



Article

Phenolic Compounds and Antioxidant Properties of Puruí (*Alibertia edulis*, Rubiaceae), an Edible Dark Purple Fruit from the Brazilian Amazon

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Abstract: *Alibertia edulis* is known as Puruí, and its leaf tea is used in the hypoglycemic and antihypertensive treatments of the Amazon native population. This study aimed to evaluate the phytochemical composition and antioxidant properties of the Puruí pulp fruit. The hydroethanolic (LFP-E), ethyl acetate (LFP-A), and volatile concentrate (LFP-V) extracts of Puruí lyophilized fruit pulp were analyzed via LC-ESI-IT-MS, GC, and GC-MS. Moreover, total phenolic and flavonoid content (TPC and TFC) and TEAC/ABTS and DPPH assays were conducted to determine their antioxidant capacity. Compounds palmitic acid, methyl linolenate, methyl linoleate, palmitic alcohol, benzene acetaldehyde, tridecanal, and furfural were mainly identified in the LFP-V extract. Compounds caffeic and quinic acids, genipin, annonaine, 3'-7-dimethoxy-3-hydroxyflavone, 4'-hydroxy-5,7-dimethoxyflavone, 6-hydroxy-7-epigardoside methyl ester, baicalin, and phloretin-2-O-apiofuranosyl-glucopyranoside were mainly identified in the LFP-E and LFP-A extracts. For LFP-E and LFP-A extracts, TPC values were 5.75 ± 0.75 and 66.75 ± 3.1 mg GAE/g; TFC values were 1.14 ± 0.65 and 50.97 ± 1.2 mg QE/g; DPPH assay showed EC₅₀ values of 1021.65 ± 5.9 and 133.60 ± 3.9 µg/mL; and TEAC/ABTS assay showed values of 28.36 ± 3.7 and 142.26 ± 2.2 µM TE/g. *Alibertia edulis* fruits are significant sources of phenolic compounds, also showing significant antioxidant capacity. The Puruí fruit seems promising for developing innovative and healthy products for the nutritional food market.

Keywords: Puruí fruit; flavonoids and phenolic compounds; volatile constituents; TEAC/ABTS and DPPH Antioxidant properties



Citation: Carvalho, N.C.C.; Monteiro, O.S.; da Rocha, C.Q.; da Silva, J.K.R.; Maia, J.G.S. Phenolic Compounds and Antioxidant Properties of Puruí (*Alibertia edulis*, Rubiaceae), an Edible Dark Purple Fruit from the Brazilian Amazon. *Nutraceuticals* **2023**, *3*, 529–539. <https://doi.org/10.3390/nutraceuticals3040038>

Academic Editor: Miquel Mulero

Received: 30 August 2023

Revised: 16 October 2023

Accepted: 25 October 2023

Published: 1 November 2023



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1. Introduction

Rubiaceae represents one of the five most species-rich families of flowering plants, numbering about 13,000, classified into about 620 genera, more than 40 tribes, and three subfamilies. The family occurs on all continents, but most taxa are in tropical or subtropical areas, and the species occupy many types of habitat in different biogeographical regions. The diversity in the family is significant, with a span of lifeforms from small, weedy herbs to large rainforest trees, flower types adapted to a wide range of pollinators, different fruit types with many kinds of dispersal mechanisms, and accumulation of different chemical compounds in the plants [1,2]. The genus *Alibertia* belongs to the subfamily Ixoroideae, tribe Gardenieae, subdivided into two well-defined and strongly supported lineages by phylogenetic analyses. The first group comprises several species of *Alibertia*, including the type species *Alibertia edulis*, three species of *Borojoa*, and the species *Randia tessmannii*. On the other hand, the second group consists of the species of *Amaioua*, *Duroia*, *Kutchubaea*, *Ibetrulia*, and *Genipa*, traditionally referred to as the genus *Alibertia*. In addition, the first group is

mainly confined to humid rainforest areas and can be distinguished morphologically from the second group by possessing relatively larger fruits and corollas [2,3].

Alibertia edulis (Rich.) A. Rich. ex DC. [syn. *A. acuminata* (Benth.) Sandwith, *A. panamensis* L. Riley, *A. utilis* A. Rich., *Amaioua utilis* Baill., *Borojoa lanceolata* (Cham.) Cuatrec., *Cordia edulis* (Rich.) Kuntze, *Garapatica edulis* (Rich.) Poir., among others) is a small Amazonian tree known as “Puruí”, 3 to 4 m high, typical of secondary vegetation, semi-open areas, and poor sandy soil, with large leaves, white flowers, globose fruits, having sweetish pulp, and a dark purple color when fully mature (see Figure 1) [4]. Puruí fruits are used to make jams, juices, and soft drinks by the native riverside population of the Amazon River [5].



Figure 1. Dark purple fruits of *Alibertia edulis*.

The decoction of *A. edulis* leaves has been used in Brazilian folk medicine by indigenous communities due to its antihypertensive, hypoglycemia, and diuretic effects: aqueous extract of *A. edulis* leaves (AEAE) is used to treat diabetes at the Platinum and Amazon Basins transition zone in southwestern Mato Grosso state, Brazil [6]; AEAE showed acute and subacute toxicity in rats [7]; AEAE presented hypotensive, antihypertensive, hemolytic, and potent diuretic effects in mice [8,9]; AEAE demonstrated no cytotoxicity, genotoxicity, and mutagenicity at the doses tested in rats [10]; and rutin present in *A. edulis* leaves decoction acted on human platelet aggregation by inhibiting cyclooxygenase and thromboxane [11]. Some phytochemical works with the leaves and stems of *A. edulis* were described earlier: a new oleanane compound and nine known triterpenes were isolated from its leaves [12], and new antifungal terpenoid glycosides were found in its stems [13].

Concerning other *Alibertia* species, chemical and biological studies were previously reported: caffeic acid esters, triterpenes, fungi toxic non-glycosidic iridoids, and a new ent-kaurane diterpene from the leaves of *A. macrophylla* K. Schum. [14–16]; triterpenes, phenolic compounds, antifungal iridoids, and cytotoxic flavonoids from the aerial parts of *A. myrciifolia* Spruce ex. K. Schum [17,18]; triterpenes, acylated flavonol glycosides, and lignans glycosides from the leaves of *A. sessilis* K. Schum [19,20].

