Universidade Estadual de Londrina

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Phytochemical study of Brazilian and Asiatic fruits and evaluation of their anti-glycation and antioxidant potential in search of a new cosmetic ingredient

Londrina
2023

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Thesis submitted to the program in Chemistry at the State University of Londrina, as a partial requirement for the degree of doctorate.

Supervisor: Profa Dra. Maria Luiza Zeraik

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Ficha de identificação da obra elaborada pelo autor, através do Programa de Geração Automática do Sistema de Bibliotecas da UEL

A613p FERRARI, ANNA BEATRIZ SABINO .

Phytochemical study of Brazilian and Asiatic fruits and evaluation of their anti-glycation and antioxidant potential in search of a new cosmetic ingredient / ANNA BEATRIZ SABINO FERRARI. - Londrina, 2024.
87 f.

Orientador: Maria Luiza Zeraik.
Tese (Doutorado em Química) - Universidade Estadual de Londrina, Centro de Ciências Exatas, Programa de Pós-Graduação em Química, 2024.
Inclui bibliografia.

1. Anti-aging - Tese. 2. Protection fator - Tese. 3. Bioactivities - Tese. 4. Natural compounds - Tese. I. Zeraik, Maria Luiza . II. Universidade Estadual de Londrina. Centro de Ciências Exatas. Programa de Pós-Graduação em Química. III. Título.

CDU 54

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Londrina, _____de _____de _____.

I dedicate this work to my mother, Hilda, and my father, Ademir, who believed in my journey and provided unconditional support, always with great love and wisdom, guiding every step of my way.

Acknowledgment

I am immensely grateful to my advisor, Dr. Maria Luiza, for welcoming me with open arms, providing me with the opportunity to carry out this work, and for all the teachings she imparted to me during these seven years of master's and doctoral studies.

Special thanks to Symrise and to Rodrigo and Martina for believing in my potential to carry out such a significant project, marking the beginning of a new era of partnerships between the university and the company. This collaboration holds great importance not only for our laboratory but also for the State University of Londrina.

To my co-advisor, Dr. Guilherme Zocollo, for all the support provided during the final stages of this work, and for all the valuable teachings that I will carry forward into all my future projects. I am thankful for your friendship and guidance, which have contributed significantly to my personal growth.

To my colleagues at the State University of Londrina, especially to the members of Labfitobio, for all the support throughout my journey in graduate school. I would also like to express my gratitude to Embrapa Soja for their support in the final stages of my research, and particularly to technician Moisés and Geovane for being my mentor and assisting me during crucial stages of my work's development.

To my boyfriend, Gabriel, for all his support during my doctoral studies, for every weekend he spent with me at UEL to finalize experiments, and for every instance he assisted me with everything within his reach.

Finally, I would like to express my gratitude to the Postgraduate Program at the State University of Londrina and to all the professors who contributed to my academic formation and were essential in completing this stage of my life. Special thanks to Dr. Nilton Syogo Arakawa, Dra. Suzana Lucy Nixdorf, Dr. Eduardo Jorge Pilau and Dr. Valdecir Farias Ximenes for accepting the invitation to be part of my defense committee and for their contribution to this work.

Ferrari, A.B.S. Phytochemical study of Brazilian and Asiatic fruits and evaluation of their antiglycation and antioxidant potential in search of a new cosmetic ingredient. 2023. 70 p. Thesis project presented to the Postgraduate Program in Chemistry, at the State University of Londrina, as a partial requirement for obtaining the doctorate degree.

ABSTRACT

Produtos naturais têm sido reconhecidos como uma fonte valiosa de compostos que podem apresentar atividade antiglicante, como aqueles que possuem propriedades antioxidantes. Dessa forma, várias espécies como *Araucaria angustifolia* (pinheiro), *Campomanesia phaea* (cambuci), *Psidium cattelianum* (araçá), *Eugenia uniflora* (pitanga), *Psidium guajava* (goiaba) e *Nephelium lappaceum* (rambotã) têm apresentado compostos com atividades antioxidantes, sendo assim consideradas candidatas potenciais com atividades antiglicantes para uso em cosméticos. Neste estudo, analisamos as atividades antioxidantes (ensaios DPPH, ABTS˙+ e ORAC), fator de proteção solar (método de espectrofotometria), estabilidade de cor (método de cor CIE Lab) e atividades antiglicantes (ensaio de albumina sérica bovina - BSA/MGO) dessas espécies. As fracionações cromatográficas dos extratos foram avaliadas por HPLC-UV/DAD e os compostos analisados por espectrometria de massa (UHPLC-QTOF-MS). *Araucaria angustifolia* (casca), *Nephelium lappaceum* (casca) e *Campomanesia phaea* (polpa) apresentaram atividade antiglicante eficiente com etanol 70:30 para as duas primeiras e acetato de etila para a última (55,40; 62,4; 52,8% de inibição da reação de glicação, respectivamente), além de atividades antioxidantes (EC50 = 3,5 ± 0,2; 5,1 ± 0,01; e 56,0 ± 0,6 μg mL-1, respectivamente). A cor desses três extratos se mostrou estável e o extrato da casca de rambotã apresentou o maior fator de proteção solar (FPS: 6,87). O geraniin foi identificado como o inibidor de glicação mais potente no extrato etanólico das cascas de N. lappaceum. Extraídos do pinheiro, a proantocianidina e a epicatequina foram correlacionadas com o efeito inibitório sobre a formação de AGEs, sendo esta a primeira vez que isso é relatado na literatura. No extrato de cambuci, foram identificados o ácido quínico, quercetina metoxi-deoxi-hexosídeo e quercetina-O-pentosídeo, com potencial antiglicante. Consequentemente, as atividades antiglicantes identificadas nesses extratos são promissoras na mitigação das manifestações de envelhecimento cutâneo, apresentando, assim, um potencial substancial para pesquisas avançadas e desenvolvimento de novos cosméticos anti-envelhecimento.

Palavras-chave: Anti-envelhecimento, Fator de proteção, Bioatividades, Compostos naturais, HPLC-UV, UHPLC-QTOF-MS.

Ferrari, A.B.S. Estudo fitoquímico de frutas brasileiras e asiáticas e avaliação de seu potencial antiglicante e antioxidante em busca de um novo ingrediente cosmético. 2023. 70 p. Projeto de tese apresentado ao Programa de Pós-Graduação em Química, na Universidade Estadual de Londrina, como requisito parcial para obtenção do título de doutor.

RESUMO

Produtos naturais têm sido reconhecidos como uma fonte valiosa de compostos que podem apresentar atividade antiglicante, como aqueles que possuem propriedades antioxidantes. Dessa forma, várias espécies como *Araucaria angustifolia* (pinheiro), *Campomanesia phaea* (cambuci), *Psidium cattelianum* (araçá), *Eugenia uniflora* (pitanga), *Psidium guajava* (goiaba) e *Nephelium lappaceum* (rambotã) têm apresentado compostos com atividades antioxidantes, sendo assim consideradas candidatas potenciais com atividades antiglicantes para uso em cosméticos. Neste estudo, analisamos as atividades antioxidantes (ensaios DPPH, ABTS˙+ e ORAC), fator de proteção solar (método de espectrofotometria), estabilidade de cor (método de cor CIE Lab) e atividades antiglicantes (ensaio de albumina sérica bovina - BSA/MGO) dessas espécies. As fracionações cromatográficas dos extratos foram avaliadas por HPLC-UV/DAD e os compostos analisados por espectrometria de massa (UHPLC-QTOF-MS). *Araucaria angustifolia* (casca), *Nephelium lappaceum* (casca) e *Campomanesia phaea* (polpa) apresentaram atividade antiglicante eficiente com etanol 70:30 para as duas primeiras e acetato de etila para a última (55,40; 62,4; 52,8% de inibição da reação de glicação, respectivamente), além de atividades antioxidantes (EC50 = 3,5 ± 0,2; 5,1 ± 0,01; e 56,0 ± 0,6 μg mL-1, respectivamente). A cor desses três extratos se mostrou estável e o extrato da casca de rambotã apresentou o maior fator de proteção solar (FPS: 6,87). O geraniin foi identificado como o inibidor de glicação mais potente no extrato etanólico das cascas de N. lappaceum. Extraídos do pinheiro, a proantocianidina e a epicatequina foram correlacionadas com o efeito inibitório sobre a formação de AGEs, sendo esta a primeira vez que isso é relatado na literatura. No extrato de cambuci, foram identificados o ácido quínico, quercetina metoxi-deoxi-hexosídeo e quercetina-O-pentosídeo, com potencial antiglicante. Consequentemente, as atividades antiglicantes identificadas nesses extratos são promissoras na mitigação das manifestações de envelhecimento cutâneo, apresentando, assim, um potencial substancial para pesquisas avançadas e desenvolvimento de novos cosméticos anti-envelhecimento.

Palavras-chave: Anti-envelhecimento, Fator de proteção, Bioatividades, Compostos naturais, HPLC-UV, UHPLC-QTOF-MS.

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SUMMARY

1. Introduction

Natural products are well-known sources of polyphenolic compounds, particularly flavonoids. Brazil has favorable geography and climatic conditions for fruits production, being the third largest producer of common fruits in the world, and shows rich biodiversity, highlighting the abundance of native fruits with worldwide potential for consumption and development of new drugs and cosmetics. Therefore, the Brazilian biodiversity has several species that contain bioactive compounds with potential to be a new ingredient cosmetic (Silva et al., 2022).

The properties from bioactive compounds, in special the antioxidant activities have been reported to inhibit the glycation reaction that is a non-enzymatic reaction that occurs between a carbohydrate and a molecule with a free amino group. This reaction can result in significant accumulation of AGEs (advanced glycation end products) in long-lived macromolecules, such as collagen, where the amino acids lysine and arginine can undergo glycation and form cross-links, reducing skin elasticity, increasing the appearance of wrinkles, and causing healing deficiencies (Cervantes-Laurean et al., 2006; Freitas et al., 2020).

The AGEs have been identified as key players in the progression of diabetes and diabetes-induced complications once a time that hyperglycemia is the principal etiology of diabetic complications. The literature has been reported deleterious effects of AGEs in irreversible damage to the structural and functional integrity of proteins through intermolecular and intramolecular crosslinks. Adjacent AGE molecules have the capability to crosslink with each other and with certain proteins that alter the structure and interfere with the functional properties of proteins. Due to this covalent cross-link formation, the biologically active proteins and enzymes are deactivated, become resistant to proteolytic digestion (Khalid et al., 2022).

In addition, another complication of the glycation reaction is the accumulation of AGEs in the skin, mainly by binding to the collagen protein, altering they structural, biochemical, and physical properties. The concentration of AGE-modified collagen increases in tissues with increasing age and may contribute to the reduction in elasticity of artery, heart and lung tissues that occurs as organisms age. Therefore, a lot of studies of the compounds with antiglycation activities have been reported (Cervantes-Laurean et al., 2006).

A a few natural products with antiglycation activity, such as quercetin, epigallocatechin gallate, myricetin and naringenin (Chinchansure et al., 2015). They have some common structural features, as possessing an aromatic ring with hydroxy group substituents (phenols) as part of the molecular structure. Thus, it is proposed that phenols are important for the antiglycation activity of natural derivatives. However, much remains to be discovered, as there are many classes of secondary metabolites that have never been tested and studied for the potential for inhibition of advanced glycation reaction (Cervantes-Laurean et al., 2006; L. De Freitas et al., 2020).

In this way, five species are originated from Brasil (*Araucaria angustifolia, Campomanesia phaea, Psidium catelianum, Eugenia uniflora and Psidium guajava) and* one of South-East Asia (*Nephelium lappaceum)* were studied in search of bioactive compounds for use in cosmetics with potential to inhibit the glycation reaction.

1.1 Studied species

1.1.1 *Araucaria angustifolia* (pine)

Araucaria angustifolia from Araucariaceae family, popularly known as Paraná pine, Brazilian pine or simply "Araucaria" (Figure 1), is a native gymnosperm of the Atlantic Forest in Brazil and has great economic, cultural, and social importance. It is concentrated in the Southern Brazilian States (Paraná, Santa Catarina and Rio Grande do Sul), with some representative forests in the Southeastern Brazilian States (São Paulo, Minas Gerais and Rio de Janeiro) (Corrêa et al., 2016; Soares & Mota, 2004).

Figure 1. (a) *Araucaria angustifolia* tree; (b) Pine hanging on the branch of the tree; (c) Pine cut in half.

Fonte: Adapted from google images (2023).

The pine harvest takes place from April to June (in Brazil) and the edible part of the seed, known as Brazilian pine or pine in Portuguese language, is consumed by different forms. The Brazilian pine seed bark is not consumed because it is very hard, nonetheless the literature have been reported its use to product edible films based on its flour (Daudt et al., 2017). The seeds are usually cooked in water or roasted and after they are crushed and the flours of raw or cooked pine are used in the preparation of regional dishes, cakes, breads and cookies. Both of this part have a high nutritional value with known antioxidant compounds, such as amentoflavon and mono-*O*-methylamentoflavone (Corrêa et al., 2016).

This species is appreciated for the high quality of its seed barks, and different parts of Araucaria angustifolia have been employed in Brazilian folk medicine to treat various ailments. They are known in popular culture as preventative measures against diseases, such as the treatment of rheumatism and infusions of the nodes, which are used orally for the treatment of kidney diseases and sexually transmitted infections (Corrêa et al., 2016; Zilli Ruiz et al., 2021). According to Zortéa-Guidolin et al. (2014), studies have shown that pine has a slower digestibility, which can lead to the gradual release of glucose, resulting in a low glycemic response and potentially reducing the risk of diabetes.

Residual pine barks, which represent about 20% of the weight have also been attracting interest due to their antioxidant properties and potential biological activity (Silva et al., 2014). In addition, Silva et al. (2014) reported the efficacy of pine bark

extract (70% ethanol in water) to decrease the postprandial glycemic levels in rats after starch administration. Authors claimed that the extract may be potentially used to suppress postprandial hyperglycemia in diabetic patients due to its inhibitory properties (Freitas et al., 2018).

Silva and collaborators (2014) revealed that the tannin, present in pine, is an effective inhibitor of both the human salivary and the porcine pancreatic α-amylases, and also an inhibitor in the decrease of blood glucose levels in rats after administration of starch (da Silva et al., 2014). In this way, the hydroalcoholic extracts obtained from the pine barks are promising to inhibit the glycation reaction, since the decrease in blood glucose levels may be directly related to the glycation reaction.

However, there is still a lack of comprehensive studies in the literature that demonstrate the antiglycation potential of pine barks and the correlations between the compounds responsible for this activity.

1.1.2 *Campomanesia phaea* (cambuci)

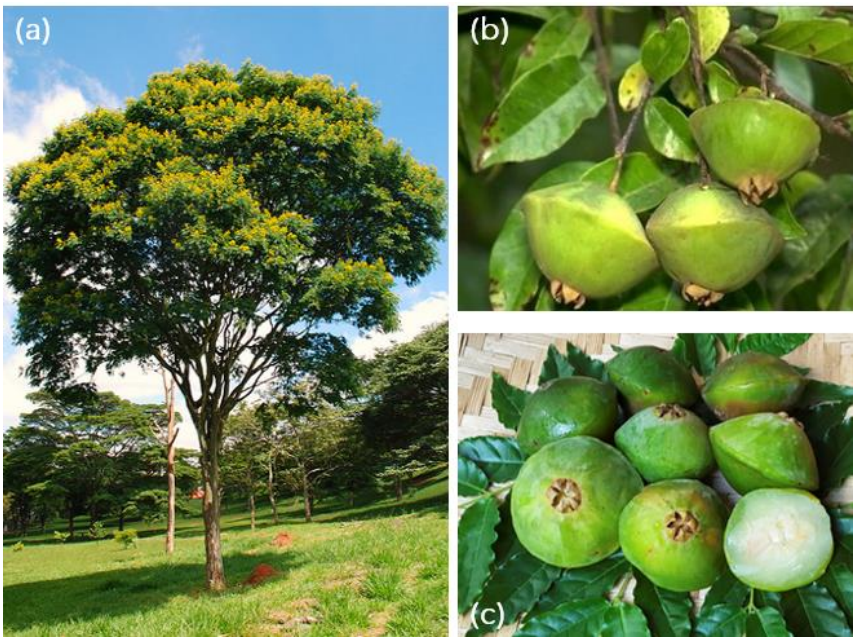
Campomanesia phaea, from Myrtaceae family, is a species native Brazilian Atlantic Rainforest, naturally occurs in state of São Paulo and in smaller numbers in the states of Rio de Janeiro and Minas Gerais (Figure 2). The fruit, popularly known in Brazil ascambuci, contain a fleshy and soft pulp when ripe, a thin skin and persistent green coloration. All cambuci parts (bark, pulp and seed) are edible, prepared and consumed by different forms such as juices, ice creams, and jellies. The chemical fruits profiles present compounds with antioxidant properties, acting as anti-inflammatory, anti-diabetic and antimicrobial, as well as, protective effects on the cardiovascular system (Spricigo et al., 2021).

Donado-Pestana and collaborators demonstrated the potential health-beneficial actions of the polyphenols from cambuci, preventing the development of obesity-associated metabolic complications, such as glucose intolerance, and meta inflammation, as well as antioxidant and antidiabetic properties in in vitro assays (Donado-Pestana et al., 2021).

According to Gonçalves et al., (2010), the pulp of cambuci is an source of bioactive compounds, such as quercetin and its derivates, kaempferol and cyanidin. In

addition, the pulp extract presented a high capacity to inhibit enzymes of carbohydrate metabolism that have been related to the glycation reaction.

Figure 2. (a) *Campomanesia phaea* tree; (b) Cambuci fruit hanging on the branch of the tree; (c) Cambuci cut in half.



Fonte: Adapted from google image (2023).

1.1.3 *Psidium cattelianum* (araçá)

Psidium cattleianum, from Myrtaceae family, is a Brazilian native species that can be found from Bahia to Rio Grande do Sul states, and also in the country of Uruguay. In Brazil, its fruitful season occurs twice a year, the first from September to October and the second in December. The fruit (Figure 3), known as araçá, is consumed in nature or processed (sweets, jams and juices). The araçá has high potential to the agri-food sector (Pereira et al., 2018).

Figure 3. (a) *Psidium cattelianum* tree; (b) Araçá fruit hanging on the branch of the tree; (c) Araçá cut in half.

Fonte: Adapted from google images (2023).

Research workshave been reported high content of phenolic compounds present on araçá (pulp and bark) with high antioxidant capacity, attributed to compounds vitamin C and phenolic compounds, mainly epicatechin and gallic acid. In vivo studies, rats fed with powdered freeze-dried araçá showed remarkable decrease on parameters altered by oxidative stress induced by cisplatin. Animals showed low levels of glucose, LDL cholesterol, oxidized LDL cholesterol and total cholesterol when compared with control animals cisplatin-induced animals not feed with araçá (Biegelmeyer et al., 2011; Pereira et al., 2018).

Other studies have been reported with the araçá extracts as a potential inhibitor of digestive enzymes, such as alfa-glucosidase e alfa-amylase, being an alternative to the modulation of hyperglycemia. Nevertheless, this activity can be related with some of the phenolic compounds present in araçá extracts, such as quercetin, myricetin and epicatechin, since these compounds are responsible for inhibiting specific enzymes (Pereira et al., 2018).

