International Journal of Food Science +Technology

Institute of Food Science +Technology ifst

Original article

First accurate profiling of antioxidant anthocyanins and flavonols of *Tibouchina urvilleana* and *Tibouchina mollis* edible flowers aided by fractionation with Amberlite XAD-7

Natalia Solarte,[1] María Jesús Cejudo-Bastante,[2]* Nelson Hurtado[1] & Francisco J. Heredia[2]

1 Departamento de Química, Grupo de Investigación en Productos de Importancia Biológica (GIPIB), Universidad de Nariño, San Juan de Pasto, Colombia

2 Food Colour and Quality Laboratory, Facultad de Farmacia, Universidad de Sevilla, Sevilla, Spain

(Received 22 October 2021; Accepted in revised form 21 January 2022)

Summary A purification and fractionation process of the edible flowers of *Tibouchina mollis* and *Tibouchina urvilleana* followed by the first attempt to the anthocyanin and flavonol characterisation and identification by UHPLC-DAD-ESI-MS were developed. *T. urvilleana* exhibited a higher monomeric anthocyanin content, mainly due to the presence of the 3-*O*-(6′-*p*-coumaroyl)-glucoside derivatives of malvidin and petunidin. Quercetin-3-*O*-hexoside was the major flavonol identified in *T. urvilleana*, and the lack of myricetin derivatives was also exhibited. The anthocyanin and flavonol profile of *T. mollis* was more miscellaneous, characterised by the occurrence of cyanidin-3-*O*-glucoside followed by the 3-*O*-(6′-*p*-coumaroyl)-glucoside and 3-*O*-glucoside derivatives of malvidin and petunidin as anthocyanins, and myricetin, quercetin, and 3-*O*-hexosides of kaemperol and quercetin as flavonol compounds. Therefore, the anthocyanin and flavonol profile, through a process based on purification and fractionation, could be a useful tool to ensure the authenticity of the *Tibouchina*. Furthermore, the purification process made the antioxidant activity increase, which is greatly correlated to the reduction capacity.

Keywords Antioxidant capacity, edible flowers, flavonoids, purification, UHPLC-DAD-ESI-MS.

Introduction

Nowadays, the growth of new natural foods with health benefits, together with that providing a gastronomical innovation, is increasing. In this sense, apart from the traditional ornamental usages because of their attractive colour, edible flowers are being considered as an opportunity for diversification in the agroalimentary market for both reasons. *Tibouchina* belongs to the *Melastomataceae* family, the seventh family of flower plants more diverse worldwide, with 180 genera and 300–350 distributed throughout the rain forests of Mexico, the Caribbean, and South America (Ruilova & Marques, 2016; Fernández-Sánchez *et al*., 2020). Colombia has a high diversity at neotropical level, with approximately 62 genus and 900 species of *Tibouchina*, also commonly known as mayo, pucasacho, pucayanta (Nariño), sietecueros (Antioquia y Quindio), Shenagufa (Putumayo), San Juanito, and Castaño (Cauca) (Almeda *et al*., 2015).

*Correspondent
E-mail: mjcejudo@us.es

In terms of the potential health-enhancing properties, some antioxidant properties and biological activities, commonly related to the pigment profile, have been ascribed to these edible flowers. Among them, it is noteworthy the antimicrobial activity of the leaves of *Tibouchina candolleana* (Durães Vieira *et al*., 2019), antioxidant and anti-inflammatory activities of *Tibouchina pulchra* and *Tibouchina granulosa* (Esposito & Domingos, 2014; Sobrinho *et al*., 2017), and anti-inflammatory activities of *Tibouchina urvilleana* (Pérez-Castorena, 2014; Gowthami & Naga Lakshmi, 2021). Further health-related properties as anti-leishmanial, anti-inflammatory, and antimicrobial activities were ascribed to *Tibouchina paratropica* (Tracanna *et al*., 2015) and antioxidant activity and cytotoxicity in THP-1 cell parasitic activity to *Tibouchina francavillana* and *Tibouchina lhotzkyana* (Bomfim *et al*., 2022).

Related to the pigment profile, among the authors that deepen the anthocyanin profile, Rezende *et al*. (2019) assigned petunidin and malvidin derivatives, with different substitution patterns, as the only anthocyanins of *T. pulchra* (Cham.) Cogn, whereas

doi:10.1111/ijfs.15600

other derivatives of malvidin and malvidin 3-(*p*-coumarylglucoside)-5-acetylxyloside stood out as major anthocyanins of *Tibouchina lepidota* (Bonpl.) Baill (Hendra & Keller, 2016), and *T. urvilleana* (Pérez-Castorena, 2014), respectively. Peonidin 3-sophoroside, peonidin 3-sambubioside, malvidin 3,5-diglucoside, and malvidin 3-(*p*-coumaroyl)-sambubioside-5-glucoside were identified in *Tibouchina ciliaris* (Vent.) Cogn (Rezende *et al*., 2019) and other complex antho cyanins such as malvidin 3-(*p*-coumaryl-glucoside)-5-(acetyl-xyloside) and malvidin 3-*O*-(6-*O*-*p*-coumaryl-β-D-glucopyranoside)-5-*O*-(2-*O*-acetyl-β-D-xylopyranosyl) were detected in *T. granulosa* (Desr.) Cogn., and *Tibouchina semidecantra* Cogn. (Rezende *et al*., 2019). In the same framework described for anthocyanins, *T. pulchra* (Cham.) Cogn were rich in hexoside derivatives of quercetin, myricetin, and kaempferol (Motta *et al*., 2005), quercetin-3-*O*-glucoside was established as the major flavonol of *T. lepidota* (Bonpl.) Baill (Hendra & Keller, 2016), and different quercetin derivatives were identified in *Tibouchina semidecandra* L. (Sirat *et al*., 2010). However, the genus *Tibouchina* spp. has been very scarcely studied in terms of the anthocyanin and flavonol profiles, which could be due to the lack of the application of fractionation techniques that favor the identification of a large number of flavonoid compounds.

