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1 # Metabolite profiling and cytotoxic activity of Andean potatoes: polyamines and
2 glycoalkaloids as potential anticancer agents in human neuroblastoma cells *in*
3 *vitro*

4

5 María Luciana Lanteri[a]*, María Ximena Silveyra[a], Mónica Mariela Morán[a],
6 Stéphanie Boutet[b], Deyvis-Dante Solis-Gozar[b], François Perreau[b], Adriana
7 Balbina Andreu[a]

8

9 [a]Instituto de Investigaciones Biológicas, Facultad de Ciencias Exactas y
10 Naturales, Universidad Nacional de Mar del Plata, CONICET, CC 1245, 7600
11 Mar del Plata, Argentina
12 [b]Université Paris-Saclay, INRAE, AgroParisTech, Institut Jean-Pierre Bourgin
13 (IJPB), 78000, Versailles, France

14

15 *Corresponding author
16 E-mail address: lanteri@gmail.com (M.L. Lanteri)

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24 **Abstract**

25 Andean potatoes (*Solanum tuberosum* L. ssp. *andigena*) are a good source of
26 dietary antioxidant polyphenols. We have previously demonstrated that
27 polyphenol extracts from Andean potato tubers exerted a dose-dependent
28 cytotoxic effect in human neuroblastoma SH-SY5Y cells, being skin extracts
29 more potent than flesh ones. In order to gain insight into the bioactivities of
30 potato phenolics, we investigated the composition and the *in vitro* cytotoxic
31 activity of total extracts and fractions of skin and flesh tubers of three Andean
32 potato cultivars (Santa María, Waicha, and Moradita). Potato total extracts were
33 subjected to liquid-liquid fractionation using ethyl acetate solvent in organic and
34 aqueous fractions. We analyzed both fractions by HPLC-DAD, HPLC-ESI-
35 MS/MS, and HPLC-HRMS. Results corroborated the expected composition of
36 each fraction. Organic fractions were rich in hydroxycinnamic acids (principally
37 chlorogenic acid isomers), whereas aqueous fractions contained mainly
38 polyamines conjugated with phenolic acids, glycoalkaloids, and flavonoids.
39 Organic fractions were not cytotoxic against SH-SY5Y cells, and indeed, some
40 increased cellular metabolism compared to controls. Aqueous fractions were
41 cytotoxic and even more potent than their respective total extracts. Treatment
42 with a combination of both fractions showed a similar cytotoxic response to the
43 corresponding extract. According to correlation studies, it is tempting to
44 speculate that polyamines and glycoalkaloids are crucial in inducing cell death.
45 Our findings indicate that the activity of Andean potato extracts is a combination

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46 of various compounds and contribute to the revalorization of potato as a
47 functional food.
48
49 **Keywords:** cytotoxicity; fractionation; food; nutraceuticals; phenolic compound;
50 *Solanum tuberosum*.
51
52 **1. Introduction**

53 Potato (*Solanum tuberosum*) is the main non-cereal food crop worldwide.
54 It is the most significant contributor to daily dietary antioxidants (Chun et al.,
55 2005), and considered a functional food. Potato is classified into two
56 subspecies, *andigena* and *tuberosum*, the former characterized by a great
57 diversity of tuber forms and colors (Hijmans & Spooner, 2001).

58 Potatoes are rich in many phytochemicals derived from the specialized
59 metabolism with diverse structures and biological functions. Phenylpropanoids
60 are a group of thousands of compounds, including hydroxycinnamic acids
61 (HCCs) (Ezekiel et al., 2013). Flavonoids are divided into six main subclasses:
62 flavanols, flavonols, flavones, isoflavones, flavanones, and anthocyanins (Lewis
63 et al., 1998). Polyamines occur primarily as putrescines, spermines, and
64 spermidines, and can be conjugated with phenolic acids (Huang et al., 2017).
65 Glycoalkaloids are plant steroids, being chaconine and solanine the main ones
66 found in potatoes (Friedman, 2006).

67 In plants, phytochemicals participate in diverse processes like protection
68 against biotic and abiotic stresses. They also exhibit diverse health-promoting
69 effects such as antiinflammatory, anticarcinogenic, proapoptotic, and

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70 antioxidant activities (Friedman, 1997). Taking into account the growing interest
71 in studying natural bioactive compounds, potato tubers and their industrial
72 byproducts appear as promising sources suitable for application in the food and
73 pharmaceutical industries. Metabolites can induce antitumoral effects based on
74 the type, concentration, and how they interplay. Some studies have determined
75 that polyphenol extracts from fruits and vegetables, including potatoes, provide
76 antiproliferative activity against multiple cancer cell lines with null or minimum
77 effect against normal cells (Bontempo et al., 2013; Vizzotto et al., 2014; Ombra
78 et al., 2015; Kubow et al., 2017). Specifically, extracts of potato tubers inhibited
79 proliferation in the prostate (Reddivari et al., 2007; Bontempo et al., 2013),
80 stomach (Hayashi et al., 2006), liver (Wang et al., 2011), and colon (Madiwale
81 et al., 2011; Kubow et al., 2017) cancer cells. Further, potato extracts rich in
82 anthocyanins (Bontempo et al., 2013) or glycoalkaloids (Friedman et al., 2005;
83 Nogawa et al., 2019) showed a dose-dependent antitumor activity in cervical,
84 breast, and leukemia cancer cells.

85 Previously, we studied the cytotoxic effect of Andean potato polyphenol
86 extracts against human neuroblastoma and hepatocarcinoma cell lines.
87 Treatments resulted in a dose-dependent viability reduction in all cell types
88 (Martínez et al., 2018; Silveyra et al., 2018). However, we do not know which
89 compound(s) exert(s) these biological activities. So far, few studies have
90 addressed potato bioactivity and nutraceutical composition in fractions or semi-
91 purified extracts (Sánchez Maldonado et al., 2014; Chaparro et al., 2018;
92 Nogawa et al., 2019).

93 This work aimed to fraction and to characterize the metabolites in
94 polyphenol extracts of skin and flesh Andean potato cultivars (Santa María,

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95 Waicha, and Moradita) and analyze their cytotoxic effects against human
96 neuroblastoma SH-SY5Y cells. We applied liquid-liquid fractionation and mass
97 spectrometry to identify the compounds responsible for the bioactivity.
98

99 2. Materials and methods

100 2.1. *Plant material*

101 Moradita, Waicha, and Santa María potato cultivars were selected based
102 on previous work from our laboratory (Silveyra et al., 2018) and other groups
103 (Calliope et al., 2018). The skin and flesh colors are purple and yellow for
104 Moradita, pink and yellow for Waicha, and intensely red for Santa María. Potato
105 cultivars were grown in a field located in Yavi Department (22°6'4"S,
106 65°35'44"O, 3377 MAMSL), Jujuy, Argentina, during the 2012/2013 campaign.
107 All cultivars were planted in random plots and harvested at the end of their
108 respective cycles. We pooled skin and flesh of freshly harvested tubers to
109 generate a representative sample for each cultivar. The material was
110 immediately frozen in liquid nitrogen and stored at −80°C until analysis. Frozen
111 potato pieces were freeze-dried and finely powdered.

112 2.2. *Extraction and fractionation of total extracts (TEs)*

113 Lyophilized tissues (2 g DW) from potato tuber skin or flesh were
114 incubated overnight with 40 mL 100% methanol at 4°C in darkness with
115 constant agitation (Figure 1). The methanolic extracts were centrifuged at
116 6000g for 20 min at 4°C, and thoroughly dried in a rotary evaporator (Senco)
117 followed by a vacuum concentrator (Martin Christ). We obtained ethanolic
118 extracts by adding 2.6 mL ethanol 80% (v/v) and centrifugation at 13000g for 10

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119 min. Liquid-liquid fractionation of the ethanolic extracts was done according to
120 the protocol described in Oki et al. (2002). Briefly, we separated 0.8 mL as total
121 extracts (TEs), and we added 40 µL of trifluoroacetic acid and 1.6 mL of distilled
122 water to the remaining volume (1.8 mL). The ethanol was removed by
123 evaporation in a rotary vacuum concentrator. Then, four consecutive extractions
124 were done with 1.8 mL ethyl acetate each. The resulting organic fractions
125 (OFs), aqueous fractions (AFs), and TEs were dried in a vacuum concentrator
126 and dissolved in methanol 30% v/v (except for HPLC-ESI-MS/MS and HPLC-
127 HRMS analysis) with proportional volumes according to the DW of each fraction
128 (0.5 mL for TEs, and 1.125 mL for OFs and AFs) (Figure 1).

129 **2.3.** *Analysis by HPLC-DAD*

130 Quantification of HCCs and anthocyanidins, present in TEs and fractions
131 of each cultivar and tissue, was carried out using a Shimadzu LC-Solution
132 system equipped with a diode array detector (DAD) as described in Silveyra et
133 al. (2018). All peaks and standards were integrated according to the baseline.
134 We expressed the levels of metabolites as µg/g DW in three biological
135 replicates.

