Natural Product Communications
Volume 15(5): 1–7

DOI: 10.1177/1934578X20922637
journals.sagepub.com/home/npx

Anthocyanin Profile and Antioxidant Property of Anti-asthma Flowers of *Cordyline terminalis* (L.) Kunth (Agavaceae)

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Abstract

Cordyline terminalis flower is traditionally used to treat asthma and the purple color of the flower is suggestive of anthocyanins. The purpose of this study was to characterize and determine the antioxidant property of anthocyanins from *C. terminalis* purple flowers. Five anthocyanins, cyanidin 3,5-di-*O*-β-glucopyranoside (2.6 ± 0.2 mg/g fr. wt) (**1**), peonidin 3,5-di-*O*-β-glucopyranoside (2.8 ± 0.3 mg/g fr. wt) (**2**), cyanidin 3-*O*-β-(6"-*O*-*E*-*p*-caffeoylglucopyranoside)-5-*O*-β-glucopyranoside (3.2 ± 0.2 mg/g fr. wt) (**3**), cyanidin 3-*O*-β-(6"-*O*-*E*-*p*-coumaroylglucopyranoside)-5-*O*-β-glucopyranoside (6.2 ± 0.4 mg/g fr. wt) (**4**), and peonidin 3-*O*-β-(6"-*O*-*E*-*p*-coumaroylglucopyranoside)-5-*O*-β-glucopyranoside (9.8 ± 0.2 mg/g fr. wt) (**5**), were isolated from the flowers of *C. terminalis* by a combination of chromatographic techniques. Their structures were established by UV-visible, NMR, and ESI-MS. The extract exhibited appreciable antioxidant activity (IC50 ± SD = 13.1 ± 0.8 μg/mL) against quercetin (IC50 ± SD = 4.5 ± 0.4 μg/mL) compared to the individual anthocyanins (IC50 ± SD = 13.8 ± 0.5 to 16.4 ± 0.7 μg/mL) when measured using the 2,2-diphenyl-1-picryl-hydrazyl method. *Cordyline terminalis* flowers extract may be justified for use and standardization as herbal remedy for asthma.

Keywords

flavonoids, phenolics, anti-asthma, phytochemicals, antioxidants, anthocyanins, *Cordyline terminalis*, flowers

Received: February 27th, 2020; Accepted: April 6th, 2020.

As many as 334 million people worldwide may have asthma, and it is predicted that this rate will increase by 20% over the next 20 years.[1] Currently the most important medicines on market are corticosteroids and β-2 agonists which are effective only in managing asthma symptoms but not as a curative therapy. In addition, there are concerns regarding the undesirable side effects due to prolonged use of the current medicines, especially by children.[2] These medicines are also not affordable and easily accessible, particularly to the economically disadvantaged asthma patients in developing countries.[3,4] In this regard, herbal therapy plays an important complementary role in the management of asthma.

Asthma is an allergic inflammatory condition of the airways associated with oxidative stress through enhancement of the production of cytokines.[5,6] Many herbs that contain antioxidant phytochemicals can relieve inflammation.[7] Anthocyanins are phytochemicals in the class of flavonoids responsible for most of the red to blue colors exhibited by flowers and other parts of the plant. They possess strong anti-inflammatory potential which is attributed to their outstanding antioxidant and free radical scavenging activities.[8,9] Through in vivo studies, the beneficial effects of anthocyanins against asthma have been demonstrated.[10,11] Clinical studies also showed that anthocyanin extracts significantly reduced and suppressed asthma.[12,13] However, the therapeutic effect of anthocyanins is mainly attributed to their antioxidant potential which considerably depends on the composition of anthocyanins.[14] Therefore, in order to develop anthocyanin-based remedy for asthma, it is imperative to know the anthocyanin content and antioxidant potential of the source of anthocyanins.

Cordyline terminalis (L.) Kunth (Agavaceae), synonyms with *Cordyline fruticosa* and *Dracaena terminalis*, is an important ornamental plant native to tropical Asia, Australia, and the Pacific