Therefore, the aim of this work is to use fractionation techniques that provide a more exhaustive characterisation of the phenolic profile, to open up the range of *Tibouchina* species to be described to make them industrially valuable. In this respect, *Tibouchina mollis* and *T. urvilleana* are species not previously studied. *T. mollis* grows at an altitude between 2500 and 3400 m above the mean sea level and it could reach up to 8 m in height. It is characterised by its stems and branches with abundant thin orange-coloured rhytidomes, pinkish petals up to 1.5 cm long, and capsular fruit with yellowish-brown seeds. *T. urvilleana* is a medium-sized shrub of around 2–3 m in height. It has an upright, moderately branched habit and persistent leaves, whose flowers are purplish blue-violet, and a hairy calyx that grows at the ends of the stem (Almeda *et al*., 2015).

Overall, the present work deals with the accurate chemical characterisation of different species of wild-grown Colombian *Tibouchina* (*T. urvilleana* and *T. mollis*), since, to the best of our knowledge, any detailed study concerning anthocyanins and flavonols compounds has been previously performed in these edible flowers. To achieve this, a methodology of purification and fractionation with Amberlite XAD-7 was carried out, that permits accurate identification of anthocyanin and flavonols fractions by UHPLC-DAD-ESI-MS. The evaluation of the antioxidant activity and Folin–Ciocalteau reduction capacity (FCRC) of the pure anthocyanin and flavonols fractions was undergone to assess these edible flowers as a potential source of health-related compounds.

Material and methods

Chemical and reagents

All solvents (Folin Ciocalteau reagent, methanol, acetonitrile, and water), standards (cyanidin-3-*O*-glucoside, gallic acid, and Trolox), acetic acid, sodium bicarbonate, and ethanol were provided by Sigma–Aldrich (St. Louis, MO, USA).

Plant material

T. mollis and *T. urvilleana* samples were harvested in San Fernando and Nariño (Colombia), respectively. Both plantations were located at an altitude of 2800 m and 2472 m above sea level, respectively. An example of the flowers is stored in the Universidad de Nariño (code numbers 45801 and 45802 for *T. mollis* and *T. urvilleana*, respectively). Although these plants produce flowers throughout the year, the samples were collected in the main flowering period (April–May), after 3 years of planting. The flowers with similar colouration were harvested and, a simple random model was followed for obtaining a representative set of samples. After homogenisation, the magenta petals of each variety were considered and maintained at –4 °C until analysis.

Obtaining the extracts

The 295 g of fresh petals were macerated with 0.5 L of methanol:acetic acid (19:1) for 24 h at 21 °C. After the separation of the supernatants, the analysis was undergone until colour removal of the raw material (Osorio *et al*., 2012). Once the organic solvent was removed at 35 °C using a rotary evaporator (Heidolph, Schwabach, Germany), the crude extract was dissolved in distilled water (1 g mL^{-1}) and then lyophilised at –80 °C and –0.012 mbar for 48 h (Labconco, MO, USA). Lyophilised samples were stored at 4 °C until analysis.

Removal of hydrocolloids and proteins

A 20 g of sample was dissolved with 60 mL of a mixture of water and 96% ethanol (1:2), and they were continuously stirred for 20 min. Subsequently, the mucilages were removed and the sample was centrifugated at 6037.2 g and filtered (Whatman No. 1 filter paper) (Mosquera *et al*., 2020). Then, the organic solvent was eliminated at 30 °C by evaporation (Heidolph, Schwabach, Germany). The crude fractions isolated from *T. urvilleana* and *T. mollis* (CFM and CFU) were then lyophilised and kept at 4 °C until analysis.

Anthocyanin and flavonol fractionation with Amberlite XAD-7 column

The ion-exchange chromatography was used for flavonoid fractionation. Briefly, two-column volumes of acidic deionised water (pH 3 adjusted with formic acid) and then three column volumes of water were passed for conditioning through a column (30×2 cm^2) with a non-ionic polymeric adsorbent, Amberlite XAD-7 (Rohm and Haas, Darmstadt, Germany). Once CFM and CFU (4.2 g), separately, were added to the column, the sugars were removed bypassing deionised water (1.25 L) through the column. Subsequently, 1 L of a mixture of methanol:acetic acid (19:1, v/v) was eluted to obtain the purified fractions (Hurtado & Charfuelan, 2019). Afterward, the organic solvent was eliminated under vacuum (40 °C) and the aqueous phase was lyophilised, obtaining the enriched in anthocyanins and flavonoids fraction of *T. mollis* (PFM) and *T. urvilleana* (PFU).

