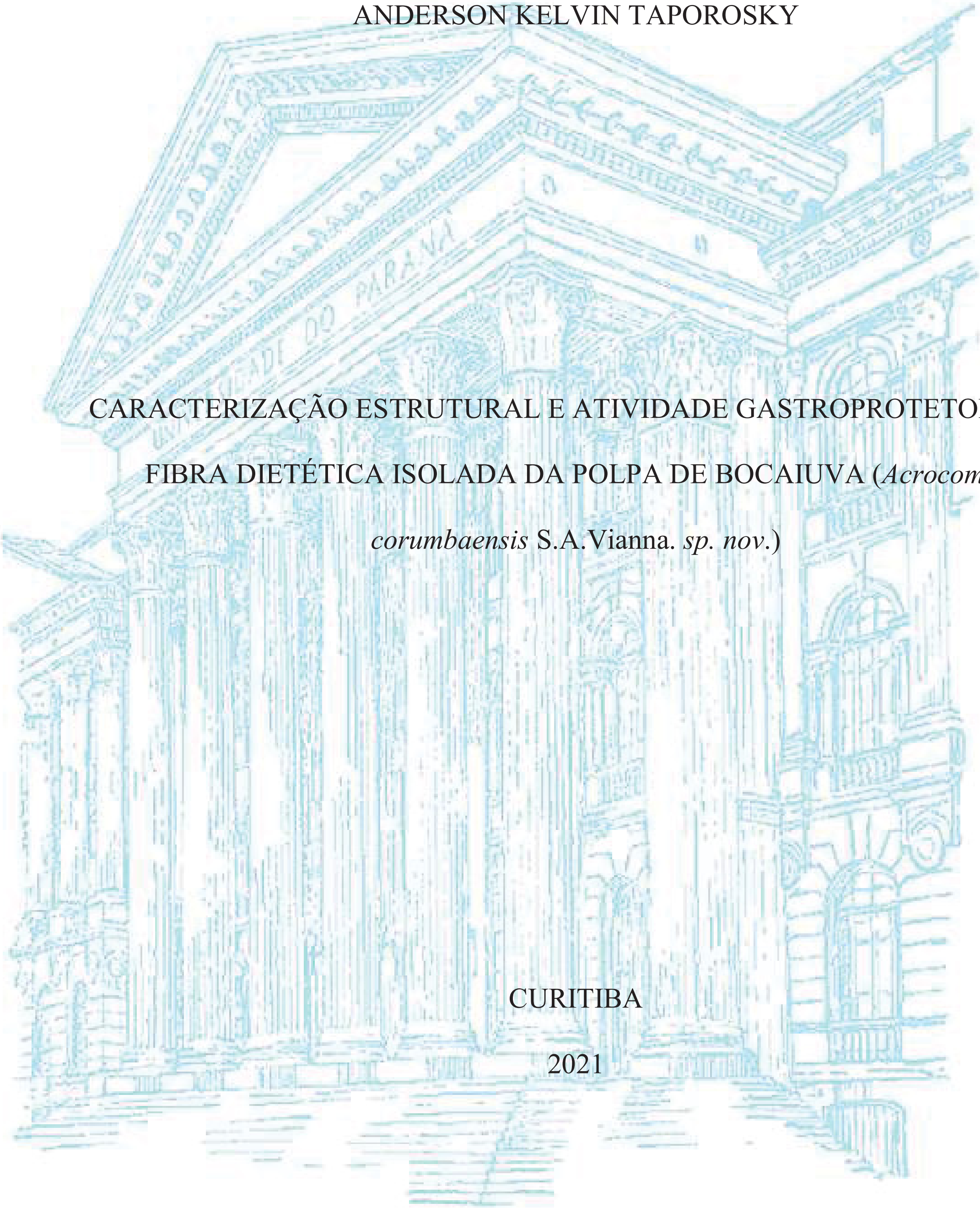

UNIVERSIDADE FEDERAL DO PARANÁ

ANDERSON KELVIN TAPOROSKY

CARACTERIZAÇÃO ESTRUTURAL E ATIVIDADE GASTROPROTETORA DE FIBRA DIETÉTICA ISOLADA DA POLPA DE BOCAIUVA (*Acrocomia corumbaensis* S.A.Vianna. *sp. nov.*)

CURITIBA

2021

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Orientadora: Profa. Dra. Lucimara Mach Côrtes Cordeiro

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ATA Nº506

ATA DE SESSÃO PÚBLICA DE DEFESA DE MESTRADO PARA A OBTENÇÃO DO GRAU DE MESTRE EM CIÊNCIAS (BIOQUÍMICA)

No dia vinte e nove de setembro de dois mil e vinte e um às 14:00 horas, na sala on-line, on-line, foram instaladas as atividades pertinentes ao rito de defesa de dissertação do mestrando **ANDERSON KELVIN TAPOROSKY**, intitulada**: CARACTERIZAÇÃO ESTRUTURAL E ATIVIDADE GASTROPROTETORA DE FIBRA DIETÉTICA ISOLADA DA POLPA DE BOCAIUVA (*Acrocomia corumbaensis* S.A.Vianna. *sp. nov.*)**, sob orientação da Profa. Dra. LUCIMARA MACH CORTES CORDEIRO. A Banca Examinadora, designada pelo Colegiado do Programa de Pós-Graduação CIÊNCIAS (BIOQUÍMICA) da Universidade Federal do Paraná, foi constituída pelos seguintes Membros: LUCIMARA MACH CORTES CORDEIRO (UNIVERSIDADE FEDERAL DO PARANÁ), DIOGO RICARDO BAZAN DUCATTI (UNIVERSIDADE FEDERAL DO PARANÁ), THAISA MORO CANTU JUNGLES (PURDUE UNIVERSITY). A presidência iniciou os ritos definidos pelo Colegiado do Programa e, após exarados os pareceres dos membros do comitê examinador e da respectiva contra argumentação, ocorreu a leitura do parecer final da banca examinadora, que decidiu pela APROVAÇÃO. Este resultado deverá ser homologado pelo Colegiado do programa, mediante o atendimento de todas as indicações e correções solicitadas pela banca dentro dos prazos regimentais definidos pelo programa. A outorga de título de mestre está condicionada ao atendimento de todos os requisitos e prazos determinados no regimento do Programa de Pós-Graduação. Nada mais havendo a tratar a presidência deu por encerrada a sessão, da qual eu, LUCIMARA MACH CORTES CORDEIRO, lavrei a presente ata, que vai assinada por mim e pelos demais membros da Comissão Examinadora.

CURITIBA, 29 de Setembro de 2021.

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LUCIMARA MACH CORTES CORDEIRO
Presidente da Banca Examinadora

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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação CIÊNCIAS (BIOQUÍMICA) da Universidade Federal do Paraná foram convocados para realizar a arguição da Dissertação de Mestrado de **ANDERSON KELVIN TAPOROSKY** intitulada: **CARACTERIZAÇÃO ESTRUTURAL E ATIVIDADE GASTROPROTETORA DE FIBRA DIETÉTICA ISOLADA DA POLPA DE BOCAIUVA (*Acrocomia corumbaensis* S.A.Vianna. *sp. nov.*)**, sob orientação da Profa. Dra. LUCIMARA MACH CORTES CORDEIRO, que após terem inquirido o aluno e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.
A outorga do título de mestre está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

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AGRADECIMENTOS

Destino meus sinceros agradecimentos a todos aqueles que de alguma forma contribuíram para o meu aperfeiçoamento acadêmico, muito obrigado.

Primeiramente a Deus, o criador do universo, por até aqui ter me sustentado.

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Mais uma vez, muito obrigado!

“O temor do Senhor é o princípio do conhecimento; os tolos desprezam a sabedoria e a instrução.”

Provérbios 1:7

NOTA EXPLICATIVA

Esta dissertação está estruturada no formato de artigo de acordo com as normas do Programa de Pós-Graduação em Ciências – Bioquímica e do Sistema de Bibliotecas (SiBi) da Universidade Federal do Paraná (UFPR). O conteúdo da dissertação é: introdução, revisão bibliográfica, justificativa, objetivos, artigos científicos, conclusões e referências. Os artigos científicos possuem revisão bibliográfica, materiais, metodologias, resultados, discussão, agradecimentos e referências.

RESUMO

A bocaiuva, trata-se de um fruto de palmeiras (*Acrocomia corumbaensis* S.A.Vianna. *sp. nov*.,), que tem sido amplamente explorada por extrativistas da região do Cerrado brasileiro, um bioma que abriga um patrimônio rico de recursos genéticos de grande relevância econômica e social, razão pela qual se tem como objetivo do presente trabalho a caracterização química estrutural das fibras dietéticas extraídas da bocaiuva bem como a avaliação da atividade anti-inflamatória, antinociceptiva e gastroprotetora em experimentação *in vivo*. A bocaiuva foi coletada na região de Corumbá, Mato Grosso do Sul, Brasil. Os frutos foram colhidos maduros, despolpados manualmente, desidratados por estufa, moídos e tamisados para obtenção da farinha da polpa da bocaiuva, com rendimento de 18%. A farinha foi deslipidificada pelo método de Soxhlet, cujo teor de lipídios foi de 31,6%. A farinha deslipidificada foi submetida ao método AOAC 991.43 para extração das fibras dietéticas, resultando nas frações FBS (fibra solúvel) e FBI (fibra insolúvel). A fração FBS é composta principalmente por uma glucomanana acetilada com proporção de 4,3:1 (manose:glucose) e grau de acetilação de 24%. As hemiceluloses foram solubilizadas com sol. de hidróxido de potássio a 10% a partir da fração FBI, originando duas novas frações (FBIKS e FBIKP). Os resultados de caracterização da fração FBIKS indicam a mistura de uma arabinogalactana e de uma provável arabinoxilana. A fração FBS não demonstrou efeito anti-inflamatório e antinociceptivo em modelo animal de dor/inflamação induzida pela injeção intraplantar de formalina, contudo, a mesma fração apresentou significativa ação gastroprotetora *in vivo*, em modelo de úlcera gástrica induzida por etanol.

Palavras-chaves: Bocaiuva; palmeira; polissacarídeos; fibra dietética; glucomanana; atividade gastroprotetora.

ABSTRACT

Bocaiuva is a fruit of palm trees (*Acrocomia corumbaensis* SAVianna. Sp. Nov.), which has been completely exploited by extractivists from the region of the wrong Brazilian Cerrado, a biome with a rich genetic diversity and great due economic and social, which is why the aim of this work is the structural chemical characterization of dietary fibers extracted from bocaiuva as well as the evaluation of anti-inflammatory, antinoceptive and gastroprotective activities *in vivo* experimentation. Bocaiuva was collected in the region of Corumbá, Mato Grosso do Sul, Brazil. The fruits were harvested ripe, manually pulped, dehydrated in an oven, milled and sieved to give the bocaiuva pulp flour with a yield of 18%. The flour was defatted by the Soxhlet method, and a lipid content of 31.6% was found. The defatted flour was submitted to the AOAC 991.43 method to extract dietary fibers, resulting in FBS (soluble fiber) and FBI (insoluble fiber) fractions. The FBS fraction was mainly composed of an acetylated glucomannan with a ratio of 4.3:1 (mannose:glucose) and a degree of acetylation of 24%. The hemicelluloses were solubilized with 10% potassium hydroxide solution from the FBI fraction, originating two new fractions (FBIKS and FBIKP). The characterization results of the FBIKS fraction indicated a mixture of an arabinogalactan and a probable arabinoxylan. The FBS fraction did not demonstrate anti-inflammatory and antinociceptive effects in an animal model of pain/inflammation induced by intraplantar formalin injection, however, the same fraction showed significant gastroprotective action in vivo, in an ethanol-induced gastric ulcer model.

Keywords: Bocaiuva; Palm tree; polysaccharides; Dietary fiber; glucomannan; gastroprotective activity.

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STRUCTURAL CHARACTERIZATION AND GASTROPROTECTIVE ACTIVITY OF SOLUBLE DIETARY FIBER EXTRACTED FROM *Acrocomia corumbaensis* FRUIT PULP

FIGURE 2 – ^{13}C-NMR spectrum of fraction FBS in D_2O at 70 ºC, the chemical shifts are expressed as δ ppm

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FIGURE 4 – HPSEC elution profile of FBS fraction. Refractive index detector. Elution volume of dextran standards of molecular weight 487 kDa, 266 kDa, 124 kDa, 72.2 kDa, 40.2 kDa, 17.2 kDa and 9.4 kDa (left to right) were employed to construct the calibration curve.

FIGURE 5 – Effect of oral and intraperitoneal treatment with soluble dietary fiber (BFS fraction) from bocaiuva pulp on ethanol-induced gastric lesion in rats. The animals were pretreated with vehicle (C: water 1 mL/kg), Sucralfate (S = 100 mg/kg), and Soluble dietary fiber from bocaiuva pulp (BFS = 0.01, 0.1, 1 and 10 mg/kg) by oral or intraperitoneal route. After 60 (for v.o.) or 30 (for i.p.) minutes, the animals were orally treated with ethanol (1 mL/animal). The results are expresses as mean ± SEM (n = 6-8). Statistical comparison was performed using analysis of variance (ANOVA) followed by Bonferroni's test. Differences from control group (* $p < 0.05$ and** $p < 0.01$).

FIGURE 6 – Effect of oral and intraperitoneal treatment with soluble dietary fiber (BFS fraction) on mucus content (A) and reduced glutathione levels (B). The animals were pretreated with vehicle (C: water 1 mL/kg), Sucralfate (S = 100 mg/kg), and Soluble dietary fiber from bocaiuva pulp (BFS = 0.01, 0.1, 1 and 10 m/k) by oral or intraperitoneal route. After 60 (for v.o.) or 30 (for i.p.) minutes, the animals were orally

treated with ethanol (1 mL/animal). The results are expressed as mean ± SEM (n = 6-8). Statistical comparison was performed using analysis of variance (ANOVA) followed by Bonferroni's test. Differences from control group (* $p < 0.05$, *** $p < 0.01$ and **** $p < 0.001$), # $p <0.05$ when compared to naïve group (N).

SUPPL. FIGURE 1 – Effect of administration of BFS fraction on neurogenic phase (A and C) and inflammatory phase (B and D) of nociception induced by 2.5% formalin in mice. The animals were treated with vehicle (V, 10 mL/kg, i.p.) or BFS fraction (orally, A and B or intraperitoneally, C and D). The results are expressed as mean ± SEM (n = 6-8). Statistical comparison was performed using analysis of variance (ANOVA) followed by Bonferroni's test.

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FIGURA 4 – Perfil de eluição das frações FBIKS100R, FBIKS100R50R e FBIKS100R50E quando analisadas por cromatografia de gel permeação.

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STRUCTURAL CHARACTERIZATION AND GASTROPROTECTIVE ACTIVITY OF SOLUBLE DIETARY FIBER EXTRACTED FROM *Acrocomia corumbaensis* FRUIT PULP

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CHEMICAL STRUCTURE, SOURCES AND HEALTH EFFECTS OF GLUCOMANNANS

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LISTA DE SIGLAS E ABREVIATURAS

Frações obtidas de *Acrocomia corumbaensis*

FB	Farinha de bocaiuva
FBD	Farinha de bocaiuva deslipidificada
FBI	Fibra insolúvel da bocaiuva
FBIK	Resíduo celulósico
FBIKS	Sobrenadante do extrato alcalino da fibra insolúvel da bocaiuva
FBIKSPF	Precipitado de Fehling do sobrenadante alcalino da fibra insolúvel da bocaiuva
FBIKSSF	Sobrenadante de Fehling do sobrenadante alcalino da fibra insolúvel da bocaiuva
FBIKS100R	Sobrenadante do extrato alcalino da fibra insolúvel da bocaiuva retido em membrana com limite de exclusão de 100 kDa
FBIKS100R50R	Sobrenadante do extrato alcalino da fibra insolúvel da bocaiuva retido em membrana com limite de exclusão de 100 kDa e 50 kDa
FBIKS100R50E	Sobrenadante do extrato alcalino da fibra insolúvel da bocaiuva retido em membrana com limite de exclusão de 100 kDa e eluido em 50 kDa
FBIKS100E	Sobrenadante do extrato alcalino da fibra insolúvel da bocaiuva eluido em membrana com limite de exclusão de 100 kDa
FBIKS100E50R	Sobrenadante do extrato alcalino da fibra insolúvel da bocaiuva eluido em membrana com limite de exclusão de 100 kDa e retido em 50 kDa
FBIKS100E50E	Sobrenadante do extrato alcalino da fibra insolúvel da bocaiuva eluido em membrana com limite de exclusão de 100 kDa e 50 kDa

FBIKP	Precipitado do extrato alcalino da fibra insolúvel da bocaiuva
FBS	Fibra solúvel da bocaiuva
FBSPF	Precipitado de Fehling da fibra solúvel da bocaiuva
FBSSF	Sobrenadante de Fehling da fibra solúvel da bocaiuva
FBS100R	Fibra solúvel da bocaiuva retida em membrana com limite de exclusão de 100 kDa
FBS100E	Fibra solúvel da bocaiuva eluida em membrana com limite de exclusão de 100 kDa
BF	Bocaiuva flour
DBF	Deffated bocaiuva flour
BFS	Soluble dietary fiber (bocaiuva)
BFI	Insoluble dietary fiber (bocaiuva)

Compostos químicos e termos associados

Ac_2O	Acetic anhydride (anidrido acético)
TCA	Trichloroacetic acid (ácido tricloroacético)
D_2O	*Deuterated water (água deuterada)*
DTNB	5,5′-dithiobis-(2-nitrobenzoic acid)(5,5′-ditiobis- (ácido 2-nitrobenzoico)
KOH	Potassium hidroxide (hidróxido de potássio)
$NaBH_4$	Boroidreto de sódio
NaN_3	Sodium azide (azida de sódio)
$NaNO_2$	Sodium nitrite (nitrito de sódio)
TFA	Trifluoracetic acic (Ácido trifluoroacético)

TRIS-HCl	Tris(hydroxymethyl)aminomethane hydrochloride, Trizma hydrochloride, TRIS hydrochloride, Tromethane hydrochloride (Cloridrato de tris (hidroximetil) aminometano, cloridrato de Trizma, cloridrato de TRIS, cloridrato de trometano)
v.o.	Oral administration (administração por via oral)
i.p.	Intraperitoneal administration (administração por via intraperitoneal)

Técnicas de análise e termos associados

COSY	Correlation Spectroscopy (Espectroscopia de Correlação)
DEPT	Distortionless Enhancement by Polarization Transfer (Aprimoramento sem Distorção por Transferência da Polarização)
GC-MS	Gas Cromagraphy-Mass Spectrometry (Cromatografia Gasosa Acoplada a Espectometria de Massa)
HPSEC	High Pressure Size Exlusion Chromagraphy (Cromatografia de exclusão estérica de alto desempenho)
HSQC	Heteronuclear Single Quantum Correlation Spectroscopy (Espectrometria de Correlação Heretonuclear Quântica Simples)
MALLS	Multi-Angle Laser Light Scattering (Espalhamento de luz laser em Múltiplos Ângulos)
NMR	Nuclear Magnetic Resonance (Ressonância Magnética Nuclear)

Demais compostos relatados

KGM	Glucomannan from *Amorphophallus konjac* (Glucomanana extraída de *Amorphophallus konjac*)

SUMÁRIO

1. INTRODUÇÃO

A bocaiuva (*Acrocomia corumbaensis* S.A.Vianna. *sp. nov.),* também conhecida popularmente como bacaúva, bocaiuveira, coco-babão, imbocaia, macaúba e macaíba, tem sido explorada por extrativistas da região do Cerrado, como nos estados do Mato Grosso do Sul, Mato Grosso, Minas Gerais e Goiás, sendo uma forma de subsistência para agricultores que residem na área de ocorrência natural da espécie (DO AMARAL et al., 2019).

Apesar da exploração da bocaiuva ocorrer fortemente pelo extrativismo silvestre, a indústria brasileira tem envidado esforços na empregabilidade de frutas para fabricação de produtos alimentícios, resultando na busca por substâncias biologicamente ativas e de baixo custo (CARDOSO et al., 2016), como por exemplo, as fibras dietéticas.

Fibra dietética ou alimentar trata-se de um conceito nutricional que se refere a uma ampla variedade de polissacarídeos, tais como celulose, hemiceluloses e pectinas, os quais não são digeridos por enzimas endógenas do trato gastrointestinal humano, não havendo, portanto, hidrólise nem absorção dos mesmos junto ao intestino delgado (TURNER & LUPTON, 2011).

Diversos estudos apontam os benefícios do consumo de fibras dietéticas na saúde humana, podendo atuar na redução do risco de ocorrência de doença arterial coronariana, acidente vascular cerebral, hipertensão arterial, diabetes *mellitus*, além de complicações gastrointestinais como constipação, hemorroidas, hérnia hiatal, diverticulite e câncer de cólon, podendo inclusive contribuir na prevenção e tratamento da obesidade (SILVA, 2019).