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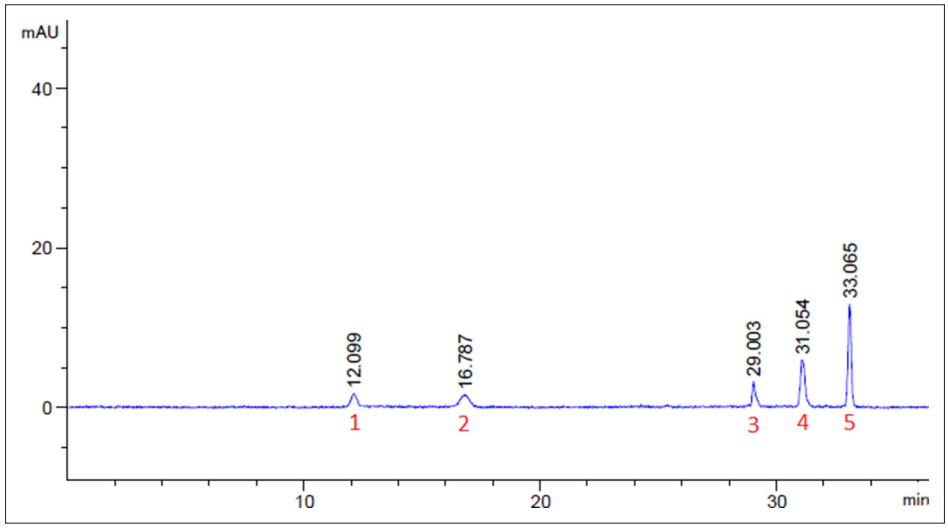

Figure 1. HPLC profile of *Cordyline terminalis* extract.

Islands but nowadays grown around the world.[15] The purple flowers of *C. terminalis* are traditionally used to treat asthma and growths in the nose and the purple color is suggestive of anthocyanins.[16] However, the phytochemistry of *C. terminalis* plant is largely unknown.

The aim of this paper is therefore to report the composition and antioxidant activity of anthocyanins of the purple flowers of *C. terminalis* for the first time.

Results and Discussion

The HPLC profile (Figure 1) of the extract of *C. terminalis* purple flowers detected at 520 ± 20 nm revealed a total of 5 anthocyanins (**1-5**). The on-line UV-visible spectra of all the 5 anthocyanins show λ_{max} at 514 to 523 nm and $A_{440}/A_{Vis\text{-}max}$ ratio of 17% to 18% (Table 1), in accordance with 3,5-substituted cyanidin or peonidin derivatives.[17] The presence of local $\lambda_{Acyl\text{-}max}$ around 313 nm in the UV region of the spectra for **4** and **5** indicated *p*-coumaric acylation and around 329 nm for **3** indicated caffeic acylation.[18] The $A_{UV\text{-}max}/A_{Vis\text{-}max}$ of 0.70, 0.77, and 0.81 for pigments **3**, **4**, and **5**, respectively (Table 1), indicate monoacylation.[17]

The downfield region of ^{1}H NMR spectrum of **5** (Table 2) showed a 3H ABX system at δ 9.10 (d, 0.6 Hz, H-4), δ 7.11 (d, 2.0 Hz, H-6), and δ 7.08 (dd, 0.6, 1.9 Hz, H-8); a 3H AMX system at δ 8.29 (d, 2.2 Hz, H-2'), δ 7.15 (d, 8.7 Hz, H-5'), and δ 8.37 (dd, 2.2, 8.7 Hz, H-6'). These signals together with a cross-peak at δ 4.1 (OCH_3)/δ 149.6 (C3') in the HMBC spectrum were in accordance with the aglycone peonidin. The rest of the downfield ^{1}H NMR signals were assigned to *p*-coumaroyl acyl moiety as follows: δ 7.38 (d, 8.6 Hz; H-2^I, 6^I), δ 6.85 (d, 8.6 Hz; H-3^I, 5^I), δ 6.29 (d, 15.9 Hz; H-α^I), δ 7.47 (d, 15.9 Hz; H-β^I). The coupling constants of H-α^I and H-β^I (15.9 Hz) of *p*-coumaroyl revealed the *E*-configuration of the aromatic acid. The chemical shifts of the carbons with the corresponding protons were assigned from the HSQC experiment and those for the quaternary carbons were assigned using the HMBC spectrum.

In the sugar region of the 1D ^{1}H NMR spectrum of **5**, the 2 anomeric protons, at δ 5.49 (H-1", 7.7 Hz) and at δ 5.25 (H-1‴, 7.8 Hz), revealed the presence of 2 sugar units with β-configurations due to the large coupling constants. The DQF-COSY and TOCSY spectra of the aliphatic region showed 13 proton signals, which indicated that the 2 sugar units were hexoses. Starting from the anomeric proton at δ 5.49 (H-1", 7.7 Hz), the observed cross-peak at δ 5.49/3.79 ppm in the DQF-COSY spectrum, supported by the cross-peaks in the HSQC spectrum, permitted the assignment of H-2". The

Table 1. HPLC and UV-Vis Data Recorded for **1-5** Anthocyanins Isolated From *Cordyline terminalis* Flowers.