Anthocyanins quantification

Samples were diluted with aqueous buffers pH 1.0 and 4.5 according to the method proposed by Liu *et al.* (2019). After 15 min, the absorbance measurements were spectrophotometrically recorded at 520 and 700 nm (Merck, Spectroquant® Pharo 300, USA). The content of anthocyanins was determined as follows: $[A]\ (mg\ g^{-1}) = ((Abs_{520} - Abs_{700})_{pH1} - (Abs_{520} - Abs_{700})_{pH4.5})(DF)(V)(MW)/(\varepsilon)(L)(W)$, where DF is the dilution factor, V is the volume (mL) of the extracts, and Mw and ε are the molecular weight and the molar extinction coefficient of cyanidin-3-*O*-glucoside (449.2 g mol^{-1} and 26900 L/(mol cm) in H_2O), L is the optical path length (1 cm), and W is the weight of the dry extract (g). Results were expressed as mg cyanidin-3-*O*-glucoside per 100 g dry weight.

Folin–Ciocalteau reduction capacity

The samples were prepared by adding methanol (100 μL) and Folin–Ciocalteu reagent (900 μL). Once waiting 5 min at 25 °C, sodium bicarbonate solution (750 μL) was added. After stirring and leaving to stand the solution for 90 min at 25 °C, a spectrophotometer (Merck, Spectroquant® Pharo 300, USA) was used for measuring at 765 nm (Rosero *et al.*, 2019). FCRC was expressed as mg gallic acid per g of dry weight.

Analysis of anthocyanins and flavonols by UHPLC-DAD-ESI-MS

Separation and identification of flavonoids (anthocyanins and flavonols) were carried out in a UHPLC Dionex Ultimate 3000 RS (Thermo Fisher Scientific™), provided with a quaternary pump, a UV–vis diode-array detector, an automatic injector, and ChemStation software (Palo Alto, USA). An Xbridge BEH C_{18} column (100×2.1 mm, 2.5 μm particle size) was thermostatted at 25 °C, and 0.1% TFA in water (eluent A) and acetonitrile (eluent B) at a flow rate of 0.8 mL min^{-1} were used. The injection of the volume was 100 μL. Anthocyanin and flavonols were separated using the following method: initially 90% A, from 0% to 10% B in 3.25 min, from 10% to 15% B in 12.5 min and maintained for 5 min, from 15% to 18% B in 5 min, from 18% to 30% B in 20 min, from 30% to 35% B in 5 min, and from 35% to 10% B in 7 min. Anthocyanins and flavonols were monitored at 520 and 360 nm, respectively and quantification was performed using the standard curves for cyanidin-3-O-glucoside and quercetin, respectively. The mass spectra were obtained using Absciex 3200 Q-trap electrospray ionisation mass spectrometer system (ESI/MS^n) (Foster City, CA, USA) equipped with a triple quadrupole linear ion trap mode, in positive ion mode using a scan range from m/z 50 to 1000. A flow rate of 40 mL min^{-1} nitrogen and 700°C of drying temperature were used. SIM mode was used to acquire mass spectra with the software AB Sciex Analyst® (version 1.6.2).

Determination of antioxidant capacity

Phosphate-buffered saline was used for diluting the $ABTS^+$ solution, and stored at dark and 25°C. It was adjusted to pH 7.4 once reaching an absorbance around 0.70 at 734 nm after approximately 16 h. Afterward, 30 μL of the sample was mixed with 3 mL of the diluted solution of $ABTS^+$, and the measurement at the absorbance at 734 nm were undergone after stirring and standing for 6 min (UV–Vis Spectroquant® Pharo 300, USA) (Rosero *et al.*, 2019). TEAC was expressed as mmol Trolox equivalents per g dry weight.

Determination of antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl

An aliquot of each sample (0.1 mL) was added to 3.9 mL of a 6.49×10^{-5} mol L^{-1} **2,2-**diphenyl-1-picrylhydrazyl (DPPH) in methanol (Cai *et al.*, 2005). The absorbance was monitored at 515 nm using a UV–Vis Spectroquant® Pharo 300 (USA) until the solution reached a silver colour (steady-state) (30 min). A calibration curve for methanolic DPPH was constructed for each antioxidant extract to calculate the %DPPH remaining in the reaction: $\%DPPH_{REM} = (C_{DPPHt=30min}/C_{DPPHt=0min} \times 100)$. The $\%DPPH_{REM}$ was plotted versus the antioxidant extract concentration, and used to calculate the efficient concentration (EC_{50}) (the concentration of antioxidant (μg/mL) required to decrease the DPPH initial concentration by 50%).

Statistical analysis

Univariate analysis of variance (ANOVA) and Fisher's least significant differences (LSD) procedure ($P < 0.05$) were applied using Statgraphics Centurion 16.1.15 software. All measurements were carried out in triplicate.

Results and discussion

Anthocyanins and flavonols identification and quantification

CFU and CFM accounted for an average of 18.3% and 13.4% of each flower's weight, respectively. After the removal of hydrocolloids and proteins and the pass through the XAD-7 Amberlite, 1.28 and 1.98 g of PFU and PFM were obtained, corresponding to 30% and 30.5% of the crude extracts' weight, respectively. The chromatographic profile of the anthocyanins and flavonols in PFM as representative is shown in Fig. 1, and the peak assignment of both species (PFM and PFU) is shown in Table 1.

