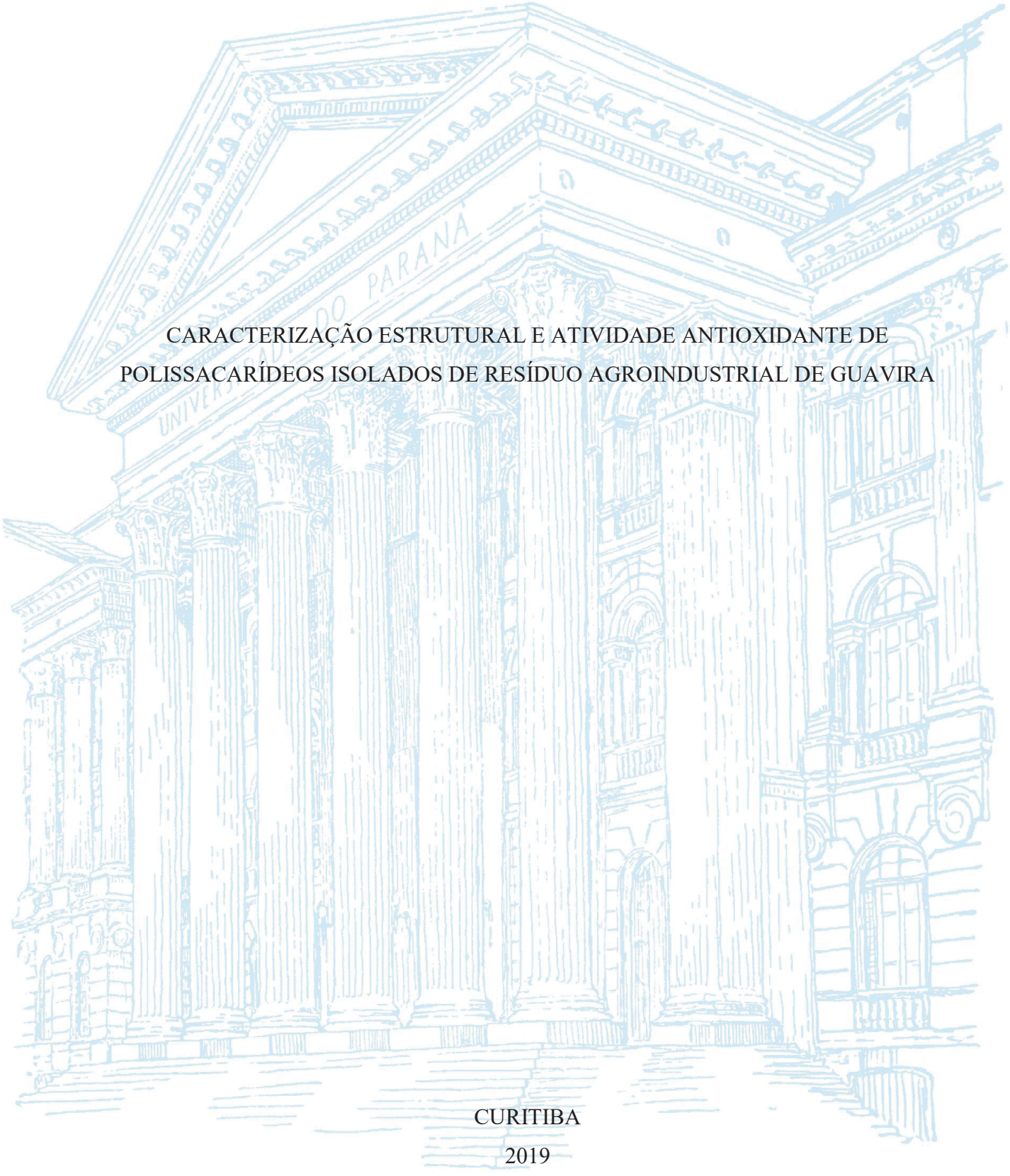
UNIVERSIDADE FEDERAL DO PARANÁ

VANESSA SUZANE SCHNEIDER



CARACTERIZAÇÃO ESTRUTURAL E ATIVIDADE ANTIOXIDANTE DE POLISSACARÍDEOS ISOLADOS DE RESÍDUO AGROINDUSTRIAL DE GUAVIRA

CURITIBA

2019

VANESSA SUZANE SCHNEIDER

CARACTERIZAÇÃO ESTRUTURAL E ATIVIDADE ANTIOXIDANTE DE POLISSACARÍDEOS ISOLADOS DE RESÍDUO AGROINDUSTRIAL DE GUAVIRA

Dissertação apresentada ao programa de pós-graduação em Ciências – Bioquímica, Departamento de Bioquímica e Biologia Molecular, Setor de Ciências Biológicas, Universidade Federal do Paraná, como requisito parcial para a obtenção do título de Mestre em Ciências – Bioquímica.

Orientadora: Profa. Dra. Lucimara Mach Cortes Cordeiro
Co-orientadoras:
Dra. Georgia Erdmann do Nascimento
Dra. Sheila Maria Brochado Winnischofer

CURITIBA
2019

Universidade Federal do Paraná. Sistema de Bibliotecas.
Biblioteca de Ciências Biológicas.
(Giana Mara Seniski Silva – CRB/9 1406)

Schneider, Vanessa Suzane
Caracterização estrutural e atividade antioxidante de polissacarídeos isolados de resíduo agroindustrial de Guavira. / Vanessa Suzane Schneider. – Curitiba, 2019.
79 p.: il. ; 30cm.

Orientador: Lucimara Mach Cortes Cordeiro
Coorientadores: Georgia Erdmann do Nascimento
Sheila Maria Brochado Winnischofer
Dissertação (mestrado) - Universidade Federal do Paraná, Setor de Ciências Biológicas. Programa de Pós-Graduação em Ciências - Bioquímica.

1. Polissacarideos 2. Antioxidantes 3. Polpa de frutas 4. Resíduos - Subprodutos I. Título II. Cordeiro, Lucimara Mach Cortes III. Nascimento, Georgia Erdmann do IV. Winnischofer, Sheila Maria Brochado, 1976 V. Universidade Federal do Paraná. Setor de Ciências Biológicas. Programa de Pós-Graduação em Ciências - Bioquímica.
CDD (22. ed.) 572.566

MINISTÉRIO DA EDUCAÇÃO
SETOR SETOR DE CIENCIAS BIOLOGICAS
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO CIÊNCIAS
(BIOQUÍMICA) - 40001016003P2

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em CIÊNCIAS (BIOQUÍMICA) da Universidade Federal do Paraná foram convocados para realizar a arguição da dissertação de Mestrado de **VANESSA SUZANE SCHNEIDER** intitulada: **CARACTERIZAÇÃO ESTRUTURAL E ATIVIDADE ANTIOXIDANTE DE POLISSACARÍDEOS ISOLADOS DE RESÍDUO AGROINDUSTRIAL DE GUAVIRA**, após terem inquirido a aluna e realizado 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.

CURITIBA, 24 de Abril de 2019.

LUCIMARA MACH CORTES CORDEIRO
Presidente da Banca Examinadora (UFPR)

MARCELLO IACOMINI
Avaliador Interno (UFPR)

FABÍOLA IAGHER
Avaliador Externo (UFPR)

Para a minha amada família.

AGRADECIMENTOS

Em primeiro, sou grata a Deus, por sempre me dar forças, ter me cuidado e guiado até aqui e ter colocado no meu caminho pessoas que fizeram diferença neste meu aprendizado.

À minha família linda: Wolmar, Ester e Keity, que para cada passo que eu dei tem me dado apoio, incentivo e amor inesgotáveis.

Ao Heder, meu parceiro, que foi meu porto seguro nos momentos de crise, me dando forças para continuar, por acreditar no meu potencial e sempre me lembrar de usar EPIs.

À Ni e ao Márcio, pelas conversas, por serem esse casal tão lindo e inspirador, por cuidarem de mim e por me receberem de braços abertos sempre.

Aos leõezinhos da JELSM, por todos os momentos de comunhão e diversão compartilhados.

Às minhas amigas queridas que alegram os meus dias, de perto ou de longe: Alejandra, Brienna, Camila, Dagna, Fernanda, Jessica, Katiane, Leidi e Thaís.

À Prof.ª Dr.ª Lucimara M. C. Cordeiro, pelos valiosos conselhos que irão contribuir na minha vida pessoal e profissional, por toda paciência, atenção e ensino.

À Prof.ª Dr.ª Sheila M. B. Winnischofer e à Dr.ª Georgia E. do Nascimento, por toda contribuição na realização desse trabalho.

Ao Prof. Dr. Marcello Iacomini pelas correções do projeto e relatório e por aceitar participar da banca examinadora desta dissertação.

À Prof.ª Fabíola Iagher por aceitar participar da banca examinadora desta dissertação.

À Prof.ª Elisvânia F. dos Santos, por ter gentilmente cedido o material de estudo.

Ao pessoal do grupo de Química de Carboidratos dos labs 247, 250, 252 e Anexo, em especial Daniela, Giuliana, Hellen, Kahlile, Marise, Pedro, Philippe e Shayane que são pessoas que serei sempre grata pela amizade, convivência e por terem me ajudado sempre que precisei. Obrigada de coração, seus lindos!

Ao grupo de Oxidações pelos empréstimos de equipamentos; e especialmente à Ju Bark e ao Diego, que me ajudaram e tiraram diversas dúvidas nos experimentos do cultivo celular.

Aos professores do Departamento de Bioquímica e Biologia Molecular da UFPR, pelo ensino, por todo o conhecimento que adquiri durante esse período.

Ao Thiago, secretário do Programa de Pós Graduação em Ciências (Bioquímica) por ser prestativo e ajudar com minhas dúvidas.

As técnicas Elis, Rosane e Keylla pelas análises de composição e GPC.

Ao Prof. Bruno e ao técnico Thiago, pelas análises de metilação no GC-MS.

Ao Prof. Dr. Guilherme Sassaki pelas análises de RMN.

Ao Dr. Arquimedes Santana pelas inúmeras dúvidas solucionadas ao longo do mestrado.

Às agências financiadoras, Capes, Fundação Araucária e CNPq pela bolsa e apoio financeiro.

A todos o meu sincero muito obrigada!

"O cientista não estuda a natureza porque ela é útil;
ele a estuda porque se deleita nela, e se deleita nela porque ela é bela.
Se a natureza não fosse bela, não valeria a pena ser conhecida,
e se não valesse a pena ser conhecida,
a vida não valeria a pena ser vivida."

Henry Poincaré

RESUMO

Guavira é uma fruta nativa do bioma Cerrado, com efeitos biológicos de sua polpa bem estabelecidos, como atividade antimicrobiana, antiinflamatória, antinociceptiva e antioxidante. Apesar de possuir potencial tecnológico, os subprodutos oriundos da produção da polpa da guavira, tais como cascas e sementes, não são aproveitados. Neste trabalho, pectinas e hemiceluloses foram extraídos por extração aquosa e alcalina, respectivamente, de resíduo proveniente da indústria de polpa de guavira (constituído por casca, semente, pedúnculo e resquícios de polpa), e analisados por GC-MS, GPC e RMN. O extrato aquoso (CPW) apresentou-se composto por uma homogalacturonana altamente metoxilada (DM = 90%) e outros polissacarídeos pécticos ricos em arabinanas. A arabinana foi purificada e apresentou-se composta por Ara (85%), Rha (3,3%), Gal (7,7%) e GalA (4%). Análises de RMN mono e bidimensionais desta arabinana demonstraram a presença de unidades terminais compostas por β-L-Ara*f*, cuja ocorrência tem sido pouco relatada na literatura. Os polissacarídeos hemicelulósicos foram purificados a partir do extrato alcalino, e caracterizados como sendo glucuronoxilanas, com grupos 4-*O*-metil-D-Glc*p*A ligados no *O*-2 das unidades de β-D-xilose (1→4)-ligadas. Além disso, a atividade antioxidante das pectinas presentes em CPW foi avaliada, sendo que esta fração apresentou ação antioxidante pelo sequestro de radicais ABTS e o tratamento de células de fibroblastos murinos NIH 3T3 com CPW por 48h notavelmente diminuiu os níveis de espécies reativas de oxigênio (ROS) e citotoxicidade induzida por H_2O_2, indicando que os polissacarídeos do bagaço de guavira foram capazes de exercer atividade antioxidante, induzindo proteção contra condições pró-oxidantes.

Palavras-chave: *Campomanesia sp*. Subproduto de indústria de polpa. Arabinana. Glucuronoxilana. Atividade antioxidante.

ABSTRACT

Guavira is a native fruit from the Cerrado biome, with established biological effects on its pulp, such as antimicrobial, anti-inflammatory, antinociceptive and antioxidant activity. Despite their technological potential, by-products from the pulp production of guavira, such as bark and seeds, are not used. In this work, pectins and hemicelluloses were extracted by aqueous and alkaline extraction, respectively, from residue from guavira pulp industry (containing remaining of pulp, peel, seeds and peduncle), and analyzed by GC-MS, GPC and NMR. The aqueous extract (CPW) was composed of a highly methoxylated homogalacturonan (DM = 90%) and other arabinan-rich pectic polysaccharides. Arabinan was purified and was composed of Ara (85%), Rha (3.3%), Gal (7.7%) and GalA (4%). Mono and bidimensional NMR analyzes of this arabinan demonstrated the presence of terminal units composed of β-L-Ara*f*, whose occurrence has been scarcely reported in the literature. Hemicellulosic polysaccharides were purified from the alkaline extract, and characterized as glucuronoxylans, with 4-*O*-methyl-D-Glc*p*A groups linked to *O*-2 of the units of (1→4)-β-D-xylan. In addition, antioxidant activity of the pectins present in CPW fraction was evaluated, and this fraction exhibited antioxidant action by the inhibition of ABTS radicals. Besides, the treatment of murine fibroblast cells NIH 3T3 with CPW for 48 h remarkably decreased levels of Reactive Oxygen Species (ROS) and H_2O_2-induced cytotoxicity, indicating that guavira bagasse polysaccharides were able to exert intracellular antioxidant activity, inducing protection against pro-oxidant conditions.

Keywords: *Campomanesia sp*. fruit pulp industry by-product. Arabinan. Glucuronoxylan. Antioxidant activity.

LISTA DE FIGURAS

REVISÃO BIBLIOGRÁFICA

ARTIGO 1

ARTIGO 2

LISTA DE TABELAS

REVISÃO BIBLIOGRÁFICA

ARTIGO 2

LISTA DE SÍMBOLOS E SIGLAS

α - Alfa

β - Beta

δ - Deslocamento químico

4-*O*-Me-α-D-GlcpA - 4-*O*-Metil-α-D-ácido glucurônico

^{1}H - Núcleo de hidrogênio

^{13}C - Núcleo de carbono

ABTS - Ácido 2,2'-azino-bis (3-etilbenzotiazolina-6-sulfônico)

Ara - Arabinose

$BaCO_3$ - Carbonato de bário

COSY - *Correlation Spectroscopy*

D_2O - Óxido de deutério

DCFH-DA - 2-7- dichloro-fluorescin-diacetate

DE - Grau de esterificação

DEPT - *Distiortionless enhancement by polarization transfer*

DF- Dietary fibre

DMEM - Meio de Eagle modificado por Dulbecco

DMSO - Dimetilsulfóxido

f- Furanosídico

Gal - Galactose

GalA - Ácido galacturônico

GC-MS - Cromatografia gasosa acoplada à espectrometria de massa

Glc - Glucose

GlcA - Ácido glucurônico

GLC - Cromatografia líquido – gasosa

GPC - *Gel Permeation Chromatography*

GPF - *Guavira pomace flour* (farinha do resíduo da guavira)

H_2O_2 - Peróxido de hidrogênio

H_2SO_4 - Ácido sulfúrico

HCl - Ácido clorídrico

HG - Homogalacturonana

HM - *High methoxyl pectin* – pectina de alta metoxilação

HSQC - *Heteronuclear Single Quantum Correlation Spectroscopy*

HSQC-DEPT - *Heteronuclear Single Quantum Correlation Spectroscopy - Distortionless Enhancement by Polarization Transfer*

kDa - Quilodalton

KOH - Hidróxido de potássio

LM - *Low methoxyl pectin* – pectina de baixa metoxilação

Man - Manose

Me - Grupamento metil ($-CH_3$)

MeI - Iodeto de metila

MeOH - Metanol

$NaBD_4$ - Boroidreto de sódio deuterado

$NaBH_4$ - Boroidreto de sódio

NaOH - Hidróxido de sódio

MTT - Brometo de 3-(4,5-dimetil-2-tiazolil)-2,5-difenil-2H-tetrazólio

Mw - Massa molar

NIH 3T3 - Linhagem celular de fibroblastos murinos

p - Piranosídico

PBS - Tampão fosfato salino

per-*O*-metilado - Parcialmente *O*-metilado

ppm - Partes por milhão

RI - Índice de refração

RMN / NMR - Ressonância Magnética Nuclear

Rha - Ramnose

RMN-^{13}C - Ressonância magnética nuclear de carbono 13

RMN-^{1}H - Ressonância magnética nuclear de hidrogênio

ROS - *Reactive oxygen species* (Espécies reativas de oxigênio)

rpm - Rotações por minuto

SFB - Soro fetal bovino

TCA - Ácido Tricloroacético

TFA - Ácido Trifluoroacético

TOCSY - *Total Correlation Spectroscopy*

UA - Ácido Urônico

v/v - Volume/volume

Xyl - Xilose

LISTA DE SIGLAS DAS FRAÇÕES

CPK - fração polissacarídica obtida após extração alcalina com KOH a 10%, sob refluxo, do resíduo de guavira

CPK-PF - fração precipitada de Fehling, após tratamento com reativo de Fehling da fração CPK

CPK-SF - fração sobrenadante de Fehling, após tratamento com reativo de Fehling da fração CPK

CPW - fração polissacarídica obtida após extração aquosa, sob refluxo, do resíduo de guavira

CPW-SF - fração sobrenadante de Fehling, após tratamento com reativo de Fehling da fração CPW

CPW-SF-30E - fração eluída na ultrafiltração em membrana de 30 kDa, após extração aquosa e tratamento com Fehling

CPW-SF-30R - fração retida na ultrafiltração em membrana de 30 kDa, após extração aquosa e tratamento com Fehling

CPW-SF-50R - fração retida na ultrafiltração em membrana de 50 kDa, após extração aquosa e tratamento com Fehling

CPW-SF-100R - fração retida na ultrafiltração em membrana de 100 kDa, após extração aquosa e tratamento com Fehling

SUMÁRIO

1 INTRODUÇÃO

O Brasil apresenta flora rica e diversificada em espécies de frutíferas, muitas delas com potencial tecnológico. A flora do cerrado, em especial, possui diversas espécies com grande potencial de exploração agroindustrial, que são tradicionalmente utilizadas pela população local (ALMEIDA, 1998). Entre tais espécies nativas que apresentam um papel econômico promissor à população e às indústrias locais está a guavira.