Comps	On-line HPLC				
	Rt (min)	$\lambda_{Vis\text{-}max}$ (nm)	$\lambda_{UV\text{-}max}$ (nm)	$A_{440}/A_{Vis\text{-}max}$ (%)	$A_{UV\text{-}max}/A_{Vis\text{-}max}$
1	12.10	514	276	18	0.54
2	16.78	516	276	17	0.52
3	29.00	521	281 329	18	0.70
4	31.05	523	281 (293), 313	17	0.77
5	33.07	521	279 (295), 313	17	0.81

Table 2. ^{1}H (600.13 MHz) and ^{13}C (150.92 MHz) NMR Spectral Data for Compounds **1-5.**

	^{1}H δ (ppm) J (Hz)					^{13}C δ (ppm)				
	1	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
2						165.1	164.9	164.8	165.1	165.2
3						146.5	146.5	145.6	145.7	145.6
4	9.23 d 0.8	9.28 d 0.7	9.00 d 0.8	9.04 d 0.8	9.10 d 0.7	136.2	136.6	135.3	135.9	136.6
5						156.7	156.7	156.5	156.6	156.8
6	7.16 s	7.18 d 2.0	7.08 d 2.0	7.10 d 2.0	7.11 d 2.0	105.8	105.9	106.0	106.0	106.1
7						169.5	169.7	169.7	169.7	169.8
8	7.18 s	7.24 dd 0.8, 1.9	6.99 dd 0.8, 2.0	7.03 dd 0.8, 2.0	7.08 dd 0.8, 2.0	97.3	97.6	97.4	97.4	97.5
9						157.3	157.4	157.2	157.0	157.3
10						113.3	113.5	113.1	113.1	113.1
1'						120.9	120.9	121,0	120.9	120.7
2'	8.17 d 2.3	8.37 d 2.2	8.10 d 2.4	8.11 d 2.3	8.29 d 2.2	118.4	115.4	118.5	118.5	115.4
3'						147.4	149.6	147.5	147.5	149.6
4'						156.5	157.2	156.3	156.4	157.2
5'	7.14 d 8.7	7.17 d 8.7	7.11 d 8.8	7.11 d 8.8	7.15 d 8.7	117.4	117.7	117.5	117.5	117.7
6'	8.45 dd 2.3, 8.7	8.46 dd 2.2, 8.7	8.36 dd 2.4, 8.8	8.37 dd 2.3, 8.8	8.37 dd 2.2, 8.7	129.1	129.7	128.8	129.0	129.6
OMe		4.13 s			4.10 s		56.9			56.8
1"	5.38 d 7.8	5.40 d 7.8	5.48 d 7.7	5.47 d 7.7	5.49 d 7.7	104.0	104.1	102.9	102.9	103.3
2"	3.78 m	3.757 m	3.82 dd 7.7, 9.1	3.82 dd 7.7, 9.1	3.79 dd 7.7, 8.9	74.8	74.61	74.7	74.7	74.7
3"	3.62 t 9.1	3.62 t 9.2	3.67 t 9.0	3.67 t 9.0	3.67 t 9.0	77.9	78.2	78.0	78.0	78.1
4"	3.51 dd 8.6, 9.4	3.48 dd 9.2, 10.0	3.60 dd 9.0, 9.6	3.60 dd 9.0, 9.6	3.59 dd 9.1, 9.7	71.3	71.4	71.9	71.9	71.9
5"	3.71 m	3.71 m	4.00 m	3.97 m	3.95 dd 5.0, 9.7	78.9	79.0	75.7	75.7	75.8
6A"	4.05 m	4.05 dd 2.2, 12.0	4.58 m	4.56 m	4.56 d 4.9	62.5	62.4	64.2	64.2	64.1
6B"	3.81 m	3.80 m								
1‴	5.25 d 7.8	5.25 d 7.8	5.26[a]	5.24[a]	5.25 d 7.8	102.7	102.8	103.2	103.2	103.1
2‴	3.76 m	3.765 m	3.86 dd 7.6, 9.2	3.83 dd 7.8, 9.3	3.83 dd 7.8, 9.3	74.6	74.56	74.6	74.6	74.8
3‴	3.65 m	3.64 t 9.2	3.65 t 9.2	3.64 t 9.2	3.64 t 9.2	78.3	78.2	77.9	77.9	77.9
4‴	3.55 dd 8.1, 9.6	3.54 dd 9.1, 9.8	3.51 dd 9.0, 9.7	3.52 dd 9.0, 9.7	3.52 dd 9.1, 9.7	71.2	71.3	71.3	71.3	71.3
5‴	3.65 m	3.65 m	3.71 m	3.67 m	3.67 ddd 2.1, 6.1, 9.7	78.3	78.6	78.8	78.8	78.9
6A‴	4.04 m	4.04 dd 2.1, 12.0	4.11 dd 2.2, 11.9	4.06 dd 2.2, 11.9	4.06 dd 2.2,12.0	62.3	62.4	62.7	62.6	62.6
6B‴	3.83 m	3.83 m	3.87 dd 6.0, 11.9	3.80 dd 6.0, 11.9	3.81 m					
Coumaroyl/cafferoyl										
α			6.28 d 15.9	6.31 d 15.9	6.29 d 15.9			114.6	114.6	114.6
β			7.37 d 15.9	7.47 d 15.9	7.47 d 15.9			146.8	147.0	147.0
1								127.3	126.7	126.6
2			6.79 b	7.38 d 8.6	7.38 d 8.6			115.3	131.5	131.4
3				6.85 d 8.6	6.85 d 8.6			146.0	116.8	116.8
4								149.7	161.2	161.2
5			6.79 b	6.85 d 8.6	6.85 d 8.6			116.4	116.8	116.8
6			7.02 b	7.38 d 8.6	7.38 d 8.6			123.4	131.5	131.4
C = O								169.2	168.9	168.9