Sobre o assunto, é sabido que a alimentação perfaz papel fundamental na prevenção e tratamento de doenças contemporâneas, atuando de forma eficaz na saúde humana pela ação de compostos bioativos bem como de fibras alimentares (OLIVA, 2018). Dentre os hábitos dietéticos não condizentes com uma boa saúde humana, destacam-se o alto consumo de açúcares refinados e gordura saturadas, bem como o baixo consumo de fibras alimentares, fatores os quais contribuem para o desenvolvimento de doenças crônicas não transmissíveis e demais complicações patológicas como por exemplo o surgimento de úlcera gástrica cuja etiologia é heterogênea, tendo como principais fatores o tabagismo, consumo de álcool, estresse, utilização crônica de anti-inflamatórios não esteroidais (AINES) bem como associação ao *Helicobacter pylori* (BRANDÃO et al., 2019). Define-se úlcera gástrica a perda de tecido na região do estômago, condição que resulta em feridas as quais são expostas à ação das substâncias cloridropépticas, podendo serem classificadas como primária quando da ausência de outras doenças sistêmicas subjacentes, ou secundária quando relacionada a doenças agudas, tais como sepse, queimaduras extensas, acidose, hipoglicemia, choque, uso de anti-inflamatórios não esteroidais e corticoides (SILVA et al., 2018).

Dado a presente problemática, observa-se que a literatura tem sugerido que atividades biológicas de polissacarídeos complexos, os quais são constituintes das fibras dietéticas, podem estar relacionadas a mecanismos de proteção gástrica e intestinal, visto que diversos estudos têm demonstrado seu potencial cicatrizante de feridas bem como atividade antiulcerogênica (LEÓDIDO et al., 2017).

Cabe ressaltar que as atividades biológicas de polissacarídeos estão atribuídas as suas características químicas, tornando-se necessário sua análise estrutural cuja finalidade visa compreender seus possíveis mecanismo de ação (SILVA, 2017). Diversas são as atividades biológicas ainda a serem exploradas em meio a vasta diversidade de estruturas químicas das fibras dietéticas disponíveis na natureza, corroborando assim para a evolução e desenvolvimento de novas terapias possíveis de prevenção e tratamento de patologias.

As propriedades químicas da bocaiuva foram avaliadas a partir da farinha da polpa obtida pela despolpa manual e mecânica da fruta. Dentre os nutrientes quantificados, o teor de fibra alimentar total (g/100g) foi de 26,31±0,06 (manual) e 22,76±0,14 (mecânica) (DO AMARAL et al., 2019). Contudo, nenhum estudo de caracterização química estrutural dessas fibras foi relatado na literatura.

Isto posto, em virtude de a atual literatura não conter estudos de caracterização e atividade biológica das fibras dietéticas presentes no fruto da bocaiuva, estes poderão demonstrar promissor efeito no tratamento de complicações patológicas através de possíveis atividades de seus polissacarídeos, como por exemplo a atividade antiulcerogênica.

2. REVISÃO DE LITERATURA

2.1 Bocaiuva

O Cerrado brasileiro representa um bioma que abriga um rico patrimônio de recursos genéticos de grande relevância econômica e social. Dentre as diversas espécies vegetais que compõem esse bioma, merecem destaque as palmeiras, que são plantas monocotiledôneas pertencentes a família *Arecaceae*, disposta em mais de 2700 espécies de mais de 240 gêneros, apresentam frutos de potencial interesse para alimentação humana e outras aplicações como formulação de cosméticos e biocombustível (LOPES, 2014). Em meio a diversidade das palmeiras, uma nova espécie foi recentemente relatada por VIANNA et al., 2017, pertencente a um gênero encontrado nas Américas a então denominada *Acrocomia corumbaensis* foi localizada na região de Corumbá, Mato Grosso do Sul, Brasil.

A bocaiuva, trata-se de um fruto de palmeiras, constituído por casca, polpa mucilaginosa e fibrosa de coloração amarelo-alaranjada, e tegumento duro e denso, além de amêndoa, sendo frutificada pela palmeira em cachos com cerca de 10 a 12 Kg. Os frutos maduros apresentam coloração amarelo-esverdeada bem como exalam odor característico ao se desprenderem dos cachos e caírem ao solo, demonstrando assim o ponto ideal da maturação conforme observado na Figura 1 (SANJINEZ et al., 2011; GALVANI e FERNANDES, 2010).

Figura 1: Frutos da bocaiuva (GALVANI e FERNANDES, 2010)

Tradicionalmente a comunidade extrativista utiliza folhas e sementes da bocaiuva como laxante e contra afecções no trato respiratório, já a polpa é utilizada para fabricação de farinha. Além de ser consumida *in natura* compondo diversas receitas caseiras como refrescos, sorvetes, bolos e pães, a farinha também representa um produto típico da região do cerrado (GALVANI et al., 2005; CONTE, 2008).

A literatura relata que tanto a farinha quanto a polpa fresca (Figura 2) da bocaiuva apresentam alto teor de ácidos graxos insaturados com predominância do ácido oleico, além de serem ricas em vitamina A, despertando assim interesse na indústria alimentícia brasileira na utilização desses compostos como matéria prima para o desenvolvimento de diversos produtos alimentícios (CARDOSO et al., 2016; HIANE et al., 2005; SANJINEZ et al., 2011).

Figura 2: Polpa da bocaiuva (GALVANI e FERNANDES, 2010)

Em um artigo publicado por CARDOSO et al., 2016, observou-se alta complexidade química em resíduos da bocaiuva (endocarpo, fibra e casca) quando da identificação de mais de 150 compostos para bio-óleos, corroborando para outros achados que evidenciam que além de ofertar nutrientes, a bocaiuva possui níveis satisfatórios de carotenoides, tocoferóis e fenólicos, com propriedades antioxidantes. Contudo não houve citação acerca do potencial de fibras dietéticas presentes no fruto (NUNES et al., 2017).

2.2 Fibras dietéticas

O termo fibra dietética, que também é conhecido como fibra alimentar, se refere a classe de polissacarídeos não digeríveis pelas enzimas do trato gastrointestinal humano (TURNER & LUPTON, 2011). No Brasil a Resolução da Diretoria Colegiada N° 360, de 23 de dezembro de 2003 define fibra alimentar como qualquer material comestível que não seja hidrolisado pelas enzimas endógenas do trato digestivo humano (BRASIL, 2003).

Polissacarídeos são polímeros naturais constituídos de monossacarídeos, podendo ser homopolissacarídeos ou heteropolissacarídeos, os quais podem apresentar diferentes tipos de ligações glicosídicas, variar em relação ao tamanho da cadeia polissacarídica, possuir ramificações em sua cadeia principal bem como substituição por outros compostos. Esses polímeros podem ser encontrados em várias espécies como algas, vegetais, animais, fungos e bactérias. Nas plantas podem ser extraídos de diferentes partes como por exemplo exsudatos, sementes, frutos, folhas, raízes e tubérculos (CUNHA, PAULA & FEITOSA, 2009).

Uma vez ingeridas, as fibras dietéticas alcançam o intestino grosso onde algumas podem ser fermentadas pelas bactérias intestinais que constituem a microbiota. Esta se refere a população de microrganismos que compõe o trato gastrointestinal a qual exerce função fundamental na homeostase do organismo, onde a depender da composição desse ecossistema, implicará em saúde ou doença para o indivíduo. Muitas patologias possuem relação direta ou indireta para com alterações na homeostase intestinal, onde fatores como dieta, tabagismo, poluição, sedentarismo entre outros podem afetar esse equilíbrio dos microrganismos e desenvolver um quadro de disbiose intestinal. Por outro lado, o consumo de uma dieta nutricionalmente adequada, rica em fibras, promove a manutenção de espécies bacterianas benéficas ao organismo preservando assim o equilíbrio da microbiota (GOMES & DA COSTA, 2020).

O consumo de fibras dietéticas permite a promoção à saúde humana pela melhora de indicadores com por exemplo lipídios séricos e glicemia, além da modulação do sistema imunológico, e tem sido associada a prevenção de doenças tais como doença cardíaca coronária, acidente vascular cerebral, hipertensão, diabetes mellitus e obesidade (ANDERSON et al., 2009).

Dentre os efeitos terapêuticos das fibras dietéticas relatados na literatura, podemos citar a ação gastroprotedora observada para glucomananas extraídas da *Cyrtopodium andersonii* (PARENTE et al., 2014), galactomanoglucana extraída de frutos da palmeira *Syagrus oleracea* (DA SILVA & PARENTE, 2010), homogalacturonana extraída do maracujá (ABBOUD et al., 2019), arabinogalactana extraída da acerola, abacaxi e caju (DE SOUSA SABINO et al., 2020), arabinana extraída da quinoa (CORDEIRO et al., 2012) e arabinoxilana da cana de açúcar (MELLINGER-SILVA et al., 2011).

Diversos são os tipos de fibras dietéticas encontradas na natureza cujas propriedades de interação com o metabolismo humano dependem de sua estrutura química. Nas plantas, com exceção da inulina e galactomanana que são polissacarídeos de reserva em algumas espécies vegetais, os polissacarídeos que constituem as fibras dietéticas estão presentes como componentes estruturais da parede celular vegetal, como a celulose, hemiceluloses e pectinas (KAY, 1982).

2.3 Parede celular vegetal

A parede celular se refere a uma matriz ordenada e dinâmica presente no reino vegetal, sendo responsável pela proteção a patógenos, manutenção hídrica, forma e rigidez das plantas. Composta predominantemente por polissacarídeos, sua estrutura também dispõe de proteínas, compostos fenólicos e minerais, cuja composição da parede varia de acordo com as necessidades de cada espécie (FARINAS, 2011).

Os polissacarídeos que compõem a parede celular vegetal se organizam em estruturas classificadas como celulose, hemicelulose e pectina. A celulose é um homopolímero de moléculas de glucose unidas por ligações glicosídicas do tipo β-(1→4), cujas cadeias paralelas interagem por ligações de hidrogênio o que confere resistência a parede. Hemiceluloses compreendem um grupo heterogêneo de polissacarídeos, tais como heteroxilanas, mananas, glucomananas, arabinanas e galatanas os quais se ligam firmemente à superfície das microfibrilas de celulose formando uma complexa rede através de ligações cruzadas. Já as pectinas são polissacarídeos ricos em ácido galacturônico, contendo geralmente outros açúcares, como por exemplo a ramnose, arabinose e galactose, podendo ligar-se iônicamente a cátions bivalentes (DA SILVA, FRANCO & GOMES, 1997).

As paredes celulares desempenham as mesmas funções físicas durante o crescimento, contudo o fazem com diferentes tipos de moléculas, sendo proposto assim dois modelos distintos de paredes primárias, a parede tipo I que representa as plantas dicotiledôneas, e parede tipo II abrangendo plantas monocotiledôneas conforme observado nas Figuras 3 e 4 (CARPITA & GIBEAULT, 1993).

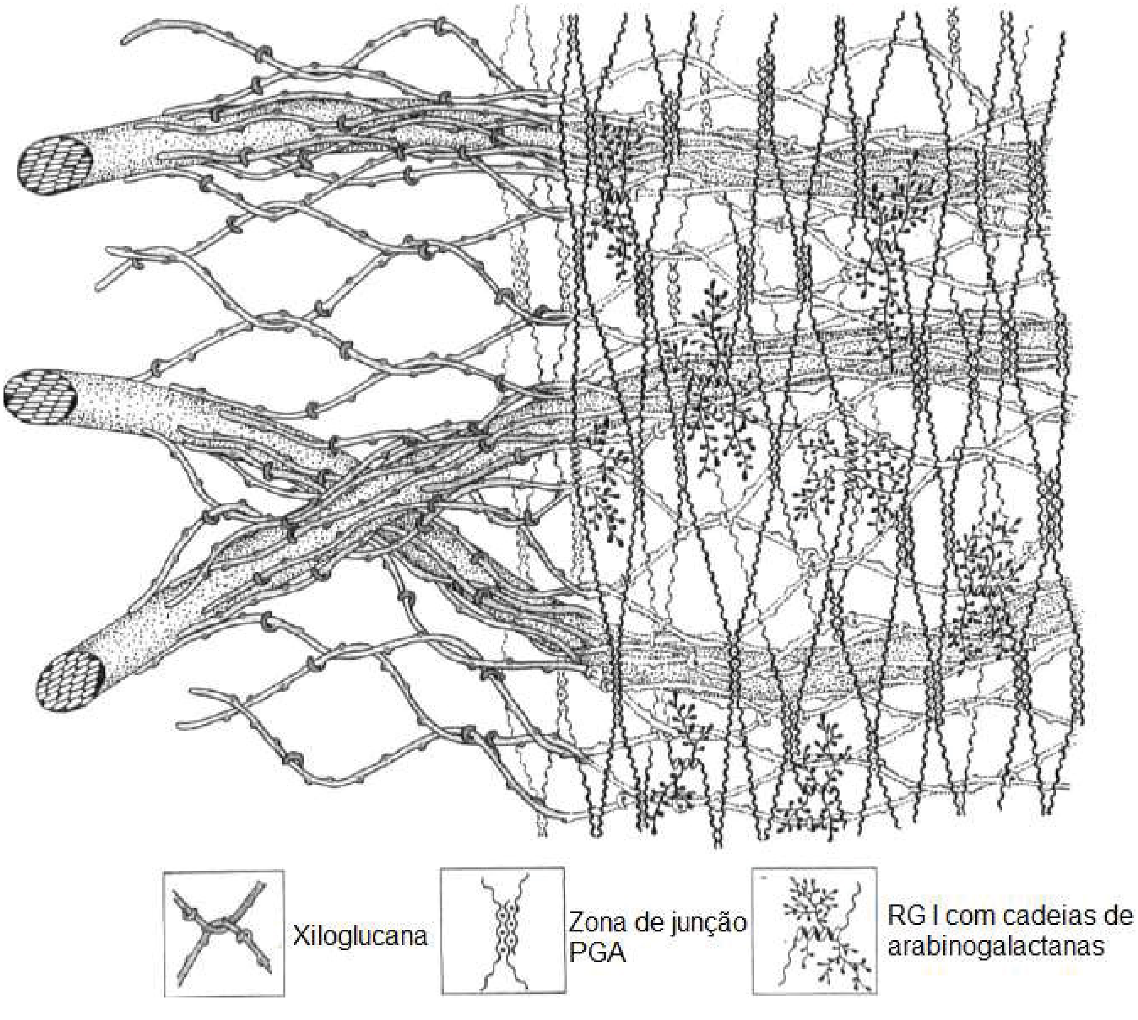

Figura 3: Parede celular tipo I presente na maioria das plantas com flores. PGA = Ácido poligalacturônico; RG I = Ramnogalacturonana do tipo I (Adaptada de CARPITA & GIBEAULT, 1993)

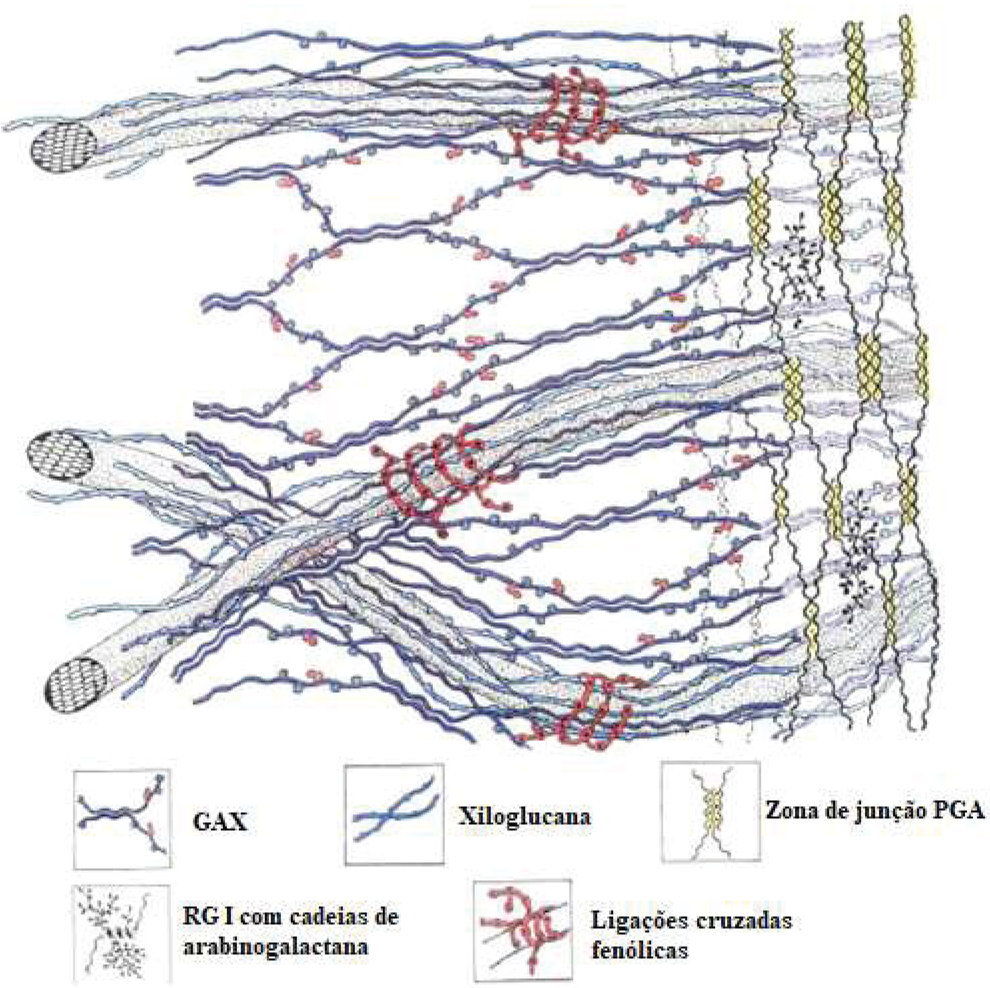

Figura 4: Parede celular tipo II da família Poaceae. GAX = Glucoronoarabinoxilana; PGA = Ácido poligalacturônico; RG I = Ramnogalacturonana do tipo I (Adaptada de CARPITA & GIBEAULT, 1993)

Algumas distinções podem ser observadas na arquitetura das paredes celulares dos tipos I e II propostas por CARPITA & GIBEAULT, 1993. Na parede do tipo I há um equilíbrio entre celulose, hemicelulose e pectina, sendo o domínio celulose-hemicelulose composto por xiloglucanas com diferentes tipos de ramificações. Já a parede celular do tipo II é pobre em pectina, e apresenta a arabinoxilana como principal hemicelulose que podem se ligar entre si por compostos fenólicos.

Os componentes hemicelulósicos da parede vegetal abrangem uma variedade de polissacarídeos de cadeia linear ou ramificada, composta por unidades de D-galactose, D-manose, D-glucose e L-arabinose. Particularmente as mananas, que também representam um dos principais polissacarídeos de hemiceluloses, podem ser dividivas em quatro subfamílias, a saber, a manana linear, galactomanana, galactoglucomanana e glucomanana (MOREIRA & FILHO, 2008).

2.4 Glucomananas

Glucomananas são formadas por unidades β-D-manopiranose e β-D-glucopiranose, onde a razão manose:glucose (1:1 a 30:1) varia de acordo com a fonte e parte da planta (SHI et al., 2018). Apesar da maioria das glucomananas serem compostas predominantemente por manose, na *Artemisia sphaerocephala* Krasch é observado ligeira proporção superior de glucose (1:1,3) (GUO et al., 2012).

As unidades de glucose e manose da cadeia principal na maioria das glucomananas são unidas por ligações glicosídicas do tipo β-D-(1→4), contudo algumas variações também foram relatadas na literatura, como no caso de *Aloe barbandensis* Miller (SHI et al., 2018) que apresentou →4)-β-D-Man*p*-(1→3)-β-D-Manp-(1→4)-β-D-Glc*p*-(1→ e de *Bletilla striata* (WHANG et al., 2014), que teve →2)-α-D-Man*p*-(1→4)-β-D-Glc*p*-(1→. Algumas glucomananas também podem apresentar ramificações em sua cadeia principal, sendo observado estes ramos ligados em O-2, O-3, O-6 nas unidades de β-D-Man*p* e O-3 e O-6 nas unidades de β-D-Glc*p* (SHI et al., 2018; ZHU, 2018).

Outra característica de glucomananas é a presença de grupos acetil, cuja posição varia de acordo com sua origem botânica (ZHU, 2018). Por exemplo, a glucomanana extraída de *Amorphophallus konjac* (KGM) tem de cinco a dez por cento das unidades substituídas por grupos acetil, mas a literatura relata variações entre 1,6 e 29,9% dependendo da fonte, como nos casos de *Dendrobium nobile* (ZHANG et al., 2020) e *Aloe barbandensis* Miller (SHI et al., 2018), respectivamente. A posição dos grupos acetil também varia de acordo com a planta, sendo relatada em O-2, O-3 e O-6, O-2,3, O-3,6 e O-2,3,6 nas unidades de β-D-Man*p* (GAO & NISHINARI, 2004).