136 **2.4.** *Analysis by HPLC-ESI-MS/MS for the relative quantification and HPLC-*
137 *HRMS for the annotation of compounds*

138 The dry pellets of TEs, OFs, or AFs were dissolved in
139 methanol/H_2O/acetone/trifluoroacetic acid (40/32/28/0.05, v/v). Then, 50 ng of
140 apigenin (Extrasynthese, Genay, France) was added as an internal standard,
141 and extracts were filtered through Whatman TM filter paper grade GF/A. After
142 this, extracts were introduced in the ESI source of a U-HPLC Acquity equipped

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143 with a PDA detector (Waters, Milford, USA). Separation was achieved on a
144 reverse-phase column Uptisphere C18 ODB, 150x2.1 mm (Interchim, Motluçon,
145 France) using a flow rate of 0.4 mL/min and a binary gradient: (A) acetic acid
146 0.1% (v/v) in water, and (B) acetic acid 0.1% (v/v) in acetonitrile. The gradient
147 program was 0-2 min 95% A, 2-4 min 90% A, 4-17 min 40% A, 17-23 min 100%
148 A, and 23-25 min 95% A. The analysis was performed on a Xevo TQS triple
149 quadrupole mass spectrometer (Waters, Milford, USA) operating in different
150 scanning modes to identify the compounds and full scan for the semi-
151 quantification. Relevant parameters were set as follows: capillary 2.70 kV,
152 extractor 3 V, source block, and desolvation gas temperatures 150°C and
153 600°C, respectively. Nitrogen was used to assist nebulization and desolvation
154 (7 Bar and 1000 L/h, respectively), and argon was used as collision gas for the
155 MS/MS mode at 2.83×10^{-3} mBar. Levels of metabolites are expressed in µg
156 apigenin equivalents/g DW based on the extracted ion chromatogram of each
157 compound. The same samples were analyzed by HPLC-HRMS to confirm the
158 annotation using standards or the accurate mass.

159 **2.5.** *Total phenolics*

160 The total phenolic content of TEs was determined by the Folin-Ciocalteu
161 method and expressed as µg 5-chlorogenic acid (5-CGA) equivalents/mL in
162 three biological replicates, according to Martínez et al. (2018). Blank samples
163 consisted of 30% methanol (v/v).

164 **2.6.** *Cytotoxic measurements and microscopy*

165 Human neuroblastoma SH-SY5Y cells (ATCC CRL-2266) were
166 maintained in DMEM/F-12 (Sigma-Aldrich) as described in Silveyra et al.

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167 (2018). After 18 h of treatments with different fractions, cell cultures were
168 photographed under an Olympus CKX41 inverted microscope equipped with an
169 Olympus Q-Color 3 camera (Figure 1). The % cell viability was obtained using
170 the MTT assay (Sigma-Aldrich) and normalizing to the values in the absence of
171 treatments. We presented all data as mean ± SD. The extracts were tested in
172 triplicate, and the experiment was performed at least three times.

173 **2.7.** *Statistical analysis*

174 We used GraphPad software (Prism) to perform the statistical analysis
175 for cell viability assays using One way ANOVA multiple comparisons followed
176 by Dunnet´s test. We considered significant differences at P values ≤0.05
177 between treatments and the control with 30% methanol (v/v). Pearson product-
178 moment correlation coefficients (R^2 values) were calculated using Microsoft
179 Excel with the mean values of metabolites levels and % cell viability.

180

181 **3. Results**

182 **3.1.** *Fractionation of TEs: recovery and purity of OFs and AFs*

183 To estimate the fractions' recovery and purity, we calculated the % of
184 compounds (HCCs or anthocyanidins) related to the total amount obtained for
185 each TE by HPLC-DAD analysis (Table 1). Results indicate that the
186 fractionation of TEs was successful because the OFs were rich in HCCs, and
187 the AFs contained the most of the anthocyanidins. The % recovery of HCCs in
188 OF was ≥51.1% in all the fractions, whereas the % recovery of anthocyanidins
189 in AF was ≥87.5%. In addition, no more than 18.6% of recovered HCCs
190 remained in the AFs, whereas a maximum of 21.7% of the recovered

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191 anthocyanidins contaminated the OFs. In short, AFs were purer than OFs
192 (Table 1).

193 OF+AF values are theoretical and represent the sum of individual OF
194 and AF. These values are a reference to know how close the values of the sum
195 of the fractions are compared to the TE values. OF+AF values were between
196 65.5% and 122% of the corresponding TE (Table 1).

197 **3.2.** *HPLC-DAD chromatograms and composition of TEs, OFs, and AFs*

198 We quantified 12 individual metabolites (six HCCs and six
199 anthocyanidins) in each cultivar, tissue, and fraction with HPLC-DAD analysis.
200 The composition of each extract and its fractions is displayed in the
201 chromatograms (Supplemental Figures 1-3); however, we could not identify
202 some of the peaks. Profiles at 320 nm for HCCs in all TEs were similar to those
203 obtained in OFs and differed from those obtained in AFs (Supplemental Figures
204 1-3). Besides, the anthocyanidin profiles at 510 nm were similar between TEs
205 and AFs (Supplemental Figures 1-3). We expected this because HCCs were
206 recovered in OFs, and anthocyanins were recovered in AFs. The HPLC-DAD
207 chromatograms display different mAU scales because some samples were
208 diluted before injection. The corrected values multiplied by the dilution factor are
209 reported in Tables 1 and 2.

210 For each cultivar, skin extracts showed higher levels and more diversity
211 of compounds than the corresponding flesh extracts (Table 2). Concerning
212 HCCs in TEs, Santa María skin had the highest total concentration (2453.61
213 µg/g DW with 2056.91 µg/g DW 5-CGA), followed by Moradita skin (1809.54
214 µg/g DW with 1484.15 µg/g DW 5-CGA). Waicha skin had a total concentration
215 of 666.28 µg/g DW, with 217.28 µg/g DW 5-CGA, 189.63 µg/g DW 4-CGA and

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216 176.73 µg/g DW 3-CGA. Among flesh extracts, Santa María contained the
217 highest total HCCs concentration (1663.41 µg/g DW), followed by Waicha and
218 Moradita (373.98 µg/g DW and 104.43 µg/g DW, respectively). Again, all the
219 HCCs were represented in Waicha flesh (Table 2).

220 Regarding the anthocyanidins, Santa María skin presented the highest
221 abundance (581.95 µg/g DW total, 468.29 µg/g DW pelargonidin). Waicha skin
222 total concentration of anthocyanidin was 60.41 µg/g DW, with 34.80 µg/g DW
223 corresponding to pelargonidin. Moradita skin concentration was 158.21 µg/g
224 DW, composed primarily of 142.76 µg/g DW petunidin. Finally, Santa María
225 flesh total concentration was 345.37 µg/g DW, comprised principally of 241.63
226 µg/g DW pelargonidin. No anthocyanidins were found in Waicha nor Moradita
227 flesh extracts (Table 2).

228 **3.3.** *Tentative identification of metabolites by HPLC-ESI-MS/MS and HPLC-*
229 *HRMS*

230 In order to gain information about the nature of anthocyanins and the
231 presence of other compounds, we performed untargeted metabolomics for
232 potato extracts. Table 3 shows the HPLC-ESI-MS/MS data for 30 compounds
233 organized by retention time (RT) and divided into six families. We confirmed the
234 annotation by comparison with databases and literature (Lewis et al., 1998;
235 Fossen et al., 2003; Ieri et al., 2011; Eichhorn et al., 2011; Navarre et al., 2013;
236 López-Cobo et al., 2014), by using authentic standards for HCCs, and by
237 HPLC-HRMS accurate mass for six compounds (Table 3). We propose the
238 identification of two miscellaneous (sinapine, salicylic acid glucoside, one
239 remains unknown), four polyamines (caffeoyl putrescine, feruloyl putrescine, bis
240 dihydrocaffeoyl spermidine, tris dihydrocaffeoyl spermine), four HCCs (3-CGA,

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241 4-CGA, 5-CGA, cis-5-CGA), ten anthocyanins (four peonidin derivatives, one
242 cyanidin derivative, five pelargonidin derivatives, plus two pelargonidin hexoses
243 without identification), two non-anthocyanin flavonoids (eriodictyol hexose, rutin,
244 plus three putative flavonoids with phenolic acids), and two glycoalkaloids
245 (solanine, chaconine). Moreover, we tentatively annotated isomers (identical
246 mass spectra and different RT), which are the case of the four CGA isomers (3-
247 CGA, 4-CGA, 5-CGA, cis-5-CGA) and the six pelargonidin derivatives indicated
248 as Form 1 (F1) and Form 2 (F2) (Table 3).

249 **3.4.** *Semi-quantitative HPLC-ESI-MS/MS analysis*

250 Total ion current chromatograms (TICs) show that the profiles of TEs are
251 similar to the profiles of AFs, except between RT 3 and 6 min, where TICs of
252 TEs are similar to those of OFs (Supplemental Figures 4-6). Therefore, OFs
253 were rich in the four HCCs, whereas AFs were rich in other compounds.