b, broad peak; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet.

[a] From COSY, overlapped by water signal.

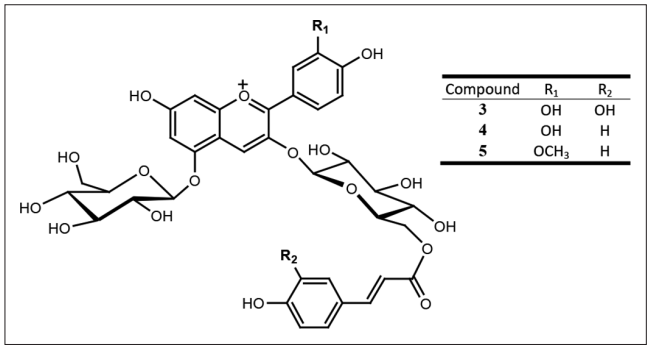

Figure 2. Structures of cyanidin 3-*O*-β-(6"-*O*-*E*-*p*-caffeoylglucopyranoside)-5-*O*-β-glucopyranoside (**3**), cyanidin 3-*O*-(6"-*E*-*p*-coumaroylglucopyranoside)-5-*O*-β-glucopyranoside (**4**), and peonidin 3-*O*-β-(6"-*E*-*p*-coumaroylglucopyranoside)-5-*O*-β-glucopyranoside (**5**).

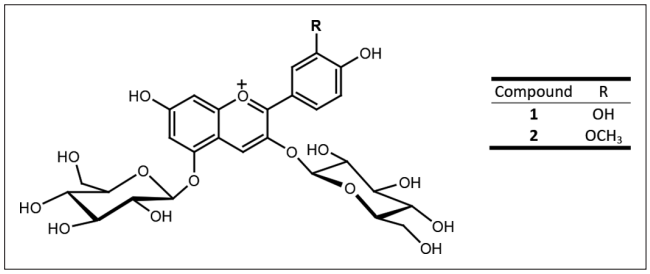

Figure 3. Structures of cyanidin 3,5-di-*O*-β-glucopyranoside (**1**) and peonidin 3,5-di-*O*-β-glucopyranoside (**2**).

chain of coupled protons H-3", H-4", H-5", and H-6A" and H-6B" were thereafter assigned in a similar fashion (Table 2). In cases where several sugar protons showed similar chemical shifts, the assignment was assisted by the TOCSY experiment. The chemical shifts and the coupling constants (Table 2) were in accordance with a β-glucopyranosyl. A cross-peak at δ 5.49/145.6 ppm in the HMBC spectrum between H-1" and C-3 of the aglycone showed that this glucosyl unit was connected to the 3-position of the aglycone. By using the doublet at δ 5.25 (7.8 Hz) as the starting point in the DQF-COSY spectrum together with the cross-peaks in the TOCSY spectrum, it was likewise possible to assign all the chemical shifts for the second monosaccharide moiety, β-glucopyranosyl (Table 2). A cross-peak at δ 5.25/156.8 ppm in the HMBC spectrum between H-1‴ and C-5 of the aglycone showed that this sugar unit was connected to the 5-position of the aglycone. All the ^{13}C shifts of the 2 sugar units were assigned from the HSQC experiment. The cross-peak at δ 4.56/168.9 ppm (H-6A"/C = O[I]) in the HMBC spectrum confirmed the linkage between the 3-β-glucopyranosyl and coumaroyl moiety to be at the 6"-hydroxyl. The HR-ESIMS spectrum of **5** showed a $[M]^+$ ion of m/z 771.2155 (calcd 771.2136 amu), corresponding to the molecular formula $C_{37}H_{39}O_{18}$, confirming the identification of **5** as peonidin 3-*O*-*β*-(6"-*E*-*p*-coumaroylglucopyranoside)-5-*O*-β-glucopyranoside (Figure 2).