Previously, the antioxidant activity of the aqueous extract of *A. edulis* (AEAE) leaves and rutin, used as the positive control, was evaluated via DPPH radical scavenging and the ferric ions reducing power assays. AEAE produced significant DPPH scavenging activity ($69.91 \pm 0.37\%$ at $150 \mu\text{g}/\text{mL}$), smaller than rutin ($74.94 \pm 0.12\%$ at $10 \mu\text{g}/\text{mL}$). Similarly, the ferric ions reducing ability of AEAE was also significant ($103.05 \pm 0.13 \mu\text{M Fe}^{2+}$) and smaller than the positive control ($148.91 \pm 0.17 \mu\text{M Fe}^{2+}$) [8].

The present work aimed to investigate the phenolic and volatile compounds of the lyophilized fruits of *Alibertia edulis* and analyze the antioxidant capacity of its dark violet fruits.

2. Materials and Methods

2.1. Chemicals

The reagents, solvents, and standard compounds were purchased from Sigma-Aldrich Chemical Company (St. Louis, MI, USA and Milwaukee, WI, USA; São Paulo, Brazil).

2.2. Plant Material and Extractions

The ripe fruits of *Albertia edulis* (Puruí) were collected on the banks of the Amazon River in the municipality of Santarém, state of Pará, Brazil, geographic coordinates 02°25'12.3" S/54°44'23.3" W. The plant was collected following Brazilian laws concerning the protection of biodiversity (SisGen n° AA45C16), and the botanical material for taxonomic purposes was identified and deposited in the herbarium of Emílio Goeldi Museum, Belém, PA, Brazil, under number MG228053. The lyophilized "Puruí" fruit pulp (LPF, 50 g) was exhaustively extracted with *n*-hexane (500 mL), and then the residue was extracted with hydroethanolic solution (300 mL, ethanol 70%), yielding the LPF-E extract. Subsequently, the LPF-E extract was fractionated with ethyl acetate (300 mL) to furnish the LPF-A extract. The solvents of the LPF-E and LPF-A extracts were eliminated in a rotary evaporator, and the dry extracts were kept in a desiccator until used in chemical and biological tests. The lyophilized pulp fruit (LPF, 10 g) was also subjected to a micro distillation-extraction system (Likens and Nickerson type) to obtain its volatile concentrate, using *n*-pentane (99% HPLC grade, 3 mL) as the solvent.

2.3. Chemical Characterization of *A. edulis* Extracts

A clean-up was performed to remove contaminants for the HPLC-ESI-IT-MS/MS and FIA-ESI-IT/MS analyses. LPF-E (hydroethanolic) and LPF-A (ethyl acetate) extracts were purified via solid-phase extraction (SPE) using Phenomenex Strata C18 cartridges (500 mg, stationary phase) that were previously activated with MeOH (5 mL) and equilibrated with MeOH and H₂O (5 mL, 1:1, *v/v*). The purified extracts were eluted from cartridges using the same MeOH/H₂O solution, filtered through a 0.22 µm PTFE filter, and dried. Then, the extracts were diluted to 10 µg/mL using the HPLC solvent. Aliquots of 20 µL were injected directly into the LC-ESI-IT-MS.

The LPF-E and LPF-A extract analyses were performed on an LC-ESI-IT-MS mass spectrometer LCQ Fleet Thermo Scientific, using a Kinetex C18 (4.6 × 100 mm, 100 Å and 5 µm) analytical column for the LC separation. Linear gradient elution from two mixtures was used in the mobile phase: 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) at room temperature. A gradient elution starting from 10 to 100% of B for 10 min was used with a flow of 1.0 mL/min. ESI-MS analysis reviewed samples from the HPLC system online in negative ion mode, in series, with a UV detector. For the FIA-ESI-IT-MS analysis, direct flow infusion of the samples was performed on a Thermo Scientific LTQ XL linear ion trap analyzer equipped with an electrospray ionization (ESI) source (Thermo, San Jose, CA, USA). A stainless steel capillary tube was used at 280 °C, a spray voltage of 5.00 kV, a capillary voltage of 90 V, a tube lens of 100 V, and a flow of 5 µL/mL. Complete scan analysis was recorded in the range from 100 to 1000 *m/z*.

Multiple-stage fragmentations via electrospray ionization mass spectrometry (ESI-MS_n) were obtained using the collision-induced dissociation (CID) method against the helium for ion activation. The first event was a full-scan mass spectrum to acquire data on the ions in that *m/z* range. The second scan event was an MS/MS experiment performed using a data-dependent scan on the [M-H] molecules from the compounds of interest at a collision energy of 30% and an activation time of 30 ms. The produced ions were subjected to further fragmentation in the same conditions until no more fragments were observed. The different compounds in the chromatographic profiles of the hydroalcoholic (LPF-E) and ethyl acetate (LPF-A) extracts were identified by comparing their retention times and MS spectra with the literature data.

The volatile concentrate extract (LPF-V) of the lyophilized fruit pulp of *A. edulis* was submitted to GC and GC-MS analysis. It was performed on a GCMS-QP2010 Ultra

system (Shimadzu Corporation, Tokyo, Japan) equipped with an AOC-20i auto-injector and the GCMS-Solution software containing standards libraries [21,22]. A Rxi-5ms (30 m × 0.25 mm; 0.25 µm film thickness) silica capillary column (Restek Corporation, Bellefonte, PA, USA) was used. The conditions of analysis were as follows: injector temperature: 250 °C; oven temperature programming: 60–240 °C (3 °C min⁻¹); helium as the carrier gas, adjusted to a linear velocity of 36.5 cm s⁻¹ (1.0 mL min⁻¹); split mode injection (split ratio 1:20) of 1.0 µL of the *n*-pentane solution; electron ionization at 70 eV; ionization source and transfer line temperatures of 200 and 250 °C, respectively. The mass spectra were obtained by automatically scanning every 0.3 s, with mass fragments in the 35–400 *m/z*. The retention index was calculated for all volatile components using a homologous series of C₈–C₄₀ *n*-alkanes according to the linear equation of van den Dool and Kratz (1963) [23]. Individual components were identified by comparing their retention indices and mass spectra (molecular mass and fragmentation pattern) with those existing in the GCMS-Solution system libraries [21,22]. The quantitative data regarding the volatile constituents were obtained using a GC2010 Series gas chromatograph, operated under similar conditions to the GC-MS system. A flame ionization detector (GC-FID) was used to quantify the relative amounts of individual components via peak-area normalization. Chromatographic analyses were performed in duplicate.

