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Antioxidant activity of *Annona crassiflora*: Characterization of major components by electrospray ionization mass spectrometry

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Abstract

The polar components of *Annona crassiflora* pulp, peel and seeds ethanolic extracts were investigated by direct infusion electrospray ionization mass spectrometry (ESI-MS) both in the negative ion mode. Characteristic ESI mass spectra with many diagnostic ions were obtained for the extracts, serving for fast and reliable information. The technique provided information of component structures revealing the presence of important bioactive components widely reported as potent antioxidants such as ascorbic acid, caffeic acid, quinic acid, ferulic acid, xanthoxylin, rutin, caffeoyltartaric acid, caffeoyl glucose and $[\text{quercetin+hexose+pentose}-\text{H}]^{-1}$ This is the first report on the composition by ESI-MS of araticum peel and seed ethanolic extracts demonstrating excellent antioxidant activity.

Keywords: Araticum; Electrospray ionization mass spectrometry; Total phenols; Antioxidant activity; DPPH

1. Introduction

The Cerrado is the second largest biome in South America, after the Amazon rainforest. It occupies almost 25% of Brazil (Proença, Oliveira, & Silva, 2000). Its very rich flora, which is just beginning to be studied, is estimated to be comprised of about 1000 species of trees, 3000 species of herbs and shrubs, and about 500 of climbers (Mendonça et al., 1998). In the last 30 years, progressive mechanization with improved techniques for clearing and fertilizing the land have contributed to the accelerated destruction of the natural vegetation, and it is estimated that 40% of the cerrado biome has already been deforested (Ratter, Ribeiro, & Bridgewater, 1997). The *Anonnaceas* family has a great variety of exotics fruits which are apparently rustic and have typical form such as conde fruit (*Annona squamosa*), graviola fruit (*Annona muricata*) and araticum of cerrado or marolo (*Annona crassiflora*) (Silva & Tassara, 2001). Many members of annonaceae are used in folk medicine for antiparasitic or antitumoral treatment of intestinal diseases. In recent years, many interesting compounds termed tetrahydrofuranic acetogenins (or annonaceous acetogenins) have been reported and have gained organic chemist's and biochemist's attention because of their novel structure and wide-range of bioactivities (Araya, 2004). *Annona crassiflora* is a tree that bears a typical fruit known as araticum of cerrado or cerradão. Its fruits are highly consumed "in natura" by native people or used to prepare juice, ice-cream or jelly. The fruits are sold in regional markets and have no commercial value in Brazil. The oil from the seeds are used against scalp infections and in the folk medicine, the leaves and seeds infusion are used against diarrhea and as antitumoral (Almeida, Proença, Sano, & Ribeiro, 1994; Lorenzi,

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1988). There are very few studies examining the relationship between the biological activities and the ethnobotanical uses by traditional herbal medicine in order to validate them. Recently, the antioxidant activity of araticum (*Annona crassiflora*) was evaluated by using in vitro models and the results indicated that ethanolic extracts of araticum seeds and peel have compounds possessing excellent antioxidant activity so high as others know antioxidant plant products. There was a noticeable correlation between total polyphenols and free-radical DPPH scavenging activity ($R^2 = 0.821$) (Roesler, Malta, Carrasco, & Pastores, 2006). The role of antioxidants in preventing oxygen radical and hydrogen peroxide induced cytotoxicity and tissue damage in various human diseases is becoming increasingly recognized. The importance of the antioxidants constituents of plant materials in the maintenance of health and protection against heart diseases and cancer is also raising interest among scientists, food manufacturers and consumers as the trend for the future is toward functional food with specific health effects (Loliger, 1991). Typical compounds that possess antioxidant activity include phenols, phenolic acids and their derivatives, flavonoids, tocols, phospholipids, amino acids and peptides, phytic acid, ascorbic acid, pigments, and sterols. Phenolic antioxidant are primary antioxidants which act as free-radical terminators (Xing & White, 1996). Electrospray (ESI) is a soft and wide-ranging ionization technique that has revolutionized the way the molecules are ionized and transferred to mass spectrometers for mass and property measurements as well as structural characterization (Fenn, Mann, Meng, Wong, & Whitehouse, 1989). ESI has therefore greatly expanded the applicability of mass spectrometry to a variety of new classes of molecules with thermal instability, high polarity and mass (Catharino et al., 2005). ESI-MS proves very fast and versatile employing little sample preparation to yield immediate compositional information of the most polar ESI-ionisable compounds. These unique features of direct infusion ESI-MS have recently been applied for fingerprinting of complex mixtures such as bee propolis (Sawaya et al., 2004), beer (Araujo et al., 2005), wine (Cooper & Marshall, 2001; Catharino et al., 2006), whisky (Møller, Catharino, & Eberlin, 2005) and vegetable oil (Catharino et al., 2005; Wu, Rodgers, & Marshall, 2004). Therefore the objective of the present work is to explore the ability of the fast and versatile ESI-MS technique with direct infusion to characterize the ethanolic extracts of araticum which demonstrated high antioxidant activity.

