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Anthocyanins in inflorescences of *Tarenaya rosea* (Vahl ex DC.) Soares Neto & Roalson (Cleomaceae)

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Abstract

Anthocyanins are plant pigments of economic interest, due to their use as natural colorants, as well as their pharmacological application. Analysis of inflorescences of *Tarenaya rosea* (Vahl ex DC.) Soares Neto & Roalson by high performance liquid chromatography coupled to diode array detector and electrospray ionization mass spectrometry (HPLC-DAD/ESIMS) revealed the presence of acylated cyanidins and a peonidin. Ten anthocyanins acylated with *p*-coumaric, caffeic, ferulic, sinapic or *p*-hydroxybenzoic acid were detected. The major peak was identified as cyanidin 3-(*p*-coumaroyl)(*p*-coumaroyl) diglucoside-5-glucoside. Two anthocyanins, (cyanidin 3-(*p*-hydroxybenzoyl) diglucoside-5-glucoside and cyanidin 3-(glycopyranosyl-caffeoyl) diglucoside-5-glucoside), were detected only in the inflorescences when compare to other analyses previously performed with the species. The study of the anthocyanin content in plant species is an important step in the development of strategies for the commercial exploitation of these pigments. The results obtained in the present work showed the diversity of anthocyanins in the inflorescences of *T. rosea*.

Keywords: acylated anthocyanins. *Tarenaya rosea*. Cyaniding. Medicinal plant. Peonidin. Restingas.

Introduction

Tarenaya rosea (Vahl ex DC.) Soares Neto & Roalson is a Brazilian herbaceous annual species known as “mussambê cor-de-rosa”, frequently found in coastal sandy plains (*restingas*), ecosystems intensely affected by human impact[1]. This species was formely named *Cleome rosea* ex DC. (2). Several protocols of plant cell tissue culture have already been established with *T. rosea*[2-8]and its medicinal potential has been evaluated from *in vivo* and *in vitro* materials, with promising results[9-11]. In addition, due to its attractive pink inflorescences the species is employed as ornamental (**FIGURE 1A**). The pink coloration is provided by anthocyanins, water-soluble plant flavonoids responsible for scarlet to blue colors in flowers, fruits, leaves and storage organs. Some of these pigments are used to color food as substitutes for synthetic red dyes[12] and great attention has been focused on their multifaceted pharmacological potential[13,14]. The diversity of anthocyanins in extracts obtained from stems of field-grown plants and callus cultures of *T. rosea* has already been identified in a previous work[5]. The increasing commercial and pharmacological interest in

plant pigments is currently leading to several studies related to their isolation and identification in different plant species. In continuation to our study of investigating anthocyanins presented in *T. rosea,* this work was undertaken to characterize the anthocyanins in inflorescences by high performance liquid chromatography coupled to diode array detector and electrospray ionization mass spectrometry (HPLC-DAD/ESIMS).

Material and Methods

Plant material and extract preparation

Inflorescences of *T. rosea* were collected from natural populations in an area of restinga vegetation located at Maricá, RJ, Brazil (22º58´01" S and 42º58´36" W) according to the Brazilian legislation on access to the biodiversity (SISBIO 17889-1 and CGEN 207/2014). A voucher specimen was deposited in the Herbarium of the Rio the Janeiro State University, Rio de Janeiro, Brazil (HRJ7185). The plant material was macerated for 24 h at 4 ºC with methanol acidified with 1% (v/v) HCl (MeOH-HCl) and the extractive solution were prepared at the final ratio of 1 g of fresh weight/2 cm^3 of solvent.

HPLC-DAD/ESIMS analysis

Anthocyanin analysis was performed with a Shimadzu HPLC system (MassLynx software, LC-10Advp binary gradient pump, SIL-10Advp autosampler, SPD-M10Avp diode-array detector and SCL-10Avp system controller). Samples were analyzed under a gradient using a Supelco C18 column (250 x 4.6 mm i.d., 5 μm particle size) with injection volume of 20 μL. The flow rate was 1 cm^3 min^{-1} and detection was performed at 525 nm. Mobile phase A was 7.5% formic acid in acetonitrile (v/v) and mobile phase B was 7.5 % formic acid in water (v/v). The gradient used was as follows: 3% A for 1 min, 3-15 % A for 11 min, 15-25 % A for 12 min, 25-30 % A for 4 min, and 30 % A for 7 min before returning to the initial conditions. The diode-array detector was set to an acquisition range of 200-600 nm at a spectral acquisition rate of 1.56 scans s^{-1} (peak width 0.2 min) and it was coupled to a Waters ZQ single quadrupole mass spectrometer. Mass spectra were achieved by electrospray ionization in positive mode scanning from *m/z* 100 to *m/z* 1500. The capillary temperature and voltage used were 100 ºC and 3 kV, respectively. In order to obtain a better spectra profile, three voltages were applied to the cone (30V, 50V and 70V). Nitrogen was used as sheath gas at a flow rate of 400 dm^3 h^{-1} at 250 ºC.