1.1.4 *Eugenia uniflora* (pitanga)

Eugenia uniflora, belonging the family Myrtaceae, is a species originally from Brazil (Figure 4). The tree grows in the tropical and subtropical regions, its fruit, known as pitanga, is distinguished by its peculiar red color, flavor and fragrance. Pitanga is mainly marketed in the form of pulp, widely used by the Brazilian industry for juice production (Massarioli et al., 2013).

Figure 4. (a) *Eugenia uniflora* tree; (b) Pitanga fruit hanging on the branch of the tree; (c) Pitanga cut in half.

Fonte: Adapted from google images (2023).

Literature report the presence of therapeutic compounds in *Eugenia* species, especially phenolic compounds such anthocyanins, responsible for their coloration. According to the anthocyanin contents of purple and red fleshed pitanga (136.6 mg.100 g^{-1})are higher than pulps of grape (*Vitis vinifera*) (30.9 mg100 g^{-1}), and açaí fruit (*Euterpe oleracea*) (22.8 mg 100 g^{-1}), which is from the same genus as pitanga (Bagetti et al., 2011).

Peixoto and collaborators observed the potential of pitanga to be used on control of diabetes. It can also reduce insulin resistance, improving pancreatic β-cell function, stimulating insulin secretion through the protection of pancreatic β-cells against oxidative damage, and inhibiting apoptosis (Peixoto Araujo et al., 2021).

In addition, another study reported that the juice extract of *Eugenia jambolanum* was observed to possess anti-inflammatory, anti-diabetic, antibacterial and

gastroprotective activities. *Eugenia dysenterica* (also known as cagaita) encapsulated fruit extract was reported to have antimicrobial, antioxidant and antiglycation activities (Karasawa & Mohan, 2018).

Eugenia uniflora presented a good effect in the human health, with reported presence of high amounts of catechins, flavonols, and proanthocyanidins, compounds known for their antioxidant activity (Biegelmeyer et al., 2011). However, the antiglycant potential of this specie has not been reported in literature.

1.1.5 *Psidium guajava* (guava)

Psidium guajava L. belonging to the family Myrtaceae, is native to the tropical region of the American continent, with a probable center of origin in the region between southern Mexico and northern South America. It is a small tree with yellow fruits (Figure 5). Today, it is widespread throughout all regions and in Brazil it can be found in Amazon, Caatinga, Cerrado, Atlantic Forest, Pampa (Pereira et al., 2003; Proença et al., 2022).

Brazil is among the largest guava producers in the world and the majority of the country's production is destined for the food industry to produce sweets, juices, jellies and frozen pulps. As result of the fruiting process, there is a discard of the leaves, seeds, part of the barks and pulp fraction not separated in the physical depumping process (Hsieh et al., 2007; Schnitzler, 2012).

Figure 5. (a) *Psidium guajava* tree; (b) Guava fruit hanging on the branch of the tree; (c) Guava cut in half.

Fonte: Adapted from google images (2023).

Their leaves or leaf product, such as guava leaf drinks, have long been utilized as a folk's medicine as an effective astringent and hemostatic, being a superior therapeutic for diabetes and enteritis. It is reported that this fruit is rich in vitamin C and polyphenolic compounds, such as tannins, phenolics and flavonoids (Hsieh et al., 2007). In addition, the literature has reported potent antioxidant activity in guava leaf extracts and attributed it to the phenolic compounds, such as phenolic acids, gallic acid and ferulic acid (Hsieh et al., 2007; Wu et al., 2009a).

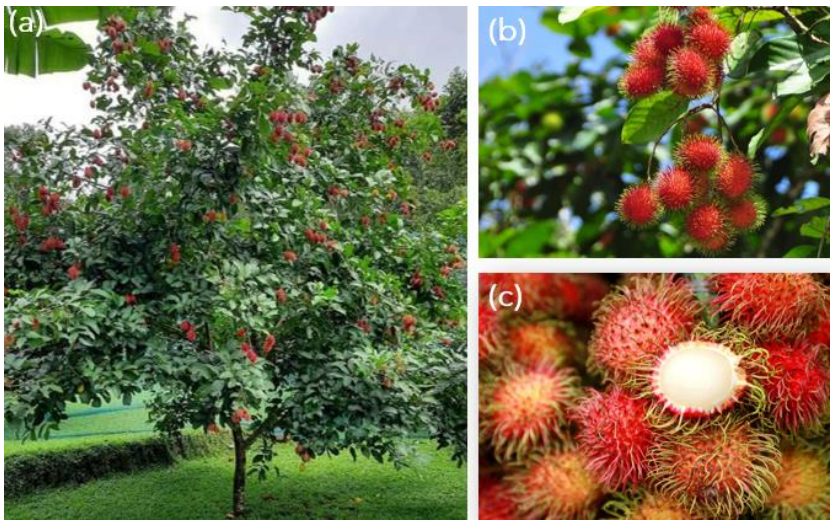
However, many of these previous studies focused on the effects of guava leaf, which was not edible for most consumers. In this way a lot of studies has been evaluated to analyzed the antiglycation activities of the guava barks. According to Mukhtar et al., (2006) the ethanolic extract from guava barks showed a considerable and statistically significant hypoglycemic effect, which may be due to potentiation of insulin release from pancreatic cells.

Other studies have demonstrated that guava leaf and bark extracts also had hypoglycemic effects on experimental models' drug-induced to severe conditions of diabetes. However, despite the presence of several antioxidant compounds and some reports of the prevention of diabetes complications, the antiglycant potential of guava pulp and bark is still little discussed (Mukhtar et al., 2006; Wu et al., 2009b).

1.1.6 *Nephelium lappaceum* (rambutan)

Nephelium lappaceum, from Sapindaceae family, is native to South-East Asia, and popular in Vietnam, Philippines, Indonesia, Thailand, Burma, Sri Lanka and India. Its name is derived from the Malay word rambut, meaning hair, concerning the soft thorns that cover the fruit surface, and in Brazil is known as rambutan (Figure 6) (Tindall 1994).

Figure 6. (a) *Nephelium lappaceum* tree; (b) Rambutan fruit hanging on the branch of the tree; (c) Rambutan cut in half.



Fonte: Adapted from google images (2023).

The fruit of rambutan can be consumed in fresh or as processed fruit. Rambutan barks have been proven to be a rich source of phenolic compounds with great antioxidant activity, low pro-oxidant and non-toxic capacity (Viet & Nguyen, 2017). Khonkarn et al. (2010) studied the cytotoxicity of this species using KB (human epidermal carcinoma of the mouth with HeLa contaminant) and Caco-2 (human colorectal adenocarcinoma) cells and shows that the methanol extract of rambutan presented a strong cell growth being a non-toxic extract (Khonkarn et al. (2010).

In addition, literature researches reported that the extracts of rambutan fruit, mainly from the bark, possess phytochemical compounds that exhibit antioxidant, antimicrobial, antidiabetic, antiviral, anti-inflammatory, anti-hypoglycemic and anti-proliferative effects in vitro and in vivo (Aguilar, 2019; Andrade et al., 2009).

The rambutan is constituted by the following parts: 27.4% total weight, 13.2% bark, 11.7% pulp, 2.53% seed and 1.60% embryo. The most consumed part is the pulp, causing the production a significant amount of bark and seed waste. However, the rambutan bark also exhibits a rich chemical composition with fiber as cellulose, hemicellulose, and lignin. Furthermore, Aguilar and collaborators reported that rambutan bark has a high content of phenolic compounds and present antioxidant activity (Aguilar, 2019).

Compared to rambutan seed extracts, the bark contains a greater amount of antioxidant compounds, these are mainly polyphenolic compounds, known for their

biological activities, including geraniin, corilagin, and ellagic acid, a group of ellagitannins, and among them the major compound present in the rambutan bark is geraniin (Aguilar, 2019).

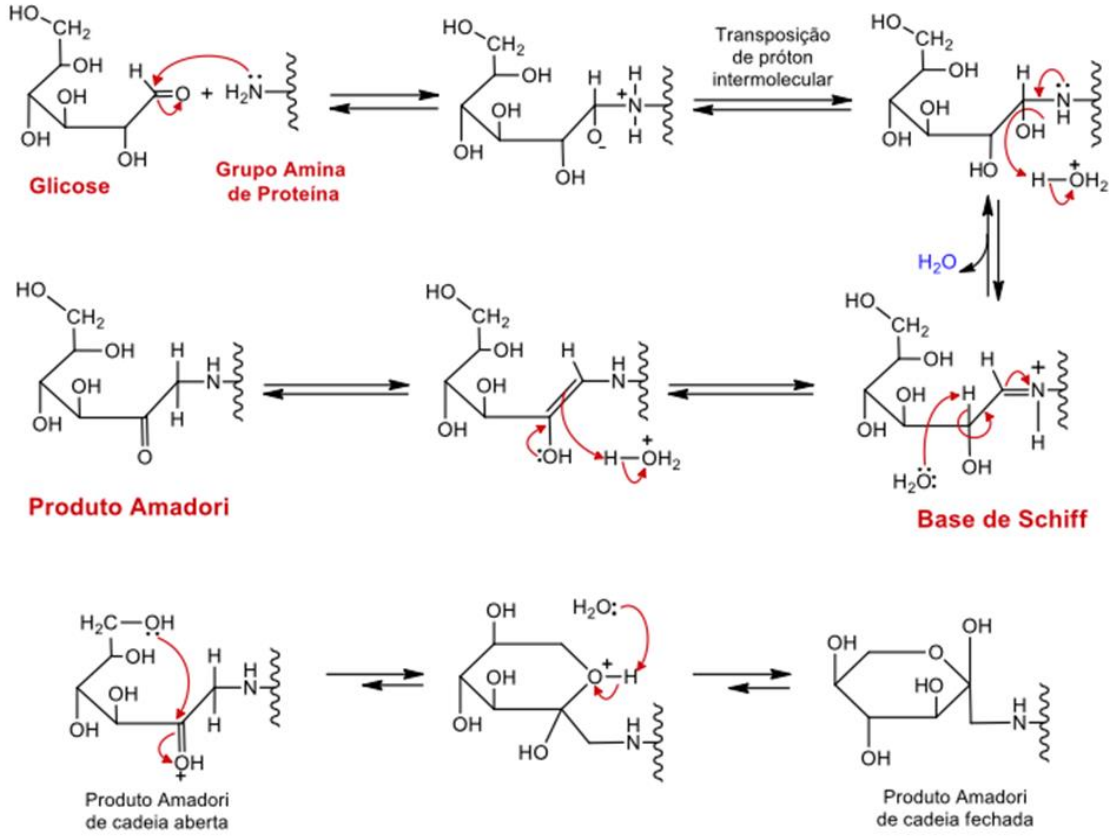
Additionally, Soeng et al. (2015) reported that rambutan seed extract exhibited a high content of α- glucosidase inhibitory activity, presenting an effect in reducing the blood glucose level and the body weight of mice induced with alloxan tetrahydrate. So, according to this results the rambutan could present a high activity to inhibit the glycation present in the skin aging.

1.2 Glycation Reaction

Glycation represents a non-enzymatic reaction that primarily involves free amino groups in proteins, notably lysine, and reducing sugars such as glucose. While this process unfolds gradually, the resulting products of this reaction, known as advanced glycation end products (AGEs), gradually accumulate in the skin over time, leading to alterations in the skin's physical, biomechanical properties (including stiffening and loss of elasticity), and biological properties (such as the modulation of matrix synthesis and degradation by cells) during the aging process.

The classical pathway of the Maillard reaction, also called glycation is a non-enzymatic reaction between the carbonyl group of reducing sugars and a free amino group of proteins (Maillard reaction, Figure 7), leading to the formation of a Schiff base, which is the first product of the glycation reaction that is relatively fast and highly reversible. The next step involves conversion of a thermodynamically unstable Schiff base into a stable reversible Amadori product. The proteins resulting from Amadori reactions are referred as glycated proteins or Maillard reaction products. Finally, the Amadori products undergoes a series of dehydration and fragmentation reactions, resulting in a variety of carbonyl compounds, including methylglyoxal (MGO), glyoxal (GO), glucosones, 3-deoxyglucosone (3-DG) and others. These carbonyl compounds are more reactive than the original sugar and act as reaction propagators, leading to the formation of advanced glycation end-products (AGEs) (Cervantes-Laurean et al., 2006).

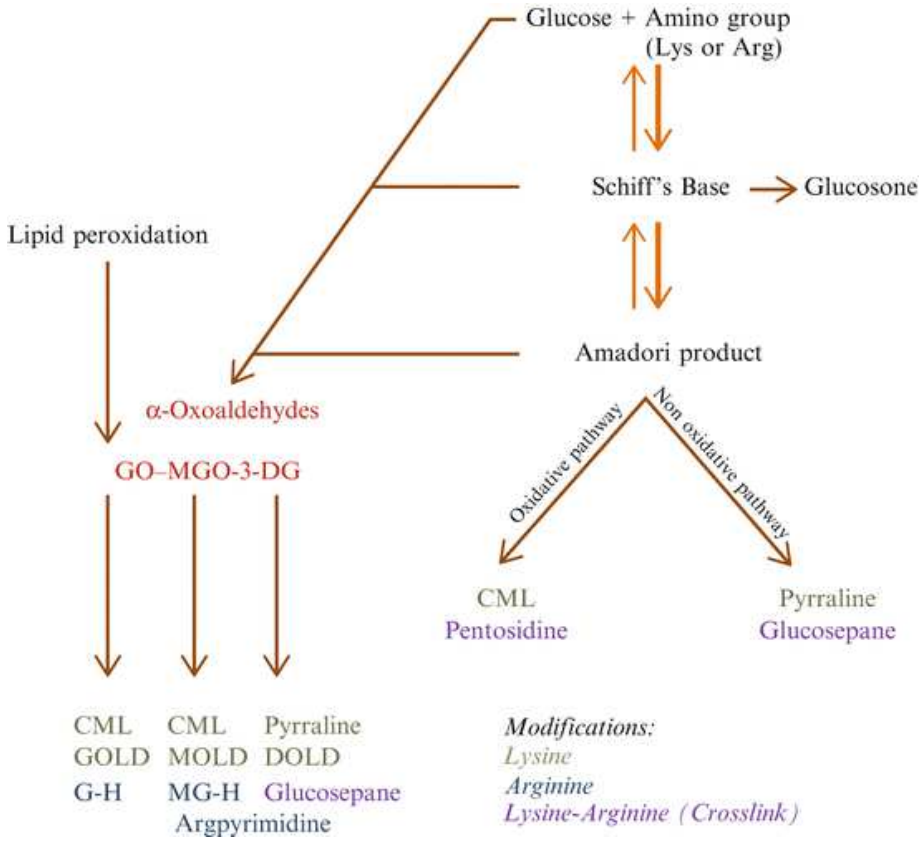
Figure 7. Glycation Reaction by classic pathway



Font: Seara, et al. (2008)

Alternative mechanisms of AGE formation include the called "carbonyl stress" pathway (Figure 8), in which the oxidation of lipids or sugars generates highly reactive intermediate dicarbonyl compounds (8). Glycolysis and glucose autoxidation, for example, produce methylglyoxal and glyoxal, which interact with amino acids to form AGEs. These dicarbonyl compounds are up to 20,000 times more reactive than glucose and are the main intermediates in the formation of AGEs (9). AGEs formed from the oxidation of sugars or lipids can also be called, respectively, products of glycoxidation or advanced lipid oxidation. It should be noted that, during some of the reactions that lead to the formation of AGEs, reactive oxygen species (ROS) are generated and compete in parallel with oxidative stress and structural and functional damage to macromolecules (Pageo, Pennacchi, and Asselineau, 2017).

Figure 8. Representation of the alternative mechanisms of glycation reaction by the carbonyl stress.



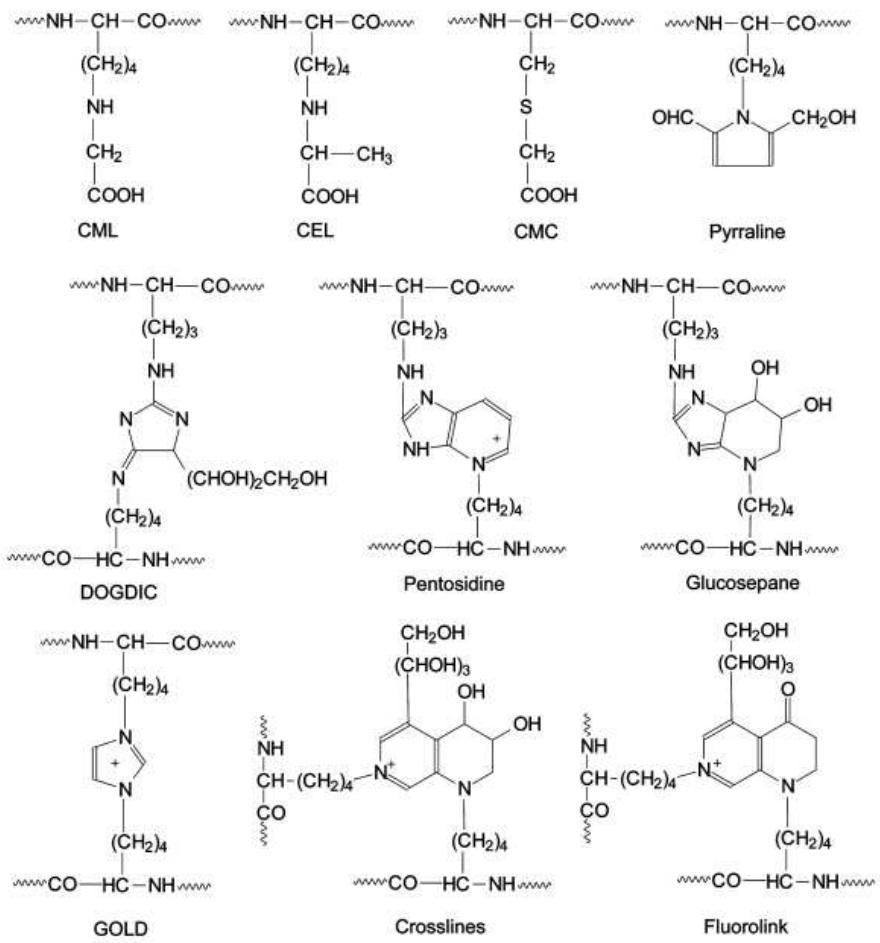
**CML* carboxymethyl-lysine, *CEL* carboxyethyl-lysine, *GOLD* glyoxal-lysine dimer, *MOLD* methylglyoxal-lysine dimer, *DOLD 3*-deoxyglucosone-lysine dimer, *G-H; G*-hyydroimidazolone, *MG-H MG*-hydroimidazolone, *GO* glyoxal, *MGO* methylglyoxal,*3-DG 3*-deoxyglucosone.

Font: Adapted from Pageo, Pennacchi, and Asselineau (2017).

Advanced glycation end-products (Figure 9) accumulate on long-lived proteins, such as collagen, altering structural, biochemical and physical properties. The concentration of AGE-modified collagen increases in tissues with increasing age and may contribute to the reduction in elasticity of artery, heart and lung tissues that occurs as organisms age. In turn, an age-related reduction in elasticity may lead to the age-dependent decline in physiological functions such as cardiac index, vital capacity, renal blood flow and maximum oxygen uptake (Cervantes-Laurean et al., 2006).