Esta planta cresce em forma de pequenos arbustos e, de forma característica, seus frutos se apresentam arredondados e de coloração verde-amarelada, sendo a polpa amarelada e suculenta. A utilização dos frutos de guavira pelas indústrias aumentou devido à produção de polpas congeladas, ocasionando aumento de subprodutos, como o bagaço, constituído principalmente por cascas e sementes. Apesar de possuir aplicações em potencial, o subproduto da guavira não é utilizado comercialmente. O número de pesquisas com resíduos do processamento de frutas (como a casca e a semente) na busca por substâncias naturais, biologicamente ativas e de baixo custo, tem aumentado ao longo dos últimos anos. Em alguns casos, foi relatado que a concentração de nutrientes nos resíduos das frutas é inclusive maior do que na polpa (IGNAT et al., 2011; AYALA-ZAVALA et al., 2011).

Dentre os compostos presentes nas frutas e em seus resíduos, aqueles com propriedades de reduzir o nível do estresse oxidativo têm despertado o interesse da comunidade científica, principalmente pelo seu conhecido papel na diminuição do efeito de processos oxidativos que ocorrem naturalmente no organismo, e consequentemente do risco de diversas doenças cardivasculares, neurodegenerativas e câncer. Alguns desses compostos são minerais, vitaminas, compostos fenólicos e fibras alimentares.

As fibras alimentares, por sua vez, também apresentam outros efeitos benéficos para a saúde humana, relacionados com a promoção de um melhor funcionamento do sistema digestivo, principalmente devido ao aumento da saciedade e ao volume e peso da matéria fecal. Quimicamente, essas fibras abrangem principalmente os polímeros de carboidratos, que resistem à hidrólise por enzimas digestivas no intestino delgado de mamíferos, mas podem ser fermentados por bactérias do intestino grosso (WILLIAMS et al, 2017).

Nos últimos anos, diversas pesquisas confirmaram que polissacarídeos de fontes naturais possuem vários efeitos terapêuticos benéficos e propriedades promotoras de saúde. Suas propriedades biológicas são afetadas por suas características estruturais, tais como composição química, massa molar, grau de ramificação, tipo e configuração da ligação

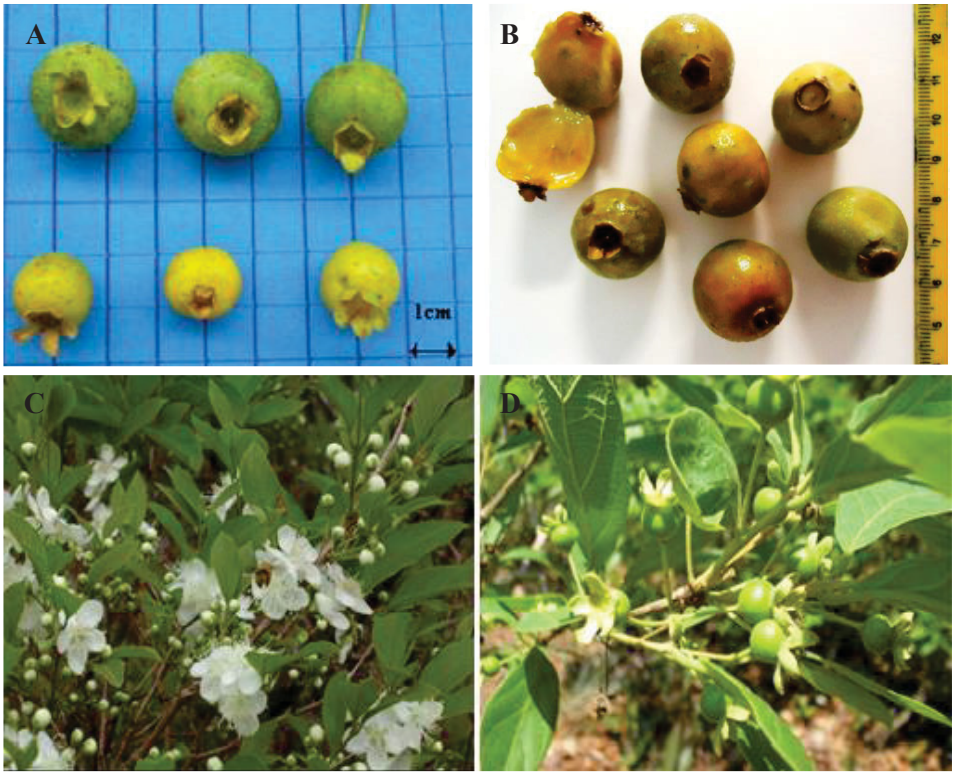
glicosídica (WANG et al., 2016). Desta forma, comprova-se a importância de investigar a estrutura, ou seja, a química fina de polissacarídeos e realizar a sua correlação com atividades biológicas. O conhecimento sobre a estrutura química de seus polissacarídeos e determinação de atividade antioxidante pode abrir novas perspectivas para a exploração do subproduto da guavira em diferentes aplicações tecnológicas e nutracêuticas.

2 REVISÃO BIBLIOGRÁFICA

2.1 GUAVIRA E O USO DE RESÍDUOS AGROINDUSTRIAIS / SUBPRODUTOS

As espécies *Campomanesia adamantium* (Cambess.) O. Berg e *Campomanesia pubescens* (D.C.), popularmente conhecidas como guavira ou gabiroba-do-campo, são subarbustos nativo do cerrado, medindo entre 0,50 e 1,5 m e ocorrendo geralmente em forma de moitas (LIMA et al., 2011). A produção é de 30 a 100 frutos por planta, com dimensões de 1 a 3 cm de comprimento por 2 a 3 cm de diâmetro (Figura 1). Os frutos são arredondados e de coloração verde-amarelada, sendo a polpa amarelada, suculenta, envolvendo numerosas sementes (LORENZI et al., 2006). O florescimento ocorre de forma intensa no período entre agosto e novembro, sendo o pico em setembro e a frutificação de setembro a dezembro (SILVA et al., 2001).

FIGURA 1 - EXEMPLARES DE FRUTOS DE *C. PUBESCENS* (A) E DE *C. ADAMANTIUM* (B) E FLORESCIMENTO DE *C. PUBESCENS* (C E D). Adaptado de PERIOTTO & GUALTIERI (2017) e FERNANDES et al (2015).



A guavira é popularmente conhecida pelo alto teor de vitamina C, sendo o fruto apreciado *in natura* e também utilizado na confecção de geléias, sorvetes, refresco, doces, pudins, licores, batidas ou curtidos na cachaça (DOUSSEAU et al., 2011). Já a infusão de folhas, casca dos frutos e galhos da guavira é popularmente utilizada como antiinflamatória, antidiarréica, anti-séptica das vias urinárias e para o tratamento da gripe (Lorenzi et al., 2006; PAVAN et al., 2009; PASCOAL et al., 2011).

Alguns efeitos biológicos documentados em literatura para essas espécies se encontram na Tabela 1. Dentre estes, destacam-se os relatos de Alves et al. (2013) e Fernandes et al. (2015), que verificaram efeito antioxidante do extrato hidroalcoólico do resíduo de guavira (sementes e casca) em ensaios de eliminação de radicais DPPH e ABTS de forma ainda mais eficaz que o extrato de polpa.

TABELA 1 - ATIVIDADES BIOLÓGICAS DE *C. ADAMANTIUM* E *C. PUBESCENS*

Espécie	Parte da planta	Atividades biológicas	Referência
C. adamantium	extrato hexânico da folha	atividade anti-bacteriana	SÁ et al., 2018
C. adamantium	extrato metanólico da polpa	atividade anti-inflamatória e antinociceptiva	VISCARDI et al., 2017
C. adamantium	extrato hidroalcoólico da polpa e de casca/semente	efeito hepatoprotetivo e atividade antioxidante em HepG2	FERNANDES et al., 2015
C. adamantium	extrato hidroalcoólico de casca	anti-inflamatória, anti-hiperalgésico e antidepressiva	SOUZA et al., 2014
C. adamantium	extrato metanólico de resíduo (casca e semente) e polpa	atividade antioxidante	ALVES et al., 2013
C. adamantium	extrato etanólico da folha	atividade antioxidante	PASCOAL et al., 2011
C. adamantium	extrato de acetato de etila da folha	anti-*Mycobacterium tuberculosis*	PAVAN et al., 2009
C. pubescens	extrato hexânico dos frutos	atividade antiproliferativa em linhagens de células tumorais humanas	CARDOSO et al., 2013
C. pubescens	extrato etanólico da folha, caule, raiz e fruto	atividade antimicrobiana e antioxidante	CHANG et al., 2011

A demanda por frutos de guavira pelas indústrias aumentou devido à produção de polpas congeladas, ocasionando aumento de subprodutos gerados. Apesar de possuir

aplicações em potencial, o subproduto da indústria de polpa da guavira, constituído por casca, semente e pedúnculo, não é utilizado comercialmente.

A utilização da fruta como um todo traz benefícios econômicos aos produtores, gera um impacto benéfico para o meio ambiente, além de levar a uma maior diversidade de produtos voltados para uso humano (AYALA-ZAVALA et al, 2011). No entanto, apesar de subprodutos de frutos serem produzidos mundialmente em larga escala e de serem estabelecidos como fontes ricas de compostos funcionais, eles são uma fonte rentável pouco explorada, levando em consideração que atualmente poucos subprodutos são industrialmente processados, como a casca de frutas cítricas, resíduos de tomate e bagaço de maçã (GALANAKIS, 2012).

A alta quantidade de compostos bioativos presentes nos subprodutos de frutos pode ser utilizada como aditivo alimentar natural, principalmente considerando-se que os resíduos agroindustriais são ricos em fibras alimentares.

2.2 FIBRAS ALIMENTARES E POLISSACARÍDEOS

Os frutos são uma fonte importante de fibras alimentares, que são constituídas principalmente por polissacarídeos da parede celular. De acordo com o Codex Alimentarius Comission (2009), os polissacarídeos não-amídicos de origem vegetal são considerados como fibra alimentar quando os "polímeros de carboidratos apresentam 10 ou mais unidades monoméricas, que não são hidrolisadas pelas enzimas endógenas no intestino delgado de humanos". Os polissacarídeos da parede celular são categorizados em três grupos principais: pectinas, hemiceluloses e celulose.

A celulose é o polissacarídeo da parede celular de plantas mais abundante, formando microfibrilas cristalinas mecanicamente fortes (COSGROVE, 2005). Associadas a essa estrutura estão as hemiceluloses. Os polímeros de hemicelulose são bastante heterogêneos com relação à composição e estrutura química, sendo compreendidos por unidades de D-xilose, D-galactose, D-glucose, L-arabinose, D-manose, ácido D-glucurônico e ácido 4-*O*-metilglucurônico. Os monossarídeos da cadeia principal são unidos por ligações do tipo β-(1→4), uma característica comum aos polissacarídeos hemicelulósicos (ALBERSHEIM et al., 1996; EBRINGEROVÁ, HROMÁDKOVÁ & HEINZE, 2005).

Como exemplo de polímeros hemicelulósicos pode-se citar xilanas, mananas e xiloglucanas. As xilanas possuem cadeias lineares com unidades D-xilopiranose β-(1→4)

ligadas e, dependendo da fonte e metodologia de extração, podem ser encontradas na forma de arabinoxilanas, glucuronoxilanas e glucuronoarabinoxilanas, quando anexadas a cadeias laterais curtas de L-arabinose, ácido D-glucurônico e/ou ácido 4-*O*-metilglucurônico. Grupos laterais como grupos acetil, fenólicos, ácidos ferúlico e cumárico também podem ser encontrados (HROMÁDKOVÁ, KOŠT'ÁLOVÁ & EBRINGEROVÁ, 2008; CAFFALL & MOHNEN, 2009).

As pectinas formam uma matriz que dá firmeza à rede formada pela associação de celulose e hemicelulose na parede celular (COSGROVE, 2005). Elas incluem três classes principais de polímeros ácidos: homogalacturonanas, ramnogalacturonanas do tipo I e ramnogalacturonanas do tipo II (YAPO, 2011).

Homogalacturonanas são os polissacarídeos pécticos mais abundantes, sendo formadas por uma cadeia linear de resíduos de α-D-GalA ligados (1→4), podendo ser parcialmente metil esterificadas em C-6 ou acetil-esterificadas em *O*-2 ou *O*-3 (YAPO, 2011). O grau de esterificação (DE) das pectinas indica a proporção de grupos carboxílicos metil-esterificados, classificando-as como pectinas de alta metoxilação (HM – *High methoxyl*) quando apresentam 50% ou mais de grupos carboxílicos metil-esterificados, e de baixa metoxilação (LM – *Low methoxyl*) quando apresentam menos que 50%.

Em ramnogalacturonanas do tipo I, as unidades da cadeia principal de α-D-GalA são intercaladas por resíduos de L-ramnose α-(1→2)-ligados, podendo conter cadeias laterais neutras, principalmente arabinanas, galactanas ou arabinogalactan ligadas ao *O*-4 (YAPO, 2011).

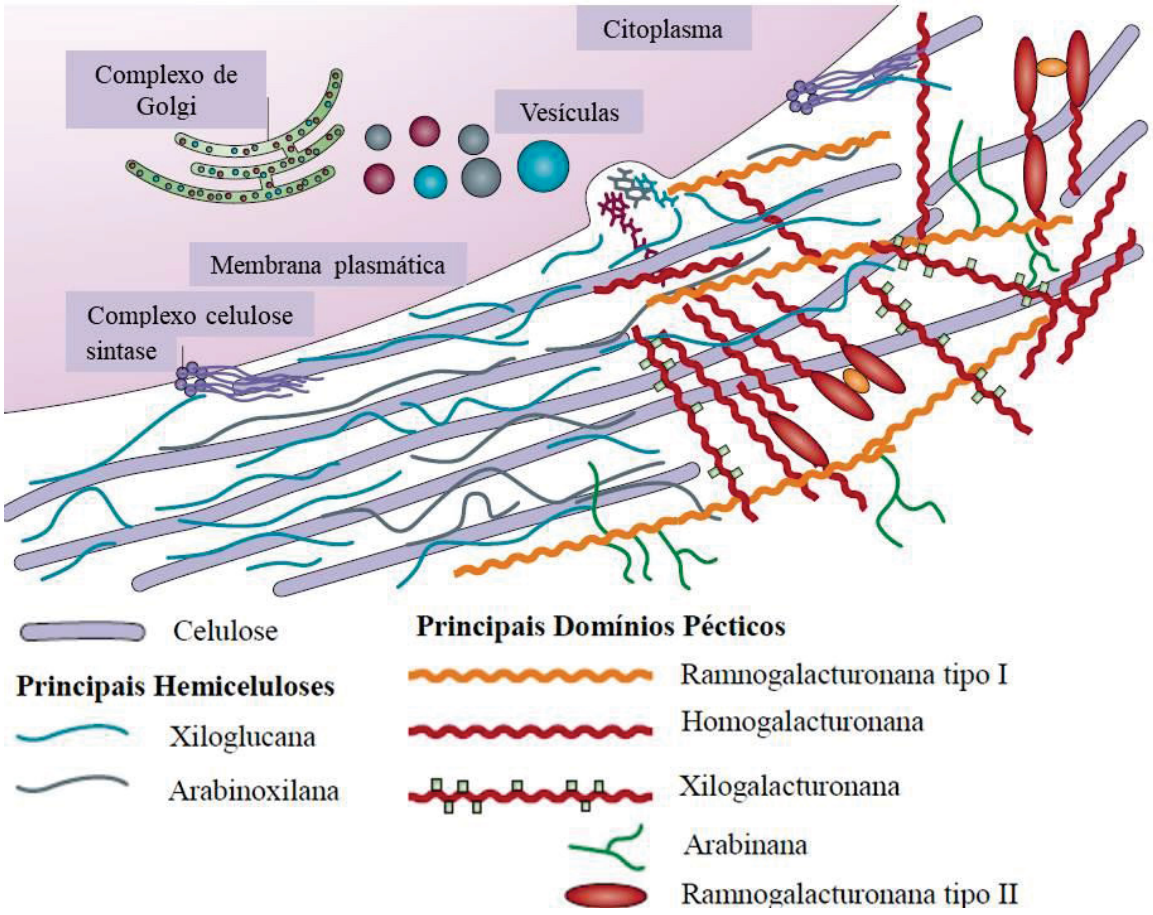
As arabinogalactanas além de compor as cadeias laterais das ramnogalacturonanas do tipo I também podem ser encontradas de forma independente, podendo ser, desta forma, consideradas polissacarídeos pécticos ou hemicelulósicos. Podem ser classificadas em arabinogalactanas do tipo I e do tipo II. Arabinogalactanas do tipo I possuem uma cadeia principal de (1→4) β-D-galactanas, com unidades de arabinose ligadas na posição *O*-3 das unidades de D-galactose. Arabinogalactanas do tipo II são constituídas por um amplo grupo de cadeias curtas de (1→3) e (1→6) β-D-galactanas conectadas umas às outras por pontos de ramificação em *O*-3 e *O*-6 e apresentam grande parte das posições *O*-3 e *O*-6 restantes ocupadas por unidades de L-Ara*f*. Além disso, as ramificações de arabinogalactanas do tipo II podem conter unidades de ácido D-glucurônico e 4-*O*-metil-D-glucurônico (ASPINALL, 1969; CARPITA & GIBEAUT, 1993).

Ramnogalacturonanas do tipo II são as pectinas estruturalmente mais complexas, sendo compostas por unidades de α-D-GalpA (1→4) ligadas e com cerca de 11-12 diferentes

tipos de unidades glicosil interligadas, em mais de 20 ligações glicosídicas distintas (YAPO, 2011).

Juntos, esses polissacarídeos formam a estrutura principal da parede celular, garantindo a integridade e o suporte das plantas (Figura 2) (COSGROVE, 2005). Além disso, também possuem aplicações diversificadas devido a suas propriedades físico-químicas, que permitem sua aplicação em diferentes segmentos da indústria (HARRIS & SMITH, 2006), ou devido a seus efeitos biológicos, possibilitando aplicações terapêuticas (PAULSEN, 2001; ZONG, CAO, & WANG, 2012). Tais aplicações dependem da composição e estrutura química do polissacarídeo.

FIGURA 2 - ESTRUTURA DA PAREDE CELULAR PRIMÁRIA (adaptado de COSGROVE, 2005).



Fibras alimentares solúveis, relativas às pectinas, podem aumentar a viscosidade do conteúdo digerido dependendo de sua estrutura química e peso molecular, que afetam a conformação desses polímeros em solução. Em consequência há uma redução da resposta glicêmica devida ao retardamento do esvaziamento gástrico e liberação de nutrientes, bem

como pela inibição da ação da α-amilase, regulando assim a glicose sanguínea, que por sua vez está associada ao desenvolvimento de resistência à insulina e, por consequência, da diabetes tipo 2 (WILLIAMS et al, 2017). Outro efeito verificado é a redução do risco e ocorrência de obesidade, hipercolesterolemia, doença cardíaca coronária, hemorroidas e câncer de cólon (DHINGRA et al., 2012). Além disso, fibras alimentares podem ser substratos para fermentação por bactérias no intestino grosso, produzindo vários compostos finais (como ácidos graxos de cadeia curta), energia e biomassa, levando à manutenção da microflora intestinal e melhoria do sistema imunológico (WILLIAMS et al, 2017).

Entre as vantagens de se incorporar subprodutos ricos em fibras em produtos alimentícios, pode-se ainda citar o fato de serem de baixo custo e não calóricos, podendo substituir parcialmente a farinha, a gordura ou o açúcar, como intensificadores da retenção de água e óleo, bem como aumentar a estabilidade da emulsão ou oxidação (ELLEUCH et al., 2011).