2. Materials and methods

2.1. Reagents and standards

Solvents, reagents and standards were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA).

2.2. Plant material

Araticum fruits (*Annona crassiflora*) were obtained from Erlow Farm, Km 07 Br 070 Goiânia Brazil, in the center west of Brazil. Fruit harvests were conduct twice and special care was taken to avoid damaged fruits. Fruits were transported to the University of Campinas (UNICAMP) and stored at 5 °C until used within 2 months.

2.3. Ethanolic extraction

Undamaged fruits were selected and peel, pulp and seeds were manually separated. About 100 g of each part of the fruit (pulp, peel and seeds) were cut in small parts, mixed and extracted twice with 300 mL of aqueous ethanol (5:95, v/v, water: ethanol) using a household mixer for 20 min. The extracts were filtered through cotton membrane and the residue was re-extracted under the same conditions. The residues were kept for further evaluations. The resultant material was subjected to vacuum rotary evaporation at 40 °C to remove ethanol. The concentrated ethanolic extracts were lyophilized and stored at −18 °C in amber glass bottles until used.

2.4. Determination of total phenols assay

Estimation of the global phenol content in the extracts was performed by the Folin–Ciocalteu method that involves reduction of the reagent by phenolic compounds, with concomitant formation for a blue complex, its intensity at 760 nm increases linearly with the concentration of phenols in the reaction medium as described by (Swain & Hillis, 1959). Ethanolic extracts were dissolved in methanol to obtain a concentration of 0.5 mg/mL. Samples (0.5 mL) were passed through a 0.45 μm membrane filter and mixed with 2.5 mL of 10 fold diluted Folin–Ciocalteu reagent and 2.0 mL of 7.5% sodium carbonate solution. After the mixture had been allowed to stand for 5 min at 50 °C, the absorbance was measured at 760 nm. In this study, gallic acid was used as spectrophotometric standards and the total phenolic content of the fruit extracts were expressed as gallic acid equivalents (GAE/100 g). Estimation of the phenolic compounds was carried out in triplicate and mean values reported.

2.5. Electrospray ionization mass spectrometry

For fingerprinting ESI-MS analysis a hybrid high-resolution and high-accuracy (5 ppm) Micromass Q-TOF mass spectrometer (Micromass, Manchester, UK) was used. The general conditions were: source temperature of 100 °C, capillary voltage of 3.0 kV and cone voltage of 40 V. For measurements in the negative ion mode, ESI(−)-MS, 10.0 μL of concentrated NH_4OH were added to the sample mixture having a total volume of 1000 μL yielding 0.1% as final concentration. For measurements in the positive ion mode ESI(+)-MS, 10.0 μL of concentrated formic acid

were added giving a final concentration of 0.1%. ESI-MS was preformed by direct infusion with a flow rate of 10 μL min^{-1} using a syringe pump (Harvard Apparatus). Mass spectra were acquired and accumulated over 60 s and spectra were scanned in the range between 50 and 1000 *m/z*. Structural analysis of single ions in the mass spectra from pulp, peel and seeds extracts was performed by ESI-MS/MS. The ion with the *m/z* of interest was selected and submitted to 15–45 eV collisions with argon in the collision quadrupole. The collision gas pressure was optimized to produce extensive fragmentation of the ion under investigation.