254 The semi-quantification results (µg apigenin equivalents/g DW) of the 30
255 compounds detected by mass spectrometry in TEs are shown in Table 4.
256 Among families, we observed that HCCs, polyamines, and glycoalkaloids were
257 found in higher concentrations than flavonoids in potato tubers. In general,
258 HCCs presented higher concentrations in the skin than in the flesh (as was also
259 found by HPLC-DAD). Among polyamines, caffeoyl and feruloyl putrescine
260 levels were similar between skin and flesh. On the contrary, bis dihydrocaffeoyl
261 spermidine was more abundant in the skin than in the flesh, and tri
262 dihydrocaffeoyl spermine was only found in skin. Miscellaneous metabolites
263 (sinapine, salicylic acid glucoside and unknown) were generally more abundant
264 in flesh than skin. Among anthocyanins, peonidin and pelargonidin derivatives
265 were found in both skin and flesh of Santa María, and in skin of Waicha and

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266 Moradita. Non-anthocyanin flavonoids were principally found in Santa María
267 skin and flesh. Among glycoalkaloids, solanine and chaconine levels were more
268 abundant in the skin than flesh, and absent in Waicha flesh (Table 4).

269 **3.5.** *Total phenolics and selection of cytotoxic doses of TEs*

270 To calculate the doses for the treatments in cells, we performed the
271 Folin-Ciocalteu reaction with the different TEs. As expected, results showed that
272 the skin extracts had more phenolic concentration than the flesh extracts.
273 Among cultivars, Santa María total phenolic content is the highest, followed by
274 Moradita and Waicha (Table 5).

275 We then used doses to an approximate 50% of cytotoxic activity in
276 human neuroblastoma SH-SY5Y cells based on the MTT assays previously
277 reported by our group (Silveyra et al., 2018). However, Figure 2 showed a minor
278 effect on % cell viability than expected. Therefore, we decided to continue with
279 100 μg/mL Santa María skin, 400 μg/mL Santa María flesh, 10 μg/mL Waicha
280 skin, 300 μg/mL Waicha flesh, 30 μg/mL Moradita skin, and 60 μg/mL Moradita
281 flesh.

282 **3.6.** *Treatments of cells with TEs, OFs, and AFs: microscopy*

283 We treated SH-SY5Y cells with the TEs, fractions, and the sum OF+AF.
284 Before measuring cell viability by MTT assay, we observed cells under the
285 microscope. Representative photographs of cells treated during 18 h with
286 Waicha skin and flesh (10 and 300 μg/mL, respectively) are shown in Figure 3.
287 Waicha was chosen to visualize the morphological changes because the skin
288 extract exerted the highest cytotoxic activity with the lowest phenolic
289 concentration (Figure 2).

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290 The typical morphology of SH-SY5Y cells is neuronal, with a starry form.
291 They grow adhered to the substrate and in a minor quantity in suspension. Both
292 control and methanol treatments showed a pattern consistent with normal cell
293 morphology (Figure 3). Cells treated with TEs from Waicha skin and flesh
294 showed morphological changes compared to controls: they were reduced in
295 size, had a rounded form, and were aggregated in cumulus. However, cells
296 were similar to the controls in treatments with OFs from both tissues.
297 Treatments with AFs exerted a similar effect to those with OF+AF, showing cell
298 damage in both skin and flesh extracts (Figure 3).

299 **3.7.** *Cytotoxic assays with TEs, OFs, and AFs: cell viability*

300 After visualizing the cells, we quantified the cytotoxic effect by the MTT
301 assay. All treatments with TEs resulted in a cell viability ≤50% (Figure 4).
302 Treatment with OFs did not cause cell death and in Waicha provoked an
303 increment in cellular metabolism compared to controls. AFs were cytotoxic in all
304 cases, even superior to the corresponding TE in Santa María flesh. Except for
305 Santa María flesh, treatments with OF+AF showed diminished cell viability, with
306 values similar to the respective TEs (Figure 4). Values obtained for Waicha skin
307 and flesh are consistent with the phenotypes observed in Figure 3.

308 **3.8.** *Correlation analysis between the content of metabolites and cell viability*

309 In order to reveal the contribution of compounds to the cytotoxic activity,
310 we analyzed the Pearson correlation coefficients between the mean values of
311 metabolites levels and of % cell viability (Figure 5). We were particularly
312 interested in the compounds present in the AFs. Notably, three polyamines
313 (feruloyl putrescine, bis dihydrocaffeoyl spermidine, and tris dihydrocaffeoyl

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314 spermine) (Figure 5A) and two glycoalkaloids (solanine and chaconine) (Figure
315 5B) exerted negative and significant correlation ($R^2 \geq 0.5743$). The correlation
316 was more evident for feruloyl putrescine ($R^2=0.9265$) and chaconine
317 ($R^2=0.8334$). In addition, the sum of the content of the three polyamines and the
318 two glycoalkaloids negatively correlated with cell viability ($R^2=0.8122$, Figure
319 5C). Results indicate that a higher content of those five metabolites would be
320 accompanied by lesser cell viability, or in other words, by a higher cytotoxic
321 activity. Correlation analysis for the other compounds in AFs was not significant
322 (data not shown).

323

324 ## 4. Discussion

325 This work deepens into the cytotoxic activity of phenolic extracts from
326 Andean potatoes via liquid-liquid fractionation in order to identify the
327 compounds responsible for that activity.

328 TEs of skin and flesh of three Andean potato cultivars were successfully
329 fractionated into OFs and AFs. Results of HPLC-DAD (and HPLC-ESI-MS/MS)
330 corroborated the expected composition of both OFs and AFs. Fractionation was
331 good but not optimal (considering the remaining compounds in each fraction),
332 being AFs purer than OFs. This result indicates that more incubation time
333 and/or more repetitions of successive extractions with ethyl acetate could be
334 required for a better fractionation. Further, the differences between the sum of
335 compounds in OF+AF compared to TE values could be attributed to slight
336 differences in resuspension volumes of the samples after complete dryness,
337 and/or to errors during HPLC-DAD measurement (coming from the equipment
338 or from dilutions performed to avoid column saturation).

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339 HPLC-DAD results indicate that the main metabolite in all potato cultivars
340 and tissues is 5-CGA, with a significant presence of CA in pigmented tissues.
341 This result is consistent with previous reports that indicate that 5-CGA is the
342 most abundant phenolic acid in potatoes, followed by CA (Navarre et al., 2011;
343 Deusser et al., 2012). HPLC-ESI-MS/MS results indicate that the main
344 metabolites are 5-CGA, 3-CGA (accurate mass and RT confirmed with
345 standards), and caffeoyl putrescine; whereas tris dihydrocaffeoyl spermine and
346 chaconine levels are also significantly in skin.

347 Agreeing with the literature (Bontempo et al., 2013; Valiñas et al., 2017),
348 our analysis revealed that red tuber tissues (such as Santa María skin and
349 flesh) contain mainly pelargonidin and peonidin derivatives, whereas purple
350 tuber tissues (such as Moradita skin) harbors mostly derivatives of petunidin,
351 malvidin (both found only by HPLC-DAD) and peonidin. No anthocyanidins were
352 detected in Waicha and Moradita fleshes, which is expected since they lack
353 pigmentation. The discrepancy between anthocyanidin/anthocyanin
354 compositions by HPLC-DAD/HPLC-ESI-MS/MS could be due to differences in
355 sample treatments (resuspension solvent) and/or chromatographic conditions
356 (column, mobile phases). Some differences could be also attributed to the acid
357 hydrolysis prior to HPLC-DAD since all aglycones are recognized as only one
358 peak (e.g. pelargonidin). In contrast, no acid hydrolysis is needed for mass
359 spectrometry analysis because each peak is semi-quantitated (HPLC-ESI-
360 MS/MS) and individually identified (HPLC-HRMS).

361 HPLC-DAD chromatograms showed that besides the quantified
362 compounds, there are some unidentified peaks. HPLC-DAD technique is limited
363 due to the lack of authentic standards for compound identification by

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364 comparison with absorption spectra and RT. By contrast, HPLC-ESI-MS/MS
365 and HPLC-HRMS offer a more complete and accurate identification of
366 metabolites. It should be noted that the levels of specific metabolites (e.g. CGA
367 isomers) by HPLC-ESI-MS/MS are lesser than the ones obtained by HPLC-
368 DAD. However, cis-5-CGA could only be quantified by mass spectrometry.
369 Notably, metabolomic profiles by mass spectrometry have not been described
370 for any of the genotypes included in our study. Also, few comparisons have
371 been made between potato tuber skin and flesh (López-Cobo et al., 2014;
372 Oertel et al., 2017; Valiñas et al., 2017; Nogawa et al., 2019). To conclude, in
373 our investigations, both techniques are complementary and necessary.

374 HPLC-DAD analysis underestimates the content of total phenolics
375 because it only quantifies some of the present compounds. Folin-Ciocalteu
376 assay overestimates the content of total phenolics because it reacts with
377 compounds like vitamins, aminoacids, and thiol derivatives (Everette et al.,
378 2010). Nevertheless, we found a significant positive Pearson correlation
379 between HPLC-DAD and Folin-Ciocalteu data (R^2=0.8612).