Anthocyanins. Almost all the identified anthocyanins were detected for the first time in *Tibouchina* genus in this study. As evidenced in Table 1, the major anthocyanins corresponded to different compounds depending on the species of *Tibouchina*. Thus, the major anthocyanins in PFU were significantly ($P < 0.05$) compounds **6** and **7**, which accounted for 80% of the total area (42% and 41%, respectively). These compounds displayed pseudomolecular ions $[M]^+$ at 625 and 639 m z^{-1} units and product ions at 317 and 331 m z^{-1} units, respectively, provoked by the loss of a fragment with 308 m z^{-1} corresponding to *p*-coumaroyl-glucose units (Cejudo-Bastante *et al.*, 2011a). As a result, compounds **6** and **7** corresponded to petunidin-3-*O*-(6′-*p*-coumaroyl)-glucoside and malvidin-3-*O*-(6′-*p*-coumaroyl)-glucoside, respectively. Concerning PFM, the significantly ($P < 0.05$) major anthocyanin was ascribed to compound **2**, and represented 46% of the total area. In the light of the mass spectra ($[M]^+$ at 449 m z^{-1} and a product ion at 287 m z^{-1} units (loss of a glucose unit, $[M\text{-}162]^+$), compound 2 was assigned to cyanidin-3-*O*-glucoside. This fact is of specific relevance since cyanidin-3-*O*-glucoside was the significantly ($P < 0.05$) most abundant anthocyanin in this variety of *Tibouchina*, contributing to the particularity of this feedstock. It is highlighted that, in PFM, compound 6 followed by compounds **3**, **5**, and **7** accounted for approximately 50% of the total area of PFM. The mass spectrum of the compounds **3** and **5** revealed a pseudomolecular ion at 479 and 493 m z^{-1} units $[M]^+$, with product ions at 317 and 331 m z^{-1} (loss of a glucose unit, $[M\text{-}162]^+$), confirming the identification of compounds 3 and 5 as 3-*O*-glucosides of petunidin and malvidin (Cejudo-Bastante *et al.*, 2011b). It was statistically established that the minor anthocyanin compounds were ascribed to compounds **1** and **4** in both species, although compound **3** was not detected in PFU (Table 1). The pseudomolecular ions of compounds **1** and **4** were observed at *m*/*z* 465 and 463 units $[M]^+$ and product ions were detected at 303 and 301 m z^{-1} units, respectively (Table 1). Those fragments appear to have been formed by a hexose bond breakdown ($[M\text{-glucose}]^+$), confirming compounds 1 and 3 as delphinidin-3-*O*-glucoside and peonidin-3-*O*-glucoside, respectively. The existence of acyl groups at position 3 of the aglycone and the attribution of a simple acylation was corroborated by UV–Vis spectroscopy since Abs_{440nm}/Abs_{max} ratio was higher than 0.3 and $A_{max\text{-}acyl}/Abs_{max}$ ratio ($A_{max\text{-}acyl}$ is the maximum absorbance in the region between 310 and 360 nm) ranged between 0.5 and 0.7 for all the anthocyanins identified (Jordheim *et al.*, 2016). What stands out is the fact that, while *T. mollis* presented a wide qualitative profile of anthocyanins, that of the *T. urvilleana* was limited only to two or three anthocyanins, a fact that

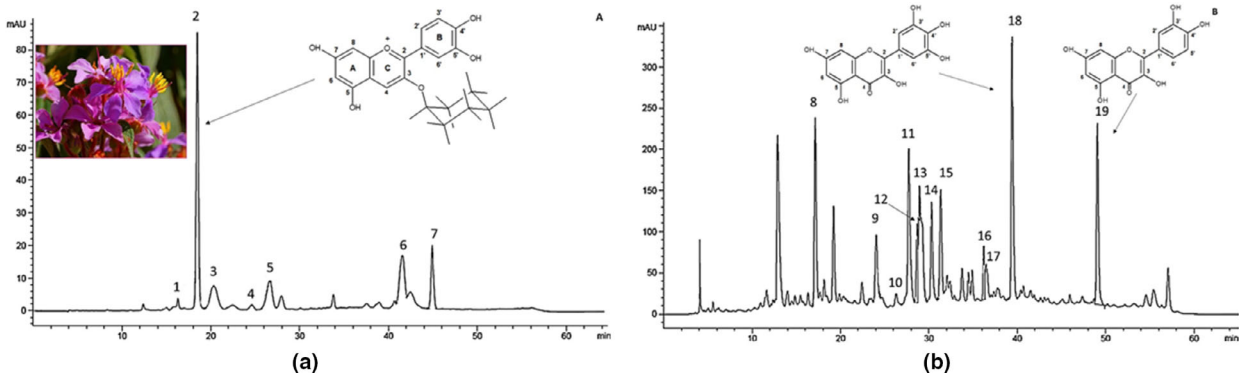

Figure 1 UHPLC-DAD chromatogram corresponding to the extract enriched in polyphenols of *Tibouchina mollis* (PFM): (a) 520 nm and (b) 360 nm.

Table 1 Peak assignment, identification, retention times (R_t), mass spectra, mean concentration (mg/g), and standard deviations of the anthocyanins and flavonols identified in the flavonoid enriched fractions of *Tibouchina urvilleana* (PFU) and *Tibouchina mollis* (PFM) by UHPLC-DAD-ESI-MS