2.6. Determination of radical scavenging activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) has been widely used to evaluate the free-radical scavenging of natural antioxidants (Bondet, Brand-Williams, & Berset, 1997; Brand-Williams, Cuvelier, & Berset, 1995; Sanchez-Moreno, Laurrauri, & Saura-Calixto, 1998). The scavenger activity of main compounds of araticum extracts (quercetin, caffeic acid, ascorbic acid, ferrulic acid and rutin) was tested against the DPPH radical as well as other compounds for comparative porpoise (gallic acid and alfa-tocopherol). Ethanolic solutions in different concentrations were prepared by adding 1000 μL of DPPH (0.004% w/v) and the final volume was brought to 1200 μL with ethanol. Final concentrations of test materials in the cuvettes were in the range of 1.0–100 μM. Each tube was incubating for 30 min at room temperature in the dark. The control was prepared as above without any extract, and ethanol was used for the baseline correction. The DPPH' solution was freshly prepared daily, stored in a flask covered with aluminum foil, and kept in the dark at 4 °C between measurements. The percent decrease in absorbance was recorded for each concentration and percent quenching of DPPH' was calculated on the basis of the observed decrease in absorbance of the radical. Changes in the absorbance of the samples were measured at 517 nm. Radical scavenging was expressed as the inhibition percentage and was calculated using the following formula (Yen & Duh, 1994).

$$\%\ \text{Inhibition} = ((A_{\text{DPPH}} - A_{\text{Extr.}})/A_{\text{DPPH}}) \times 100$$

where A_{DPPH} is the absorbance value of the DPPH' blank sample and $A_{\text{Extr.}}$ is the absorbance value of the test solution. $A_{\text{Extr.}}$ was evaluated as the difference between the absorbance value of the test solution and the absorbance value of its blank. The IC_{50} values are reported as final concentration of extract in the cuvettes defined as μg/mL of dried extracts required to decrease the initial DPPH concentration by 50%.

3. Results and discussion

Recently, the total phenol and the antioxidant activity of ethanolic extracts of araticum pulp, peel and seeds had been reported in various in vitro models (Roesler et al., 2006). The results showed that araticum extracts contain extremely high contents of total phenols. The highest amount was 111.42 g kg^{-1} dry matter for peel ethanolic extract and 136.98 g kg^{-1} dry matter for the seed ethanolic extract. Overall, the lowest concentration was found in the ethanolic pulp extract at 31.08 g kg^{-1} dry matter. Considering the high total phenol content of araticum extracts compared to recent results of fruits extracts published in the literatures such as pomegranate (Singh, Chidambar Murthy, & Jayaprakasha, 2002), apple, plum and pear (Imeh & Khokhar, 2002), guava (Jimenez, Rincon, Pulido, & Fulgencio, 2001) and berries (Benvenuti, Pellati, Melegari, & Bertelli, 2004) – (Table 1), the antioxidant activity

Table 1
Total phenol contents of fruit extracts recently published

	Total phenols	Reference
Rubus fruticosus L. (Blackberry)	289.3 ± 55.8[a]	Benvenuti et al. (2004)
Rubus idaeus L. (Raspberry)	177.5 ± 52.2 [a]	Benvenuti et al. (2004)
*Ribes nigrum*L. (Black currant)	639.8 ± 112.9 [a]	Benvenuti et al. (2004)
Ribus rubrum L. (Red currant)	417.9 ± 72.6 [a]	Benvenuti et al. (2004)
Aronia melanocarpa Elliott (Black chokeberry)	690.2 ± 8.8 [a]	Benvenuti et al. (2004)
Psidium guajava peel	58.7 ± 4.0[b]	Jimenez et al. (2001)
Psidium guajava pulp	26.3 ± 0.8 [b]	Jimenez et al. (2001)
Malus pumila (Apple Red Delicious)	2866 ± 102[c]	Imeh and Khokhar (2002)
Pyrus communis (Pear Forelle)	1194 ± 83 [c]	Imeh and Khokhar (2002)
Prunus domestica (Plum Royal Garnet)	2643 ± 112 [c]	Imeh and Khokhar (2002)
Punica granatum peel (Pomegranate – ethanolic extraction)	18.0[d]	Singh et al. (2002)
Punica granatum seed (Pomegranate – ethanolic extraction)	2.1 [d]	Singh et al. (2002)
Annona crassiflora peel (Araticum – ethanolic extraction)	111.42 ± 8.57 [b]	Roesler et al. (2006)
Annona crassiflora peel (Araticum – ethanolic extraction)	136.98 ± 7.56 [b]	Roesler et al. (2006)
Annona crassiflora peel (Araticum – ethanolic extraction)	31.08 ± 1.23 [b]	Roesler et al. (2006)

[a] Total polyphenols expressed as mg of gallic acid per 100 g fresh weight (FW).
[b] Total polyphenols expressed as g of gallic acid per kg dry matter (dm).
[c] Total polyphenols expressed as mg of catechin per 100 g dry matter (dm).
[d] Total polyphenols expressed as % w/w of tannic acid – dry matter (dm).