2.3 ATIVIDADE ANTIOXIDANTE DE POLISSACARÍDEOS

A condição de estresse oxidativo é definida como uma alteração no equilíbrio entre defesas antioxidantes e a produção de espécies reativas de oxigênio (ROS), como ânions superóxido (O_2^-), peróxido de hidrogênio (H_2O_2), radical hidroxila ($OH^\bullet$), assim como de espécies reativas de nitrogênio (RNS) (GIORDANO, 2016).

Embora a oxidação causada por radicais livres possa ter efeitos benéficos, a sua quantidade excessiva pode causar alguns problemas relacionados à saúde, como câncer, processos degenerativos e desenvolvimento imediato de processos inflamatórios no trato gastrointestinal (KIM et al., 2012).

Agentes antioxidantes podem restringir a oxidação de lipídios ou outras moléculas ao inibir a iniciação ou propagação das reações oxidativas em cadeia (VELIOGLU et al., 1998). O potencial de vegetais e seus elementos antioxidantes na manutenção da saúde têm aumentando o interesse entre cientistas e fabricantes de alimentos, principalmente devido à alta demanda por alimentos funcionais com efeitos específicos à saúde. Dentro deste contexto, vários estudos têm demonstrado o potencial de polissacarídeos de origem vegetal com ação antioxidante. Yan et al. (2010), por exemplo, averiguaram que polissacarídeos de *Astragalus mongholicus* aumentaram enzimas de atividade antioxidante em soro de camundongos e diminuíram níveis de lipídeos peroxidados, enquanto que Kong et al. (2010) observaram que

polissacarídeos da polpa de lichia (*Litchi chinensis* Sonn.) exibiram atividade sequestradora de radicais livres de maneira dose-dependente. Polissacarídeos pécticos e extrato metanólico de pó de guaraná (*Paullinia cupana*) exibiram uma alta capacidade de sequestro de radical DPPH (DALONSO & PETKOWICZ, 2012).

Esses trabalhos demonstram a importância e a aplicabilidade de polissacarídeos na defesa de organismos vivos contra danos oxidativos. Os mecanismos envolvidos, porém, não foram completamente elucidados. Em geral, o efeito antioxidante tem sido relacionado a grupos funcionais como -OH, -COOH e C=O encontrados em polissacarídeos, além de grupos funcionais aniônicos e catiônicos, como os ácidos urônicos (WANG et al., 2016). Outros pesquisadores, porém, sugerem que tais atividades são atribuídas aos compostos fenólicos e proteicos atrelados à matriz extracelular, especialmente quando as análises foram realizadas utilizando polissacarídeos brutos ou semi-purificados (WANG et al., 2016).

Além das propriedades citadas anteriormente, as fibras alimentares possuem ação antioxidante. Fibras antioxidantes podem ser definidas como um produto contendo quantidades significativas de antioxidantes naturais, tais como compostos fenólicos, associados à matriz de fibras. A determinação de sua ação antioxidante complementa a caracterização de tais fibras e auxilia a avaliação de seus efeitos e aplicações na saúde como um ingrediente (SAURA-CALIXTO, 2011).

Atualmente muitos métodos de química são amplamente utilizados para examinar o potencial antioxidante. No entanto, eles não levam em conta as condições fisiológicas das células, a biodisponibilidade da molécula antioxidante, bem como o metabolismo celular geral (WOLFE & LIU, 2007). Portanto, tem havido um interesse crescente no uso de sondas fluorescentes para monitorar ROS em sistemas *in vitro*, pois são sensores excepcionais para detecção de ROS (YAZDANI, 2015). Previamente em nosso grupo de pesquisa foi verificada a redução de níveis de espécies reativas e do efeito citotóxico induzido por H_2O_2 em células de fibroblasto murino (NIH 3T3) a partir de tratamento com polissacarídeo péctico de acerola (*Malpighia emarginata*), rico em arabinana, com o uso da sonda DCFH-DA (KLOSTERHOFF et al., 2017).

Desta forma testamos neste trabalho se fibras alimentares extraídas do resíduo agroindustrial de guavira apresentavam efeito antioxidante, avaliado pelo método ABTS, assim como pelo efeito antioxidante intracelular contra o estresse oxidativo induzido por H_2O_2.

3 OBJETIVOS

3.1 OBJETIVO GERAL

Purificar e caracterizar estruturalmente polissacarídeos extraídos de resíduo agroindustrial de guavira (*Campomanesia* sp) e avaliar seu potencial antioxidante.

3.2 OBJETIVOS ESPECÍFICOS

- Obter polissacarídeos de resíduo agroindustrial de guavira através de extração aquosa e alcalina;
- Purificar as frações polissacarídicas;
- Realizar a caracterização da estrutura química;
- Verificar atividade antioxidante em experimentos *in vitro,* tanto em método de sequestro de radicais livres como em linhagem celular de fibroblasto murino (NIH 3T3) submetido ao estresse oxidativo com H_2O_2.

ARTIGO I

Antioxidant dietary fibres from guavira pomace, a byproduct from fruit pulp industry

Antioxidant dietary fibres from guavira pomace, a byproduct from fruit pulp industry

Vanessa Suzane Schneider[a], Juliana Müller Bark[a], Sheila M. B. Winnischofer[a, b,c], Elisvânia Freitas Dos Santos[d], Marcello Iacomini[a, b], Lucimara M. C. Cordeiro[a, b*]

[a] *Postgraduate Program in Biochemistry Sciences, Sector of Biological Sciences, Federal University of Paraná, Curitiba, PR, 81531-990, Brazil.*

[b] *Department of Biochemistry and Molecular Biology, Federal University of Paraná, Curitiba, Brazil.*

[c] *Postgraduate Program in Cell and Molecular Biology, Federal University of Paraná, Curitiba, Brazil.*

[d] *Department of Food Technology and Public Health, Federal University of Mato Grosso do Sul, Campo Grande, MS, Brazil.*

[*] **Corresponding author**. Phone: +55 (41) 33611655; Fax: +55 (41) 32662042;

E-mail address: lucimaramcc@ufpr.br

ABSTRACT

Campomanesia sp is a fruit plant spread along the Brazilian Cerrado with established biological effects on its pulp, such as antimicrobial, anti-inflammatory, antinociceptive and antioxidant activity. Polysaccharides from Guavira Pomace Flour were obtained by aqueous extraction, analysed by sugar composition, GPC and NMR spectroscopy analysis. A fraction containing a mixture of arabinan-rich pectic polysaccharides, highly-methoxylated homogalacturonan (DM = 90%) and small amounts of arabinogalactan was obtained. In the present study, *in vitro* trolox equivalent antioxidant capacity (TEAC) of the extract was evaluated, as well as its quantitative phenolic and protein composition. MTT and DCFH-DA assay were performed to assess, respectively, changes in cell viability and the potential intracellular antioxidant activity against H_2O_2-induced oxidative stress in murine fibroblast cell line (NIH 3T3). The incubation of the cells with the aqueous extract for 48h remarkably decreased the levels of Reactive Oxygen Species (ROS) and the H_2O_2-induced cytotoxic effect. The results indicate that polysaccharides from guavira fruit pomace were able to exert intracellular antioxidant activity in murine fibroblasts, inducing a protection against pro-oxidant conditions.

Keywords: *Campomanesia sp*.; fruit pulp industry by-product; pectin; antioxidant activity.

1 INTRODUCTION

In the last years, an increasing demand on fruit production, trade and consumption has been noticed on the domestic and international markets. This generates, as consequence, a substantial amount of byproducts from processing, which consist on the fruit skin and other undesirable fruit elements (AYALA-ZAVALA et al., 2011).

The high amount of bioactive compounds present in fruit byproducts can be used as natural food additives, especially considering that agroindustrial waste is rich in dietary fibres (DF). Non-amidic polysaccharides from plant sources are recognised as Dietary Fibre if the "carbohydrate polymers presents 10 or more monomeric units, which are not hydrolysed by the endogenous enzymes in the small intestine of humans" (CODEX ALIMENTARIUS COMISSION, 2009). Food products may incorporate fibre-rich byproducts as inexpensive, non-caloric bulking agents for partial substitute of flour, fat or sugar, as enhancers of water and oil retention and to increase emulsion or oxidative stabilities (ELLEUCH et al., 2011). Moreover, DF presents beneficial human health effects related to promoting better functioning of the digestive system, mainly due increased satiety and the volume and weight of faecal matter. It also reduces the risk and occurrence of obesity, hyperglycaemia, hypercholesterolaemia, constipation, coronary heart disease, haemorrhoids and colon cancer (DHINGRA et al., 2012; WILLEM VAN DER KAMP et al, 2010). Furthermore, DF can be substrates for fermentation by bacteria in the large intestine, producing various end compounds (such as short chain fatty acids), energy and biomass leading to the maintenance of the gut microflora and improvement of the immune system (WILLIAMS et al, 2017).

Antioxidant capacity is another important property of DF as they may contribute significantly to health effects. Antioxidant DF can be defined as a product containing significant amounts of natural antioxidants, such as phenolic compounds, associated with the fibre matrix (SAURA-CALIXTO, 2011) and thus add antioxidant properties to fibre benefits.

In this perspective, exotic fruits and their byproducts may be valuable sources of antioxidant DF useful for food industry and human health. Guavira, also known as guabiroba-do-campo, is a plant widely distributed in the Brazilian Cerrado. Its fruit is appreciated *in natura* and may be used for the preparation of jellies, ice cream and soft drinks. The pulp of the fruit depicts most of the technological applicability due to its juiciness, fibre and mineral content, while its peel and seeds are considered industrial waste. Alves et al. (2013) and Fernandes et al. (2015) observed that hydroalcoholic extract from guavira residue (seeds and peel) presented antioxidant effect on DPPH and ABTS radical scavenging assays, even more

effectively than pulp extract. Guavira was, thus, considered a Brazilian fruit rich in antioxidant compounds.

In this work, we tested if dietary fibres extracted from pomace flour obtained as agroindustrial waste of a guavira pulp industry presented antioxidant effect. Trolox equivalent antioxidant capacity (TEAC) was assessed, along with antioxidant effect evaluation against H_2O_2-induced oxidative stress in NIH 3T3 cells. The DF containing fraction was also chemically characterized.

2 MATERIAL AND METHODS

2.1. PLANT MATERIAL

The guavira fruit pomace (containing remaining of pulp, peel, seeds and peduncle) was acquired from a fruit pulp industry (SR Ouro Verde Industry) in Bodoquena, at the State of Mato Grosso do Sul, Brazil, where the fruits were collected. The pomace may be constituted by a mixture of two species of guavira (*C. adamantium* and *C. pubescens*) commonly found in Midwestern Brazil. Guavira Pomace Flour (GPF) was oven-dried and fragmented in industrial blender.

2.2 EXTRACTION OF DIETARY FIBRES

The nonpolar compounds of GPF were extracted using methanol-chloroform (1: 1, v/v) in Soxhlet apparatus. DF were, in turn, extracted from the deffated residue with boiling water under reflux for 2 h (x 9; 1 L each), followed by filtration. The extract had its volume decreased by evaporation under reduced pressure and subsequently polysaccharides were precipitated in excess ethanol, collected by centrifugation (12,000 × g, 20 min), dialysed and freeze-dried, providing fraction CPW. DF yield was expressed as percentage based on the weight of GPF subjected to extraction (106.3 g).

2.3 SUGAR COMPOSITION

Neutral monosaccharide components of CPW and their ratios were assessed by hydrolysis with 2 M TFA for 8 h at 100 ºC, with subsequent to conversion to alditol acetates by $NaBH_4$ reduction and acetylation with anhydride-pyridine (1:1,v/v, 1 ml) at room temperature for 14 h. The alditol acetate analysis was carried out using GC–MS (Saturn 2000R–3800 gas chromatograph coupled to an Ion-Trap 2000R mass spectrometer, Varian, Walnut Creek, CA, USA), using a DB-225 column (30 m × 0.25 mm i.d.) programmed from 50 to 210 ºC at 40 ºC/min, with He as carrier gas.

Uronic acid content was spectrophotometrically determined using the modified sulfamate/m-hydroxybiphenyl method (Filisetti-Cozzi & Carpita, 1991), employing galacturonic acid as standard.

2.4 NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY

HSQC-DEPT NMR correlation map was acquired at 70ºC on a Bruker AVANCE III 400 NMR spectrometer, operating at 9.5 T, observing ^{13}C at 100.61 MHz and ^{1}H at 400.13 MHz, equipped with a 5-mm multinuclear inverse detection probe with z-gradient. The degree of methyl esterification of homogalacturonan present in CPW fraction was determined by ^{1}H NMR according to Grasdalen, Bakoy and Larsen (1988). The sample was dissolved in D_2O with chemical shifts expressed as δ PPM, using the resonances of CH_3 groups of acetone (δ_C 30.2/δ_H 2.22) as internal reference.

2.5 DETERMINATION OF HOMOGENEITY AND MOLECULAR WEIGHT

The homogeneity and molecular weight of CPW water-soluble polysaccharides were determined by gel permeation chromatography (GPC). The procedure was carried out as previously described by Leivas, Iacomini and Cordeiro (2015). Briefly, the sample was dissolved in the mobile phase at a concentration of 1 mg/mL, filtered in 0.22 μm membrane (Millipore) and analysed on a Waters chromatograph equipped with four Ultrahydrogel column connected in series (2000, 500, 250, 120; with size exclusion of 7×10^6 Da, 4×10^5 Da, 8×10^4 Da and 5×10^3 Da; Milford, MA, USA), coupled to a Waters 2410 (Milford, MA,

USA) differential refractometer (RI) detector. The mobile phase used was 0.1 mol/L sodium nitrite containing 0.2 g/L sodium azide at a flow rate of 0.6 mL/min and at 25 ºC. To obtain the molecular weight, standard dextrans (487 kDa, 266 kDa, 124 kDa, 72.2 kDa, 40.2 kDa, 17.2 kDa and 9.4 kDa, from Sigma) were employed to obtain the calibration curve.

2.6 TOTAL PHENOLIC COMPOUNDS QUANTIFICATION

Total phenolic compounds quantification was carried out using the Folin–Ciocalteu method (Singleton & Rossi, 1965), adapted to microplates. This method involves the reduction of the reagent by the phenolic compounds present in the sample and the formation of a blue complex. In sum, twenty microliters of CPW fraction at 10 mg/mL were pipetted to each well of a microplate. 100 µL of Folin–Ciocalteu reagent (diluted tenfold) and 75 µL of 7.5% sodium carbonate solution were added. The absorbance was read after 40 min incubation in the dark at 740 nm. A calibration curve of gallic acid at concentrations ranging from 20 to 120 µg/mL was built and the results were expressed as gallic acid equivalents (mg GAE per 100 g of sample dry weight).

2.7 PROTEIN QUANTIFICATION

Protein content of CPW was determined using Bradford method (Bradford, 1976). A calibration curve of albumin at concentrations ranging from 2 to 10 µg/mL was built and the results were expressed as g protein/100 g of sample.

2.8 TROLOX EQUIVALENT ANTIOXIDANT CAPACITY (TEAC) ASSAY

TEAC method adapted to microplates was used for the assessment of antioxidant capacity of CPW fraction, according to Al-Duais (2009). Trolox, a water-soluble analogue of vitamin E, was used as standard for antioxidant capacity determinations. This method is based on the production of $ABTS^{\bullet+}$ by mixing 6.62 mg $K_2S_2O_8$ in 10 mL water with 38.4 mg ABTS in 10 mL water. After 16 h at room temperature in the dark, $ABTS^{\bullet+}$ solution was diluted with pH 7.4 phosphate buffer solution to obtain an initial absorbance of 0.70 ± 0.10 at 734 nm. The calibration curve was prepared with phosphate buffer from fresh 2.5 mmol/L Trolox standard

solutions at the following dilutions: 0.0125, 0.050, 0.100, 0.150, 0.200 mmol/L. Next, 20 µL of the standard solutions or the sample (at concentrations ranging from 50-1000 µg/mL) were mixed with 220 µL of fresh $ABTS^{\bullet+}$ solution. $ABTS^{\bullet+}$ scavenging effect (%) was calculated according to the following equation: $ABTS^{\bullet+}$ scavenging effect (%) = (1− absorbance of sample/absorbance of control) × 100. All data were measured in three replications.

2.10 CELLULAR ANTIOXIDANT ACTIVITY (CAA) OF CPW

2.10.1 Cell culture

NIH 3T3 murine fibroblast cell line was kindly provided by Prof. Mari C. Sogayar (Cell and Molecular Therapy Center –NUCEL/NETCEM, Faculty of Medicine, University of São Paulo-FMUSP). The cell line was cultured in DMEM high glucose (Sigma-Aldrich) medium supplemented with 10% fetal bovine serum (Gibco), penicillin (100 U/mL), streptomycin (100 µg/mL), which was replaced three times a week, and cells were subcultured at maximum 90% confluence. Cells were maintained at 37 °C in humidity-saturated atmosphere containing 5% CO_2.

2.10.2 Cell viability assay

Cell viability of NIH 3T3 murine fibroblasts was measured by colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method. In sum, 5×10^3 cells/well were cultured on 96-well plates and grown at 37 °C in humidity-saturated atmosphere containing 5% CO_2.

For CPW viability assays, cells were treated with this fraction at 10 and 100 µg/mL for 24, 48 and 72 h. The cell viability after H_2O_2-induced toxicity was also carried out. After the culture times of 24, 48 and 72 h, cells were exposed to 100 µM H_2O_2 for 15 min.

Polysaccharide-containing culture medium or H_2O_2 solution was removed after treatment period and replaced with 200 µL of MTT (0.5 mg/mL). After 3 h incubation (37 °C, 5% CO_2), the formazan crystals formed on viable cells were dissolved in 200 µL of dimethyl sulfoxide (DMSO) and absorbance read at 545 nm. Results were expressed as percentage of control (culture medium) or vehicle control (milliQ water used to dissolve the extract at 10

μg/mL concentration) assigned as 100% of viability. Vehicle control of 10 μg/mL and 100 μg/mL concentrations were not statistically different (data not shown).

2.10.3 Treatment procedure

For treatment, 5×10^3 cells/well were firstly cultured in 96-well plates and incubated for 24 h at 37 ºC. Thereafter, cells were treated with 10 μg/mL of CPW for 48 h, polysaccharide-containing culture medium was removed, and cells were exposed to 100 μM H_2O_2 for 15 min. MTT method was performed as described above as also the evaluation of intracellular levels of reactive oxygen species (ROS).

2.10.4 Intracellular ROS measurement

Intracellular ROS levels were evaluated using DCFH-DA (2',7'-Dichlorofluorescin diacetate, Sigma-Aldrich). Cells were seeded on black 96-well plates, treated with CPW and exposed to H_2O_2 as previously described, and then incubated with 5 mM DCFH-DA at 37 ºC for 30 min. Fluorescence was measured on a spectrofluorometer (Infinite M200, Tecan Trading AG, Switzerland) with a 485 nm excitation and a 520 nm emission.

2.11 DATA ANALYSIS

All data are expressed as mean ± SEM of three independent experiments in sextuplicate. Comparisons were performed by one-way analysis of variance (ANOVA) followed by Tukey's. Difference of $p < 0.05$ was considered to be statistically significant.