As glucomananas já foram extraídas de plantas monocotiledôneas como nos bulbos de *Hippeastrum hybridum, Narcissus poeticus, N. tazetta, N. tazetta var chinesis, Lycoris squamigera, Lilium longiflorum e Lilium maximowiczii (*TOMODA et al., 1985; ZHAUYNBAEVA et al., 2003; ZHAUYNBAEVA, MALIKOVA & RAKHIMOV, 2003; TOMODA et al., 1980; TOMODA et al., 1983; TOMODA et al., 1978; KATO et al., 1976*), de pseudo-bulbos de Cyrtopodium andersonii* e *C. cardiochilum (*PARENTE et al., 2014; BARRETO & PARENTE, 2006*)*, de tubérculos como *Amorphophallus konjac* e *Bletilla striata* (MAEDA et al., 1980; CESCUTTI et al., 2002; KATSURAYA et al., 2003; PENG et al., 2014; ZHANG et al., 2014; WANG et al., 2014*),* de sementes de *Phoenix dactylifera,* das folhas de *Aloe barbandensis Miller (SHI et al., 2018;* CAMPESTRINI et al., 2013; CHOKBORIBAL et al., 2015; CHUN-HUI et al., 2007; MANDAL & DAS, 1980*),* das folhas e mucilagem de *Dendrobium huoshanense* (HISIEH et al., 2008), do rizoma de *Curculigo orchioides* (WANG et al., 2017), bem como de plantas dicotiledôneas como em sementes de *Artemisia sphaerocephala Krash* e *Bryonia lacinosa (*ISHRUD et al., 2001; GUO et al., 2012; SINGH & MALVIYA, 2006*),* e de partes aéreas da *Salvia officinalis* (CAPEK, 2009). Estruturas correlatas também foram obtidas a partir de algumas espécies de palmeiras, como a galactoglucomanana da *Acrocomia acuelata* (DA SILVA et al., 2009), e galactomanoglucanas da *Arecastrum romanzoffiazum* e *Syagrus oleracea* (DA SILVA et al., 2010; DA SILVA & PARENTE, 2010;), polímeros que apresentam maior proporção de glucose sobre manose em sua cadeia principal.

Glucomananas além de exercerem efeitos benéficos ao metabolismo humano na qualidade de fibras dietéticas, também desempenham diversas atividades biológicas de promoção à saúde, como ação prebiótica (ZHAI et al., 2018; KANG et al., 2019; ARIESTANTI et al., 2019), redução da constipação (HAN et al., 2017), ação gastroprotetora (PARENTE et al., 2014), promoção à saúde hepática (VÁZQUEZ - VELASCO et al., 2017), redução do colesterol (SOOD, BAKER & COLEMAN, 2008; ZALEWSKI & SZAJEWSKA, 2019), controle glicêmico (DEVARAJ et al., 2019; ZHOU et al., 2019), supressão tumoral (ZHAN et al., 2014; FENG et al., 2019; LI et al., 2020), anti-osteoporose (WANG et al., 2017), anti-fibrose (WANG et al., 2014), além de exercer atividades anti-inflamatória (BARRETO & PARENTE, 2006; PARENTE et al., 2014), antioxidante (CHUN-HUI et al., 2007; HUANG et al., 2016; LUO et al., 2016) e de modulação do sistema imunológico (BARRETO & PARENTE, 2006; HISIEH et al., 2008; PENG et al., 2014; HUANG et al., 2016; DENG et al., 2018; HUANG et al., 2018; CHEN et al., 2018; LI-ZHI et al., 2020).

Dentre as diversas propriedades terapêuticas atribuídas as glucomananas, podemos destacar a ação gastroprotetora relatada por PARENTE et al., 2014 que avaliou esse potencial de ação a partir da fibra extraída de *Cyrtopodium andersonii*.

2.5 Arabinoxilanas

Arabinoxilanas são polissacarídeos neutros não-amiláceos encontrados principalmente em grãos e cereais (MORALES-ORTEGA et al., 2013) e representam o principal polissacarídeo constituinte de hemiceluloses em plantas monocotiledôneas, como por exemplo, arroz, trigo, cevada, milho, centeio e aveia. Sua estrutura química varia de acordo com a fonte, mas predominantemente é disposta em uma cadeia linear constituída por β-D-Xyl*p* com ligações glicosídicas do tipo (1→4), com uma estrutura substituída com unidades α-L-Ara*f*, podendo ser substituído nas posições O-2 e O-3 (CHEN et al., 2019). Os resíduos de arabinose são unidos por ligações glicosídicas do tipo (1→3) ou (1→2), onde alguns resíduos podem estar esterificados com a ácido ferúlico, como acontece nas monocotiledôneas comelinóides. A cadeia de xilose pode ser monosubstituída ou dissubstituída de acordo com o número de resíduos de arabinose, além disso, outros substituintes menores podem ser observados, como o ácido glucurônico e a galactose, conforme exposto na Figura 5 (MORALES-ORTEGA et al., 2013). A proporção entre arabinose e xilose é bastante variada, quesito que repercute na estrutura molecular do polissacarídeo e consequentemente em sua atividade biológica. Algumas arabinoxilanas podem ser extraídas com água, já outras dependem de uma extração alcalina que envolve a quebra de uma ligação éster (KISZONAS et al., 2013).

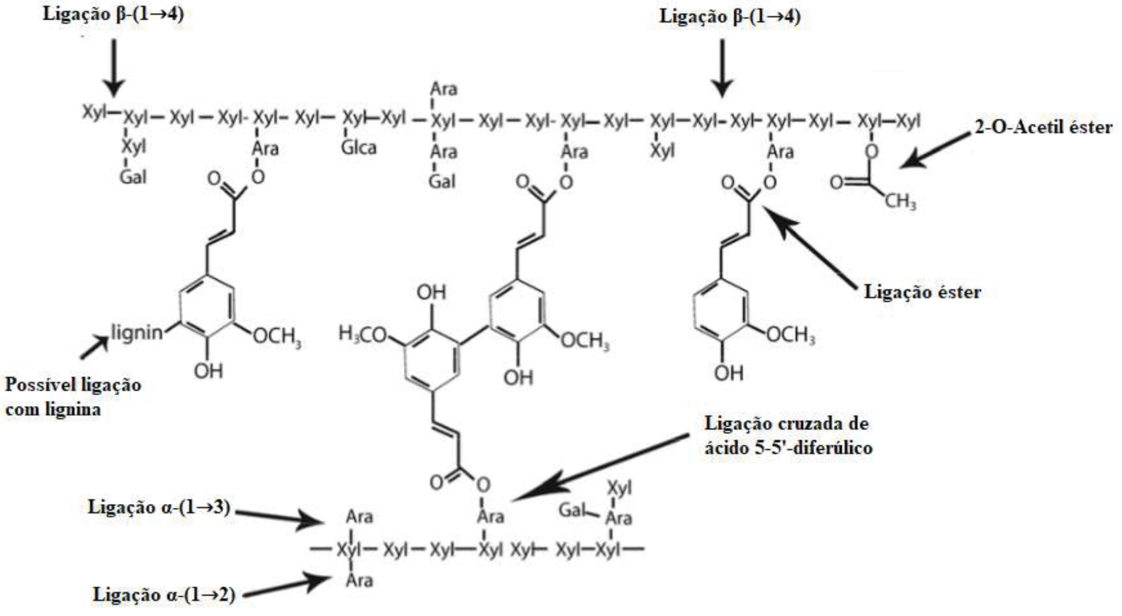

Figura 5: Principais características estruturais das arabinoxilanas. Ara = arabinose; Xyl = xilose; Gal = galactose; GlcA = Ácido glucurônico (Adaptada de KISZONAS et al., 2013)

Por também representar uma fibra dietética, as arabinoxilanas desempenham função prebiótica pela interação com a microbiota intestinal (CHEN et al., 2019), além disso, apresentam diversas propriedades terapêuticas, como por exemplo, regulação do metabolismo da glucose (CHRISTENSEN et al., 2013), melhora da sensibilidade à insulina (NEYRINCK et al., 2012), redução do colesterol plasmático (TONG et al., 2014), redução do peso corporal e massa adiposa (SURIANO et al., 2017), imunomodulação (CAO et al., 2011) e atividade antioxidante (YUWANG et al., 2018).

3. JUSTIFICATIVA

A avaliação de atividades biológicas de polissacarídeos componentes de frutas, demonstram grande valia em âmbito científico para a elucidação de possíveis aplicações na saúde humana. A perspectiva de novas terapias de prevenção e tratamento de patologias, visam a promoção à saúde pública e o bem-estar social.

Contudo vale ressaltar que as atividades biológicas observadas nos mais diversos componentes vegetais, muitas vezes são específicas e variam de acordo com a suas estruturas químicas, motivo pelo qual se faz necessário a caracterização estrutural de polissacarídeos para que haja a real compreensão de suas estruturas químicas e possíveis interações com o organismo humano.

Isto posto, a caracterização química e avaliação de atividade biológica de fibras dietéticas extraídas da bocaiuva, se justifica uma vez que essa fruta é consumida pela população e até o momento não existem relatos junto a literatura científica sobre a estrutura e atividade biológica de suas fibras dietéticas.

4. OBJETIVOS

4.1 Objetivo Geral

Purificar e caracterizar estruturalmente as fibras dietéticas extraídas da polpa da bocaiuva (*Acrocomia corumbaensis* S.A. Vianna. *sp. nov*.), além de avaliar seu potencial gastroprotetor.

4.2 Objetivos específicos

- Obter as fibras dietéticas da polpa de bocaiuva pelo método AOAC 991.43;
- Purificar os polissacarídeos presentes na porção das fibras solúveis e insolúveis presentes na bocaiuva;
- Realizar a caracterização da estrutura química fina dos polissacarídeos obtidos;
- Verificar a atividade antinociceptiva da fibra solúvel em experimento *in vivo*.
- Verificar a atividade anti-inflamatória da fibra solúvel em experimento *in vivo*.
- Verificar a atividade gastroprotetora da fibra solúvel em experimento *in vivo*.

ANEXO I

STRUCTURAL CHARACTERIZATION AND GASTROPROTECTIVE ACTIVITY OF SOLUBLE DIETARY FIBERS EXTRACTED FROM BOCAIUVA (*Acrocomia corumbaensis)* FRUIT PULP

Structural characterization and gastroprotective activity of soluble dietary fibers extracted from bocaiuva (A*crocomia corumbaensis*) fruit pulp

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Abstract

Soluble dietary fibers from *Acrocomia corumbaensis* fruit pulp were extracted and characterized. An acetylated glucomannan (4.3:1 Man:Glc ratio, degree of acetylation 24% and M_w of 1.82×10^5 g/mol) was obtained. Its gastroprotective activity was evaluated in the ethanol induced gastric ulcer model. Antinociceptive and anti-inflammatory activity were evaluated, however the fraction did not present any effect when compared with control. Oral and intraperitoneal administration of the glucomannan at different concentrations significantly decreased the gastric lesion area, prevented the depletion of GSH levels and preserved the mucus content in the gastric mucosa, thus demonstrating to be a promising therapeutic agent against gastric ulcer.

Keywords: Bocaiuva, glucomannans, chemical structure, gastroprotective activity

1. Introduction

Bocaiuva (*Acrocomia corumbaensis* S.A. Vianna. *sp. nov.*), is a fruit from the Brazilian Cerrado, explored by extractivists serving as a means of their subsistence as well as generating interest to the Brazilian industry in the search for biologically active substances and low cost like dietary fiber (CARDOSO et al., 2016; SANJINEZ et al., 2011; GALVANI e FERNANDES, 2010). The study by DO AMARAL et al., 2019 evaluated the nutritional composition of bocaiuva, demonstrating a high fiber content in the fruit pulp, which results varied between 26.31±0.06 (g/100 g) for manual pulping and 22.76 ± 0.1420 (g/100 g) for mechanical pulping.

Several studies have pointed out to the benefits of dietary fiber consumption on human health, acting effectively in reducing the risk of occurrence of chronic non-communicable diseases and metabolic complications such as obesity, inflammation and gastric ulcer, as trials have demonstrated the potential of polysaccharides in wound healing and also antiulcer activity (LEÓDIDO et al., 2017; SILVA, 2019). Among the public health problems, we can mention peptic ulcer, a pathology that refers to an injury that affects the mucosa or muscle layer of the gastric or duodenal region, forming a cavity with an acute or chronic inflammatory response, resulting in symptoms such as abdominal pain, burning sensation, heartburn and indigestion (MORA, 2014).

Given the present problem, literature suggests that the biological activities of polysaccharides may be related to gastric and intestinal protection mechanisms, as several studies have demonstrated their wound healing potential as well as antiulcerogenic and anti-inflammatory activities (LEÓDIDO et al., 2017). It is noteworthy that the biological activities of polysaccharides are attributed to their chemical characteristics, among other factors, requiring their structural analysis, whose objective is to understand their possible mechanism of action and therapeutic effects for the development of new medicinal therapies (SILVA, 2017).

In this sense, the chemical characterization and evaluation of the biological activity of dietary fibers extracted from bocaiuva corroborate the scientific advance in the search for new solutions in the treatment of illnesses, given that, despite being a fruit widely used by population, the current literature does not report studies on the topic in question. The present work aims to extract dietary fibers from *Acrocomia corumbaensis*, as well as to evaluate the potential anti-inflammatory, antinociceptive and gastroprotective activity.

2. Material and methods

2.1 Plant material

Bocaiuva (*Acrocomia corumbaensis* S.A. Vianna. sp. nov.) (VIANNA, 2017) was collected in Corumbá city, Mato Grosso do Sul, in the Antônio Maria Coelho Community, at geographic coordinates (19°17'29.07"S; 57° 36'0.05"O). The fruits were picked ripe and manually pulped. Bocaiuva flour (BF) was obtained from dehydration of the pulp in an oven with air circulation (40 °C) for 24 hours. Afterwards, dried pulp was fragmented in an industrial blender (FAKÒ, Brazil) and sieved (20 Mesh).

2.2 Extraction of dietary fibers

The BF was defatted with methanol and chloroform in a 1:1 ratio in a Soxhlet apparatus and subsequently air dried in the hood, resulting in the defatted bocaiuva flour (DBF fraction). Dietary fibers were extracted following the enzymatic-gravimetric official method (AOAC method 991.43). Briefly, DBF was suspended in distilled water

(20:1, g:L), then submitted to sequential enzymatic digestion, starting with 200 µL α-amylase (A3403, Sigma Aldrich Corp., St Louis, MO) at 90 °C for 2 hours with constant stirring. Then, the suspension was cooled to 60 °C and 200 µL amyloglucosidase (A7095, Sigma Aldrich Corp., St. Louis, MO) was added and incubated for 90 min. The pH was adjusted to 7.0 with a 10% KOH solution and 350 µL protease (P1236, Sigma Aldrich Corp., St. Louis, MO) was added to the suspension and incubated for 2 hours. Finally, the sample was heated to 100 °C to inactivate the enzymes.

The enzyme-treated suspension was then centrifuged (3,500 rpm, 25 min, 4 ºC) and the supernatant (containing soluble dietary fibers) separated from the precipitate (containing insoluble dietary fibers). The supernatant was concentrated in a rotaevaporator, and then excess of ethanol (3:1) was added. The resulting solution was kept under for 24 hours under refrigeration and centrifuged again to collect the precipitated polysaccharides. Then the resulting material was dissolved in water and dialyzed for 2 days (12-14 kDa MWCO membrane, Spectra Por®). The material was lyophilized to give soluble dietary fiber (BFS) fraction. The yield was determined and expressed as a percentage based on the weight of BF (Fig. 1).

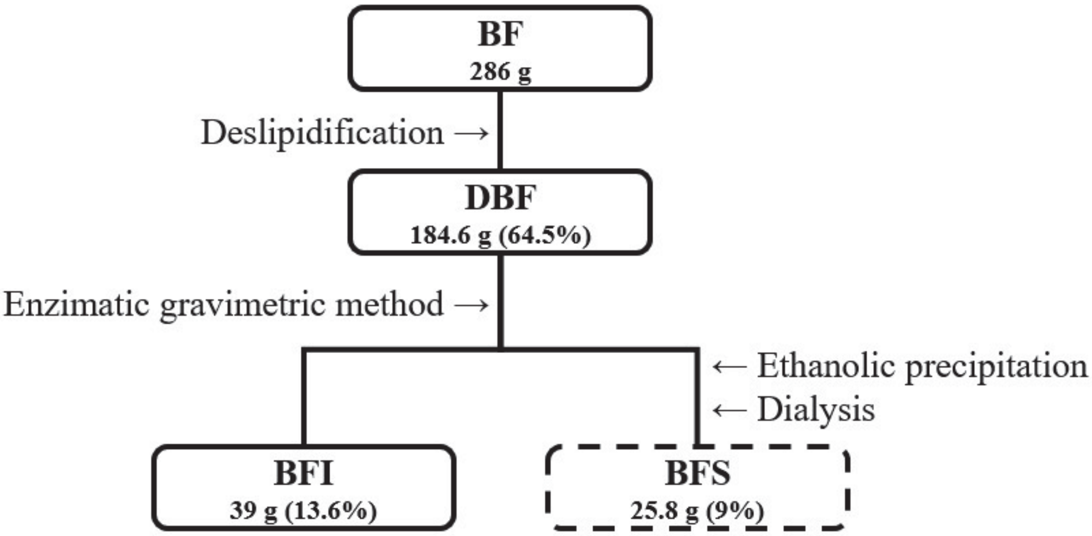


Fig. 1. Scheme of extraction and yields of polysaccharides from soluble dietary fiber fraction from *Acrocomia corumbaensis* fruit pulp.

2.3 Determination of monosaccharide composition

The composition of neutral monosaccharides was determined by total acid hydrolysis with 2M TFA for 8 h at 100 °C, followed by conversion to alditol acetates through reduction with $NaBH_4$ (WOLFROM and THOMPSON, 1963b) and acetylation with acetic anhydride and pyridine (WOLFROM and THOMPSON, 1963a). The alditol acetates were identified and quantified by GC-MS analysis. This was performed using a Varian Sarturn 4000R - model 3800 gas chromatographer coupled to a Varian Ion - Trap 4000R mass spectrometer, equipped with a VF-1 fused silica capillary column (30 mx 0. 25 mm) and analytical 5.0 helium at a flow rate of 1 mL/min as carrier gas. The capillary column was held at 50 ºC (held for 1.0 min), followed by a gradual increase of 40 ºC/min up to 220 ºC, being maintained isothermally until the end of the analysis.

The quantification of uronic acids was based on the colorimetric method of FILISETTI-COZI & CARPITA, 1991.

2.4 Determination of homogeneity and molar mass

The homogeneity and molar mass determination were carried out by HPSEC. A Wyatt Technology apparatus, equipped with a high-performance steric exclusion chromatographer, with four ultrahydrogel columns 2000, 500, 250 and 120 coupled in series (with limits of exclusion of 7×10^6, 4×10^5, 8×10^4 and 5×10^3 Da, respectively) and a refractive index detector, model Waters 2410 was used. As eluent, 0.1 mol/L $NaNO_2$ solution containing 0.2 g/L NaN_3 was used, with a controlled flow of 0.6 mL/min. The sample was solubilized in 0.1M $NaNO_2$ to a final concentration of 1 mg/mL and filtered through 0.22 μm membrane. The results were processed using the ASTRA software provided by the manufacturer (Wyatt Technologies). The relative molar mass of BFS was calculated based on a calibration curve using standard dextran (4.87×10^5 g/mol, 2.66×10^5 g/mol, 1.24×10^5 g/ mol, 7.22×10^4 g/mol, 4.02×10^4 g/mol, 17.2×10^4 g/mol and 9.4×10^3 g/mol, from Sigma).

2.5 Nuclear magnetic resonance spectroscopy

The NMR spectra (^1H, ^{13}C and the two-dimensional ^1H, ^{13}C HSQC) were obtained in a Bruker Avance III HD 400 MHz or Bruker Avance-III 600 MHz spectrometer. The sample was analyzed in an inverted probe of 5 mm in diameter, at 70 °C, using D_2O as solvent. Chemical shifts were expressed in ppm relative to acetone ($\delta = 30.2/2.22$ for ^{13}C and ^1H respectively) as internal standard.

2.6 General analytical methods

Quantification of total sugars was performed by the phenol-sulfuric acid method (DUBOIS et al., 1956).

The protein content was determined using the Bradford method using a calibration curve of bovine serum albumin and the results expressed in g protein/100 g of sample (BRADFORD, 1976).

Quantification of acetyl groups was performed according to the method of HESTRIN (1949), whose calibration curve was composed of a standard solution of β-D-glucose pentaacetate.

