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Original Paper

HPLC-ESI-MS/MS phenolic profile of "Nanicão Corupá" (*Musa acuminata*)

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Abstract

"Nanicão Corupá" (*Musa acuminata*) comes from Southern Brazil. The tropical climate in the region provides unique characteristics, including a sweeter flavor. This difference resulted in a Geographical Indication Recognition and Designation of Origin, recognized by the National Institute of Industrial Property (INPI) in Brazil. Considering that "Nanicão Corupá" has some peculiarities related to the climate and there are no studies evaluating this banana cultivars, the purpose of the present study was to investigate the qualitative and quantitative phenolic composition of the aerial parts of "Nanicão Corupá" by HPLC-ESI-MS/MS in comparison to 46 commercial standards of phenolic compounds. Aerial parts (flower, leaves, fruit and stem) of "Nanicão Corupá" were collected and macerated in methanolic extracts, which were partitioned with solvents of different polarities (dichloromethane and ethyl acetate). The HPLC-ESI-MS/MS analysis was performed using the sample pre-treatment, chromatographic and mass spectrometer parameters. Results demonstrated that a total of 11 phenolic compounds were identified in the analyzed samples. The majority of compounds was identified in the ethyl acetate fraction (BFEF) of banana flowers: rutin (36.06 ± 0.23) and isoquercetin (28.83 ± 5). The compounds isoquercetin, naringerin and myricitrin were identified for the first time in the *Musa* genus.

Key words: banana, Corupá, Nanicão, phenolic compounds, phytochemistry.

Resumo

O "Nanicão Corupá" (*Musa acuminata*) é proveniente do sul do Brasil. Em função do clima tropical na região a banana apresenta características diferenciadas incluindo o sabor mais doce. Essa diferença resultou em um Reconhecimento de Indicação Geográfica e Denominação de Origem, reconhecido pelo Instituto Nacional de Propriedade Industrial (INPI) no Brasil. Considerando que o Nanicão Corupá possui importantes peculiaridades e que não existem estudos que avaliaram este cultivar, o objetivo do presente estudo foi investigar a composição fenólica qualitativa e quantitativa das partes aéreas da banana (flores, folhas, frutos e caule) de Corupá por HPLC-ESI-MS/MS em comparação com 46 padrões de compostos fenólicos. As partes aéreas de Nanicão Corupá foram coletados e macerados em metanol, separadamente. Os extratos obtidos foram particionados com solventes de diferentes polaridades (diclorometano e acetato de etila). Os resultados demonstraram que um total de 11 compostos fenólicos foram identificados nas amostras analisadas. Os compostos majoritários foram

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identificados na fração de acetato de etila das flores de banana (BFEF): rutina (36,06 ± 0,23) e isoquercetina (28,83 ± 5). Os compostos Isoquercetina, Naringerina e Micricitrina foram identificados pela primeira vez no gênero *Musa*. Este é o primeiro estudo que identificou compostos fenólicos em Nanicão Corupá.
Palavras-chave: banana, Corupá, Nanicão, compostos fenólicos, fitoquímica.

Introduction

Bananas (*Musa* spp.) are the most consumed tropical fruit worldwide, being grown in approximately 130 countries. In the world, the fruit has an average consumption of 55 kg per inhabitant / per year; it is a great source of energy and is considered the cheapest, most nutritious and easily accessible fruit for consumers (Altendorf 2019).

"Nanicão Corupá" (*Musa acuminata* var. *Cavendish*) is a banana cultivar from Southern Brazil with the label "sweet by nature" due to its edaphoclimatic characteristics, mainly the geological and climatic formation of this region (SEBRAE 2018). This tropical climate makes the fruit extend its production cycle, resulting in its main qualitative characteristics: the most pronounced sweet flavor combined with the less acidity (INPI 2018). For these reasons, the fruit has a Geographical Indication Recognition (IG) and Designation of Origin (DO), recognized by National Institute of Industrial Property (INPI) in Brazil. Banana is the second most cultivated fruit in Brazil, cultivation is popular in all regions, according to IBGE data, the national harvest of 2017, referring to January of the same year, had 477,261 thousand hectares of plantation and 6,778,043 million tons of bananas produced (IBGE 2017).