3 RESULTS AND DISCUSSION

3.1 EXTRACTION AND CHARACTERIZATION OF DF FROM GUAVIRA FRUIT POMACE

GPF was defatted and the DF extracted with boiling water, obtaining fraction CPW (8.5% yield). It was mainly composed of arabinose (46.7%) and uronic acids (44.6%),

indicating the presence of arabinan-rich pectic polysaccharides. The fraction also contained minor amounts of xylose (1.3%), mannose (0.8%), glucose (1.1%) and galactose (5.5%). On gel permeation chromatography (Fig. 1), a main peak was observed, with relative M_w of 126 kDa. It had 1.8% of protein and total phenolic content of 3.2 g GAE/100 g of CPW sample, indicating that some amounts of phenolic compounds may be complexed with DF present in CPW fraction.

CPW fraction was analysed by HSQC-DEPT NMR experiment (Fig. 2). The signals at δ 100.1/4.96 and 99.3/5.14 (C-1/H-1) and at δ 70.9/5.04 and 71.7/4.67 (C-5/H-5) were assigned to carbons and hydrogens of esterified and unesterified units of α-D-Gal*p*A, respectively. Signals of acetyl carbons and methyl carbons of esterified carbonyls in Gal*p*A units appeared at δ 20.7/2.10 and 53.0/3.81, respectively. Other assignments of α-D-Gal*p*A units were seen at δ 78.8/4.46 (C-4/H-4), δ 68.6/3.99 (C-3/H-3) and δ 68.5/3.74 (C-2/H-2). These assignments are in agreement with published literature data (Nascimento, Iacomini & Cordeiro, 2017; Cantu-Jungles et al., 2015). The degree of methyl esterification (DM) was determined according to Grasdalen, Bakoy and Larsen (1988) obtaining a DM of 90%, characterizing this polymer as high-methoxyl (HM) pectin. In accordance with sugar analysis, signals of Ara units were also present in NMR spectra (Fig. 2). The chemical shifts of anomeric carbons at δ 107.8/5.08 and 107.2/5.18 confirmed that L-Ara adopted mainly α-configuration and the furanose form. The HSQC-DEPT NMR confirmed C-5 *O*-substitution of α-L-Ara*f* units, presenting inverted low field resonance at δ 67.0/3.85. Inverted signals at δ 61.5/3.82 and 61.5/3.75 can be assigned to unsubstituted C-5 of α-L-Ara*f* units (Cantu-Jungles et al., 2015; Cordeiro et al., 2012). In addition, a signal at δ 101.6/5.10 was observed and tentatively assigned to β-L-Ara*f* units, according to literature (Das et al, 2013; Shakhmatov et. al., 2014). Moreover, it was possible to identify small amounts of β-D-Gal*p* units due the signals at δ 104.5/4.60 and 103.2/4.49 (C-1/H-1) (Nascimento et al., 2015; Cipriani, et al., 2009). At last, a small signal at δ 16.9/1.26 revealed the presence of CH_3-6 of Rha*p* units (Cipriani, et al., 2009), which was not perceived in composition analysis possibly due its acid-resistant glycosidic linkage to GalA units, not allowing its detection by GC-MS.

As far as we know, there are no reports in the literature about DF from *C. pubescens* and *C. adamantium* yet. Concerning polysaccharides present in *Campomanesia* genus only one report was found in the literature. Recently, Barbieri et al. (2017) have isolated and structurally characterized a galactoglucomannan from the pulp of *C. xanthocarpa* Berg collected from Irati-Paraná/Brazil. Thus, the presence of high methyl esterified homogalacturonan and arabinan-rich pectic polysaccharides in *Campomanesia* genus is

reported for the first time in this study. Structurally diverse arabinans have already been reported for some fruits, such as pomegranate (Shakhmatov et al., 2019), starfruit (Leivas et al., 2015), buriti (Cantu-Jungles et al., 2015) and olive (Cardoso, Silva & Coimbra, 2002). Additionally, arabinan and arabinan-rich pectins have expressed diversified bioactivities, including anti-fatigue (Klosterhoff et al., 2018), gastro-protective (Cordeiro et al., 2012), as well as immunological (Dourado et al., 2004; Mandal et al., 2013) and anticoagulant activities (Fernandez et al., 2013). Moreover, an arabinan-rich pectic polysaccharide isolated from acerola (*Malpighia emarginata*) in our research group exhibited a significant intracellular antioxidant activity in the cell culture model used in the present study (Klosterhoff et al., 2017).

3.3 TEAC ASSAY

Many plant polysaccharides are explored as potential antioxidants and/or free radical scavengers. The free radical scavenging potential of the guavira DF present in CPW fraction was tested chemically prior to introduction into cellular antioxidant assay. As shown in figure (Fig. 3A), the inhibitory effect of CPW on ABTS radicals was found to be concentration dependent. At the lowest concentration it presented a far lower scavenging activity than Trolox. At the highest concentration, however, the scavenging rate of CPW was 67.4%, similar to Trolox at 50 μg/mL concentration (63% of scavenging activity). Likewise, Ai et al. (2017) noticed approximately 60% of ABTS radical scavenging effect of a pectic polysaccharide from *Graptopetalum paraguayense* leaves at 1.0 mg/mL. This result indicated that CPW has potential on free radical scavenging and could help preventing oxidative stress.

3.4 CELLULAR ANTIOXIDANT ACTIVITY ASSAY

3.3.1 Effects of CPW on NIH 3T3 cell line viability

Since CPW expressed *in vitro* antioxidant activity, it was tested in cellular antioxidant activity assay, which better represents the complexity of biological systems. NIH 3T3 cells were treated with CPW at 10 and 100 μg/mL for 24 h, 48 h and 72 h, and cytotoxic effect was evaluated by MTT method. Vehicle effect (milliQ water) was also carried out (Fig. 4). The results showed that no differences were observed between the viability of vehicle and control (culture medium only). Moreover, CPW did not show negative effects on cell viability at 10 and 100 μg/mL concentrations when compared to control group. Although a decrease of metabolically active cells has been observed at 100 μg/mL concentration in all the tested times, no significative difference was observed by statistical analysis. Provided that CPW had no effects on viability on NIH 3T3 cells using both concentrations, the subsequent experiments were performed with the lowest (10 μg/mL) concentration during 48 h of treatment. Compared to control, this treatment preserved cell viability at 100%.

3.3.2 Cytoprotective activity of CPW against H_2O_2-induced toxicity

Firstly, cells were exposed to 100 μM H_2O_2 during 15 min after different culture periods. H_2O_2-induced toxicity effectively promoted oxidative stress on NIH 3T3 cells, decreasing its viability to 56%, 61% and 71% at 24, 48 and 72 h of culture, respectively (Fig. 5A). In sequence, cytoprotective effect of CPW was tested. Cells were treated with CPW at 10 μg/mL and, after 48 h, the medium containing the fraction was removed, followed by cell exposure to 100 μM H_2O_2-induced toxicity (15 min). Comparing to control, cell viability decreased to 65% when exposed to H_2O_2. The pretreatment of cells with CPW for 48 h effectively protected the cells against the toxic effects of H_2O_2, increasing the cell viability to 77.6% (Fig. 5B). Thereafter, intracellular ROS level was measured with the aim to evidence whether CPW exhibits intracellular antioxidant capacity.

3.3.3 Effect of CPW on intracellular ROS levels

Following pretreatment with CPW (10 μg/mL) for 48 h, intracellular ROS production in NIH 3T3 cells was detected using 2',7'-dichlorofluorescein diacetate (DCFH-DA) probe. DCFH-DA is a non-fluorescent compound that can be oxidized by ROS to generate the fluorescent compound DCF. As expected, H_2O_2-induced toxicity promoted an increase in the intracellular ROS levels compared with control (Fig. 6). Nevertheless, the group pretreated with CPW (10 μg/mL) for 48 h statistically lowered ROS levels, when compared to the control and also compared with cells exposed to H_2O_2 (100 μM) toxicity. These results demonstrated the protective effect of guavira DFs against the oxidative stress induced by the H_2O_2 in NIH 3T3 cells.

Some studies have used the DCFH-DA probe to investigate the intracellular antioxidant capacity of different polysaccharides after damage induced by H_2O_2. Polysaccharides from Goji berry fruit (*Lycium barbarum*) decreased cell apoptosis and ROS levels in human lens epithelial cell lines (SRA01/04) (QI et al., 2014), while Chowdhury et al., (2014) observed a significant reduction of the levels ROS in human fibroblasts (WI38) after treatment with polysaccharide from the bacteria *Bacillus megaterium*. Similarly, the reduction of reactive species levels and the cytotoxic effect induced by H_2O_2 in murine fibroblast cells (NIH 3T3) were observed after treatment with polysaccharide fraction from acerola (*Malpighia emarginata*) (Klosterhoff et al., 2017).

Alves et al. (2013) and Fernandes et al. (2015) have previously reported that hydroalcoholic extract from guavira pomace was more effective in antioxidant protection than compounds from their pulp. Here we have shown that guavira pomace dietary fibres also presented antioxidant activity, by scavenging the ABTS radical *in vitro*, as well as by protecting fibroblast cells from pro-oxidant condition induced by H_2O_2.

Importantly, concerning the applicability in human consumption, guavira peel can be considered safe since it was observed that it did not cause any lethality or changes in the general behavior in rats in both acute and subacute toxicity studies (Souza et al., 2014). These facts evidence that guavira pomace is a valuable source of antioxidant dietary fibres, therefore, this underexploited agroindustrial byproduct could have great application in food and nutraceutical industry.

ACKNOWLEDGMENTS

The authors are grateful to CAPES-PROEX 0587/2018 (Grant number 23038.000817/2018-93 and CNPq (Grant numbers 404717/2016-0, 310332/2015-0) for financial support. The author VSS received a fellowship from CAPES - Fundação Araucária (Process 19-2015). The authors are grateful to NMR Center of UFPR for recording the NMR spectrum.

REFERENCES

AI, L.; et al. Antioxidant hydrocolloids from herb *Graptopetalum paraguayense* leaves show anti-colon cancer cells and anti-neuroinflammatory potentials. **Food Hydrocolloids**, v. 73, p. 51-59, 2017.

AL-DUAIS, M., MÜLLER, L., BÖHM, V. et al. Antioxidant capacity and total phenolics of *Cyphostemma digitatum* before and after processing: use of different assays. **Eur Food Res Technol**, v. 228, p. 813, 2009.

ALVES, A.M.; ALVES, M.S.O.; FERNANDES, T.O.; NAVES, R.V.; NAVES, M.M.V. Caracterização física e química, fenólicos totais e atividade antioxidante da polpa e resíduo de gabiroba. **Rev Bras Fruticultura**, v. 35, p. 837-844, 2013.

AYALA-ZAVALA, J. F.; VEGA-VEGA, V.; ROSAS-DOMÍNGUEZ, C.; PALAFOX-CARLOS, H.; VILLA-RODRIGUEZ, J. A.; WASIM SIDDIQUI, MD.; et al. Agro-industrial potential of exotic fruit byproducts as a source of food additives. **Food Research International**, v. 44, p. 1866–1874, 2011.

BARBIERI, S.F.; RUTHES, A. C.; PETKOWICZ, C. L. O.; GODOY, R. C. B.; SASSAKI, G. L.; SANTANA-FILHO, A. P.; SILVEIRA, J. L. M. Extraction, purification and structural characterization of a galactoglucomannan from the gabiroba fruit (*Campomanesia xanthocarpa* Berg), Myrtaceae family. **Carbohydrate Polymers**, v. 174, p. 887–895, 2017.

BRADFORD, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, **Anal. Biochem**, v. 72, p. 248–254, 1976.

CANTU-JUNGLES, T.M.; ALMEIDA, C. P.; IACOMINI, M.; CIPRIANI, T. R.; CORDEIRO, L. M.C. Arabinan-rich pectic polysaccharides from buriti (*Mauritia flexuosa*): An Amazonian edible palm fruit. **Carbohydrate Polymers**, v. 122, p. 276–281, 2015.

CARDOSO, S. M.; SILVA, A. M. S.; COIMBRA, M. A. Structural characterisation of the olive pomace pectic polysaccharide arabinan side chains. **Carbohydrate Research**, v. 337, p. 917–924, 2002.

CIPRIANI, T. R.; MELLINGER, C. G.; BERTOLINI, M. L. C.; BAGGIO, C. H.; FREITAS, C. S.; MARQUES, M. C. A.; GORIN, P. A. J.; SASSAKI, G. L.; IACOMINI, M. Gastroprotective effect of a type I arabinogalactan from soybean meal. **Food Chemistry**, v. 115, n. 2, p. 687-690, 2009.

CHOWDHURY, R. S. et al. Bacterial fucose-rich polysaccharide stabilizes MAPKmediated Nrf2/Keap1 signaling by directly scavenging reactive oxygen species during hydrogen peroxide-induced apoptosis of human lung fibroblast cells. **PloS one**, v. 9, n. 11, p. e113663, 2014.

CODEX ALIMENTARIUM COMMISSION. ALINORM 09/32/26, definition of dietary fiber. p. 27-54 and Appendix II, 2009.

CORDEIRO, L. M. C.; REINHARDT, V. D.; BAGGIO, C. H.; WERNER, M. F. D.; BURCI, L. M.; SASSAKI, G. L.; IACOMINI, M. Arabinan and arabinan-rich pectic polysaccharides from quinoa (*Chenopodium quinoa*) seeds: structure and gastroprotective activity. **Food Chemistry**, v. 130, n. 4, p. 937-944, 2012.

DAS, D. et al. A new arabinoxylan from green leaves of *Litsea glutinosa* (Lauraeae): Structural and biological studies. **Carbohydrate Polymers**, v. 92, p. 1243–1248, 2013.

DHINGRA, D.; MICHAEL, M.; RAJPUT, H.; PATIL, R. Dietary fibre in foods: A review. **Journal of Food Science and Technology-Mysore**, v. 49, n. 3, p. 255–266, 2012.

DOURADO, F.; MADUREIRA, P.; CARVALHO, V.; COELHO, R.; COIMBRA, M. A.; VILANOVA, M.; MOTA, M.; GAMA, F. M. Purification, structure and immunobiological activity of an arabinan-rich pectic polysaccharide from the cell walls of *Prunus dulcis* seeds. **Carbohydrate Research**, v. 339, p. 2555–2566, 2004.

ELLEUCH, M.; BEDIGIAN, D.; ROISEUX, O.; BESBES, S.; BLECKER, C.; ATTIA, H. Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. **Food Chemistry**, v. 124, p. 411−421, 2011.

FERNANDES, T.O.; ÁVILA, R.I.; MOURA, S.S.; RIBEIRO, G.A.; NAVES, M.M.V.; VALADARES, M.C. *Campomanesia adamantium* (Myrtaceae) fruits protect HEPG2 cells against carbon tetrachloride-induced toxicity. **Toxicol Rep**, v. 2, p. 184-193, 2015.

FERNANDEZ, P. V.; QUINTANA, I.; CEREZO, A. S.; CARAMELO, J. J.; POL-FACHIN, L.; VERLI,H.; et al. Anticoagulant activity of a unique sulfated pyranosic (1→3)-beta-l-arabinan through direct interaction with thrombin. **Journal of Biological Chemistry**, v. 288, p. 223–233, 2013.

FILISETTI-COZZI, T. M. C. C.; CARPITA, N. C. Measurement of uronic acids without interference from neutral sugars**. Analytical Biochemistry**, v. 197, n. 1, p. 157-162, 1991.

GRASDALEN, H.; EINAR BAKOY, O.; LARSEN, B. Determination of the degree of esterification and the distribution of methylated and free carboxyl groups in pectins by ^{1}H-N.M.R spectroscopy, **Carbohydr. Res.**, v. 184, p. 183–191, 1988.

KLOSTERHOFF, R.R.; BARK, J.M.; GLÄNZEL, N.M.; IACOMINI, M.; MARTINEZ, G.R.; WINNISCHOFER, S.M.B.; CORDEIRO, L.M.C. Structure and intracellular antioxidant activity of pectic polysaccharide from acerola (*Malpighia emarginata*). **Int J Biol Macromol**, v.106, p. 473-480, 2017.

KLOSTERHOFF, R.R. et al. Anti-fatigue activity of anarabinan-rich pectin from acerola (*Malpighia emarginata*). **International Journal of Biological Macromolecules** v. 109, p. 1147–1153, 2018.

LEIVAS, C.L.; IACOMINI, M.; CORDEIRO, L.M.C. Structural characterization of a rhamnogalacturonan I-arabinan-type I arabinogalactan macromolecule from starfruit (*Averrhoa carambola* L.), **Carbohydr. Polym**., v. 121, p. 224–230, 2015.

MANDAL, E. K.; MANDAL, S.; MAITY, S.; BEHERA, B.; MAITI, T. K.; ISLAM, S. S. Structural studies of an immunostimulating gluco-arabinan from seeds of *Caesalpinia bonduc*. **Carbohydrate Polymers**, v. 92, p. 704–711, 2013.

NASCIMENTO, G.E.; CORSO, C.R.; WERNER, M. F. P.; BAGGIO, C. H.; IACOMINI, M.; CORDEIRO, L.M.C. Structure of an arabinogalactan from the edible tropical fruit tamarillo (*Solanum betaceum*) and its antinociceptive activity. **Carbohydrate Polymers**, v. 116, p. 300–306, 2015.

NASCIMENTO, G.E.; IACOMINI, M.; CORDEIRO, L.M.C. New findings on green sweet pepper (*Capsicum annum*) pectins: Rhamnogalacturonan and type I and II arabinogalactans. **Carbohydrate Polymers**, v. 171, p. 292–299, 2017.

QI, B.; JI, Q.; WEN, Y.; LIU, L.; GUO, X.; HOU, G.; WANG, G.; ZHONG, J. *Lycium barbarum* polysaccharides protect human lens epithelial cells against oxidative stress–induced apoptosis and senescence, **PLoS One 9**, e110275, 2014.

SAURA-CALIXTO, F. Dietary Fiber as a Carrier of Dietary Antioxidants: An Essential Physiological Function. **J. Agric. Food Chem**, v. 59, p. 43–49, 2011.

SINGLETON, V.L.; ROSSI, J.A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, **Am. J. Enol. Vitic**, v. 16, p. 144–158, 1965.

SHAKHMATOV, E.G.; et al. Structural studies of arabinan-rich pectic polysaccharides from *Abies sibirica* L. Biological activity of pectins of *A. sibirica*. **Carbohydrate Polymers**, v. 113, p. 515–524, 2014.

SHAKHMATOV, E.G.; et al. Structural studies of biologically active pectin-containing polysaccharides of pomegranate *Punica granatum*. **International Journal of Biological Macromolecules**, v. 122, p. 29–36, 2019.

SOUZA. J.C.; PICCINELI, A.C.; AQUINO, D.F.; et al. Toxicological analysis and antihyperalgesic, antidepressant, and anti-inflammatory effects of *Campomanesia adamantium* fruit barks. **Nutr Neurosci**, v. 0, p. 23-31, 2014.

WILLEM VAN DER KAMP, J.; et al. Dietary Fibre: New Frontiers for Food and Health, 2010. In: SAURA-CALIXTO, F. Dietary Fiber as a Carrier of Dietary Antioxidants: An Essential Physiological Function. **J. Agric. Food Chem**., v. 59, p. 43–49, 2011.

WILLIAMS, B. A.; GRANT, L. J.; GIDLEY, M. J.; MIKKELSEN, D. Gut Fermentation of Dietary Fibres: Physico-Chemistry of Plant Cell Walls and Implications for Health. **Int. J. Mol. Sci**, v. 18, p. 2203, 2017.