Figure 9. Representative advanced glycation end-products (AGEs). Oxidative decomposition of the Amadori product or reaction of tissue proteins with reactive carbonyl and dicarbonyl compounds can lead to the formation of advanced glycation end-

products (AGEs). AGEs include Nε -(carboxymethyl) lysine (CML), Nε -(carboxylethyllysine) (CEL), S-(carboxymethyl) cysteine (CMC), pyrraline, 3-deoxyglucosone-derived imidazolium cross-link (DOGDIC), pentosidine, glucosepane, glyoxal lysine dimer (GOLD), crosslines, and fluorolink (Zhang et al., 2009).



Font: Cervantes-Laurean et al. (2006).

Another problem caused by the glycation is its contribution to the cellular mechanisms of insulin resistance that worsen cases of type 2 diabetes. Insulin is a hormone that lowers blood glucose levels in the body acting through insulin receptors on target cells. In addition, glycated insulin in pancreatic cells or in the blood makes it harder to maintain homeostatic levels of glucose and to stimulate lipogenesis. Finally, several studies have shown that glycated albumin and methylglyoxal (a glycation intermediary) decrease the biological responses triggered when insulin binds to its receptor on the target cell (Zhang et al., 2009).

Currently, there are many therapeutic options to reduce AGEs available, including AGE cross-link breakers, AGE inhibitors, already clinically approved drugs, nutrition and phototherapy, whereas only a few of them have been clinically evaluated (Jud & Sourij, 2018).

The first form to inhibit or prevent glycation reaction is modifying or blocking the amino acid side chains by bioactive compounds, before the Schiff base/Amadori product formation. For example, acetylsalicylic acid prevents protein glycation by transferring an acetyl group to lysine, and the acetylated lysine cannot react with glucose, and consequently avoids the formation of AGEs. Natural products with an ability to acetylate proteins could be potential glycation inhibitors (Chinchansure et al., 2015; Jud & Sourij, 2018).

The second form to inhibit or prevent glycation reaction and AGEs formation is reducing oxidative stress, that occurs during the Schiff base/Amadori product formation, this is the first step in the glycation reaction, where intervention can be carried out. Various inhibitors are known to act at this stage of the reaction, e.g. amines, polyamines, and small peptides. Some natural products, like gallic acid, (+)-catechin and quercetin are also known for inhibit Amadori product formation (Chinchansure et al., 2015).

Oxidative stress promotes the glycation reaction and acts synergistically to increase the accumulation of AGEs. It also upregulates the expression of RAGE (receptor advanced glycation end-products), which is the principal receptor in AGEs induced pathogenesis. The AGE–RAGE interaction results in ROS production and activation of a pro-inflammatory pathway. Thus, reducing oxidative stress may inhibit AGE formation. Many plant-derived products, especially dietary antioxidants, are known to possess antiglycation activity (Chinchansure et al., 2015; Jud & Sourij, 2018).

The third form to inhibit or prevent glycation reaction occurs when the reaction has done with the cross-link breakers breaking the link between AGEs and extracellular molecules. Members of this class include thiazolium derivatives, like N-phenacyl thiazolium bromide (PTB) and alagebrium or ALT-711, and pyridinium derivatives, like TRC4186 and TRC4149. Although the exact mechanism of the AGE cross-link breakers is not fully understood, it seems that AGE cross-link breakers break diketone compounds up. So far, clinical data are only available for alagebrium and TRC4186. Another clinically investigated AGE cross-link breaker is TRC4186 (Jud & Sourij, 2018).

The most known compound capable to inhibit the glycation reaction is aminoguanidine. It is a nucleophilic agent with two key reaction centers: the nucleophilic

hydrazine group —$NHNH_2$ and the dicarbonyl-directing guanidino group —NH-C(=NH) NH_2. According to Lewis & Harding, (1990) this compound bind the proteins (first form) and protects them directly or indirectly against attack by sugar, in which prevents glycation by binding to the protein. In addition, Thornalley, (2003) reported that the two amino groups of aminoguanidine can reacts rapidly with α, β, -dicarbonyl compounds such as methylglyoxal, glyoxal, and *3*-deoxyglucosone and prevent the formation of advanced glycation end products (AGEs).

In another way, the antioxidant compounds from natural products act in the second stage of the reaction. According to Narda et al., (2018) the AGE modified biomechanical properties leading to loss of elasticity and increased stiffening; changes that promote the appearance of wrinkles and after this point it not easy to revert the process.

1.2.1 Role of AGEs during skin aging

Aging is the progressive accumulation of damage to an organism over time leading to disease and death. Over the past few years, there has been significant research dedicated to understanding the pathophysiology of ageing and identifying potential interventions to combat age-related illnesses. Various theories have emerged to explain the ageing process, with particular focus on advanced glycation end products (AGEs) in recent investigations (Gkogkolou and Böhm, 2012).

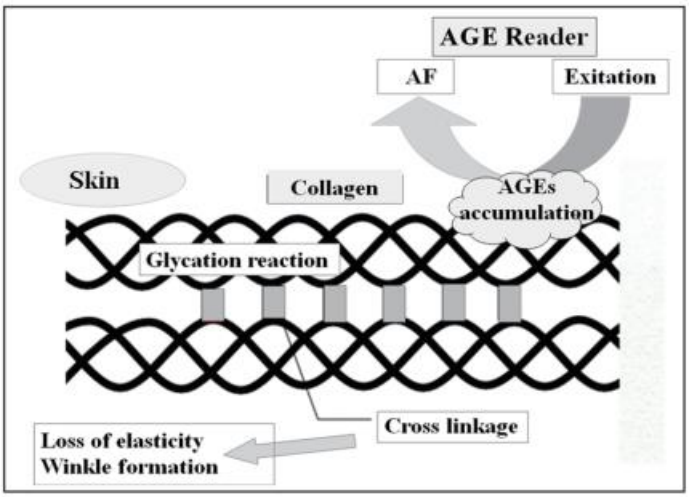
The sources of AGEs in the skin are primarily divided into endogenous production and exogenous intake. Endogenous AGEs are generated and accumulate in the skin during normal physiological metabolism as part of aging, or in diseases linked to inflammatory responses or chronic metabolic disorders. Exogenous AGEs stem from dietary sources, cigarette smoke, ultraviolet light exposure, and air pollution (Chen et al., 2022).

Studies have suggested that AGEs accumulate subsequent to their production within the human body, resulting in the degradation of skin tissues by modulating gene expression, disrupting protein structures, binding to RAGEs, mediating various signaling pathways, and influencing the apoptosis and differentiation of skin-related cells. AGEs impact all layers of the skin, precipitating inflammation, aging, yellowing, and other related concerns (Gkogkolou and Böhm, 2012; Chen et al., 2022).

In addition, AGEs and collagen form cross-links (Figure 10), resulting in protein browning and fiber deformation. Elastin fibers become thinner, less rigid, and lose their

biological properties. Glycosylated vimentin contributes to the impairment of fibroblasts' contraction ability and the inability to maintain the basic cell shape. AGEs bind to RAGE receptors, modulating gene expression, and orchestrating a cascade of signaling pathways (Gkogkolou and Böhm, 2012; Chen et al., 2022; Zgutka et al., 2023).

Figure 10. Glycation of collagen and AGEs accumulation in the skin (Ichihashi et al., 2011).



Font: Ichihashi et al. (2011).

As mentioned above, Aging promotes the formation and accumulation of AGEs in various parts of the body, triggering metabolic, appearance, and biomechanical changes in organs. Inhibiting AGE formation can limit oxidative and inflammatory damage in tissues, improving quality of life during aging. Dietary rich in antioxidants compounds have been associated with functional and physiological improvements in individuals with and without age-related diseases. These lifestyle interventions help reduce AGE formation and accumulation, mitigating cellular and organic changes associated with aging(Zgutka et al., 2023).

1.2.2 Antioxidant compounds with antiglycation activity

Research studies show only a few natural compounds with both activities antiglycant and antioxidant. These compounds have an aromatic ring with hydroxy group

substituents as part of the molecular structure, such as the phenols and flavonoids (Desmouliè, 2018; L. De Freitas et al., 2020).

Antioxidant compounds are known to inhibit the glycation reaction during the Schiff base/Amadori product, this is the first step of the glycation. Their antiglycation potential was caused by their antioxidant activities and was related to their abilities to trap reactive carbonyl species, such as MGO (Chinchansure et al., 2015).

In addition, advanced glycation of proteins from glucose or ascorbate has been shown to often require a free radical oxidation reaction. Even though it is not always required, oxygen does frequently accelerate the glycation when it is present (Tessier, 2010). Therefore, some antioxidants compounds present antiglycation activities as shown in Table 1.

Table 1. Compounds with antiglycation activity.

Compound	Activities
b-Carotene	Inhibitory effects on the formation of AGEs; prevention of secondary structural changes in BSA due to thermal glycation
(+)-Catechin	Protective activity against glycation, tryptophan damage, browning and main-chain fragmentation of proteins incubated with glucose
-Epicatechin	Protective activity against glycation, tryptophan damage, browning, and main-chain fragmentation of proteins incubated with glucose
Epigallocatechin gallate	Decreased AGE-stimulated gene expression; inhibits the AGE-mediated activation and DNA binding activity of NF-kB by suppressing the degradation of its inhibitory protein IkBa in the cytoplasm
7-Hydroxyflavone	Protective activity against glycation, tryptophan damage, browning, and main-chain fragmentation of proteins incubated with glucose
Kaempferol	AGE-related NF-kB activation and its pro inflammatory genes through the suppression of AGE-induced NADPH oxidase activation

Morin	Protective activity against glycation, tryptophan damage, browning, and main-chain fragmentation of proteins incubated with glucose
Myricetin	Protective activity against glycation, tryptophan damage, browning, and main-chain fragmentation of proteins incubated with glucose
Naringenin	Protective activity against glycation, tryptophan damage, browning, and main-chain fragmentation of proteins incubated with glucose4

Font: Chinchansure et al., (2015)

In 1990 was identified that aminoguanidine and rutin could inhibit the collagen-linked in diabetic rats. In addition, currently the literature have been described by the establish the relative efficacy of various antioxidant compounds such as flavonoids and rutin metabolites as inhibitors of glucose autoxidation, glycation and specific AGE formation, estimated as CML and pentosidine formation and glucose-induced collagen-linked fluorescence, under conditions of hyperglycemia using glucose and type I collagen as in vitro models (Jung et al., 2015).

Quercetin is an important flavonol present in many plant species. *Psidium guajava* leaf extract, for example, inhibited glycation processes in an albumin/glucose model system and was identified as one of the active compounds with over 80% inhibitory effect. Chinchansure and collaborators related that this compound prevented oxidative stress conditions induced by stress in streptozotocin (STZ) and were found to increase insulin release (Chinchansure et al., 2015).

Other flavonoids with antiglycation activities are morin and myricetin. These compounds exhibited suppression of AGE formation. Catechins and their proanthocyanidins polymers have also been reported to exhibit an inhibitory effect on the formation of AGEs in a BSA/glucose model. Their antiglycation potentials were caused by their antioxidant activities and were related to their abilities to trap reactive carbonyl species, such as MGO (Chinchansure et al., 2015; Yim et al., 1995).

In this way, despite there being reports in the literature researches about compounds responsible for inhibiting skin aging associated with glycation. However, there are still many species that have not been studied and that require special attention

because they have easy access for collection and a large production chain available for industrialization.

2. General objective

The objective of this project is carrying out the phytochemical studies of six species: *Araucaria angustifolia, Campomanesia phaea, Psidium cattleianum, Eugenia uniflora, Psidium guajava* (natives from Brazil), and *Nephelium lappaceum* (Asian origin), in search of bioactive compounds and/or extracts with antiglycation, protection factor and antioxidant activities for manufacturing new cosmetics.

2.1 Specific objective

• Collection and preparation of fruits extracts of the species *Araucaria angustifolia, Campomanesia phae, Psidium cattleianum, Eugenia uniflora, Psidium guajava, and Nephelium lappaceum;*

• Study of the antioxidant, protection factor and antiglycation activities using different extractors solvents and selection of extracts with greater bioactive potential;

• Development of chromatographic methods by HPLC-UV/DAD to separate the compounds present in extracts selected;

• Fractionation of the bioactive extracts by HPLC-UV/DAD preparative;

• Use of the UHPLC-QTOF-MS technique to identify chemical structures of the compounds of interest in each bioactive extract;

• Establish a relationship between biological activity and its chemical constituents.

3. Methods

3.1 Selection of the Species

In this project were studied 6 species, being 5 from Brazil, and 1 from Asian. *Araucaria angustifolia* (Authorization number: A94CC9D), *Campomanesia phaea* (Authorization number: AC2F458), and *Psidium cattleianum* (Authorization number: AA6456D) were selected because they already have promising results for the glycation assay, previously obtained by the LabFitoBio, UEL; furthermore, several antioxidant compounds were related in their composition, according to the literature. *Psidium guajava* (Authorization number: AA6456D) and *Nephelium lappaceum* (Authorization number: AD359B5) were selected because these species belonging to list of China compliant, which demonstrate that they are allowed for use in cosmetics in China. *Eugenia uniflora* (Authorization number: AF1FFD4) was selected due to presence of antioxidant compounds previous related in the literature, easy collection access, and belonging to the Myrtaceae family, like the other four species.

3.2 Collection and preparation of fruits

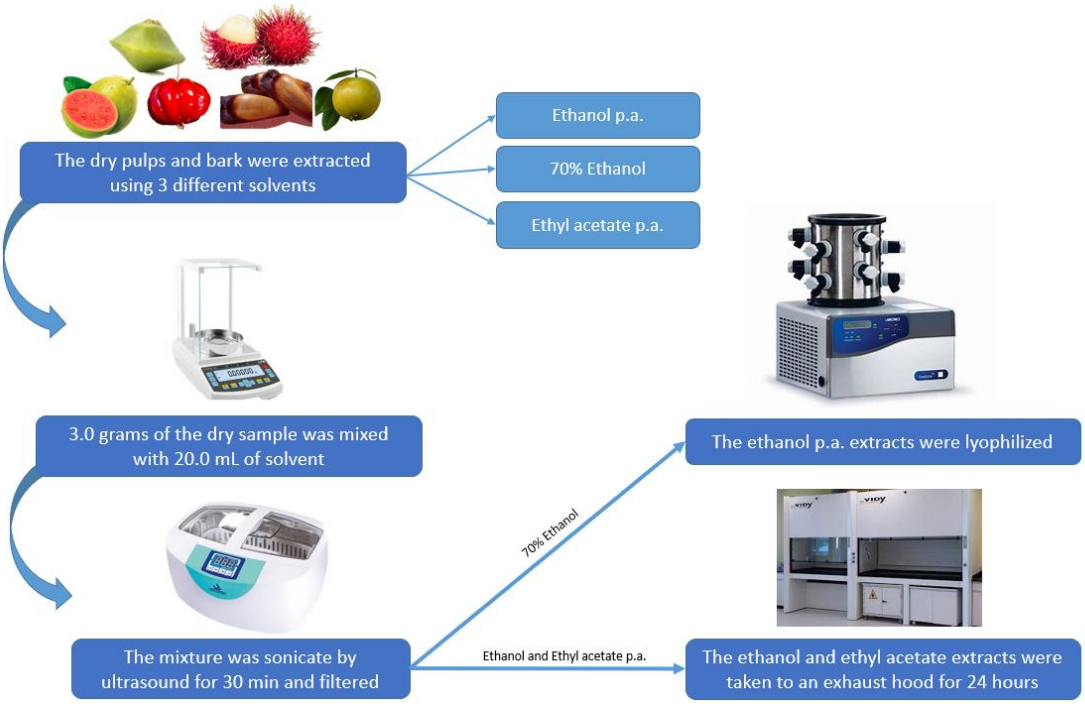
The fruits of *Psidium cattleianum* were collected in Recanto Flora Londrina Viveiro Florestal, in the city of Londrina Paraná in 02/10/2019; *Psidium guajava* and *Eugenia uniflora* were collected in the campus of the State University of Londrina (UEL), Paraná, in 30/01/2020; *Campomanesia phaea* was purchased from Institute for Socio-Environmental Entrepreneurship, located in the city of Osasco in 17/01/2020; *Araucaria angustifolia* and *Nephelium lappaceum* were purchased in the local markets of the cities of Londrina and Belém, respectively. After acquire the fruits, they were brought to LabFitoBio-UEL, sanitized and then separated into bark, pulp and seed, with exception of pitanga (*Eugenia uniflora*) that was did a mix (pulp and bark). The seeds were discarded. The pulp was frozen and the barks were oven dried at 40 °C for 24 hours.

3.3 Preparation of extracts

For the preparation of the extracts (Figure 11), the pulps and fruit barks were extracted by maceration using 3.0 grams of the dry and ground samples and 20.0 mL of different solvents, pure ethanol (EtOH P.A. – Dinâmica), 70:30 ethanol:water (70%

EtOH) or ethyl acetate (EtOAc P.A. – Dinâmica), left to sonicate ultrasound for 30 min. After this step, the solution was filtered to remove the supernatant (extract). Repeat the procedure for two more consecutive times. The EtOAc and EtOH extracts were concentrated under reduced pressure to total withdrawal and then taken to an exhaust hood for a period of 24 hours. The 70% EtOH extract was concentrated under reduced pressure to the total of the ethanol withdrawal and then lyophilized for a total withdrawal of the water present. The extraction method was adapted from the Brazilian Pharmacopoeia (2011).

Figure 11. Preparation of the extracts of the fruits studied with different solvents: ethanol p.a., 70% ethanol and ethyl acetate.



Font: Author herself.

3.4 Selection of active extracts with antiglycation, protection factor and antioxidant activities.

The evaluation of the in vitro antiglycation, protection factor and antioxidant activities (DPPH, $ABTS^{\cdot+}$ and ORAC assays) for all the extracts were carried out with different extractors solvents (total of 45 extracts tested), in order to obtain an initial screening and make the selection of the active extracts to proceed to the next steps. LabFitoBio has a procotol that considers an active extract with result above 50% of inhibition of the radical or enzymes.

3.5 Antiglycation activity assay

Antiglycation activity was determined by the bovine serum albumin (BSA) assay, according to the method described by Ramkissoon and co-workers (2013), with some modifications. BSA (Sigma) (1 mg mL^{-1}) will be prepared in 10 mmol L^{-1} sodium phosphate buffer (Sigma), pH 7.4, containing 150 mmol L^{-1} sodium chloride (Sigma). Methylglyoxal (MGO - Sigma) (5 mmol L^{-1}) and the extracts (150 µg mL^{-1}) should be added to the BSA solution, and the mixtures incubate at 37°C for three days. Samples was incubated in the presence and absence (negative control) of aminoguanidine (10 mmol L^{-1}), used as positive standard. After incubation, fluorescence (FL) of samples was measured at the excitation maximum of 370 nm and an emission maximum of 440 nm, and the percentage inhibition of AGEs formation was calculated by $[(FL_{CN} - FL_{bCN}) - (FL_s - FL_{bS})]/(FL_{CN} - FL_{bCN}) \times 100$, where FL_{CN} and FL_{bCN} are the fluorescence intensities of the negative control mixture and it blank, respectively, and FLs and FL_{bs} are the fluorescence intensities of the extract and its blank, respectively. Aminoguanidine (Sigma) was used as standard.