2.4. Antioxidant Capacity of the Fruit Pulp of *A. edulis*

2.4.1. Determination of Total Phenolics Content (TPC)

The total phenolic content (TPC) of the LPF-E and LPF-A extracts was determined according to the Folin-Ciocalteu colorimetric procedure [24,25]. A calibration curve with gallic acid was prepared at 1, 2, 4, 6, 8, and 10 µg/mL. The acid gallic solutions' aliquots (500 µL) were mixed with Folin-Ciocalteu reagent (250 µL, 1 N) and sodium carbonate (1250 µL, 75.0 g/L). After 30 min of reaction, absorbance was read at 760 nm in a spectrophotometer UVVisible (Shimadzu, UV 1800, Shimadzu Corporation, Tokyo, Japan) at 25 °C and in a dark environment. The LPF-E and LPF-A extracts were solubilized in methanol at initial concentrations of 20,000 µg/mL (for the extract) and 10,000 µg/mL (for the fractions) to induce an absorbance between 0.3 and 0.7. According to the Folin-Ciocalteu procedure [24,25], the total phenolic content (TPC) was expressed as gallic acid equivalents in mg per g of extract (mg GAE/g).

2.4.2. Determination of Total Flavonoids Content (TFC)

The total flavonoid content of the LPF-E and LPF-A extracts was determined according to the aluminum chloride colorimetric procedure [26]. A calibration curve with the quercetin standard at concentrations of 0.625, 1.25, 2.5, 5, 10, and 20 µg/mL was prepared. The aliquots of the quercetin solutions (1000 µL) were mixed with aluminum chloride (1000 µL, 2%). After 30 min of reaction, absorbance was read in a UV/Visible spectrophotometer (Shimadzu, UV 1800, Shimadzu Corporation, Tokyo, Japan) at 420 nm, in a dark environment, and at 25 °C. The LPF-E and LPF-A extracts were solubilized in methanol at an initial 6000 µg/mL concentration. The total flavonoid content was expressed as quercetin equivalents in mg per g of extract (mg QE/g).

2.4.3. DPPH Radical Scavenging Assay

The LPF-E and LPF-A extracts were evaluated via the DPPH radical-scavenging assay [25]. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable dark violet free radical with maximum absorption at 517 nm, reduced by antioxidants. A stock solution of DPPH (0.5 mM) was prepared in ethanol. The solution was diluted to approximately 60 µM, measuring an initial absorbance of 0.62 ± 0.02 at 517 nm (Shimadzu, UV 1800, Shimadzu Corporation, Tokyo, Japan) at room temperature. Absorbance was measured at the start of the reaction, every 5 min during the first 30 min, and then at 30 min intervals until constant absorbance values were observed (plateau of reaction, 2 h). LPF-E extract (50 µL) was mixed with 1950 µL of methanolic DPPH solution (0.5 mM). The standard

curves were prepared with concentrations of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Sigma-Aldrich, St. Louis, MO, USA) of 1, 2, 4, 6, 8, and 10 $\mu\text{g}/\text{mL}$. The results were expressed as the equivalent concentration of 50% (EC_{50}) and milligrams of Trolox equivalent per gram of the extract (mgTE/g).

2.4.4. ABTS Radical Cation Assay

The LPF-E extract was evaluated via ABTS radical cation assay [25,27]. ABTS radical cation was obtained by mixing 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) solution (7 mM, 5000 μL) with potassium persulfate solution (140 mM, 88 μL) followed by incubation for 16 h in the dark at room temperature. Afterward, ABTS radical cation solution was diluted in ultrapure water until reaching the absorbance of 0.70 ± 0.02 at 734 nm (ABTS work solution). Aliquots (20 μL) of LPF-E extract prepared in ultrapure water were added to ABTS work solution (2020 μL), and the absorbance was measured in a spectrophotometer (Shimadzu, UV 1800, Shimadzu Corporation, Tokyo, Japan). The results were expressed in Trolox Equivalent Antioxidant Capacity (TEAC, $\mu\text{MTE}/\text{g}$). The TEAC value was calculated by measuring the area under the curve and plotting the percentage inhibition of the absorbance as a function of time. The area under the curve was calculated from a sample dilution, showing a final percentage inhibition between 20% and 80%.

2.5. Statistical Analysis

Samples were assayed in triplicate, and the results are shown as means \pm standard deviation. Analysis of variance was conducted, and the differences between variables were tested for significance by a Tukey test. Differences at $p < 0.05$ were considered statistically significant. The IC_{50} 's values were calculated via nonlinear regression using the GraphPad program (version 5.0, Intuitive Software for Science, San Diego, CA, USA).

3. Results and Discussion

3.1. Composition of Fruit Pulp Extracts of *A. edulis*

The hydroethanolic extract (LPF-E) from the hydrophilized fruit pulp of *A. edulis* yielded 9.0%. The fractionation of LPF-E extract (4 g), with *n*-hexane and ethyl acetate, furnished the LPF-H (1.9%) and LPF-A (0.9%) extracts, respectively. The chemical profiles of the LPF-E and LPF-A extracts were analyzed using the LC-ESI-IT/MS technique (m/z 100–1000 Da), and the fragmentation data recorded are shown in Table 1. The main secondary metabolites identified were two phenolic acids, two iridoids, one aporphine alkaloid, three flavones, and one dihydrochalcone (see Table 1 and Figure 2).

Table 1. Mass spectral characteristics of secondary compounds detected via LC-ESI-IT-MS in the *Alibertia edulis* fruit pulp extracts at negative ionization mode*.