Figure Legends

Fig. 1. GPC elution profile of pectic polysaccharides present in CPW fraction, obtained from *Campomanesia* sp. (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 plot the calibration curve.

Fig. 2. $^{1}H/^{13}C$ HSQC-DEPT correlation map of CPW fraction. Sample was dissolved in deuterium oxide and data collected at probe temperature of 70 ºC. Inverted signals in DEPT experiment are marked with an asterisk (*).

Fig. 3. Scavenging effects of CPW on $ABTS^{\bullet+}$ (A). Trolox is used as reference (B).

Fig. 4. Effect of CPW on NIH 3T3 cell viability. Cells were treated with CPW (10 and 100 μg/mL) or vehicle (milliQ water) or control (medium only) for 24, 48 and 72 h. The proportion of viable cells was measured by MTT assay. Data are presented as mean ± SEM from three independent experiments, each in sextuplicate. Statistical comparison was performed using one-way (ANOVA) followed by Tukey's.

Fig. 5. Cell viability after H_2O_2-induced toxicity. Cells were cultured for 24, 48 and 72 h and then exposed to 100 μM H_2O_2 for 15 min (A). Cytoprotective effect of CPW on NIH 3T3 cell viability after H_2O_2-induced toxicity. Cells treated with either CPW (10 μg/mL) or control for 48 h prior to H_2O_2 (100 μM) addition for 15 min. The proportion of viable cells was evaluated by MTT assay. Data are presented as mean ± SEM from three independent experiments, each in sextuplicate. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test

was performed and * $p < 0.05$, ***$p < 0.001$ considered significantly different from control and ## $p < 0.05$, ### $p < 0.001$ considered significantly different from control + H_2O_2.

Fig. 6. ROS measurement. Cells were pretreated with CPW (10 μg/mL) or control for 48 h prior to H_2O_2 (100 μM) exposure for 15 min. The levels of ROS were measured by addition of DCFH-DA probe. Data are presented as mean ± SEM from three independent experiments, each in sextuplicate. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was performed and * $p < 0.05$ considered significantly different from control and ### $p < 0.001$ considered significantly different from control + H_2O_2.

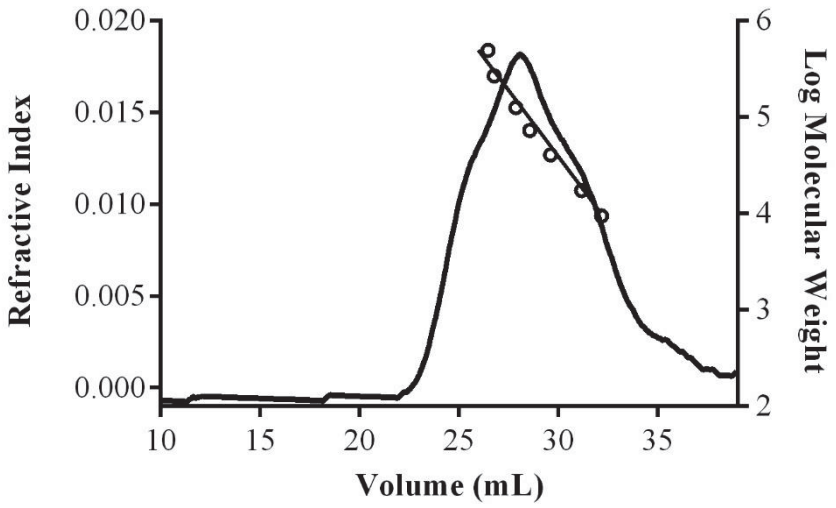

Refractive Index
0.020
0.015
0.010
0.005
0.000
10
15
20
25
30
35
Volume (mL)
6
5
4
3
2
Log Molecular Weight

Figure 1

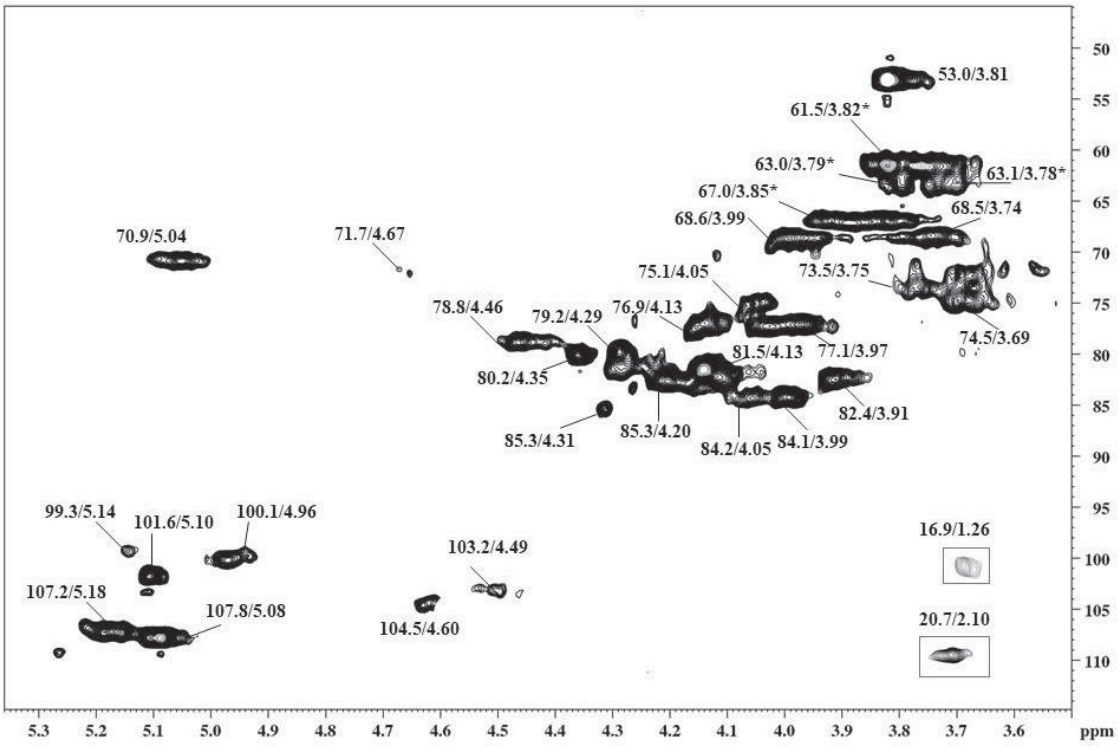

53.0/3.81
61.5/3.82*
63.0/3.79*
63.1/3.78*
67.0/3.85*
68.5/3.74
68.6/3.99
70.9/5.04
71.7/4.67
73.5/3.75
75.1/4.05
78.8/4.46
79.2/4.29
76.9/4.13
74.5/3.69
81.5/4.13
77.1/3.97
80.2/4.35
82.4/3.91
85.3/4.31
85.3/4.20
84.2/4.05
84.1/3.99
99.3/5.14
101.6/5.10
100.1/4.96
103.2/4.49
16.9/1.26
107.2/5.18
107.8/5.08
104.5/4.60
20.7/2.10
5.3 5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 ppm
50 55 60 65 70 75 80 85 90 95 100 105 110

Figure 2

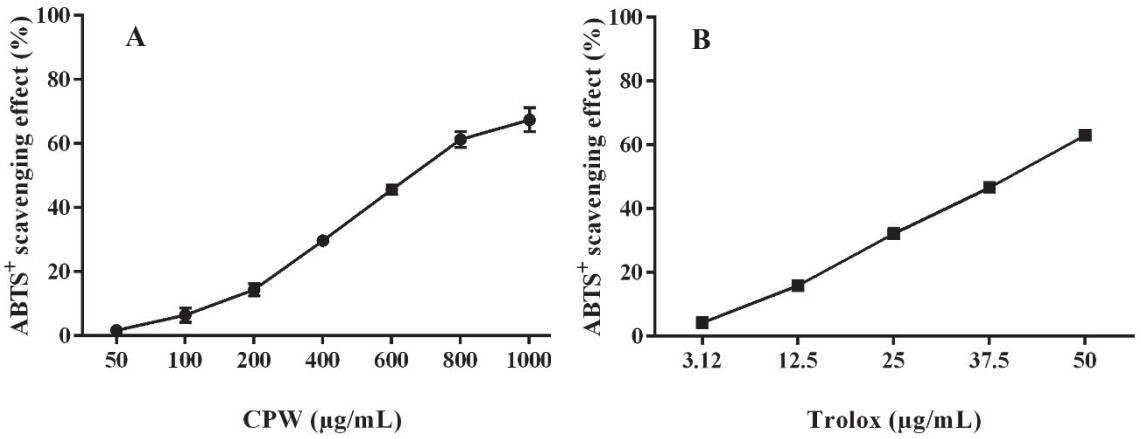

A
ABTS+ scavenging effect (%)
100
80
60
40
20
0
50
100
200
400
600
800
1000
CPW (µg/mL)
B
ABTS+ scavenging effect (%)
100
80
60
40
20
0
3.12
12.5
25
37.5
50
Trolox (µg/mL)

Figure 3

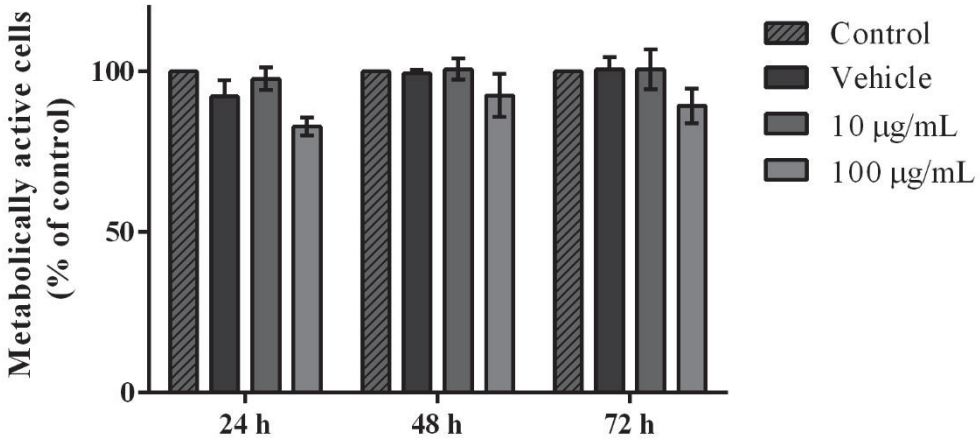

Metabolically active cells
(% of control)
100
50
0
24 h
48 h
72 h
Control
Vehicle
10 µg/mL
100 µg/mL

Figure 4

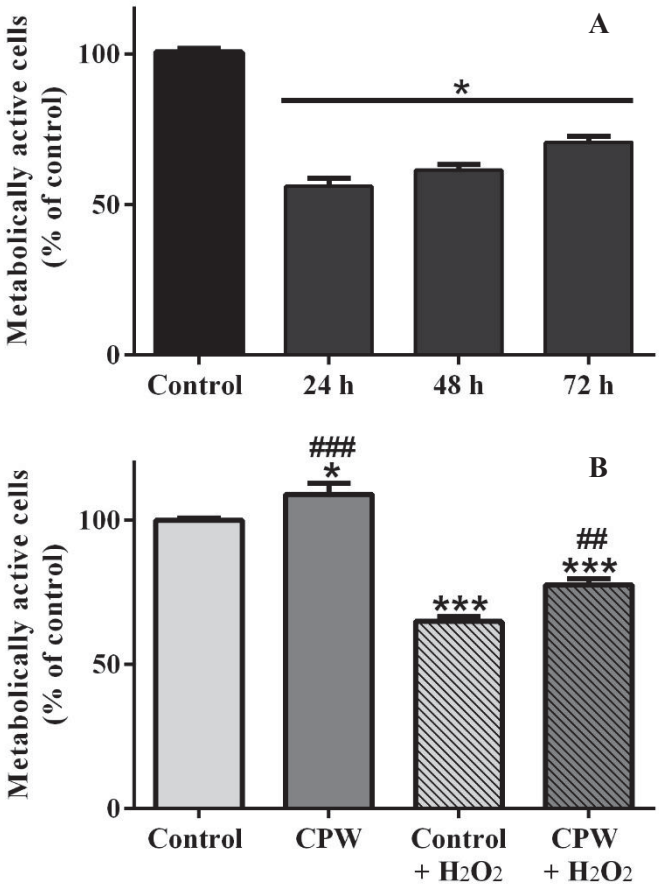

A
Metabolically active cells (% of control)
100
50
0
*
Control
24 h
48 h
72 h
B
Metabolically active cells (% of control)
100
50
0
###
*
***
##
***
Control
CPW
Control + H2O2
CPW + H2O2

Figure 5

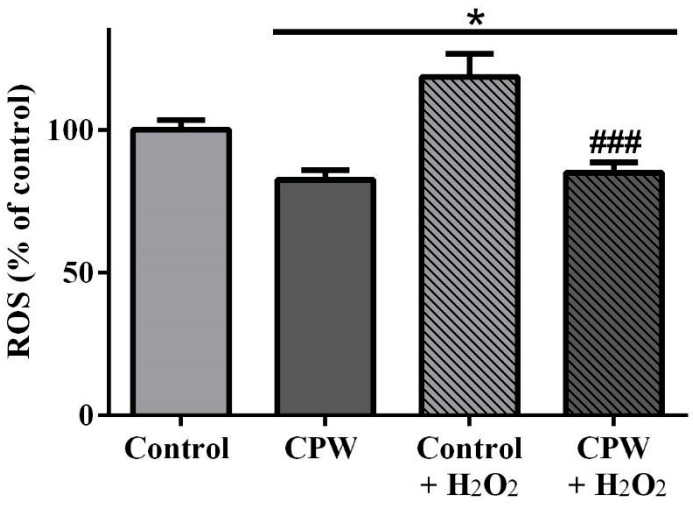


Figure 6

ARTIGO II

β-L-Araf-containing arabinan and glucuronoxylan from guavira fruit pomace

β-L-Ara*f*-containing arabinan and glucuronoxylan from guavira fruit pomace

Vanessa Suzane Schneider[a], Marcello Iacomini[a, b], Lucimara M. C. Cordeiro[a, b*]

[a] *Postgraduate Program in Biochemistry Sciences, Sector of Biological Sciences, Federal University of Paraná, Curitiba, PR, 81531-990, Brazil.*

[b] *Department of Biochemistry and Molecular Biology, Federal University of Paraná, Curitiba, Brazil.*

[*] **Corresponding author**. Phone: +55 (41) 33611655; Fax: +55 (41) 32662042;

E-mail address: lucimaramcc@ufpr.br

ABSTRACT

Guavira is a plant that belongs to Myrtaceae family, being widespread in the Brazilian Cerrado. In this study, pectic and hemicellulosic polysaccharides from guavira pomace, an agroindustry residue from pulp industry, were structurally characterized using GPC, monosaccharide composition, methylation and NMR experiments. Pectic polysaccharides were composed of a highly methoxylated homogalacturonan (DM = 90%) and other arabinan-rich pectic polysaccharides. Arabinan was purified, presenting Ara (85%), Rha (3.3%), Gal (7.7%) and GalA (4%). Mono and bidimensional NMR analyses of this arabinan demonstrated the presence of terminal β-L-Ara*f* units, whose occurrence has been scarcely reported in the literature. Hemicellulosic fraction contained a glucuronoxylan, with 4-*O*-methyl-D-Glc*p*A group linked to *O*-2 of a (1→4)-β-D-xylan, presenting one 4-*O*-methyl-D-Glc*p*A for every six xylose units.

Keywords: *Campomanesia sp*.; fruit pulp industry by-product; arabinan; glucuronoxylan.

1 INTRODUCTION

In recent years, the awareness on the direct link between dietary non-starch polysaccharides and a wide range of potential health benefits has increased and recognized the importance of polysaccharides in the fields of therapeutics and nutrigenomics (KUMAR et al., 2012). Moreover, physicochemical properties of polysaccharides play a substantial role in determining some fruit characteristics, such as texture and flavour that are important for consumers and food industry (LADANIYA, 2008). The health benefits and industrial applications of polysaccharides are correlated to their chemical structures (monosaccharide composition, molar mass, degree of branching, type and configuration of the glycosidic linkage). In the light of these considerations, information about polysaccharide structure and organization is fundamental to understand nutritional and technological aspects of fruits.

Campomanesia pubescens and *C. adamantium* are native species found in Brazil, abundantly distributed in the Brazilian Cerrado. Belonging to Myrtaceae family, they are popularly known as guavira or guabiroba-do-campo. Guavira fruit is consumed *in natura* and used as sweet, ice cream, soft drinks, liqueurs ingredients and as flavourings in alcoholic distillates (DOUSSEAU et al., 2011), what depicts its economic value and sociocultural importance in Midwestern Brazil.

In a previous study Schneider et al (unpublished) reported that a pectic fraction (CPW) obtained from guavira pomace displayed antioxidant activity in $ABTS^{\bullet+}$ scavenging *in vitro* assay, as well as protected NIH 3T3 murine fibroblast cells from oxidative stress induced by H_2O_2 cytotoxic effect. Since CPW may have unusual structural motifs, like the presence of β-L-Ara*f* units, in this work we performed the purification of polysaccharides from CPW fraction and determined their fine chemical structure. The evaluation of chemical strutures of hemicellulosic polysaccharides was also determined.

2 MATERIAL AND METHODS

2.1 PLANT MATERIAL

Guavira fruit pomace (containing remaining of pulp, peel, seeds and peduncle) was acquired from a pulp industry (SR Ouro Verde Industry) in Bodoquena, at the State of Mato

Grosso do Sul, Brazil, where the fruits were collected. The pomace may be constituted by a mixture of two species of guavira (*Campomanesia adamantium* and *C. pubescens*) commonly found in Midwestern Brazil. Guavira Pomace Flour (GPF) was oven-dried and fragmented in industrial blender.

2.2 EXTRACTION AND PURIFICATION OF PECTIC POLYSACCHARIDES

GPF (106.3 g) was defatted with chloroform-methanol (1: 1, v/v) in Soxhlet apparatus. Pectins, present as water soluble polysaccharides (CPW), were extracted as described by Schneider et al. (unpublished) and shown schematically in Fig. 1. Briefly, pectins were extracted from the deffated residue with boiling water under reflux for 2 h (x 9; 1 L each), followed by filtration. After concentration, polysaccharides were precipitated in excess ethanol, collected by centrifugation (12,000 × g, 20 min), dialysed and freeze-dried, providing fraction CPW.

Fraction CPW was then treated with Fehling's solution (JONES & STOODLEY, 1965), resulting in a soluble fraction (CPW-SF) and a Cu^{+2}-precipitated fraction (CPW-PF), which were separated by centrifugation (12,000 × g, 20 min at 10°C). Both fractions were neutralized with acetic acid, dialyzed against tap water and deionized with cation exchange resin. Fraction CPW-SF (1.14 g) was posteriorly purified by sequential ultrafiltration through membranes with 100, 50 and 30 kDa cut-offs (Ultracel, Millipore). All the extraction and purification procedures are summarized in Fig. 1.