The identification and quantification of new compounds in natural products, through sensitive and precise methods, is a technological challenge. The use of separation techniques such as high-performance liquid chromatography (HPLC) and capillary electrophoresis, coupling to mass spectrometry are fundamental for the qualitative and quantitative identification of bioactive compounds in environmental, biological, food and pharmaceutical samples (Ribani *et al*. 2004). Studies have already identified some phenolic compounds in banana species, as gallocatechin in banana peels (Pereira 2010) and tannins in green banana fruit, which have already been described as antioxidants and antiulcer agents (Costa & Brito 1997). In addition, literature data evidenced a significant antioxidant activity for nine banana peel varieties (Baskar *et al*. 2011; Rebello *et al*. 2014). Phenolic compounds are, in general, potent antioxidants agents and free radical scavengers protecting against superoxide anion radicals, hydroxyl radicals, and hydroperoxides that induce cancers, Alzheimer's, and Parkinson's diseases, as well as aging and heart conditions (Baskar *et al*. 2011).

The present work aims to investigate the qualitative and quantitative phenolic composition of the aerial parts of "Nanicão Corupá" by HPLC-ESI-MS/MS in comparison to 46 commercial standards of phenolic compounds. It is important to highlight that this is the first work that evaluates the presence of phenolic compounds in this specific and special banana type from Brazil.

Material and Methods

Plant material

Aerial parts (flower, leaves, fruit and stem) of *Musa acuminata* var. *Cavendish* were collected from a cultivator in Corupá, Santa Catarina - Brazil 26°25'31"S, 49°14'35"W 2.IV.2019 in 2019 (April), a species previously identified by SEBRAE, whose specimen is cataloged under the registration number SCS452- Corupá, SISGEN: AD5308F. The plant material was divided into flower (900 g), fruit peel (2,000 g), leaves petiole (500 g), leaves blade (1,200 g), stem/aerial stem (2,000 g) and stem peduncle (2,000 g). These fresh materials were extracted by macerating in methanol for 7 days at room temperature (1:5 v/v). The extracts were filtered and concentrated in a rotary evaporator under reduced pressure at 50 ºC, giving the respective methanolic extracts: Banana Flower Methanolic Extract (BFME), Banana Fruit Peel Methanolic Extract (BFPME), Banana Leaves Petiole Methanolic Extract (BLPME), Banana Leaves Blade Methanolic Extract (BLBME), Banana Aerial Stem Methanolic Extract (BASME) and Banana Stem Peduncle Methanolic Extract (BSPME). All the methanolic extracts were successively partitioned with solvents of different polarities (dichloromethane and ethyl acetate) to obtain the respective dichloromethane fractions (Banana Flower Dichloromethane Fraction (BFDF), Banana Fruit Peel Dichloromethane Fraction (BFPDF), Banana

Leaves Petiole Dichloromethane Fraction (BLPDF), Banana Leaves Blade Dichloromethane Fraction (BLBDF), Banana Aerial Stem Dichloromethane Fraction (BASDF) and Banana Stem Peduncle Dichloromethane Fraction (BSPDF) and ethyl acetate fractions (Banana Flower Ethyl acetate Fraction (BFEF), Banana Fruit Peel Ethyl acetate Fraction (BFPEF), Banana Leaves Petiole Ethyl acetate Fraction (BLPEF), Banana Leaves Blade Ethyl acetate Fraction (BLBEF), Banana Aerial Stem Ethyl acetate Fraction (BASEF) and Banana Stem Peduncle Ethyl acetate Fraction (BSPEF). The proportion of material and solvent were 1:4 (v/v) with two partitions each fractionation.