LPF-E and LPF-A Extracts	Compounds	RT (min)	[M-H] ⁻ (m/z)	MS ⁿ Fragments (m/z)
1	Caffeic acid ^a	1.7	179	136
2	Quinic acid ^a	1.8	191	173
3	Genipin ^{a,b}	10.2	225	207, 175, 147
4	Annonaine ^{a,b}	10.1	264	249, 219, 191
5	3',7-Dimethoxy-3-hydroxyflavone ^{a,b}	15.7	297	265, 149
6	4'-Hydroxy-5,7-dimethoxyflavone ^{a,b}	16.3	299	281, 255
7	6-Hydroxy-7-epigardoside methyl ester ^{a,b}	6.5	403	241
8	Baicalin ^a	18.1	445	269, 167, 101
9	Phloretin-2-O-apiofuranosyl-glucopyranoside ^{a,b}	15.3	567	419, 273

* Compounds identified by comparing retention times and MS data with those of reference compounds.

^a Compounds identified in LPF-E extract (hydroethanolic). ^b Compounds identified in LPF-A extract (ethyl acetate).

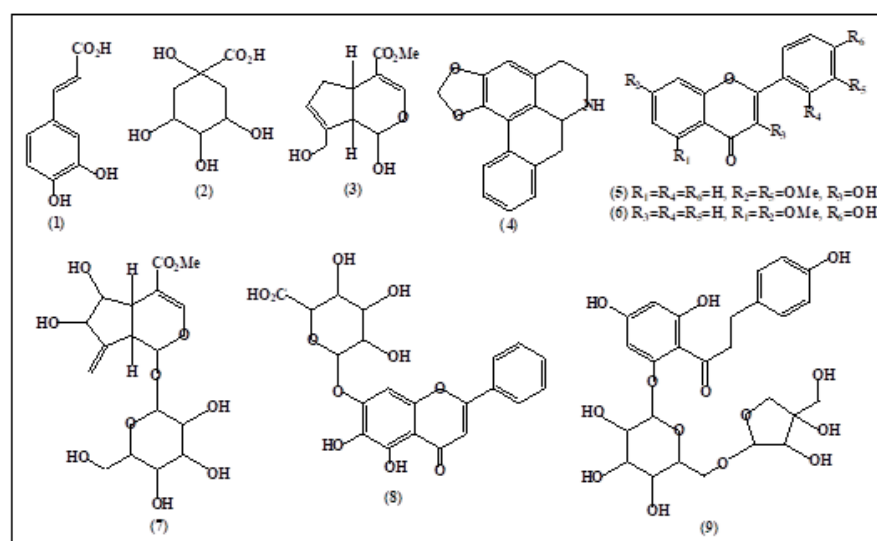


Figure 2. Constituents identified in the hydroethanolic (LPF-E) and ethyl acetate (LPF-A) extracts from the fruit pulp of *Alibertia edulis* (see Table 1).

Compounds 1 (m/z 179) and 2 (m/z 191) undergo decarboxylation (loss of CO_2 , 44 Da), producing the fragments m/z 135 and m/z 173, corresponding to caffeic and quinic acid, respectively [28,29]. Compound 3 (m/z 225), characterized by genipin, produced the ion-fragments in m/z 207 and m/z 175, corresponding to H_2O molecule loss and the cleavage of methylol group on C-11, and the primary moiety, the ion at m/z 225, may have undergone a successive loss of OH radical on C-1 and the methyl ester group on C-4, to produce the ion m/z 147 [30]. Compound 4 (m/z 264) suggests the initial loss of an amino group (NH, m/z 249) and successive losses of substituent groups, such as CH_2O (m/z 219) and CO (m/z 191), which occur until MS^4 fragmentation, compatible with the aporphine alkaloid annonaine [31].

Compound 5 (m/z 297) refers to 3'-7-dimethoxy-3-hydroxyflavone, which loses a methoxyl (CH_3O) group, producing the ion-fragment m/z 265, followed by a retro Diels-Alder (RDA) mechanism to produce the ion m/z 149 [32]. Compound 6 (m/z 299), characterized by 4'-hydroxy-5,7-dimethoxyflavone, produced ion fragments in m/z 281 and m/z 255, corresponding to H_2O and CO_2 molecule losses. Compound 7 (m/z 403) was pointed out as the iridoid glycoside 6-hydroxy-7-epigardoside methyl ester, which generates the ion-fragment m/z 241 upon losing the hexose moiety [13]. Genipin (3) and 6 β -hydroxy-7-epigardoside methyl ester (7) are chemical markers of *Alibertia edulis*, derived from geniposide, a glycosylated iridoid from *Genipa americana* L. [33]. Compound 8 (m/z 445) was identified as baicalin (7-*O*-glucuronide-5,6,7-trihydroxyflavone), characterized by the presence of glucuronic acid at C-7 of the A ring, which, when fragmented, produces baicalin aglycone (m/z 269) resulting from the loss of the glucuronide portion, in addition to m/z ions 167 and 101, formed from baicalin aglycone via a retro Diels-Alder (RDA) mechanism [34]. Compound 9 (m/z 567) was characterized as phloretin-2-*O*-apiofuranosyl-glucopyranoside. This dihydrochalcone disaccharide undergoes the loss of a pentose unit ($\text{C}_5\text{H}_8\text{O}_5$, 148 Da) to produce the ion m/z 419 and then the loss of a glucose unit ($\text{C}_6\text{H}_{10}\text{O}_4$, 146 Da) to generate the dihydrochalcone aglycone (m/z 273) [35].

A new oleanane (3 β ,19 α ,23,24-tetrahydroxy-12-oleanen-28-oic) acid was isolated with nine known triterpene compounds from the leaves of *A. edulis* [12]. New antifungal terpenoid glycosides were identified in their stems [13]. The decoction of the aqueous extract of their leaves has been used as an antihypertensive, hypoglycemic, diuretic, antioxidant, and acting on human platelet aggregation properties [6–11]. Previously, chemical and biological studies from other *Alibertia* species have also been published: Caffeic acid esters, di- and triterpenes, and fungi toxic non-glycosidic iridoids from leaves and stems of *Alibertia macrophylla* [14–16]; flavonol, lignans glycosides, and terpenoids from the leaves and

stems of *Alibertia sessilis* [19,20]; and cytotoxicity of flavonoids, antifungal iridoids, and triterpenes isolated from leaves and stems of *Alibertia myrciifolia* and *A. rigida* [17,18].