2.7 Evaluation of gastroprotective activity of BFS fraction

2.7.1 Animals

Female Wister rats weighing between 180 – 200 g were obtained from the vivarium of Federal University of Paraná. They were housed in plastic cages with a maximum of 5 rats per cage on soft bedding and free access to water and food (Nuvi-Lab CR-1, Quimtia S/A, Brazil) and kept under laboratory condition, 12 h light/dark cycle and controlled temperature (22 ± 2 °C). All animal protocols were conducted in agreement with the 8th edition of “Guide for the Care and Use of Laboratory Animals” (National Research Council, 2011) and approved by the Committee of Animal Experimentation of the Federal University of Paraná (CEUA/ BIO – UFPR) under approval number 1357-R.O. 03/2020.

2.7.2 Acute hemorrhagic gastric lesions induced by ethanol in rats

Gastric lesions were induced by oral administration of absolute ethanol as previously described (ROBERT et al., 1979), with minor modifications. After 18 h of fasting, the animals were orally pretreated with water (Control [C] at 1 mL/kg), sucralfate (positive control [S] at 100 mg/kg) or BFS fraction (BFS at 0.01, 0.1, 1 and 10 mg/kg). In another experiment, instead being administered by oral gavage, BSF was intraperitoneally administered (at 0.1 mg/kg). One hour after the oral treatment or 30 minutes after the intraperitoneal administration, all animals received absolute ethanol (1 mL/rat). After 1 h of ethanol administration, the animals were euthanized by thiopental overdose (100 mg/kg, i.p.), then, the stomachs were removed, opened along the smaller curvature, cleaned with saline and photographed to measure the extent of lesions (mm^2) using a computerized planimetry software (ImageJ® 3.0).

After this procedure, the glandular portion of the stomach was divided in two parts and weighted for determination of reduced glutathione (GSH) and wall mucus levels on the tissue.

2.7.3 Measurement of reduced glutathione (GSH) and wall mucus levels on gastric tissue

The samples of stomach from ethanol-induced lesions were homogenized with cold 200 mM potassium phosphate buffer (pH 6.5) in a volume equal to 3 times the weight of fresh gastric tissue to determine reduced glutathione (GSH) levels as previously described (SEDLAK & LINDSAY, 1968). Aliquots of samples were mixed with 12.5% trichloroacetic acid before and centrifuged at 3,000 rpm for 15 min at 4 °C. To the supernatant of the samples, 400 mM TRIS-HCl buffer (pH 8.5) and 10 mM 5,5′-dithiobis-(2-nitrobenzoic acid) were added in a 96-well plate to perform the colorimetric reaction. The absorbance was read at 412 nm and the results were expressed in µg of GSH/g of tissue.

The determination of gastric mucus content was performed according the reported method reported by CORNE et al., (1974). Briefly, glandular segments from stomach were complexed with a solution of 0.1% Alcian Blue dye during 2 h. Then, the tissue was washed with 250 mM sucrose twice for 15 and 45 min respectively, and the complex mucus-dye was extracted adding 500 mM of magnesium chloride and stirred for 2 h. The solution extracted was mixed with the same ether volume and centrifuged for 10 min at 3600 rpm. The aqueous layer was separated to measure the absorbance at 580 nm and the results were expressed in µg Alcian blue/g of glandular tissue.

2.8 Evaluation of anti-inflammatory and antinoceptive activity of fraction BFS

The anti-inflammatory and antinoceptive activity of BFS fraction were evaluated by the formalin test. *Mus musculus* mice were treated one hour before the experiment with vehicle (control [saline]) or BFS fraction (v.o. or i.p.). Then, 20 µL of 2.5% formalin was injected intraplantarly into the region of the right pelvic limb and the time in which each animal exhibited certain behaviors such as licking, biting or shaking the affected area was timed. The timing took place in two moments, in the first five minutes after the application of formalin, to assess pain sensitivity in the neurogenic phase, and in a second moment, covering the interval between fifteen and thirty minutes, to assess pain sensitivity in the inflammatory phase (HUNSKAAR & HOLE, 1987).

2.9 Statistical analysis

Results were expressed as mean ± standard error of mean (SEM) and the statistical significance ($p < 0.05$) was determined using one-way analysis of variance (ANOVA) followed by Bonferroni's test ($n = 48$ animals) using GraphPad Prism® software version 6.0 (San Diego, CA, USA).

3. Results

3.1 Chemical characterization of soluble dietary fiber fraction of bocaiuva pulp

The bocaiuva flour (BF) was defatted, giving a lipid content of approximately 31.6%, higher than that reported by DO AMARAL et al., 2019 (26.27%). The dietary fiber extraction by the enzymatic gravimetric method was performed on defatted bocaiuva flour (DBF), resulting in soluble (BFS) and insoluble (BFI) dietary fiber fractions (Fig. 1), with yields of 9.0% and 14%, respectively. Thus, the amount of total dietary fiber was 23%, resembling that reported by DO AMARAL et al., (2019), which was of 26.3%.

Interestingly, the monosaccharide composition of BFS (Table 1) showed mainly mannose and glucose, suggesting the presence of mannan/glucomanan instead of pectic polysaccharides, as expected for fruit pulps. Other monosaccharides were also observed in minor amounts, such as galactose, arabinose, xylose and fucose. Uronic acids were not detected by the colorimetric method of FILISETTI-COZI & CARPITA, 1991. It presented 1.2% of protein and 82% of total sugar.

Table 1. Monosaccharide composition of fraction obtained from bocaiuva (*Acrocomia corumbaensis* S.A.Vianna. *sp. nov.*)

Fraction	**Neutral sugars** [a]						**Uronic acid** [b]
	Glc	**Man**	**Gal**	**Ara**	**Xyl**	**Fuc**	
BFS	16.0	70.3	3.3	6.7	0.5	3.2	nd[c]

[a] % of peak area relative to total peak area, determined by GC–MS.

[b] Determined using the *m*-hydroxybiphenyl method FILISETTI-COZZI & CARPITA, 1991.

[c] Not detected.

The monosaccharide composition is in line with the attributions of signals observe in the ^{13}C-NMR (Fig. 2) and ^{1}H, ^{13}C HSQC-Dept (Fig. 3) spectra, suggesting the presence of a glucomannan in BFS fraction. The anomeric signal at δ 102.3/4.51 has been assigned to β-D-Glc*p* units, beyond the signals at δ 73.1/3.35 (C-2/H-2), δ 74.9/3.63 (C-3/H-3), δ 78.7/3.65 (C-4/H-4) (ZHAUYNBAEVA et al., 2003; KATSURAYA et al., 2003; HUA et al., 2004; HSIEH et al., 2008; CAPEK et al., 2009; GUO et al., 2012; PARENTE et al., 2014; WHANG et al., 2014; ZHANG et al., 2014; LUO et al., 2016; WANG et al., 2017; SHI et al., 2018; ZHANG et al., 2020.). Other signals were attributed to β-D-Man*p* units, at δ 100.1/4.74 (C-1/H-1), δ 70.2/4.10 (C-2/H-2), δ 71.7/3.77 (C-3/H-3), δ 76.5/3.81 (C-4/H-4) (ISHRUD et al., 2001; ZHAUYNBAEVA et al., 2003; KATSURAYA et al., 2003; HUA et al., 2004; CHUN-HUI et al., 2007; HSIEH et al., 2008; CAPEK et al., 2009; GUO et al., 2012; CAMPESTRINI et al., 2013; PARENTE et al., 2014; WHANG et al., 2014; ZHANG et al., 2014; LUO et al., 2016; WANG et al., 2017; ZHANG et al., 2020.). The signal at δ 75.1/3.54 corresponds to superimposed C-5/H-5 of β-D-Glc*p* and β-D-Man*p* units, while the signal at δ 60.5 correspond to superimposed C-6 from β-D-Glc*p* and β-D-Man*p*. Signals at δ 20.2/2.10 and at δ 20.4/2.18 were assigned to acetyl groups (ISHRUD et al., 2001; HUA et al., 2004; HSIEH et al., 2008; GUO et al., 2012; CAMPESTRINI et al., 2013; PARENTE et al., 2014; XING et al., 2014; HUANG et al., 2016; LUO et al., 2016; WANG et al., 2017; SHI et al., 2018; DENG et al., 2018; ZHANG et al., 2020.). The degree of acetylation was determined by the method of HESTRIN (1949), resulting in 24% acetylation degree.

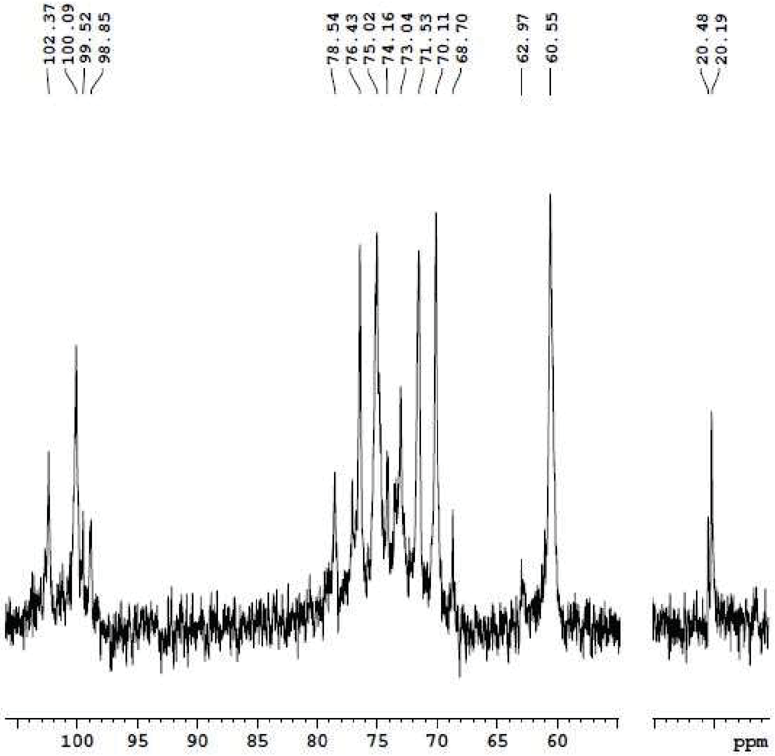


Fig. 2. ^{13}C-NMR spectrum of fraction FBS in D_2O at 70 ºC, the chemical shifts are expressed as δ ppm

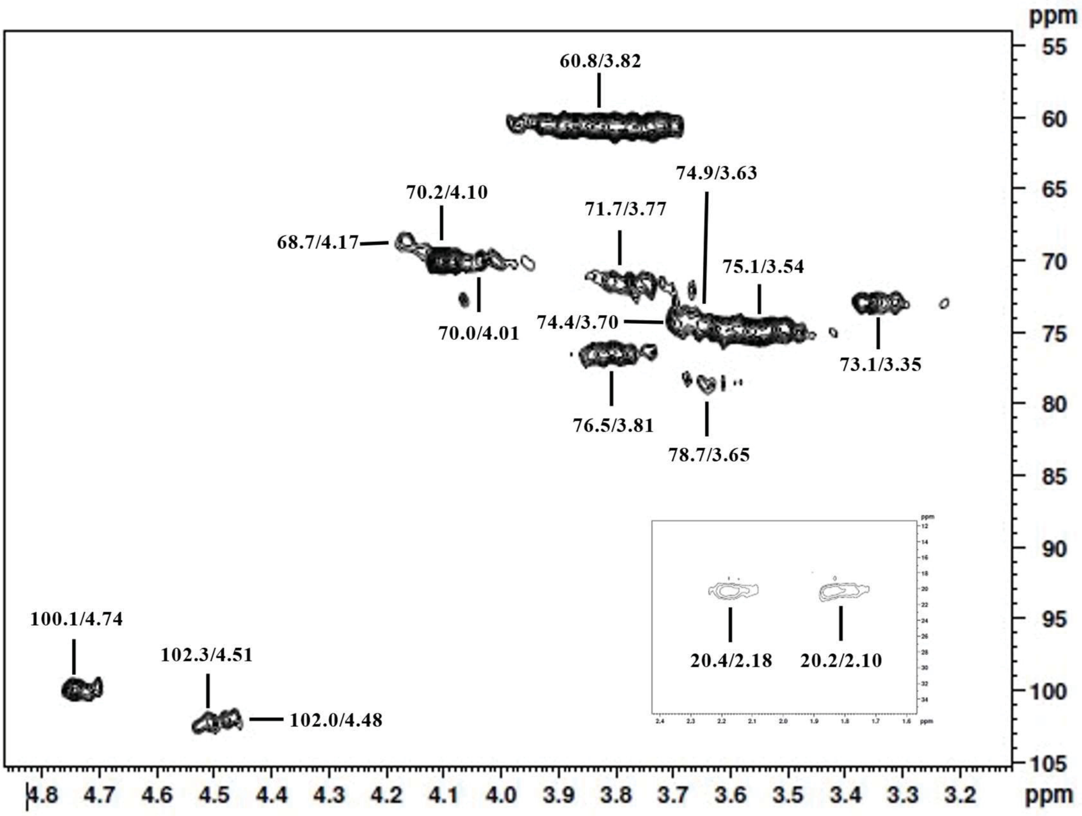


Fig. 3. HSQC correlation map of BSF fraction in D_2O at 70 ºC, the chemical shifts are expressed as δ ppm.

The HPSEC elution profile of BFS is shown in Fig. 4, presenting a main peak at 45.8 min with M_w of 1.82 × 10^5 g/mol.

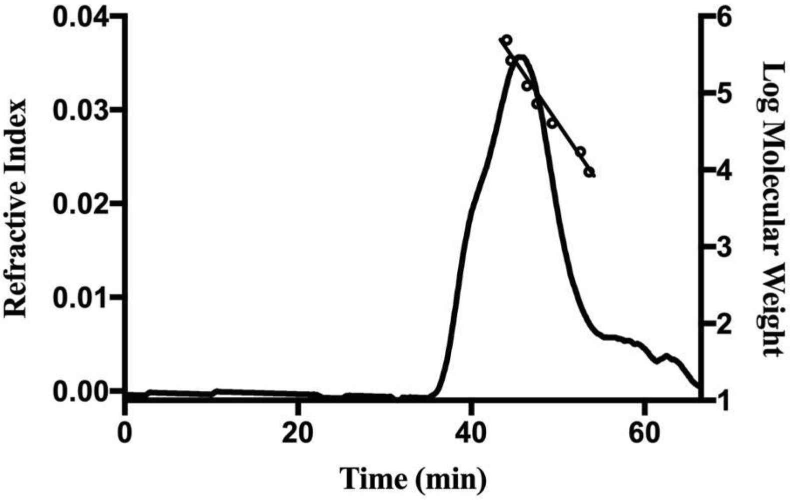


Fig. 4. HPSEC elution profile of FBS fraction. Refractive index detector. Elution volume of dextran standards of molecular weight 487 kDa, 266 kDa, 124 kDa, 72.2 kDa, 40.2 kDa, 17.2 kDa and 9.4 kDa (left to right) were employed to construct the calibration curve.

3.2 Evaluation of the gastroprotective effect of soluble dietary fiber fraction (BFS) from bocaiuva pulp

Our results showed that oral pre-treatment with soluble dietary fibers extracted from *A. corumbaensis* (BFS fraction) at doses 0.01, 0.1, and 1 mg/kg decreased the acute hemorrhagic gastric lesions induced by ethanol by 72.2, 68.5 and 54.2% respectively when compared to the control group (C: 145.0 ± 20.52 mm^2) (Fig. 5). As expected, the positive group pretreated with Sucralfate (100 mg/kg), significantly reduced the ulcer area by 57.5% (S: 61.60 ± 23.38 mm^2), when compared to the control group. The gastroprotective effect of BFS has not been observed on the higher dose (BFS: 10 mg/kg).

Considering the mechanism of action of conventional antiulcer options available to treat peptic ulcer disease, the most common options focus on decreasing aggressive factors such as inhibition of gastric acid and pepsin secretion or increase protective factors like improving the mucus coating of gastric mucosa (KUNA et al., 2019). In this sense, it was evaluated the effect of pre-treatment with BFS by the intraperitoneal route (Fig. 5). It was observed that this treatment also significantly decreased the gastric lesion area by 65.8% when compared to the control group (C: 145.0 ± 20.52 mm^2), confirming the systemic effect of BFS and discarding the possibility of its gastroprotective effect by the mechanical barrier only.

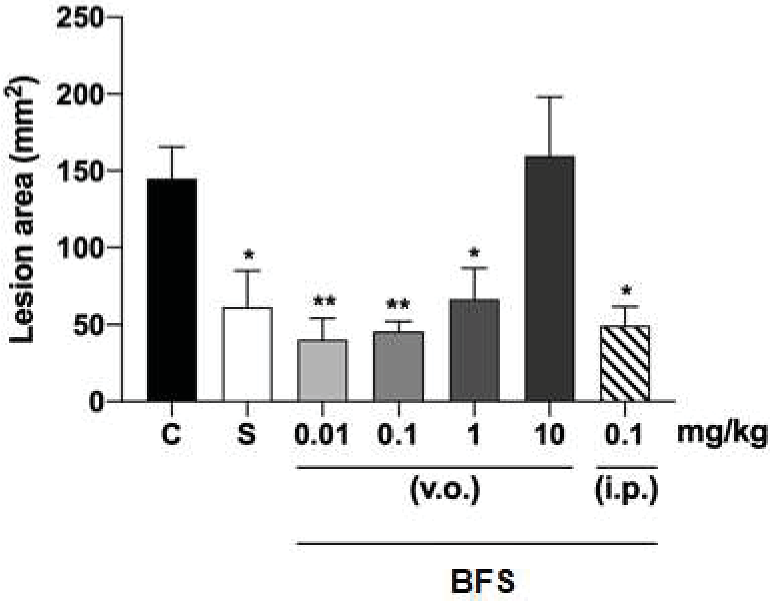


Fig. 5. Effect of oral and intraperitoneal treatment with soluble dietary fiber (BFS fraction) from bocaiuva pulp on ethanol-induced gastric lesion in rats. The animals were pretreated with vehicle (C: water 1 mL/kg), Sucralfate (S = 100 mg/kg), and soluble dietary fiber from bocaiuva pulp (BFS = 0.01, 0.1, 1 and 10 mg/kg) by oral or

intraperitoneal route. After 60 (for v.o.) or 30 (for i.p.) minutes, the animals were orally treated with ethanol (1 mL/animal). The results are expressed as mean ± SEM (n = 6-8). Statistical comparison was performed using analysis of variance (ANOVA) followed by Bonferroni's test. Differences from control group (* $p < 0.05$ and** $p < 0.01$).

The oral ethanol administration is known to induce several damages on the gastric tissue, to decrease the glycoprotein content, and to increase oxidative stress and inflammatory response. (GUGLIANDOLO et al., 2021). Concerning the protective factors of mucosal tissue, mucus represents one of the most significant physiological factors of gastric protection. BFS administrated by oral route at doses of 0.01, 0.1, 1 and 10 mg/kg, significantly preserved the mucus content when compared to the control group (C: 1742 ± 351.5 µg Alcian blue/g of tissue). In the same way, BFS administrated by the intraperitoneal route was also able to inhibit the depletion of mucus levels when compared to the non-treated group (Fig 6A).

Another important adverse effect of ethanol instillation is the infiltration of neutrophils on gastric tissue, during this process occurs generation of reactive oxygen species and oxidative stress (DE SOUZA et al., 2019), to prevent damage in the gastric mucosal, an endogenous antioxidant system (reduced glutathione – GSH and the enzymes superoxide dismutase and catalase) maintain the gastric homeostasis. Results showed that the oral pre-treatment with BFS at doses of 0.01 and 0.1 mg/kg significantly prevented the depletion of GSH levels by 55.0 and 52.1% respectively, when compared to the control group (C: 430.0 ± 59.51 5 µg GSH/g of tissue). The intraperitoneal BFS administration also showed a non-significant trend to increase the GSH levels (Fig 6B).