380 The assays for selecting cytotoxic doses in human neuroblastoma SH-
381 SY5Y cells show a lesser activity of the extracts than expected, taking into
382 account previous studies from our laboratory (Silveyra et al., 2018). This result
383 could be due to the use of new stock of cells and/or changes in the reagents
384 used for cell culture.

385 Together, results obtained by Folin-Ciocalteu and the assay of cytotoxic
386 doses in SH-SY5Y cells, it can be concluded that a higher concentration of total
387 phenolics does not imply a higher cytotoxic activity. For example, among the
388 skins of the three cultivars, Waicha has the lesser total phenolics content (3640

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389 µg/mL) and requires the lesser concentration (10 µg/mL) to induce cell
390 mortality. Santa María skin has the highest level of total phenolics (16480
391 µg/mL) but requires the highest dose (100 µg/mL) to exert approximate 50%
392 cell viability. A similar result was obtained for inhibiting cell proliferation in
393 human breast cancer by potato extracts (Leo et al., 2008). Therefore, levels of
394 specific compounds and/or interactions between different phytochemicals could
395 be essential in inducing cytotoxic activity.

396 Concerning assays with SH-SY5Y cells, we observed a similar pattern for
397 the TEs and their fractions in all the tested cultivars and tissues. OFs, rich in
398 HCCs, were not cytotoxic in any case. In Waicha skin and flesh, they even
399 provoked an increase in mitochondrial activity. Since treatments with 200-400
400 µg/mL of commercial 5-CGA were cytotoxic (Silveyra et al., 2018), we speculate
401 that the absence of activity in OFs could be due to lesser doses of 5-CGA or to
402 other components that antagonize the effect of 5-CGA. Conversely, AFs were
403 cytotoxic in all cases, even in Waicha and Moradita fleshes which lack
404 anthocyanins. Although numerous studies support the cytotoxic activity of
405 potato extracts rich in anthocyanins against cancer cells (Reddivari et al., 2007;
406 Bontempo et al., 2013; Vizzotto et al., 2014; Ombra et al., 2015), our study
407 indicates that other components could be involved in inducing cell death.
408 Results showed that treatments with OF+AF were cytotoxic with values near but
409 not identical to the corresponding TEs. This result could be due to experimental
410 errors generated by variations in the concentration when OFs and AFs are
411 combined instead of TEs.

412 We used an untargeted mass spectrometry-metabolomics approach in
413 order to gain information about bioactive compounds. Based on our

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414 observations, we can hypothesize that a combination of three polyamines
415 (feruloyl putrescine, bis dihydrocaffeoyl spermidine and tris dihydrocaffeoyl
416 spermine) and two glycoalkaloids (solanine and chaconine) are mainly
417 responsible for the observed cytotoxic activity. This result reveals the
418 nutraceutical properties and interactions of metabolites in a complex food
419 matrix, like the potato. Since Santa María AF had major cytotoxic activity than
420 the corresponding TE, we could even suggest that other compounds antagonize
421 their effects. The next step could involve solid-phase fractionation to specifically
422 recover glycoalkaloids, as was described by Sánchez Maldonado et al. 2014.
423 Chaconine and solanine commercial standards induced 50% cytotoxicity
424 against five mammalian cell lines at 2.9 μM and 18 μM, respectively (Nogawa et
425 al., 2019). In addition, polyamines conjugated with phenolic acids (also called
426 phenolamides) have been shown to exert anticancer properties in various
427 cellular models (Roumani et al., 2020). In sum, future experiments should be
428 directed to study the response of SH-SY5Y cells to treatments with different
429 doses of individual or subgroups of bioactive metabolites. It would be interesting
430 to determine individual compounds with cytotoxic activity by isolation with
431 preparative HPLC, and the mechanisms of antitumoral activity. The cytotoxic
432 effects of potato extracts involved the induction of apoptosis by pathways
433 dependent and independent of caspases in prostate cancer cell lines (Reddivari
434 et al., 2007). Other researchers have observed that extracts of Vitelotte cultivar
435 cause dose-dependent inhibition of proliferation and induction of apoptosis in
436 breast, cervix, prostate, and leukemia cancers (Bontempo et al., 2013).

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437 This work contributes to potato revalorization as a functional food. It
438 enlarges the knowledge of potato polyphenols' bioactivity as promising sources
439 of natural products with an added value for the food industry and human health.
440

441 5. Conclusions

442 The present study demonstrates that a complex interplay of polyphenolic
443 compounds contributes to potato benefits. It also suggests that conjugated
444 polyamines and glycoalkaloids are critical in inducing cytotoxicity against human
445 neuroblastoma cells *in vitro*. Our results are of fundamental interest due to the
446 nutritional, biological, and industrial contribution. Further studies are needed to
447 identify and isolate the active compounds, determine their bioavailability, and
448 confirm their neuroprotection *in vivo*.
449

450 Contributions

451 M.L.L. and M.X.S. designed and instructed the research work. M.L.L., M.X.S.,
452 M.M.M., S.B., D-D.S-G. and F.P. performed the experiments. M.L.L. wrote the
453 manuscript. M.L.L., M.X.S., and A.B.A. provided funding for this work. All
454 authors commented on the manuscript and approved the final article.
455

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478

479 **Conflict of interest statement**

480 The authors declare that there is no conflict of interest regarding the publication
481 of this article.

482

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483 **Supporting information description**

484 **Supplemental Figure 1.** HPLC-DAD chromatograms of TE, OF, and AF from
485 Santa María skin and flesh. Profiles for HCCs were obtained at 320 nm and at
486 510 nm for anthocyanidins. 5-CGA, chlorogenic acid; CA, caffeic acid; FA,
487 ferulic acid; Cy, cyanidin; Pl, pelargonidin; Pn, peonidin; Mv, malvidin.

488

489 **Supplemental Figure 2.** HPLC-DAD chromatograms of TE, OF, and AF from
490 Waicha skin and flesh. Profiles for HCCs were obtained at 320 nm and at 510
491 nm for anthocyanidins. 5-CGA, chlorogenic acid; CA, caffeic acid; CouA,
492 coumaric acid; FA, ferulic acid; Dp, delphinidin; Cy, cyanidin; Pt, petunidin; Pl,
493 pelargonidin; Pn, peonidin; Mv, malvidin.

494

495 **Supplemental Figure 3.** HPLC-DAD chromatograms of TE, OF, and AF from
496 Moradita skin and flesh. Profiles for HCCs were obtained at 320 nm and at 510
497 nm for anthocyanidins. 5-CGA, chlorogenic acid; CA, caffeic acid; CouA,
498 coumaric acid; FA, ferulic acid; Dp, delphinidin; Cy, cyanidin; Pt, petunidin; Pl,
499 pelargonidin; Pn, peonidin; Mv, malvidin.

500

501 **Supplemental Figure 4.** TIC chromatograms of TE, OF, and AF from Santa
502 María skin and flesh. HPLC-ESI-MS/MS in positive ion mode.

503

504 **Supplemental Figure 5.** TIC chromatograms of TE, OF, and AF from Waicha
505 skin and flesh. HPLC-ESI-MS/MS in positive ion mode.

506

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507 **Supplemental Figure 6.** TIC chromatograms of TE, OF, and AF from Moradita
508 skin and flesh. HPLC-ESI-MS/MS in positive ion mode.
509

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640

641 **Figure captions**

642 **Figure 1.** Simplified scheme for the obtention of potato extracts, the protocol of
643 fractionation, and other assays done. TFA, trifluoroacetic acid.

644

645 **Table 1.** The total content of HCCs and anthocyanidins in TE, OF, AF, and
646 OF+AF from tuber skin and flesh of Santa María, Waicha, and Moradita
647 cultivars. Levels of metabolites were determined by HPLC-DAD and expressed
648 as µg/g DW. Values of OF+AF are theoretical and correspond to the sum of OF

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649 and AF in each case. Percentages between parentheses represent the recovery
650 to the corresponding TE. ND, not detected.

651

652 **Table 2.** Content of individual HCCs and anthocyanidins in TE, OF, AF, and
653 OF+AF from skin and flesh of the three Andean potato cultivars. Levels of
654 metabolites were determined by HPLC-DAD and expressed as μg/g DW. ND,
655 not detected.

656

657 **Table 3.** Identification data of the 30 compounds detected by HPLC-ESI-MS/MS
658 in potato samples. #, peak number; *, validated by standard; caf, caffeoyl;
659 coum, coumaroyl; cy, cyanidin; deoxyhex: deoxyhexose; ESI, electrospray
660 ionization; fer, feruloyl; F1, form 1; F2, form 2; glc, glucose; hex, hexose; pl,
661 pelargonidin; pn, peonidin; RT, retention time; rut, rutinoside.

662

663 **Table 4.** Content of individual compounds by HPLC-ESI-MS/MS in potato
664 samples. Levels of metabolites were expressed as μg apigenin equivalents/g
665 DW. ND, not detected.

666

667 **Table 5.** Total phenolics content in TE by the Folin-Ciocalteu assay. Results are
668 expressed as μg 5-CGA equivalents/mL.