3.6 DPPH assay (antioxidant assay)

The percentage of antioxidant activity of each extract was assessed by DPPH free radical assay. The measurement of the DPPH radical scavenging activity was performed according to methodology described by Brand-Williams et al. (1995). The samples were reacted with the stable DPPH radical (Sigma) in an ethanol solution [0.5 mM in ethanol (Dinâmica)]. The reaction mixture consisted of adding 200 µL of sample prepared in different concentrations in ethanol (0.4; 10; 25; 50; 75 and 100.0 µg mL^{-1})., 800 µL of of DPPH radical solution. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep violet to light yellow)

were read [Absorbance (Abs)] at 517 nm after 30 min of reaction using a UV-VIS spectrophotometer. The mixture of ethanol (200 µL) and sample (800 µL) serve as blank. The control (quercetin) solution was prepared by mixing ethanol (200 µL) and DPPH radical solution (800 µL). The results were expressed as 50% of the maximum response (EC_{50}). Quercetin (Sigma) was used as standard, and the DPPH scavenger activity was calculated as follows: %DPPH scavenging = [(Abs.Control - Abs.sample) /Abs.Control] x 100.

3.7 *ABTS*$^{\cdot+}$ scavenging activity (antioxidant assay)

The $ABTS^{\cdot+}$ (2,20-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)) cation radical (Sigma) reported by Re and co-workers (1999), with some modifications was produced by reacting 1 mL $ABTS^{\cdot+}$ solution (10 mmol L^{-1}) with 430 µL of 8.17 mmol L^{-1} ammonium persulphate (Dinamica), and holding the mixture in the dark at room temperature for about 16 h. After this, the solution was diluted 60-fold with water to reach an absorbance of 0.6 units. The assays should be conducted by adding 200 µL $ABTS^{\cdot+}$ solution to 800 µL of samples prepared in different concentrations in ethanol (0.4; 10; 25; 50; 75 and 100.0 µg mL^{-1}). The mixture was incubated for 30 min in the dark, and the $ABTS^{\cdot+}$ scavenging activity will be determined spectrophotometrically at 755 nm in a microplate reader. The results were expressed as 50% of the maximum response (EC_{50}). Quercetin (Sigma) was used as standard, and the $ABTS^{\cdot+}$ scavenger activity was calculated as follows: %$ABTS^{\cdot+}$ scavenging = [(Abs.Control - Abs.sample) /Abs.Control] x 100.

3.8 Oxygen radical capacity assay (ORAC- antioxidant assay)

The ORAC assay reported by Huang and co-workers (2002), with some modifications was also used to evaluate the antioxidant capacity of the fruit extracts. The AAPH (azobis-amidinopropane - Sigma) reagent solution (88.0 mmol L^{-1}) was prepared in glycine buffer (Sigma) (pH 8.3). The fluorescein solution (1.0 10^{-4} mol L^{-1}) was prepared in glycine buffer (pH 8.3) and maintained at 4° C in the dark. The working solution of fluorescein (6.45 10^{-8} mol L^{-1}), was prepared daily, diluting the stock solution in glycine buffer (pH 8.3). In black-walled 96-well plates, 186 µL of fluorescein solution

was added to 4.0 µL of standard (quercetin – Sigma) or extracts to be tested in different concentrations (2.50-0.08 mg mL^{-1}, diluted in glycine buffer, pH 8.3). The plate was capped and incubated with stirring for 15 min at 40 ° C. After this step, the reaction started with 10 µL of AAPH solution (88.0 mmol L^{-1}). After this step, the reaction was incubated under agitation for 95 min at 40° C. After incubation, the plate was held at room temperature for 5 min to cool, and the fluorescence was measured in a microplate reader. Excitation filters at 485 nm and emission filters at 528 nm were used. Final fluorescence measurements were expressed relative to the reading control. For the control assays, the extracts tested were replaced by the corresponding volume of glycine buffer (pH 8.3), under identical conditions (value taken as 100%). The absorption capacity of the oxygen radical by the extracts were expressed in terms of EC_{50} (concentration required to obtain a 50% of quercetin antioxidant effect). The radical scavenging activity is generally quantified in terms of inhibition percentage of the pre-formed free radical by antioxidants, and the EC_{50} is a typically employed parameter to express the antioxidant capacity and to compare the activity of different extracts.

3.9 Protection factor

Protection factor was calculated based on the spectrophotometric method proposed by Mansur et al. (1986). The extracts were prepared in different concentrations in ethanol P.A (Dinamica) (3.0 µg mL^{-1}; 1.0 µg mL^{-1}; and 15 µg mL^{-1} to rambutan, cambuci and pine, respectively). The absorption spectra of samples in solution were obtained in the range of 290 to 450 nm, using 1 cm quartz cell, and ethanol as a blank. The absorption data were obtained in the range of 290 to 320 nm, every 5 nm, and 3 determinations were made at each point. Benzophenone (Sigma) was used as a positive control in the concentration of 15 µg mL^{-1}. In addition, Mansur et al. (1986), developed a mathematical equation which substitutes the in vitro method proposed by Sayre et al., (1979), using UV spectrophotometry, following equation 1:

$$SPF_{spectrophootometric} = CF \times \sum_{290}^{320} EE\,(\lambda) \times I\,(\lambda) \times Abs\,(\lambda) \qquad \text{(1) Equation}$$

Where: EE – erythemal effect spectrum; I –solar intensity spectrum; Abs - absorbance of sunscreen product ; CF – correction factor (= 10). The values of EE x I are constants according to the range of spectrum (290 nm = 0.0150; 295 0.0817 nm = 0.0817;

300 nm = 0.2872; 305 nm = 0.3278; 310 nm = 0.0839; 320 nm = 0.0180) (Mansur et al., 1986).

3.10 Colorimetric assays by CIE Lab method

Colorrimetric assay was evaluated using a Minolta® CR-400 colorimeter with illuminant D65 and 10° angle of view to determine the color parameters (L*, a*, b*) according to the CIE (Comission of Internacional of the ilumination) Lab system (Kato et al., 2023). Two samples of each extract were prepared, both at a concentration of 0.1 % (m/m). One solution was left on the LabFitoBio bench at room temperature, and the other was kept at 40ºC on the stove. After a period of 90 days, the samples were analyzed, and the coloration results were compared with those obtained initially.

3.11 Analysis of extracts selected by HPLC-UV/DAD

The selected extracts were analyzed by high performance liquid chromatography (HPLC) in order to obtain a previous separation of their chemical constituents. The experiments were conducted in a diode arrangement detector system with a C_{18} column as stationary phase (250 mm long x 4.6 mm diameter, 5.0 μm). The solvent system for exploratory analysis of the chemical profile of the fractions was a mixture of H_2O (A) and Acetonitrile (Sigma) (B), both with 0.1% formic acid, in the gradient mode from 5 to 100% B in 60 min, with a linear gradient, flow rate of 1.0 mL min^{-1}. The chromatogram was recorded, and the UV spectra of the individual peaks will be monitored in the 200-600 nm wavelength range.

3.12 Fractionation of the extracts by HPLC-UV/DAD preparative

In order to identify the bioactive compounds presents in each extract, the samples were fractionated by preparative scale by HPLC-UV/DAD . Approximately 20 mg of

each extract were dissolved at 25 mL of Milliq water and 25 mL of acetonitrile, filtered in a 0.45 μm diameter membrane, and injected into a Waters 2489 HPLC equipped with a PDA detector model 350 RI, and a reverse phase semi preparative column (SunFire ^{TM} Prep C8 OBD TM 5 μm; 19 x 100 mm Column – Waters). The flow was adjusted to 5 mL min^{-1} and 500 μL was injected. The solvent A was water and solvent B was acetonitrile with formic acid (0.1%). The gradient varied linearly from 5% to 95% B (v/v) in 60 min with the UV-Vis detector at 280 nm. Triplicate of injection were performed and fractions were collected and dried out according to the time of the peaks. It was collected 9 fractions of rambuta, 18 of pine and 20 of cambuci.

3.13 Identification of the metabolites of the extracts by UHPLC-QTOF-MS

In order to elucidate the structures of the compounds presente in the fractions obtained in the item 3.1.1 the analyses by UHPLC-QTOF-MS (Shimadzu LC coupled with Bruker Compass/HyStar - Bruker Daltonics, Billerica, MA) were performed. The chromatographic separation was performed using a Waters Acquity UHPLC BEH (150 x 2.1 mm, 1.7 μm) column, operating at 40 °C. Water (solvent A) and acetonitrile (solvent B) were used for mobile phase, both with 0.1 % of formic acid. The gradient ranged from 5 to 95 % of B in 20 min with flow of 0.4 mL min^{-1} and injection volume of 5.0 μL per sample. Nitrogen was used as the desolvation gas. The desolvation temperature was set at 350 °C with a flow rate of 500 L h^{-1} and temperature source of 120 °C. The capillary voltage was 20 kV. The collision energy was varied in the 1–110 eV range. The spectrometer operated with MSE centroid programming using a tension ramp from 20 to 40 V. The sample cone voltage of 20 and 32 V. The mass range detected was from 50 to 1180 Da. The generated data were processed using Data Analysis (v. 5.1). The comparison of the peaks within chromatogram was based ± 0.05 minutes of retention time tolerance and ± 0.05 Da for the exact mass.

4. Results

4.1 Bioassay screening of the extracts using different solvents

The fruits from *Campomanesia phaea, Psidium cattleianum, Eugenia uniflora, Psidium guajava*, and *Nephelium lappaceum* were separated into bark and pulp, according to the descriptions in the item 3.2. Then, the seeds from *Araucaria angustifolia,* and the barks and pulps from the others species studied were extracted with three different solvents (EtOH P.A., 70% EtOH, and EtOAc, item 3.3). The difference of solvent polarity determines the difference of type, composition, and phytochemical compounds extracted of the each sample (Widyawati et al., 2014). All the 33 extracts (Table 2, 3 and 4) obtained were subjected to antioxidant, protection factor and anti-glycant assays. Tables 2, 3 and 4 present the antioxidant ($DPPH^{\cdot}$, $ABTS^{\cdot+}$ and ORAC scavenging assays) and antiglycant activities, in addition, the yield of the extracts prepared with different solventes.

Table 2. $DPPH^{\cdot}$, $ABTS^{\cdot+}$ and ORAC assays of the ethanol P.A. extracts, their percentage of inhibition on the formation of advanced glycation end products (AGEs), and extracts yield.

Nº	Fruits	Parts	DPPH	$ABTS^{\cdot+}$	ORAC	% inhibition of glycation	Yield
			EC_{50} (µg mL^{-1}) *			300 µg mL^{-1}	%
1	Guava Red	Bark	>100	>100	>100	0	26.88
2	Yellow Araçá	Bark	92.42 ± 0.97	61.05 ± 2.19	>100	0	3.18
3	Rambutan	Bark	11.90 ± 0.08	2.84 ± 0.15	24.7 ± 0.10	37.9 ± 0.25	17.76
4	Cambuci	Bark	>100	>100	>100	0	42.65
5	Pine	Bark	2.55 ± 0.02	2.18 ± 0.11	30.76 ± 0.91	55.4 ± 0.15	9.33
6	Guava Red	Pulp	>100	>100	>100	0	13.34
7	Yellow Araçá	Pulp	>100	>100	>100	0	23.40
8	Rambutan	Pulp	>100	>100	>100	0	16.82
9	Cambuci	Pulp	75.25 ± 1.64	58.67 ± 2.51	>100	0	10.78
10	Pine	Pulp	>100	>100	>100	0	1.89
11	Pitanga	Mix	>100	>100	>100	0	26.88
	Quercetin		2. ± 0.03	1.39 ± 0.001	2.5 ± 0.001		
	Aminoguanidine					89 ± 1.79	

*$DPPH^{\cdot}$, 2,2-diphenyl-1-picrylhydrazyl radical; $ABTS^{\cdot+}$+,2,20-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) cation radical; ORAC- Oxygen radical Absorbance Capacity. EC_{50}, effective concentration where 50% of its maximal effect is observed; quercetin was used as positive control in antioxidants activities, and aminoguanidine (AG) (10 mmol L^{-1}) was used as positive control in antiglycant assay. Experiments were performed in triplicate.

Some fruits extracted with ethanol PA showed significant yield values, with percentages above 20%, such as, 22.6; 42.65; 22.28; 23.40 and 26.88% for red guava,

cambuci, red araçá, yellow araçá and pitanga, respectively. These values must be considered, since the objective of this work is an industrial application, then, a high yield is an extremely important point for large scale production.

In relation to the antioxidant tests ($DPPH^-$, $ABTS^{\cdot+}$ and ORAC) the fruit extratcs that presented high activity, were the rambutan bark, with values of 11.90 ± 0.08 μg mL^{-1} (for $DPPH^-$ assay), 2.84 ± 0.15 μg mL^{-1} (for $ABTS^{\cdot+}$ assay), 24.7 ± 0.10 μg mL^{-1} (for ORAC assay) and pine bark, with values of 2.55 ± 0.02 μg mL^{-1} (for $DPPH^-$ assay), 2.18 ± 0.11 μg mL^{-1} (for $ABTS^{\cdot+}$ assay), and 30.76 ± 0.91 μg mL^{-1} (for ORAC assay), respectively.

In addition, for the antiglycant assay, these same fruit extratcs (rambutan and pine barks) were the only ones that showed positive results with values of 37.9 μg mL^{-1} for rambutan and 55.4 μg mL^{-1} for pine.

In this way, previous research reported that 70% ethanol can dissolve polar compounds, such as sugar, amino acid, glycoside compounds, phenolic compounds with low molecular weights. In addition, literature studies show theses compouds with a promissor antiglycating activity (Sri Widyawati et al., 2014). So, all extracts were evaluated with this solvent (70% ethanol) and the results obtained were presented in the Table 3.

Table 3. $DPPH^-$, $ABTS^{\cdot+}$ and ORAC assays of 70% ethanol (v/v) extracts, their percentage of inhibition on the formation of advanced glycation end products (AGEs), and extracts yield. *

Nº	Fruits	Parts	DPPH⁻	ABTS·+	ORAC	% inhibition of glycation	Yield
			EC_{50} (μg mL^{-1}) *			300 μg mL^{-1}	%
1	Guava Red	Bark	>100	>100	>100	0	60.87
2	Yellow Araçá	Bark	>100	53.67 ± 2.03	>100	4.6 ± 1.01	37.57
3	Rambutan	Bark	4.45 ± 0.02	2.92 ± 0.07	24.5 ± 0.5	62.4 ± 0.63	31.85
4	Cambuci	Bark	64.42 ± 1.25	41.04 ± 0.87	>100	0	46.61
5	Pine	Bark	3.90 ± 0.07	2.48 ± 0.13	28.58 ± 0.30	59.8 ± 0.58	10.46
6	Red Guava	Pulp	>100	>100	>100	0	14.36
7	Yellow Araçá	Pulp	>100	98.3 ± 4.13	>100	0	26.63
8	Rambutan	Pulp	>100	>100	>100	0	26.51
9	Cambuci	Pulp	58.54 ± 2.11	41.21 ± 0.74	>100	0	14.67
10	Pine	Pulp	>100	>100	>100	0	8.94
11	Pitanga	Mix	>100	>100	>100	0	13.57
	Quercetin		2. ± 0.03	1.39 ± 0.001	2.5 ± 0.001		
	Aminoguanidine					89. ± 1.79	

*DPPH˙, 2,2-diphenyl-1-picrylhydrazyl radical; ABTS˙+˙+,2,20-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) cation radical; ORAC- Oxygen radical Absorbance Capacity. EC_{50}, effective concentration where 50% of its maximal effect is observed; quercetin was used as positive control in antioxidants activities, and aminoguanidine (AG) (10 mmol L^{-1}) was used as positive control in anti-glycant assay. Experiments were performed in triplicate.

The presence of water significantly increased the yield of the extracts. The antioxidant and antiglycant capacity also improved, since the antioxidant compounds also have greater polar characteristics and may have been extracted in greater amounts. The rambutan and pine bark extracts continued presenting the best results, with high antioxidant and antiglycation activities. The values obtained for rambutan bark extract were 4.45 ± 0.02 µg mL^{-1}; 2.92 ± 0.07 µg mL^{-1}; 24.5 ± 0.5 µg mL^{-1} for the $DPPH^{-}$, $ABTS^{\cdot+}$ and ORAC assays, respectively, and 62.4 µg mL^{-1} for glycation test; the values of 3.90 ± 0.07 µg mL^{-1}; 2.48 ± 0.13 µg mL^{-1}; 28.58 ± 0.30 µg mL^{-1} were obtained for pine bark extract in the $DPPH^{-}$, $ABTS^{\cdot+}$ and ORAC assays, respectively, and 59.8 µg $mL^{-1}$for glycation test.

Furthermore, an extraction was carried out with the solvent ethyl acetate, to obtain an extraction with more non-polar compounds. The results are shown in Table 4.

Table 4. $DPPH^{\cdot}$, $ABTS^{\cdot+}$ and ORAC assays of the ethyl acetate extracts, their percentage of inhibition on the formation of advanced glycation end products (AGEs), and extracts yield.

Nº	Fruits	Parts	DPPH	ABTS˙+	ORAC	% inhibition of glycation	Yield (%)
			EC_{50} (µg mL^{-1}) *			300 µg mL^{-1}	%
1	Guava Red	Bark	>100	61.46 ± 0.1	>100	15.0 ± 0.58	2.63
2	Yellow Araçá	Bark	53.27 ± 0.66	>100	>100	49.3 ± 1.98	2.79
3	Rambutan	Bark	10.6 ± 0.90	6.36 ± 0.21	>100	16.7 ± 2.54	5.76
4	Cambuci	Bark	>100	>100	>100	10.8 ± 0.9	3.06
5	Pine	Bark	5.01 ± 0.10	1.52 ± 0.02	>100	54.7 ± 0.64	5.37
6	Red Guava	Pulp	>100	>100	>100	0	0.25
7	Yellow Araçá	Pulp	>100	>100	>100	0	6.81
8	Rambutan	Pulp	>100	>100	>100	0	0.30
9	Cambuci	Pulp	82.71 ± 2.93	54.87 ± 0.65	>100	52.8 ± 2.01	2.93
10	Pine	Pulp	>100	>100	>100	0	5.93
11	Pitanga	Mix	74.09 ± 3.94	73.83 ± 1.23	>100	4.2 ± 1.2	0.70
	Quercetin		2. ± 0.03	1.39 ± 0.001	2.5 ± 0.001		
	Aminoguanidine					89.0 ± 1.79	

*DPPH˙, 2,2-diphenyl-1-picrylhydrazyl radical; ABTS˙+˙+,2,20-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) cation radical; ORAC- Oxygen radical Absorbance Capacity. EC_{50}, effective concentration where 50% of its maximal effect is observed; quercetin was used as positive control in antioxidants activities, and aminoguanidine (AG) (10 mmol L^{-1}) was used as positive control in anti-glycant assay. Experiments were performed in triplicate.

The antioxidant assays (DPPH˙ and $ABTS^{\cdot+}$) obtained for ethyl acetate extracts showed a potential to rambutan and pine bark with 10.6 ± 0.90, 6.36 ± 0.21 for rambutan; and 5.01 ± 0.10, 1.52 ± 0.02 µg mL^{-1} for pine, respectively. However to ORAC assay the extracts do not present activities, this result can be correlated by the chemical and biological modes of action of the compounds (Borlinghaus et al., 2020).

For the glycation reaction, the activities above 50% of inhibition were obtained to pine bark (54.7 ± 0.64 %) and cambuci pulp extracts (52.8 ± 2.01 %). Pine bark extracts also presented high antiglycation activities with ethanol P.A (55.4 ± 0.15 µg mL^{-1}) and ethanol 70% (59.8 ± 0.58 %). However the activities e increased to pine and cambuci when extracted with ethyl acetate (-9.6 ± 1.04 % to ethanol P.A. and -1.4 ± 0.59 % to ethanol 70% for cambuci pulp extracts).