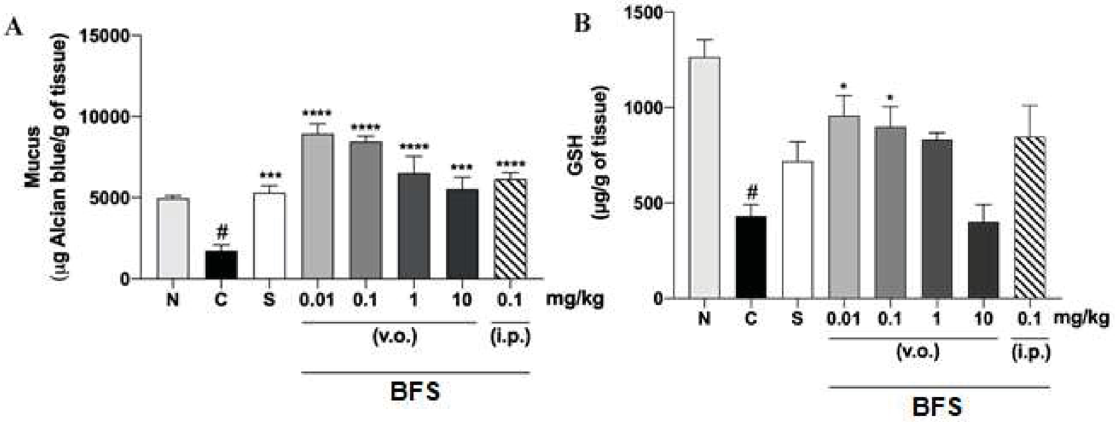
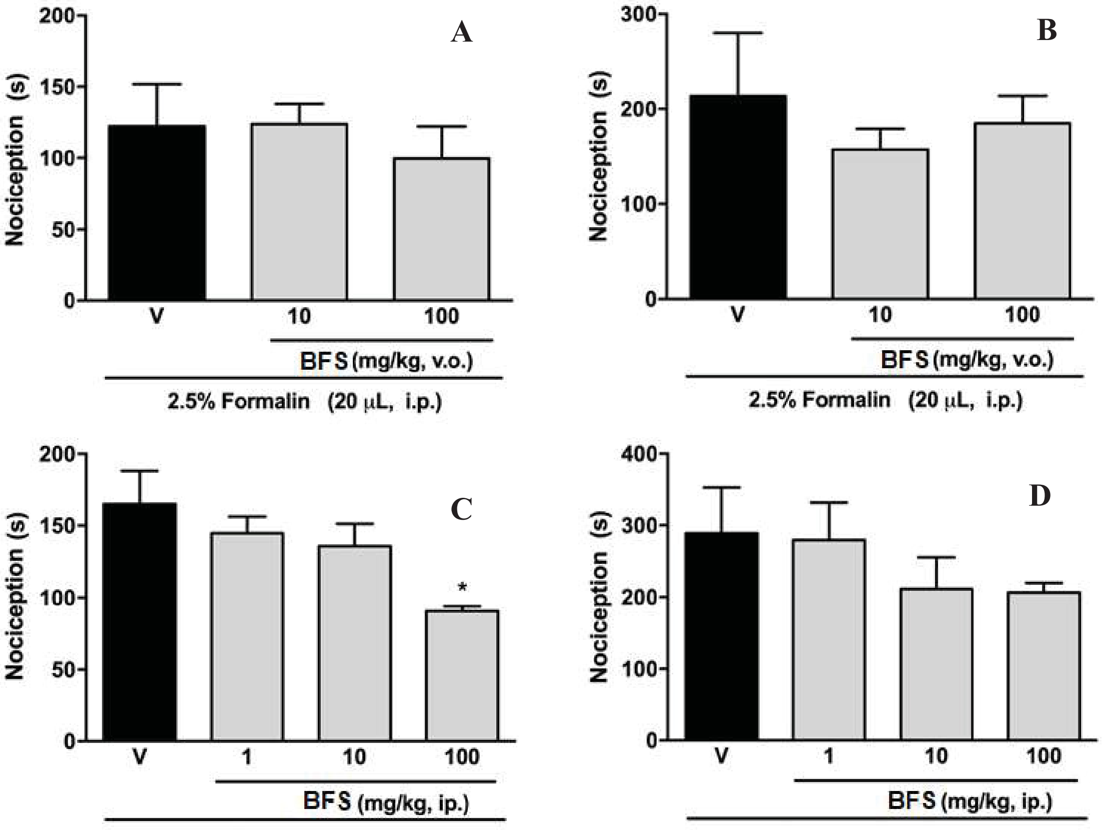


Fig. 6. Effect of oral and intraperitoneal treatment with soluble dietary fiber (BFS fraction) on mucus content (A) and reduced glutathione levels (B). The animals were pretreated with vehicle (C: water 1 mL/kg), Sucralfate (S = 100 mg/kg), and Soluble dietary fiber from bocaiuva pulp (BFS = 0.01, 0.1, 1 and 10 m/k) by oral or intraperitoneal route. After 60 (for v.o.) or 30 (for i.p.) minutes, the animals were orally treated with ethanol (1 mL/animal). The results are expressed as mean ± SEM (n = 6-8). Statistical comparison was performed using analysis of variance (ANOVA) followed by Bonferroni's test. Differences from control group are denoted by an esterix (* $p < 0.05$, *** $p < 0.01$ and **** $p < 0.001$), # $p < 0.05$ when compared to naïve group (N).

3.3 Evaluation of anti-inflammatory and antinociceptive effect of soluble dietary fiber fraction (BFS) from bocaiuva pulp

Glucomannans may present several biological activities, such as gut microbiota modulation and prebiotic action (ARIESTANTI et al., 2019), reduction of constipation (HAN et al., 2017), gastroprotective effect (PARENTE et al., 2014), liver health (VÁZQUEZ -VELASCO et al., 2017), cholesterol reduction (HO et al., 2017), glycemic control (DEVARAJ et al., 2019), antitumor effects (FENG et al., 2019), antioxidant activity (LUO et al., 2016), anti-inflammatory effect (BARRETO & PARENTE, 2006), modulation of immune system (LI-ZHI et al., 2020), anti-osteoporosis (WANG et al., 2017) and antifibrosis activity (WANG, et al., 2014). In order to verify the anti-inflammatory and antinociceptive activity of glucomannan from bocaiuva pulp, BFS fraction was subjected to the formalin test in mice. Although different BFS doses have been administered, both orally and intraperitoneally (Suppl. Fig. 1), in general, the results demonstrated that it not had anti-inflammatory nor antinociceptive activity in this model, however, the dose of 100 mg/kg i.p. (Fig. C) showed antinociceptive activity when compared to the group control.



Suppl. Fig. 1. Effect of administration of BFS fraction on neurogenic phase (A and C) and inflammatory phase (B and D) of nociception induced by 2.5% formalin in mice. The animals were treated with vehicle (V, 10 mL/kg, i.p.) or BFS fraction (orally, A and B or intraperitoneally, C and D). The results are expressed as mean ± SEM (n = 6-8). Statistical comparison was performed using analysis of variance (ANOVA) followed by Bonferroni's test.

4. Discussion

Glucomannans (GM) are polysaccharides formed by β-D-Man*p* and β-D-Glc*p* units with varying proportions, with mannose being the predominant monosaccharide in most plants, whose variation is from 1:1 to 30:1 (Man:Glc ratio) (SHI et al., 2018). The Man:Glc ratio of BFS was 4.3:1, value close to that observed in GM extracted from the trunk of *Dendobrium officinale*, whose ratio was 4.5:1 (HUANG et al., 2016). Galactoglucomannans/galactomannoglucans have also been reported in pulp fruit from palm trees, such as in *Acrocomia aculeata* (DA SILVA et al., 2009), *Arecastrum romanzoffiazum* (DA SILVA et al., 2010) and *Syagrus oleracea* (DA SILVA & PARENTE, 2010). The ratio of Gal:Man:Glc varied according to the species, being 1:3.3:1.2 for *A. aculeata,* 1:3.7:5.3 for *A. romanzoffiazum* (DA SILVA et al., 2010) and 1:1.1:1.2 for *S. oleracea* (DA SILVA et al., 2009, 2010, DA SILVA & PARENTE, 2010).

GM generally contains acetyl groups, whose position varies depending on their botanical source (ZHU, 2018), with values between 1.6 and 29.9% (ZHANG et al., 2020; SHI et al., 2018). The degree of acetylation of BFS was 24%, a result similar to GM from *Dendobrium huoshanense* leaf (25%) (HISIEH et al., 2008), *Narcissus tazetta var chinesis* bulb (22.7%) (TOMODA et al., 1980) and *Phoenix dactylifera* seeds (22%) (ISHRUD et al., 2001). Unfortunately, the acetyl content of galactoglucomannans/galactomannoglucans from the palm fruit pulp of *A aculeata*, *A. romanzoffiazum* and *S. oleracea* were not reported (DA SILVA et al., 2009, 2010, DA SILVA & PARENTE, 2010).

Regarding the molar mass, BFS presented a M_w of 1.82 × 10^5 g/mol, similar to that found in *A. romanzoffiazum,* which had a M_w of 1.5 × 10^5 g/mol (DA SILVA et al., 2010). Higher M_w of 3.5 × 10^5 g/mol and 4.1 × 10^5 g/mol have been reported for *A. aculeata* (DA SILVA et al., 2009) and *S. oleracea* (DA SILVA & PARENTE, 2010), respectively.

The results of the biological activity showed that BFS (0.1 and 0.01 mg/kg) presented gastroprotective activity in rats associated with the preservation of the mucus of gastric mucosa as well as inhibits the depletion of GSH, whose action potential, in this experiment, was superior to the pharmacological option (sucralfate) with a similar mechanism of action. In a similar *in vivo* experiment, glucomannan from *Cyrtopodium andersonii* (PARENTE et al., 2014) had a gastroprotective effect in male Swiss mice by reducing the area of gastric injury induced by ethanol through oral administration of GM at higher concentration (100 mg/kg). The galactomannoglucan from the fruit pulp of *S. oleracea* also inhibited the gastric lesions induced by ethanol in male Swiss mice at 100 mg/kg. Although the mucus amount has not been quantified in this study, the authors suggested that the gastroprotective action exerted by the polysaccharide was mainly related to the stimulus to the production of mucus, which represents a crucial factor for the protection of the gastric mucosa (DA SILVA & PARENTE, 2010). As reported above, FBS increased the amount of the mucus of gastric mucosa as well as inhibited the depletion of GSH (Fig. 6A, B).

Regarding anti-inflammatory activity of glucomannans, it has been reported that a glucomannan from *Cyrtopodium andersonii* reduced the inflammation and inhibited the edema formation similar to the reference compounds indomethacin and dexamethasone in the paw edema induced by subplantar carrageenan injection. The GM at 100 mg/kg demonstrated the anti-inflammatory action in the initial phase of inflammation, which is related to the release of histamine, serotonin and other similar substances, and exerted less effect in the delayed phase of inflammation that is related to the action of prostaglandins (PARENTE et al., 2014). Galactomannoglucan from the fruit pulp of palm tree *A. romanzoffianum* also showed anti-inflammatory activity at a dose of 100 mg/kg in the carrageenan-induced mouse paw edema. (DA SILVA & PARENTE, 2010). Another study evaluated the anti-inflammatory potential of glucomannan extracted from *Cyrtopodium cardiochilum* in an acetic acid-induced vascular permeability in male BALB/c albino mice. The polysaccharide inhibited the acetic acid-induced increase in vascular permeability, a condition typical of an early inflammatory response (BARRETO & PARENTE, 2006).

It is worth to note that BFS did not had effect on inflammation induced by intraplantar injection of formalin (Suppl. Fig. 1). The biological activities are attributed to polysaccharide structures, including the degree of acetylation, as observed in the study by FENG et al., 2019, which observed that different degrees of acetylation of konjac GM resulted in different immunomodulatory activities. It can be observed that FBS has some distinct structural features as compared with the tested glucomannans, such as the Man:Glc ratio and the degree of acetylation, which was superior to *C. andersonii* (14.6%) and *C. cardiochilum* (4.2%) glucomannans. Unfortunately, the degree of acetylation of GM from *Arecastrum romanzoffianum* was not determined (BARRETO & PARENTE, 2006, DA SILVA & PARENTE, 2010, PARENTE et al., 2014).

5. Conclusion

The present study allowed the structural characterization of a glucomannan with a degree of acetylation of 24%, which represents the soluble dietary fiber fraction extracted from *Acrocomia corumbaensis* fruit pulp. It was also observed that this fraction presented gastroprotective activity *in vivo*, thus being a promising therapeutic agent in the management of gastric pathologies.

Although the fruit is widely exploited by extractivists and industry, there are still no reports of its commercial use for medicinal purposes, however, more studies are still needed for a better understanding of their biological activities, in addition to the evaluation of the ideal dose according to each context, as well as for the structural characterization of the insoluble fibers present in the bocaiuva pulp.

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ANEXO II

CHEMICAL STRUCTURE, SOURCES AND HEALTH EFFECTS OF GLUCOMANNANS

Chemical structure, sources and health effects of glucomannans

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ABSTRACT

Nature has a diversity of polysaccharides which exert their function in the environment and can interact with human metabolism in different ways, improving human health. Among the polysaccharides that constitute the dietary fibers, glucomannan is of interest due to its wide use by the food industry and their beneficial effects on the human health. This review summarizes the sources, chemical structure and health effects of glucomannans such as gut microbiota modulation and prebiotic action, reduction of constipation, gastroprotective effect, liver health, cholesterol reduction, glycemic control, antitumor effects, antioxidant activity, anti-inflammatory effect, modulation of immune system, anti-osteoporosi and antifibrosis activity.

Keywords: glucomannans; chemical structure; sources; health effects.

1. Introduction

Nature has a diversity of polysaccharides which exert their function in the environment and can interact with human metabolism in different ways, improving human health (DA SILVA et al., 2020). Besides starch and glycogen, that are broken down by the action of digestive enzymes, supplying energy for the body, the majority of food polysaccharides are not hydrolyzed by these enzymes, so they can be metabolized by the intestinal microbiota, then called as dietary fibers. The main types of dietary fibers are present as cell wall components, from plants, mushrooms and algae. Thus, dietary fibers are composed of a wide diversity of polysaccharide chemical structures, leading to a variety of interactions and metabolic responses through their consumption (TURNER & LUPTON, 2011). Since is well recognized that biological activities of polysaccharides are attributed to their respective chemical structures, the structural characterization of dietary fibers is essential for understanding their interaction with human metabolism.

Among the polysaccharides that constitute the dietary fibers, glucomannan is of interest due to its wide use by the food industry and also due to their beneficial effects on the human health (TESTER & AL-GHAZZEWI, 2017). This review summarizes the sources, chemical structure and health effects of glucomannans.

2. Occurrence and chemical structure of glucomannans

Glucomannans were already extracted from plants belonging to different families and genera, as described in Table 01.

Table 01. Plants sources of glucomannans

Family	**Plant species**	**Plant portion**	**Reference**
Amaryllidaceae	*Hippeastrum hybridum*	Bulb	TOMODA et al., 1985
	Narcissus poeticus	Bulb	ZHAUYNBAEVA et al., 2003
	Narcissus tazetta	Bulb	ZHAUYNBAEVA, MALIKOVA & RAKHIMOV, 2003
	Narcissus tazetta var chinesis	Bulb	TOMODA et al., 1980
	Lycoris squamigera	Bulb	TOMODA et al., 1983
Araceae	*Amorphophallus konjac*	Tuber and flour	MAEDA et al., 1980; CESCUTTI et al., 2002; KATSURAYA et al., 2003
	Amorphophallus oncophyllus	Porang flour	WARDHANI et al., 2019
Arecaceae	*Phoenix dactylifera*	Seeds	ISHRUD et al., 2001
Asphodelaceae	*Aloe barbandensis Miller*	Leaves pulp, leaves, gel and skin	SHI et al., 2018; CAMPESTRINI et al., 2013; CHOKBORIBAL et al., 2015; CHUN-HUI et al., 2007; MANDAL & DAS, 1980;
Asteraceae	*Artemisia sphaerocephala Krash*	Seeds	GUO et al., 2012
Curcubitaceae	*Bryonia lacinosa*	Seeds	SINGH & MALVIYA, 2006
Hipoxidaceae	*Curculigo orchioides*	Rhizome	WANG et al., 2017
Laminaceae	*Salvia officinals*	Aerial parts	CAPEK, 2009

Liliaceae	*Lilium longiflorum*	Bulb	TOMODA et al., 1978
	Lilium maximowiczii	Bulb	KATO et al., 1976
Orchidaceae	*Dendrobium denovianum*	Powder	DENG et al., 2018
	Dendrobium huoshanense	Leaf, stem cell walls and mucilage	HSIEH et al., 2008
	Dendrobium nobile	Smashed stems	ZHANG et al., 2020
	Dendrobium officinale	Stem	XING et al., 2014; HUANG et al., 2016; HUA et al., 2004; LUO et al., 2016
	Cyrtopodium andersonii	Pseudobulbs	PARENTE et al., 2014
	Cyrtopodium cardiochilum	Pseudobulbs	BARRETO & PARENTE, 2006
	Bletilla striata	Tuber and powder	PENG et al., 2014; ZHANG et al., 2014; WANG et al., 2014

These polysaccharides are formed by β-D-mannopiranosys and β-D-glucopyranose units with varying proportions, with mannose being the predominant monosaccharide in most plants, whose variation is from 1:1 to 30:1 (M:G ratio) (SHI et al., 2018). However, some exception may occur, such as in *Artemisia sphaerocephala Krasch*, which presents a higher proportion of glucose (ratio M:G of 1:1.3) (GUO et al., 2012). Within this class of hemicellulose, polysaccharides with other substituting sugars are also found, such as galactoglucomannans (MOREIRA & FILHO, 2008) as those found in *Acrocomia acuelata* (DA SILVA et al., 2009) and galactomannanoglucans, which are polymers that contain more glucose than mannose in its main chain, as found in *Arecastrum romanzoffiazum* and *Syagrus aleracea* (DA SILVA et al., 2010; DA SILVA & PARENTE, 2010).

Most glucomannans are formed by →4)-linked β-D-Man*p* and →4)-β-D-linked Glc*p* units at the main chain, some variations were also reported, as in the case of *Aloe barbandensis Miller* (SHI et al., 2018) which presented →4)-β-D-Man*p*-(1→3)-β-D-Man*p*-(1→4)-β-D-Glc*p*-(1→ and of *Bletilla striata* (WHANG et al., 2014), which had →2)-α-D-Man*p*-(1→4)-β-D-Glc*p*-(1→. Differences can also be observed in the branching degree of the main chain, where some polysaccharides have no branches, while others have branches at *O*-2, *O*-3, *O*-6 in the Man*p* units and/or at *O*-3 and *O*-6 in the Glc*p* units (SHI et al., 2018; ZHU, 2018).

Glucomannans generally contain acetyl groups, whose position varies depending on their botanical source (ZHU, 2018). For example, the glucomannan extracted from *Amorphophallus konjac* (KGM) has from 5-10% of the units substituted by acetyl groups, but the literature reports variations between 1.6 and 29.9% depending on the source, as in the cases from *Dendrobium nobile* (ZHANG et al., 2020) and *Aloe barbandensis Miller* (SHI et al., 2018), respectively. The position of the acetyl groups also varies according to the plant, being reported at *O*-2, *O*-3 and *O*-6, at *O*-2,3, *O*-3,6 and *O*-2,3,6 Man*p* units (GAO & NISHINARI, 2004).

The structural characteristics of glucomannans reported in literature are summarized in Table 2.

Table 02. Structural characteristics of glucomannans from different sources.