2.3 EXTRACTION AND PURIFICATION OF HEMICELLULOSIC POLYSACCHARIDES

The remaining residue after CPW extraction was treated with aq. 10% KOH under reflux at 100 °C for 2 h (×6, 1 L each), in order to solubilize hemicellulosic polysaccharides. Thereafter, it was filtered, neutralized with acetic acid and dialyzed against tap water (Cellulose Spectrum labs 12–14 kDa cut-off), giving alkali soluble polysaccharides (CPK fraction) (Fig. 1). Then, CPK was treated with Fehling's solution, as described above, giving CPK-SF and CPK-PF fractions (Fig. 1). At last, CPK-SF was submitted to 10%

trichloroacetic acid precipitation, giving soluble (CPK-SF-STCA) and insoluble (CPK-SF-PTCA) fractions.

2.4 SUGAR COMPOSITION

Neutral monosaccharide components of polysaccharides and their ratios were assessed by hydrolysis with 2 M TFA for 8 h at 100 ºC, with subsequent to conversion to alditol acetates by $NaBH_4$ reduction and acetylation with anhydride-pyridine (1:1, v/v, 1 ml) at room temperature for 14 h. The alditol acetate analysis was carried out using GC–MS (Saturn 2000R–3800 gas chromatograph coupled to an Ion-Trap 2000R mass spectrometer, Varian) or GLC (Thermo Trace GC 3), using a DB-225 column (30 m × 0.25 mm i.d.) programmed from 50 to 210 ºC at 40 ºC/min, with He as carrier gas (GC–MS) or programmed from 100 to 250 ºC at 60 ºC/min, with He/N as carrier gas (GLC).

Uronic acid content was spectrophotometrically determined by modified sulfamate/m-hydroxybiphenyl method (FILISETTI-COZZI & CARPITA, 1991), using galacturonic or glucuronic acid as standard.

2.5 METHYLATION ANALYSIS OF POLYSACCHARIDES

The polysaccharides were *O*-methylated according to the method of Ciucanu & Kerek (1984), using powdered NaOH in DMSO-MeI. The per-*O*-methylated polysaccharide was later submitted to methanolysis in 3% HCl–MeOH (80 ºC, 2 h), then hydrolysed with H_2SO_4 (0.5 M, 12 h) and neutralized with $BaCO_3$. The sample was then submitted to reduction and acetylation as described above for sugar composition, except that the reduction was performed using $NaBD_4$. The partially *O*-methylated alditol acetates produced were examined by capillary GC–MS (Shimadzu, model TQ 8040). A SH-Rtx-5MS capillary column (30 m × 0.25 mm i.d.), held at 100 ºC during injection and programmed at 7 ºC /min up to 280 ºC and held at this temperature for 5 min, was used for separation. The partially *O*-methylated alditol acetates were identified by their typical electron impact breakdown profiles and retention times (SASSAKI et al, 2005).

2.6 NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY

^{3}C $\{^{1}H\}$ and HSQC-DEPT NMR spectra were acquired at 70 ºC on a Bruker AVANCE III 400 NMR spectrometer, operating at 9.5 T, observing ^{13}C at 100.61 MHz and ^{1}H at 400.13 MHz, equipped with a 5-mm multinuclear inverse detection probe with z-gradient. The samples were dissolved in D_2O or DMSO with chemical shifts expressed as δ PPM, using the resonances of CH_3 groups of acetone (δ_C 30.2/δ_H 2.22) or DMSO (δ_C 39.7/δ_H 2.60) as internal references.

2.7 DETERMINATION OF HOMOGENEITY AND MOLECULAR WEIGHT OF POLYSACCHARIDES

The homogeneity and molecular weight of polysaccharides were determined by gel permeation chromatography (GPC). The procedure was carried out as previously described by Leivas, Iacomini and Cordeiro (2015). Briefly, the sample was dissolved in the mobile phase at a concentration of 1 mg/mL, filtered in 0.22 μm membrane (Millipore) and analysed on a Waters chromatograph equipped with four Ultrahydrogel column connected in series (2000, 500, 250, 120; with size exclusion of 7×10^6 Da, 4×10^5 Da, 8×10^4 Da and 5×10^3 Da; Milford, MA, USA), coupled to a Waters 2410 (Milford, MA, USA) differential refractometer (RI) detector. The mobile phase used was 0.1 mol/L sodium nitrite containing 0.2 g/L sodium azide at a flow rate of 0.6 mL/min and at 25 ºC. To obtain the molecular weight, standard dextrans (72.2 kDa, 40.2 kDa, 17.2 kDa, 9.4 kDa and 5.0 kDa, from Sigma) were employed to obtain the calibration curve.

3 RESULTS AND DISCUSSION

3.1 EXTRACTION AND PURIFICATION OF PECTIC POLYSACCHARIDES

GPF was defatted and pectins were extracted with boiling water (fraction CPW, 8.6% yield) (Fig. 1). Monosaccharide analysis of the fraction CPW (Table 1), together with the NMR spectral data (Schneider, unpublished), indicated the presence of a high methoxy homogalacturonan and arabinan-rich pectic polysaccharides. Moreover, an unusual signal at 101.6/5.10 was observed in the HSQC correlation map of this fraction and tentatively assigned to β-L-Ara*f* units, according to literature (DAS et al, 2013; SHAKHMATOV et. al., 2014). In order to confirm this assignment and determine the fine chemical structure of the guavira pomace polysaccharides, CPW fraction was further purified. Since it had asymmetrical peak on GPC analysis, this fraction was treated with Fehling's solution, giving soluble (CPW-SF) and Cu^{+2}-insoluble (CPW-PF) polysaccharides. As expected, homogalacturonan complexed with Cu^{+2} ions and precipitated, as observed by the monosaccharide analysis (Table 1) which had 82% of uronic acids. Its ^{13}C NMR spectrum (Fig. 3.A) exhibited intense signals typical of unesterified homogalacturonan (δ 174.9 and δ 99.0, attributed to carboxyl groups (C-6) and anomeric carbon, respectively), as previously observed by Popov et al. (2011). The remaining assignments were seen at δ 68.6 (C-2), δ 69.3 (C-3), δ 78.2 (C-4) and δ 71.5 (C-5). Also, it had anomeric signal at δ 107.6 attributed to α-L-Ara*f* units, present in 18% in this fraction (Table 1).

Concerning fraction CPW-SF, it also contained arabinose and uronic acids as main monosaccharides, but unlike fraction CPW-PF, its amount of arabinose was greater than uronic acids. It also contained small amounts of galactose (Table 1). Interestingly, its ^{13}C NMR spectrum (Fig. 3B) exhibited the signal at δ 101.6 from β-L-Ara*f* units. Other signals at δ 107.7 and δ 107.3 corresponded to anomeric carbons of α-L-Ara*f* units and a signal at δ 104.5 is from β-D-Gal*p* units (CORDEIRO et al., 2012; CIPRIANI et al., 2009). These assignments suggest that arabinan and small amounts of arabinogalactan may constitute the structural domains of pectic polysaccharides present in CPW-SF fraction. Leivas et al. (2015) and Cantu-Jungles et al. (2014) also observed that arabinogalactans do not complex with Cu^{2+} and remain soluble in Fehling treatment.

When analysed by gel permeation chromatography, CPW-SF displayed a heterogeneous elution profile (Fig. 2), thus it was further fractionated by sequential ultrafiltration through membranes with 100 kDa (giving fraction CPW-SF100R), 50 kDa (fraction CPW-SF50R), and 30 kDa (fractions CPW-SF30R and CPW-SF30E) cut-offs (Fig. 1). In monosaccharide composition analysis, all fractions remained with an arabinose-rich profile, being also composed by minor amounts of other neutral sugars (Table 1). Although fractions presented similar spectra (data not shown), they presented different elution profiles, indicating the presence of polysaccharides with analogous structure but different molar mass. Thus, CPW-SF30R which had a homogeneous GPC profile (Fig. 2), with M_w of 25 kDa, was chosen for further structural characterization and analysed by methylation and 2D NMR experiments. On methylation analysis (Table 2) it mainly gave derivatives of Ara (82.1%), which was in agreement with monosaccharide analysis. High amounts of 2,3-Me_2-Ara-ol acetate were noticed, indicating (1→5)-linked arabinan. Its main chain was branched at *O*-3, due to the presence of 2-Me-Ara-ol acetate. The derivative 2,5-Me_2-Ara-ol acetate indicated (1→3)-linked Ara*f* units, probably present as side chains. Terminal Ara*f* was also observed, in amounts that corresponded to the branching points. Moreover, low amounts of galactose derivatives were found in methylation analysis (Table 2), such as 2,4,6-Me_3-Gal-ol-acetate, 2,3,4-Me_3-Gal-ol-acetate, 2,3,6-Me_3-Gal-ol-acetate, 2,6-Me_2-Gal-ol-acetate, 2,3-Me_2-Gal-ol-acetate, 2,4-Me_2-Gal-ol-acetate and 2-Me-Gal-ol-acetate indicating the presence of 3-*O*-, 6-*O*-, 4-*O*-, 3,4-di-*O*-, 4,6-di-*O*-, 3,6-di-*O*-, 3,4,6-tri-*O*-substituted Gal*p* units, probably arising from a galactan. The arabinan is probably attached to a type I rhamnogalacturonan (RG-I). Since low amounts of uronic acids (4.0%) were observed in monosaccharide analysis, the fraction has not been carboxyl reduced prior to methylation, leading to absence of GalA and Rha derivatives in this linkage analysis. However, weak signals of these units corresponding to RG-I were observed by NMR analysis (Fig. 4, discussed below).

2D NMR experiments (HSQC-DEPT, COSY and TOCSY) have been performed in CPW-SF30R, allowing the assignment of all ^{1}H and ^{13}C signals (Table 3 and Fig. 4), which were in agreement with literature data (DAS et al, 2013; DOURADO et al., 2006; CARDOSO et al., 2002; CARDOSO et al., 2007; HABIBI, 2004; SHAKHMATOV et. al., 2014). Intense anomeric signals were assigned to the following Ara units, terminal β-L-Ara*f*, (1→3)-α-L-Ara*f*, (1→3,5)-α-L-Ara*f*, (1→5)-α-L-Ara*f* and terminal α-L-Ara*f*, represented as residues A-E, respectively. Units B to E are normally found in pectic arabinans, while units A have scarcely been reported in the literature. Regarding this unit, the upfield anomeric signal at δ 101.6 and downfield C-2, C-3 and C-4 at δ 76.6, 74.8, 82.1, respectively, indicated its β-configuration

and furanose conformation. The occurrence of these terminal β-L-Ara*f* units have been found in few sources up to date, for instance in *Litsea glutinosa* leaves, a tree distributed throughout India (DAS et al, 2013); olive (*Olea europaea*, L.) pomace (CARDOSO et al., 2002) and pulp (CARDOSO et al., 2007); and *Abies sibirica* L. (Siberian fir) wood greenery (SHAKHMATOV et. al., 2014). Thus, this work expands the finding of terminal β-L-Ara*f* units-containing arabinans, adding guavira pomace as a source of this unusual structural motif. Moreover, this is the first study about polysaccharide chemical structure characterization of guavira fruits.

3.2 EXTRACTION AND PURIFICATION OF HEMICELLULOSIC POLYSACCHARIDES

The residue obtained after the aqueous extraction was submitted to 10% aq. KOH extraction. The alkali-extracted polysaccharides (fraction CPK) are mainly composed of xylose (63.6%) and minor amounts of arabinose, glucose, galactose and uronic acids (Table 1). ^{13}C NMR spectrum of fraction CPK (Fig. 5A) presented five intense signals at δ 101.6 (C-1), δ 76.4 (*O*-substituted C-4), 73.7 (C-3), 72.7 (C-2) and 63.0 (C-5), characteristic of (1→4)-β-D-linked xylan (CANTU-JUNGLES et al, 2017; CORDEIRO, DE ALMEIDA, & IACOMINI, 2015; NASCIMENTO et al., 2013). A heterogeneous profile was evidenced on GPC analysis (data not shown) and thus, CPK was treated with Fehling' solution (Fig. 1). Xylan precipitated with Cu^{+2} and was found in CPK-PF fraction, which was composed of 86% of Xyl units (Table 1), with minor amounts of uronic acids and arabinose. Its HSQC correlation map (Fig. 5B) had the five main signals of 4-linked β-D-Xyl*p* units, as well signals of 4-*O*-methyl-α-D-Glc*p*A and 2,4-linked β-D-Xyl*p* units (Table 3). The integration of anomeric signals in its ^{1}H NMR spectrum (Fig. 5C) showed a molar ratio of 4-linked Xyl*p* units (at δ 4.48), 2,4-linked Xyl*p* units (at δ 4.64) and 4-*O*-Me-Glc*p*A units (at δ 5.25) estimated to be 5:1:1, respectively. Thus, indicating the presence of six Xyl units per GlcA residue. The NMR data are in agreement with the structures of (4-*O*-methyl-α-D-glucurono)-β-D-xylans already found in the literature, for instance, in plants such as *Cudrania tricuspidata* roots (SHI, DONG & DING, 2014), grape stalks (PROZIL et al, 2012), bamboo (PENG et al, 2011), *Artemisia sphaerocephala* Krasch seeds (GUO et al, 2011) and *Agave sisalana* leaves (MARQUES et al, 2010). Similar molar ratio was found in palm of *Phoenix*

dactylifera L. (BENDAHOU et al, 2007) and *Argania spinosa* seeds (HABIBI & VIGNON, 2005).

In the past decades, several agricultural residues/by-products have been used as sources of xylans, such as straw, sorghum, sugar cane, corn stalks and cobs, hulls and husks from starch production (JAYAPAL et al, 2013; EBRINGEROVÁ & HROMÁDKOVÁ, 1999). Regarding its application, after being hydrolysed to xylooligosaccharides (XOS), xylans can be employed as prebiotics, selectively stimulating beneficial gut microbiota growth (SAMANTA et al., 2015). Thus, guavira pomace could be a promising xylan source, suitable for applications in biomedical as well as industry field.

ACKNOWLEDGMENTS

The authors are grateful to CAPES-PROEX 0587/2018 (Grant number 23038.000817/2018-93 and CNPq (Grant numbers 404717/2016-0, 310332/2015-0) for financial support. The author VSS received a fellowship from CAPES - Fundação Araucária (Process 19-2015). The authors are grateful Prof. Dr. Elisvânia F. dos Santos (Federal University of Mato Grosso do Sul) for providing the guavira pomace and to NMR Center of UFPR for recording the NMR spectrum.

REFERENCES

BENDAHOU, A. et al. Isolation and structural characterization of hemicelluloses from palm of *Phoenix dactylifera* L. **Carbohydrate Polymers**, v. 68, p. 601–608, 2007.

CANTU-JUNGLES, T. M.; MARIA-FERREIRA, D.; SILVA, L. M.; BAGGIO, C. H;, WERNER, M. F. D.; IACOMINI, M.; et al. Polysaccharides from prunes: Gastroprotective activity and structural elucidation of bioactive pectins. **Food Chemistry**, v. 146, p. 492–499, 2014.

CANTU-JUNGLES, T.M.; et al. Isolation and characterization of a xylan with industrial and biomedical applications from edible açaí berries (*Euterpe oleraceae*). **Food Chemistry**, v. 221, p. 1595–1597, 2017.

CAPEK, P. & MATULOVÁ, M. An arabino(glucurono)xylan isolated from immunomodulatory active hemicellulose fraction of *Salvia officinalis* L. **International Journal of Biological Macromolecules**, v. 59, p. 396– 401, 2013.

CARDOSO, S. M.; SILVA, A. M. S.; COIMBRA, M. A. Structural characterisation of the olive pomace pectic polysaccharide arabinan side chains. **Carbohydrate Research**, v. 337, p. 917–924, 2002.

CARDOSO, S. M.; et al. Structural ripening-related changes of the arabinan-rich pectic polysaccharides from olive pulp cell walls. **J. Agric. Food Chem.**, v. 55, n. 17, p. 7124-7130, 2007.

CIPRIANI, T. R.; MELLINGER, C. G.; BERTOLINI, M. L. C.; BAGGIO, C. H.; FREITAS, C. S.; MARQUES, M. C. A.; GORIN, P. A. J.; SASSAKI, G. L.; IACOMINI, M. Gastroprotective effect of a type I arabinogalactan from soybean meal. **Food Chemistry**, v. 115, n. 2, p. 687-690, 2009.

CIUCANU, I.; KEREK, F. A simple and rapid method for the permethylation of carbohydrates. **Carbohydrate Research**, v. 131, n. 2, p. 209-217, 1984.

CORDEIRO, L. M. C.; DE ALMEIDA, C. P.; IACOMINI, M. Unusual linear polysaccharides: (1→5)-α-L-Arabinan, (1→3)-(1→4)-α-D-glucan and (1→4)-β-D-xylan from pulp of buriti (*Mauritia flexuosa*), an edible palm fruit from the Amazon region. **Food Chemistry**, v. 173, p. 141–146, 2015.

CORDEIRO, L. M. C.; REINHARDT, V. D.; BAGGIO, C. H.; WERNER, M. F. D.; BURCI, L. M.; SASSAKI, G. L.; IACOMINI, M. Arabinan and arabinan-rich pectic polysaccharides from quinoa (*Chenopodium quinoa*) seeds: structure and gastroprotective activity. **Food Chemistry**, v. 130, n. 4, p. 937-944, 2012.

DAS, D. et al. A new arabinoxylan from green leaves of *Litsea glutinosa* (Lauraeae): Structural and biological studies. **Carbohydrate Polymers**, v. 92, p. 1243–1248, 2013.

DOURADO, F. et al. NMR structural elucidation of the arabinan from *Prunus dulcis* immunobiological active pectic polysaccharides. **Carbohydrate Polymers**, v. 66, p. 27–33, 2006.

DOUSSEAU, S. et al. Ecofisiologia da germinação de sementes de *Campomanesia pubescens*. **Cienc. Rural** [online], vol.41, n.8, p. 1362-1368, 2011.

EBRINGEROVÁ, A., & HROMÁDKOVÁ, Z. Xylans of industrial and biomedical importance. **Biotechnology and Genetic Engineering Reviews**, v. 16, p. 325–346, 1999.

FILISETTI-COZZI, T. M. C. C.; CARPITA, N. C. Measurement of uronic acids without Interference from neutral sugars. **Analytical Biochemistry**, v. 197, n. 1, p. 157-162, 1991.

GUO, Q. et al. Structure characterization of high molecular weight heteropolysaccharide isolated from *Artemisia sphaerocephala* Krasch seed **Carbohydrate Polymers**, v. 86, p. 742– 746, 2011.

HABIBI, Y. et al. An arabinogalactan from the skin of *Opuntia ficus-indica* prickly pear fruits. **Carbohydrate Research**, v. 339, p. 1201–1205, 2004.

HABIBI, Y. & VIGNON, M. R. Isolation and characterization of xylans from seed pericarp of *Argania spinosa* fruit. **Carbohydrate Research**, v. 340, p. 1431–1436, 2005.

JAYAPAL, N. et al. Value addition to sugarcane bagasse: Xylan extraction and its process optimization for xylooligosaccharides production. **Industrial Crops and Products**, v. 42, p. 14– 24, 2013.

JONES, J. K. N.; STOODLEY, R. J. Fractionation using copper complexes. **Methods in Carbohydrate Chemistry**, v. 5, p. 36-38, 1965.

KUMAR, V.; SINHA, A. K.; MAKKAR, H. P.; DE BOECK, G.; BECKER, K. Dietary roles of non-starch polysaccharides in human nutrition: A review. **Critical Reviews in Food Science and Nutrition**, v. 52, n. 10, p. 899–935, 2012.