Table 2
Antioxidant activity of Araticum ethanolic extracts and respective compounds identified by ESI(−)-MS/MS

	TP ($g\,kg^{-1}$)	IC 50 ($\mu g\,mL^{-1}$)		Mainly compounds (ESI-MS)
		DPPH	Lipid peroxidation	
A. crassiflora pulp	31.08	148.82	8.62	Sugar and organic acids ions
A. crassiflora peel	111.42	48.82	4.44	Ascorbic acid, caffeic acid, quinic acid, ferulic acid, xanthoxylin and rutin
A. crassiflora seed	136.98	31.14	1.72	Ascorbic acid, caffeic acid, quinic acid, ferulic acid, xanthoxylin, caffeoyltartaric acid, caffeoyl glucose, quercetin and rutin

of araticum extracts was determined by using in vitro methods. Free-radical scavenging potentials of araticum peel and seed extracts at different concentrations were tested by the DPPH method and seed, peel and pulp presented IC_{50} of 31.14 $\mu g\,mL^{-1}$, 48.82 $\mu g\,mL^{-1}$ and 148.82 $\mu g\,mL^{-1}$, respectively. In addition, the inhibition of lipid peroxidation by using rat liver microsomes as an oxidative system was also evaluated. Fifty percent inhibition of lipid peroxidation of microsomes to thiobarbituric acid reactive species (TBARS) by hydroxyl radicals, generated by $FeCl_3$ system, requires 1.72 $\mu g\,mL^{-1}$ of seed ethanolic extract, 4.44 $\mu g\,mL^{-1}$ of peel ethanolic extract and 8.62 $\mu g\,mL^{-1}$ of pulp etanolic extract. The results are summarized in Table 2. Reports on antioxidant activity of other plant extracts such as *Emblica officinalis* (Jose & Kuttan, 1995), *Phoenix dactylifera* (Vayalil, 2002) and *Punica granatum* (Singh et al., 2002) require a IC_{50} of 3.4 $mg\,mL^{-1}$ and 2.2 $mg\,mL^{-1}$ and around 100 ppm, respectively of extracts solution. Expressing the antioxidant activity of the extract solution instead of the final cuvettes concentration as mentioning before, the IC_{50} for seed, peel and pulp was 0.043 $mg\,mL^{-1}$ (43 ppm), 0.12 $mg\,mL^{-1}$ or 115 ppm and 0.243 $mg\,mL^{-1}$ or 243 ppm (Roesler et al., 2006). The ESI-MS Fingerprints technique with direct infusion was used to characterize the presence of compounds with potent free-radical scavenging activity in araticum fruit. The extracts were analyzed by direct insertion both in the negative and positive ion modes. However, ESI(+)-MS fingerprints produce by far the most characteristic mass spectra; hence only the ESI(−)-MS data will be presented and discussed. This method in the negative ion mode provides a sensitive and selective method for the identification of polar organic compounds with acidic sites, such as the phenolic, organic acids and sugars compounds found in araticum. Deprotonated forms of the compounds of interest were then selected and dissociated and their ESI-MS/MS were compared to those of standards. The ESI-MS fingerprints of the samples of araticum extracts (Fig. 1) show