Preparations of the samples and analysis of phenolic compounds by HPLC-ESI-MS/MS

The analyses were conducted in an Agilent® 1,200 chromatograph, with a Phenomenex® Synergi 4 µ Polar-RP 80 A column (150 mm × 2 mm ID, particle size of 4 µm) at a temperature of 30 °C. The eluent solvents were A (MeOH/H_2O in ratio of 95:5, v v^{-1}) and B (formic acid 0.1%) as follows: 1st stage - 10% solvent A and 90% B (isocratic mode) for 5 minutes; 2nd stage - linear gradient of solvents A and B (from 10% to 90% of A) for 2 minutes; 3rd stage - 90% of A and 10% of B (isocratic mode) for 3 minutes; 4th stage - linear gradient of solvents A and B (from 90% to 10% of A) for 7 minutes with a flow rate of 250 µL.min^{-1} in the mobile phase. The injected volume in all the analyses was 5 uL.

The liquid chromatograph was coupled to a mass spectrometry system consisting of a hybrid triple quadrupole/linear ion trap mass spectrometer Qtrap® 3200 (Applied Biosystems/MDS SCIEX, USA) with TurboIonSpray® as the ionization source, in positive ionization mode. The source parameters used were: ion spray interface at 400 °C; ion spray voltage of 4,500 V; curtain gas, 10 psi; nebulizer gas, 45 psi; auxiliary gas, 45 psi; collision gas, medium. The software Analyst® (version 1.5.1) was used to record and process the data. Pairs of ions were monitored in MRM (Multiple Reaction Monitoring) mode.

HPLC-ESI-MS/MS analysis was performed using the sample pre-treatment, chromatographic and mass spectrometer parameters previously described by Schulz *et al*. (2015). The samples were prepared by dissolving 50 mg of the material (ethyl acetate fractions) in a 5 mL solution of hydrochloric acid at pH 2. These solutions were extracted three times with 2 mL of ethyl ether each time and the three extracts were combined. After drying the combined extract, it was stored in a sealed container at -20 °C. Prior to the analyses, the dried material was solubilized in 1 mL of MeOH and centrifuged at 12,000 rpm for 120s.

For the identification and quantification of the compounds, 46 standard phenolic compounds, commercially purchased (Sigma-Aldrich) and with high purity grade (almost all > 97%) (4-aminobenzoic acid, 4-hydroxymethylbenzoic acid, apigenin, aromadendrin, caffeic acid, carnosol, catechin, chlorogenic acid, chrysin, cinnamic acid, coniferaldehyde, ellagic acid, epicatechin, epigallocatechin, epigallocatechin gallate, eriodictyol, ferulic acid, fustin, galangin, gallic acid, hispudulin, isoquercetin, kaempferol, mandelic acid, methoxyphenylacetic acid, myricetrin, naringerin, naringin, *p*-anisic acid, *p*-coumaric acid, pinocembrin, protocatechuic acid, quercetin, resveratrol, rosmarinic acid, rutin, salicylic acid, scopoletin, sinapaldehyde, sinapic acid, syringaldehyde, syringic acid, taxifolin, umbelliferone, vanillic acid and vanillin) dissolved in methanol (0.02 to 6 $mg.L^{-1}$) were analyzed under the same conditions as those described above.

Results and Discussion

After complete drying of samples, the extracts and fractions yields were calculated (Tab. 1). The leaves blade methanolic extract (BLBME) presented the major yield (1.45%), followed by the flower methanolic extract (BFME) (1.42%). The dichloromethane fractions mostly present better yields than ethyl acetate fractions, specially the leaves petiole dichloromethane fraction (BLPDF) (30.39%) and the leaves blade dichloromethane fraction (BLBDF) with 19.51%. The dichloromethane extracts an acceptably wide range of nonpolar analytes, corroborating with the higher concentration in these samples. This is possible because bananas have a greater amount of nonpolar and volatile compounds, such as, for example, terpenes (Facundo *et al*. 2012). In fact, some studies have identified the presence of terpenes compounds in different banana varieties, such as sesquiterpenes (β-bisabolene, nerolidol) identified in *M. acuminata* Colla (Fingolo *et al*. 2013), eugenol, α-ocimene, α-cedreno, α-pinene identified in *M. acuminata*, "Nanicão" (Facundo *et al*. 2012), and α-ocimene, limonene identified in *Musa acuminata* (Qamar & Shaikh 2018).