According to the literature, ethyl acetate has been recognized as efficient in the extraction of alkaloids, aglycones, sterols, terpenoids, and flavonoids, whereas ethanol demonstrates efficacy in obtaining sugar, amino acid, and glycoside compounds (Sri et al., 2014). The pine extract was derived from the bark, resulting in a lower sugar content compared to the extract obtained from cambuci pulp. Consequently, it is plausible to propose that cambuci exhibits superior antiglycation properties when extracted with ethyl acetate due to its lower sugar content.

In this way, the extracts that presented higher activities (glycation inhibition values above 50%) were selected to chromatography analysis in order to identify the chemical structures of the compounds responsible for this activity.

4.1.1 Selection of bioactive extracts

According to the results obatined in section 4.1.1 and the protocol of LabFitoBio, which considers a bioactive compound values above 50% of inhibition, the extracts from *Nephelium lappaceum* (rambutan bark), *Araucaria angustifolia* (pine bark), *Campomanesia phaea* (cambuci pulp), and *Psidium cattleianum* (araçá bark) were selected as the most active antiglycants and antioxidant extracts.

However, unfortunately, the extraction of araçá bark could not be carried out again because it was not in the fruiting period, so this sample was not selected for the continuation of this work. The fruit has also limited access and not having a large-scale production chain, important points for industrial production. Table 5 resume the results previously obtained on this work to the selected fruits.

Table 5. DPPH˙, $ABTS^{\cdot+}$ assay of the extracts and their percentage of inhibition on the formation of advanced glycation end products (AGEs).

Fruit	Part	Solvent	DPPH	ABTS˙+	% inhibition of Glycation	Initial mass (g)	Final Mass (g)	Yield (%)
			EC_{50} (μg mL^{-1})*		300 μg mL^{-1}			
Rambutan	Bark	70% Ethanol	4.45± 0.02	2.92 ± 0.07	62.4 ± 0.63	80	22.4	31.85
Pine	Bark	70% Ethanol	3.90 ± 0.13	2.48 ± 0.13	59.8 ± 0.58	150	17.25	10.46
Cambuci	Pulp	Ethyl acetate	82.71 ± 2.33	54.87± 0.65	52.8 ± 2.01	80	1.0	2.93
Quercetin			1.9 ± 0.001	1.7 ± 0.001				
Aminoguanidine					87.5 ± 0.01			

*DPPH˙, 2,2-diphenyl-1-picrylhydrazyl radical; ABTS˙+˙+,2,20-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) cation radical; EC_{50}, effective concentration where 50% of its maximal effect is observed; quercetin was used as positive control in antioxidants activities, aminoguanidine (AG) (10 mmol/L) was used as positive control. Experiments were performed in triplicate.

4.2 Color assay of the selected extracts

The color in food is the first attribute to be evaluated by consumers, and its influence on the approval or rejection of any food without taking into account other characteristics, is well-known. In the cosmetic industry, it's no different, colors that go against from consumers' common sense may induce the sensation of expired or spoiled cosmetics, which can directly influence the industrial feasibility of a product (Gonza´lez et al., 2011).

Color measurement in the food industry is made by sensory evaluation (visual) or instrumental evaluation, which can be achieved by tristimulus colorimeters, spectrophotometers or spectroradiometers. This instrument is based on the measurement of the colour from the spectral radiance reflected by the object, by an optical probe, just as the eye of a human observer does in real life (Terrab et al., 2004).

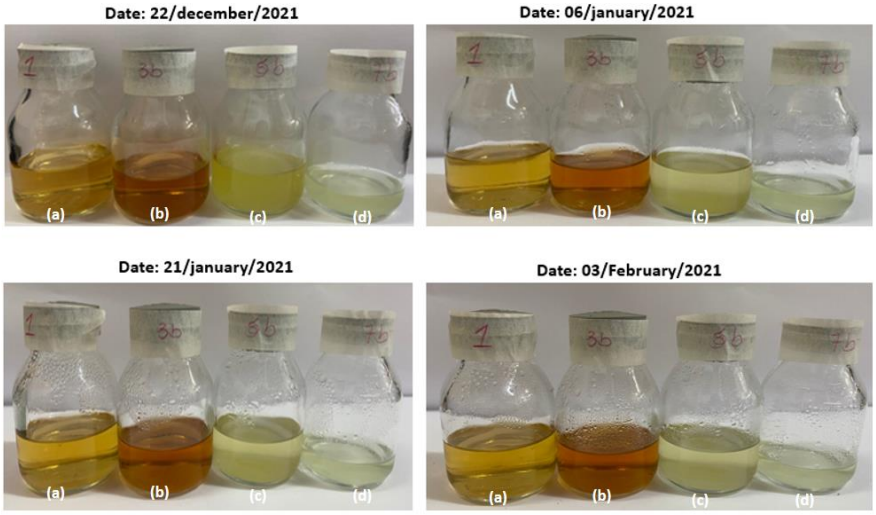
The CIE Lab color space is designed to approximate human vision, where the variable L∗ aspires to perceptual uniformity and the a∗ (redness/greenness) and b∗ (yellowness) to the coordinates of colors.

Table 8 shows the results of the color of two system and Figure 12 presents the photos that were taken during the 90 days of the color assay.

Table 8. Results of Lab system to the extracts selected.

Fruit	Part	Solvent	Room temperature		40ºC at	
			22/December/2021	03/February/2022	22/December/2021	03/February/2022
Rambutan	Bark	70% Ethanol	L = 48.67 ± 0.01[a] A = 1.09 ± 0.09 [a] B = 13.95 ± 0.05[a]	L = 50.01 ± 0.07 [a] A = 1.25 ± 0.05 [a] B = 14.92 ± 0.04 [a]	L = 48.67 ± 0.01[a] A = 1.09 ± 0.09 [a] B = 13.95 ± 0.05[a]	L = 51.10 ± 0.13 [a] A = 1.35 ± 0.12 [a] B = 15.51 ± 0.09 [a]
Pine	Bark	70% Ethanol	L = 46.93 ± 0.07 [a] A = 7.25 ± 0.05 [a] B = 19.04 ± 0.12 [a]	L = 40.89 ± 0.12 [a] A = 7.7 ± 0.04 [a] B = 20.01 ± 0.9 [a]	L = 46.93 ± 0.07 [a] A = 7.25 ± 0.05 [a] B = 19.04 ± 0.12 [a]	L = 41.01 ± 0.09 [a] A = 8.0 ± 0.84 [a] B = 20.07 ± 0.25 [a]
Araçá	Bark	Ethyl acetate	L = 51.60 ± 0.06 [a] A = -1.93 ± 0.13 [a] B = 13.78 ± 0.09 [a]	L = 51.01 ± 0.45 [a] A = -2.13 ± 0.09 [a] B = 15.01 ± 0.5 [b]	L = 51.60 ± 0.06 [a] A = -1.93 ± 0.13 [a] B = 13.78 ± 0.09 [a]	L = 51.97 ± 0.65 [a] A = -2.20 ± 0.17 [a] B = 15.99 ± 0.36 [b]
Cambuci	Pulp	Ethyl acetate	L = 51.27 ± 0.04 [a] A = -1.96 ± 0.05 [a] B = 8.41 ± 0.7 [a]	L = 50.27 ± 0.09 [a] A = -2.01 ± 0.07 [a] B = 9.10 ± 0.13 [a]	L = 51.27 ± 0.04 [a] A = -1.96 ± 0.05 [a] B = 8.41 ± 0.7 [a]	L = 50.78 ± 0.09 [a] A = -2.08 ± 0.15 [a] B = 9.89 ± 0.25 [a]

Figure 12. Photos of the extract's solution from 22 of December and 03 of February: (a) Rambutan, (b) Pine, (c) Araçá, and (d) Cambuci.



Font: Author herself.

To rambutan bark extract at room temperature (25º C) the L*, a* and b* values were l = 48.67 ± 0.01, a = 1.09 ± 0.09, b = 13.95 ± 0.05 initialy, and L = 50.01 ± 0.07, a = 1.25 ± 0.05, b = 14.92 ± 0.04 after 90 days. When the solution was heated (40 º C), the rambutan extract showed an increase in values of L*, a* and b* to 51.10 ± 0.13, 1.35 ± 0.12 and 15.51 ± 0.09, respectively. In the other words, the temperature increase the

values of Lab, and according to Sirisompong et al. (2011) this result may suggest an increase of the yellow pigments.

Dias et al., (2018) used CIE Lab color space to discriminate the fruit color of the different cambuci accessions. They reported the color parameter L ranged from 56.30 to 60.27, a* ranged from -1.12 to -4.76 and the b* ranged from 6.60 to 14.07. On this study the results obtained to cambuci pulp extracts were similar, and the values between the 90 days of exposure showed no significant difference in the color parameter, presenting l = 48.67 ± 0.01, a = 1.09 ± 0.09, b = 13.95 ± 0.05 to the intial measured, and l = 50.01 ± 0.07, a = 1.25 ± 0.05, b = 14.92 ± 0.04, after 90 days.

Araçá bark extract presented values of l = 51.60 ± 0.06, a = -1.93 ± 0.13, and b= 13.78 ± 0.09 initialy, and l = 51.01 ± 0.4, a = -2.13 ± 0.09, b = 15.01 ± 0.5 after 90 days at room temperature; with increasing temperature to 40º C the extract presented l = 51.97 ± 0.65, a = -2.20 ± 0.17, b = 15.99 ± 0.36. Gonzalez et al. (2011) studied araçá and described that the l* values presented differents color characteristics stages of maturity,showing values of 55.09 ± 5.66 when the bark is mature and the negative values of a* when the araçá bark is green.

Malta et al., (2022) evaluate color of cooked pine (*A. angustifolia*) almond collected from five separate locations distributed over a wide area; the color parameters L*, a*, and b* were statistically differents (l= 68.43 ± 0.02, a= 5.51 ± 0.04 and b= 16.23 ± 0.0), the color variation in most samples was not remarkable to the human eye.

In this study was obtained the values of l = 46.93 ± 0.07, a = 7.25 ± 0.05, b= 19.04 ± 0.12 to initialy samples of pine bark extract, l = 40.89 ± 0.12, a = 7.7 ± 0.04, and b = 20.01 ± 0.9 after 90 days at room temperature, and l = 41.01 ± 0.09, a = 8.0 ± 0.84, b = 20.07 ± 0.25 after 90 days at 40ºC.

4.3 Sun protection fator

The effectiveness of extracts or product in absorbing ultraviolet radiation is assessed through the Sun Protection Factor (SPF). This factor means how long a person using sunscreen can stay in the sun compared to a person without any sun protection, without getting sunburned (Wagemaker, et al., 2011).

According to the Resolution- RDC No. 30, dated June 1, 2012 of ANVISA (National Health Surveillance Agency) which approves the Mercosur Technical Regulation on Sunscreens in Cosmetics and provides other measures, a cosmetic can only

be considered to have protective activity when it exhibits an SPF higher than 6. Table 9 presents the values of SPF found in the sample selected (ANVISA, 2012).

Table 9: Determination of in vitro protection factor of *Nephelium lappaceum* (rambutan bark), *Araucaria angustifolia* (pine bark), *Campomanesia phaea* (cambuci pulp) extracts.

Fruit extracts	Concentration	FPS
Rambutan	3 μg mL^{-1}	6.83 ± 0.88
Pine	1 μg mL^{-1}	4.99 ± 0.12
Cambuci	15 μg mL^{-1}	3.99 ± 0.02
Benzophenone	15 μg mL^{-1}	6.96 ± 0.56

The determination of SPF values relies on the absorbance data acquired during experimentation. As per Beer's Law, the concentration of active extract exhibits a linear correlation with its absorbance (Galo and Colombo, 2019). Thus, in cases where the extracts, such as pine (FPS 4.99) and cambuci (FPS 3.99) failed to meet the minimum thresholds established by ANVISA, a potential strategy for enhancement their protection factor could involve increasing their concentrations to reach values that exceed the stipulated reference parameters.

Rambutan extract exhibited the highest SPF values at 6.83 at 3 μg mL^{-1} with concentration 5 times lower compared to the standard of protection sun (benzophenone). The benzophenone-3, despite providing effective protection against UVA and UVB rays, has its application limited due to the adverse effects it can cause, such as skin and eye irritation. Therefore, there is potential interest in researching new organic compounds with photoprotective properties that are not associated with these usage issues (Lima et al. 2019)

Adhayanti, et al. (2018), analyzed the rambutan ethanolic extract in different concentrations and obtained a range of FPS of 6.47 to 17.53 (concentration of 50, 100, 150 and 200 μg mL^{-1} with values of 6.47 ± 0.45, 9.26 ± 0.28, 13.01 ± 0.33, and 16.17 ± 0.63, respectively).

The main compounds that can be associated to SPF improvements when using rambutan extract is ellagic acid corilagin, geraniin, apigenin, quercetin, catechin, and anthocyanins. These compounds contain conjugated bonds and chemical groups responsible for absorbing UV radiation across various wavelengths. For example,

quercetin and anthocyanins directly absorb UV radiation, restraining skin damage caused by UV-B radiation (Mota et al., 2020).

Moreover, ellagitannins and catechin are acknowledged as potent antioxidants capable of minimizing the impact of free radical damage associated with UV radiation exposure. Several studies have demonstrated the protective effect of apigenin in the prevention of skin carcinogenesis induced by UV radiation. The antioxidant properties of these metabolites might also contribute to stabilizing organic synthetic UV filters, such as EHMC (ethylhexyl methoxycinnamate). Than, their presence minimizes EHMC photodegradation, thereby enhancing the UV protection effect (Mota et al., 2020).

According to Mota et al., (2020) the addition of rambutan extract in the formulation showed the potential to reduce the use of synthetic photoprotectors by about 64% of the total synthetic organic filters used to achieve the SPF value of 26.3. In addition, the sunscreen formulation supplemented with rambutan extract containing 1.00% showed a potential to minimize the risk of synthetic agent toxicity and a 45% of the reduction in the cost of sunscreen production.

4.4 Productive chain

Considering that the focus of this study in partnership with Symrise enterprise is to find an extract or compound with antiglycation potential for use in cosmetics, the production chain of the selected fruits is an extremely important factor; without a good production chain, the industrialization of the active ingredient becomes unfeasible.

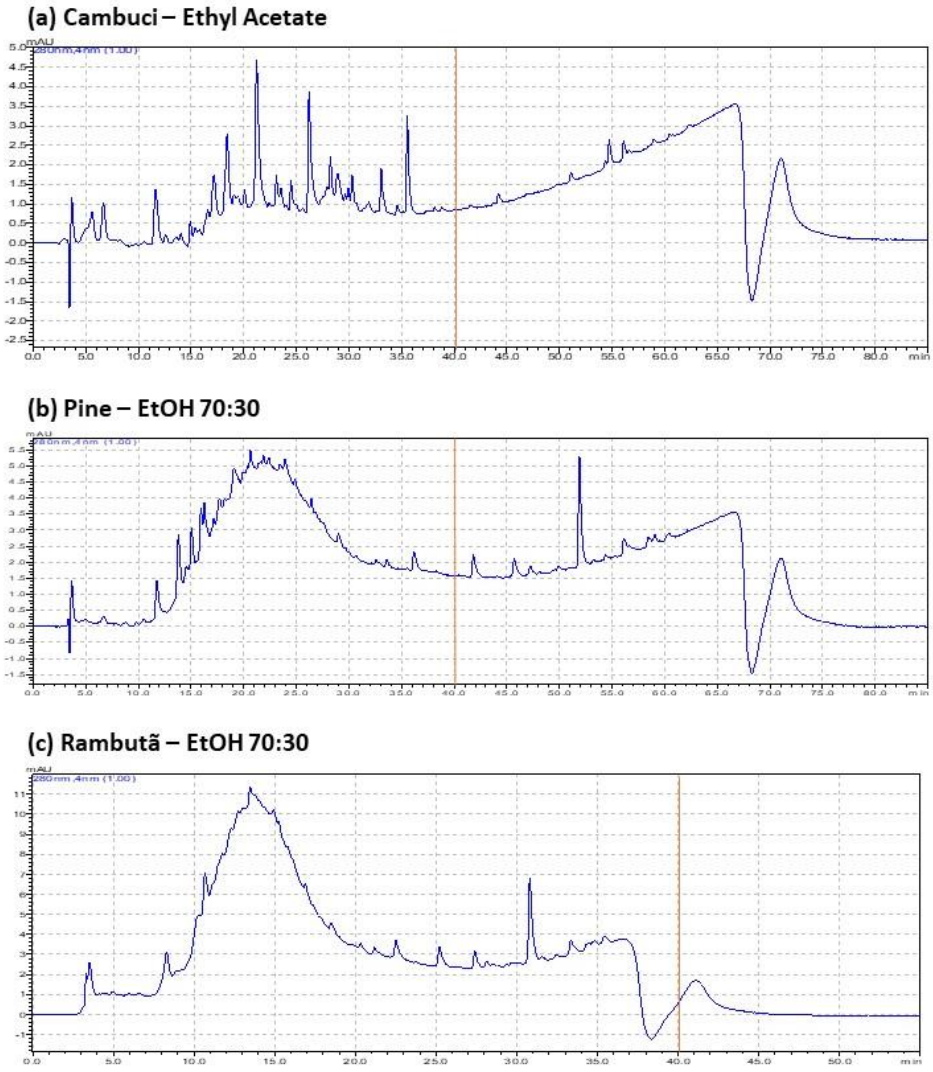
Consequently, after selecting the four extracts with the highest antiglycation potential (rambutan, cambuci, araça and pine extracts), a study was conducted in the literature regarding their production chains. It was found that despite the promising results of the araçá extract, it cannot be found in significant quantities, making its continuation in the project unfeasible. Thus, the work was continued with the extracts of rambutan, cambuci and pine.

4.5 Fractionation of the bioactive selected extracts

After the analysis of the activity results and selection of the most promising extracts, bio-guided fractionation was carried out, which consists of identifying natural compounds with biological activity in a controlled manner, aiming to focus the studies only on compounds that truly exhibit the desired activity of interest. Thus, the extracts that showed promising results achieved by fractionation were followed by the study of their antiglycation potential.

Initially, the extracts of cambuci, rambutan, and pine were subjected to an exploratory chromatographic analysis by HPLC-UV/DAD, monitored at 280 nm with a run time of 60 minutes, to verify the chemical profile of the extracts and evaluate chromatographic resolution, peak intensity and quantity, as well as separation efficiency. Figure 13 shows the obtained chromatograms.

Figura 13: Chromatographic profiles in exploratory gradient (HPLC-UV/DAD) of the extracts (a) cambuci with ethyl acetate, (b) pine with 70% ethanol and (c) rambutan with 70% ethanol [Phenomenex® C_{18}-Luna column (250 mm x 4.6 mm, 5 µm); gradient elution mode 5-100 %B in 60 minutes, ACN:H_2O (0.1% formic acid), λ=280 nm, injection volume: 20 µL].



The obtained chromatograms were analyzed using chromatographic optimization calculations to determine new conditions, primarily evaluating the run time, elution mode (isocratic or gradient), and percentage of acetonitrile. The methodology described by SHAH, B. et al., (2012) was applied, in which the choice between using an isocratic or gradient mode depends mainly on the number of components to be separated and eluted.