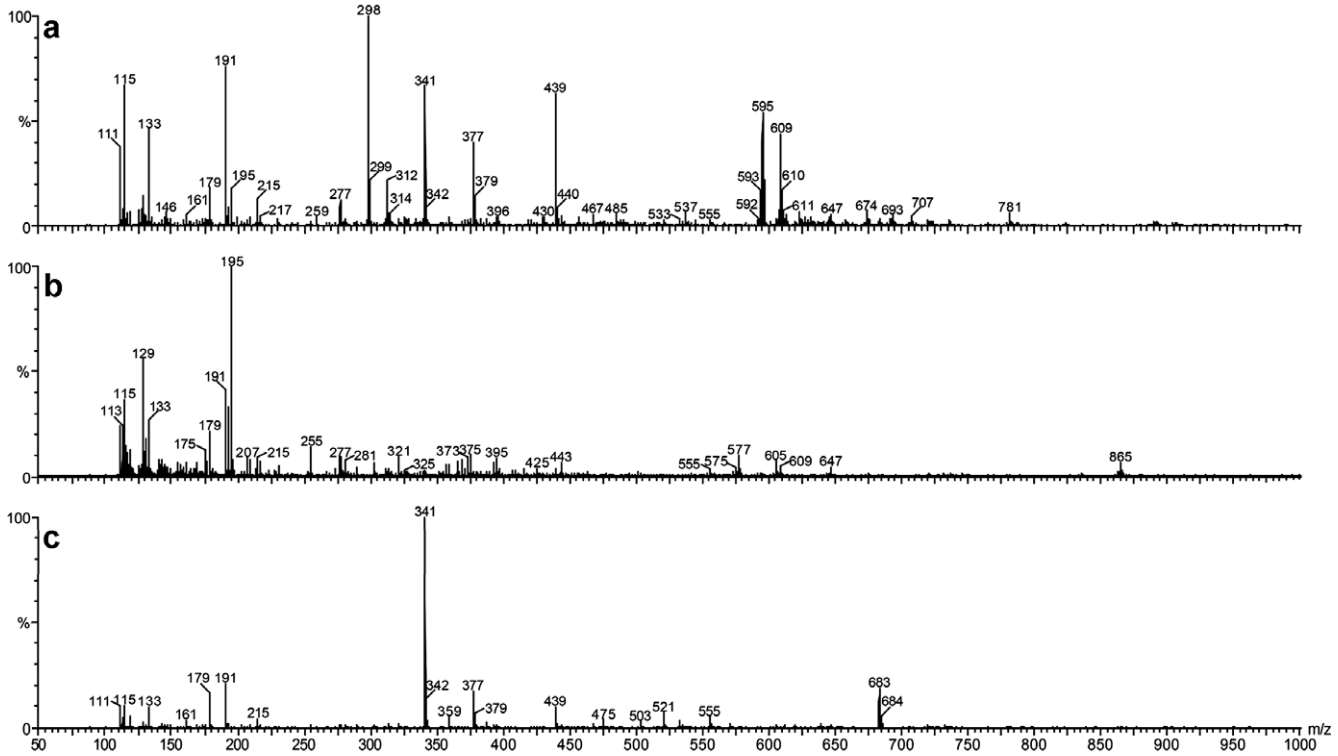

Fig. 1. ESI-MS fingerprints of araticum: (a) seed; (b) peel and (c) pulp.

