

## Flavonoids in the *Annona* genus: Chromatographic and Spectral Methods of Analysis – A Review

### Flavonoides no Gênero *Annona*: Métodos Cromatográficos e Espectrais de Análises – Revisão

Isabela de Souza Pinto Pereira,<sup>a,\*</sup>  Maria Raquel Garcia Vega<sup>a</sup> 

<sup>a</sup> Laboratório de Ciências Químicas, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Av. Alberto Lamego 2000, CEP 28016-811 Campos dos Goytacazes-RJ, Brasil.

\*E-mail: [isabela65spp@gmail.com](mailto:isabela65spp@gmail.com)

Recebido em: 4 de Maio de 2021

Aceito em: 25 de Outubro de 2021

Publicado online: 17 de Março de 2022

The Annonaceae family comprises about 2500 species and 109 recognized genera. *Annona* is one of the most represented genera in the number of species in the family, with approximately 120 species distributed in tropical America and Africa. Flavonoids are considered chemotaxonomic markers, due to the specificity in some species, genera and families, as in the case of Annonaceae members. Studies on natural products have increased in recent years, according to the advantages associated with certain techniques, such as high performance liquid chromatography coupled to mass spectrometry (HPLC-MS) used to determine the chemical profile of plant species. A bibliographic survey was carried out on the search site Scifinder using the keywords *Annona*, flavonoids, HPLC and UPLC. The first article was published in 2007 and the research was carried out up to December 2020. Results obtained from this review revealed that the major class of flavonoids identified by HPLC-MS of the studied species are glycosylated flavonols, which may indicate this class as a possible chemotaxonomic marker for the *Annona* genus. An analysis of the techniques and parameters used in the assessed studies indicate the exclusive use of the reverse phase, acidified systems in the mobile phase, diode array detectors (DAD) and mass spectrometer (MS), and the type of ionization at atmospheric pressure most used is the ESI in negative mode. The bibliographic review data presented herein reinforce the importance of using hyphenated techniques, a fast and efficient alternative in the discovery of chemical profiles, and of the detection and quantification of compounds of interest in plant species.

**Keywords:** Annonaceae; *Annona*; flavonoids; HPLC-MS; UPLC-MS.

### 1. Introduction

According to Leboeuf *et al.* *Annona* is one of the most represented genera regarding the number of Annonaceae family species, comprising approximately 120 species distributed in America and tropical Africa. In Brazil, the *Annona* genus is found in the Amazon, Caatinga, Cerrado, Atlantic Forest and Pantanal biomes.<sup>1,2</sup>

The secondary metabolite classes most detected in species belonging to the *Annona* genus are alkaloids, acetogenins, steroids, diterpenes, flavonoids and peptides<sup>1,3</sup>. Given the chemical and biological importance of flavonoids, this study aimed to emphasize this class.

Flavonoids belong to a class of compounds widely distributed in the plant kingdom and represent one of the most important and diverse classes of secondary metabolites among natural products. They are found in abundance in angiosperms and exhibit great structural diversity.<sup>4</sup>

In plants, flavonoids are involved in a variety of biological processes that are important for their survival such as: attraction of animal vectors for pollination and seed dispersal, stimulation of *Rhizobium* bacteria for nitrogen fixation, promotion of pollen tube growth, reabsorption of mineral nutrients from senescent leaves, control the action of plant hormones and inhibit enzymes. Furthermore, flavonoids are also known to increase tolerance for a variety of abiotic stressors, for being defensive agents against herbivores and pathogens, and as allelopathic agents with other plant species. The flavonoids are evidently extremely useful to plants, and it is not surprising, therefore, that species, as angiosperms, invest significant amounts of metabolic energy into the production of these compounds.<sup>5</sup>

Flavonoids are considered chemotaxonomic markers, due to their specificity in some species, genera and families, such as Annonaceae members. In the review presented in PhD Thesis,<sup>6</sup> until 2018, 46 isolated flavonoids were reported in *Annona* species. The development of phylogenetic relationships and evolution studies, for example, are hindered by a lack of studies focusing on flavonoids present in the Annonaceae family. Therefore, faster and more efficient identification analyses, such as those carried out applying HPLC-MS, are an alternative to increase the

knowledge concerning the chemical constituents belonging to this class in *Annona* members.

In the literature we can find several biological studies with species of the *Annona* genus. According to a bibliographic survey carried out by Paes<sup>7</sup>, 26 species have been studied biologically. The most reported biological activities in species of this genus are pesticide, cytotoxic, antitumor, antidepressant, larvicide, anti-oxidant, antiparasitic, anxiolytic, antifungal, antinociceptive, molluscide, anticonvulsant, anti-inflammatory, bactericidal, among others.

Many *Annona* species are used in traditional medicine for the treatment of various diseases. The main objective of this work is to show the importance of using fast and efficient analytical techniques in the identification of bioactive compounds of interest as shown in the reviews by Anaya-Esparza and Leite *et al.*,<sup>8,9</sup> that focuses on traditional uses, phytochemistry studies and biological activities of *Annona* species.

High performance liquid chromatography (HPLC) has significantly evolved in terms of equipment, chromatographic supports and stationary phases, therefore improving analysis efficiency, besides allowing coupling to other equipment (hyphenated techniques), permitting more complete analyte assessments.

Ultra-performance liquid chromatography (UPLC), represents an evolution of this technique, also termed ultra-high performance liquid chromatography (UHPLC) in the literature. These modern liquid chromatography advances also indicate a trend in the technological area with the promotion and concern of green chemistry, applying lower amounts of solvent and resulting in less waste production. Table 1 displays the main characteristics of each of these techniques.

The mass spectrometer acts as a detector capable of providing information on the compounds eluted by the

chromatographic column. Table 2 describes the characteristics of the most applied detectors in recent decades.<sup>13</sup>

The advantages related to hyphenated techniques, such as HPLC-MS, have increased studies on natural products aiming to determine the chemical profile of specific species.

This work aims to carry out a bibliographic survey on flavonoids identified by HPLC-MS in species belonging to the *Annona* genus, due to their species specificity, characterizing this chemical class as a chemotaxonomic marker.

## 2. Flavonoid Detection by HPLC-MS

Several types of detectors have been used to identify and/or detect flavonoids in liquid chromatography analyses, such as electrochemical detection, fluorescence, diode array (UV-vis), NMR and MS. The most commonly applied today are the diode array (UV-vis) and MS.

Flavonoids have a combined aromatic character, which allows for absorption at relatively long wavelengths, increasing the selectivity of quantitative and spectrophotometric methods. Therefore, different classes of flavonoids exhibit characteristic spectra. In flavonoid analyses, the UV-vis spectrum exhibits two absorption maximums, band I and band II. Band II absorbs between 240-285 nm and is assigned to ring A. Band I absorbs between 300-550 nm, corresponding to ring B, and is considered the most important, as it provides selective information, since all flavonoids absorb between 240-285 nm.<sup>14</sup>

The flavan-3-ol, flavanone, flavanone and isoflavone classes present only band II absorption (269-279 nm) due to a lack of conjugation between rings A and B. The flavonol and flavone classes, however, present band I absorption (300-380 nm) (Figures 1 and 2), while anthocyanidins exhibit band I absorption between 460 and 550 nm. Therefore,

**Table 1.** Main HPLC and UPLC differences and characteristics<sup>10-12</sup>

	HPLC	UPLC/UHPLC
<b>Chromatographic columns</b>	15-20 cm x 2-5 mm	5-10 cm x 1-2.1 mm
<b>Particle size of the stationary phase</b>	3 - 5 $\mu$ m	$\leq$ 2 $\mu$ m
<b>Silica</b>	Silica, alumina, C18, C8, phenyl, cyano, polar groups	C18, C8, phenyl, cyano, polar groups
<b>Pressure</b>	100-200 bar (~1400-3000 psi)	400-1000 bar (~6000-15000 psi)
<b>Detectors</b>	UV-vis, diode-array detector (DAD), mass spectrometry (MS), fluorescence, refractive index (RI), electrochemical	UV-vis, diode -array detector (DAD), mass spectrometry (MS)