669

670 **Figure 2.** Cytotoxic activity of TEs from skin and flesh of Santa María, Waicha,
671 and Moradita cultivars against human neuroblastoma SH-SY5Y cells. Doses
672 are expressed as μg 5-CGA equivalents/mL. Values represent the average ±

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673 SD from at least three experiments with three technical repetitions. One way
674 ANOVA multiple comparisons was followed by Dunnet´s test. ****, significant
675 differences with P <0.0001; Control, non-treated cells; Methanol, cells treated
676 with methanol ≤3% v/v.
677
678 **Figure 3.** SH-SY5Y cells were treated for 18 h with TE, OF, AF, and OF+AF
679 from Waicha skin (10 μg/mL) and flesh (300 μg/mL). Control, non-treated cells;
680 Methanol, cells treated with methanol ≤3% v/v. Cells were examined under
681 inverted microscopy (10X). The bar represents 200 μm.
682
683 **Figure 4.** Cytotoxic activity of TE, OF, AF, and OF+AF from skin and flesh of
684 Santa María, Waicha, and Moradita cultivars against human neuroblastoma SH-
685 SY5Y cells. Doses are expressed as μg 5-CGA equivalents/mL. Values
686 represent the average ± SD from at least three experiments with three technical
687 repetitions. One way ANOVA multiple comparisons was followed by Dunnet´s
688 test. **, significant differences with P<0.005; ***, significant differences with
689 P<0.001; ****, significant differences with P<0.0001; Control, non-treated cells;
690 Methanol, cells treated with methanol ≤3% v/v.
691
692 **Figure 5.** Correlation analysis between the content of individual metabolites and
693 % cell viability in human neuroblastoma SH-SY5Y cells. A, Pearson correlation
694 coefficients for the polyamines feruloyl putrescine, bis dihydrocaffeoyl
695 spermidine, and tris dihydrocaffeoyl spermine. B, Pearson correlation
696 coefficients for the glycoalkaloids solanine and chaconine. C, Pearson

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697 correlation coefficients for the sum of the three polyamines and the two
698 glycoalkaloids.

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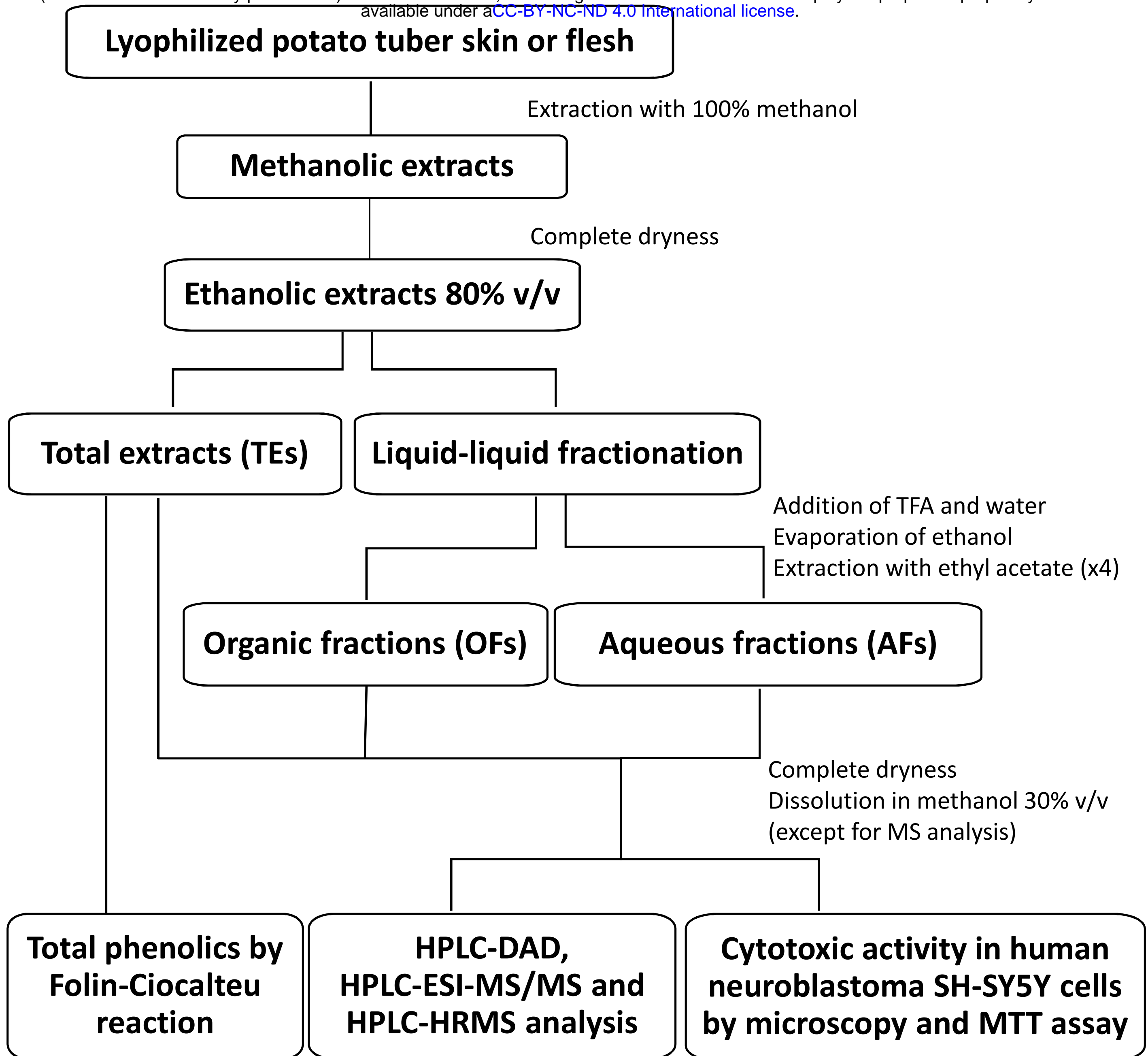

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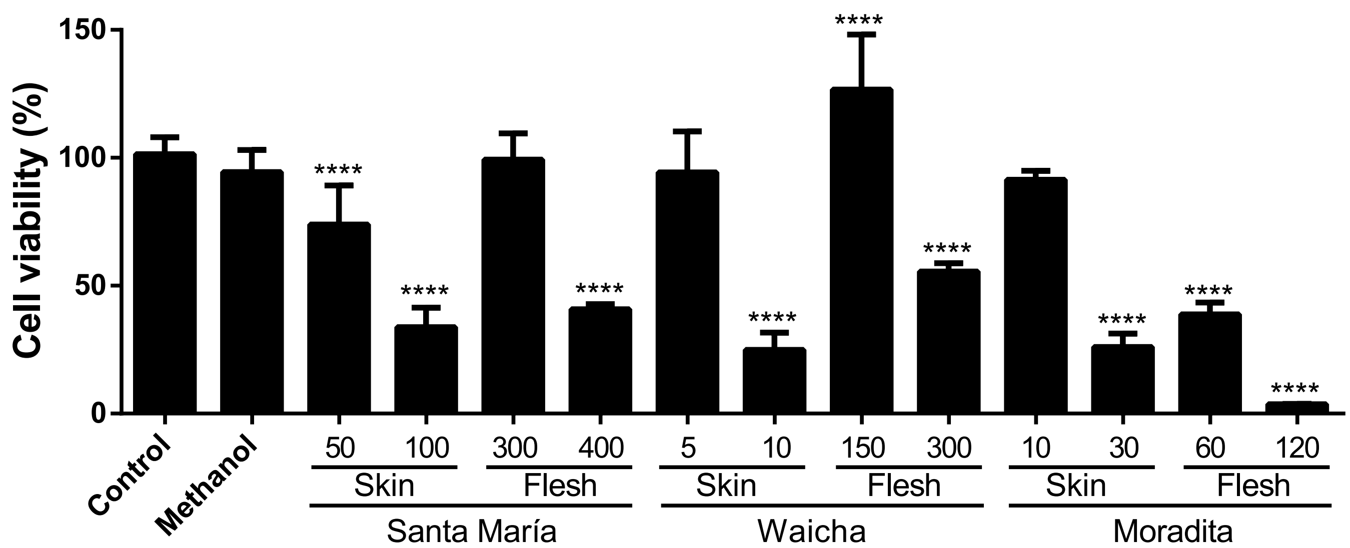

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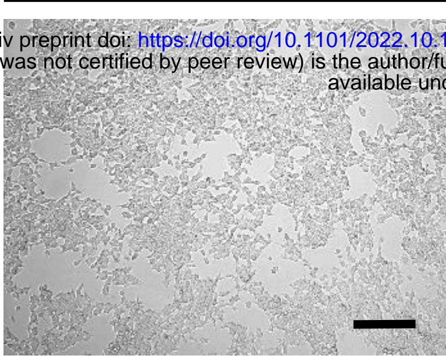
Control