similarities and some important differences. Fig. 1c shows an ESI(−)-MS typical for pulp extracts. Only sugars and organic acids anions are observed as [malic acid-H_2O−H]$^-$ of *m*/*z* 115, [malic acid−H]$^-$ of *m*/*z* 133, [hexose−H_2O−H]$^-$ of *m*/*z* 161, [hexose−H]$^-$ of *m*/*z* 179, [2 hexose−H_2O−H]$^-$ of *m*/*z* 341, [3 hexose−2H_2O−H]$^-$ of *m*/*z* 503, [3 hexose−H_2O−H]$^-$ of *m*/*z* 521 and [4 hexose−2H_2O−H]$^-$ of *m*/*z* 683. Therefore, the small content of total phenols (31.08 g kg^{-1}), lack of detection of bioactives and the predominance of sugars and acids in the pulp extract possibly explains its non-antioxidant activity results by the in vitro models DPPH and inhibition of lipid peroxidation. In the fingerprint of the peel extracts (Fig. 1b), the following components were identified in their deprotonated forms: anhydric malic acid (*m*/*z* 115), malic acid (*m*/*z* 133), ascorbic acid (*m*/*z* 175), caffeic acid (*m*/*z* 179), quinic acid (*m*/*z* 191), ferulic acid (*m*/*z* 193), xanthoxylin (*m*/*z* 195) and rutin (*m*/*z* 609). The ESI-MS fingerprints of the seed extracts (Fig. 1a) identify a much greater variety of major components wich include: anydric malic acid (*m*/*z* 115), malic acid (*m*/*z* 133), ascorbic acid (*m*/*z* 175), caffeic acid (*m*/*z* 179), quinic acid (*m*/*z* 191), ferulic acid (*m*/*z* 193), xanthoxylin (*m*/*z* 195), caffeoyltartaric acid (*m*/*z* 312), caffeoyl glucose (*m*/*z* 341), [quercetin+hexose+pentose−H]$^-$ of *m*/*z* 595 and rutin (*m*/*z* 609). Besides these common components, other ions reveal the presence of unique components for the seed extracts, that is caffeoyltartaric acid (*m*/*z* 312), caffeoyl glucose (*m*/*z* 341) and [quercetin+hexose+pentose] of *m*/*z* 595. The richer composition of the seed extract particularly the presence of phenolic components possibly explain its higher antioxidant activity as compared to the peel and pulp extracts of araticum. The summary of the main compounds found in each part of the araticum fruit is shown in Table 3. In addition, an association between the antioxidant activity of araticum and its compounds is presented in Table 2. The investigation by direct infusion electrospray ionization mass spectrometry (ESI-MS) provided important information of biactive components present in the araticum extracts that are widely reported as potent antioxidants probably explaining the antioxidant activity of the extracts (Gülçin, 2006; Kim et al., 2006; Kweon, Hwang, & Sung, 2001; Roche, Dufour, Mora, & Dangles, 2005). The major compounds found were malic acid, ascorbic acid, caffeic acid, quinic acid, ferulic acid, xanthoxylin, caffeoyltartaric acid, caffeoyl glucose, [quercetin+hexose+pentose−H]- and rutin. The scavenger activity against DPPH radical (IC_{50}) of the main araticum compounds is reported in Table 4. In addition, Table 4 also displays recently published data about some other compounds and its derivatives presented in the araticum extracts. On the whole, araticum major compounds were very effective scavenger and the best results were obtained by caffeic acid IC50 = 1.9 mg mL^{-1}, quercetina IC50 = 3.1 mg mL^{-1} and rutin IC50 = 7.5 mg mL^{-1} respectively. It is important to point out that results for scavenger abilities against DPPH are in good agreement with previous data reported for these compounds (Baratto et al., 2003; Kim et al., 2006; Saleem, Kim, Jin, & Lee, 2004 & Kweon et al., 2001). Saleem et al., 2004 reported excellent results for quercetin IC50 = 4.75 mg mL^{-1} and dimethyl ester of caffeoyltartaric acid IC50 = 9.67 mg mL^{-1}. According to (Kweon et al., 2001), great results were given by epigallocatechin IC50 = 3.7 μM, 3-*O*-caffeoyl-1-methylquinic acid IC50 = 6.9 μM, 5-*O*-caffeoyl-4-methylquinic acid IC50 = 8.8 μM, chlorogenic acid IC50 = 12.3 μM and caffeic acid 13.7 μM. Concerning the results found by (Kim et al., 2006), the lowest IC50 was found for caffeic acid IC50 = 3.2 mg mL^{-1}, quinic acid derivatives (methyl 3,5-di-*O*-caffeoyl-4-*O*-(3-hydroxy-

Table 3
Compounds identified in ethanolic extracts of araticum using ESI(−)-MS/MS

ESI-MS ions (*m*/*z*)					
Compound	*A. crassiflora*			Deprotonated ions [M−H]$^-$ *m*/*z*	MS/MS ions *m*/*z*
	Seed	Peel	Pulp		
[Malic acid−H_2O−H]$^-$	√	√	√	115	–
[Malic acid−H]$^-$	√	√	√	133	115
[Hexose−H_2O−H]$^-$	n.d.	n.d.	√	161	89
Ascorbic acid	√	√	n.d.	175	143
Caffeic acid	√	√	n.d.	179	179; 135
[Hexose−H]$^-$			√	179	162; 89
Quinic acid	√	√	n.d.	191	173; 127; 111; 93; 85
Ferulic acid	√	√	n.d.	193	193; 178; 149; 134
Xanthoxylin	√	√	n.d.	195	–
Caffeoyltartaric acid	√	n.d.	n.d.	311	179
Caffeoyl glucose	√	n.d.	n.d.	341	179
[2 Hexose−H_2O−H]$^-$	n.d.	n.d.	√	341	162; 89
[3 Hexose−2H_2O−H]$^-$	n.d.	n.d.	√	503	162; 89
[3 Hexose−H_2O−H]$^-$	n.d.	n.d.	√	521	162; 89
[Quercetin+hexose+pentose]$^-$	√	n.d.	n.d.	595	301
Rutin hydrate	√	√	n.d.	609	301
[4 Hexose−2H_2O−H]$^-$	n.d.		√	683	162; 89

√: detected; n.d.: not detected.

