), FY(1), YP(1),GP(1), PL(1), AW(1), IW(1), LYP(1), VK(1), IA(1), IP(1), RP(1), AP(2), KR(1), VP(2), RA(1), YA(1), AA(1), GF(2), FR(1), IF(2), VG(1), IG(2), GI(1), GA(1), GR(1), DA(1), GV(1), GK(2), GE(1), GG(4), QG(1), LG(1), TG(5), EA(1), NG(4), VR(1),	GP(1), VA(4), KA(1), AP(2), LP(2), VP(2), LL(1), VV(2), VPL(1), IP(1), RP(1), KP(1), YP(1), GA(1), IA(1), RA(1), TA(1), QP(1), FL(1), WV(1), EK(1), AL(1), SL(1), VR(1), AA(1), PL(1), WN(1), WT(1), AW(1), YT(2), AD(2), AE(1), AH(1), AT(3), AV(2), DP(1), DQ(1), EI(1), ET(1), EV(2),

	VAV(1), TTN(1), DG(1), SF(2), KF(1), KL(1), KA(1), EY(1), KP(1), IAP(1), EI(1), EV(2), VE(1), LQ(1), AH(1), PQ(1), EW(1), EK(1), KE(1), TF(4), AI(1), LSW(1), AV(2), FYN(1), DY(1), DF(1), FQ(1), TGP(1), LTGP(1), TLS(1), ST(1), YN(2), LGV(1), QP(1)	EW(1), EY(1), FN(2), FQ(1), FR(1), GE(1), GF(2), GG(4), GI(1), GV(1), GY(4), HE(1), HI(2), HV(1), IH(1), IW(1), KE(1), KF(1), KR(1), KS(2), KT(1), LI(1), LT(4), NA(2), NG(4), NL(3), NT(2), NV(3), PI(1), PK(1), PN(3), PQ(1), PS(2), PV(1), QG(1), QN(1), QT(2), RH(1), RI(1), SF(2), SI(2), SV(1), SW(2), TD(1), TF(4), TG(5), TH(1), TI(1), TK(4), TL(3), TN(1), TS(1), TT(5), TV(2), VD(1), VE(1), VF(1), VG(1), VI(1), VK(1), VL(2), VN(1), VT(1), WD(2), YA(1), YD(1), YL(1), YN(2), YS(1)
Pepsin and trypsin Allce	76 peptides	124 peptides
	LY(2), IY(1), LVL(2), VW(1), VY(1), VAA(1), YG(1), GP(1), IW(2), GPV(1), VK(1), LA(1), RA(2), YA(1), AA(3), VG(2), GL(1), AG(1), GR(1), KG(1), GS(2), GV(1), GQ(1), GK(1), GT(3), GG(2), TG(3), EG(1), NG(3), VR(1), QK(1), DG(2), NY(1), NF(1), RR(1), AR(1), VIY(1), EY(1), VE(1), VE(1), LQ(1), TF(1), AV(3), GLY(1), VLY(1), VGP(1), TP(1), YE(1), IL(3), WA(2), VVGP(1), RG(1), ST(3)	GP(1), VA(2), MA(2), LA(1), VV(5), TP(1), RA(2), WA(2), TA(1), SL(1) GL(1), VR(1), AA(3), WS(1), YT(1), AD(1), AG(1), AS(3), AT(1), AV(3), DR(1), EG(1), EY(1), GG(2), GV(1), IL(3), IM(1), IW(2), KG(1), KK(1), KV(2), LT(1), LV(4), MN(1), MQ(2), NE(1), NF(1), NG(3), NL(1), NN(1), NQ(1), NT(1), NV(1), NV(3), NY(1), PI(1), PV(1), QA(1), QD(1), QN(1), QS(1), QY(1), RG(1), RK(2), RN(2), RR(1), SV(1), TF(1), TG(3), TI(2), TV(4), TY(1), VD(1), VE(1), VG(2), VI(1), VK(1), VL(3), VM(2), VN(2), VT(3), VW(1), VY(1), YA(1), YD(1), YD(1), YE(1), YG(1), YI(1), YR(1), YS(1), GPV(1), VAAA(1)
Pepsin and Trypsin Allsa	87 peptides	127 peptides