					Mean concentration (mg/g)	
Peak No.	**Compound**	**R_t (min)**	**Mass spectra**		**PFU**	**PFM**
Anthocyanins			*m/z* $[M]^+$	Product ion		
1	Delphinidin-3-*O*-glucoside	14.97	465	303	0.44 ± 0.09[a]	0.05 ± 0.01[a]
2	Cyanidin-3-*O*-glucoside	17.84	449	287	0.56 ± 0.10[b]	3.62 ± 0.25[e]
3	Petunidin-3-*O*-glucoside	19.72	479	317	nd	0.99 ± 0.02[c]
4	Peonidin-3-*O*-glucoside	23.67	463	301	3.58 ± 1.03[c]	0.13 ± 0.02[b]
5	Malvidin-3-*O*-glucoside	25.49	493	331	7.79 ± 1.76[d]	0.93 ± 0.05[c]
6	Petunidin-3-*O*-(6′-*p*-coumaroyl)-glucoside	41.53	625	317	13.49 ± 2.32[f]	1.24 ± 0.04[d]
7	Malvidin-3-*O*-(6′-*p*-coumaroyl)-glucoside	44.85	639	331	27.85 ± 4.42[e]	0.89 ± 0.08[c]
Flavonols			*m/z* $[M]^-$	Product ion		
8	Quercetin-3-*O*-hexoside	17.12	463	301	1.42 ± 0.08[e]	8.46 ± 0.38[g]
9	Myricetin-3-*O*-glucoside	24.02	479	317	nd	4.28 ± 0.05[e]
10	Myricetin-3-*O*-glucoronide	24.68	493	317	nd	0.61 ± 0.01[a]
11	Kaempferol-3-*O*-hexoside	27.70	447	285	0.43 ± 0.06[b]	8.56 ± 0.96[g]
12	Isorhamnetin-3-*O*-glucuronide	28.65	491	315	0.55 ± 0.11[c]	2.77 ± 0.39[d]
13	Quercetin-3-*O*-hexoside	28.90	463	301	17.83 ± 3.46[f]	nd
14	Quercetin-3-*O*-glucoside	30.29	463	301	0.46 ± 0.04[b]	4.39 ± 0.50[e]
15	Quercetin-3-*O*-glucuronide	31.32	477	301	1.07 ± 0.05[d]	5.05 ± 0.11[f]
16	Kaempferol-3-*O*-glucoside	36.19	477	285	0.34 ± 0.01[a]	2.04 ± 0.26[c]
17	Kaempferol-3-*O*-glucoronide	36.45	461	285	nd	1.76 ± 0.38[b]
18	Myricetin	39.39	317	*	nd	11.06 ± 1.62[h]
19	Quercetin	49.04	301	*	1.00 ± 0.06[d]	8.17 ± 0.71[g]

*Fragmentation was not achieved. By chemical families, different letters in the same column denote significant differences ($P < 0.05$) among compounds for each sample by LSD test.

could enable to set the anthocyanin profile as a fingerprint to authenticate the genus of *Tibouchina*.

Flavonols. As far as we know, most of these flavonols have been firstly identified in *Tibouchina* in this study, such as the 3-*O*-glucoside derivatives of myricetin and kaempferol, and the 3-*O*-glucuronide derivatives of isorhamnetin and kaempferol. The fragmentation pattern of compounds **8**, **13**, **14**, **15,** and **19** was similar, that is, an ion at 301 m z^{-1} unit, which corresponded to the quercetin aglycon. Compounds **8**, **13**, and **14** exhibited a pseudomolecular ion $[M]^-$ 467 m z^{-1} unit and a product ion at 301 m z^{-1} unit, corresponding to the breakdown of an *O*-hexoside bond. Compound **14** was assigned as quercetin-3-glucoside after a comparison of the retention time of the commercial standard and mass data. Compounds **8** and **13** were finally assigned as quercetin-3-hexosides. Regarding compound 15, a pseudomolecular ion $[M]^-$ 477 m z^{-1} unit and a product ion at 301 m z^{-1} unit was observed, whose loss of a fragment with 176 m z^{-1} was attributed to glucuronide acid, identifying it as quercetin-3-*O*-glucuronide. Rezende *et al*. (2019) previously described these quercetin derivatives in the Brazilian tree *T. pulchra* (Cham.) Cogn. In addition, the *m/z* 301 unit of compound 19 made it identified as the quercetin aglycon, confirmed with the chromatographic data of the commercial standard. In addition, similar MS ions were observed in compounds **9**, **10**, and **18**, with a fragmentation daughter of 317 m z^{-1} units, assigned to a myricetin aglycon. The ion $[M]^-$ 479 and 493 m z^{-1} units, with a product ions at 317 m z^{-1} unit, made attribute the compounds **9** and **10** as myricetin-3-*O*-glucoside and myricetin-3-*O*-glucuronide, corresponding to the loss of the same fragment of the compounds **14** and **15**, respectively (162 and 176 m z^{-1} units) (Table 1). Thus, compound **18** was attributed to the myricetin flavonol (Motta *et al*., 2005). Conversely, the MS ion of 285 m z^{-1} unit of the compounds **11**, **16**, and **17** ascribed to kaempferol aglycon. Taking into account the similar fragmentation pattern of the myricetin and quercetin-derived products aforementioned described, that is, the loss of 162 and 176 m z^{-1} units, these compounds were assigned for the first time to *Tibouchina* as kaempferol-3-*O*-hexoside, kaempferol-3-*O*-glucoside, and kaempferol-3-*O*-glucuronide, respectively. Finally, isorhamnetin-3-*O*-glucuronide (compound **12**) was confirmed by the identification of a pseudomolecular ion at *m/z* 491 and a product ion at 315 m z^{-1} unit, which appears to have been generated by the loss of a glucuronide acid moiety $[M\text{-}176]^+$, previously detected in *T. granulosa* (Sobrinho *et al*., 2017).