**Table 2.** Main ionization methods, mass analyzers and MS detectors<sup>13</sup>

Ionization Methods	Mass Analyzers	Detectors
Electrospray Ionization (ESI)	Quadrupole (Q)	Electron Multiplier (EM)
Atmospheric Pressure Chemical Ionization (APCI)	Time-of-Flight (TOF)	Photographic plates
Atmospheric Pressure Photon Ionization (APPI)	Ion trap (IT)	Faraday cups

UV-vis spectra aid in distinguishing between different flavonoid classes and become a very useful technique for flavonoid analysis when applied in combination with HPLC and MS. UV-vis detection sensitivity depends on the target flavonoid and the instrument, and generally between 0.02 and 10 ng of injected mass is sufficient.<sup>14</sup>

### 2.1. *Annona* genus flavonoid detection by HPLC-MS

Flavonoids can be found in their free form (aglycone) or linked to one or more sugar units (heterosides/glycosides). Structural flavonoid analysis requires advanced analytical techniques, such as NMR spectrometry, MS and/or X-ray crystallography, and all require properly purified samples in large amounts. In addition, they are difficult to isolate due to the complexity of compounds present in the extracts. Therefore, hyphenated techniques such as LC-MS or LC-NMR help to circumvent these disadvantages. HPLC-MS is a fast methodology, exhibiting high sensitivity and requiring small amounts of samples.<sup>15</sup>

In general, the most common type of flavonoids in plants comprise glycosylates, which may be O-glycoside or C-glycoside. The difference is that the sugar unit is linked to

the oxygen atom of the hydroxyl group of the aglycone in O-glycosides, while in C-glycosides the sugar unit is directly linked to the carbon chain by a C-C bond. O-glycosylated flavonoids predominate in the C-3 and C-7 positions, with O-glycosylation also occurring in the C-3 and C-5 positions of anthocyanidins. C-glycosylated flavonoids prevail in the C-6 and C-8 positions (Figure 3).<sup>15</sup>

In a mass spectrometry analysis, the sugar unit is the first portion to be fragmented. In the case of O-glycosylated flavonoids, low energy is required to break the hemiacetal CO bond, which normally causes the release of the entire sugar unit, resulting in hexose (162 u), deoxyhexose (146 u) and pentose (132 u) losses. In C-glycosylated flavonoids, on the other hand, high amounts of energy are required to break the strong C-C bond, with the predominance of intraglycoside fragmentation. In this case, fragments of the sugar units alongside losses of water (18 u), CO (28 u) and/or CO<sub>2</sub> (44 u) molecules are noted (Figure 3).<sup>17</sup>

### 3. Literature Review

In view of the importance of hyphenated techniques in

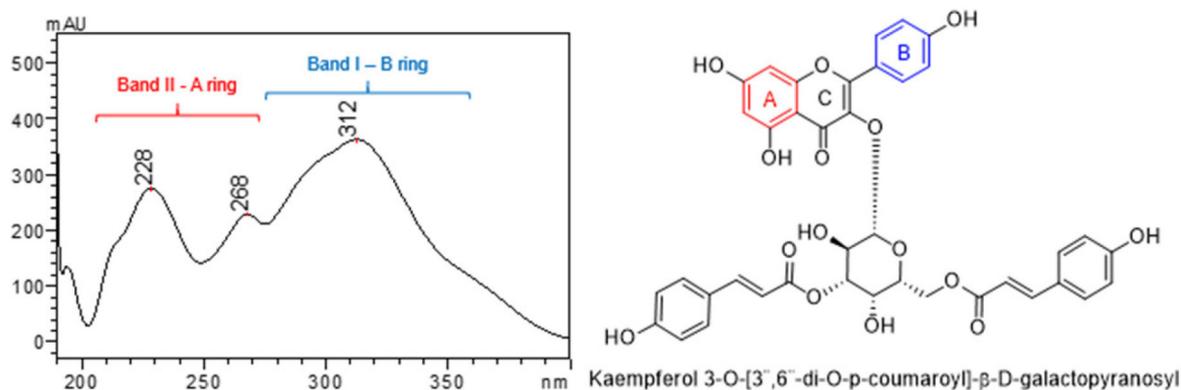


Figure 1. UV-vis spectrum with absorption bands represented by the respective colors of the rings of a glycosylated flavonol<sup>6</sup>

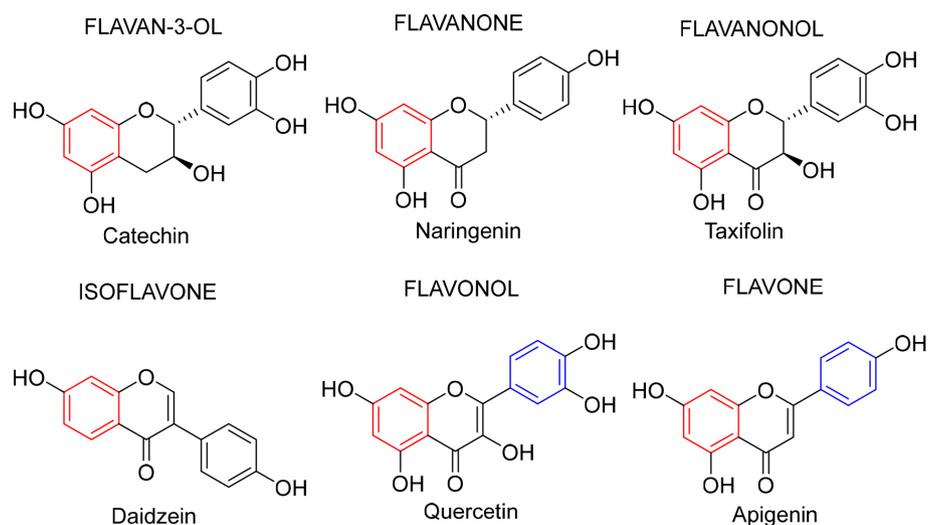
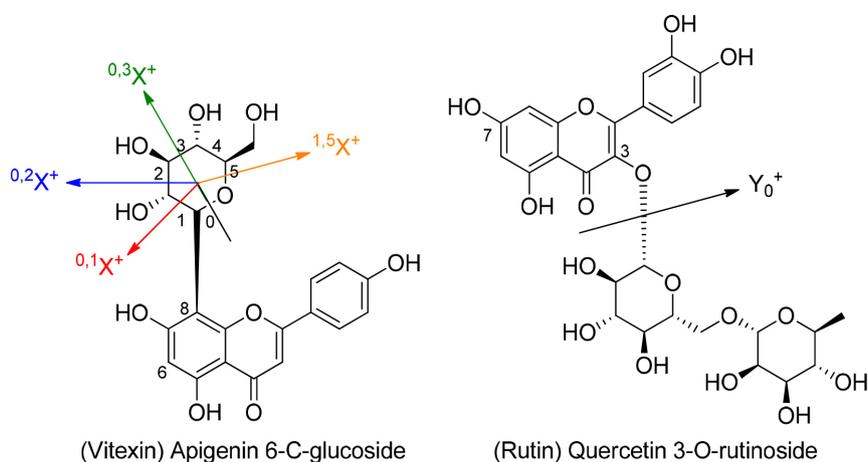


Figure 2. Ring A represented by the red color in relation to band II and ring B represented by the blue color in relation to band I in the UV-vis absorption spectrum of the main classes of flavonoids



**Figure 3.** Structure and proposals for fragmentation in O and C-glycosylated flavonoids identified in the *Annona* genus. Nomenclature of the fragments follows Domon and Costello<sup>16</sup>

flavonoid identification and considering the chemotaxonomic markers of the Annonaceae family, a bibliographic survey was carried out concerning the *Annona* genus using the Scifinder search site. The keywords *Annona*, flavonoids, HPLC and UPLC were used in the search. The first article founded is from the year 2007 and the research was carried out up to December 2020. Data is presented from the most sophisticated to the simplest method of analysis, as summarized in Table 3.

Figure 4 indicates the percentage of the total number of parts of the plants mentioned in Table 3, represented by n. In some studies, more than one plant part was analyzed, for example, García-Salas *et al.*,<sup>22</sup> who analyzed *A. cherimola* pulp, skin and fruit seeds. Figure 5 indicates the percentage of the total number of flavonoids mentioned in Table 3, represented by n. For n, equal flavonoids, this quantitative assessment aims to represent the major flavonoid classes observed in HPLC analyses carried out for *Annona* species.