Among the ethyl acetate fractions, the best yields were observed in the stems - the stem

Table 1 – Yield of extracts and fractions of the aerial parts of "Nanicão Corupá".

Banana's aerial parts	Collected material (g)	Methanolic extract (g/%)[a]	Dichloromethane fraction (g/%)[b]	Ethyl acetate fraction (g/%)[b]
Flower	900	12.84/1.42	2.98/11.06	0.13/1.01
Leaves blade	1200	6.98/0.58	2.12/ 30.39	0.18/2.58
Leaves petiole	500	7.28/1.45	1.42/19.51	0.39/5.35
Green fruit peel	2000	7.12/0.35	0.08/1.12	0.33/4.63
Stem peduncle	2000	5.98/0,20	1.04/17.40	0.72/12.04
Aerial stem	2000	6.01/0.30	0.36/5.98	0.45/7.48

[a] = Yield (g) calculated based on material obtained in the crude extract

[b] = Yield (%) calculated from the mass (g) on the crude extract

peduncle ethyl acetate fraction (BSPEF) with 12.04% and the aerial stem ethyl acetate fraction (BASEF) with 7.48%. However, besides the best yields, only one phenolic compound was identified by HPLC-ESI-MS/MS in both fractions, ρ-coumaric acid with 0.89 ± 0.18 $\mu g.g^{-1}$ and 1.27 ± 0.25 $\mu g.g^{-1}$, respectively (Tab. 2). This compound was identified in almost all samples analyzed of flower ethyl acetate fraction (BFEF) (6.11 ± 1.34 $\mu g.g^{-1}$), banana leaves blade ethyl acetate fraction (BLBEF) (4.44 ± 0.24 $\mu g.g^{-1}$); leaves petiole ethyl acetate fraction (BLPEF) (4.65 ± 1.57 $\mu g.g^{-1}$); except in fruit peel ethyl acetate fraction (BFPEF).

In total, 11 phenolic compounds were identified in the analyzed samples by the direct comparation with standards (Fig. 1). Although the BFEF was the fraction with the lowest extraction yield, it was also the sample with the highest number of identified compounds, eight phenolic compounds: rutin (36.06 ± 0.23 $\mu g.g^{-1}$), isoquercetrin (28.83 ± 0.5 $\mu g.g^{-1}$), protocatechuic acid (17.44 ± 5.81 $\mu g.g^{-1}$), ferulic acid (4.19 ± 1 $\mu g.g^{-1}$), myricitrin (3.73 ± 0.36 $\mu g.g^{-1}$), vanilic acid (1.81 ± 0.19 $\mu g.g^{-1}$) and sinapic acid (0.41 ± 0.09 $\mu g.g^{-1}$). The second fraction with the highest number of compounds found was BLBEF with six compounds identified myricitrin (4.31 ± 0.27 $\mu g.g^{-1}$), rutin (3.89 ± 0.09 $\mu g.g^{-1}$), syringic acid (2.98 ± 0.19 $\mu g.g^{-1}$), naringerin (1.47 ± 0 $\mu g.g^{-1}$) and caffeic acid (0.8 ± 0.05 $\mu g.g^{-1}$), beside p-coumaric acid (4.44 ± 0.24 $\mu g.g^{-1}$) as already cited. In BLPEF were recognized three phenolic compounds myricitrin (3.28 ± 0.09 $\mu g.g^{-1}$) and ferulic acid (0.65 ± 0.04 $\mu g.g^{-1}$) and in BFPEF only ferulic acid was identified with 0.72 ± 0.24 $\mu g.g^{-1}$.