	IR(1), GP(1), LY(2), IY(1), LVL(2), VW(1), VY(1), VAA(1), YG(1), GP(1), IW(2), GPV(1), IA(1), RP(1), LA(1), RA(1), YA(1), AA(1), VG(2), IG(1), GA(2), GL(1), AG(1), GR(2), GS(2), GV(1), MG(1), GQ(1), GK(1), GT(3), GE(1), GG(2), TG(4), EG(1), NG(5), VR(1), QK(1), DG(2), NY(1), NF(1), KF(1), YK(1), RR(1), AR(1), KA(1), VIY(1), EY(1), VE(1), LQ(1), AI(2), AV(2), GLY(1), VLY(1), TP(1), YE(1), IL(3), MM(1), WA(2), RG(1) , GTG(1), ST(3), AVV(1),	GP(1), VA(1), MA(2), KA(1), LA(1), VV(5), TP(1), SP(1), RP(1), GA(1), IA(1), RA(1), WA(2), TA(1), SL(1), GL(1), VR(1), AA(3), WS(1), AG(1), AS(3), AT(1), AV(2), DR(1), EG(1), EY(1), GE(1), GG(2), GV(1), IL(3), IM(1), IR(1), IW(2), KF(1), KV(1), LT(2), LV(2), MG(1), MM(1), MN(1), MQ(2), MR(1), NF(1), NG(5), NL(1), NQ(1), NT(1), NV(3), NY(1), PI(1), PK(1), PV(2), QD(1), QN(1), QS(1), QY(1), RG(1), RI(1), RK(1), RN(2), RR(1), SV(3), TG(4), TI(1), TN(1), TS(1), TT(1), TV(2), TY(1), VD(1), VE(1), VG(2), VI(2), VL(3), VM(2), VN(2), VT(2), VW(1), VY(1), YA(1), YD(1), YE(1), YG(1), YI(1), YK(1), YR(1), YS(1), GPV(1)
Pepsin and Trypsin Coles	110 peptides	140 peptides
	RL(3), AVP(1), LY(3), VF(2), LVL(2), VW(1), RF(1), PR(1), LF(2), YG(4), LLP(1), VFK(1), PGL(1), GP(1), VK(1), GW(1), IP(2), RP(1), AP(1), VP(2), RA(1), YA(1), AA(2), FR(2), GA(1), GL(2), GH(1), HL(1), GR(3), KG(2), FG(1), GS(1), GQ(2), GK(1), GT(1), HG(1), GE(3), GG(1), QG(1), SG(3), LG(2), GD(4), NG(5), PG(1), PAP(1), YGG(1), DG(4), NY(2), KL(2), CF(1), LLF(2), LN(3), HP(1), HK(1), AI(1), AV(3), VVF(1), VLY(3), AVL(1), DY(1), DF(2), IL(1), ST(1),YN(1), LR(2)	GP(1), VA(1), MA(1), AP(1), PA(2), LP(1), VP(2), LL(6), VV(1), IP(2), RP(1), HP(1), GA(1), RA(1), TA(2), FL(2), WV(1), HL(1), AL(1), SL(1), GL(2), WRS(1), AA(2), WR(1), WQ(2), AV(3), DN(1), DP(1), EH(1), FR(2), GE(3), GG(1), GH(1), GW(1), II(1), IL(1), KG(2), KI(1), KS(1), KY(1), LH(1), LI(2), LN(3), LT(3), LV(6), MQ(2), ND(1), NG(5), NH(2), NL(3), NN(1), NT(3), NW(1), NY(2), PF(1), PG(1), PS(3), PW(1), QG(1), QL(1), QN(1) QS(2), QT(1), QV(1), RL(3), RN(1), RN(1), SV(2), TD(3), TH(1), TL(2), TN(1), TV(1), VF(2), VI(1), VK(1), VL(4), VM(2), VW(1), YA(1), YG(4), YL(1), YN(1)

[a] Inhibitory amino acids sequences;

[b] Number of repetitions in which the inhibitory sequence was found.

Supplementary Table 2. Prediction of all ACE and DDP-IV inhibitory peptides released from plant lectins after sequential *in silico* digestion using pepsin and trypsin. The peptides were obtained from the BIOPEP database.