Results and Discussion

Ten anthocyanins were found in the inflorescences of *T. rosea* and fragment ions at *m/z* 287 and *m/z* 301 allowed the identification of the aglycones cyanidin and peonidin, respectively. The anthocyanins are acylated with *p*-coumaric, caffeic, ferulic, sinapic or *p*-hydroxybenzoic acids, with prevalence of *p*-coumaroyl derivatives, that constitutes the most common acylation type among known anthocyanins[15]. Acylated anthocyanins were also identified in extracts obtained from stems of field-grown plants as well as from callus cultures of *T. rosea*[5]. Analysis of inflorescences of another *Cleomaceae* species, *C. hassleriana*, revealed the presence of acylated cyanidins and pelargonidins [16]. Moreover, studies with species from the related family Brassicaceae reported the occurrence of polyacylated anthocyanins with prevalence of the same aromatic acids found in *T. rosea* [17-19]. Acylated anthocyanins have commercial importance since they are more stable in neutral aqueous solutions and thus more suitable for use as food and beverage colorants[20].

The presence of peonidins was not observed in previous studies with extracts obtained from stems of field-grown plants of *T. rosea*, but two peonidins were identified on callus cultures that were initiated with stem explants [5]. The bioconversion of cyanidin to peonidin by methylation of the B-ring of the aglycon influenced by the type and concentration of growth regulators used to establish the *in vitro* culture is suggested and it was also reported in strawberry cell cultures [21].

The major peak in the chromatogram from the inflorescences was observed at the retention time of 25.90 min (**FIGURE 1B**) and has been identified as cyanidin 3-(*p*-coumaroyl)(*p*-coumaroyl) diglucoside-5-glucoside (**TABLE 1**) based on the fragment ions displayed at *m/z* 1065; 903; 449; 287. Although this anthocyanin has also been identified in stem extracts, its presence was detected in small percentage of the total anthocyanins [5].

FIGURE 1: Inflorescences features of *Tarenaya rosea*. Morphological aspect (a) and HPLC anthocyanin profile at 525 nm (b). The peaks numbers refer to Table 1. *Bar* = 1.0 cm.