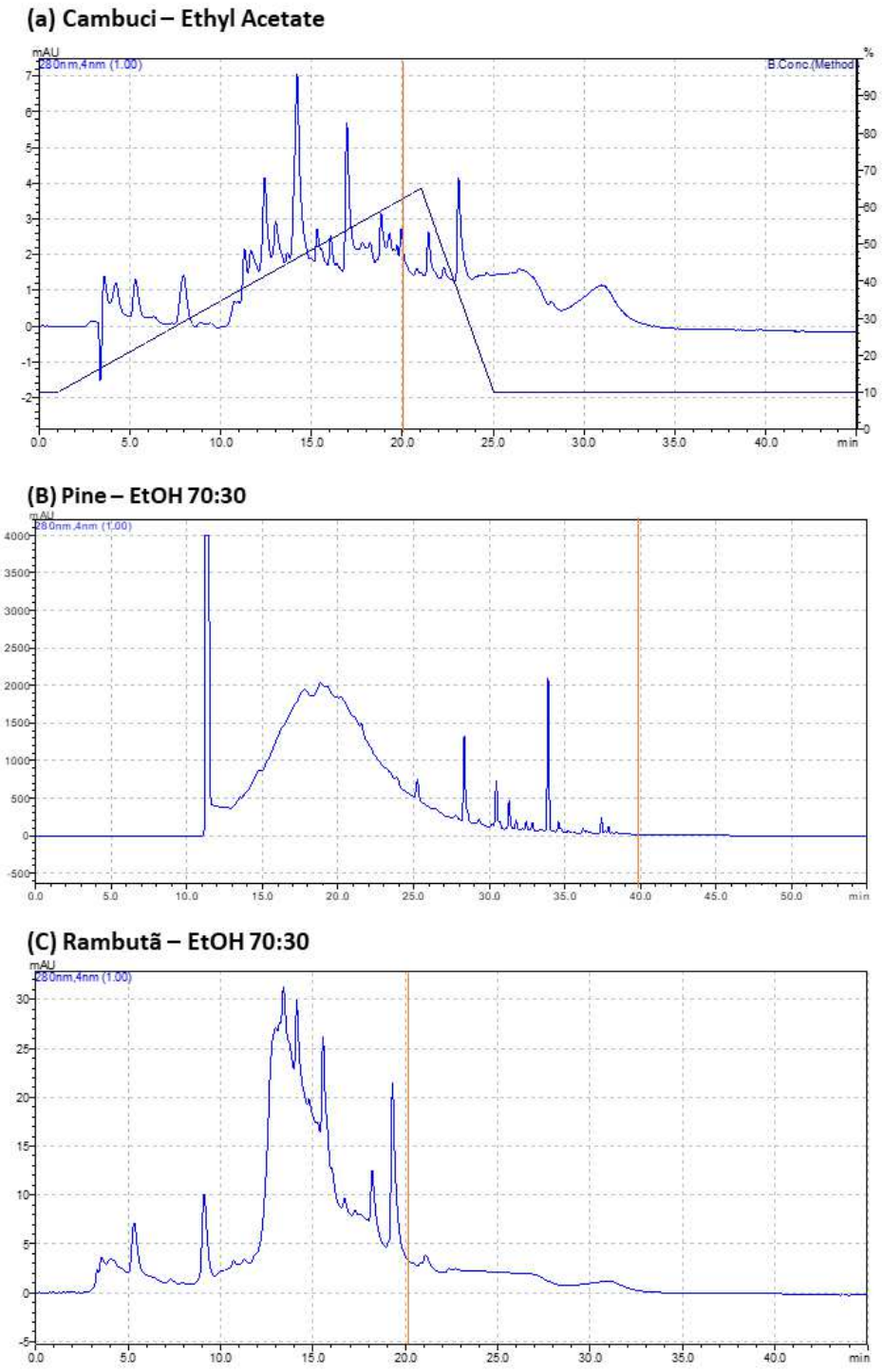
In orde to carry out the chromatographic optimization was calculate the relationship between the total time of the exploratory gradient (tg), and the retation time (retention time of the last peak - and the first peak) (Δtr/tg). The values obtained for each chromatogram are represented in Table 10.

Table 10: Values of retention time, gradient and mode of exploratory gradient obtained for each chromatogram.

	tr (last peak)	tr (first peak)	Δ tr (min)	tg (min)	Δ tr/tg	Mode
Cambuci	39.0	4.5	34.5	60	0.575	Isocratic
Rambutan	30.0	4.5	25.5	60	0.425	Isocratic
Pine	60.0	4.5	55.5	60	0.92	Isocratic

In addition, subsequently, specific acetonitrile percentages (B) were assigned to each chromatogram of the extracts: cambuci at 10-55% B, rambutan at 10-65% B, and pine at 12-95% B. These adjustments led to the generation of the chromatograms represented in Figure 14.

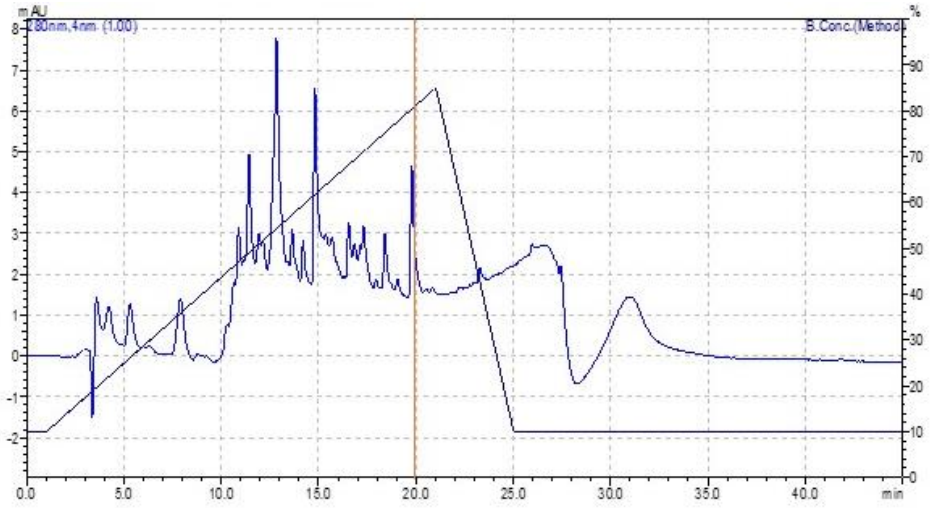
Figura 14: Chromatographic profiles in mode gradient (HPLC-UV/DAD) of the extracts (a) cambuci with ethyl acetate, (b) pine with 70% ethanol and rambutan with 70% ethanol [Phenomenex® C_{18}-Luna column (250 mm x 4.6 mm, 5 μm); gradient elution mode 10-65 %B in 20 minutes to cambuci, 12-95%B in 40 minuts to pine and 10-65%B in 20 minutes to rambutan, ACN:H_2O (0.1% formic acid), λ=280 nm, injection volume: 20 μL].



However, the cambuci extract exhibited an unsatisfactory separation of the peaks in the chromatogram, notably revealing the coelution of certain peaks during the column cleaning phase subsequent to the initial 20-minute run time, as showed in Figure 14-a. Consequently, to address this issue, a novel approach was implemented by elevating the acetonitrile concentration from 2.2 mL (gradient mode 10-65% B over 20 minutes) to 3.75 mL (gradient mode 10-85% B over 20 minutes) per minute. This adjustment resulted

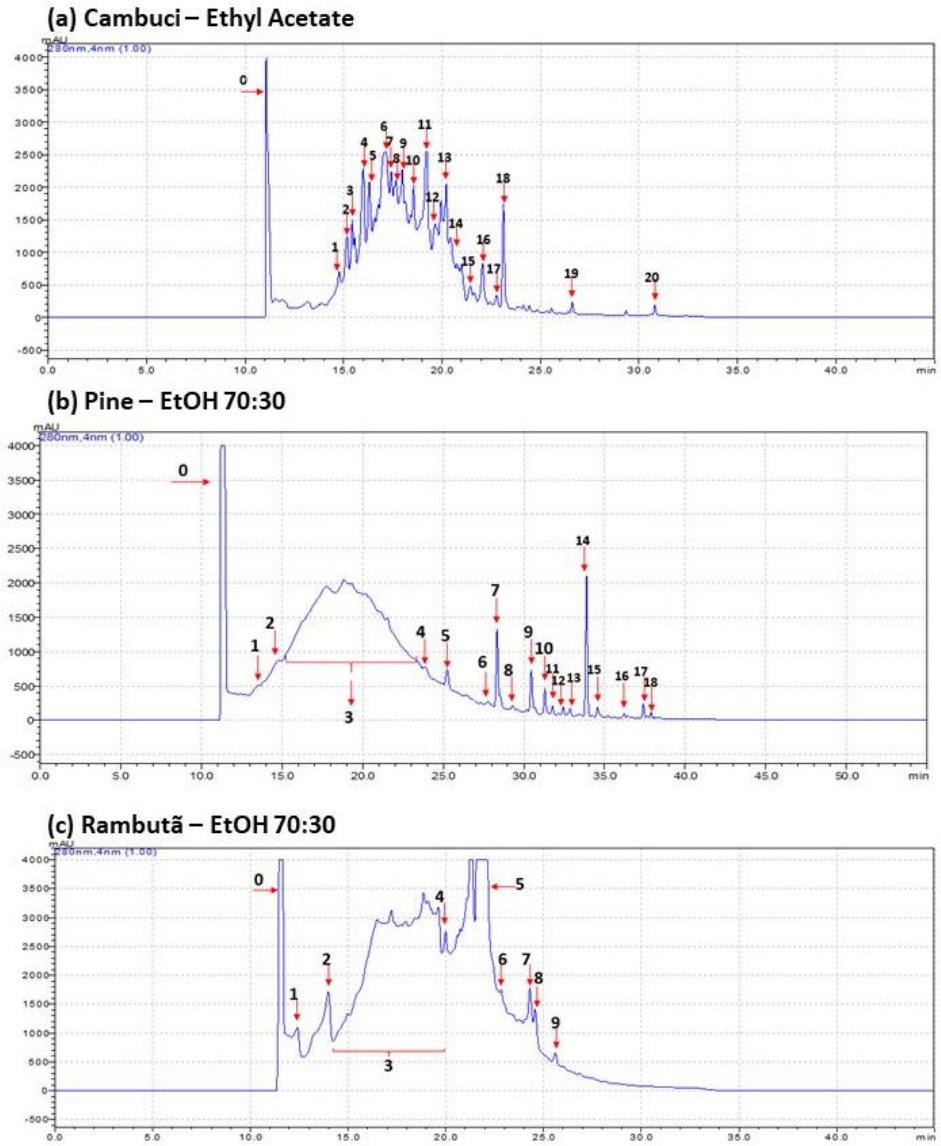
in an augmentation of the eluent's potency, marked by an intensified proportion of component B over time, thereby ensuring the complete elution of peaks within the allotted 20 minute chromatographic runtime, as delineated in Figure 15.

Figura 15: Chromatographic profiles in mode gradient (HPLC-UV/DAD) of the extracts of cambuci with ethyl acetate [Phenomenex® C_{18}-Luna column (250 mm x 4.6 mm, 5 μm); gradient elution mode 10-85 %B in 60 minutes, ACN:H_2O (0.1% formic acid), λ=280 nm, injection volume: 20 μL].



After developed and optimized the methods, preparative-scale liquid chromatography was initiated to fractionate the extracts, resulting in the collection of 20 fractions from cambuci extract, 18 from pine extract, and 9 of the rambutan bark extract. Fractionation was executed in accordance with the retention time of individual peaks, all meticulously collected manually. Figure 16 shows the three chromatograms, with numerical annotations representing the specific peaks from each fraction collected, observed at a wavelength of 280 nm.

Figura 16: Chromatographic profiles by preparative HPLC-UV/DAD of the extracts (a) cambuci with ethyl acetate, (b) pine with 70% ethanol and rambutan with 70% ethanol [Phenomenex® C_{18}-Luna column (250 mm x 4.6 mm, 5 μm); gradient elution mode 10-85 %B in 60 minutes to cambuci, 12-95%B in 40 minuts to pine and 10-65%B in 20 minutes to rambutan , ACN:H_2O (0.1% formic acid), λ=280 nm, injection volume: 20 μL].



Comparing the chromatograms acquired at the analytical scale (Figure 14) and the preparative scale (Figure 16), discernible discrepancies in retention time and chromatographic resolution are evident. Nonetheless, the fundamental chromatographic profile remained similar. The amount of the sample injected during the HPLC analysis was 20 µL, while in preparative-scale liquid chromatography (LC), it was increased to 500 µL. This mass and injection volume in the preparative-scale analysis resulted in discernible shifts in peak retention times. Nevertheless, despite these variations, a chromatographic consistency persisted when juxtaposing both scales, facilitating the continuation of the study. Table 11 presents the mass values and their respective yields for each collected fraction.

Table 11: Mass values obtained of the yields of each collected fraction.

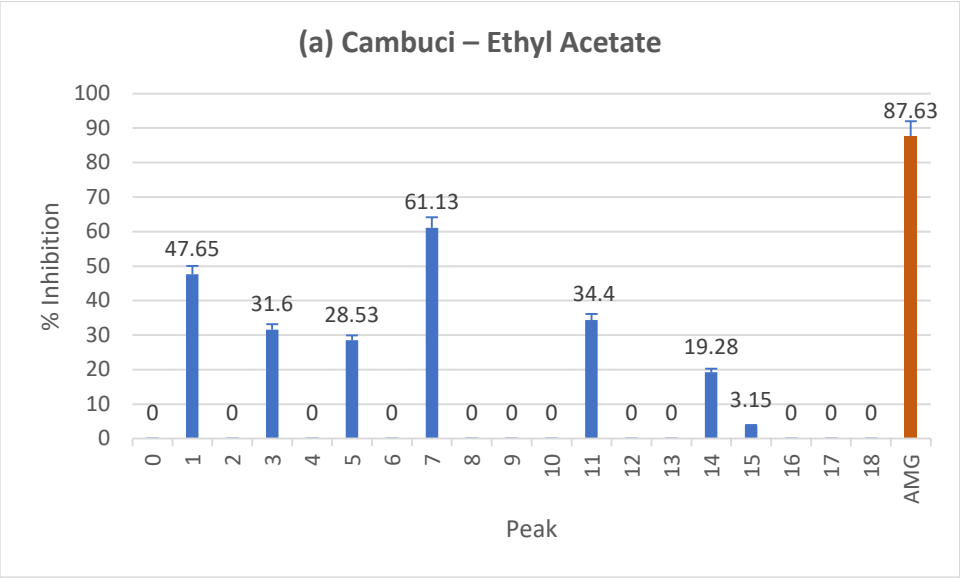
	Yield (mg)		
Peak	Cambuci	Rambutan	Pine
0	20.3	374.4	60.3
1	1.5	18.2	2.9
2	0.5	67.0	2.8
3	0.6	4.5	2.6
4	1.2	66.5	41.1
5	1.8	47.8	2.8
6	3.6	57.1	0.8
7	0.6	28.4	1.0
8	0.1	6.7	1.
9	0.7	28.4	1.0
10	0.4		0.8
11	1.4		0.5
12	0.1		0.3
13	0.2		0.4
14	0.4		0.5
15	0.1		0.5
16	0.2		0.1
17	0.1		0.3
18	0.3		0.1
19	0.3		
20	0.5		

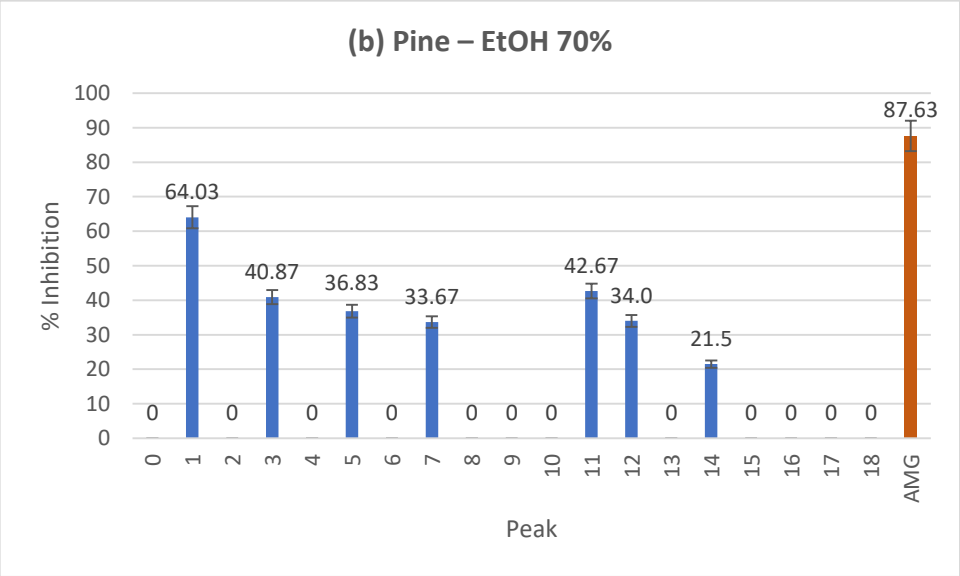
The collected fractions for the three extracts were dried and subjected to antiglycation analysis to evaluate their ability to inhibit the formation of AGEs, and UHPLC-QTOF analysis for the identification of bioactive metabolites with antiglycation potential were performed.

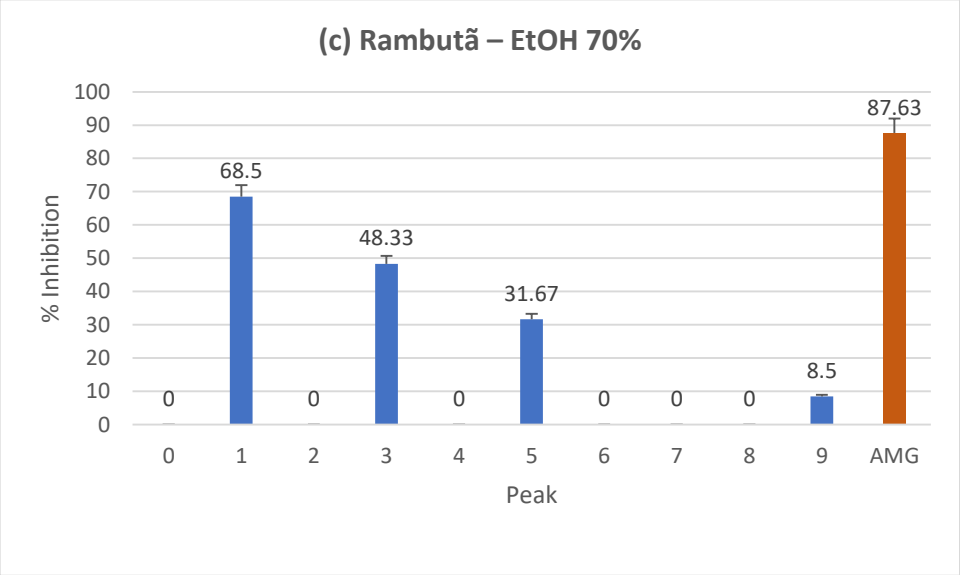
4.6 Evaluation of the antiglycation activity of the collected fractions

The antiglycation activity of each fraction obtained from rambutam, cambuci and pine extracts we evaluated by their ability to inhibit the formation of AGEs using a BSA/MGO system, with aminiguanidine used as a standard. The results were expressed as a percentage of inhibition of glycation (Fig. 17).