**Table 3.** HPLC-MS flavonoid studies carried out for the *Annona* genus

Equipment	Stationary phase (column and particle dimensions), flow, temperature	Mobile phase (v/v)	Flavonoids	Specie / plant parts	Ref.
HPLC-PDA-ESI (-/+)-Quad-MS	Luna C18 (25 cm x 4.6 mm, 5 μm), 1.0 mL/min, 22 °C	0,1% FA:ACN 85:15 - 40 min to reach FA:ACN 30:70 and return to the initial conditions in exactly 5 min	Luteolin / Quercetin	<i>A. sylvatica</i> / leaves	18
HPLC-ESI(-)-triQ-TOF-MS/MS	Shim-pack XR-ODS III (150 x 2.0mm, 2.2 μm), 0.40 mL/min, 40 °C	A: 0,1% FA in H <sub>2</sub> O; B: MeOH; 0–1 min, 5% B; 1–4 min, 5–60% B; 4–7 min, 60–70% B; 7–10 min, 70–100% B; 10–10.50 min, 100% B; 10.50–11 min, 100–5% B; 11–15 min, 5% B	Catechin / Epicatechin / Rutin / Quercetin / Naringenin / Apigenin	<i>A. crassiflora</i> / fruits	19
HPLC-ESI-DAD-MS/MS	C 18 (150 x 3.0 mm, 3 μm), 200 μL/min	A: 0.1% FA in H <sub>2</sub> O; B: 0.1% FA in ACN; 0–10 min 5% B, 10–13 min linear increase to 95% B, 13–20 min hold 95% B, 21 min linear decrease 5% B and 22 min coming back to the initial conditions.	Catechin / Quercetin 3-O-rutinoside-7-O-glucoside / Epicatechin / Quercetin 3-O-rutinoside-7-O-pentoside / Quercetin 3-O-rutinoside / kaempferol-3-galactoside-7-rhamnoside / quercetin 3-O-glucoside / kaempferol-3-O-glucoside / Apigenin 8-C-glucoside / luteolin-3-galactoside-7-rhamnoside / luteolin -3-glucoside-7-rhamnoside	<i>A. cherimola</i> and <i>A. atemoya</i> / leaves	20
HPLC-ESI-MS/MS	C 18 (150 x 2.0 mm, 2.2 μm), 200 μL/min	A: 0.1% FA in H <sub>2</sub> O; B: MeOH; 0–1 min, 5% B; 1–4 min, 5–60% B; 4–7 min, 60–70% B; 7–10 min, 70–100% B; 10–10.50 min, 100% B; 10.50–11 min, 100–5% B; 11–15 min, 5% B.	Catechin / epicatechin / rutin / quercetin / naringenin	<i>A. crassiflora</i> / peel and seed	21

**Table 3.** HPLC-MS flavonoid studies carried out for the *Annona* genus (cont.)

Equipment	Stationary phase (column and particle dimensions), flow, temperature	Mobile phase (v/v)	Flavonoids	Specie / plant parts	Ref.
HPLC-DAD-ESI (-)-QTOF-MS	Poroshell 120 EC-C18 (4.6 x 100 mm, 2.7 µm), 0.8 mL/min, 25 °C	A: 0,5% AA; B: ACN; 0 min, 5% B; 2 min, 7% B; 4 min, 9% B; 7 min, 12% B; 8 min, 15% B; 9 min, 16% B; 12 min, 18% B; 14 min, 20% B; 15 min, 22% B; 16 min, 25% B; 18 min, 28% B; 19 min, 30% B; 20 min 31% B; 21.50 min, 32% B; 25 min, 100% B; 30 min, 100% B; 32 min, 5% B.	<b>Pulp:</b> Luteolin-glucopyranoside / Procyanidin dimer type A (1) / Procyanidin dimer type B (5) / Procyanidin trimer type B (2) / Procyanidin tetramer type B (3) / Catechin / Epicatechin; <b>Peel:</b> Procyanidin dimer type A (2) / Procyanidin dimer type B (5) / Procyanidin trimer type B (3) / Procyanidin tetramer type B (4) / Catechin / Epicatechin / Calabricoside A (2) / Rutin (2) / Quercetin hexoside / Catechin di-glucopyranoside (2) / Hesperidine; <b>Seeds:</b> Procyanidin dimer type B (3) / Procyanidin trimer type B (1) / Catechin / Epicatechin / Rutin (2) / Neoferocitrin / Hesperidine / Neoponcirin / Miconioside A	<i>A. cherimola</i> / Pulp, peel and seeds	22
HPLC-DAD-ESI (-)-MS <sup>a</sup>	Phenomenex Gemini C18 (250 x 3.0 mm, 5 µm), 0.4 mL/min	A: ACN; B: 0,1% AA; 25% A (10 min); 25% A (20 min); 50% A (40 min); 100% A (42–47 min); 20% A (49–55 min)	Proanthocyanidin dimer type B (3) / Catechin / Rutin / Quercetin-3-O-hexoside / Quercetin-3-O-glucuronide	<i>A. cherimola</i> / fruits	23
HPLC-ESI(-)-Q-TOF-MS/MS	Poroshell 120 SB-Aq (100 x 2.1 mm i.d., 2.7 µm) 0.45 mL/min, 40 °C	A: 0,1% FA in H <sub>2</sub> O; B: 0,1% FA in ACN; 0–1 min, 5% B; 1–10 min, from 5% to 18% B; 10–13 min, from 18% to 70% B; 13–15 min, from 70% to 100% B; 15–17 min, 100% B; and 3 min of post time at 5% B to column re-equilibration	Catechin / Epicatechin / Vicenin-2 / Vitexin / Rutin / Naringenin / Luteolin	<i>A. crassiflora</i> / fruit peel	24
HPLC-DAD-ESI (-/+)-ITMS	Discovery C18 (250 mm x 4.6 mm, 5 µm), 1.0 mL/min, 30 °C	MeOH/H <sub>2</sub> O: 10–50% (0–50 min), 50–100% (50–60 min), 100–10% (60–70 min)	Epicatechin / Procyanidins dimers (4) / Procyanidins trimers (6)	<i>A. cherimola</i> / fruit pulp	25
UPLC-PDA-ESI (-)-MS/MS	Waters Atlantis T3 (50 x 4.6 mm, 3 µm), 30 °C	A: 0,2% AA in H <sub>2</sub> O; B: 0,2% AA in ACN; 0 min, 2% B; 4.5 min, 22% B; 6.5 min, 22% B; 7.5 min, 90% B; 8.5 min, 90% B; 9.5 min, 2% B; 11 min, 2% B	Catechin / Epicatechin / Epigallocatechin gallate / Dihydroxyquercetin / Procyanidin B2 / Procyanidin trimer	<i>A. squamosa</i> / fruit pulp	26
HPLC-ESI-QTOF-MS	C18 (250 x 4.6 mm, 5 µm), 1.0 mL/min	ACN and 0.1% aqueous solution of AA (16:84, v/v).	Quercetin-3-O-robinobioside / Rutin / Quercetin-3-O-β-D-glucoside / Kaempferol-3-O-robinobioside / Kaempferol-3-O-rutinoside	<i>A. squamosa</i> / leaves	27
HPLC-ESI-TOF-MS	Poroshell 120 EC-C18 (4.6 mm x 100 mm, 2.7 µm), 0.8 mL/min, 25 °C	A: 1% AA; B: ACN; 0 min, 0.8% B; 2.5 min, 0.8% B; 5.5 min, 6.8% B; 11 min, 14.4% B; 17 min, 24% B; 22 min, 40% B; 26 min, 100% B, 30 min, 100% B; 32 min, 0.8% B; 34 min, 0.8% B	Procyanidin dimer type B (6) / Procyanidin trimer type B (3) / Procyanidin tetramer type B (2) / Procyanidin dimer type A / Catechin / Epicatechin / Quercetin-3-O-rutinoside-7-O-glucoside (2) / Calabricoside A (2) / Rutin (2) / Quercetin hexoside (2) / Catechin diglucopyranoside / Catechin diglucopyranoside / Kaempferol hexoside (2) / Kaempferol 3-O-β-D-(6"-O-p-coumaroyl) galactopyranoside (3)	<i>A. cherimola</i> / leaves	28
RP-HPLC-ESI(-)-QTOF-MS/MS	Hypersil ODS (250 x 4.6 mm, 5.0 mm)	-	Catechin / Epicatechin / Proanthocyanidins (procyanidin type B)	<i>A. cherimola</i> / pericarp	29
HPLC-DAD-ESI (-/+)-Duo ion trap-MS/MS	LiChroCARTs 250-4 LiChrosphers 100 RP-18 (250 x 4.0 mm, 5.0 µm), 1.0 mL/min	A: 0,05% TFA; B: MeOH; 0 min - 80% A, 20% B; 20 min - 20% A, 80% B; 25 min - 20% A, 80% B	Rutin xyloside / Rutin / Kaempferol-3-O-rutinoside	<i>A. cherimola</i> / leaves	30