As evidenced in Table 1, the flavonol profile differed depending on the species of *Tibouchina*. Thus, PFM

showed a more complete flavonol profile than PFU (Table 1). It was also statistically established that the major flavonols in PFM were the compounds **8**, **11**, **18**, and **19**, encompassing more than a half of the flavonol peak area, followed by compounds **9**, **14**, and **15** which represented 20%. Intriguingly, the only non-detected flavonol in PFM (quercetin-3-*O*-hexoside) corresponded to the major flavonol quantified in PFU, with 80% of the total area. Other compounds such as the myricetin (compounds **9**, **10**, and **18**) and kaempferol (compounds **11**, **16**, and **17**) derivatives also contributed to distinguish among both species of flowers, in the light of the absence and the lower content in PFU compared to PFM, respectively. Thus, those compounds, quercetin-3-*O*-hexoside, myricetin derivatives, and kaempferol-3-*O*-hexoside, could represent a fingerprint for assuring the specificity of both species of the genus *Tibouchina*.

Total anthocyanin, folin–ciocalteau reduction capacity and antioxidant activity

The total content of anthocyanins significantly ($P < 0.05$) differed, being far superior to the anthocyanin amount in CFU to CFM (Tabla 2). These differences could be attributable to the presence in CFM of sugars, organic acids, or other non-retained compounds by the resin. Moreover, the purification process with XAD-7 Amberlite provoked an enrichment of anthocyanins in both flowers, above all in *T. mollis*, as indicated the significant ($P < 0.05$) differences in PFU and PFM compared to their respective crude fractions. Although the anthocyanin content of PFM was 12-fold higher than that of CFM (Table 2), the amount of anthocyanins was significantly ($P < 0.05$) higher in PFU than in PFM. Regarding other species, Janna *et al*. (2007) reported a lower content of monomeric anthocyanin in *T. semidecandra* L. *Borago officinalis*, and *Calendula officinalis* than *T. mollis*, contrarily to *Tagetes erecta* that presented a similar amount (Benvenuti *et al*., 2016). However, *T. urvilleana* exhibited twofold content of monomeric anthocyanins of *Pelargonium peltatum* and *Petunia* × *hybrid* or *blue Viola* × *wittrockiana*.

Both species of *Tibouchina* were remarkable sources of antioxidant capacity, and significant ($P < 0.05$) differences of antioxidant capacity by TEAC and DPPH and FCRC were found among samples (CFU, CFM, PFU, and PFM) (Table 2). It was also statistically established that the purified fractions (PFU and PFM) exhibited a 9-fold higher antioxidant capacity by TEAC and FCRC than the respective crude extracts (CFU and CFM), depicting the efficiency of the purification procedure (Rosero *et al*., 2019).

Despite PFU showing higher monomeric anthocyanin content than PFM, the retention of polyphenol content (FCRC) was significantly ($P < 0.05$) higher in PFM (Table 2). The antioxidant capacity of anthocyanins is closely linked to their molecular structure and redox properties, thus the higher number of hydroxyl groups in the B-ring of anthocyanins, the higher the antioxidant capacity (Kongpichitchoke *et al*., 2015). The delphinidin derivative is one of the most antioxidant anthocyanins due to the occurrence of three hydroxyl substituents in the B-ring (Zhang *et al*., 2011). Considering this fact, the lower FCRC and TEAC values of PFU could be attributed to their high percentage (52%) of malvidin derivatives (with only one OH-group in the B-ring) in comparison with the 75% of cyanidin, petunidin, and delphinidin derivatives (with two and three OH-groups in the B-ring, respectively) of PFM. In addition, the existence of myricetin and quercetin derivatives (35%), with three and two hydroxyl groups in the B-ring, respectively, could greatly contribute to the higher reduction capacity of PFM against PFU (Table 2) (Semwal *et al*., 2016). Moreover, the antioxidant activity (TEAC) was strongly correlated to the FCRC data, as shown by the high R^2 values ($R^2 = 0.9994$) derived from the univariate linear correlation analysis applied. These findings were also observed in different pigment sources (Betancourt *et al*., 2017; Rosero *et al*., 2019; Mosquera *et al*., 2020). Nonetheless, a less strong relationship was found between the FCRC and DPPH

Table 2 Mean values and standard deviation ($n = 3$) of the content of monomeric anthocyanins, Folin–Ciocalteu reduction capacity (FCRC), and antioxidant activity by TEAC and DPPH of the crude and the flavonoid enriched fractions of *Tibouchina urvilleana* (CFU and PFU) and *Tibouchina mollis* (CFM and PFM), respectively

Sample	Monomeric anthocyanins (mg cyanidin-3-*O*-glucoside 100 g^{-1} dry weight)	FCRC (mg gallic acid g^{-1} dry weight)	TEAC (mmol Trolox g^{-1} dry weight)	DPPH EC_{50} (μg antioxidant mL^{-1})
CFU	24.33 ± 0.08^{c}	22.39 ± 0.80^{a}	1.43 ± 0.08^{a}	14.62 ± 0.17^{d}
CFM	1.19 ± 0.01^{a}	22.40 ± 0.81^{a}	1.70 ± 0.04^{b}	8.82 ± 0.15^{c}
PFU	26.63 ± 0.12^{d}	194.79 ± 1.16^{b}	13.07 ± 0.02^{c}	4.93 ± 0.02^{b}
PFM	13.89 ± 0.24^{b}	216.09 ± 1.91^{c}	14.83 ± 0.26^{d}	4.54 ± 0.01^{a}

Different letters in the same column denote significant differences ($P < 0.05$) among samples by ANOVA test.

(EC_{50}) ($R^2 = 0.742$), attributable to the competitive reaction kinetics between antioxidants and the substrate (Capanoglu *et al*. (2018); Rosero *et al*., 2019;.