Figure 17: Results of the antiglycation assay for the fractions obtained from the extract of cambuci, pine and rambutan.


(a) Cambuci – Ethyl Acetate
% Inhibition
Peak
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 AMG
0 47.65 0 31.6 0 28.53 0 61.13 0 0 0 34.4 0 0 19.28 3.15 0 0 0 87.63


(b) Pine – EtOH 70%
% Inhibition
Peak
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 AMG
0 64.03 0 40.87 0 36.83 0 33.67 0 0 0 42.67 34.0 0 21.5 0 0 0 0 87.63


(c) Rambutã – EtOH 70%
% Inhibition
Peak
0 1 2 3 4 5 6 7 8 9 AMG
0 68.5 0 48.33 0 31.67 0 0 0 8.5 87.63

Peak 1 from rambutan extract, 7 from cambuci extract, and 1 from pine extract exhibited elevated antiglycation activity with values of 68.5, 64.03, and 61.13% AGEs inhibition, respectively. When the fraction are compared to the total extracts listed in Table 5 (section 4.1.1), these extracts demonstrated antiglycation values of 62.4 for rambutan, 52.8 for cambuci, and 55.4% glycation inhibition for pine. These results suggest that the isolated compound can provid a higher antiglycant activies.

According to Carvalho, Silveira, Matos, (2022), the robust biological efficacy of a crude extract or fraction can be described to various compounds possessing low or moderate activities. In cases where these compounds coexist within the same extract, they may exert synergistic effects, amplifying the biological potency of the sample. This methodological approach is recognized as a synergistic bio-guided study. However, the isolation or fractionation of these compounds may disrupt such interactions, resulting in decreased biological activity. Isolated compounds may consequently fail to exhibit satisfactory performance (Carvalho, Silveira, Matos, 2022).

To rambutan extract, a study conducted by Palanisamy et al. (2011) revealed that the crude extract displayed lower antiglycation capacity compared to the isolated compound geraniin, demonstrating inhibition values of 43% and 96%, respectively. Geranin, renowned in literature for its antiglycation activity, effectively inhibits glycation reactions during the Schiff base formation stage (Chinchansure, et al., 2013).

The antiglycating activity is reported for the first time for the pine fruits. There are no reports in the literature about the inhibition of AGEs by the pine extract. However, this activity may potentially be associated with the existence of phenolic compounds in the extract. Freitas et al. (2017) identified 13 phenolic compounds in the chromatogram peaks of pine nut, including catechin, epicatechin, and quercetin-3-*O*-glucoside, recognized for their antiglycation activity in the initial reaction phase involving Schiff base formation (Chinchansure, et al., 2013; Freitas et al. 2017).

Literature indicates that cambuci fruit has substantial quantities of glycosylated quercetin derivatives, acknowledged for their antiglycation activity, leading to significant R-glucosidase inhibition and moderate to low R-amylase inhibition. Additionally, other antioxidant compounds, such as proanthocyanidins, carotenoids, and vitamin C, have been reported in its composition, these may contribute to the observed antiglycating activities (Gonçalves, et al. 2010).

4.7 Identification of the metabolites present in the fractionation of the bioactive extracts by UHPLC-QTOF-MS

4.7.1 *Nephelium lappaceum* (rambutan)

In order to identify the compounds present in the *Nephelium lappaceum*, the hydroalcoholic bark extract and fractions were analyzed in negative ionization mode. The total ion chromatogram (TIC) obtained for rambutan extract is presented in Figure 18.

In the supporting information of this study, the mass spectra of all peak collected during the fractionations of the extracts were presented, in addition to the annotation of the chemical structures and exact mass tables.

The experimental mass and the calculated mass of the compounds detected can be founding in Table 12. In addition, the structures of the identified compounds in the *Nephelium lappaceum* extract by UHPLC-QTOF-MS were presented in Figure 19.

Figure 18. Total ion chromatogram (TIC) of the *Nephelium lappaceum* (rambutan) 70% ethanol (barks) extract by UHPLC-QTOF-MS in negative mode, with the enumerated peaks of the compounds identified and shown in Table 12.

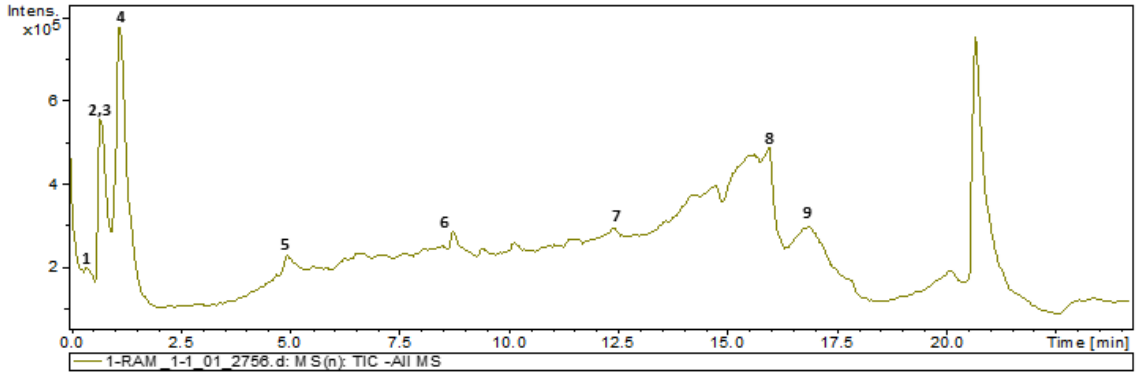
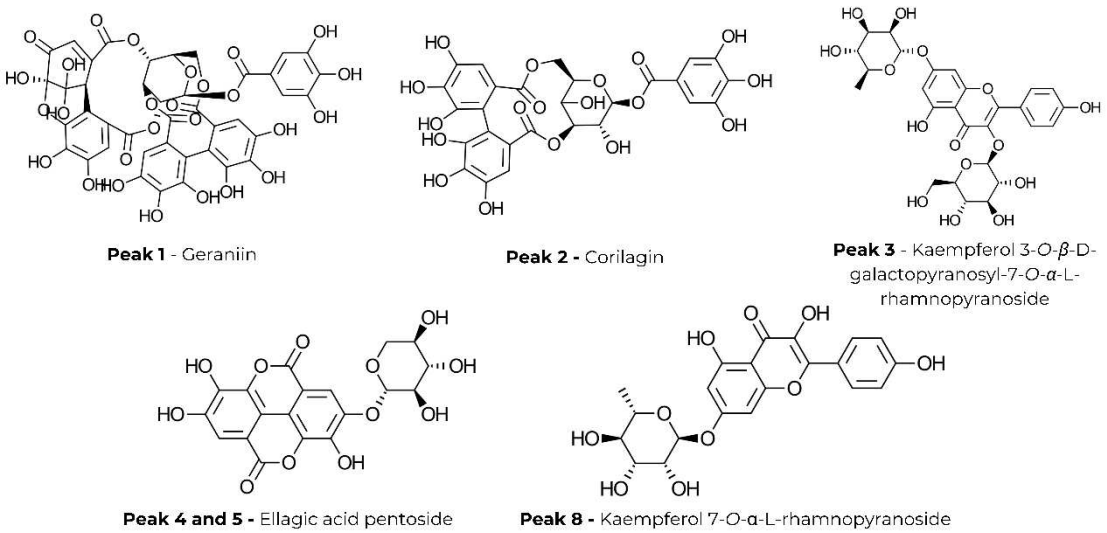


Table 12. Proposal for the identification of compounds present in the 70% methanol extract of the bark of *Nephelium lappaceum* (rambutan), UHPLC-QTOF-MS analysis.

Peak number	Retention time (min)	Measured (*m/z*)	Calculated (*m/z*)	Formula	Error (ppm)	Compound
0	11.25					Not identified

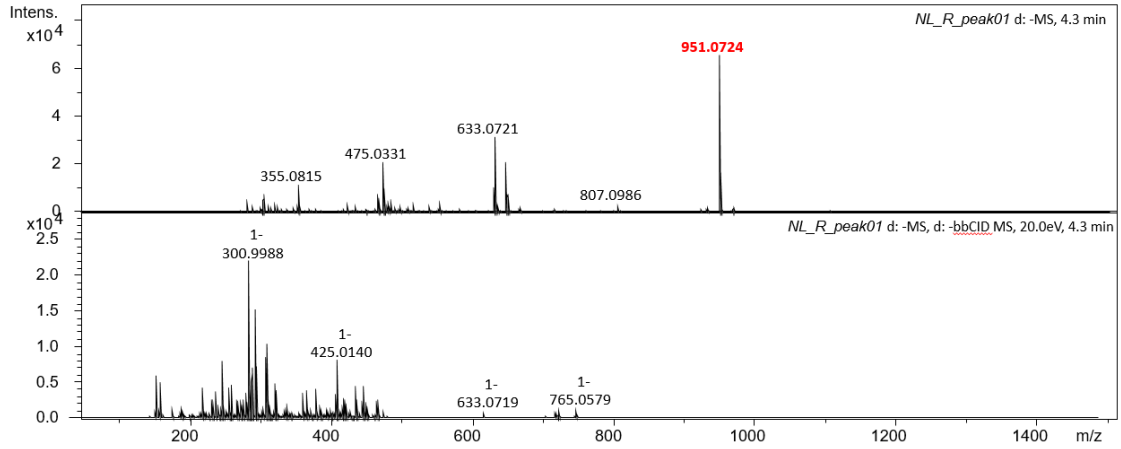
1	12.24	951.0724	951.0817	$C_{41}H_{28}O_{27}$	-0.5	Geraniin
2	14.07	633.0735	633.0806	$C_{27}H_{22}O_{18}$	-5.2	Corilagin
3	18.5	593.1532	593.1506	$C_{27}H_{29}O_{15}$	4.4	Kaempferol 3-*O*-*β*-*D*-galactopyranosyl-7-*O*-*α*-L-rhamnopyranoside
4	20.0	433.0417	433.0485	$C_{19}H_{14}O_{12}$	-7.2	Ellagic acid pentoside
5	22.21	433.0417	433.0485	$C_{19}H_{14}O_{12}$	-3,3	Ellagic acid pentoside
6	23.0					Not identified
7	24.50					Not identified
8	24.75	447.0948	447.1056	$C_{21}H_{20}O_{11}$	6.0	Kaempferol 7-*O*-*α*-*L*-rhamnopyranoside
9	25,85					Not identified

Figure 19. Structures of the compounds proposal identified on the 70% ethanol extract of the bark of *Nephelium lappaceum* (rambutan) in negative mode, obtained by UHPLC-QTOF-MS analysis.



Geraniin (Peak 1) is an ellagitannin with molecular ions at measured *m/z* 951.0724 and calculated *m/z* 951.0817 ($C_{41}H_{28}O_{27}$). Studies identified the geraniin such as one of the major phenolic from *N. lappaceum* bark, with ellagic acid and corilagin (Palanisamy, et al., 2011). On the glycation reaction, this compound has the ability to prevent the formation of AGEs, furthermore, possesses *α*-glucosidase and *α*-amylase inhibitory activity, aldol reductase inhibition activity (Phang, et al., 2018).

Figure 20: Total ion chromatogram (TIC) of geraniin (peak 1) present in 70% ethanol extract of the bark of *Nephelium lappaceum* (rambutan) in negative mode, obtained by UHPLC-QTOF-MS analysis.



Peak 2 was tentatively identified as a corilagin, with a precursor ion at *m/z* 633.0735 and calculated *m/z* 633.0806 ($C_{27}H_{22}O_{18}$). The compound is a member of the tannin family and has been widely reported, such as inhibitor of a-glucosidase, antiglycation, anti-hyperalgesia, anti-fibrosis, antiviral, antihypertensive, anti-inflammatory activities (Yisimayili, et al., 2019).

However, in the literature, there is no report of a study that directly relates the metabolite corilagin with antiglycant activity. However, according to Phang, Palanisamy and Kadir (2018), the activity of geraniin could be accredited to the metabolite corilagin. It is postulated that geraniin is converted to secondary metabolites such as, corilagin, gallic acid, ellagic acid and brevifolincarboxylic acid, as well as decarboxylated products.

Lee et al., (2020) detected the presence of kaempferol 3-*O*-*β*-D-galactopyranosyl-7-O-α-L-rhamnopyranoside in crude extracts of *N. lappaceum* seeds, identified by the precursor ion at *m/z* 593.1505. In addition, according to Sheng, et al., (2017) kaempferol has been reported to inhibit antiglycation activities by 70.7%, using the BSA-MGO methodology.

In this study, peak 3 was identified as kaempferol 3-*O*-*β*-*D*-galactopyranosyl-7-O-*α*-*L*-rhamnopyranoside at *m/z* 593.1532 ($C_{27}H_{29}O_{15}$,) with an exact calculated mass of 593.1506 and peak 8, was identified as a kaempferol 7-*O*-*α*-*L*-rhamnopyranoside, with a precursor ion at *m/z* 447.0948 ($C_{21}H_{19}O_{10}$).

Compounds 4 and 5 were identified as ellagic acid pentoside with a molecular ion based on their characteristic at *m/z* 433.0417 and the exact calculated mass of *m/z* 433.0485 ($C_{19}H_{14}O_{12}$).

The mass spectra of all these compounds tentatively identified in the rambutan barks extracts were presented in the supporting information.

Monrroy et al., (2020) reported the presence of ellagic acid on rambutan barks extracts and its effect on antiglycation activity. In addition, Ahmad et al., (2022) suggested that ellagic acid may exert its protective effect against alloxan-induced diabetes by decreasing lipid peroxidation and by inhibition of early, intermediate products and AGE formation from glycation.

In this way, these compounds identified in the rambutan barks extract confirm the antiglycation activity exhibited on this studied, and according to the literature the ability of these compounds have been reported to control the development of diabetic complications by inhibiting aldose reductase activity and the formation of advanced glycation end-products (Yongliang et al., 2017).

4.7.2 *Araucaria Angustifolia* (pine)

In order to identify the compounds present in the *Araucaria angustifolia* (pine), the hydroalcoholic bark extract and fractions were analyzed in negative ionization mode. The total ion chromatogram (TIC) obtained for pine extract was presented in Figure 20, while the calculated mass of the compounds detected can be founding in Table 13. In addition, the structures of the tentatively identified compounds in the *Araucaria angustifolia* extract by UHPLC-QTOF-MS were presented in Figure 21.

Figure 21. Total ion chromatogram (TIC) of the *Araucaria angustifolia* (pine) bark extract by UHPLC-QTOF-MS in negative mode, with the enumerated peaks of the compounds identified and shown in Table 13.

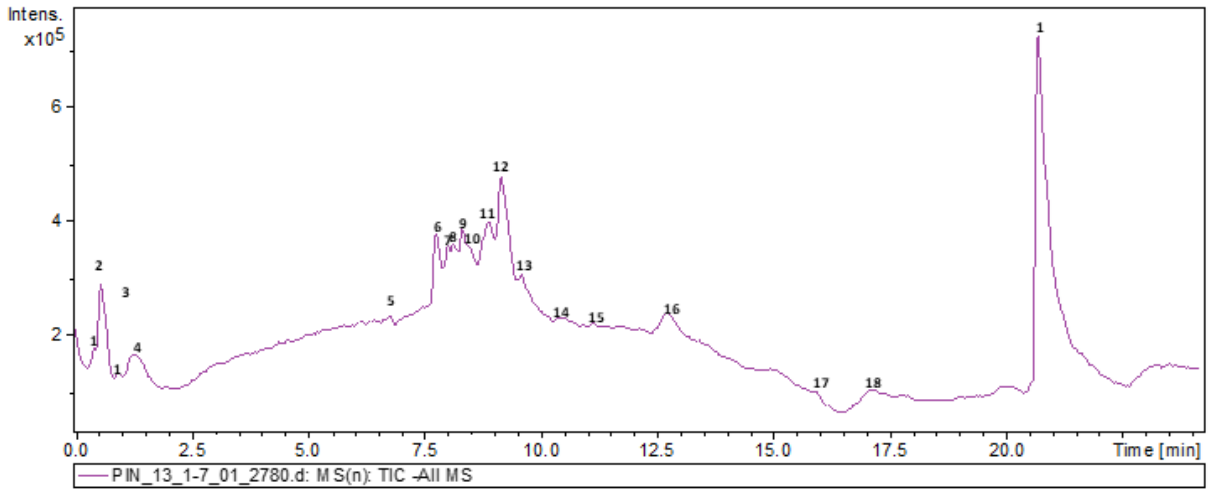
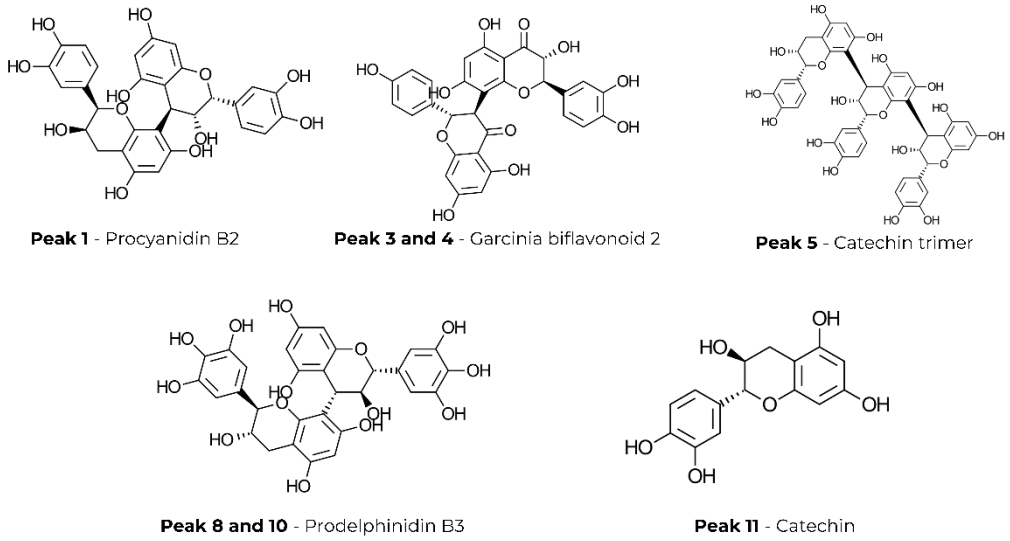


Table 13. Proposal for the identification of compounds present in the 70% ethanol extract of the bark of *Araucaria angustifolia* (pine), UHPLC-QTOF-MS analysis.