**Table 3.** HPLC-MS flavonoid studies carried out for the *Annona* genus (cont.)

Equipment	Stationary phase (column and particle dimensions), flow, temperature	Mobile phase (v/v)	Flavonoids	Specie / plant parts	Ref.
LC-DAD-MS MALDI-MS	-	-	Quercetin-O-dihexoside / Quercetin-O-hexosyl-pentoside / Rutin / Quercetin-3-O- $\beta$ -glucopyranoside / Kaempferol-O-deoxyhexosyl-hexoside / Kaempferol-O-hexoside / Quercetin-O-pentoside / Kaempferol-O-pentoside / Catechin / Epicatechin / Procyanidin dimer type A (4) / Procyanidin dimer type B (3) / Procyanidin trimer type B (5) / Procyanidin trimer type A (2)	<i>A. crassiflora</i> / leaves	31
HPLC-DAD-ESI (-)-IT/MS	Synergi Hydro-RP C18 (150 x 3.0 mm, 4 $\mu$ m), 0.4 mL/min, 25 $^{\circ}$ C	A: 2% AA in H <sub>2</sub> O; B: 0.5% AA in H <sub>2</sub> O and MeOH 10:90	Dihydrokaempferol-hexoside	<i>A. muricata</i> / pulp	32
HPLC-ESI(-)-MS/MS	Shimadzu Shimpack XR-ODS III (150 x 2 mm, 5 $\mu$ m), 0.35 mL/min	A: 0,1% FA in H <sub>2</sub> O; B: 0,1% FA in ACN; 15–40% (0–7 min); 40–90% (7–8 min); 90–90% (8–9 min); 90–15% (9–10 min)	(Epi)catechin (2) / Procyanidins type B2 (2) / Procyanidin type C1 / Quercetin-3-glucoside / Kaempferol-3-O-rutinoside / Kaempferol-7-O-glucoside	<i>A. crassiflora</i> / fruit peel	33
HPLC-ESI(-)-Q-TOF-MS/MS	Agilent Zorbax (50 x 2.1mm, 1.8 $\mu$ m), 0.35 mL/min	A: 0,1% FA in H <sub>2</sub> O; B: MeOH; 2% B (0 min); 98% B (0–15 min); 100% B (15–17 min); 2% B (17–18 min); 2% B (18–22 min)	Procyanidin type B2 (2) / Procyanidin type C1 (2) / (Epi)catechin (2) / Quercetin-diglucoside (2) / Quercetin-glucosyl-pentoside (2) / Quercetin-glucoside / Rutin (2) / Quercetin-rhamnoside / Kaempferol-rhamnoside / Quercetin / Quercetin-xyloside-rutinoside	<i>A. muricata</i> / leaves	34
HPLC-DAD-ESI (-/+)-MS <sup>n</sup>	C18 Spherisorb ODS2 (25.0 x 0.46 cm, 5 $\mu$ m), 0.92 mL/min	A: 1% FA; B: MeOH; Initial 5% B; 15% B - 3 min; 25% B - 13 min; 30% B - 25 min; 35% B - 35 min; 45% B - 39 min; 45% B - 42 min; 55% B - 47 min; 75% B - 56 min; 100% B - 60 min	Catechin / Epicatechin / Procyanidin derivative / Catechin derivative / Quercetin-hexoside-rhamnosyl-pentoside / Quercetin-di-O-hexoside / Quercetin-di-O-hexoside / Rutin-O-pentoside / Quercetin-3-O-galactoside / Quercetin-3-O-glucoside / Quercetin-3-O-rutinoside / Quercetin-3-O-robinobioside / Quercetin-3-O-pentoside / Kaempferol-3-O-hexoside / Quercetin-rhamnosyl-hexoside + Kaempferol-rhamnosyl-hexoside / Quercetin-hexosyl-rhamnoside / Quercetin-pentosyl-rhamnoside / Kaempferol-3-O-rutinoside	<i>A. muricata</i> / leaves	35
UPLC-PDA-ESI(+)-MS	Zorbax Eclipse XDB-C18 (250 x 4.6 mm, 5 $\mu$ m), 1.0 mL/min	A: 1% FA in H <sub>2</sub> O; B: ACN; 95–5% A (0–60 min)	Quercetin-3-O-rutinoside / Kaempferol-3-O-rutinoside	<i>A. muricata</i> / leaves	36
HPLC-PDA	Capcell Pak C18 UG120 (250 x 4,6 mm, 5 $\mu$ m), 1.0 mL/min, 30 $^{\circ}$ C	A: 0,1% TFA in H <sub>2</sub> O; B: MeOH; 18% B (0-20 min); 18-22% B (20-25 min); 22% B (25-50 min); 22-100% B (50-80 min)	(+)-Catechin / (-)-Epicatechin / Procyanidin type B1 / Procyanidin type B2 / Procyanidin type C1	<i>A. atemoya</i> / fruits	37
			(-)-Epicatechin / Procyanidin type B1 / Procyanidin type B2 / Procyanidin type C1	<i>A. cherimola</i> / fruits	
HPLC-DAD	C18 Spherisorb ODS2 (25.0 x 0.46 cm, 5 $\mu$ m), 0.9 mL/min	A: 5% FA; B: MeOH; Initial 5% B; 15% B - 3 min; 25% B - 13 min; 30% B - 25 min; 35% B - 35 min; 45% B - 39 min; 45% B - 42 min; 55% B - 47 min; 75% B - 56 min; 100% B - 60 min	Quercetin-3-O-galactoside / Quercetin-3-O-glucoside / Quercetin-3-O-rutinoside / Kaempferol-3-O-rutinoside / Quercetin / Kaempferol	<i>A. muricata</i> / leaves	38