Table 4
Free radical scavenging activity of araticum compounds and other natural antioxidants by DPPH

	DPPH free radical scavenging activity					
	Kim et al. (2006)	Kweon et al. (2001)	Saleem et al. (2004)	Baratto et al. (2003)	Experiments data	
	IC_{50} (μg/mL)	IC_{50} (μM)	IC_{50} (μg/mL)	IC_{50} (μM)	IC_{50} (μg/mL)	IC_{50} (μM)
Quinic acid derivatives						
Methyl 5-*O*-caffeoyl-3-*O*-sinapoylquinate	8.3 ± 0.2					
Ethyl 5-*O*-caffeoyl-3-*O*-sinapoylquinate	6.1 ± 0.1					
Methyl 5-*O*-caffeoyl-4-*O*-sinapoylquinate	8.5 ± 0.4					
Ethyl 5-*O*-caffeoyl-4-*O*- sinapoylquinate	9.5 ± 0.3					
Methyl 3,5-di-*O*-caffeoyl-4-*O*-(3-hydroxy-3-methyl) glutaroylquinate	4.4 ± 0.1					
Ethyl 5-*O*-caffeoylquinate	7.1 ± 0.4					
3,5-Dicaffeoylquinic acid	5.6 ± 0.1					
4,5-Dicaffeoylquinic acid	5.9 ± 0.2					
Quinic acid derivatives						
3-*O*-(3′-Methylcaffeoyl)quinic acid		16.0 ± 0.34				
5-*O*-Caffeoyl-4-methylquinic acid		8.8 ± 0.06				
3-*O*-Caffeoyl-1-methylquinic acid		6.9 ± 0.07				
Caffeic acid derivatives						
Dimethyl ester of caffeoyltartaric acid			9.67 ± 1.97			
Dimethyl ester of caffeoyltartronic acid			10.71 ± 1.63			
Monomethyl ester of caffeoyltartronic acid			14.17 ± 4.15			
Galloyl quinic derivatives						
Methyl ester of caffeic acid			13.13 ± 0.96			
5-*O*-Galloyl quinic				18.7 ± 2.1		
3,5-*O*-Digalloyl quinic				7.1 ± 0.8		
3,4,5-*O*-Trigalloyl quinic				3.9 ± 0.6		
Quercetin	5.9 ± 0.7		4.75 ± 0.57		3.1 ± 0.1	9.2 ± 0.1
Quercetin 3-*O*-glucopyranoside	16.6 ± 1.0					
Caffeic acid	3.2 ± 0.1	13.7 ± 0.1			1.9 ± 0.1	10.4 ± 0.7
Ascorbic acid	5.5 ± 0.1	49.5 ± 0.35	6.49 ± 1.07		7.7 ± 0.3	43.5 ± 1.6
Ferulic acid		36.5 ± 0.23			9.9 ± 0.2	51.3 ± 0.9
Rutin					7.5 ± 0.1	12.4 ± 0.1
Chlorogenic acid		12.3 ± 0.12				
Gallic acid				11.2 ± 0.9	1.4 ± 0.1	9.6 ± 0.3
Alpha-tocopherol		40.6 ± 0.29	12.64 ± 0.42		7.0 ± 0.1	14.8 ± 0.1
Epigallocatechin		3.7 ± 0.03				

3-methyl) glutaroylquinate IC50 = 4.4 mg mL^{-1}, 3,5-dicaffeoylquinic acid IC50 = 5.6 mg mL^{-1} and 4,5-dicaffeoylquinic acid IC50 = 5.9 mg mL^{-1}) and quercetin IC50 = 5.9 mg mL^{-1}. In conclusion, the results of the present work indicate the presence of compounds possessing excellent antioxidant activity from peel and seed of araticum fruits with the seeds as the best source of the antioxidants exhibiting higher activity as compared to peel and pulp. Besides, it is highly important to emphasize that the araticum fruit may be considered a very cost-effective natural antioxidant if considered the following relevant aspects: very low IC50; seed and peel are remains of human consumption and often dismissed; the extraction process is extremely simple and fast and the possibility of even more effective extracts by optimisation of extraction, purification and isolation of the identified phenolic compounds. As a consequence, the present results may encourage additional and more in-depth studies on the pharmacological and functional properties of araticum extracts in order to evaluate its possibility as a natural source for the development of a dietary supplement or functional food due to its high antioxidant activity.

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