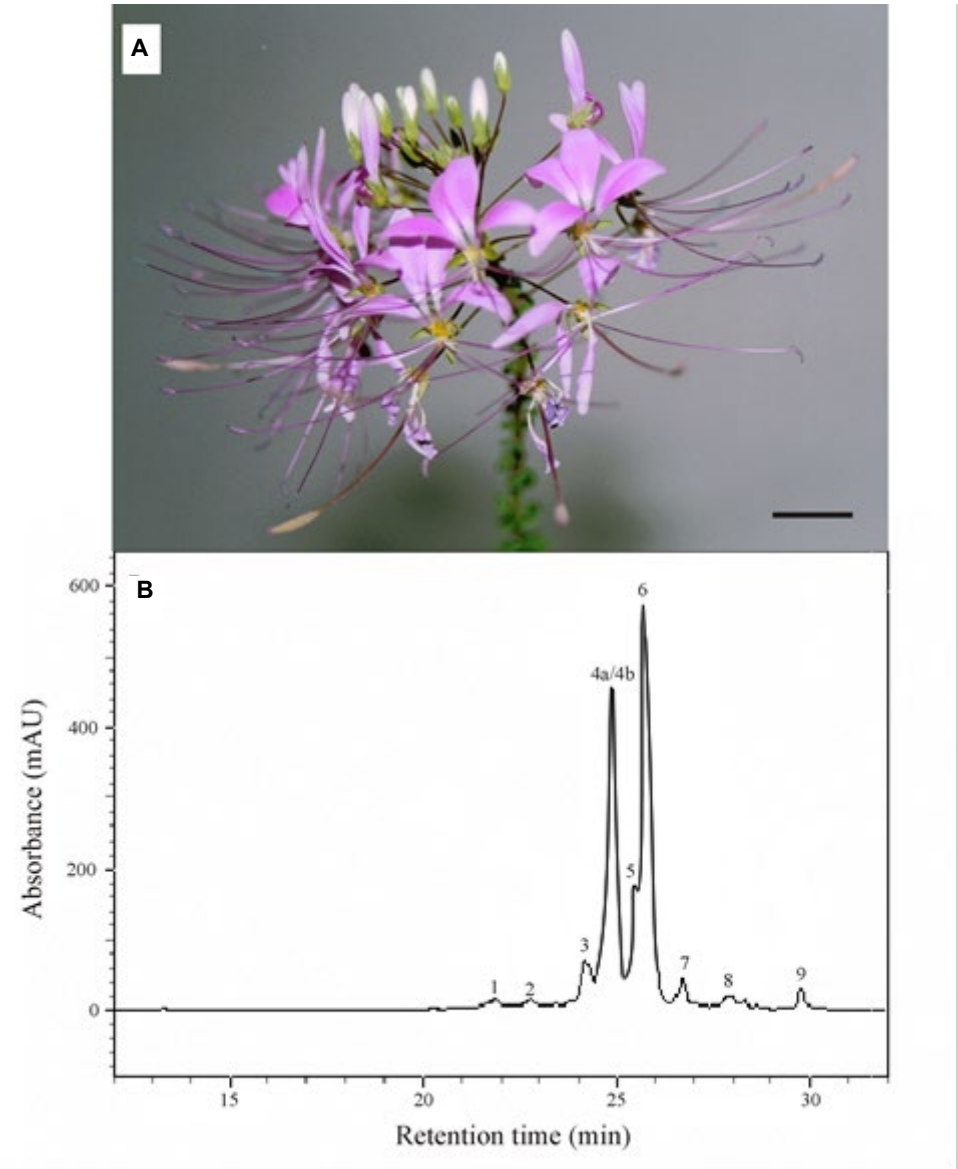

TABLE 1: Characterization of anthocyanins from inflorescences of *Tarenaya rosea* using HPLC-DAD/ESIMS.

Peak	t_R (min)	$[M]^+$ (*m/z*)	Fragment ions *m/z*)	Proposed identity
1	21.79	979	817/449/287	cyanidin 3-(sinapoyl)diglucoside-5-glucoside
2	22.90	1081	919/449/287	cyanidin 3-(caffeoyl)(*p*-coumaroyl)diglucoside-5-glucoside
3*	24.27	1125	963/449/287	cyanidin 3-(feruloyl)(feruloyl)diglucoside-5-glucoside
4a*	24.87	1125	963/449/287	cyanidin 3-(feruloyl)(feruloyl)diglucoside-5-glucoside
4b	24.87	1095	933/449/287	cyanidin 3-(*p*-coumaroyl)(feruloyl)diglucoside-5-glucoside
5**	25.63	1065	903/449/287	cyanidin 3-(*p*-coumaroyl)(*p*-coumaroyl)diglucoside-5-glucoside
6**	25.90	1065	903/449/287	cyanidin 3-(*p*-coumaroyl)(*p*-coumaroyl)diglucoside-5-glucoside
7	26.64	989	827/449/287	cyanidin 3-(*p*-hydroxybenzoyl)diglucoside-5-glucoside
8	28.05	1079	917/463/301	peonidin 3-(*p*-coumaroyl)(*p*-coumaroyl)diglucoside-5-glucoside
9	29.76	1003	841/449/287	cyaniding 3-(glycopyranosyl-caffeoyl)diglucoside-5-glucoside

*Isomers of cyanidin 3-(feruloyl)(feruloyl)diglucoside-5-glucoside; **Isomers of cyanidin 3-(*p*-coumaroyl)(*p*-coumaroyl)diglucoside-5-glucoside.

Two anthocyanins coelute at 24.87 min, one of them showed fragment ions at *m/z* 1095; 933; 449; 287 and was identified as cyanidin 3-(*p*-coumaroyl)(feruloyl) diglucoside-5-glucoside. The other anthocyanin showed fragment ions at *m/z* 1125; 963; 449; 287 and was identified as cyanidin 3-(feruloyl)(feruloyl) diglucoside-5-glucoside. The structure of the latter compound is similar to a cyanidin previously detected in stem extracts of *T. rosea*[5]. In addition, similarly to previous findings in extracts from stem and callus cultures, some isomeric anthocyanins (sugar moiety can present different hexoses with the same molecular weight, such as galactose and glucose) were observed in inflorescences of *T. rosea* (peaks 3 - 4a and peaks 5 - 6). In such cases, the compounds were identified by their retention times.

Considering the anthocyanins reported in inflorescences of *T. rosea*, six of them were detected in extracts obtained from stems and five of these anthocyanins were also observed in callus cultures of *T. rosea*. Despite the similarities among the pigments identified in these materials, two anthocyanins were found only in inflorescences. The MS fragmentation pattern of these compounds showed fragment ions at *m/z* 989, 827, 449; 287 and at *m/z* 1003, 841, 449; 287. They were identified as cyanidin 3-(*p*-hydroxybenzoyl) diglucoside-5-glucoside and cyanidin 3-(glycopyranosyl-caffeoyl) diglucoside-5-glucoside, respectively.

The variety in anthocyanin production from different organs of *T. rosea* is a desirable characteristic, since small differences in chemical structure can have critical impacts on color and tinctorial strength[22] as well as in their biological activities[23]. In addition, a great number of biotechnological strategies have been applied to *in vitro* production of anthocyanins[24] considering the increased interest in the scale-up production of these pigments to pharmaceutical, food, cosmetic, and chemical industries.

Conclusion

The results achieved in the present work provide additional information on anthocyanin content in *T. rosea*. These data contribute to demonstrate the diversity of these pigments in the species and it will be very important for further commercial exploitation.

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