The FCRC and antioxidant capacity of the different *Tibouchina* flower species have been very scarcely reported. Only Sirat *et al*. (2010) described a remarkable antioxidant activity of pure isolated polyphenol fraction of *T. semidecandra* L., mainly due to quercetin and quercitrin. Therefore, the antioxidant activity of purified flavonoid fractions from *T. urvilleana* and *T. mollis* has been evaluated for the first time, demonstrating that these edible flowers have a considerable amount of bioactive compounds, in line with other fruits and flowers.

Conclusions

The purification and subsequent fractionation of the extracts make it possible to identify a large extent of anthocyanin and flavonols for the first time in the edible flowers of *T. urvilleana* and *T. mollis*, namely kaempferol-3-*O*-glucuronide and the 3-*O*-glucoside derivatives of myricetin and kaempferol, as flavonols, and almost all the identified anthocyanins. The profile, content, and nature of anthocyanins and flavonols showed a marked variability with the species of edible flower. It is proposed the identification of cyanidin-3-*O*-glucoside and the 3-*O*-(6′-*p*-coumaroyl)-glucoside derivatives of petunidin and malvidin, as anthocyanins, and quercetin-3-*O*-hexoside and myricetin derivatives, as flavonols, as marked compounds to warranty the differentiation and authenticity of *Tibouchina* species. Furthermore, *Tibouchina* was demonstrated to be a potent antioxidant activity source, highly correlated with FCRC. Consequently, the industrial exploitation of the edible flowers of *Tibouchina* genus, wild-grown in approximately ten of the thirty-two departments of Colombia, could be an interesting endeavor from several perspectives beyond its solely ornamental use (agroalimentary, cosmetic, and pharmaceutical). For that purpose, previous market studies which include the feasibility of its intensive cultivation, the control of flower development, or the industrial costs for warranting the flavonoid isolation, are needed.

Acknowledgments

The authors are grateful to University of Nariño (VIPRI) and Biology Service (SGI, Universidad de Sevilla) for technical assistance.

Author contribution

Natalia Solarte: Investigation (equal); Writing – original draft (equal). **María Jesús Cejudo Bastante:** Investigation (equal); Writing – review & editing (equal). **Nelson Hurtado:** Conceptualization (equal); Writing – original draft (equal). **Francisco J. Heredia:** Supervision (equal); Writing – review & editing (equal).

Conflicts of interest

The author(s) declared no potential conflicts of interest.

Ethical statement

Ethics Approval Was Not Required For This Research.

Data availability statement

Data are available within the article.

References

Almeda, F., Mendoza-Cifuentes, H., Penneys, D.S., Michelangeli, F.A. & Alvear, M. Tibouchina aspera Aubl. Vol. 1.1. In *Catálogo de plantas y líquenes de Colombia*, Bernal, R., Gradstein, S.R. & Celis, M. Ed. 2015, Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá.

Benvenuti, S., Bortolotti, E. & Maggini, R. (2016). Antioxidant power, anthocyanin content and organoleptic performance of edible flowers. *Scientia Horticulturae*, **199**, 170–177.

Betancourt, C., Cejudo-Bastante, M.J., Heredia, F.J. & Hurtado, N. (2017). Pigment composition and antioxidant capacity of betacyanins and betaxanthins fractions of *Opuntia dillenii* (Ker Gawl) Haw cactus fruit. *Food Research International*, **101**, 173–179.

Bomfim, E.M.S., Coelho, A.A.O.P., Silva, M.C., Marques, E.J. & Vale, V.L.C. (2022). Phytochemical composition and biological activities of extracts from ten species of the family Melastomataceae Juss. *Brazilian Journal of Biology*, **82**, 1–6.

Cai, Y.Z., Sun, M. & Corke, H. (2005). Characterization and application of betalain pigments from plants of the *Amaranthaceae*. *Trends in Food Science & Technology*, **16**, 370–376.

Capanoglu, E., Kamiloglu, S., Ozkan, G. & Apak, R.(2018). Evaluation of antioxidant activity/capacity measurement methods for food products. *Measurement of Antioxidant Activity & Capacity: Recent Trends and Applications*. John Wiley & Sons Ltd.

Cejudo-Bastante, M.J., Hermosín-Gutiérrez, I. & Pérez-Coello, M.S. (2011a). Micro-oxygenation and oak chip treatments of red wines: Effects on colour-related phenolics, volatile composition and sensory characteristics. Part I: Petit Verdot wines. *Food Chemistry*, **124**, 727–737.

Cejudo-Bastante, M.J., Hermosín-Gutiérrez, I. & Pérez-Coello, M.S. (2011b). Micro-oxygenation and oak chip treatments of red wines: Effects on colour-related phenolics, volatile composition and sensory characteristics. Part II: Merlot Wines. *Food Chemistry*, **124**, 738–748.

Durães Vieira, E.R., de Assis Júnior, S.L., Alvarenga, M., Santos, G.C. & Barroso, G.A. (2019). First Record of Membracis juncta (Walker) (Hemiptera: Membracidae) in *Tibouchina candolleana* (Mart. ex DC.) (Melastomataceae). *Florida Entomologist*, **102**, 275–277.