Phenolic compounds tend to be stored in dermal tissues in various plant parts, due to their significant role in protecting against ultraviolet radiation, acting as attractants in fruits dispersal and as defense constituents against predators and pathogens (Strack 1997). These could be a possible explanation for the results of phenolic compounds obtained in this study.

Yingyuen *et al*. (2020) described the identification and purification of a leaf extract from *M. balbisiana* with 5.6% of rutin. This is the major compound found in "Nanicão Corupá" and represents 0.72% of BFEF. Rutin is a well-known compound with important antioxidant activity, found in differing among varieties of *Musa* spp. peel (Passo-Tsamo *et al*. 2015; Vu *et al*. 2018).

Pothavorn *et al*. (2010) have already described the presence of aglycone myricetin in sad of *M. acuminata*. Drapal *et al*. (2018) also described the presence of this compound in leaves of *Musa* sp. In the present work, we identified the glycosylated derivative in BLBEF, BFEF and BLPEF. This find can be related to the geological and climatic formation of the Corupá region (SEBRAE 2018) and the extended production cycle of "Nanicão Corupá".

Bennett *et al*. (2010) identified ferulic acid in the fruit cell wall and Mahouachi *et al*. (2014), described the identification of this compound in the leaf power of *M. acuminata*. It was also identified in rhizomes of *Musa* AAB cv. Nanjanagudu Rasabale, grown in India as well as caffeic acid. The phenolic compounds sinapic, siryngic, caffeic and also the ferulic acid were described in *Musa Red Yade* variety (Passo-Tsamo *et al*. 2015) and

Table 2 – Phenolic compounds identified in "Nanicão Corupá" by HPLC-ESI-MS/MS.

Compound	RT*	Calculated Mass	Experimental mass $[M - H]^-$	MS/MS (*m/z*)	BLPEF[c]	BLBEF[b]	BFEF[a]	BSPEF[e]	BASEF[f]	BFPE[d]
Protocatechuic acid	8.01	151.75	152.92	109			17.44± 5.81			
ρ-coumaric acid	10.33	164.04	162.92	93	4.65± 1.57	4.44± 0.24	6.11± 1.34	0.89±0.18	1.27±0.25	
Vanilic acid	10.13	168.14	166.92	108			1.81± 0.19			
Caffeic acid	9.32	180.16	178.92	135		0.8± 0.05				
Ferulic acid	10.71	194.18	192.95	134	0.65± 0.04		4.19± 1			0.72±0.24
Siringic acid	9.97	198.17	196.93	121.1		2.98± 0.19				
Sinapic acid	10.86	224.63	223.01	148.8			0.41± 0.09			
Myricitrin	11.23	318.23	316.99	151	3.28±0.09	4.31±0.27	3.73±0.36			
Isoquercitrin	10.89	464.38	463.15	300			28.83± 0.5			
Naringerin						1.47± 0				
Rutin	10.71	610.52	609.24	300.1		3.89± 0.09	36.06± 0.23			
Total compound					**3**	**6**	**8**	**1**	**1**	

[a] = Banana Flower Ethyl acetate Fraction (BFEF); [b] = Banana Leaves Blade Ethyl acetate Fraction (BLBEF); [c] = Banana Leaves Petiole Ethyl acetate Fraction (BLPEF); [d] = Banana Fruit Peel Ethyl acetate Fraction (BFPEF); [e] = Banana Stem Peduncle Ethyl acetate Fraction (BSPEF); [f] = Banana Aerial Stem Ethyl acetate Fraction (BASEF). * = Passo-Tsamo *et al.*, described these compounds in Musa Red Yade variety. *RT: min.