**Table 3.** HPLC-MS flavonoid studies carried out for the *Annona* genus (cont.)

Equipment	Stationary phase (column and particle dimensions), flow, temperature	Mobile phase (v/v)	Flavonoids	Specie / plant parts	Ref.
HPLC-DAD	C18 ODS (250 x 4.6 mm, 5 µm), 1.0 mL/min	MeOH/H <sub>2</sub> O 93:7	Quercetin	<i>A. squamosa</i> / seeds	39
HPLC-DAD	C18 (4,6 mm x 25 cm, 5 µm), 30 °C	A: 0.6% monosodium orthophosphate buffer; B: ACN	Rutin (3) / Isoquercetrin (2) / Epicatechin (2) / Catechin derivative (2)	<i>A. senegalensis</i> / leaves	40
HPLC-UV	C18, 1.0 mL/min	30% ACN and 70% phosphate buffer 0.025M, pH 2.5	Kaempferol	<i>A. muricata</i> / leaves	41
HPLC-DAD	C18 (4.6 mm x 250 mm, 5 µm), 1.0 mL/min	A: ACN; B: AA; 9% A - 10 min; 25% A - 11 min; 70% A - 16 min; 9% A - 31 min	Catechin / Epicatechin	<i>A. squamosa</i> / fruits	42
HPLC-DAD	LiChrospher 100 RP-18 (250 x 4 mm, 5 mm), 1.0 mL/min, 40 °C	A: 0.1% H <sub>3</sub> PO <sub>4</sub> in H <sub>2</sub> O; B: 0.1% H <sub>3</sub> PO <sub>4</sub> in ACN; 5% B (0 min); 95% B (60 min); 10 min isocratic elution	Flavonoids	<i>A. crassiflora</i> / leaves	43
HPLC-DAD	C18 (250 x 4.6 mm, 5 µm), 0.7 mL/min	A: 1% FA in H <sub>2</sub> O; B: ACN; 13% B - 10 min; 20% B - 20 min; 30% B - 30 min; 50% B - 40 min; 60% B - 50 min; 70% B - 60 min; 20% B - 70 min; 10% B - 80 min	Catechin / Epicatechin / Rutin / Isoquercitrin / Quercitrin / Quercetin / Kaempferol	<i>A. muricata</i> / leaves	44
HPLC-DAD	Dionex C18 (250 x 4.6 mm, 5 µm), 0.8 mL/min, 24 °C	A: 0.05% TFA in H <sub>2</sub> O; B: 0.05% TFA in MeOH; 0-10 min, 10%-25% B; 10-15 min, 25%-40% B; 15-35 min, 40%-75% B; 35-36 min, 75-15% B.	Catechin	<i>A. muricata</i> / leaves	45
HPLC	-	-	Flavonoid	<i>A. squamosa</i> / fruits	46
HPLC-DAD	C18 Hypersil BDS (150 x 4.6 mm, 5 µm), 1.0 mL/min	A: 0.1% FA in H <sub>2</sub> O; B: 0.1% FA in ACN; 0 - 20 min, 20% - 50% B; 20 - 25 min, 50% - 70% B; 25 - 30 min, 70% - 80% B; 30 - 35 min, 80% - 20% B, 35 - 40 min, 20% B,	Rutin	<i>A. muricata</i> / leaves	47
UPLC-DAD	C18 (2.1 x 50 mm, 1.7 µm).	A: 0.1% AA in H <sub>2</sub> O; B: MeOH; C: ACN; initial 90% A, 5% B and 5% C; at 6 min to 76% A, 12% B and 12% C; at 11 min to 36% A, 32% B and 32% C; back to the initial gradient at 12 min until the run was finished.	Rutin / Naringenin	<i>A. muricata</i> / leaves	48
HPLC-DAD	C18 (200 x 15 mm, 5 µm), 1.0 mL/min	A: 2% AA in H <sub>2</sub> O; B: ACN; 96% to 88% A for 20 min; 88% to 80% A for 10 min; 80% to 50% A for 15h min; 50% to 96% A for 15 min.	Rutin / Nicotiflorin / Narcissin	<i>A. cherimola</i> / leaves	49
HPLC-MS	-	-	quercetin-3-O-β-galactopyranoside / quercetin-3-O-β-glucopyranoside / isorhamnetin-3-O-β-galactopyranoside / quercetin-3-O-β-D-apio-furanosyl-(1-2)-galactopyranoside	<i>A. nutans</i> / leaves	50

**Table 3.** HPLC-MS flavonoid studies carried out for the *Annona* genus (cont.)

Equipment	Stationary phase (column and particle dimensions), flow, temperature	Mobile phase (v/v)	Flavonoids	Specie / plant parts	Ref.
HPLC	C18 (4.6 × 250 mm, 5 μm); 1.0 mL/min	ACN and 0.1% AA in H <sub>2</sub> O 16:84 v/v	Quercetin-3-O-robinobioside / rutin / quercetin-3-O-β-D-glucoside / kaempferol-3-O-robinobioside / kaempferol-3-O-rutinoside	<i>A. squamosa</i> / leaves	51
LC-PDA	C18 (4.6 mm x 25 cm, 5 μm), 1.0 mL/min, 22 °C	A: sodium acetate 2 mM; B: ACN; 10% B -10 min; 15% B - 5 min; 100% B - 15 min	Rutin	<i>A. crassiflora</i> / seeds	52
HPLC	C18 (4.6 x 50 mm, 5 mm), 1.0 mL/min, 35 °C	A: 50 mM sodium phosphate in 10% MeOH, pH 3.3; B: 70% MeOH; 0-15 min 100% A; 15-45 min 70% A; 45-65 min 65% A; 65-70 min 60% A; 70-95 min 50 % A; 95-100 min 0 % A	Luteolin / Homoorientin / Quercetin / Tangeretin / Genistein / Glycitein / (+)-Taxifolin / (+)-Catechin / (+)-Galocatechin / (+)-Epicatechin gallate / Daidzein	<i>A. muricata</i> / leaves	53
HPLC	C18 (4.6 x 50 mm, 5 mm), 1.0 mL/min, 35 °C	A: 50 mM sodium phosphate in 10% MeOH, pH 3.3; B: 70% MeOH; 0-15 min 100% A; 15-45 min 70% A; 45-65 min 65% A; 65-70 min 60% A; 70-95 min 50 % A; 95-100 min 0 % A	Quercetin / Robinetin / Apigenin-6-C-glucoside / Vitexin / Luteolin 3',7-di-O-glucoside	<i>A. muricata</i> / leaves	54
HPLC-DAD	C18 Spherisorb ODS2 (25.0 x 0.46 cm, 5 μm), 0.9 mL/min	A: H <sub>2</sub> O/FA 19:1; B: MeOH; Initial 5% B; 15% B - 3 min; 25% B - 13 min; 30% B - 25 min; 35% B - 35 min; 45% B - 39 min; 45% B - 42min; 55% B - 47 min; 75% B - 56 min; 100% B - 60 min	Quercetin-3-O-galactoside / Quercetin-3-O-glucoside / Quercetin-3-O-rutinoside / Kaempferol-3-O-rutinoside	<i>A. muricata</i> / leaves	55
HPLC-DAD	C18 (4.6 x 150 mm, 5 μm), 0.6 mL/min	A: 2% FA in H <sub>2</sub> O; B: ACN; 17% B - 10 min; 20% B - 20 min; 30% B - 30 min; 50% B - 40 min; 60% B - 50 min; 70% B - 60 min; 20% B - 70 min; 10% B - 80 min	Catechin / Epicatechin / Rutin / Quercitrin / Quercetin / Luteolin	<i>A. coriacea</i> / leaves	56

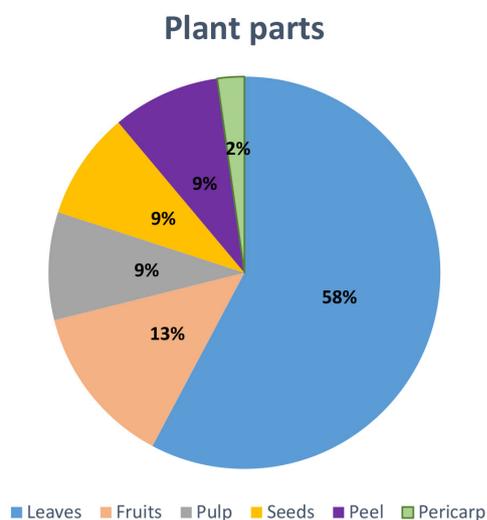
ACN (Acetonitrile); MeOH (Methanol); H<sub>2</sub>O (Water); FA (Formic acid); AA (Acetic acid); TFA (Trifluoroacetic acid). The numbers in parentheses represent equal identified compounds.

The data presented in Table 3 and Figures 4 and 5, are the result of the analysis of studies published between 2007 and December 2020, with 39 references involving nine species belonging to the *Annona* genus, with *A. muricata*, *A. cherimola*, *A. crassiflora* and *A. squamosa* as the most studied. Most of the flavonoids identified in these species were present in leaves (58%). The most reported flavonoid classes were flavonols (43%), procyanidins (28%) and flavan-3-ol (17%), respectively.