	ACE	**DDP-IV**	**ACE + DDP-IV**
All lectins	1,207 peptides	2,025 peptides	1,488 peptides
	RL[a](14[b]), FGK(1), RY(1), VF(7), LW(1), VW(4), FP(4), LVR(1), PR(3), GP(9), GEP(2), VK(8), LKA(1), AF(10), LA(11), VP(10), RA(14), VG(25), GA(10), GL(23), AG(8), GR(19), FG(6), GS(19), GV(8), GQ(9), GK(17), WG(2), GE(13), GG(25), LG(16), GD(19), TG(29), EG(6), PG(4), VR(15), QK(7), DG(23), SY(16), KY(2), KF(7), KL(15), YK(6), KA(7), EV(8), VE(10), VRY(1), LEF(1), AV(27), VVF(2), DGL(3), AVL(2), VGP(2), DF(7), YV(1), ST(28), LR(5) , IR(3), LY(16), YL(1), MF(4), KW(3), LF(13), FY(10), FNQ(3), AY(2), YP(14), IW(12), IA(9), PSY(3), IP(8), YA(11), AA(19), IF(9), IL(21), YN(12), AVV(6), IIY(2), IY(3), AP(12), KR(4), IG(13), GI(8), DA(14), GT(17), QG(8),	VA(27), MA(10), AP(13), PA(6), LP(12), LL(19), VV(34), IP(8), SP(9), FP(4), HP(4), YP(14), IA(10), RA(14), TA(13), FL(12), WV(11), AL(15), SL(24), GL(21), VR(15), AA(21), WN(7), WS(8), WE(3), VGL(3), AD(18), AF(10), AH(7), AS(24), AT(26), AV(27), AY(2), DN(6), DP(10), DQ(7), EI(3), ES(7), ET(16), EW(8), FN(15), GI(11), HE(8), HF(4), HI(17), II(6), IL(18), IR(3), IW(12), KE(8), KK(5), KR(5), KS(21), KT(12), KV(14), KW(3), LH(5), LI(16), LM(1), LT(29), MF(4), MQ(9), MV(1), NA(15), NE(6), NG(29), NL(17), NM(4), NQ(6), NT(17), NV(27), PH(6), PI(10), PN(15), PQ(6), PS(19), PV(11), QF(3), QG(10), QN(12), RL(15), RN(7), SF(25), SH(4), SI(14), SK(16), SV(22), SW(15), SY(15), TD(9),	RL(29), VF(14), LW(2), VW(8), FP(8), GP(17), VK(17), AF(20), LA(22), VP(20), RA(28), VG(50), GA(17), GL(44), AG(16), GV(16), WG(4), GE(26), GG(50), TG(64), EG(12), PG(9), VR(30), SY(31), KY(5), KF(13), YK(12), YK(14), EV(15), VE(22), AV(54), YV(2), IR(6), YL(9), MF(8), KW(6), AY(4), YP(28), IW(24), IA(19), IP(16), YA(22), AA(40), IL(39), YN(24), AP(25), KR(9), GI(19), QG(18), NG(57), SF(49), TE(8), AH(13), PQ(11), EW(15), KE(8), HP(7), PH(11), TF(39), GF(20), GPA(4), VY(10), YG(16), AW(8), KG(18), MG(4), NY(12), YH(2), RG(6), GY(18), EY(11), KP(12), TQ(10), EK(4), FQ(8), PL(6), FR(8), NF(11), LN(14), PP(2), TP(12), QP(4), VPL(3), PT(2),