Source	Plant portion	Molecular weight	Man*p*/Glc*p* ratio	Linkage	Branch	DA	Acetylated position	Biological Activity	Reference
Aloe barbadensis Miller	Leaves	3.33×10^5 Da	2.8:1 – 22:1	→4)-*β*-Man*p*-(1→3)-β-Manp-(1→4)-β-Glc*p*-(1→	O-6 Man*p*	29.9%	O-2 Man*p* O-3 Man*p* O-6 Man*p* O-2,3 Man*p* O-2,6 Man*p* O-3,6 Man*p*	-	SHI et al., 2018
Aloe barbadensis Miller	Leaves pulp	-	24:1	→4)-*β*-Man*p*-(1→4)-*β*-Glc*p*-(1→	-	18.4%	O-2 Man*p* O-3 Man*p* O-6 Man*p* O-2,3 Man*p*	-	CAMPESTRINI et al., 2013
Aloe barbadensis Miller Gel Acemannan	Leaves	190 – 220 kDa	57%:22%	→4)-*β*-Man*p*-(1→4)-*β*-Glc*p*-(1→	O-6 Man*p*	-	O-2 Man*p* O-3 Man*p*	-	CHOKBORIBAL et al., 2015

Aloe barbadensis Miller	Gel and skin	3.97×10^4 Da	296:36	→4)-*β*-Man*p*-(1→4)-*β*-Glc*p*-(1→	-	-	O-6 Man*p*	Antioxidant *In vitro*	CHUN-HUI et al., 2007
Aloe barbadensis Miller	Leaves	1.5×10^4	22:1	→4)-*β*-Man*p*-(1→4)-*β*-Glc*p*-(1→	O-6 Man*p*	-	-	-	MANDAL & DAS, 1980
Aloe mucilaginous		-	-	→4)-*β*-Man*p*-(1→4)-*β*-Glc*p*-(1→	O-6 Man*p*	-	-	-	SIMÕES et al., 2012
Aloe Vera L.	Leaf gel	1.1×10^6	15:1	→4)-*β*-Man*p*-(1→4)-*β*-Glc*p*-(1→	O-2 Man*p* O-3 Man*p* O-6 Man*p*	-	-	-	CHOW et al., 2005
Amorphophallus konjac	Tuber	1.12×10^6	1.6:1	→4)-*β*-D-Man*p*-(1→4)-*β*-D-Glc*p*-(1→	O-3 Man*p* O-3 Glc*p*	-	-	-	MAEDA et al., 1980
Amorphophallus konjac	Tuber	5.83×10^5 g/mol	1.6:1	-	O-3 Man*p* O-3 Glc*p*	-	-	-	CESCUTTI et al., 2002
Amorphophallus konjac	Flour	-	1.6:1	→4)-*β*-D-Man*p*-(1→4)-*β*-D-Glc*p*-(1→	O-6 Glc*p*	-	-	-	KATSURAYA et al., 2003

Amorphophallus oncophyllus	Porang flour	-	-	-	-	-	-	-	WARDHANI et al., 2019
Artemisia sphaerocephala Krasch	Seeds	38.7 kDa	1:1.3	→4)-*β*-Man*p*-(1→4)-*β*-Glc*p*-(1→	O-6 Glc*p*	-	-	-	GUO et al., 2012
Bletilla striata	Tubers	2.35×10^5 Da	2:1	→4)-Man*p*-(1→4)-Glc*p*-(1→	O-6 Glc*p*	2.9%	O-3 Man*p* O-6 Man*p*	Immunomoduladotory *In vitro*	PENG et al., 2014
Bletilla striata	Tubers	20 kDa	3.5:1	→4)-*β*-D-Man*p*-(1→4)-*β*-D-Glc*p*-(1→	-	-	-	-	ZHANG et al., 2014
Bletilla striata	Powder	260 kDa	3:1	→2)- α-D-Man*p*-(1→4)-*β*-D-Glc*p*-(1→	-	-	-	Anti-fibrosis *In vitro*	WANG et al., 2014
Bryonia lacinosa	Seeds	-	1:1	→4)-*β*-Man*p*-(1→	O-6 Man*p*	-	-	-	SINGH & MALVIYA, 2006
Curculigo orchioides	Rhizome	4.61 kDa	-	→4)-*β*-D-Man*p*-(1→4)-*β*-D-Glc*p*-(1, 3→6)-*β*-D-Man*p*	O-3 Man*p*	-	O-3 Man*p*	Antiosteoporosis *In vitro*	WANG et al., 2017
Cyrtopodium andersonii	Pseudobulbs	5.35×10^5	3:1	→4)-*β*-Man*p*-(1→4)-*β*-Glc*p*-(1→	O-2 Man*p* O-3 Man*p*	14.6%	O-2 Man*p*	Anti-inflamatory Gastroprotective	PARENTE et al., 2014

					O-6 Man*p*			*In vivo*	
Cyrtopodium cardiochilum	Pseudobulbs	4.6×10^5	2:1	→4)-*β*-D-Man*p*-(1→4)- *β*-D-Glc*p*-(1→	-	4.2%	O-2 Man*p* O-2 Glc*p*	Immunomodulatory Anti-inflamatory *In vivo*	BARRETO & PARENTE, 2006
Dendrobium devonianum	Powder	3.99×10^5 Da	29.6:1	→4)-*β*-D-Man*p*-(1→4)- *β*-D-Glc*p*-(1→	-	18.8%	O-2 Man*p* O-3 Man*p*	Immunomodulatory *In vitro*	DENG et al., 2018
Dendrobium huoshanense	Leaf, stem cell walls and mucilage	9.7 kDa	3.8:1	→4)-*β*-D-Man*p*-(1→4)- *β*-D-Glc*p*-(1→	-	25%	O-2 Man*p* O-3 Man*p*	Immunomodulatory *In vitro*	HSIEH et al., 2008
Dendrobium nobile	Smashed stems	67.5 kDa	3:1	→4)-*β*-D-Man*p*-(1→4)- *β*-D-Glc*p*-(1→	-	1.6%	O-2 Man*p* O-3 Man*p*	-	ZHANG et al., 2020
Dendrobium officinale	Stem	168 kDa	6.9:1	→4)-*β*-D-Man*p*-(1→4)- *β*-D-Glc*p*-(1→	-	-	O-2,3 Man*p*	-	XING et al., 2014
Dendrobium officinale	Stem	394 kDa 362 kDa	5.8:1 4.5:1	→4)-*β*-D-Man*p*-(1→4)- *β*-D-Glc*p*-(1→	O-2 Man*p* O-3 Man*p* O-6 Man*p* O-3 Glc*p*	-	-	Immunomodulatory Antioxidant *In vitro*	HUANG et al., 2016

Dendrobium officinale	Stem	1.3×10^5	40.2:8.4	→4)-*β*-D-Man*p*-(1→4)- *β*-D-Glc*p*-(1→	O-6 Glc*p* O-6 Man*p* O-6 Glc*p*	-	O-2 Man*p* O-2 Glc*p*	-	HUA et al., 2004
Dendrobium officinale	Stem	8500 Da	6.2:2.3	→4)-*β*-D-Man*p*-(1→4)- *β*-D-Glc*p*-(1→	O-2 Man*p*	-	-	Antioxidant *In vitro*	LUO et al., 2016
Hippeastrum hybridum	Bulb	331000	5:2	→4)-*β*-D-Man*p*-(1→4)- *β*-D-Glc*p*-(1→	O-3 Glc*p*	13.2%	O-2 Man*p* O-6 Man*p*	-	TOMODA et al., 1985
Lilium longiflorum	Bulb	263000	5:2	→4)-*β*-D-Man*p*-(1→4)- *β*-D-Glc*p*-(1→	O-2 Man*p* O-3 Man*p*	3.2%	O-6 Man*p* O-2,6 Man*p* O-3,6 Man*p* O-2,3,6 Man*p* O-6 Glc*p* O-2,3,6 Glc*p*	-	TOMODA et al., 1978
Lilium maximowiczii	Bulb	-	2:1	→4)-*β*-D-Man*p*-(1→4)- *β*-D-Glc*p*-(1→	O-3 Man*p*	-	-	-	KATO et al., 1976
Lycoris squamigera	Bulb	1800000	7:2	→4)-*β*-D-Man*p*-(1→4)- *β*-D-Glc*p*-(1→	O-3 Man*p*	16.7%	O-2,6 Man*p*	-	TOMODA et al., 1983

Narcissus poeticus	Bulb	32000	30:1	→4)-*β*-D-Man*p*-(1→4)-*β*-D-Glc*p*-(1→	-	8.6%	-	-	ZHAUYNBAEVA et al., 2003
Narcissus tazetta	Bulb	31000	5.6:1	→4)-*β*-D-Man*p*-(1→4)-*β*-D-Glc*p*-(1→	-	12.9%	-	-	ZHAUYNBAEVA, MALIKOVA & RAKHIMOV, 2003
Narcissus tazetta var chinesis	Bulb	119000	5:1	→4)-*β*-D-Man*p*-(1→4)-*β*-D-Glc*p*-(1→	O-3 Man*p*	22.7%	O-6 Man*p* O-2,6 Man*p*	-	TOMODA et al., 1980
Phoenix dactylifera	Seeds	1×10^6	13.2:1	→4)-*β*-Man*p*-(1→4)-*β*-Glc*p*-(1→	-	22%	O-6 Man*p* O-2,3 Man*p*	-	ISHRUD et al., 2001
Salvia officinals	Aerial parts	-	1:1.3	→4)-*β*-D-Man*p*-(1→4)-*β*-D-Glc*p*-(1→	O-6 Man*p* O-6 Glc*p*	-	-	-	CAPEK, 2009

DA = Degree of acetylation

3. Health effects of glucomannans

3.1 Gut microbiota modulation and prebiotic action

The intestinal microbiota refers to the population of microorganisms that inhabit the gastrointestinal tract, which plays a fundamental role in the organism's homeostasis. Depending on the composition of this ecosystem, it may result in health or disease for the individual. Many pathologies are directly or indirectly related to changes in intestinal homeostasis, where factors such as diet, smoking, pollution, physical inactivity, among others, can affect this balance favoring the proliferation of pathogenic microorganisms and leading to intestinal dysbiosis (GOMES & DA COSTA, 2020).

The effects on intestinal microbiota of glucomannans from two different sources were evaluated by HARMAYANI, APRILIA & MARSONO (2014). They elaborated a study involving 32 Wistar rats divided into 4 groups based on food, being standard AIN-93 (diet formulated for the maintenance of adult rodents), glucomannan obtained from Porang (PGM), from konjac (KGM) and diet with inulin as a source of fiber. The intervention took place during 14 days in which the bacterial population and the chemical properties of the intestinal microbiota were subsequently analyzed. The results showed that the groups PGM and KGM inhibited the growth of *Escherichia coli*, where PGM also improved the production of short chain fatty acids, indicating these fibers as functional food options for intestinal modulation. Another study also pointed out the action of KGM oligosaccharides obtained by enzymatic action of β-mannanase on the gut microbiota in fecal batch culture (ARIESTANTI et al., 2019). The results showed that the oligo-glucomannan was selectively fermented by beneficial bacteria, in addition to increasing the production of butyric acid during fecal fermentation.

An animal model with C57BL/6J mice fed a high-lipid diet for 16 weeks was used to compared the effect of adding fibers to the diet on fatty acid metabolism and intestinal microbiota (ZHAI et al., 2018). The authors showed that the combination of 4% KGM and 4% bacterial cellulose significantly reduced the abundance of Firmicutes and *Musci spirillum*. Besides, a great increase in Bacteroidetes and *Akkermansia* in the mice intestines were observed, concluding that the proliferation of these species was beneficial to the maintenance and integrity of the colon by the production of short chain fatty acids. Another similar study distributed 30 mice in control group, high-fat diet and high-fat diet treated with KGM for 12 weeks and evaluated parameters related to body composition and intestinal microbiota. According to the study, fiber supplementation increases the diversity and abundance of gut beneficial microorganisms such as *Megasphaera elsdenii*, while reducing harmful microorganisms such as *Alistipes*, *Alloprevotella*, *Bacteroides acidifaciens* and *Parabacteroides goldsteinii* (KANG et al., 2019).

3.2 Reduction of constipation

Constipation is a common disorder in children and adolescents, where more than 90% of cases are classified as functional gastrointestinal disorders. The clinical manifestation consists in the difficulty of evacuation, which may result in incomplete evacuation, with low frequency and fecal petrification, causing several damages to the organism, where, according to the diagnostic criterion, it can be considered as a public health problem (GOMES & MORAIS, 2020).

In a review published by HAN et al., 2017 on the effectiveness of KGM in constipation, the findings revealed that KGM can increase the frequency of defecation in addition to decreasing stool hardness in children. Moreover, BEHERA & RAY, 2016 reviewed the effectiveness of KGM as a treatment for constipation in pregnant women by a combination of 1.45 g of KGM and 4.2 g of lactose, twice daily for 3 months. The treatment recovered the normal number of bowel movements alleviating constipation.

3.3 Gastroprotective effect

Peptic ulcer refers to an injury that affects the mucosa or muscle layer of the gastric or duodenal region, thus forming a cavity with an acute or chronic inflammatory response, resulting in symptoms such as abdominal pain, burning sensation, heartburn and indigestion (MORA, 2014).

The gastroprotective effect of a glucomannan extracted from *Cyrtopodium andersonii* was evaluated by PARENTE et al., 2014 in an ethanol-induced gastric ulcer model in male Swiss mice. The animals were euthanized after one hour of the ulcerogenic experiment to remove the stomachs and analyze the lesion of gastric mucosa. This fiber at 100 mg/kg induced an expressive reduction of gastric hyperemia and of the number and severity of the gastric lesions when compared with the control (water).

3.4 Liver health

The liver is the largest gland in the human body, which acts both exocrine through excretions in external channels, and endocrine due to its secretions from the bloodstream. Among its functions are the synthesis of proteins, cholesterol and bile, storage of iron and vitamins, hormonal degradation, inactivation and excretion of drugs and toxins, thus acting as a regulation center for substances in the body (DE JESUS, DE SOUSA & BARCELOS, 2014).

Some pharmacological and nutritional interactions may have side effects on the liver, such as liver damage caused by drugs, a condition that elevates blood biomarkers such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as well as liver steatosis, characterized by accumulation of fat in the liver, which makes it susceptible to ischemia injury and consequently generates liver dysfunction (DE JESUS, DE SOUSA & BARCELOS, 2014).

In an animal model with a high-lipid diet for 7 weeks, markers of cell damage in liver structures were evaluated. The animals were treated orally with AIN-93M and squid surimi, corresponding to 70% and 30% respectively, divided into 6 groups, being control, enriched with GM, enriched with GM and spirulina, and the other groups with similar diet plus 2% cholesterol and 0.4% cholic acid. Both diets enriched with GM, and with GM plus spirulina significantly reduced plasma ALT and AST, hepatic steatosis, lipogranulomas and total inflammation in contrast to control groups. The authors argued that the dietary pattern (cholesterol) aggravated the harmful effects in control diets, such as liver weight, hepatic-somatic index and damage markers and histological score, markers that were improved with the addition of GM, with no additional effect with spirulina addition (VÁZQUEZ -VELASCO et al., 2017).

3.5 Cholesterol reduction

Cholesterol plays a fundamental role for the proper functioning of human metabolism, which is synthesized in the liver and transported through plasma lipoproteins. On the other hand, high blood cholesterol levels resulting from genetic factors as well as eating habits can lead to an increase in plasma lipoproteins, in the case of LDL becoming a predictor of cardiovascular diseases, which are among the biggest causes of death in the world (WILLEIT et al., 2020).

In a systematic review and meta-analysis carried out by HO et al., 2017, the authors suggested that, although the relationship between fiber consumption and cholesterol reduction is still not well understood, studies suggested that this reduction are related to viscosity and structure/type of the dietary fiber. It is estimated that the average consumption of 5 to 10 g of soluble dietary fiber is necessary to reduce 5% of LDL cholesterol, while a lower dose of KGM (3 g) could reduce LDL more effectively (10%) (HO et al., 2017). The viscosity of KGM was pointed out by the authors as the key parameter for the cholesterol reduction. This effect was superior to other soluble dietary fibers such as beta-glucans, fibers from oats, barley, psyllium and wheat bran (HO et al., 2017).

A randomized, double-blind, placebo-controlled study evaluated 96 children aged 6 to 17 years with overweight and obesity with daily intake of 3 g of KGM or placebo (maltodextrin) during the 12-week period. The results showed that the group that consumed KGM had lower concentrations of total cholesterol and LDL in the blood (ZALEWSKI & SZAJEWSKA, 2019).

In a systematic review and meta-analysis involving 14 human clinical trials, the authors investigated the impact of KGM on total, LDL and HDL cholesterol, concluding that this fiber can beneficially affect these markers, since the results demonstrated a significant reduction in total cholesterol and LDL, not being observed for HDL (SOOD, BAKER & COLEMAN, 2008).

3.6 Glycemic control

Chronic hyperglycemia is a primary factor for the development of several pathologies, since the lack of glycemic control results in metabolic and physiological complications in the individual, thus evolving to more severe conditions such as obesity, dyslipidemias, cardiovascular diseases and type 2 diabetes mellitus (FERREIRA, 2011).

DEVARAJ et al., 2019 comments that KGM supplementation reduces blood glucose levels in healthy, diabetic and hypercholesterolemic patients, by delaying gastric emptying and regulating the rate of absorption of nutrients, thus improving insulin sensitivity. A proposed mechanism is the characteristic of KGM absorbing a large amount of liquids, transforming it into mucilage and increasing its volume, where in the middle of digestion it merges with the food cake making a layer of the cake, thus decreasing sugar absorption (DEVARAJ et al., 2019).

An *in vivo* trial with male Wistar rats observed that KGM ingestion exerts a positive effect on the maintenance of postprandial glucose homeostasis, further highlighting a greater efficacy by the consumption of KGM-supplemented wheat noodles compared to the exclusively supplemented form of fiber (ZHOU et al., 2019).

3.7 Antitumor effects

Tumor, cancer, or neoplasm are terms used to refer to a disease characterized by the uncontrolled proliferation of abnormal cells that cause damage to the body. Cancer reflects a major public health problem across the globe, being among the biggest killers in the world (SIEGEL et al., 2021).

The effects of KGM with different degrees of acetylation was tested against sarcoma S180 and melanoma B16-F10 cells. The results demonstrated that KGM with acetylation degree of 1.8 (AcKGM-1.8) changed the phenotype of tumor macrophages, where both the degree of acetylation and the structure of the polysaccharide were crucial factors for the antitumor activity. The *in vivo* intratumor administration of AcKGM-1.8 in mice effectively suppressed both tumors as well as increased the animals' survival rate. In addition, the safety of intraperitoneal administration of AcKGM-1.8 was validated, demonstrating high safety when applied at 20 mg/kg. The authors concluded that the proposed mechanisms occur by signaling type 2 Toll Like receptors (TLR2), triggering macrophage-mediated antitumor response (FENG et al., 2019).

Another study used an alendronate conjugate and glucomannan from *Bletilla striata*, a polysaccharide with binding affinity to the macrophage mannose receptor. The conjugate eliminated tumor-associated macrophages both *in vitro* (Raw 264.7 cells) and *in vivo*, markedly suppressing angiogenesis, recovered the immune response against cancer and inhibited the progression of the S180 tumor induced intraperitoneally in mice. This polysaccharide demonstrated several advantages as a vehicle for drug administration, as it is a branched polysaccharide with repeated units of mannose in its main chain, favoring its binding to macrophage mannose receptor due to the high affinity for cell targeting, in addition to the fact that the conjugate with this polysaccharide maintained its tumor-associated macrophage inhibitory activity even with pH variation (8.0 to 6.5), which did not occur with the exclusive administration of alendronate (ZHAN et al., 2014).

The selenium modified KGM oligosaccharides was prepared by the sodium selenite-nitric acid method in different concentrations, demonstrating antitumor activity in cell lines A549, HCC1937 and MHCC97H possibly through by inducing apoptosis in the mitochondrial pathway (LI et al., 2020).

3.8 Antioxidant activity

Oxidative stress is a biological condition that can be defined by the imbalance between oxidants, such as reactive oxygen and nitrogen species, and antioxidants, thus resulting in the disruption of redox signaling and consequent molecular damage, being related to the development of various pathologies (SIES, BERNDT e JONES, 2017).

An *in vitro* study published by HUANG et al., 2016 observed a cytoprotective effect of glucomannan extracted from *D. officinalle* on RAW 264.7 cells. The results showed that pretreatment with this fiber increased the cell viability of macrophages treated with hydrogen peroxide, decreasing significantly apoptosis and preserving cell morphology and structure.

Another *in vitro* study evaluated the antioxidant potential of the same fiber. The metal chelating activity of different concentrations of polysaccharide (0.05 to 0.8 mg/mL) was evaluated. The results demonstrated dose-dependent activity, whose chelation ranged from 6.3% to 85.2% from the lowest to the highest polysaccharide concentration. The authors also observed the high action of fiber in scavenging 1,1-diphenyl-2-picrylhydrazyl and hydroxyl free radicals (LUO et al., 2016).

3.9 Anti-inflammatory effect

The inflammatory process is characterized by the involvement of immune cells such as macrophages, neutrophils, eosinophils, mast cells, dendritic cells and T and B lymphocytes. However, local inflammation does not only include cell activity, but also some cytokines with a pro-inflammatory profile, among them tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), C-reactive protein (CRP), interleukins: 1 beta (IL-1β), IL-6 and IL-8. Subclinical chronic inflammation is associated with several comorbidities such as obesity, systemic arterial hypertension, and cancer (DE OLIVEIRA et al., 2020).

An animal study involving male Swiss mice evaluated the anti-inflammatory potential of glucomannan from *Cyrtopodium andersonii* in paw edema induced by subplantar injection of carrageenan. The fiber (100 mg/kg) demonstrated anti-inflammatory action in the initial stage of inflammation, which is related to the release of histamine, serotonin, and other similar substances, in addition to causing an inhibition in paw edema similar to the positive control indomethacin (25 mg/kg) and dexamethasone

(25 mg/kg). However, the polysaccharide exerted less activity in the delayed phase of inflammation which is related to the action of prostaglandins (PARENTE et al., 2014).

Another study evaluated the anti-inflammatory potential of glucomannan extracted from *Cyrtopodium cardiochilum* on acute inflammation induced by intraperitoneal administration of acetic acid (10 mL/kg) after intravenous injection of Evans' solution (10 mL/kg) in male BALB/c albino mice. The polysaccharide inhibited the increase in vascular permeability induced by acetic acid, a typical condition of an early inflammatory response (BARRETO & PARENTE, 2006).

3.10 Modulation of immune system

The immune system represents a biological defense mechanism against aggressive agents and metabolic disruptions, where amidst its functional complexity it can be modulated to promote the fight against infections, neoplasms and other pathologies (DE ABREU, 2018).