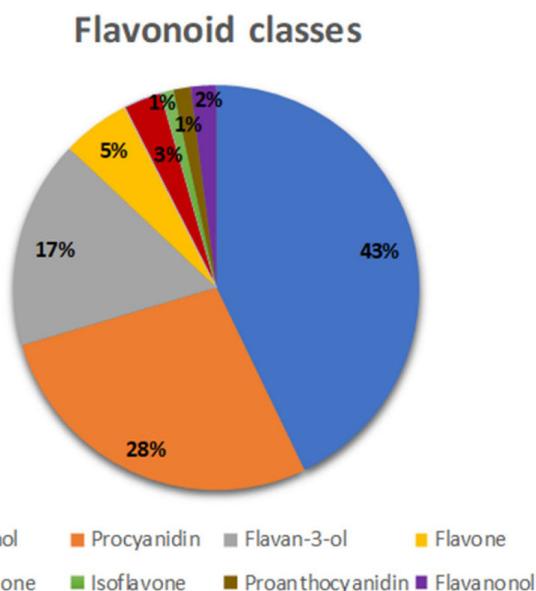
According to Table 3, most studies mention the exclusive use of the reverse phase (RP-LC), in which the majority of RP-LC analyses are performed using C18 octadecyl-silica columns. The mobile phase most frequently contains an aqueous phase and an organic phase consisting of methanol and acetonitrile, and approximately 70% are acidified

systems, usually using acetic acid or formic acid, which aids in suppressing flavonoid ionization, in addition to increasing retention. The most applied HPLC detectors were the diode array (DAD) and the mass spectrometer (MS), and the most widely applied type of ionization at atmospheric pressure was ESI in the negative mode, which displays the greatest sensitivity.

According to Santos and Salatino<sup>57</sup>, there is a chemotaxonomic importance of the presence of flavonoids in species of the Annonaceae family. Pereira<sup>6</sup> summarizes the isolated flavonoids in the *Annona* genus, most of which are in the form of glycosides, 83% of which are flavonols (76% glycosylated), 7% flavone and 4% flavan-3-ol and dihydroflavonol. The data collected hereing corroborate this profile, indicating that most flavonoids are glycosylated. The



**Figure 4.** Plant parts identified by HPLC-MS in *Annona* genus species, n = 45



**Figure 5.** Flavonoid classes identified by HPLC-MS in *Annona* genus species, n = 294

classes with the highest numbers of occurrence of glycosylated flavonoids were flavonols, flavanones and flavones with approximately 89%, 71% and 63%, respectively.

Also, in the study by Vega *et al.*,<sup>58</sup> four glycosylated flavonoids of the kaempferol type were isolated from methanol extract leaves from *Annona dioica*. For that reason, glycosylated flavonols may be considered a chemotaxonomic marker for the *Annona* genus since there has been reported a large number of occurrences in the species. With these results, we want to emphasize the importance of analysis using hyphenated techniques such as HPLC-MS to identify bioactive compounds from understudied species. For example, analysis such as dereplication can save time and the use of solvents which contributes to green chemistry by achieving goals efficiently and economically.

## 4. Final Considerations

The data from the bibliographic review presented herein reinforce the importance of using hyphenated techniques, which are a quick and efficient alternative way to characterize the chemical profile of plant species and can direct isolation studies in the search for novel compounds and/or those with biological activity. In addition, the chemotaxonomic importance of flavonoids presents in the *Annona* genus is highlighted, which may indicate glycosylated flavonols as a possible marker for this genus. This review affords information that may direct new research with species from the *Annona* genus, encouraging the use of hyphenated techniques as HPLC-MS.

## Acknowledgment

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

## References

- Leboeuf, M.; Cavé, A.; Bhaumik, P. K.; Mukherjee, B.; Mukherjee, R.; The Phytochemistry of the Annonaceae. *Phytochemistry* **1982**, *21*, 2783. [[Crossref](#)]
- Sítio Flora do Brasil; *Flora Do Brasil 2020 Em Construção. Jardim Botânico Do Rio de Janeiro*. 2019. Disponível em <<http://floradobrasil.jbrj.gov.br/reflora/listaBrasil/ConsultaPublicaUC/ConsultaPublicaUC.do>>. Acesso em 16 fevereiro 2022.
- Nugraha, A. S.; Damayanti, Y. D.; Wangchuk, P.; Keller, P. A.; Anti-infective and anti-cancer properties of the *Annona* Species: their ethnomedicinal uses, alkaloid diversity, and pharmacological activities. *Molecules* **2019**, *24*, 4419. [[Crossref](#)] [[PubMed](#)]
- Zuanazzi, J. A. S.; Montanha, J. A.; Zucolotto, S. M.; Em *Farmacognosia: Do Produto Natural Ao Medicamento*; Simões, C. M. O.; Schenkel, E. P.; Mello, J. C. P.; Mentz, L.; Petrovick, P. R., eds.; Artmed: Porto Alegre, 2017, cap. 15.
- Andersen, O. M.; Markham, K. R.; Flavonoids. Chemistry, Biochemistry and Applications, 1st. ed., CRC Press: Boca Raton, 2006.
- Pereira, I. S. P.; *Tese de Doutorado*, Universidade Estadual do Norte Fluminense Darcy Ribeiro, 2019. [[Link](#)]
- Paes, M. M.; *Tese de Doutorado*, Universidade Estadual do Norte Fluminense Darcy Ribeiro, 2016. [[Link](#)]
- Anaya-Esparza, L. M.; García-Magaña, M. de L.; Abraham Domínguez-Ávila, J.; Yahia, E. M.; Salazar-López, N. J.; González-Aguilar, G. A.; Montalvo-González, E.; *Annonas: Underutilized species as a potential source of bioactive compounds. Food Research International* **2020**, *138*. [[Crossref](#)] [[PubMed](#)]