3.2. Composition of Volatile Concentrate of *A. edulis* Fruit Pulp

Volatile constituents are responsible for the characteristic aroma and flavor of fruits and are present in various concentrations, represented by different chemical classes. The constituents of volatile concentrate (LPF-V) in the fruit pulp of *A. edulis* were analyzed via GC and GC-MS and are listed in Table 2. The yield of volatile concentrate was 0.2%. Twenty-five constituents were identified, comprising more than 95% of the volatile concentrate. The primary components above 5% were palmitic acid (20.4%), methyl linolenate (16.6%), methyl linoleate (7.1%), palmityl alcohol (5.5%), benzene acetaldehyde (5.5%), tridecanal (5.4%), and furfural (5.4%), comprising 65.9% of the volatile concentrate. The odor descriptions of palmitic acid (slightly oily), methyl linolenate (oily fruity), methyl linoleate (oily woody), palmityl alcohol (fatty floral oil), benzene acetaldehyde (green, sweet floral), tridecanal (fresh herbal), and furfural (sweet woody) point to a significant contribution in the “Puruí” aroma characteristics, corresponding to about 70% of the total identified constituents. The volatile composition of Puruí fruit is here being reported for the first time.

Table 2. Constituents of volatile concentrate of fruits pulp of *Alibertia edulis*.

Constituents	RI _C	RI _L	LPF-V (%)
Hexanal	799	801 ^a	2.1
Furfural	828	828^a	5.4
(3Z)-Hexenal	849	850 ^a	2.2
Heptanal	901	901 ^a	0.8
(2E)-Heptenal	952	947 ^a	0.4
<i>n</i> -Heptanol	958	959 ^a	1.4
Octen-3-ol	975	974 ^a	1.9
Benzene acetaldehyde	1041	1036^a	5.5
γ-Terpinene	1055	1054 ^a	0.8
Linalool	1099	1095 ^a	2.5
Naphthalene	1181	1178 ^a	0.8
Methyl salicylate	1193	1190 ^a	0.8
(2E)-Decenal	1260	1260 ^a	0.9
Safrole	1287	1285 ^a	3.6
Thymol	1291	1289 ^a	0.7
Tridecanal	1511	1509^b	5.4
Palmitic alcohol	1876	1874^a	5.5
Methyl palmitate	1925	1921 ^a	4.8
Palmitic acid	1962	1959^a	20.4
Ethyl palmitate	1993	1992 ^b	0.6
Methyl linoleate	1386	2095^a	7.1
Methyl linolenate	1395	2098^b	16.6
Phytol	1596	2106 ^b	1.6
Linoleic acid	1794	2132 ^a	2.1
Incensole	2160	2158 ^a	1.3
Total (%)			95.2

RI_C = Calculated Retention Index (Rxi-5ms column); RI_L = Literature Retention Index; Bold = Main constituents; ^a Adams 2007 [21]; ^b Mondello 2011 [22].

3.3. Antioxidant Capacity of the *A. edulis* Pulp Fruit

3.3.1. Total Phenolic Content (TPC)

The Folin-Ciocalteu assay enabled the estimation of flavonoids, anthocyanins, and other phenolic compounds in LPF-E and LPF-A extracts. The standard curve was prepared with gallic acid at 1 to 10.0 µg/mL concentrations, resulting in the linear regression equation: $y = 11.454x - 1.0986$, where $R^2 = 0.9981$ and $p = 0.0006$. The total phenolic compounds

(TPC) for the LFP-E and LFP-A extracts were 5.75 ± 0.75 mg GAE/g and 66.75 ± 3.1 mg GAE/g, respectively. Usually, fruits are classified into three categories depending on their total phenolic content: low (<1 mg GAE/g), medium (1–5 mg GAE/g), and high (>5 mg GAE/g) [36]. Therefore, LFP-E and LFP-A extracts can be considered excellent sources of phenolic compounds. Previously, the aqueous and ethyl acetate extracts of *A. edulis* leaves had been tested for their total phenolic content, whose values were significantly higher, i.e., 348 ± 2.88 mg GAE/g and 204.73 ± 6.82 mg GAE/g, respectively [8]. In addition, the fruits of *A. edulis* collected in Lavras, MG, Brazil, and extracted in a solution containing 70% methanol-water (*v/v*) have displayed a TPC value of 1.32 mg GAE/g of fresh weight [37]. The TPC value of 0.816 ± 1.16 mg GAE/g for the hydroalcoholic extract of *Alibertia sessilis* fruit has been previously reported [38].

3.3.2. Total Flavonoid Content (TFC)

The assay enabled the estimation of the flavonoid compounds in LFP-E and LFP-A extracts. The standard curve was prepared with quercetin at concentrations from 0.625 to 20.0 $\mu\text{g/mL}$, and the absorbance values were read at 760 nm. The linear regression equation was as follows: $y = 13.121x + 0.3323$ ($R^2 = 0.9971$). The total flavonoid content (TFC) for the LFP-E and LFP-A extracts were 1.14 ± 0.65 mg QE/g and 50.97 ± 1.2 mg QE/g, respectively. Previously, the aqueous and ethyl acetate extracts of *A. edulis* leaves had been tested for the total flavonoid content, whose values were 87.09 ± 6.10 mg rutin equivalents (RE)/g and 13.63 ± 0.11 mg rutin equivalents (RE)/g, respectively [8]. Moreover, the TFC value of 0.269 ± 2.45 mg QE/g for the hydroalcoholic extract of *Alibertia sessilis* fruits has been previously reported [38]. Flavonoids are the predominant class of phenolic compounds in the fruit pulp of *A. edulis*, which is confirmed by the relationship between the contents of total flavonoids and total phenolic compounds. These data are also related to the significant antioxidant activity evaluated in the reducing power tests of DPPH and ABTS reagents.

3.3.3. DPPH and TEAC/ABTS Assays

Phenolic compounds exhibit various functional properties due to their antioxidant capacity, acting as reducing agents, hydrogen donors, transition metal chelators, reactive oxygen and nitrogen species (ROS/RNS) quenchers, enzyme inhibitors involved in oxidative stress, and regulators/protectors of endogenous defense systems. Moreover, dietary phenolic compounds exert health effects, strengthening the immune system and helping prevent disorders in the individual's physical conditions caused by cancer, bacteria, and viruses [39–41].