The protective effects against cyclophosphamide-induced immunosuppression of three glucomannans (KGM and those extracted from *Aloe vera* and *Dendrobium officinalle*) were investigated by LI-ZHI et al., 2020. The authors concluded that the structural properties of polysaccharides may be relevant to their bioactivity, and that different sources of glucomannans act with immunostimulatory activity in different ways. The fiber from *D. officinalle* demonstrated significant capacity in the counting increase of CD4 and CD8 T lymphocytes in spleen, as well as in the increase of XBP-1 mRNA expression related to the secretion of IgM and IgG in serum. In the case of KGM, it significantly increased the secretions of TNF-α, IFN- γ and IL-4 by the spleen to stimulate the immune system, while *Aloe vera* fiber showed good ability to improve the expression of the T-bet mRNA in the Th1 type stimulus in T lymphocytes (LI-ZHI et al., 2020).

Another study also evaluated the influence of glucomannan from *Dendobrium officinalle*, namely 2,3-O-acetyl-1,4-β-D-glucomannan (DOP-1-1) on monocytic cell line from human leukemia (THP-1) cells. It promoted the immune response by activating NF-κB via degradation of IκB proteins by sensitizing TLR4 receptor (HUANG et al., 2018). The authors also verified increased expression of CCL4 and IP10, which are chemotactic cytokines that regulate the migration of immune cells, such as monocytes, macrophages, neutrophils and T cells. Besides, glucomannan from *D. officinalle* also had stimulatory effects on splenocytes, T-lymphocytes and B-lymphocytes, promoting the cell viability and NO production of RAW 264.7 macrophages (HUANG et al., 2016). Another glucomannan, extracted from *D. devonianum*, also demonstrated immunomodulatory effects on RAW 264.7 macrophages, due to enhanced phagocytic activity and nitric oxide release (DENG et al., 2018)

CHEN and cols. (2018) evaluated the application of KGM nanoparticles as vaccine adjuvants to promote the immune response induced by ovoalbumin in animals. As in other studies, KGM did not show cellular toxicity *in vitro* (Marc-145 cells), even those loaded with ovoalbumin. The results indicated that the proliferation of splenocytes, IgG and cytokine levels induced by the humoral immune response of ovoalbumin were positively regulated by the KGM nanoparticles, as well inducing greater production of IL-2, IL-4, IL- 10 and IFN- γ, thus suggesting a promising vaccine adjuvant.

Other scientific findings discuss the increase in the phagocytosis activity and IL-6 and TNF-α gene expression by macrophage-like J774.1 cells and mouse peritoneal macrophages induced by glucomannan from *Amorphophallus oncophyllus* esterified with octenylsuccinic anhydride. This action was related to the NF-κB and MAPK activation cascade and not by endotoxin contamination, thus classifying it as a potential immunomodulatory food (GURUSMATIKA et al., 2017).

The phagocytosis stimulating property of the glucomannan extracted from *Cyrtopodium cardiochilum* was evaluated *in vivo* in male BALB/c albino mice (BARRETO & PARENTE, 2006). The authors administered intraperitoneally 50 mg/kg of glucomannan or zimosan as a positive control, once a day for five days. After this period, colloidal carbon (10 mL/kg) was injected into the vein of the animals for subsequent collection of blood from the orbital vein and assessment of carbon clearance. The phagocytic index of the group administered with polysaccharide increased significantly, thus suggesting that it has immunomodulatory properties.

Cultured spleen cells from BALB/c mice were stimulated for a period of 72 h by glucomannan extracted from *Bletilla striata*, in concentrations of 10, 50 and 100 µg/mL, as well as 5 µg/mL lipopolysaccharides (LPS - Escherichia Coli 055:B5 Sigma-Aldrich) as positive control (PENG et al., 2014). The results showed that the polysaccharide induced the proliferation of spleen cells in a dose dependent manner, whose action was slightly lower when compared to the positive control. The effects of glucomannan from *Dendobrium huoshanense* on murine splenocytes was also evaluated by HISEH et al., 2008. The cells were stimulated with the polysaccharide in different concentrations (2; 10; 50; 100 µg/mL). The results demonstrated an effect on the growth of splenocytes in a dose-dependent manner, in addition to increased expression of cytokines IFN-γ, IL-10, IL-6 and IL-1α and hematopoietic growth factors. The authors also observed the importance of the acetyl groups for the bioactivity, since the deacetylated glucomannan was not able to induce cytokine production.

3.11 Anti-osteoporosis

Osteoporosis is a disease developed from a skeletal disorder characterized by compromised bone strength both by reduction in mass and by altered architecture, thus resulting in greater bone fragility and risk of fracture (AKKAWI & ZMERLY, 2018).

The osteoprotective effect of glucomannan extracted from *Curculigo orchioides* was evaluated. In their experimental model, the authors cultivated osteoblasts removed from the periosteum of Kunming mice (WANG et al., 2017). The polysaccharide stimulated the proliferation and differentiation of primary osteoblasts at different concentrations (C1: 2.2 µM; C2: 10.8 µM; C3: 21.7 µM; C4: 54.2 µM). While C2 presented the highest proliferation rate, C3 had the highest differentiation rate, even higher than the positive control.

3.12 Antifibrosis activity

Fibrosis is characterized by the deposition of connective tissue in reparative character to tissue damage, however this process can occur in an uncompensated way and affect the functionality of the repaired organ, causing from discomfort to severe complications to the affected individual (BELLINI & MATTOLI, 2007).

In an *in vitro* model with human mesangial cells, different concentrations of glucomannans from *Bletilla striata* were tested. It was observed that the dose of 20 µg/mL can significantly reduce the proliferation of human mesangial cells, negatively regulating the expression of TGF-β RI, TGF-β RII and SMA-α practically at the normal level after overexpression, demonstrating anti-fibrosis activity of this polysaccharide (WANG, et al., 2014).

4. Concluding remarks

It is possible to observe that the vast majority of published studies of structural characterization and/or biological activity of glucomannans are based on the polysaccharide extracted from *Amorphaphyllus konjac*. However, in this review we highlight the great diversity of this fiber sources, which can be found in different botanical families and portions of the plant of the same species, bringing together specific chemical characteristics that may match certain biological activities.

The functional properties of glucomannans prove to be a very promising field for several therapies for the maintenance of health, encouraging further studies with glucomannans from different sources and fine chemical structures.

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ANEXO III

RESULTADOS ADICIONAIS

1. Introdução

Em meio a grande diversidade de estruturas químicas dos polissacarídeos dispostos na natureza, os experimentos bioquímicos de purificação desses polímeros muitas vezes são baseados em sucessivas tentativas fundamentadas, mas hipotéticas em busca do sucesso experimental.

A escolha das estratégias experimentais é baseada em diversos fatores, tais como discussão relatada pela literatura científica, objetivos propostos e recursos disponíveis. Dentre alguns princípios que envolvem tais decisões, merece atenção o conceito da preservação estrutural do polímero, de modo que a purificação busque um resultado mais próximo a estrutura natural do composto, contribuindo assim para uma maior fidelidade a sua caracterização bem como a resultados futuros, como por exemplo atividades biológicas.

A química de carboidratos atua em determinadas situações pela tentativa e erro, metodologia de resolução de problemas em que várias tentativas são exercidas para se obter uma solução. Diante do exposto, vários outros experimentos foram realizados na tentativa da purificação dos polissacarídeos presentes na polpa da bocaiuva (*Acrocomia corumbaensis*), cuja sequência metodológica pode ser observada no esquema ilustrado na Figura 1.

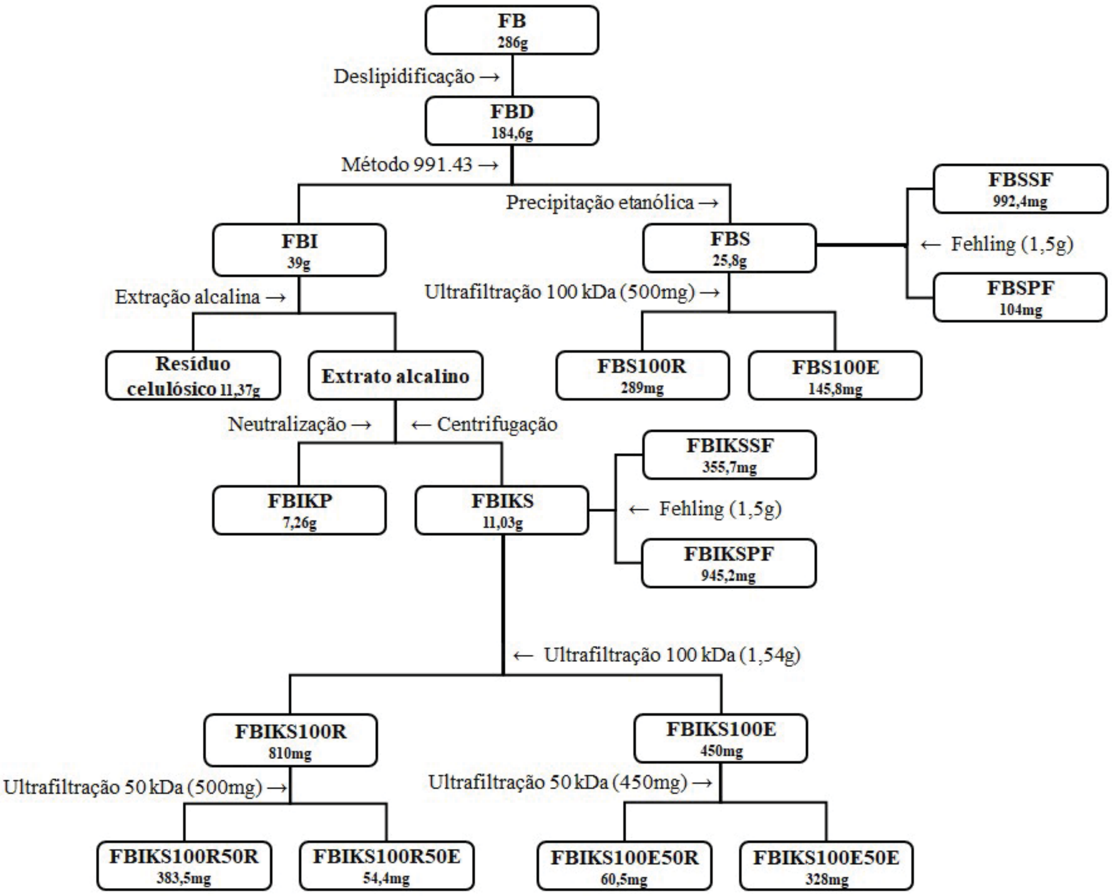

Figura 1: Esquema de purificação de fibras dietéticas extraídas da farinha de bocaiuva (*Acrocomia corumbaensis*).

2. Fibras dietéticas insolúveis da bocaiuva (*Acrocomia corumbaensis)*

Seguido do processo de deslipidificação da farinha de bocaiuva, o método enzimático gravimétrico (AOAC 991.43) permitiu a obtenção de fibras dietéticas solúveis (FBS), discutidas no anexo I, bem como das fibras dietéticas insolúveis, cuja amostra foi denominada FBI. Para a solubilização das hemiceluloses presentes na fração FBI, esta foi submetida a extração alcalina (NaOH 10%) sob refluxo, seguido de filtração, originando o extrato alcalino e o resíduo celulósico a partir do material retido. Os extratos alcalinos foram neutralizados com ácido acético, ocorrendo a formação de precipitado quando o pH foi diminuído. Desta maneira, os extratos foram centrifugados (3500 rpm / 20 min a

4 ºC), resultando assim em duas novas amostras: FBIKS (sobrenadante) e FBIKP (precipitado), ambas concentradas em rotaevaporador, dialisadas e liofilizadas.

A determinação da composição de monossacarídeos neutros foi determinada pela hidrólise ácida total com TFA 2M por 8 horas a 100 °C, seguido da conversão para alditóis acetato a partir da redução com $NaBH_4$ (WOLFROM e THOMPSON, 1963b) e acetilação com uma mistura de anidrido acético (Ac_2O) e piridina (1:1, v/v) a 100 °C por 30 minutos (WOLFROM e THOMPSON, 1963a). Os alditóis acetatos produzidos a partir da FBIKS e FBIKP foram analisados por cromatografia gasosa com espectrômetro de massa (GC-MS).

A composição monossacarídica de FBIKP mostrou a presença de arabinose (34,6%), xilose (23,4%), manose (22,1%) e glucose (19,9%). A dosagem de ácidos urônicos foi realizada pelo método de FILISETTI-COZZI & CARPITA, 1991, obtendo-se um valor de 0,33% (Tabela 1). O conteúdo de açúcar total (determinado pelo método de DUBOIS et al. 1956) e de proteínas (determinado pelo método de BRADFORD, 1976) presente nessa fração foi de 61,5% e 4,3% respectivamente.

Tabela 1. Composição monossacarídica de frações obtidas da bocaiuva (*Acrocomia corumbaensis*).

Fração	**Açúcares neutros** [a]						**Ácidos urônicos** [b]
	Glc	**Man**	**Gal**	**Ara**	**Xyl**	**Fuc**	
FBIKP	19,9	22,1	-	34,6	23,4	-	0,33
FBIKS	6,0	0,4	32,5	32,6	25,5	0,7	nd[c]

[a] % da área de pico em relação a área total de pico, determinado por GC–MS.

[b] Determinado pelo método de FILISETTI-COZZI & CARPITA, 1991.

[c] Não detectado

Os espectros de Ressonância Magnética Nuclear (RMN) – ^{13}C, DEPT- 135 e os bidimensionais HSQC foram obtidos em espectrômetro Bruker Avance-DRX-400 MHz ou Bruker Avance-III 600 MHz, do Departamento de Bioquímica e Biologia Molecular da Universidade Federal do Paraná. As amostras foram analisadas em uma sonda invertida de 5 mm de diâmetro, às temperaturas de 70 °C, utilizando como solventes D_2O ou Me_2SO-d_6, de acordo com a solubilidade dos polissacarídeos. Os deslocamentos químicos foram expressos em ppm, e foram utilizados como padrão interno os sinais de acetona (δ 30,2 ppm) para FBIKS e Me_2SO-d_6 (δ 39,7 ppm) para FBIKP.

A fração FBIKP reúne como característica a extrema viscosidade, o que motivou a utilização de Me_2SO-d_6 como solvente para análise junto ao RMN, contudo não houve espectro observável. Na tentativa de se obter uma melhor leitura de espectro ^{13}C-RMN de FBIKP, procedeu-se um clareamento da amostra pela adição de NaClO (MONOBE et al., 2008), contudo a técnica relatada como *bleaching* também não apresentou resultados e dessa forma, não foi posteriormente analisada.

Em avaliação da composição monossacarídica, a fração FBIKS apresentou percentual expressivo de arabinose (32,6%), galactose (32,5%) e xilose (25,5%), com menores quantidades de glucose (6%), ramnose (2,3%), fucose (0,7%) e manose (0,5%), além de ser identificado a ausência de ácidos urônicos na amostra (Tabela 1). As dosagens de açúcar total e proteína para essa fração foram de 55,5% e 3,3% respectivamente.

Diferente da fração citada anteriormente, para a fração FBIKS, o espectro de ^{13}C-RMN (Figura 2A) sugere a presença de dois polissacarídeos, sendo uma arabinogalactana e uma xilana, em virtude da observação de sinais característicos em δ 107,3 ppm atribuído às unidades de α-L-Ara*f* (DELGOBO *et al.*, 1999; CORDEIRO, 2012), do sinal em δ 104,1 ppm relativo às unidades de β-D-Gal*p* (TISCHER; GORIN e IACOMINI, 2002;

CIPRIANI, et al. 2009) bem como do sinal em δ 101,4 ppm atribuídos às unidades de β-D-Xyl*p*.

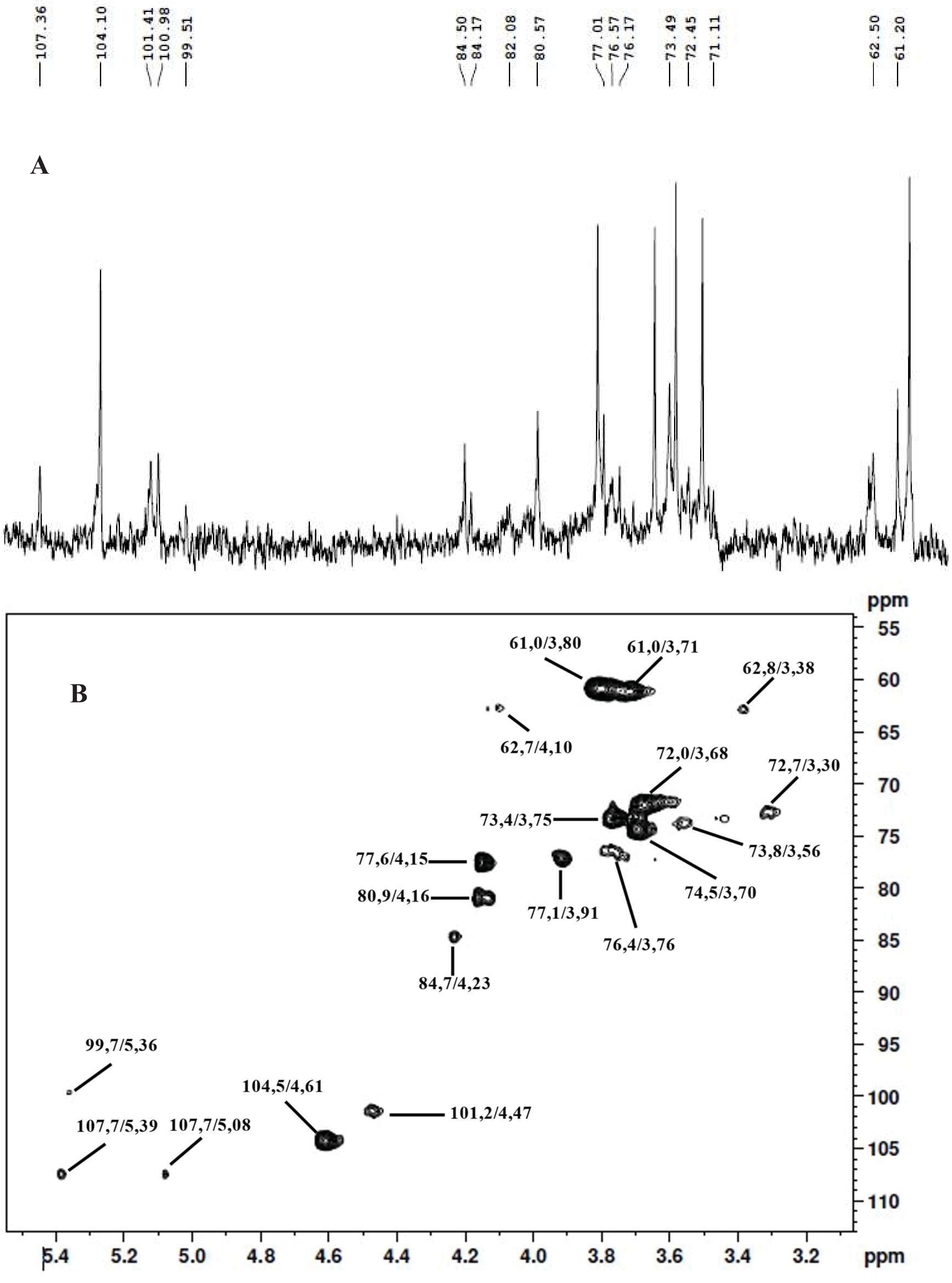

Figura 2: Análise de RMN da fração FBIKS. (A) espectro de ^{13}C-RMN; (B) mapa de correlação ^{1}H-^{13}C obtido pelo experimento HSQC. Amostra dissolvida em D_2O e analisada a temperatura de 70 ºC.

A presença desses polissacarídeos vai de encontro com os sinais observados pelo espectro de HSQC da mesma fração (Figura 2B), sendo δ 107,7/5,08-5,39 (C1/H1) para unidades de α-L-Ara*f*, δ 104,5/4,61 (C1/H1) para unidades de β-D-Gal*p* e δ 101,2/4,47 (C1/H1) para unidades de β-D-Xyl*p*. Além dos assinalamentos dos carbonos/hidrogênios anoméricos, os demais sinais, quando comparados com a literatura, reforçam a possível presença de uma arabinogalactana do tipo I (AG-I), devido a presença dos sinais em δ 72,0/3,68 (C-2/H-2), δ 73,4/3,75 (C-3/H-3), δ 77,6/4,15 (C-4/H-4 O-substituído), δ 74,5/3,70 (C-5/H-5) e δ 61,0/3,80- 61,0/3,71 (C-6/H-6) que podem ser atribuídos a unidades de β-D-Gal*p* (1→4)-ligadas da cadeia principal da AG-I. O sinal em δ 99,7/5,36 e o sinal em 16,9/1,30 podem ser atribuídos ao C-1/H-1 e grupos CH_3, respectivamente, das unidades de α-L-Rha*p* da ramnogalacturonana do tipo I onde provavelmente a AG-I está ancorada (DO NASCIMENTO, IACOMINI & CORDEIRO, 2017). Além disso, os sinais da xilana podem ser vistos em δ 72,7/3,30 (C-2), δ 73,8/3,56 (C-3), δ 76,4/3,76 (C-4) e δ 62,7/4,10-62,8/3,38 (C5), atribuídos as unidades de β-D-Xyl*p* (1→4)-ligadas da cadeia principal (DO ESPÍRITO SANTO et al., 2020, SCHNEIDER, IACOMINI & CORDEIRO, 2019). Devido a sua solubilidade em água e ausência de ácidos urônicos (Tabela 1), sugere-se a presença de uma arabinoxilana, uma vez que β-D-xilanas lineares (1→4)-ligadas são insolúveis nesse solvente.