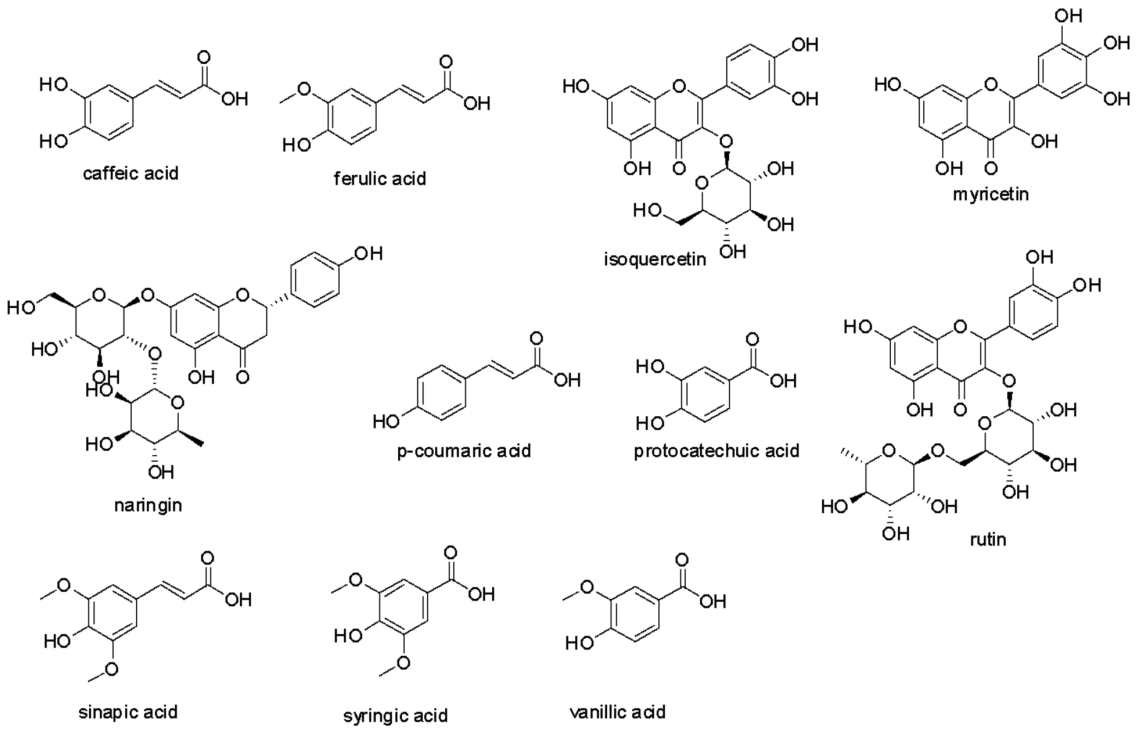

Figure 1 – Structures of the phenolic compounds identified in Banana of Corupá.

some of them in *M. acuminata* var. *Cavendish* (Russell *et al*. 2009). Besides the protocatechuic acid was quantified in four different extracts, the acetone extract was more concentrated with 153.12 ug.mg^{-1} (Kandasamy & Aradhya 2014). This compound was identified only in BFEF from "Nanicão Corupá", in a lower concentration (17.44 ± 5.81 ug.mL^{-1}).

Although some of the compounds identified in this work have already been cited in the *Musa* genus, as seen previously, *ρ*-coumaric acid, vanilic acid, myricitrin, isoquercitrin and naringerin are reported for the first time for "Nanicão Corupá". Russell *et al*. (2009) had already described the presence of some compounds in *Musa acuminata* var. *Cavendish*, as *ρ*-coumaric acid, Vanilic acid and Syringic acid. However, despite having been researched, *ρ*-coumaric acid, caffeic acid, ferulic acid and sinapic acid were not found. In addition to the previously mentioned compounds, this was also the first report demonstrating the presence of Naringerin *M. acuminata*.

The samples of "Nanicão Corupá" studied in the present work demonstrated some similarities regarding other species of the genus *Musa*. Despite this, bananas and phenolic compounds are an essential part of the human diet. Considered a source of energy, nutrients, and minerals combined with antioxidant properties of the compounds found, "Nanicão Corupá" shows the benefits for human health (Passo-Tsamo *et al*. 2015). This was the first study carried out with the "Nanicão Corupá" in which the identification, characterization and quantification of phenolic compounds were obtained. Further studies are needed to assess the possible biological properties of this species.

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