The DPPH assay has evaluated the antioxidant activity of organic and aqueous extracts from food matrices containing hydrophilic and lipophilic compounds. It is based on the ability of the sample's antioxidants to reduce the DPPH radical via electron transfers, measuring its absorption at 517 nm. A more potent radical agent generally lowers the value to a 50% effective concentration (EC_{50}). DPPH radical scavenging activity showed for the *A. edulis* LFP-E and LFP-A extracts, the EC_{50} values of 1021.65 ± 5.9 $\mu\text{g/mL}$ and 133.60 ± 3.9 $\mu\text{g/mL}$, respectively. Based on DPPH/ EC_{50} values, the fruits have been considered in three distinct categories: significant antioxidant property (DPPH/ $\text{EC}_{50} \leq 100$ $\mu\text{g/mL}$), medium antioxidant property (100 $\mu\text{g/mL} < \text{DPPH}/\text{EC}_{50} \leq 316$ $\mu\text{g/mL}$), and weak antioxidant property (DPPH/ $\text{EC}_{50} > 316$ $\mu\text{g/mL}$) [42]. Therefore, via the DPPH assay, the *A. edulis* LFP-E and LFP-A extracts showed weak-to-medium antioxidant properties, respectively. The *A. edulis* aqueous extract produced significant DPPH scavenging activity in previous work, with an estimated value of $69.91 \pm 0.37\%$ at 150 $\mu\text{g/mL}$. However, its antioxidant activity was less than that presented by the rutin standard ($74.94 \pm 0.12\%$, at 10 $\mu\text{g/mL}$) [8,37].

TEAC/ABTS is an antioxidant assay widely used to determine the antioxidant activity of extracts of food matrices, especially indicated for evaluating hydrophilic compounds. Based on the sample antioxidants' ability to reduce the ABTS radical by transferring an electron and/or hydrogen atom, it measured the absorption decreases at 734 nm [25]. The

LFP-E and LFP-A extracts of “Puruí” pulp fruit showed significant antioxidant activities by the discoloring action of the cation radical ABTS, whose results as Trolox equivalent antioxidant capacity (TEAC) values were $28.36 \pm 3.7 \mu\text{M TE/g}$ e $142.26 \pm 2.2 \mu\text{M TE/g}$, respectively.

3.3.4. Correlation between Total Phenolic Compounds and Antioxidant Activity of *A. edulis* Fruit Pulp

The values of total phenolic compounds and the antioxidant activities (DPPH and TEAC/ABTS) of LFP-E and LFP-A extracts of pulp fruit of *A. edulis* were correlated via linear regression. The results show that DPPH ($r = 0.60$; $R^2 = 0.36$) and TEAC/ABTS ($r = 0.73$, $R^2 = 0.55$) assays correlate with the soluble total phenolic compounds (TPC) and the other phenolic compounds identified by HPLC-ESI-MS/MS (See Table 1 and Figure 3).

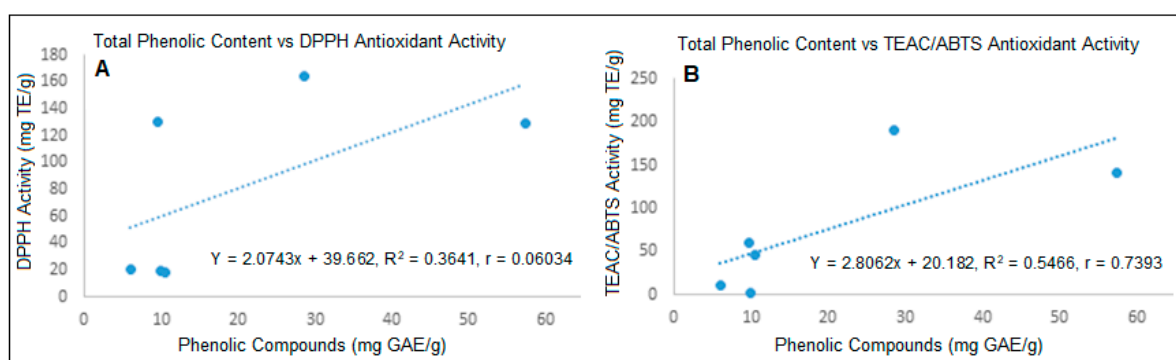


Figure 3. Correlation between the total phenolic compounds and the antioxidant activities DPPH (A) and TEAC/ABTS (B) of the *A. edulis* fruit pulp.

4. Conclusions

The present study revealed that the extracts of pulp fruit from *A. edulis* showed significant phytochemical constituents belonging to phenolic acids, flavonoids, aporphine alkaloids, and fatty acid esters classes. Many of the identified compounds are correlated with the observed antioxidant activity, suggesting that the extracts could be used in the future for the potential application of functional foods and oxidative stress control.

Author Contributions: Conceptualization, J.G.S.M.; methodology, J.G.S.M., C.Q.d.R. and J.K.R.d.S.; formal analysis and investigation, N.C.C.C., O.S.M., C.Q.d.R., J.K.R.d.S. and J.G.S.M.; writing—original draft preparation, N.C.C.C. and J.G.S.M.; writing—review and editing, C.Q.d.R. and J.K.R.d.S.; project administration, N.C.C.C., C.Q.d.R. and J.G.S.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Upon reasonable request, data from the corresponding author are made available.