Os ensaios de homogeneidade e determinação de massa molar foram realizados em HPSEC, através do aparelho da Wyatt Technology, equipado com um cromatógrafo de exclusão estérica de alta performance, com quatro colunas *ultrahydrogel* 2000, 500, 250 e 120 acopladas em série (com limites de exclusão de 7×10^6, 4×10^5, 8×10^4 e 5×10^3 Da, respectivamente), um detector de índice de refração, modelo Waters 2410, e um detector de espalhamento de *laser* multiângulo (MALLS) a 632,8 nm, modelo Dawn DSP, que promove a leitura do espalhamento de luz, que é captado em diferentes intensidades por detectores em diferentes ângulos. Como eluente, foi utilizada uma solução de $NaNO_2$ 0,1 mol/L contendo NaN_3 0,2 g/L, com fluxo controlado de 0,6 mL/min. As amostras foram solubilizadas em $NaNO_2$ 0,1M para uma concentração final de 1 mg/mL e filtradas em membranas de éster de celulose (Millipore) de porosidade 0,22 µm. Os resultados de homogeneidade e de determinação de massa molar foram analisados com o *software* ASTRA 4.70.07.

O perfil de eluição da fração FBIKS na análise por HPSEC demonstrou a presença de 2 picos principais, indicando a presença de polissacarídeos com massas molares diferentes conforme exposto na Figura 3, corroborando assim com as sugestões relatadas anteriormente.

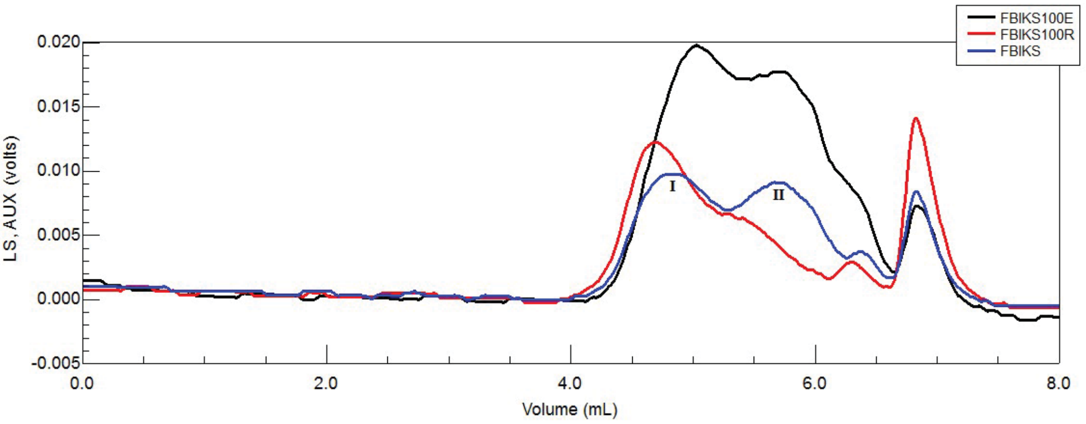

Figura 3: Perfil de eluição das frações FBIKS, FBIKS100R e FBIKS100E quando analisadas por cromatografia de gel permeação.

Com base no exposto, pode-se concluir que a fração FBIKS apresenta uma mistura de polissacarídeos. Dessa maneira, a primeira estratégia traçada para purificação desta fração, foi o processo de ultrafiltração, buscando a separação dos polissacarídeos presentes pela retenção junto a membrana de 100 kDa. O procedimento resultou nas amostras FBIKS100E (amostra eluída) e FBIKS100R (amostra retida). Os perfis de eluição na análise por HPSEC dessas frações estão apresentados na Figura 3. Pode-se observar que o polissacarídeo apresentando maior massa (pico I) ficou concentrado na fração FBIKS100R, entretanto, ainda contendo a presença do outro polissacarídeo, como evidenciado pela presença do ombro a direita do pico principal. A fração FBIKS100E, por sua vez, apresentou perfil de eluição semelhante a fração original, FBIKS. Desta maneira, ambas frações foram submetidas a ultrafiltração em membrana apresentando limite de exclusão menor (50 kDa), conforme ilustrado na Figura 1. Nesse procedimento, amostra FBIKS100R resultou em duas novas frações, sendo a fração FBIKS100R50R (retida na membrana) e FBIKS100R50E (eluída na membrana), assim como foram obtidas as frações FBIKS100E50R (retida) e FBIKS100E50E (eluída) a partir da amostra FBIKS100E. Os perfis de eluição dessas amostras no HPSEC estão mostrados nas figuras 4 e 5. Pode-se observar que as frações FBIKS100R50R, FBIKS100R50E e FBIKS100E50E ainda apresentaram um perfil de eluição heterogêneo e semelhante a fração original, não havendo dessa maneira, separação dos polímeros.

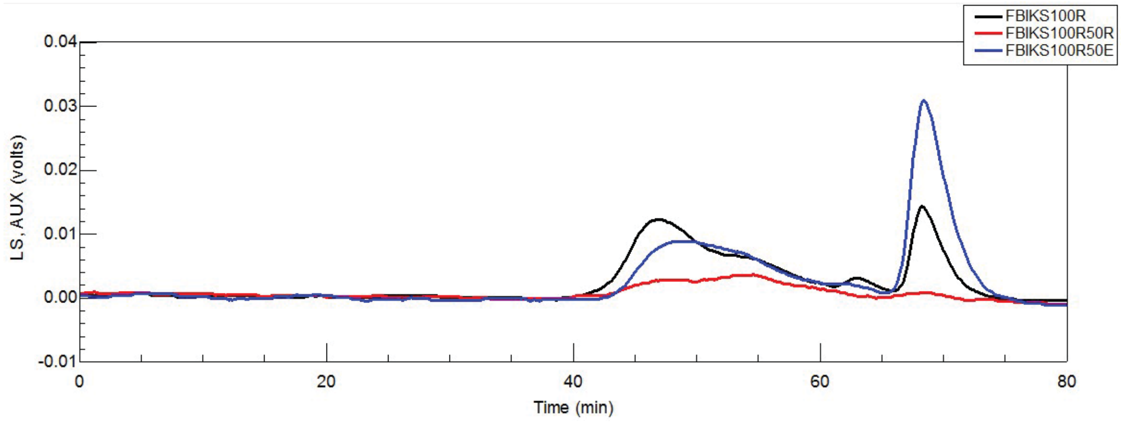

Figura 4: Perfil de eluição das frações FBIKS100R, FBIKS100R50R e FBIKS100R50E quando analisadas por cromatografia de gel permeação.

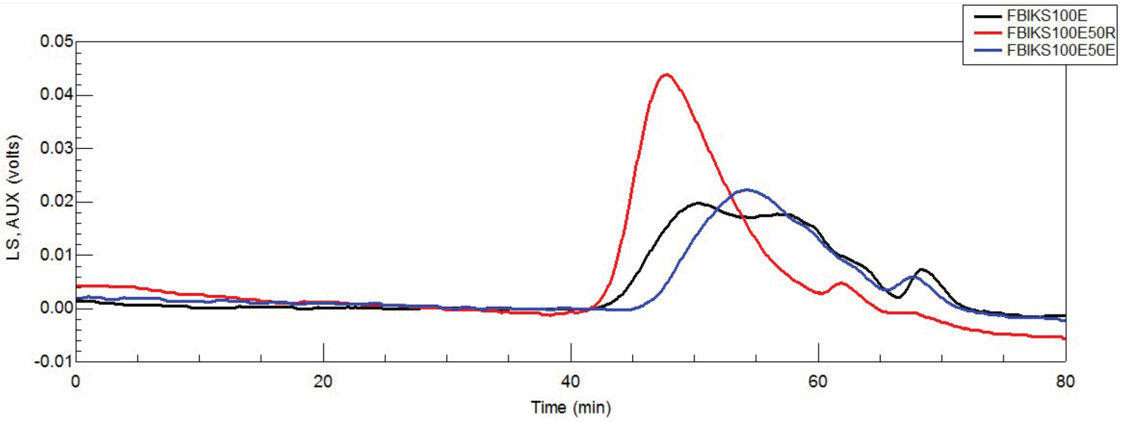

Figura 5: Perfil de eluição das frações FBIKS100E, FBIKS100E50R e FBIKS100E50E quando analisadas por cromatografia de gel permeação.

Por outro lado, pode-se observar um perfil de eluição homogêneo para a fração FBIKS100E50R. Entretanto, seu espectro de HSQC (Figura 6) ainda indicava a mistura dos polissacarídeos, como observado pela presença dos sinais 107,6/5,38 e 108,3/5,27 referentes às unidades de α-L-Ara*f*, 104,3/4,61 e 101,4/4,47 referentes às unidades de β-D-Gal*p* e β-D-Xyl*p*, respectivamente. Sendo assim, uma nova estratégia de purificação da amostra FBIKS foi empregada, utilizando-se a precipitação pelo reagente de Fehling (JONES & STOODLEY, 1965), originando duas novas frações, FBIKSPF (precipitado) e FBIKSSF (sobrenadante), a expectativa foi de que a possível xilana presente na fração, precipitasse em meio ao experimento. O espectro de ^{13}C-RMN da amostra FBIKSSF está mostrado na Figura 7 e pode-se observar sinais referentes anoméricos referentes à arabinogalactana (108.10 – 104.84) e da xilana (101.72), indicando que ambos os polissacarídeos ficaram solúveis no tratamento com o reagente de Fehling.

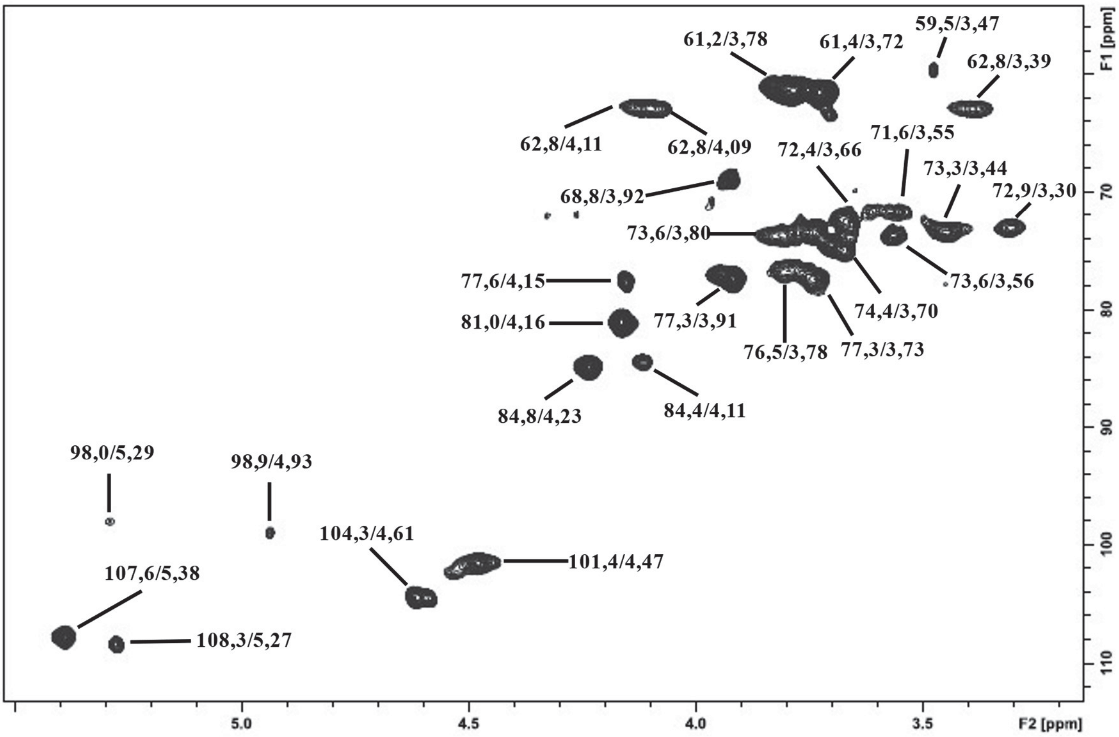

Figura 6: Análise de RMN da fração FBIKS100E50R mapa de correlação ^{1}H-^{13}C obtido pelo experimento HSQC. Amostra dissolvida em D_2O e analisada a temperatura de 70 ºC

Dessa maneira, outras estratégias de purificação devem ser testadas para a separação desses polímeros. Uma sugestão seria o tratamento da fração com enzimas específicas seguida de diálise. Como por exemplo, após digestão enzimática com galactanase, que levaria à degradação da arabinogalactana, poder-se-ia isolar a xilana, e após a digestão enzimática com xilanase, poder-se-ia purificar a arabinogalactana.

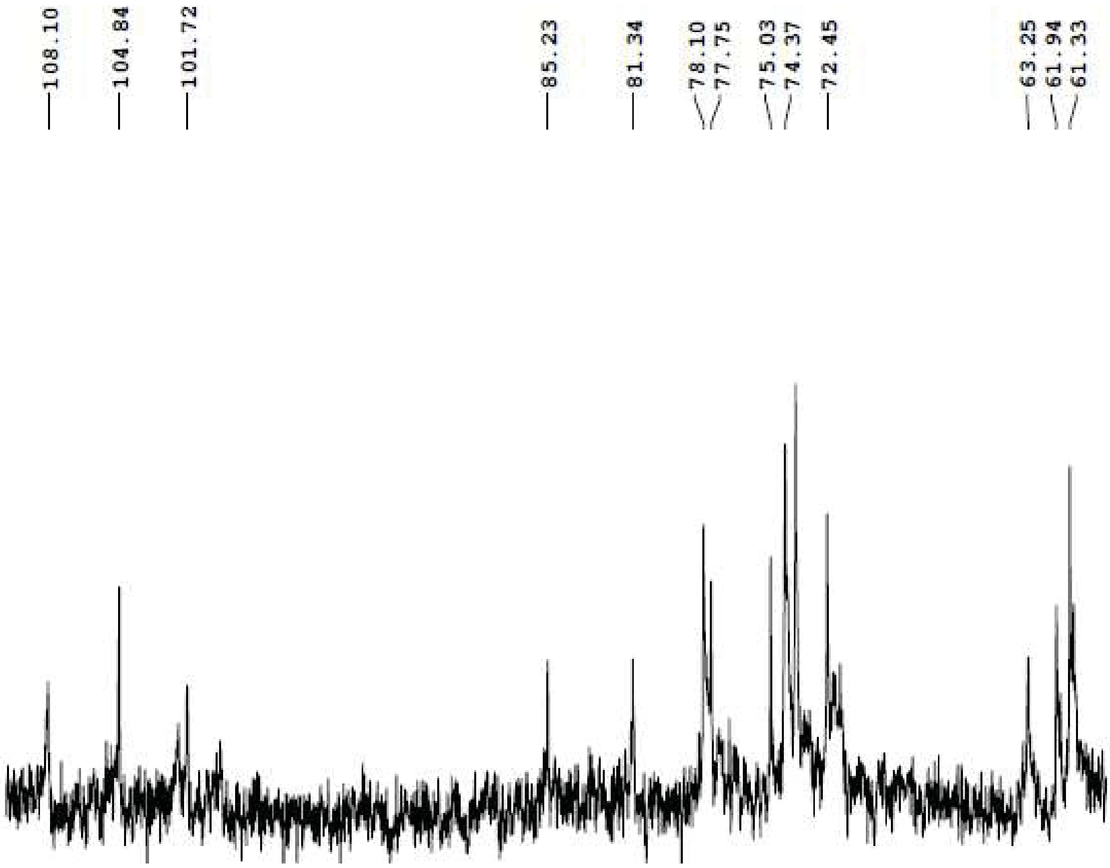

Figura 7: Espectro de ^{13}C-RMN da amostra FBIKSSF em D_2O a 70 ºC

3. Fibra dietética solúvel da bocaiuva (*Acrocomia corumbaensis*)

A fibra dietética solúvel da bocaiuva (FBS) discutida no anexo I, apresenta além da glucomanana, possíveis contaminantes, como observado pela sua homogeneidade (Figura 8), que apresenta a presença de um ombro a esquerda do pico principal, e pela composição monossacarídica, que além de glucose (16%) e manose (70,3%), apresenta outros monossacarídeos em menor proporção (Gal 3,3%; Ara 6,7%, Xyl 0,5% e Fuc 3,2%).

Seguindo os mesmos princípios adotados na purificação da fração FBIKS, a amostra FBS também foi submetida ao processo de ultrafiltração em membrana com limite de exclusão de 100 kDa, resultando em duas novas frações, FBS100R (retida na membrana) e FBS100E (eluida na membrana). Contudo, essa técnica também se demonstrou parcialmente efetiva, uma vez que as frações resultantes ainda obtiveram perfil de eluição heterogêneo, quando analisadas por HPSEC (Figura 8).

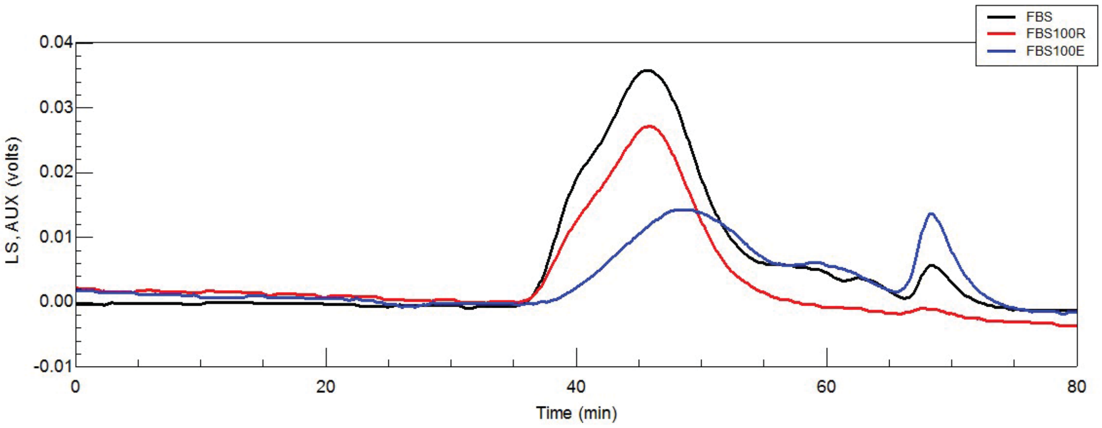

Figura 8: Perfil de eluição das frações FBS, FBS100R e FBS100E quando analisadas por cromatografia de gel permeação.

Buscando novas alternativas para purificação da fibra solúvel da bocaiuva, o método de Fehling (JOONES & STOODLEY, 1965) foi realizado junto a fração FBS. Entretanto, devido ao pH alcalino do reagente de Fehling, ocorre a deacetilação da glucomanana, e após esse tratamento, as amostras ficaram insolúveis, impossibilitando a sua análise por RMN e HPSEC. Em virtude dos atuais resultados, a purificação de FBS seguirá pelo processo de ultrafiltração, com a utilização de membranas com limites de exclusão inferiores a 100 kDa.

4. Conclusão

O presente estudo permitiu a caracterização estrutural de uma glucomanana com grau de acetilação de 24%, que representa a fração de fibra alimentar solúvel extraída da polpa do fruto de *Acrocomia corumbaensis*. Observou-se também que essa fração apresentou atividade gastroprotetora *in vivo*, sendo, portanto, um agente terapêutico promissor no manejo de patologias gástricas

A fração contendo as fibras insolúveis da bocaiúva apresenta a mistura de duas hemiceluloses, provavelmente uma arabinogalactana do tipo I e uma xilana neutra, podendo ser uma arabinoxilana.

Novas estratégias serão empregadas em busca da purificação e caracterização química desses polissacarídeos obtidos da bocaiuva (*Acrocomia corumbaensis*) para que haja o conhecimento estrutural dos polissacarídeos ali presentes, uma vez que se trata de uma fruta amplamente consumida pela população do cerrado brasileiro e que já faz parte do interesse da indústria em diversas outras aplicações.

A purificação pelo processo de ultrafiltração com membranas de corte menores as já empregadas, a identificação de possíveis ramificações junto a sua cadeia principal, bem como a determinação da posição dos grupos acetil pela técnica de metilação são exemplos de experimentos a serem executados para a fibra solúvel da bocaiuva. Já para as fibras insolúveis, a purificação pela ultrafiltração também será uma metodologia a continuar ser empregada, em buscar de uma fração purificada para sua caracterização estrutural.

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