Esposito, M.P. & Domingos, M. (2014). Establishing the redox potential of Tibouchina pulchra (Cham.) Cogn., a native tree species from the Atlantic Rain Forest, in the vicinity of an oil refinery

in SE Brazil. *Environmental Science and Pollution Research*, **21**, 5484–5495.
Fernández-Sánchez, L., Mancipe, C. & Calderón-Hernández, M. (2020). Evaluación de dos métodos de propagación para la conservación ex situ de tres melastomatáceas altoandinas. *Caldasia*, **42**, 129–141.
Gowthami, V. & Naga Lakshmi, J. (2021). A Review on *Tibouchina urvilleana* (DC) Cog. *International Journal of Pharmaceutical Sciences Review and Research*, **67**, 78–82.
Hendra, R. & Keller, P.A. (2016). Flowers in Australia: Phytochemical Studies on the Illawarra Flame Tree and Alstonville. *Australian Journal of Chemistry*, **69**, 925–927.
Hurtado, N.H. & Charfuelan, C. (2019). Contribución a la Caracterización y Evaluación de la Actividad Antioxidante de las Antocianinas del Fruto de Ivilan (*Monnina Obtusifolia* H.B.K). *Información Tecnológica*, **30**, 81–90.
Janna, O., Khairul, A. & Maziah, M. (2007). Anthocyanin stability studies in *Tibouchina semidecandra* L. *Food Chemistry*, **101**, 1640–1646.
Jordheim, M., Calcott, K., Gould, K.S., Davies, K.M., Schwinn, K.E. & Andersen, Ø.M. (2016). High concentrations of aromatic acylated anthocyanins found in cauline hairs in *Plectranthus ciliatus*. *Phytochemistry*, **128**, 27–34.
Kongpichitchoke, T., Hsu, J.-L. & Huang, T.-C. (2015). Number of Hydroxyl Groups on the B-Ring of Flavonoids Affects Their Antioxidant Activity and Interaction with Phorbol Ester Binding Site of PKCδ C1B Domain. In Vitro and in Silico Studies. *Journal of Agricultural and Food Chemistry*, **63**, 4580–4586.
Liu, W., Yang, C., Zhou, C., Wen, Z. & Dong, X. (2019). An improved microwave-assisted extraction of anthocyanins from purple sweet potato in favor of subsequent comprehensive utilization of pomace. *Food and Bioproducts Processing*, **115**, 1–9.
Mosquera, N., Cejudo-Bastante, M.J., Heredia, F.J. & Hurtado, N. (2020). Identification of new Betalains in separated betacyanin and betaxanthin fractions from Ulluco (*Ullucus tuberosus* Caldas) by HPLC-DAD-ESI-MS. *Plant Foods Human Nutrition*, **75**, 434–440.
Motta, L.B., Kraus, J.E., Salatino, A. & Salatino, M.L.F. (2005). Distribution of metabolites in galled and non-galled foliar tissues of *Tibouchina pulchra*. *Biochemistry Systematics and Ecology*, **33**, 971–981.
Osorio, C., Hurtado, N., Dawid, C., Hofmann, T., Heredia-Mira, F.J. & Morales, A.L. (2012). Chemical characterisation of anthocyanins in tamarillo (*Solanum betaceum* Cav.) and Andes berry (*Rubus glaucus* Benth.) fruits. *Food Chemistry*, **132**, 1915–1921.
Pérez-Castorena, A. L. (2014). Triterpenes and other Metabolites from Tibouchina urvilleana. *Journal of the Mexican Chemical Society*, **58**, 218–222.
Rezende, F., Ferreira, M., Clausen, M., Rossi, M. & Furlan, C. (2019). Acylated flavonoid glycosides are the main pigments that determine the flower colour of the Brazilian native tree *Tibouchina pulchra* (Cham.) Cogn. *Molecules*, **24**, 718–727.
Rosero, J.C., Cruz, S., Osorio, C. & Hurtado, N. (2019). Analysis of phenolic composition of byproducts (seeds and peels) of avocado (*Persea americana* Mill.) cultivated in Colombia. *Molecules*, **24**, 3209.
Ruilova, X.R. & Marques, I. (2016). Better common than rare? Effects of low reproductive success, scarce pollinator visits and interspecific gene flow in threatened and common species of *Tibouchina* (Melastomataceae). *Plant Species Biology*, **31**, 288–295.
Semwal, D., Semwal, R., Combrinck, S. & Viljoen, A. (2016). Myricetin: A Dietary Molecule with Diverse Biological Activities. *Nutrients*, **8**, 1–31.
Sirat, H.M., Rezali, M.F. & Ujang, Z. (2010). Isolation and identification of radical scavenging and tyrosinase inhibition of polyphenols from *Tibouchina semidecandra* L. *Journal of Agricultural and Food Chemistry*, **58**, 10404–10409.
Sobrinho, A.P., Minho, A.S., Ferreira, L.L.C., Martins, G.R., Boylan, F. & Fernandes, P.D. (2017). Characterization of anti-inflammatory effect and possible mechanism of action of *Tibouchina granulosa*. *Journal of Pharmacy and Pharmacology*, **69**, 706–713.
Tracanna, M.I., Fortuna, A.M., Contreras Cárdenas, A.V. *et al.* (2015). Anti-Leishmanial, Anti-Inflammatory and Antimicrobial Activities of Phenolic Derivatives from *Tibouchina paratropica*. *Phytotherapy Research*, **29**, 393–397.
Zhang, D., Chu, L., Liu, Y. *et al.* (2011). Analysis of the antioxidant capacities of flavonoids under different spectrophotometric assays using cyclic voltammetry and density functional theory. *Journal of Agricultural and Food Chemistry*, **59**, 10277–10285.