Acknowledgments: This research was supported by CNPQ and CAPES, scientific research agencies of the Brazilian Government. N.C.C.C. is grateful to CAPES for the doctoral scholarship.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Bremer, B.; Eriksson, T. Time tree of Rubiaceae: Phylogeny and dating the families, subfamilies, and tribes. *Int. J. Plant Sci.* **2009**, *170*, 766–793. [[CrossRef](#)]
- Delprete, P.G.; Jardim, J.G. Systematics, taxonomy and floristics of Brazilian Rubiaceae: An overview about the current status and future challenges. *Rodriguésia* **2012**, *63*, 101–128. [[CrossRef](#)]

3. Persson, C. Phylogeny of the Neotropical *Alibertia* group (Rubiaceae), with emphasis on the genus of *Alibertia*, inferred from ITS and 5S ribosomal DNA sequences. *Am. J. Bot.* **2000**, *87*, 1018–1028. [CrossRef] [PubMed]
4. The Plant List. 2021. Available online: theplantlist.org/1.1/browse/A/Rubiaceae/Alibertia (accessed on 10 July 2023).
5. Cavalcante, P.B. *Frutas Comestíveis da Amazônia. Coleção Adolpho Ducke*; Museu Paraense Emílio Goeldi: Belém, PA, Brazil, 1996.
6. Rieder, A. Plants used for diabetes in the transition zone of Platinum and Amazon Hydrographic Basins, Southwest portion of Mato Grosso, Brazil. *Planta Medica* **2013**, *79*, PF8. [CrossRef]
7. Menegati, S.E.L.T.; de Lima, F.F.; Traesel, G.K.; Souza, R.I.C.; dos Santos, A.C.; Aquino, D.F.S.; de Oliveira, V.S.; Vieira, S.C.H.; Cardoso, C.A.L.; Vieira, M.C.; et al. Acute and subacute toxicity of the aqueous extract of *Alibertia edulis* (Rich.) A. Rich. ex DC. in rats. *J. Ethnopharmacol.* **2016**, *194*, 1096–1102. [CrossRef] [PubMed]
8. Aquino, D.F.S.; Tirloni, C.A.S.; Menegati, S.E.L.T.; Cardoso, C.A.L.; Vieira, S.C.H.; Vieira, M.C.; Simonet, A.M.; Macías, F.A.; Gasparotto Junior, A. *Alibertia edulis* (L.C. Rich.) A.C. Rich—A potent diuretic arising from Brazilian indigenous species. *J. Ethnopharmacol.* **2017**, *196*, 193–200. [CrossRef]
9. Aquino, D.F.S.; Monteiro, T.A.; Cardoso, C.A.L.; Vieira, S.C.H.; Vieira, M.C.; Souza, K.P.; Amaya-Farfan, J.; Carvalho, G.C.B.C.; Moura, C.S.; Morato, P.N. Investigation of the antioxidant and hypoglycemic properties of *Alibertia edulis* (L.C. Rich.) A.C. Rich. leaves. *J. Ethnopharmacol.* **2020**, *253*, 112648. [CrossRef]
10. Tolouei, S.E.L.; Traesel, G.K.; de Lima, F.F.; de Araújo, F.H.S.; Lescano, C.H.; Cardoso, C.A.L.; Oesterreich, S.A.; Vieira, M.C. Cytotoxic, genotoxic and mutagenic evaluation of *Alibertia edulis* (rich.) a. Rich. ex DC: An indigenous species from Brazil. *Drug Chem. Toxicol.* **2018**, *43*, 200–207. [CrossRef]
11. Lescano, C.H.; de Lima, F.F.; Cardoso, C.A.L.; Vieira, S.C.H.; Monica, F.Z.; de Oliveira, I.P. Rutin present in *Alibertia edulis* extract acts on human platelet aggregation through inhibition of cyclooxygenase/thromboxane. *Food Funct.* **2021**, *12*, 802–814. [CrossRef]
12. Brochini, C.B.; Martins, D.; Roque, N.F.; Bolzani, V.S. An oleanane acid from *Alibertia edulis*. *Phytochemistry* **1994**, *36*, 1293–1295. [CrossRef]
13. da Silva, V.C.; Giannini, M.J.S.M.; Carbone, V.; Piacente, S.; Pizza, C.; Bolzani, V.S.; Lopes, M.N. New antifungal terpenoid glycosides from *Alibertia edulis* (Rubiaceae). *Helv. Chim. Acta* **2008**, *91*, 1355–1362. [CrossRef]
14. Bolzani, V.S.; Trevisan, L.M.V.; Young, M.C.M. Caffeic acid esters and triterpenes of *Alibertia macrophylla*. *Phytochemistry* **1991**, *30*, 2089–2091. [CrossRef]
15. Young, M.C.M.; Braga, M.R.; Dietrich, S.M.C.; Gottlieb, H.E.; Trevisan, L.M.V.; Bolzani, V.S. Fungitoxic non-glycosidic iridoids from *Alibertia macrophylla*. *Phytochemistry* **1992**, *31*, 3433–3435. [CrossRef]
16. da Silva, V.C.; Faria, A.O.; Bolzani, V.S.; Lopes, M.N. A new ent-kaurane diterpene from stems of *Alibertia macrophylla*. *Helv. Chim. Acta* **2007**, *90*, 1781–1785. Available online: <http://hdl.handle.net/11449/69936> (accessed on 11 January 2022). [CrossRef]
17. Militão, G.C.G.; Pessoa, C.O.; Costa-Lotufo, L.V.; de Moraes, M.E.A.; de Moraes, M.O.; Luciano, J.H.S.; Lima, M.A.S.; Silveira, E.R. Cytotoxicity of flavonoids isolated from *Alibertia myrciifolia*. *Pharm. Biol.* **2005**, *43*, 480–484. [CrossRef]
18. Luciano, J.H.S.; Lima, M.A.S.; Silveira, E.R.; Vasconcelos, I.M.; Fernandes, G.S.; de Souza, E.B. Antifungal iridoids, triterpenes and phenol compounds from *Alibertia myrciifolia* Sprunge ex. Schum. *Quim. Nova* **2010**, *33*, 292–294. [CrossRef]
19. Olea, R.S.G.; Roque, N.F.; Bolzani, V.S. Acylated flavonol glycosides and terpenoids from the leaves of *Alibertia sessilis*. *J. Braz. Chem. Soc.* **1997**, *8*, 257–259. [CrossRef]
20. da Silva, V.C.; Silva, G.H.; Bolzani, V.S.; Lopes, M.N. Isolation of lignans glycosides from *Alibertia sessilis* (Vell.) K. Schum. (Rubiaceae) by preparative high-performance liquid chromatography. *Eclat. Quim.* **2006**, *31*, 55–58. [CrossRef]
21. Adams, P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*, 4th ed.; Allured Publishing: Carol Stream, IL, USA, 2007.
22. Mondello, L. *FFNSC 2: Flavors and Fragrances of Natural and Synthetic Compounds, Mass Spectral Database*; John Wiley & Sons Inc.: Hoboken, NJ, USA, 2011.
23. Van den Dool, H.; Kratz, P. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J. Chromatogr. A* **1963**, *11*, 463–471. [CrossRef]
24. Singleton, V.L.; Rossi, J.A. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158. [CrossRef]
25. da Silva, J.K.R.; Andrade, E.H.A.; Kato, M.J.; Carreira, L.M.M.; Guimarães, E.F.; Maia, J.G.S. Antioxidant capacity and larvicidal and antifungal activities of essential oils and extracts from *Piper krukoffii*. *Nat. Prod. Commun.* **2011**, *6*, 1361–1366. [PubMed]
26. Chang, C.-C.; Yang, M.-H.; Wen, H.-M.; Chern, J.-C. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal.* **2002**, *10*, 178–182. [CrossRef]
27. Rufino, M.S.M.; Alves, R.E.; de Brito, E.S.; de Moraes, S.M.; Sampaio, C.G.; Pérez-Giménez, J.; Saura-Calixto, F.D. *Metodologia Científica: Determinação da Atividade Antioxidante Total em Frutas pela Captura do Radical Livre ABTS*; Comunicado Técnico 128; Embrapa Agroindustrial Tropical: Fortaleza, Brazil, 2007.
28. Saldanha, L.L.; Vilegas, W.; Dokkedal, A.L. Characterization of flavonoids and phenolic acids in *Myrcia bella* Cambess. using FIA-ESI-IT-MSn and HPLC-PAD-ESI-IT-MS combined with NMR. *Molecules* **2013**, *18*, 8402–8416. [CrossRef] [PubMed]
29. Masike, K.; Mhlong, M.I.; Mudau, S.P.; Nobela, O.; Ncube, E.N.; Tugizimana, S.; George, M.J.; Madala, N.E. Highlighting mass spectrometric fragmentation differences and similarities between hydroxycinnamoyl-quinic acids and hydroxycinnamoyl-isocitric acids. *Chem. Cent. J.* **2017**, *11*, 29. [CrossRef]