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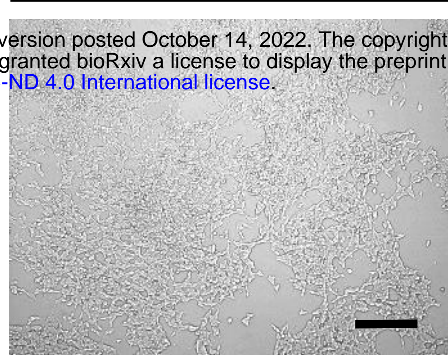
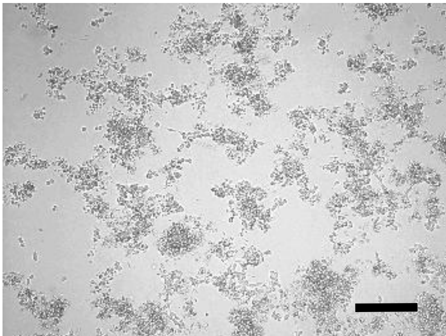


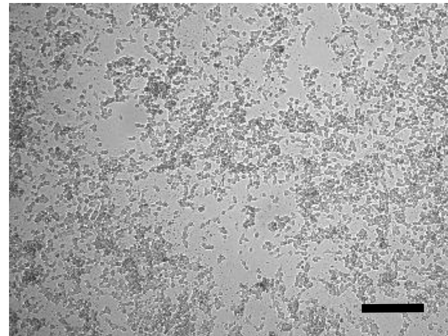
Waicha

Skin

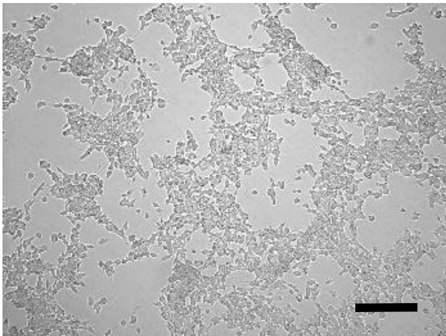
Flesh

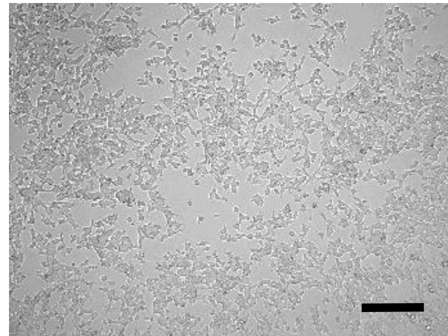
TE





OF





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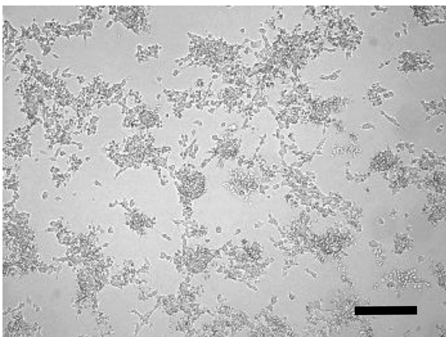
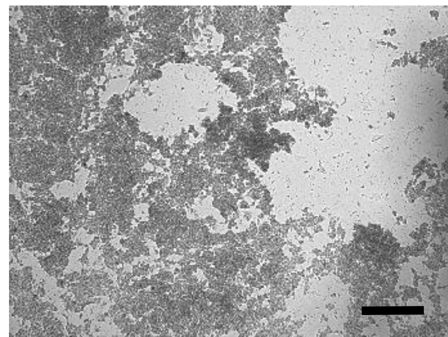
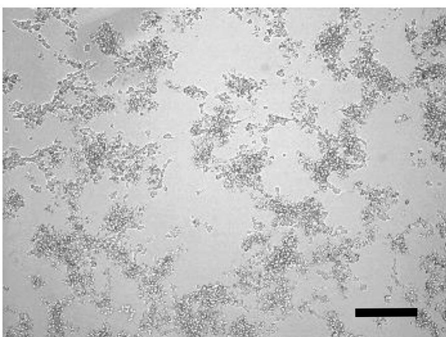
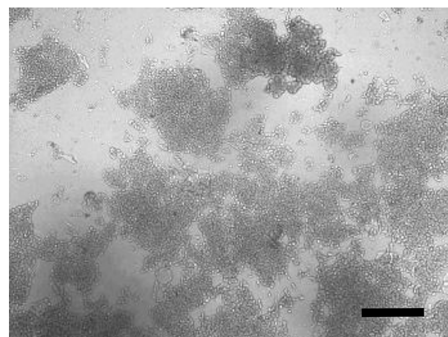




OF+AF





Cytotoxic activity-fractions.pzf:Layout 2 - Wed Jul 13 20:30:36 2022
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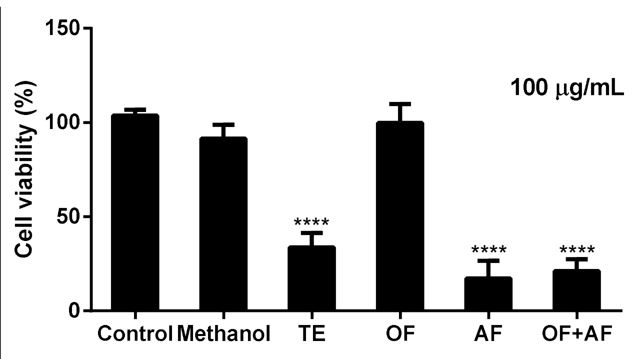
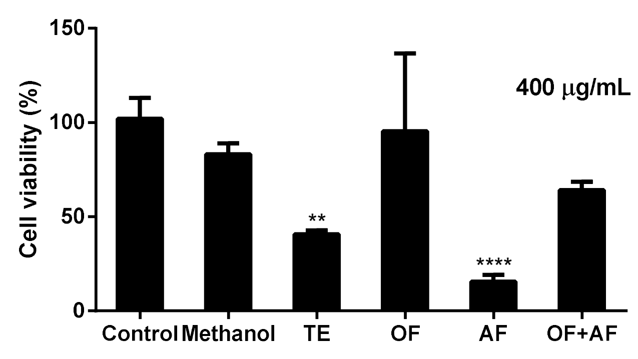
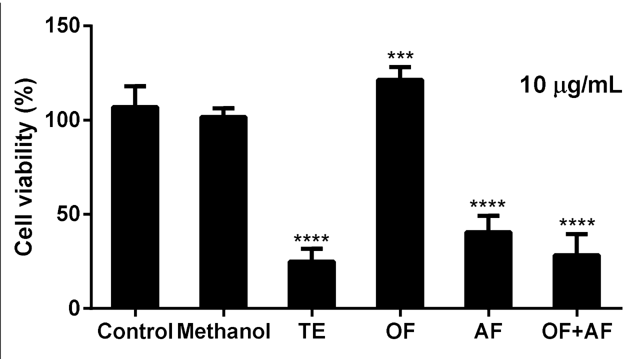
Skin | Flesh