LADANIYA, M. S. Fruit biochemistry. In M. S. Ladaniya (Ed.), Citrus fruit: Biology, technology and evaluation, p. 125–190. San Diego: **Academic Press**, 2008.

LEIVAS, C.L.; IACOMINI, M.; CORDEIRO, L.M.C. Structural characterization of a rhamnogalacturonan I-arabinan-type I arabinogalactan macromolecule from starfruit (*Averrhoa carambola* L.), **Carbohydr. Polym**., v. 121, p. 224–230, 2015.

MARQUES, G. et al. Acetylated heteroxylan from *Agave sisalana* and its behavior in alkaline pulping and TCF/ECF bleaching. Carbohydrate Polymers, v. 81, p. 517–523, 2010.

NASCIMENTO, G.E. et al. Structure of a galactoarabinoglucuronoxylan from tamarillo (*Solanum betaceum*), a tropical exotic fruit, and its biological activity. **Food Chemistry**, v. 141, p. 510–516, 2013.

PENG, P. et al. Isolation and structural characterization of hemicelluloses from the bamboo species *Phyllostachys incarnata* Wen. **Carbohydrate Polymers**, v. 86, p. 883– 890, 2011.

POPOV, S. V.; OVODOVA, R. G.; GOLOVCHENKO, V. V.; POPOVA, G. Y.; VIATYASEV, F. V.; SHASHKOV, A. S.; et al. Chemical composition and anti-inflammatory activity of a pectic polysaccharide isolated from sweet pepper using a simulated gastric medium. **Food Chemistry**, v. 124, n.1, p. 309–315, 2011.

PROZIL, S. et al. Structural characterization of polysaccharides isolated from grape stalks of *Vitis vinifera* L. **Carbohydrate Research**, v. 356, p. 252-9, 2012.

SAMANTA, A.K., et al. Xylooligosaccharides as prebiotics from agricultural by-products: Production and applications. **Bioactive Carbohydrates and Dietary Fibre**, v. 5, p. 62–71, 2015.

SASSAKI, G. L., GORIN, P. A. J., SOUZA, L. M., CZELUSNIAK, P. A., & IACOMINI, M. Rapid synthesis of partially O-methylated alditol acetate standards for GC–MS: Some relative activities of hydroxyl groups of methyl glycopyranosides on Pur-die methylation. **Carbohydrate Research**, v. 340, p. 731–739, 2005.

SHAKHMATOV, E.G.; et al. Structural studies of arabinan-rich pectic polysaccharides from *Abies sibirica* L. Biological activity of pectins of *A. sibirica*. **Carbohydrate Polymers**, v. 113, p. 515–524, 2014.

SHI, L.; DONG, Q.; DING, K. Structure elucidation and immunomodulatory activity *in vitro* of a xylan from roots of *Cudrania tricuspidata*. **Food Chemistry**, v. 152, p. 291–296, 2014.

Figure Legends

Fig. 1. Scheme of extraction and purification of of pectic (CPW) and hemicellulosic (CPK) polysaccharides from guavira pomace (*Campomanesia* sp.).

Fig. 2. GPC elution profiles of fractions CPW, CPW-SF and CPW-SF30R (refractive index detector). Elution volume of dextran standards of molecular weight 72.2 kDa, 40.2 kDa, 17.2 kDa, 9.4 kDa and 5.0 kDa (left to right) were employed to plot the calibration curve.

Fig. 3. ^{13}C NMR spectra of fractions CPW-PF (A) and CPW-SF (B). Samples were dissolved in deuterium oxide and data collected at probe temperature of 70 ºC.

Fig. 4. ^{1}H/^{13}C HSQC-DEPT (A) and (B) ^{1}H-^{1}H TOCSY correlation maps of CPW-SF30R. Sample was dissolved in deuterium oxide and data collected at probe temperature of 70 ºC. In (A) negative signals in DEPT experiment are marked with an asterisk (*) and in (B) the coupled hydrogens of units A, B and D are shown.

Fig. 5. NMR experiments of hemicellulosic fractions extracted from guavira pomace. (A) ^{13}C NMR spectrum of fraction CPK; (B) HSQC correlation map and (C) ^{1}H NMR spectrum of CPK-PF fraction, showing integration of anomeric peaks from 4-linked β-D-Xyl*p* units (at δ 4.48), 2,4-linked β-D-Xyl*p* units (at δ 4.64) and terminal 4-*O*-Me-α-D-Glc*p*A units (at δ 5.25). Samples were dissolved in deuterium oxide and data collected at probe temperature of 70 ºC.

Table 1

Monosaccharide composition of fractions obtained from guavira pomace

Fractions	Monossaccharide (%)							
	Rha	**Fuc**	**Ara**	**Xyl**	**Man**	**Glc**	**Gal**	**Uronic acid[(1)]**
CPW	tr	-	46.7	1.3	0.8	1.1	5.5	44.6
CPW-PF	-	-	18.0	-	-	-	-	82.0
CPW-SF	tr	-	70.9	-	-	-	2.7	26.3
CPW-SF100R	2.8	-	68.0	4.6	0.6	1.8	9.5	12.7
CPW-SF50R	5.0	-	81.7	2.0	0.5	1.0	9.3	0.5
CPW-SF30R	3.3	-	85.0	-	-	-	7.7	4.0
CPW-SF30E	0.4	1.7	81.7	4.7	3.6	2.7	5.2	-
CPK	-	-	18.5	63.6	0.9	6.4	8.7	1.9
CPK-PF	-	-	3.0	86.0	-	-	-	11.0

Notes: [1]Uronic acids, determined using the modified m-hydroxybiphenyl method (Filisetti-Cozzi & Carpita, 1991).

Table 2

Linkage types based on analysis of partially *O*-methyl alditol acetates of arabinan-rich pectic polysaccharide (fraction CPW-SF30R) obtained from guavira pomace.

Partially *O*-methylalditol acetate	CPW-SF30R[a]	Linkage type[b]
2,3,5-Me_3-Ara[c]	20.0	Ara*f*-(1→
2,5-Me_2-Ara	4.0	→3)-Ara*f*-(1→
2,3-Me_2-Ara	38.6	→5)-Ara*f*-(1
2,3,4,6-Me_4-Gal	5.0	Gal*p*-(1→
2-Me-Ara	19.5	→3,5)-Ara*f*-(1→
2,3,6-Me_3-Gal	2.4	→4)-Gal*p*-(1→
2,4,6-Me_3-Gal	1.2	→3)-Gal*p*-(1→
2,3,4-Me_3-Gal	4.0	→6)-Gal*p*-(1→
2,6-Me_2-Gal	0.9	→3,4)-Gal*p*-(1→
2,3-Me_2-Gal	1.1	→4,6)-Gal*p*-(1→
2,4-Me_2-Gal	1.6	→3,6)-Gal*p*-(1→
2-Me-Gal	1.7	→3,4,6)-Gal*p*-(1→

[a] % of peak area of *O*-methylalditol acetates relative to total area, determined by GC–MS.

[b] Based on derived *O*-methylalditol acetates.

[c] 2,3,5-Me_3-Ara = 2,3,5-tri-*O*-Methyl-arabinitol acetate, etc.

Table 3

^{13}C and ^{1}H NMR data of arabinan (CPW-SF30R) and glucuronoxylan (CPK-PF) from guavira pomace.

Units/assignments		**1**	**2**	**3**	**4**	**5**		**6**	**-O-CH$_3$**
						5a	**5b**		
Arabinan									
β-L-Ara*f*-(1→[a]	^{13}C	101.6	76.6	74.8	82.1	63.0		-	-
A	^{1}H	5.10	4.13	4.04	3.90	3.78	3.72	-	-
3→)-α-L-Ara*f*-(1 →[b]	^{13}C	107.0	79.9	83.9	82.4	61.3		-	-
B	^{1}H	5.17	4.35	3.99	4.15	3.81	3.75	-	-
3, 5→)-α-L-Ara*f*-(1 →[c]	^{13}C	107.5	79.5	82.1	80.6	66.6		-	-
C	^{1}H	5.08	4.28	4.09	4.27	3.91	3.85	-	-
5→)-α-L-Ara*f*-(1 →[d]	^{13}C	107.5	81.3	77.0	82.3	66.6		-	-
D	^{1}H	5.08	4.14	4.02	4.20	3.87	3.81	-	-
Glucuronoxylan									
4→)-β-D-Xyl*p*-(1 →[e]	^{13}C	101.6	72.8	73.8	76.4	63.0		-	-
A	^{1}H	4.48	3.33	3.58	3.78	4.12	3.40	-	-
2, 4→)-β-D-Xyl*p*-(1 →[e]	^{13}C	101.2	76.6	72.6	76.4	62.8		-	-
B	^{1}H	4.64	3.58	3.68	3.78	4.17	3.78	-	-
4-O-Me-α-D-Glc*p*A-(1 →[e]	^{13}C	97.7	71.4	72.6	82.2	72.4		176.2	59.3
C	^{1}H	5.25	3.60	3.78	3.29	4.28		-	3.48

[a] Das et al, 2013; Cardoso et al., 2002; Cardoso et al., 2007; Shakhmatov et. al., 2014.

[b] Shakhmatov et. al., 2014; Cardoso et al., 2007.

[c] Dourado et al., 2006; Shakhmatov et. al., 2014; Cardoso et al., 2007.

[d] Cardoso et al., 2002; Cardoso et al., 2007; Habibi, 2004; Shakhmatov et. al., 2014.

[e] Capek and Matulová, 2013; Guo et al., 2011; Habibi and Vignon, 2005; Peng et al, 2011.

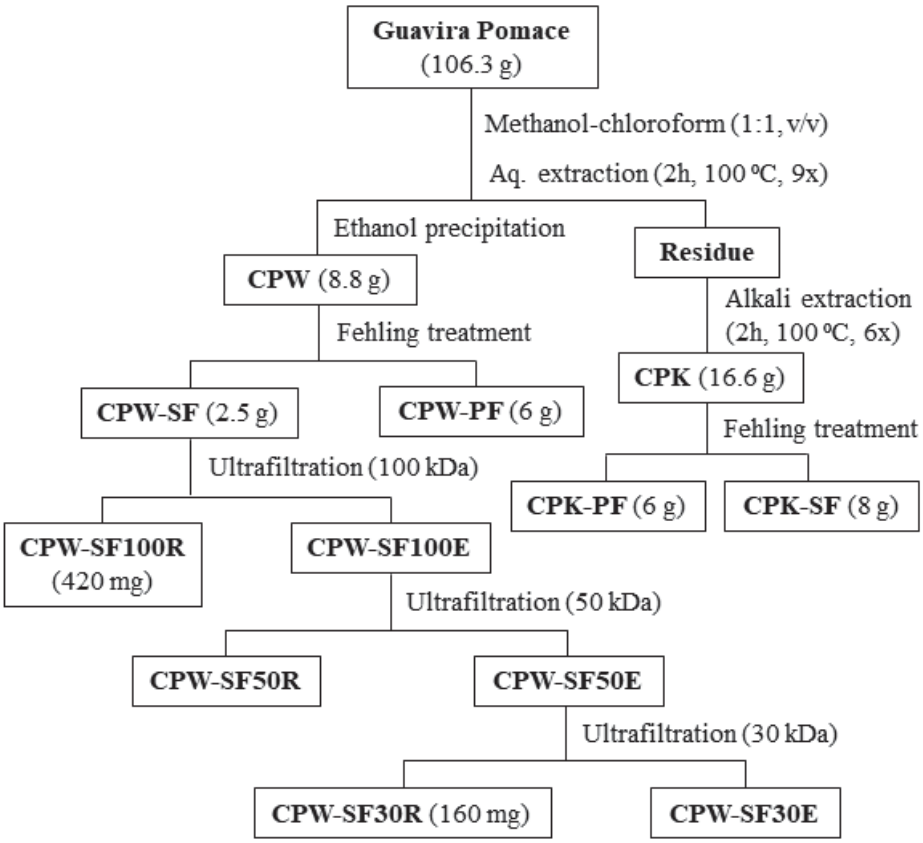

Guavira Pomace
(106.3 g)
Methanol-chloroform (1:1, v/v)
Aq. extraction (2h, 100 ºC, 9x)
Ethanol precipitation
Residue
CPW (8.8 g)
Alkali extraction
(2h, 100 ºC, 6x)
Fehling treatment
CPK (16.6 g)
CPW-SF (2.5 g)
CPW-PF (6 g)
Fehling treatment
Ultrafiltration (100 kDa)
CPK-PF (6 g)
CPK-SF (8 g)
CPW-SF100R
(420 mg)
CPW-SF100E
Ultrafiltration (50 kDa)
CPW-SF50R
CPW-SF50E
Ultrafiltration (30 kDa)
CPW-SF30R (160 mg)
CPW-SF30E

Figure 1

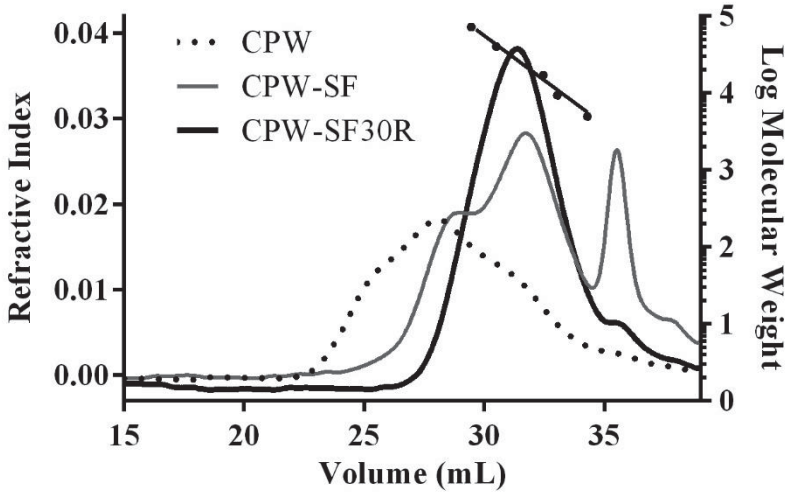

CPW
CPW-SF
CPW-SF30R
Refractive Index
0.04
0.03
0.02
0.01
0.00
15
20
25
30
35
Volume (mL)
Log Molecular Weight
5
4
3
2
1
0

Figure 2

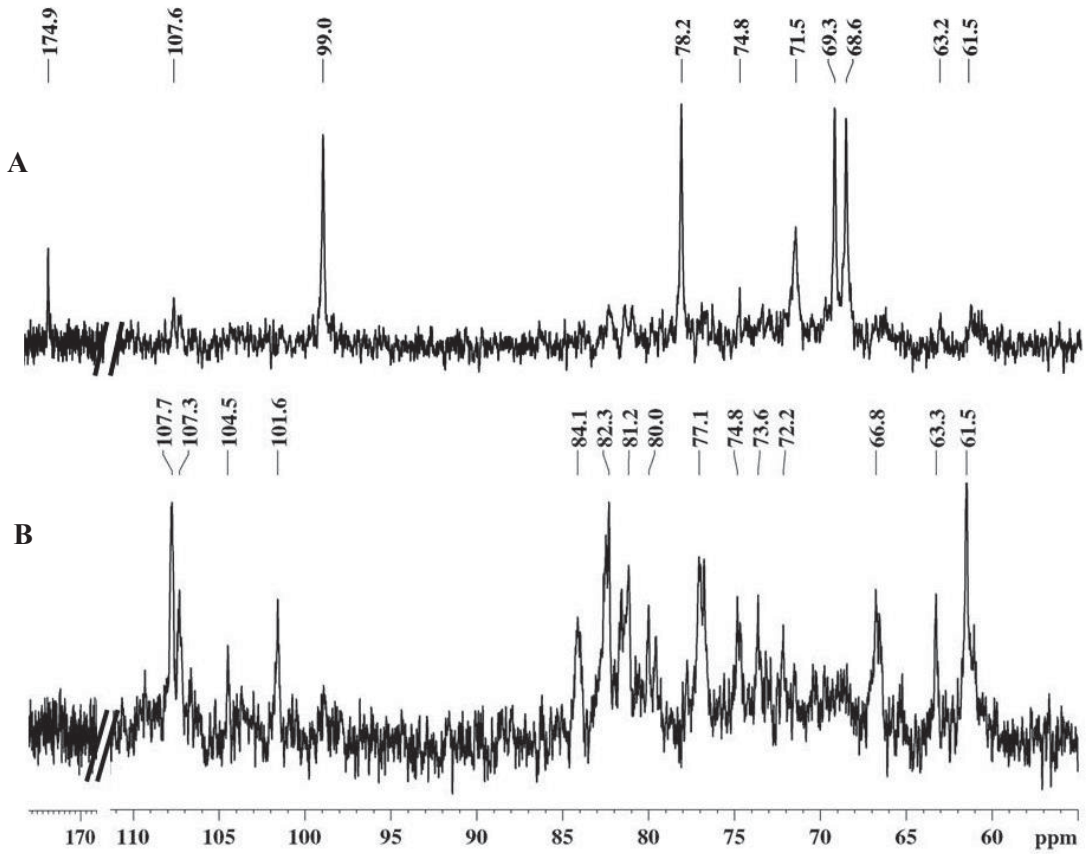
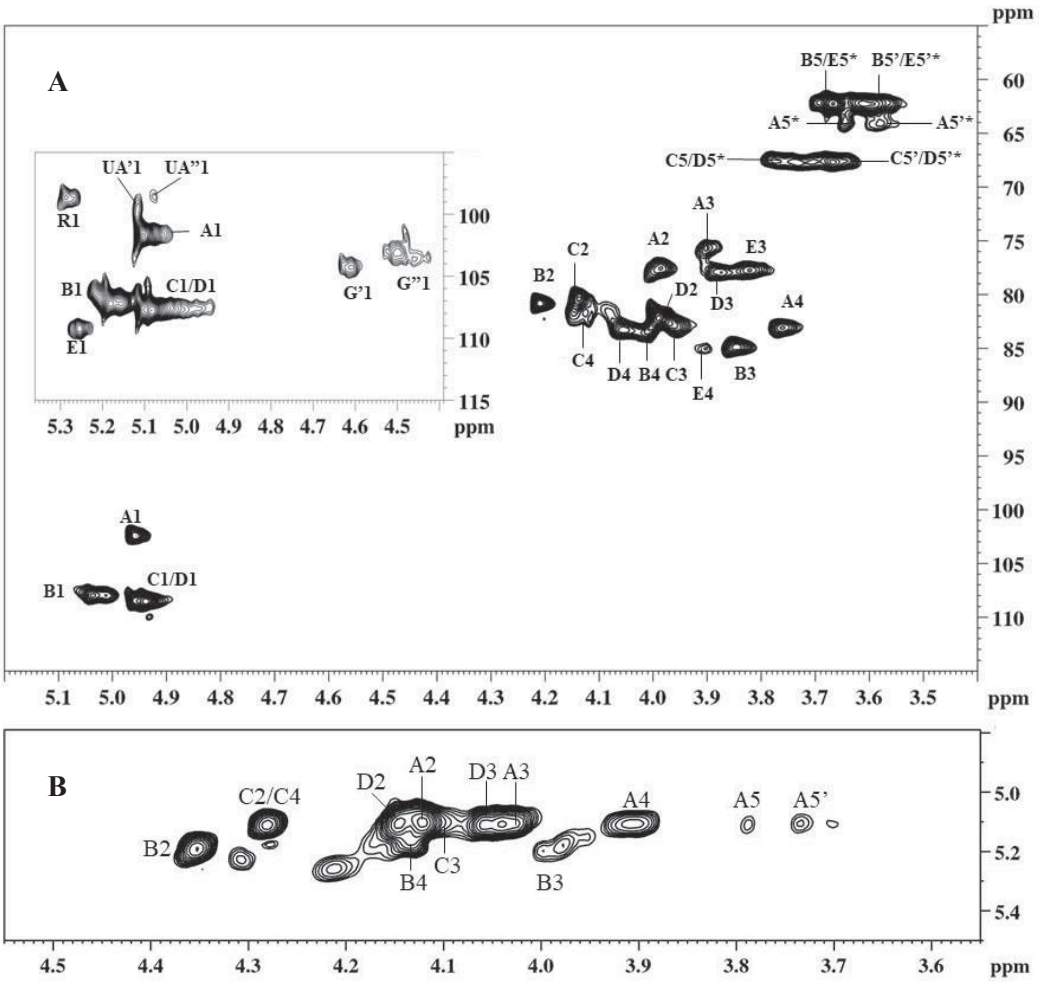