	SG(12), EA(4), NG(28), VAV(7), SHP(2), SF(24), TE(4), LQ(13), AH(6), PQ(5), EW(7), KE(7), HP(3), PH(5), TF(18), LSW(6), GF(10), GLY(3), GKV(1), GPA(2), VY(5), YG(8), AW(4), GM(1), KG(9), MG(2), HG(2), MGK(1), NY(6), NK(5), AI(9), DM(1), YH(1), MGP(1), RG(3), FGG(1), GGY(4), GY(9), NGF(1), EY(5), KP(6), IAP(3), TQ(5), EK(2), ASL(4), LEE(2), IFL(2), FYN(3), FQ(4), TLS(4), LGV(3), LVYP(2), SLVYP(2), LVL(9), VYP(2), VPK(1), PL(3), FFL(1), LAP(1), FR(4), NF(5), LN(7), PP(1), LVY(2), FVP(1), FNE(2), FNF(1), TP(5), QP(2), VPL(1), RF(2), LLP(2), YKY(1), FAL(1), PT(1), SGP(1), TTW(1), VHW(1), LYP(1), RP(3), TTN(1), EI(1), DY(2), TGP(5), LTGP(1), VAA(2), GPV(2), RR(2), AR(2), VIY(2), VLY(5), YE(2), WA(4), VVGP(1), GRP(1), MM(1), GTG(1), AVP(1), VFK(1), PGL(1), GW(1), GH(1), HL(1), PAP(1), YGG(1), CF(1), LFF(1), HK(1),	TF(21), TG(35), TH(6), TI(13), TM(1), TN(15), TR(4), TS(28), TT(24), TV(27), TY(11), VD(15), VE(12), VG(25), VH(5), VI(11), VL(31), VN(15), VS(17), YA(11), YD(11), YK(6), YN(12), GP(8), KA(7), LA(11), VP(10), VV(3), WP(1), GA(7), EP(4), NP(2), WK(2), LW(1), WF(1), WG(2), AG(8), DR(6), EG(6), EV(7), GE(13), GG(25), GV(8), KF(6), KY(3), LV(22), PG(5), PW(3), QD(3), QL(4), QS(6), TW(2), VF(7), VK(9), VQ(3), VW(4), YV(1), YY(2), TE(4), GF(10), TK(19), TP(7), GPA(2), AW(4), EY(6), KG(9), KI(3), MG(2), MK(1), MN(3), NY(6), PF(3), QE(1), RG(3), RH(5), RI(6), VT(13), VY(5), YG(8), YH(1), YL(8), YR(3), KP(6), EK(2), YT(7), AE(6), FQ(4), GY(9), HS(4), IH(5), IN(2), MI(3), NF(6), NR(1), QA(3), QQ(1), QT(10), TL(14), TQ(5), WD(12), YS(7), PP(1), FA(6), HA(2), QP(2), PL(3), FR(4), HD(1), LN(7), NN(5), PK(6), QV(2), VPL(2), HT(1), HW(1), IQ(1), ND(3), PT(1), QI(2), RP(3), WT(1), HV(1), WA(4), IM(2), QY(2), RK(2), RR(2), VM(6), YE(2), YI(2), GPV(2), VAAA(1), MM(1), MR(1), HL(1), WRS(1), WR(1), WQ(2), EH(1), GH(1), GW(1), NH(2), NW(1)	RP(6), EI(4), GPV(4), RR(4), YE(4), WA(8), MM(2), GW(2), GH(2), HL(2),

[a] Inhibitory amino acid sequence;

[b] Number of repetitions in which the inhibitory sequence was found

6. CONSIDERAÇÕES FINAIS

As lectinas compreemdem um grupo bastante diverso de proteínas capazes de se ligarem com alta especificidaas a diferentes açúcares. Desde a sua descoberta, diversas atividades biológicas foram descritas e o ramo da lectinologia ainda hoje continua sendo uma área de grande interesse na pesquisa.

A lectina de *A. esculentus* (AEL) é uma proteína terapêutica candidata a várias aplicações farmacológicas. A avaliação de risco feita para a AEL buscou reunir informações sobre a segurança de uso dessa proteina. A AEL mostrou similaridade com proteínas anti-nutricionais e alergênicas, com destaque para os inibidores de serino proteases. Embora a AEL tenha apresentado inibição de proteases intestinais como tripsina e quimotripsina, esta foi completamente digerida durante a digestão sequencial *in vitro*. Além disso, a AEL não causou efeitos adversos aos embriões de peixe-zebra. Dessa forma, os resultados indicaram que a AEL é uma proteína segura para ser utilizada com fins terapêuticos.

As lectinas oriundas de fontes vegetais apresentam um grande potencial para a geração de peptídeos bioativos com diferentes propriedades farmacológicas. Os peptídeos anti-diabéticos e anti-hipertensivos se destacaram como a principal atividade biológica encontrada nas sequências encriptadas em dez lectinas vegetais. As duas atividades encontradas foram evidenciadas pela inibição das enzimas ECA-I e DPP-IV, respectivamente. Os peptídeos apresentaram baixa solubilidade e baixa toxicidade quando analisados através de ferramentas *in silico*. Contudo, mais estudos devem ser realizados a fim de atestar a eficácia e segurança *in vivo* desses peptídeos para fins terapêuticos.