9. Leite, D. O. D.; de F. A. Nonato, C.; Camilo, C. J.; de Carvalho, N. K. G.; da Nobrega, M. G. L. A.; Pereira, R. C.; da Costa, J. G. M.; *Annona* Genus: Traditional Uses, Phytochemistry and Biological Activities. *Current Pharmaceutical Design* **2020**, *26*, 4056. [[Crossref](#)] [[PubMed](#)]
10. Ciola, R.; *Fundamentos da Cromatografia a Líquido de Alto Desempenho: HPLC*, 1a. ed., Edgard Blücher: São Paulo, 1998.
11. Maldaner, L.; Cristina, I.; Fontes, S.; UHPLC – Uma abordagem atual : desenvolvimentos e desafios recentes. *Scientia Chromatographica* **2012**, *4*, 197. [[Crossref](#)]
12. Porto, H. S. M.; *Dissertação de Mestrado*, Universidade de Coimbra, 2014. [[Link](#)]
13. Lanças, F. M.; A Cromatografia Líquida Moderna e a Espectrometria de Massas: finalmente “compatíveis”? *Scientia Chromatographica* **2009**, *1*, 35. [[Crossref](#)]
14. Villiers, A. De; Venter, P.; Pasch, H.; Recent advances and trends in the liquid-chromatography – mass spectrometry analysis of flavonoids. *Journal of Chromatography A* **2016**, *1430*, 16. [[Crossref](#)]
15. Vukics, V.; Guttman, A.; Structural characterization of flavonoid glycosides by multi-stage mass spectrometry. *Mass Spectrometry Review* **2010**, *29*, 1. [[Crossref](#)] [[PubMed](#)]
16. Domon, B.; Costello, C. E.; A Systematic Nomenclature for Carbohydrate Fragmentations in FAB-MS/MS Spectra of Glycoconjugates. *Glycoconjugate J* **1988**, *5*, 397. [[Crossref](#)]
17. Cuyckens, F.; Claeys, M.; Mass spectrometry in the structural analysis of flavonoids. *Journal of Mass Spectrometry* **2004**, *39*, 1. [[Crossref](#)] [[PubMed](#)]
18. Araujo, R. C. P.; Neves, F. A. R.; Formagio, A. S. N.; Kassuya, C. A. L.; Stefanello, M. E. A.; Souza, V. V.; Pavan, F. R.; Croda, J.; Evaluation of the anti- mycobacterium tuberculosis activity and in vivo acute toxicity of *Annona sylvatic*. *BMC Complementary and Alternative Medicine* **2014**, *14*, 209. [[Crossref](#)]
19. Arruda, H. S.; Pereira, G. A.; Morais, D. R.; Eberlin, M. N.; Pastore, G. M.; Determination of free, esterified, glycosylated and insoluble-bound phenolics composition in the edible part of araticum fruit (*Annona crassiflora* Mart.) and its by-products by HPLC-ESI-MS/MS. *Food Chemistry* **2018**, *245*, 738. [[Crossref](#)] [[PubMed](#)]
20. Mannino, G.; Gentile, C.; Porcu, A.; Agliassa, C.; Caradonna, F.; Bertea, C. M.; Chemical Profile and Biological Activity of Cherimoya (*Annona cherimola* Mill.) and Atemoya (*Annona atemoya*) Leaves. *Molecules* **2020**, *25*. [[Crossref](#)]
21. Prado, L. G.; Arruda, H. S.; Peixoto Araujo, N. M.; de Oliveira Braga, L. E.; Banzato, T. P.; Pereira, G. A.; Figueiredo, M. C.; Ruiz, A. L. T. G.; Eberlin, M. N.; de Carvalho, J. E.; Vendramini-Costa, D. B.; Pastore, G. M.; Antioxidant, antiproliferative and healing properties of araticum (*Annona crassiflora* Mart.) peel and seed. *Food Research International* **2020**, *133*, 109168. [[Crossref](#)] [[PubMed](#)]
22. García-Salas, P.; Gómez-Caravaca, A. M.; Morales-Soto, A.; Segura-Carretero, A.; Fernández-Gutiérrez, A.; Identification and quantification of phenolic and other polar compounds in the edible part of *Annona cherimola* and its by-products by HPLC-DAD-ESI-QTOF-MS. *Food Research International* **2015**, *78*, 246. [[Crossref](#)] [[PubMed](#)]
23. Spínola, V.; Pinto, J.; Castilho, P. C.; Identification and quantification of phenolic compounds of selected fruits from Madeira Island by HPLC-DAD – ESI-MS n and screening for their antioxidant activity. *Food Chemistry* **2015**, *173*, 14. [[Crossref](#)] [[PubMed](#)]
24. Arruda, H. S.; Keven, E.; Pereira, G. A.; Fernando, C.; Angolini, F.; Eberlin, M. N.; Meireles, M. A. A.; Pastore, G. M.; Effects of high-intensity ultrasound process parameters on the phenolic compounds recovery from araticum peel. *Ultrasonics - Sonochemistry* **2019**, *50*, 82. [[Crossref](#)]
25. Barreca, D.; Laganà, G.; Ficarra, S.; Tellone, E.; Leuzzi, U.; Galtieri, A.; Bellocco, E.; Evaluation of the antioxidant and cytoprotective properties of the exotic fruit *Annona cherimola* Mill. (Annonaceae). *Food Research International* **2011**, *44*, 2302. [[Crossref](#)]
26. Baskaran, R.; Pullencheri, D.; Somasundaram, R.; Characterization of free, esterified and bound phenolics in custard apple (*Annona squamosa* L) fruit pulp by UPLC-ESI-MS/MS. *Food Research International* **2016**, *82*, 121. [[Crossref](#)]
27. Song, X.; Li, K.; Cui, L.; Yu, J.; Ali, I.; Zhu, H.; Wang, Q.; Wang, X.; Wang, D.; A simple and efficient linear gradient coupled with inner-recycling high-speed counter-current chromatography mode for the preparative separation of flavonoid glycosides from leaves of custard apple. *Journal of Chromatography A* **2020**, *1615*. [[Crossref](#)]
28. Díaz-de-Cerio, E.; Aguilera-Saez, L. M.; Gómez-Caravaca, A. M.; Verardo, V.; Fernández-Gutiérrez, A.; Fernández, I.; Arráez-Román, D.; Characterization of bioactive compounds of *Annona cherimola* L. leaves using a combined approach based on HPLC-ESI-TOF-MS and NMR. *Analytical and Bioanalytical Chemistry* **2018**, *410*, 3607 [[Crossref](#)] [[PubMed](#)]
29. Chai, W.; Lin, M.; Wang, Y.; Xu, K.; Huang, W.; Pan, D.; Zou, Z.; Peng, Y.; Inhibition of tyrosinase by cherimoya pericarp proanthocyanidins: Structural characterization, inhibitory activity and mechanism. *Food Research International* **2017**, *100*, 731. [[Crossref](#)] [[PubMed](#)]
30. Falé, P. L.; Ferreira, C.; Maruzzella, F.; Florêncio, M. H.; Frazão, F. N.; Serralheiro, M. L. M.; Evaluation of cholesterol absorption and biosynthesis by decoctions of *Annona cherimola* leaves. *Journal of Ethnopharmacology* **2013**, *150*, 718. [[Crossref](#)] [[PubMed](#)]
31. Ferraz, C. R.; Silva, D. B.; Prado, L. C. S.; Canabrava, H. A. N.; Bispo-da-Silva, L. B.; Antidiarrhoeic effect and dereplication of the aqueous extract of *Annona crassiflora* (Annonaceae). *Natural Product Research* **2019**, *33*, 563. [[Crossref](#)] [[PubMed](#)]
32. Jiménez, V. M.; Gruschwitz, M.; Schweiggert, R. M.; Carle, R.; Esquivel, P.; Identification of phenolic compounds in soursop (*Annona muricata*) pulp by high-performance liquid chromatography with diode array and electrospray ionization mass spectrometric detection. *Food Research International* **2014**, *65*, 42. [[Crossref](#)]
33. Justino, A. B.; Pereira, M. N.; Vilela, D. D.; Peixoto, L. G.; Martins, M. M.; Teixeira, R. R.; Miranda, N. C.; Neide, M.; Sousa, R. M. F. De; Oliveira, A. De; Espindola, F. S.; Peel of araticum fruit (*Annona crassiflora* Mart.) as a source of antioxidant compounds with  $\alpha$ -amylase,  $\alpha$ -glucosidase and