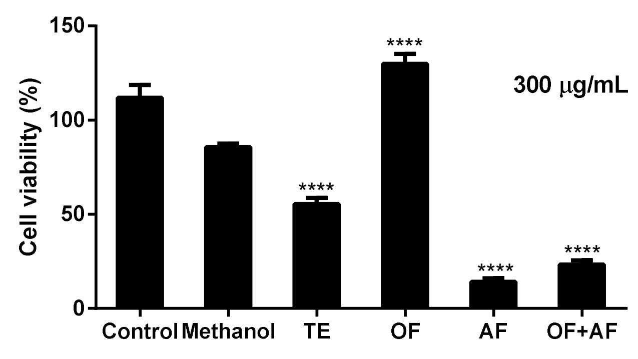
Santa María



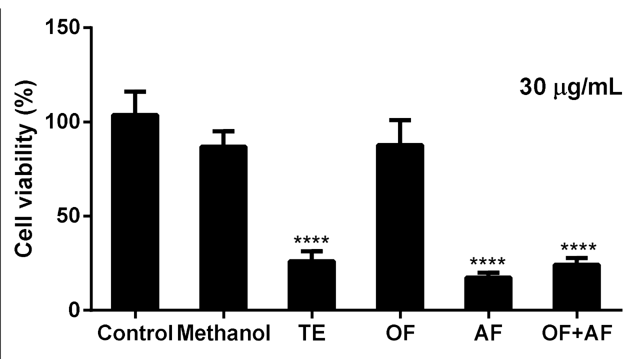


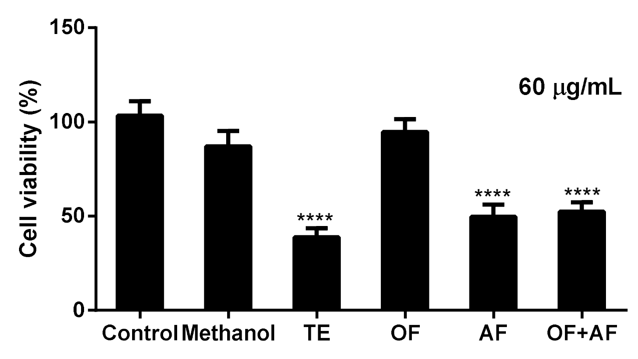
Waicha





Moradita





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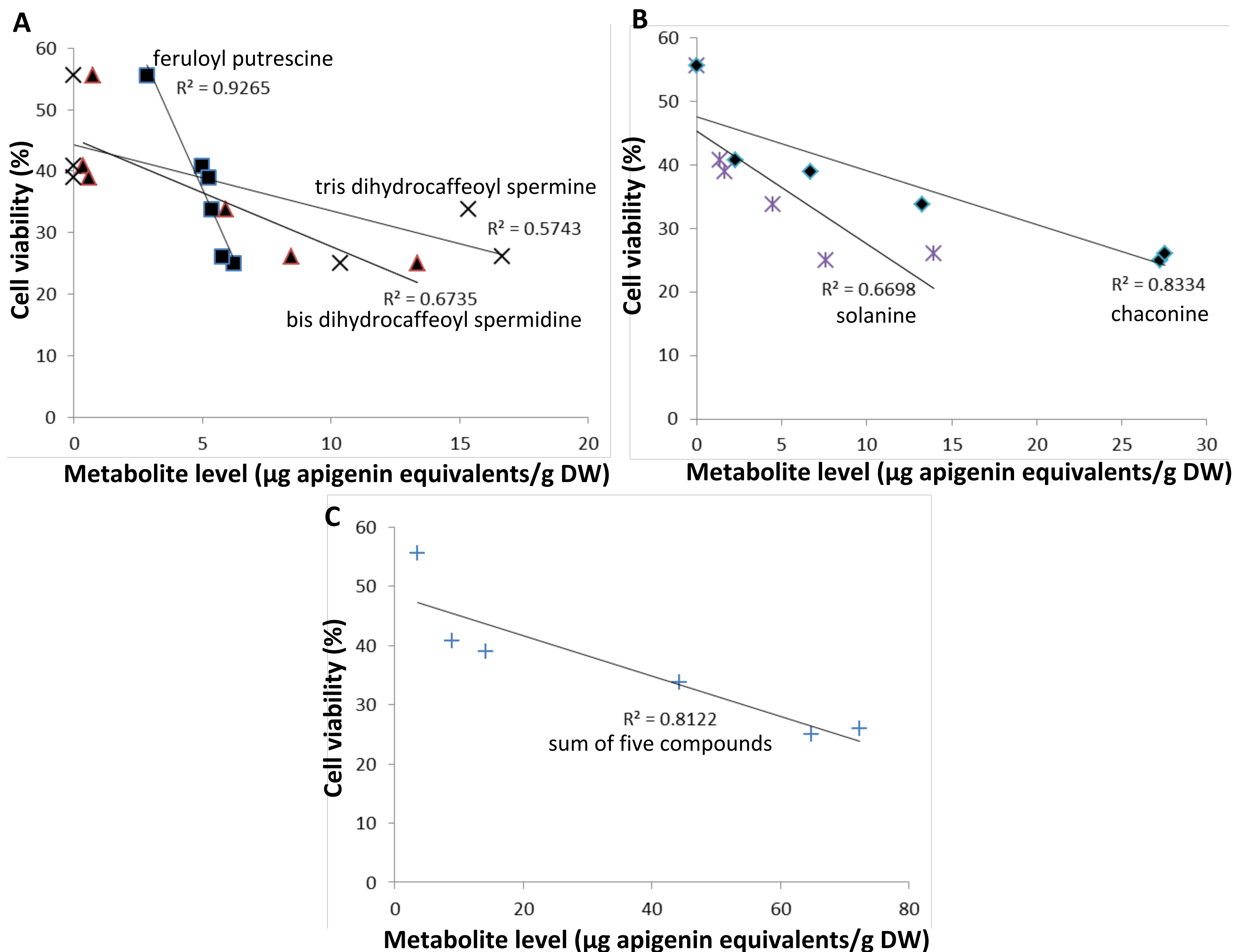

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Cultivar	Compounds	Skin				Flesh			
		TE	OF	AF	OF+AF	TE	OF	AF	OF+AF
Santa María	HCCs	2453.61 (100)	2018.67 (82.3)	264.31 (10.8)	2282.98 (93.0)	1663.41 (100)	1322.72 (79.5)	34.50 (2.1)	1357.22 (81.6)
	Anthocyanidins	581.95 (100)	89.99 (14.9)	544.55 (93.6)	631.54 (108.5)	345.37 (100)	32.03 (9.3)	390.41 (113)	422.44 (122)
Waicha	HCCs	666.28 (100)	340.54 (51.1)	96.12 (14.4)	436.66 (65.5)	373.98 (100)	267.63 (71.6)	69.50 (18.6)	337.13 (90.1)
	Anthocyanidins	60.41 (100)	13.09 (21.7)	58.78 (97.3)	71.87 (118.9)	ND	ND	ND	ND
Moradita	HCCs	1809.54 (100)	1609.11 (88.9)	228.71 (12.6)	1837.82 (101.6)	104.43 (100)	94.26 (90.3)	7.32 (7.0)	101.58 (97.3)
	Anthocyanidins	158.21 (100)	11.87 (7.5)	138.37 (87.5)	150.24 (95)	ND	ND	ND	ND

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	Santa María							
	Skin				Flesh			
Compounds	**TE**	**OF**	**AF**	**OF+AF**	**TE**	**OF**	**AF**	**OF+AF**
5-CGA	2056.91	1656.57	100.08	1756.65	1495.93	1188.89	24.34	1213.24
4-CGA	217.89	219.51	87.80	307.32	121.14	97.72	8.78	106.50
3-CGA	121.14	112.20	76.42	188.62	46.34	36.10	1.38	37.48
CA	57.67	30.40	ND	30.40	ND	ND	ND	ND
CouA	ND	ND	ND	ND	ND	ND	ND	ND
FA	ND	ND	ND	ND	ND	ND	ND	ND
Total HCCs	**2453.61**	**2018.67**	**264.31**	**2282.98**	**1663.41**	**1322.72**	**34.50**	**1357.22**
Delphinidin	ND	ND	ND	ND	ND	ND	ND	ND
Cyanidin	2.28	ND	2.28	2.28	1.46	ND	1.14	1.14
Petunidin	ND	ND	ND	ND	ND	ND	ND	ND
Pelargonidin	468.29	73.33	429.27	502.60	241.63	32.03	262.11	294.15
Peonidin	111.38	13.66	113.01	126.67	94.47	ND	99.02	99.02
Malvidin	ND	ND	ND	ND	7.80	ND	28.13	28.13
Total anthocyanidins	**581.95**	**86.99**	**544.55**	**631.54**	**345.37**	**32.03**	**390.41**	**422.44**
Total	**3035.56**	**2105.67**	**808.86**	**2914.53**	**2008.78**	**1354.75**	**424.91**	**1779.66**

	Waicha							
	Skin				Flesh			
Compounds	**TE**	**OF**	**AF**	**OF+AF**	**TE**	**OF**	**AF**	**OF+AF**
5-CGA	217.28	106.71	15.65	122.36	190.41	155.69	25.20	180.89
4-CGA	189.63	93.70	27.24	120.93	109.11	76.50	19.35	95.85
3-CGA	176.73	72.15	48.78	120.93	70.73	33.09	24.63	57.72
CA	59.37	62.65	4.46	67.11	0.21	0.10	0.28	0.37
CouA	1.49	0.79	ND	0.79	0.61	0.75	ND	0.75
FA	21.79	4.54	ND	4.54	2.91	1.50	0.04	1.54
Total HCCs	**666.28**	**340.54**	**96.12**	**436.66**	**373.98**	**267.63**	**69.50**	**337.13**
Delphinidin	ND	ND	ND	ND	ND	ND	ND	ND
Cyanidin	ND	ND	ND	ND	ND	ND	ND	ND
Petunidin	ND	ND	ND	ND	ND	ND	ND	ND
Pelargonidin	34.80	8.46	33.50	41.95	ND	ND	ND	ND
Peonidin	9.51	1.30	8.94	10.24	ND	ND	ND	ND
Malvidin	16.10	3.33	16.34	19.67	ND	ND	ND	ND
Total anthocyanidins	**60.41**	**13.09**	**58.78**	**71.87**	**ND**	**ND**	**ND**	**ND**
Total	**726.69**	**353.63**	**154.90**	**508.53**	**373.98**	**267.63**	**69.50**	**337.13**