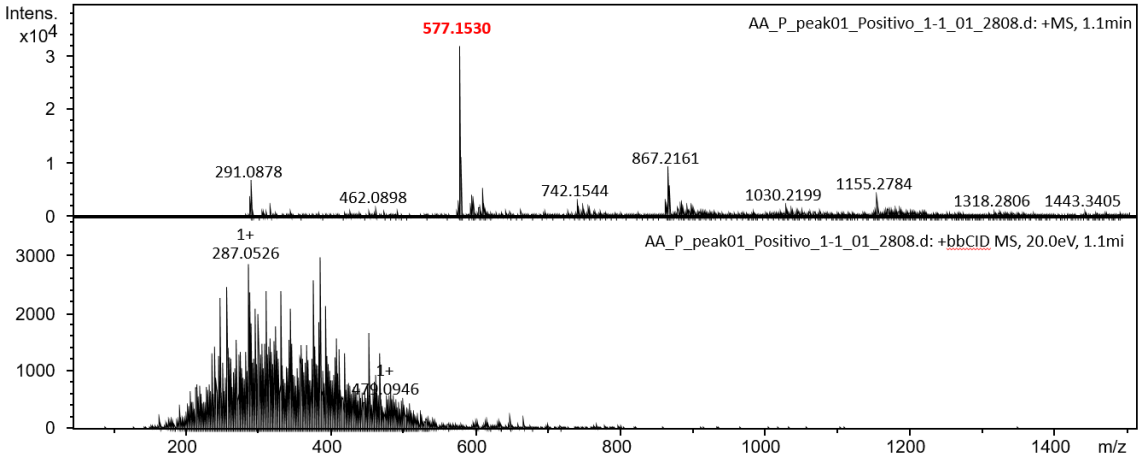
Peak number	Retention time (min)	Measured (*m/z*)	Calculated (*m/z*)	Formula	Error (ppm)	Compound
0	11.1					Not identified
1	13.4	577.1530	577.1580	$C_{30}H_{26}O_{12}$	-5.3	Not identified Procyanidin B2
2	14.0					Not identified
3	19.2	573,1043	573,1111	$C_{30}H_{22}O_{12}$	8.9	Garcinia biflavonoid 2
4	23.0	573,1043	573,1111	$C_{30}H_{22}O_{12}$	6.1	Garcinia biflavonoid 2
5	25.0	865.2037	865. 2058	$C_{30}H_{26}O_{12}$	-2.4	Catechin trimer
6	27.5					Not identified
7	28.0					Not identified
8	29.7	609.1243	609.1322	$C_{30}H_{26}O_{14}$	-4.9	Prodelphinidin B3
9	30.4					Not identified
10	31.6	609.1243	609.1322	$C_{30}H_{26}O_{14}$	-3.9	Prodelphinidin B3
11	32.0	289.0760	289.0790	$C_{15}H_{14}O_6$	-7.4	Catechin
12	33.4					Not identified
13	33.7					Not identified
14	33.9					Not identified
15	34.5					Not identified
16	37.5					Not identified
17	38.0					Not identified

Figure 22. Structure of the compounds proposal identified on the 70% ethanol extract of the bark of *Araucaria angustifolia* (pine), obtained by UHPLC-QTOF-MS analysis.



Peak 1 was Identified as a procyanidin with at *m/z* 577.1530 (Figure 23) and the calculated mass at *m/z* 577.158 ($C_{31}H_{32}O_{12}$). Condensed tannins are a category of polyhydroxyflavan-*3*-ol oligomers and polymers connected by carbon–carbon bonds among flavanol subunits. The primary classes include procyanidins, composed of chains of catechin, epicatechin, and their gallic acid esters, and prodelphinidins, which consist of gallocatechin, epigallocatechin, and their galloylated derivatives as the monomeric units.

Figure 23. Total ion chromatogram (TIC) of procyanidin (peak 1) extract by UHPLC-QTOF-MS in positive mode of procyanidin.



Peaks 3 and 4 were tentatively identified cas Garcinia biflavonoid 2, with a precursor ion obtained at *m/z* 573.1043 ($C_{30}H_{22}O_1$), with its calculated molecular mass, in the negative-ion at *m/z* 573.1111. This compound belongs to flavonols group that have been confirmed to possess a wide range of biological and therapeutic actions, such as a glycation reaction. Studies found in the literaturehe have been reported the Garcinia biflavonoid 2 as a potent antiglycation agent, in particular for inhibiting the glycation of collagen (Derbres, et al., 2012).

Peak 11 was tentatively identified as catechin with a precursor ion obtained at *m/z* 289.0760 ($C_{15}H_{14}O_6$) and with the calculated mas at *m/z* 289.0790. In addition, Peak 5 was tentatively identified as catechin trimer with a precursor ion obtained at *m/z* 865,2037 ($C_{30}H_{26}O_{12}$) and with the calculated mass at *m/z* 865.2058. This compound is a polymer of flavonoids containing three units of catechin linked by C-C bonds.

Wu et al. (2020) conducted a study comparing the inhibition of aminoguanidine (used as a standard in this study) and catechin using the BSA-glucose model. The results obtained showed that the inhibitory effects of catechins were two to three times higher than those of aminoguanidine. This result could be related to the ability of catechins to donate hydrogen atoms from the phenolic hydroxyl group, to scavenge radicals and inhibit the glycation reaction.

Peaks 8 and 10 were tentatively identified as prodelphinidin B3, with a precursor ion obtained at *m/z* 609.1243 ($C_{30}H_{26}O_{14}$) and with the calculated mass at *m/z* 609.1322. These compound belongs to the class of flavonoids, specifically they are polymers of

flavan-*3*-ols and present two asymmetric centers, allowing for various combinations of R and S configurations (4 stereoisomers), leading to different retention times.

The mass spectra of all these compounds tentatively identified in the pine barks extracts were presented in the supporting information.Freitas et al., (2018) identified a prodelphinidin B3 on seeds of *Araucaria angustifolia* by MS detection, performed in negative ion mode, using an LTQ XL linear ion trap mass spectrometer (Thermo Finnigan, San Jose, CA, USA), equipped with an ESI source. In addition, the authors analyzed the pine nut extract that contained prodelphinidin B3 and observed an inhibition in the DPPH assay of 50% at a concentration of 52 μg mL^{-1} of extract.

Antioxidant compounds are known to inhibit the glycation reaction during the Schiff base/Amadori product, during the first step of the glycation reaction where intervention can be carried out. The antiglycation potential of the antioxidant compounds may be caused by their antioxidant activities and related to the abilities to trap reactive carbonyl species, such as MGO (Chinchansure et al., 2015).

4.7.3 *Campomanesia phaea* (cambuci)

In order to identify the compounds present in the *Campomanesia phaea* (cambuci), the ethyl acetate bark extract and fractions were analyzed in negative ionization mode. Figure 24 illustrates the total ion chromatogram (TIC) acquired for the negative mode, showcasing each peak, while Table 14 provides the calculated masses of the detected compounds. Furthermore, the structures of the identified compounds found in the cambuci extract by UHPLC-QTOF-MS were showed in Figure 25.

Figure 24. Total ion chromatogram (TIC) of the *Campomanesia phaea* (cambuci) pulp ethyl acetate extract by HPLC-MS/MS in negative mode obtained by LC-MS/MS analysis.

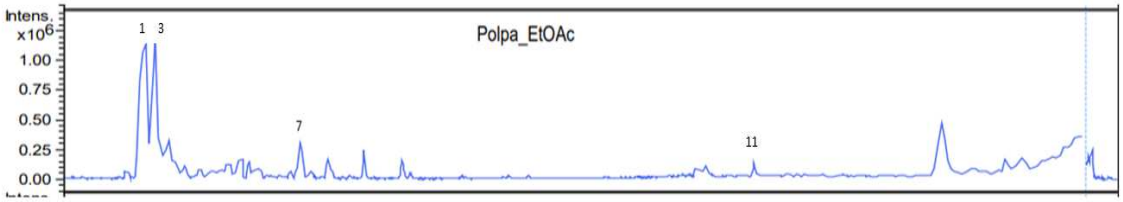
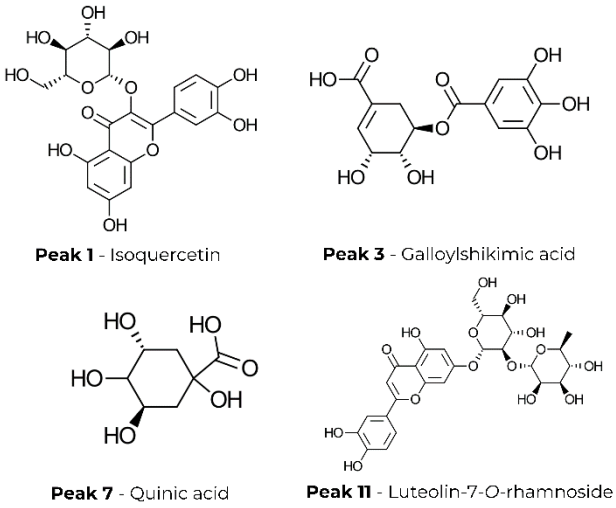


Table 14. Proposals for identification of compounds in the ethyl acetate extract of *Campomanesia phaea* (cambuci) pulp, obtained by UHPLC-QTOF-MS analysis in negative mode.

Peak number	Retention time (min)	Measured (*m/z*)	Calculated (*m/z*)	Formula	Error (ppm)	Compound
1	5.8	464.0971	464.0954	$C_{21}H_{20}O_{12}$	-3.7	Isoquercetin
3	5.10	325.0724	325.0637	$C_{14}H_{14}O_9$	-6.7	Galloylshikimic acid
7	8.1	191.0193	191.0133	$C_7H_{12}O_6$	5.2	Quinic acid
11	15.9	594.1592	594.1584	$C_{27}H_{30}O_{15}$	-4.3	Luteolin-*7*-*O*-rhamnoside

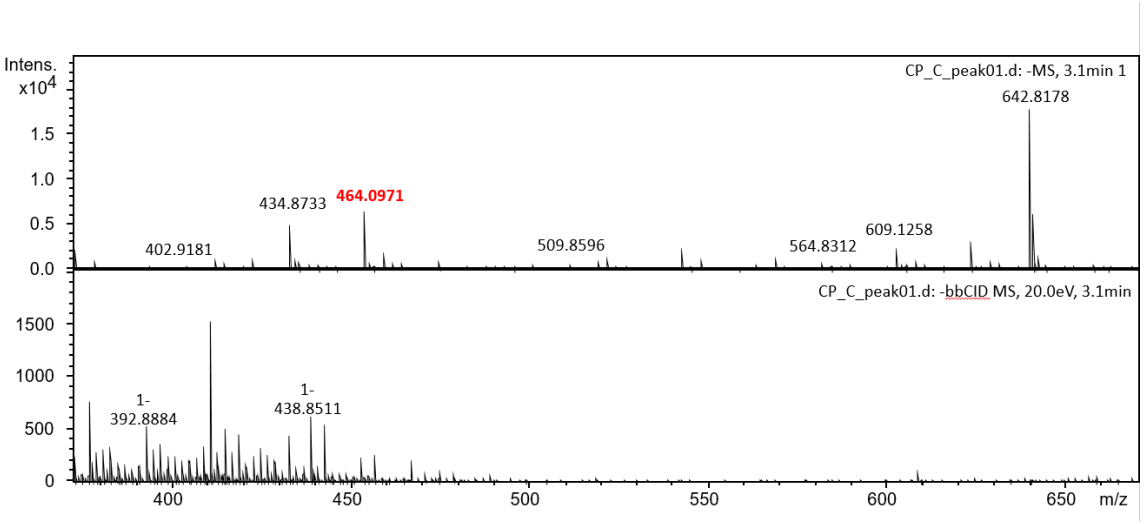
Figure 26. Structure of the compounds proposal identified on the extract fractions of ethyl acetate from *Campomanesia phaea* (cambuci) pulp (negative mode), obtained by UHPLC-QTOF-MS.



Peak 1 was identified as a isoquercetin at *m/z* 464.0971 ($C_{21}H_{20}O_{12}$) and the calculated massa at *m/z* 464.0954. Kavalek (2022) identified these compound on seed of *Camponesia canthocarpa* (Figure 27), a plant of the same gender. In addition, Zhang et al., (2020) elucidate the potential of isoquercetin of suppressing the formation of AGEs and obtained values of 74.66% at 36.58 μmol L^{-1}.

Figure 27: Total ion chromatogram (TIC) of Isoquercetin (Peak 1) present in ethyl acetate extract of the pulp of acetat extract of *Campomanesia phaea* (cambuci) in negative mode,

obtained by UHPLC-QTOF-MS analysis.



Galloylshikimic acid was tentativaley identified on peak 3 with the *m/z* 325.0724 ($C_{14}H_{14}O_9$) and the calculated massa at *m/z* 325.0637. This polyphenol has been fouded on cambuci in concentration of 5.2 ± 0.1 µg/100 mL of crud extract (Donado-Pestana et al., 2021). The impact of phenolic compounds on protein glycation (AGEs) is associated with their antioxidant capacity, as they hinder oxidation and the consequent formation of AGEs, sometimes termed autoxidation.

Al-Malki (2018) demonstrated that inhibition of overproduction of ROS by galloylshikimic acid was 50 or 100 mg/kg body weight in the diabetic rats, indicated by the reduction of glucose and glycated hemoglobin (HbA1c) levels as compared to the untreated group.

For the mass spectrum of compound 7, proposed as quinic acid at *m/z* 191.0193 ($C_{31}H_{32}O_{12}$), the signal of calculated was *m/z* 191.0133. This hydroxycinnamic acid is some of the most significant acid found in cambuci, ranking only behind shikimic acid and citric acid. In addition, according to Cui, (2009) the quinic acid is efficacy to inhibiting rat lens aldose reductase activity and the formation of AGEs (Spricigo et al., 2021; Cui, 2009).

Luteolin-7-*O*-rhamnoside was founded on peak 11 with a molecular ion at m/z 594.1592 ($C_{27}H_{30}O_{15}$), the signal of m/z calculated was 594.1584.

The mass spectra of all these compounds tentatively identified in the cambuci pulp extracts were presented in the supporting information.

Luteolin presented a similar potent inhibitory effect than aminoguanidin with 82.2% and 85.2% of glycation inhibition on MGO-BSA assay (Wu and Yen, 2005).

Literature suggested that the glycation of albumin leading to the increased production of ROS, so the highest potetion of luteolin could be associeted to these antioxidant activity and correlated their abilities to scavenge radicals on the glycation reaction (Wu and Yen, 2005).

4.8 Correlation of the antyglication activity and the compounds identified on the bioactive extracts.

Several plant extracts have undergone investigation, revealing promising antiglycation activity. The isolation of active compounds from these plants holds the potential to identify lead molecules for the development of novel antiglycation products. Some of these extracts, or their purified forms, have demonstrated additional pharmacological properties, similar to the extracts studied in this research. Hence, it is plausible to establish a correlation between the antiglycation activities, the potential of the extract, and the purified compound.

The crude extract from the rambutan bark obtained with 70% ethanol initially exhibited an antiglycation activity of 71.1 ± 0.1%. Following the fractionation process, 9 peaks were collected, among which only peaks 1, 3, 5, and 9 demonstrated antiglycation activity (80.8 ± 1.3; 48.5 ± 0.9; 32.5 ± 3.6; and 8.9 ± 4.9% AGEs inhibition, respectively) Chinchansure (2015) describes that the antiglycation activities observed in rambutan extracts are attributed to the presence of geraniin, a tannin known for its antioxidant activity and antiglycant property.

Thus, the compounds identified by mass spectrometry confirm the data found in the literature, considering that the peak showing the highest antiglycation activity is peak 1, which geraniin was tentatively identified, exhibiting an inhibition of approximately 80.8%.

Palanisamy, et al., (2011) reported a maximum inhibition activity of 96% by geraniin and 43% by *N. lappaceum* ethanolic extract, results in agreement with the experimental values obtained in this work (Palanisamy, et al., 2011).

Other studies reported the antiglycation activity of kaempferol 3-*O*-*β*-D-galactopyranosyl-7-O-α-L-rhamnopyranoside, identified in peak 3, and ellagic acid pentoside, presented in peak 5. However, these compounds might be present in smaller quantities, because these fractions presented low activity.

Regarding the pine nut, initially the crude extract from the barks exhibited an antiglycation activity of 67.4 ± 0.1%. However, the literature does not provide records of the pine nut's potential antiglycation. Therefore, the correlation of the results was based on the antiglycation potential of the isolated compounds identified in mass spectrometry.

As a result of the fractionation process from pine bark extract, of the 18 collected peaks, 7 exhibited some antiglycation activity, the most promising being those containing proanthocyanidin. Peaks 1 (proanthocyanidin with 64.1 ± 0.5% glycation inhibition), 7 (catechin with 79.5 ± 0.9% glycation inhibition), and 11 ((epi)catechin with 42.5 ± 1.1% glycation inhibition), except peak 3, identified as Garcinia biflavonoid 2 (41.4 ± 3.2% glycation inhibition).

Proanthocyanidin and epicatechin are reported to exhibit an inhibitory effect on the formation of AGEs in a BSA/glucose model, with abilities to trap reactive carbonyl species, such as MGO.

Epicatechin obtained from an ethanolic extract of *V. vitis-idaea* fruits exhibited antiglycation activity with an IC_{50} of 8.35 mg mL^{-1} (28.75 mM). An extract of *Cinnamomum zeylanicu*m with (+)-catechin , epicatechin , and procyanidin B2 have been identified as responsibles for the antiglycation activities.

In addition, Garcinia biflavonoid 2 belongs to flavonols group that have been confirmed to possess a wide range of biological and therapeutic actions, such as a glycation reaction. The lecture has been reported the Garcinia as a potent antiglycation agent, in particular for inhibiting the glycation of collagen (Derbres, et al., 2012).

According to the results of antiglycation inhibitory activity of *Campomanesia phaea* (cambuci) pulp extracts, which are presented in Table 7, the ethyl acetate crud extract inhibited 50.8 ± 0.1 % of the glycation reaction. However, for the fractions, the peak 7 exhibited the most potent inhibitory activity with 61.13%, followed by peak 1 (47.65%), peak 11 (34.4%), peak 3 (31.6%), peak 5 (28.53%) and peak 14 (19.28%).

In addition, the compounds identified in cambuci pulp extract as peak 1 (isoquercetin), 3 (galloylshikimic acid), 11 (quinic acid) and 7 (luteolin-7-*O*-rhamnoside) belong to the class of phenolic compounds, which consist of an aromatic ring with at least one hydroxyl group attached. This property enhances the antioxidant capacity of the molecules, making them capable of combating free radicals formed during glycation reactions.

5. Conclusions

The extracts from *Araucaria angustifolia* (barks), *Campomanesia phaea* (pulp), and *Nephelium lappaceum* (barks) exhibited high antiglycation, sun protection, and antioxidant properties. The compound geraniin emerged as the most potent glycation inhibitor in the ethanolic extract of *N. lappaceum* barks. In the case of pine, the presence of proanthocyanidin and epicatechin were correlated with a pronounced inhibitory effect on AGEs formation, in a BSA/glucose model, demonstrating an ability to sequester reactive carbonyl species, like MGO.In the crude extract of cambuci, the antiglycation properties can be attributed to the compounds reported in the pulp extract: quinic acid, methoxy quercetin deoxy hexoside, and quercetin-*O*-pentoside; phenolic compounds known for their antioxidant properties.

Furthermore, these investigated extracts exhibited robust stability when stored under low temperatures, with no observed degradation. Remarkably, they also displayed remarkable color stability, with no discernible alterations noted.

The accumulation of advanced glycation end products (AGEs) resulting from glycation reactions progressively impacts skin integrity, leading to physical and biomechanical changes such as stiffening and diminished elasticity, alongside alterations in biological characteristics, including matrix modulation by cells, throughout the aging process.

Consequently, the identified antiglycation activities within these extracts offer promising avenues for mitigating manifestations of cutaneous aging, underscoring their substantial potential for advanced research and the formulation of novel anti-aging cosmetic interventions.

The development of new functional products of natural, safe and anti-aging properties, capable of avoiding more serious degenerations in tissues has great importance not only in research, but especially in the process of innovation of biodiversity products.

This study reports for the first time the antiglycant activity and sun protection factor in pine bark extracts. The pine barks is a industrial residue from the seed crushing process, represent numerous tons of industrial waste. Thus, adding value to these by - products is of economic, scientific and technological interest.

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