- glycation inhibitory activities. *Bioorganic Chemistry* **2016**, *69*, 167. [[Crossref](#)] [[PubMed](#)]
34. Justino, A. B.; Miranda, N. C.; Franco, R. R.; Martins, M. M.; Silva, N. M.; Espindola, F. S.; *Annona muricata* Linn. leaf as a source of antioxidant compounds with in vitro antidiabetic and inhibitory potential against  $\alpha$ -amylase,  $\alpha$ -glucosidase, lipase, non-enzymatic glycation and lipid peroxidation. *Biomedicine and Pharmacotherapy* **2018**, *100*, 83. [[Crossref](#)] [[PubMed](#)]
  35. Mancini, S.; Nardo, L.; Gregori, M.; Ribeiro, I.; Mantegazza, F.; Delerue-matos, C.; Masserini, M.; Grosso, C.; Functionalized liposomes and phytosomes loading *Annona muricata* L. aqueous extract: Potential nanoshuttles for brain-delivery of phenolic compounds. *Phytomedicine* **2018**, *42*, 233. [[Crossref](#)] [[PubMed](#)]
  36. Kim, G.; Khoi, N.; Tran, S.; Choi, E.; Song, Y.; Song, J.; Shim, S.; Park, T.; Immunomodulatory Efficacy of Standardized *Annona muricata* (Graviola) Leaf Extract via Activation of Mitogen-Activated Protein Kinase Pathways in RAW 264.7 Macrophages. *Evidence-Based Complementary and Alternative Medicine* **2016**, *2016*. [[Crossref](#)] [[PubMed](#)]
  37. Kumazawa, S.; Ikenaga, M.; Usui, Y.; Kajiya, K.; Miwa, S.; Endo, J.; Chikasawa, C.; Suzuki, Y.; Shimoi, K.; Nakayama, T.; Comprehensive Analysis of Polyphenols in Fruits Consumed in Japan. *Food Science and Technology Research* **2007**, *13*, 404. [[Crossref](#)]
  38. Oliveira, A. P.; Ivone, S.; Pereira, D. M.; Gonçalves, R. F.; Andrade, P. B.; Valentão, P.; Exploratory Studies on the in Vitro Anti-inflammatory Potential of Two Herbal Teas (*Annona muricata* L. and *Jasminum grandiflorum* L.), and Relation with Their Phenolic Composition. *Chemistry and Biodiversity* **2017**, *14*. [[Crossref](#)]
  39. Panda, S.; Kar, A. Ā.; *Annona squamosa* seed extract in the regulation of hyperthyroidism and lipid-peroxidation in mice : Possible involvement of quercetin. *Phytomedicine* **2007**, *14*, 799. [[Crossref](#)] [[PubMed](#)]
  40. Potchoo, Y.; Richard, D.; Sakiè, E.; Guissou, I. P.; Kini, F.; Yaro, B.; Comparative phytochemical content of leaves extracts of two *Annona senegalensis* Pers: the one from Togo and the other originates from Burkina Faso. *Journal of Biological Sciences* **2008**, *8*, 577. [[Crossref](#)]
  41. Batubara, I.; Achmadi, S. S.; Nurwendari, W.; Extraction technique to separate kaempferol from Soursop (*Annona muricata*) leaves. *Journal of Chemical and Pharmaceutical Research* **2015**, *7*, 106. [[Link](#)]
  42. Ramalho, S. A.; Gualberto, N. C.; Terezinha, M.; Leite, S.; Batista, R. A.; Araújo, S. M.; Jesus, J.; Catechin and Epicatechin Contents in Wines Obtained from Brazilian Exotic Tropical Fruits. *Food and Nutrition Sciences* **2014**, *5*, 449. [[Crossref](#)]
  43. Ribeiro, I. C. D. O.; Mariano, E. G. A.; Careli, R. T.; MORAIS-COSTA, F.; Anna, F. M. D. S.; Pinto, M. S.; Souza, M. R. De; Duarte, E. R.; Plants of the Cerrado with antimicrobial effects against *Staphylococcus* spp. and *Escherichia coli* from cattle. *BMC Veterinary Research* **2018**, *14*, 32. [[Crossref](#)]
  44. Souza, D. O.; Sales, V. S.; Rodrigues, C. K. S.; Oliveira, L. R.; Lemos, I. C. S.; Delmondes, G. A.; Monteiro, A. B.; Nascimento, E. P.; Figueiredo, F. R. S. D. N.; Costa, J. G. M.; Cruz, G. M. P.; Viana, G. S. B.; Barbosa, R.; Menezes, I. R. A.; Felipe, C. F. B.; Kerntopf, M. R.; Phytochemical Analysis and Central Effects of *Annona muricata* Linnaeus: Possible Involvement of the Gabaergic and Monoaminergic Systems. *Iranian Journal of Pharmaceutical Research* **2018**, *17*, 1306. [[PubMed](#)]
  45. Souza, J. A. L. .; Silva, W. A. V. .; Bezerra, I. C. F. .; Ferreira, M. R. A. .; Soares, L. A. L.; Chemical Profiles by Thin - layer Chromatography and High - performance Liquid Chromatography of Plant Species from Northeast Brazil. *Pharmacognosy Magazine* **2018**, *14*, 437. [[Crossref](#)]
  46. Alvionita, M.; Oktavia, I.; Subandi; Muntholib. Bioactivity of Flavonoid in Ethanol Extract of *Annona squamosa* L. Fruit as Xanthine Oxidase Inhibitor. *IOP Conference Series: Materials Science and Engineering* **2019**, *546*, 6. [[Crossref](#)]
  47. Arnaud, K.; Nicodème, C.; Durand, D-N.; Martial, N.; Basile, S.; Haziz, S.; Christine, N.; Christian, K. A.; Halfane, L.; Victorien, D.; Noumavo, P.; Lamine, B-M.; Antioxidant, Anti-Inflammatory Efficacy and HPLC Analysis of *Annona muricata* Leaves Extracts from Republic of Benin. *American Journal of Plant Sciences* **2020**, *11*, 803. [[Crossref](#)]
  48. Balderrama-Carmona, A. P.; Silva-Beltrán, N. P.; Gálvez-Ruiz, J. C.; Ruiz-Cruz, S.; Chaidez-Quiroz, C.; Morán-Palacio, E. F.; Antiviral, Antioxidant, and Antihemolytic Effect of *Annona muricata* L. Leaves Extracts. *Plants* **2020**, *9*, 1650. [[Crossref](#)] [[PubMed](#)]
  49. Calzada, F.; Valdés, M.; Merlin, V. I.; Velázquez, C.; Barbosa, E.; Chávez-Soto, M.; Pina-Jiménez, E.; Ordoñez-Razo, R. M.; García-Hernández, N.; Phytochemical Analysis, Antipropulsive and Antilymphoma Activities of Leaves Extract of *Annona cherimola* Miller. *Pharmacognosy Magazine* **2019**, *16*, 317. [[Crossref](#)]
  50. Silva, N. L.; Saldanha, A. A.; Vieira, L.; da Silva, D. B.; Carollo, C. A.; Sartori, Ā. L. B.; de Azambuja Ribeiro, R. I. M.; Thomé, R. G.; dos Santos, H. B.; Soares, A. C.; de Siqueira, J. M.; Chemical composition, anti-inflammatory and antinociceptive effects of the butanolic fraction of *Annona nutans* (Annonaceae) leaves. *Natural Product Research* **2021**, *35*, 5397. [[Crossref](#)] [[PubMed](#)]
  51. Zhu, H.; Chen, L.; Yu, J.; Cui, L.; Ali, I.; Song, X.; Park, J. H.; Wang, D.; Wang, X.; Flavonoid epimers from custard apple leaves, a rapid screening and separation by HSCCC and their antioxidant and hypoglycaemic activities evaluation. *Scientific Reports* **2020**, *10*, 8819. [[Crossref](#)] [[PubMed](#)]
  52. Formagio, A. S. N.; Vieira, M. C.; Volobuff, C. R. F.; Silva, M. S.; Matos, A. I.; Cardoso, C. A. L.; In vitro biological screening of the anticholinesterase and antiproliferative activities of medicinal plants belonging to Annonaceae. *Brazilian Journal of Medical and Biological Research* **2015**, *48*, 308. [[Crossref](#)] [[PubMed](#)]
  53. George, V. C.; Kumar, D. R. N.; Suresh, P. K.; Kumar, R. A.; Antioxidant, DNA protective efficacy and HPLC analysis of *Annona muricata* (soursop) extracts. *Journal of Food Science and Technology* **2015**, *52*, 2328. [[Crossref](#)] [[PubMed](#)]
  54. George, V. C.; Kumar, N.; Rajkumar, V.; Suresh, P.; Kumar, R.; Quantitative Assessment of the Relative Antineoplastic Potential of the n-butanolic Leaf Extract of *Annona muricata* Linn. in Normal and Immortalized Human Cell Lines. *Asian Pacific Journal of Cancer Prevention* **2012**, *13*, 699. [[Crossref](#)] [[PubMed](#)]

55. Grosso, C.; Valentão, P.; Andrade, C.; Andrade, P. B.; HPLC – DAD analysis and in vitro enzyme inhibition: An integrated approach to predict herbal binary mixture behaviour employing median effect equation. *Microchemical Journal* **2015**, *119*, 176. [[Crossref](#)]
56. Junior, J. G. A. S.; Coutinho, H. D. M.; Boris, T. C. C.; Cristo, J. S.; Pereira, N. L. F.; Figueiredo, F. G.; Cunha, F. A. B.; Aquino, P. E. A.; Polyana, A. C.; Chemical characterization and cytoprotective effect of the hydroethanol extract from *Annona coriacea* Mart. (Araticum). *Pharmacognosy Research* **2016**, *8*, 253. [[Crossref](#)] [[PubMed](#)]
57. Santos, D. Y. A. C.; Salatino, M. L. F.; Foliar flavonoids of Annonaceae from Brazil: taxonomic significance. *Phytochemistry* **2000**, *55*, 567. [[Crossref](#)] [[PubMed](#)]
58. Vega, M. R. G.; Esteves-Souza, A.; Vieira, I. J. C.; Mathias, L.; Braz-Filho, R.; Echevarria, A.; Flavonoids from *Annona dioica* Leaves and their Effects in Ehrlich Carcinoma Cells, DNA-topoisomerase. *Journal of the Brazilian Chemical Society* **2007**, *18*, 1554. [[Crossref](#)]