174.9
107.6
99.0
78.2
74.8
71.5
69.3
68.6
63.2
61.5
A
107.7
107.3
104.5
101.6
84.1
82.3
81.2
80.0
77.1
74.8
73.6
72.2
66.8
63.3
61.5
B
170
110
105
100
95
90
85
80
75
70
65
60
ppm

Figure 3



A: β-L-Ara*f*-(1→ **E: α-L-Ara*f*-(1→**

B: 3→)-α-L-Ara*f*-(1 → **G: β-D-Gal*p***

C: 3,5→)-α-L-Ara*f*-(1 → **R: α-L-Rha*p***

D: 5→)-α-L-Ara*f*-(1 → **UA: α-D-Gal*p*A**

Figure 4

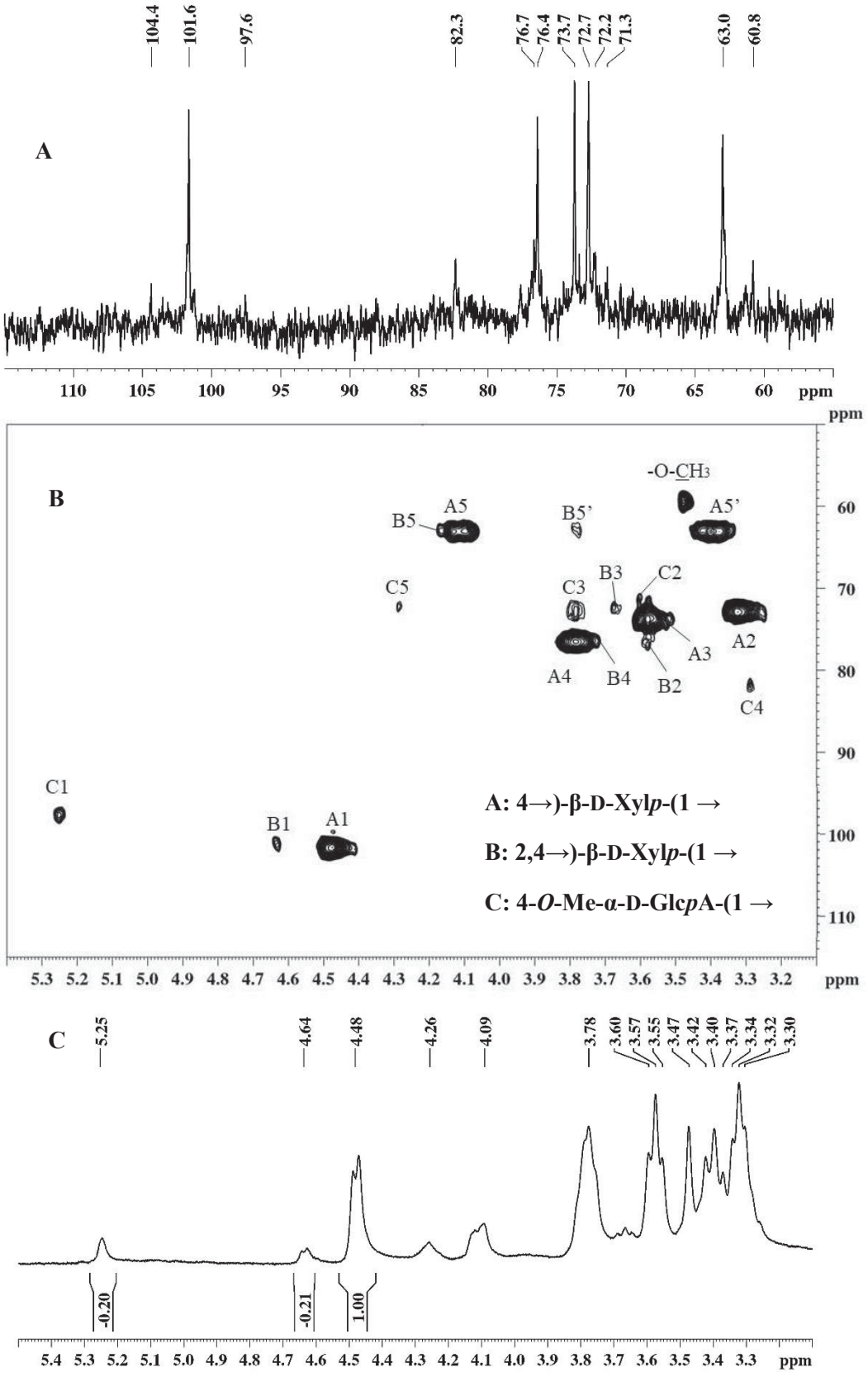

A
104.4
101.6
97.6
82.3
76.7
76.4
73.7
72.7
72.2
71.3
63.0
60.8
110 105 100 95 90 85 80 75 70 65 60 ppm
B
-O-CH3
A5
B5
B5'
A5'
C5
C3
B3
C2
A2
A3
A4
B4
B2
C4
C1
B1
A1
A: 4→)-β-D-Xylp-(1 →
B: 2,4→)-β-D-Xylp-(1 →
C: 4-O-Me-α-D-GlcpA-(1 →
ppm
C
5.25
4.64
4.48
4.26
4.09
3.78
3.60
3.57
3.55
3.47
3.42
3.40
3.37
3.34
3.32
3.30
0.20
0.21
1.00
ppm

Figure 5

CONCLUSÃO

A partir de extrações aquosa e alcalina de resíduo agroindustrial de guavira, associadas a diferentes etapas de purificação foi possível a obtenção de diferentes polissacarídeos pécticos e hemicelulósicos, componentes principais das fibras alimentares.

Para os polissacarídeos pécticos foram encontradas estruturas de uma homogalacturonana altamente metoxilada (DM = 90%) e outros polissacarídeos pécticos ricos em arabinanas. A fração purificada de arabinana, de massa molar estimada em 25 kDa, apresentou a seguinte composição: arabinose (85%), ramnose (3,3%), galactose (7,7%) e ácido galacturônico (4%). Análises de metilação e RMN mono e bidimensionais desta arabinana apontaram a presença das seguintes unidades: terminal β-L-Ara*f*, (1→3)-α-L-Ara*f*, (1→3,5)-α-L-Ara*f*, (1→5)-α-L-Ara*f* e terminal α-L-Ara*f*. A presença de terminais compostos por β-L-Ara*f* foi pouco relatada na literatura, desta forma, este trabalho apresenta a guavira como uma nova fonte para essa estrutura incomum.

Com a purificação de polissacarídeos hemicelulósicos foi possível a caracterização de uma glucuronoxilana, com grupos 4-*O*-metil-D-Glc*p*A ligados no *O*-2 das unidades de β-D-xilose (1→4)-ligadas. A integração dos sinais anoméricos no espectro de ^{1}H RMN indicou a presença de seis unidades de xilose para cada unidade de ácido glucurônico.

Além disso, foi verificada atividade antioxidante da fração péctica bruta, pelo sequestro de radicais ABTS e pelo seu tratamento em células de fibroblastos murinos (NIH 3T3), que reduziu os níveis de espécies reativas de oxigênio (ROS) e induziu proteção contra condição pró-oxidante.

REFERÊNCIAS

ALBERSHEIM, P.; DARVILL, A. G.; O'NEILL, M. A.; SCHOLS, H. A.; VORAGEN, A. G. J. An hypothesis: the same six polysaccharides are components of the primary cell walls of all higher plants. Progress in biotechnology: **Pectins and Pectinases**, v. 14, p. 47-55, 1996.

ALVES, A. M., et al. Caracterização física e química, fenólicos totais e atividade antioxidante da polpa e resíduo de gabiroba. **Rev. Bras. Frutic**., v. 35, n. 3, p. 837-844, 2013.

ALMEIDA, S.P.; PROENÇA, C.E.B.; SANO, S.M.; RIBEIRO, J.F. Cerrado: espécies vegetais úteis. **Planaltina: EMBRAPA-CPAC - DF**. 464p, 1998.

ASPINALL, G. O. Gums and mucilages. **Advances in Carbohydrate Chemistry and Biochemistry**, v. 24, p. 333-79, 1969.

AYALA-ZAVALA, J. F.; VEGA-VEGA, V.; ROSAS-DOMÍNGUEZ, C.; PALAFOX-CARLOS, H.; VILLA-RODRIGUEZ, J. A.; WASIM SIDDIQUI, MD.; et al. Agro-industrial potential of exotic fruit byproducts as a source of food additives. **Food Research International**, v. 44, p. 1866–1874, 2011.

CAFFALL K. H.; MOHNEN, D. The structure, function, and biosynthesis of plant cell wall pectic polysaccharides. **Carbohydrate Research**, v. 344, p. 1879-1900, 2009.

CARDOSO, C.A.L.; SALVADOR, M.J.; CARVALHO, J.E.; COELHO, R.G. Avaliação das atividades antiproliferativa e antioxidante em frutos de *Campomanesia pubescens***. Rev Inst Adolfo Lutz**, v. 72, n. 4, p. 309-15, 2013.

CARPITA, N. C.; GIBEAUT, D. M. Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. **Plant Journal**, v. 3, p. 1-30, 1993.

CHANG, R.; MORAIS, S.A.L.; NASCIMENTO, E.A.; CUNHA, L.C.S.; ROCHA, E. O.; AQUINO, F.J.T.; SOUZA, M.G.M.; CUNHA, W.R.; MARTINS, C.H.G. Essential Oil

Composition and Antioxidant and Antimicrobial Properties of *Campomanesia pubescens* O. Berg, Native of Brazilian Cerrado. **Lat. Am. J. Pharm**., v. 30, n. 9, p 1843-8, 2011.

CODEX ALIMENTARIUM COMMISSION. ALINORM 09/32/26, definition of dietary fiber. p. 27-54 and Appendix II, 2009.

COSGROVE, D. J. Growth of the plant cell wall. **Nature Reviews: Molecular CellBiology**, v. 6, p. 850–861, 2005.

DALONSO, N.; PETKOWICZ, C. L. Guarana powder polysaccharides: Characterisation and evaluation of the antioxidant activity of a pectic fraction. **Food Chem.**, v. 134, n. 4, p. 1804-1812, 2012.

DHINGRA, D.; MICHAEL, M.; RAJPUT, H.; PATIL, R. Dietary fibre in foods: A review. **Journal of Food Science and Technology-Mysore**, v. 49, n. 3, p. 255–266, 2012.

DOUSSEAU, S. et al. Ecofisiologia da germinação de sementes de *Campomanesia pubescens*. **Cienc. Rural**, v..41, n.8, p.1362-1368, 2011.

EBRINGEROVÁ, A.; HROMÁDKOVÁ, Z.; HEINZE, T. Hemicelluloses. **Advances in Polymer Sciences**, v. 186, p. 1–67, 2005.

ELLEUCH, M.; BEDIGIAN, D.; ROISEUX, O.; BESBES, S.; BLECKER, C.; ATTIA, H. Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. **Food Chemistry**, v. 124, p. 411−421, 2011.

FERNANDES, T. O. et al. *Campomanesia adamantium* (Myrtaceae) fruits protect HEPG2 cells against carbon tetrachloride-induced toxicity. **Toxicology Reports**, v. 2, p. 184–193, 2015.

GALANAKIS, C. M. Recovery of high added-value components from food wastes: Conventional, emerging technologies and commercialized applications. **Trends in Food Science & Technology**, v. 26, p. 68–87, 2012.

GIORDANO, M.E.; INGROSSO, I.; SCHETTINO, T.; CARICATO, R.; GIOVINAZZO, G.; LIONETTO, M.G. Intracellular Antioxidant Activity of Grape Skin Polyphenolic Extracts in Rat Superficial Colonocytes: *In situ* Detection by Confocal Fluorescence Microscopy. **Front. Physiol.**, v. 7, n. 177, 2016.

HARRIS, P. J., & SMITH, B. G. Plant cell walls and cell-wall polysaccharides: Structures, properties and uses in food products. **International Journal of Food Science and Technology**, v. 41, p. 129–143, 2006.

HROMÁDKOVÁ, Z., KOŠT'ÁLOVÁ, Z., & EBRINGEROVÁ, A. Comparison of conventional and ultrasound-assisted extraction of phenolics-rich heteroxylans from wheat bran. **Ultrasonics Sonochemistry**, v. 15, p. 1062–1068, 2008.

IGNAT, I.; VOLF, I.; POPA, V. I. A critical review of methods for characterization of polyphenolic compounds in fruits and vegetables**. Food Chemistry, Barking**, v. 126, n. 4, p. 1821-1835, 2011.

KIM, Y. J.; KIM, E.; HAHM, K.B. Oxidative stress in inflammation-based gastrointestinal tract diseases: challenges and opportunities. **J. Gastroenterol. Hepatol.**, v. 27, p. 1004–1010, 2012.

KLOSTERHOFF, R.R.; BARK, J.M.; GLÄNZEL, N.M.; IACOMINI, M.; MARTINEZ, G.R.; WINNISCHOFER, S.M.B.; CORDEIRO, L.M.C. Structure and intracellular antioxidant activity of pectic polysaccharide from acerola (*Malpighia emarginata*). **Int J Biol Macromol**, v.106, p. 473-480, 2017.

KONG, F.; ZHANG, M.; LIAO, S.; YU, S.; CHI, J.; WEI, Z. Antioxidant activity of polysaccharide-enriched fractions extracted from pulp tissue of *Litchi Chinensis* Sonn. **Molecules**, v. 15, n. 4, p. 2152-2165, 2010.

LIMA, D.F.; GOLDENBERG, R.; SOBRAL, M. O gênero Campomanesia (Myrtaceae) no estado do Paraná, Brasil. **Rodriguésia**, v. 62, p. 683–693, 2011.

LORENZI, H.; SARTORI, S.; BACHER, L.B.; LACERDA, M. Frutas Brasileiras e Exóticas Cultivadas: de consumo *in natura*. **Instituto Plantarum de Estudos da Flora**, São Paulo, 2006.

PASCOAL, A.C.R.F.; EHRENFRIED, C.A.; EBERLIN, M.N.; STEFANELLO, M.E.A.; SALVADOR, M.J. Free-radical scavenging activity, determination of phenolic compounds and HPLC-UV/DAD/ESI-MS profile of *Campomanesia adamantium* leaves. **Natural Product Communications**, v. 6, p. 969-972, 2011.

PAULSEN, B. Plant polysaccharides with immunostimulatory activities. **Current Organic Chemistry**, v. 5, p. 939–950, 2001.

PAVAN, F.R.; LEITE, C.Q.F.; COELHO, R.G.; COUTINHO, I.D.; HONDA, N.K.; CARDOSO, C.A.L.; VILEGAS, W.; LEITE, S.R.A.; SATO, D.N. Evaluation of Anti-*Mycobacterium tuberculosis* activity of *Campomanesia adamantium* (Myrtaceae). **Química Nova**, v. 32, p. 1222-1226, 2009.

PERIOTTO, F.; GUALTIERI, S. C. J. Germinação e desenvolvimento inicial de *Campomanesia pubescens* (DC.) o. berg (Myrtaceae) em diferentes substratos. **Ciência Florestal**, v. 27, n. 3, p. 743-752, 2017.

SÁ, S.; et al. Phytochemistry and antimicrobial activity of *Campomanesia adamantium* **Revista Brasileira de Farmacognosia**, v. 28, p. 303–311, 2018.

SILVA, D.B. et al. Frutas do cerrado. **Embrapa Informação Tecnológica**, Brasília, 2001.

SOUZA, J.C.; PICCINELLI, A.C.; AQUINO, D.F.S.; SOUZA, V.V.; SCHMITZ, W.O.; TRAESEL, G.K.; CARDOSO, C.A.L.; KASSUYA, C.A.L.; ARENA, A.C. Toxicological analysis and antihyperalgesic, antidepressant, and anti-inflammatory effects of *Campomanesia adamantium* fruit barks. **Nutrition Neuroscience**, v. 20, n. 1, p. 23-31, 2014.

VELIOGLU, Y.S.; MAZZA, G.; GAO, L.; OOMAH, B.D. Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables, and Grain Products. **J Agric Food Chem**, v. 46, p. 4113–4117, 1998.

VISCARDI, D.Z. et al. Anti-inflammatory, and antinociceptive effects of *Campomanesia adamantium* microencapsulated pulp. **Revista Brasileira de Farmacognosia**, v. 27, p. 220–227, 2017.

WANG, J.; HU, S.; NIE, S.; YU, Q.; XIE, M. Reviews on mechanisms of in vitro antioxidant activity of polysaccharides, **Oxidative Med. Cell. Longev**, vol. 2016, Article ID 5692852, 13 pages, 2016.

WILLIAMS, B. A.; GRANT, L. J.; GIDLEY, M. J.; MIKKELSEN, D. Gut Fermentation of Dietary Fibres: Physico-Chemistry of Plant Cell Walls and Implications for Health. **Int. J. Mol. Sci**, v. 18, p. 2203, 2017.

WOLFE, K. L.; LIU, R. H. Cellular Antioxidant Activity (CAA) Assay for Assessing Antioxidants, Foods, and Dietary Supplements. **J. Agric. Food Chem**, v. 55, p. 8896–8907, 2007.

YAN, H.; XIE, Y.; SUN, S.; SUN, X.; REN, F.; SHI, Q.; WANG, S.; ZHANG, W.; LI, X.; ZHANG. J. Chemical analysis of *Astragalus mongholicus* polysaccharides and antioxidant activity of the polysaccharides. **Carbohydr. Polym**., v. 82, n. 3, p. 636-640, 2010.

YAPO, B. M. Pectic substances: From simple pectic polysaccharides to complex pectins - A new hypothetical model. **Carbohydrate Polymers**, v. 86, p. 373– 385, 2011.

YAZDANI, M. Concerns in the application of fluorescent probes DCDHF-DA, DHR 123 and DHE to measure reactive oxygen species *in vitro*, **Toxicol. InVitro**, v. 30, p. 578–582, 2015.

ZONG, A., CAO, H., & WANG, F. Anticancer polysaccharides from natural resources: A review of recent research. **Carbohydrate Polymers**, v. 90, p. 1395–1410, 2012.