	Moradita							
	Skin				Flesh			
Compounds	**TE**	**OF**	**AF**	**OF+AF**	**TE**	**OF**	**AF**	**OF+AF**
5-CGA	1484.15	1322.36	128.86	1451.22	85.37	75.20	7.32	82.52
4-CGA	171.14	144.31	59.76	204.07	18.70	18.29	ND	18.29
3-CGA	65.04	52.03	39.43	91.46	ND	ND	ND	ND
CA	88.91	90.19	ND	90.19	ND	0.76	ND	0.76
CouA	0.31	0.23	0.66	0.89	ND	ND	ND	ND
FA	ND	ND	ND	ND	0.37	ND	ND	ND
Total HCCs	**1809.54**	**1609.11**	**228.71**	**1837.82**	**104.43**	**94.26**	**7.32**	**101.58**
Delphinidin	0.49	ND	0.49	0.49	ND	ND	ND	ND
Cyanidin	0.16	ND	0.49	0.49	ND	ND	ND	ND
Petunidin	142.76	9.11	122.28	131.38	ND	ND	ND	ND
Pelargonidin	ND	1.46	0.33	1.79	ND	ND	ND	ND
Peonidin	4.88	0.49	5.53	6.02	ND	ND	ND	ND
Malvidin	9.92	0.81	9.27	10.08	ND	ND	ND	ND
Total anthocyanidins	**158.21**	**11.87**	**138.37**	**150.24**	**ND**	**ND**	**ND**	**ND**
Total	**1967.76**	**1620.98**	**367.08**	**1988.07**	**104.43**	**94.26**	**7.32**	**101.58**

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					Validation			Data MS/MS								
#	RT (min)	Family	Putative compound	ESI	MS/MS Parent Mode	MS/MS Daughter Mode	HRMS Accurate Mass	Molecular ion form	Fragmentation of molecular ion [M+H]+ or [M+Na]+							Proposed identification
1	1.73	miscellaneous	sinapine	+		✓		[M]+ 310	292	264	132	120				sinapine
2	2.93	polyamine	caf putrescine	+	✓	✓	✓	[M+H]+ 251	234	163	145	135	117	89		caf putrescine
3	3.13	miscellaneous	unknown	+		✓		[M+H]+ 367								unknown
4	3.18	HCC	neo chlorogenic acid*	+				[M+Na]+ 377/[M-2H+Na]- 375	163	145	135	117	89			3-CGA
5	4.13	miscellaneous	salicylic acid glucoside	-		✓		[M-H]- 299	221	197	137	93				salicylic acid glucoside
6	4.25	anthocyanin	pn hex deoxyhex hex	+	✓	✓		[M]+ 771	609	463	301					pn 3-O-rut 5-O-glc
7	4.76	HCC	chlorogenic acid*	-				[M+Na]+ 377/[M-2H+Na]- 375	163	145	135	117	89			5-CGA
8	4.88	polyamine	fer putrescine	+	✓	✓		[M+H]+ 265	177	145	117	89	72			fer putrescine
9	5.08	HCC	crypto chlorogenic acid*	-				[M-H]- 353	163	145	135	117	89			4-CGA
10	5.84	HCC	chlorogenic acid cis isomer*	-				[M-H]- 353	163	145	135	117	89			cis-5-CGA
11	5.95	polyamine	bis dihydrocaf spermidine	+		✓	✓	[M+H]+ 474	474	457	236	222	166			bis dihydrocaf spermidine
12	6.4	non-anthocyanin flavonoid	putative flavonoid	+ / -				[M+Na]+ 473/[M-H]- 449	473	311	185					eriodictyol-hex
13	7.45	anthocyanin	pl hex caf deoxyhex hex	+	✓	✓		[M]+ 903	741	433	271					pl 3-O-caf-rut 5-O-glc
14	7.7	anthocyanin	pn hex caf deoxyhex hex	+	✓	✓		[M]+ 933	771	463	301					pn 3-O-caf-rut 5-O-glc
15	7.8	anthocyanin	cy deoxyhex hex deoxyhex	+	✓	✓		[M]+ 903	741	595	449	433	287			cy 3-O-rut 5-O-deoxyhex
16	8.08	polyamine	tris dihydrocaf spermine	+	✓	✓	✓	[M+H]+ 695	531	474	457	293	222			tris dihydrocaf spermine
17	8.29	anthocyanin	pl hex coum deoxyhex hex F1	+	✓	✓		[M]+ 887	725	433	271					pl 3-O-coum-rut 5-O-glc F1
18	8.3	non-anthocyanin flavonoid	putative flavonoid with phenolic acid	+ / -		✓		[M+Na]+ 927/[M-H]- 903	909	765	747	473	455	311	293	putative flavonoid with phenolic acid
19	8.53	anthocyanin	pn hex coum deoxyhex hex	+	✓	✓		[M]+ 917	755	463	301					pn 3-O-coum-rut 5-O-glc
20	8.53	anthocyanin	pl hex fer deoxyhex hex F1	+	✓	✓		[M]+ 917	755	433	271					pl 3-O-fer-rut 5-O-glc F1
21	8.81	anthocyanin	pl hex coum deoxyhex hex F2	+	✓	✓		[M]+ 887	725	433	271					pl 3-O-coum-rut 5-O-glc F2
22	8.84	anthocyanin	pn fer deoxyhex hex hex	+	✓	✓		[M]+ 947	785	463	301					pn 3-O-fer-rut 5-O-glc
23	8.84	non-anthocyanin flavonoid	putative flavonoid with phenolic acid	+ / -		✓		[M+Na]+ 927/[M-H]- 903	765	747	473	455	311	293		putative flavonoid with phenolic acid
24	9.22	non-anthocyanin flavonoid	rutin	+ / -		✓	✓	[M+Na]+ 617/[M-H- 593	471	331	309	186				rutin
25	9.63	non-anthocyanin flavonoid	putative flavonoid with phenolic acid	+ / -		✓		[M+Na]+ 927/[M-H]- 903	909	765	747	473	454	311	293	putative flavonoid with phenolic acid
26	9.79	anthocyanin	pl hex fer deoxyhex hex F2	+	✓	✓		[M]+ 917	755	433	271					pl 3-O-fer-rut 5-O-glc F2
27	12.1	anthocyanin	pl hex -306 F1	+	✓	✓		[M]+ 739	577	433	271	253				pl hex F1
28	12.29	glycoalkaloid	solanine	+ / -		✓	✓	[M+H]+ 868	866	704	558					solanine
29	12.35	anthocyanin	pl hex -306 F2	+	✓	✓		[M]+ 739	577	433	271	253				pl hex F2
30	12.42	glycoalkaloid	chaconine	+ / -			✓	[M+H]+ 852								chaconine

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		Santa María		Waicha		Moradita	
#	**Proposed identification**	**Skin**	**Flesh**	**Skin**	**Flesh**	**Skin**	**Flesh**
1	sinapine	6.98	10.55	2.01	6.81	14.47	13.96
2	caf putrescine	15.28	19.84	8.04	8.34	8.03	8.41
3	unknown	5.40	15.51	0.54	1.60	13.62	17.64
4	3-CGA	12.56	10.54	15.28	4.20	17.77	0.81
5	salicylic acid glucoside	2.14	3.06	1.58	1.40	1.90	3.37
6	pn 3-O-rut 5-O-glc	5.16	4.05	0.77	ND	1.16	ND
7	5-CGA	16.91	19.23	2.32	0.50	15.57	0.77
8	fer putrescine	5.33	4.95	6.22	2.83	5.75	5.24
9	4-CGA	3.50	4.14	3.58	0.38	3.70	ND
10	cis-5-CGA	0.54	0.19	0.09	ND	0.65	0.05
11	bis dihydrocaf spermidine	5.91	0.38	13.36	0.75	8.44	0.58
12	eriodictyol-hex	0.15	0.13	ND	ND	ND	ND
13	pl 3-O-caf-rut 5-O-glc	0.13	0.09	ND	ND	ND	ND
14	pn 3-O-caf-rut 5-O-glc	0.17	0.19	ND	ND	2.69	ND
15	cy 3-O-rut 5-O-deoxyhex	0.05	0.03	ND	ND	ND	ND
16	tris dihydrocaf spermine	15.36	ND	10.35	ND	16.67	ND
17	pl 3-O-coum-rut 5-O-glc F1	2.74	4.15	0.24	ND	0.04	ND
18	putative flavonoid with phenolic acid	5.66	5.96	0.37	ND	ND	ND
19	pn 3-O-coum-rut 5-O-glc	0.43	0.30	0.12	ND	ND	ND
20	pl 3-O-fer-rut 5-O-glc F1	0.21	0.54	0.02	ND	0.10	ND
21	pl 3-O-coum-rut 5-O-glc F2	0.43	0.45	0.05	ND	ND	ND
22	pn 3-O-fer-rut 5-O-glc	0.17	0.06	0.03	ND	0.05	ND
23	putative flavonoid with phenolic acid	0.18	0.19	0.02	ND	ND	ND
24	rutin	0.13	0.18	0.03	ND	ND	ND
25	putative flavonoid with phenolic acid	0.05	0.17	ND	ND	ND	ND
26	pl 3-O-fer-rut 5-O-glc F2	ND	0.09	ND	ND	ND	ND
27	pl hex F1	0.04	0.02	ND	ND	ND	ND
28	solanine	4.50	1.39	7.63	ND	13.96	1.67
29	pl hex F2	0.23	0.08	ND	ND	ND	ND
30	chaconine	13.28	2.28	27.27	ND	27.57	6.72
	Total	123.62	108.77	99.92	26.81	152.16	59.22

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Cultivar	Tissue	Total phenolics (µg 5-CGA equivalents/mL)
Santa María	Skin	16480
	Flesh	6280
Waicha	Skin	3640
	Flesh	990
Moradita	Skin	9160
	Flesh	1500