30. Ding, Y.; Hou, J.-W.; Zhang, Y.; Zhang, L.-Y.; Zhang, T.; Chen, Y.; Cai, Z.-Z.; Yang, L. Metabolism of genipin in rat and identification of metabolites by using ultraperformance liquid chromatography/quadrupole time-of-flight tandem mass spectrometry. *J. Evid. Based Complement. Alternat. Med.* **2013**, *2013*, 957030. [[CrossRef](#)]
31. da Silva, F.M.A.; Koolen, H.H.F.; de Almeida, R.A.; de Souza, A.D.L.; Pinheiro, M.L.B. Desrepliação de alcalóides aporfínicos e oxoaporfínicos de *Unonopsis guatteriodes* por ESI-IT-MS. *Quim. Nova* **2012**, *35*, 944–947. [[CrossRef](#)]
32. Troalen, L.G.; Phillips, A.S.; Pegg, D.A.; Barran, P.E.; Hulme, A.N. Historical textile dyeing with *Genista tinctoria* L.: A comprehensive study by UPLC-MS/MS analysis. *Anal. Meth.* **2014**, *6*, 8915–8923. [[CrossRef](#)]
33. Náthia-Neves, G.; Nogueira, G.C.; Vardanega, R.; Meireles, M.A.A. Identification and quantification of genipin and geniposide from *Genipa americana* L. by HPLC-DAD using a fused-core column. *Food Sci. Technol.* **2018**, *38* (Suppl. 1), 116–122. [[CrossRef](#)]
34. Li, J.; Wang, Y.-H.; Smillie, T.J.; Khan, I.A. Identification of phenolic compounds from *Scutellaria lateriflora* by liquid chromatography with ultraviolet photodiode array and electrospray ionization tandem mass spectrometry. *J. Pharm. Biomed. Anal.* **2012**, *63*, 120–127. [[CrossRef](#)]
35. Zhao, Y.; Li, X.; Zeng, X.; Huang, S.; Hou, S.; Lai, X. Characterization of phenolic constituents in *Lithocarpus polystachyus*. *Anal. Meth.* **2014**, *6*, 1359–1363. [[CrossRef](#)]
36. Vasco, C.; Ruales, J.; Kamal-Eldin, A. Total phenolic compounds and antioxidant capacities of major fruits from Ecuador. *Food Chem.* **2008**, *111*, 816–823. [[CrossRef](#)]
37. Guimarães, A.C.G.; de Souza Gomes, M.; Zacaroni Lima, L.; Sales, P.F.; da Cunha, M.C.; Rodrigues, L.J.; de Barros, H.E.A.; Pires, C.R.F.; dos Santos, V.F.; Natarelli, C.V.L.; et al. Application of chemometric techniques in the evaluation of bioactive compounds and antioxidant activity of fruit from Brazilian Cerrado. *J. Food Meas. Charact.* **2023**, *17*, 2095–2106. [[CrossRef](#)]
38. Becker, F.S. Desenvolvimento, Caracterização e Atividade Antioxidante de Marmelada-de-Cachorro (*Alibertia sessilis* Schum.). Ph.D. Thesis, Universidade Federal de Lavras, Lavras, MG, Brazil, 2015.
39. Cani, P.D.; Everard, A. Talking microbes: When gut bacteria interact with diet and host organs. *Mol. Nutr. Food Res.* **2016**, *60*, 58–66. [[CrossRef](#)] [[PubMed](#)]
40. Smith, R.E.; Tran, K.; Smith, C.C.; McDonald, M.; Shejwalkar, P.; Hara, K. The Role of the Nrf2/ARE antioxidant system in preventing cardiovascular diseases. *Diseases* **2016**, *4*, 34. [[CrossRef](#)]
41. Takiishi, T.; Fenero, C.I.M.; Câmara, N.O.S. Intestinal barrier and gut microbiota: Shaping our immune responses throughout life. *Tissue Barriers* **2017**, *2*, e1373208. [[CrossRef](#)]
42. Gregoris, E.; Lima, G.P.P.; Fabris, S.; Bertelle, M.; Sicari, M.; Stevanato, R. Antioxidant properties of Brazilian tropical fruits by correlation between different assays. *BioMed Res. Int.* **2013**, *2013*, 132759. [[CrossRef](#)]

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