Please refer to the supplemental material for 1D ^{1}H, HMBC, HSQC, COSY, and TOCSY NMR spectra for compound **5** and structures of compounds **1** to **5**.

The NMR spectrum of pigment **4** showed many similarities with the corresponding spectrum of **5** (Table 2). However, the aglycone was identified to be cyanidin by the chemical shift values at δ 9.04 (d, 0.8 Hz; H-4), δ 7.10 (d, 2.0 Hz; H-6), δ 7.03 (dd, 0.8, 2.0 Hz; H-8), δ 8.11 (d, 2.3 Hz; H-2'), δ 7.11 (d, 8.8 Hz; H-5'), and δ 8.37 (dd, 2.3, 8.8 Hz; H-6'). The HR-ESIMS spectrum of **4** showed a $[M]^+$ ion of m/z 757.2033 (calcd 757.198 amu), corresponding to the molecular formula $C_{36}H_{37}O_{18}$, confirming the identification of cyanidin 3-*O*-(6"-*E*-*p*-coumaroylglucopyranoside)-5-*O*-β-glucopyranoside (Figure 3). The other three pigments were identified as cyanidin 3-*O*-β-(6"-*O*-*E*-*p*-caffeoylglucopyranoside)-5-*O*-β-glucopyranoside (**3**; Figure 3), peonidin 3,5-di-*O*-β-glucopyranoside (**2**; Figure 3), and cyanidin 3,5-di-*O*-β-glucopyranoside (**1**; Figure 3), in a similar fashion.

The structures of the 5 anthocyanins from *C. terminalis* purple flowers are based on 2 of the common aglycones, cyanidin and peonidin. Cyanidin is the most widely distributed aglycone encountered in higher plants, mostly as part of cyanidin monoglycosides. It is also one of the major aglycones in the seed coat of the black soybean, blackberry, *Viola odorata*, and *Passiflora edulis*,[19-21] all of which are implicated in anti-asthma activity. Although compound **5** has a common anthocyanidin (peonidin), sugar (glucose), and aromatic acyl unit (coumaryl), establishment of the connectivity of these components by NMR to yield a Pn 3-(*p*-coumaroylglucoside)-5-glucoside has so far been reported for pigments isolated from *Impatiens walleriana* flowers only.[22] This depicts compound **5** as a scarce pigment. Peonidin-3-*O*-(cis-6-*O*-coumaryl-glucoside) and peonidin-3-*O*-(trans-6-*O*-coumaryl-glucoside) have been reported to occur in grape berries but not as 3,5-diglycosides.[23]

The knowledge of these structures may therefore help in studies on pharmacological activities and mechanisms of action relevant to asthma, which are currently limited.

The IC50 value (the concentration of sample that reduced the initial 2,2-diphenyl-1-picryl-hydrazyl [DPPH] concentration by 50%) of the extract (Table 3) shows that the extract is an effective free radical scavenger and thus may protect the body from free radical damage. The useful biological properties of anthocyanins are mainly attributed to their ability to scavenge reactive oxygen species.[24] The DPPH radical scavenging activity of anthocyanins is known to be dependent on the chemical structure of anthocyanins.[25] In particular, the degree and position of hydroxylation and methoxylation in the B-ring have a noteworthy effect on the radical scavenging ability of anthocyanins in aqueous solution.[8,26,27] The hydroxylation enhances the activity more than the methoxylation and the more the oxygenation of the B-ring the better the activity. Thus, for the common aglycones, delphinidin possesses the highest free radical scavenging activity, followed by cyanidin and peonidin, pelargonidin, malvidin, and petunidin.[14] From

Table 3. Quantitative Amounts of **1**-**5** and IC50 for Anthocyanin Extract and Quercetin.

Analyte	Amount (mg/g fr. wt)	IC50 ± SD (μg/mL)
Anthocyanin 1	2.6 ± 0.2	15.8 ± 0.7
Anthocyanin 2	2.8 ± 0.3	17.5 ± 0.8
Anthocyanin 3	3.2 ± 0.2	13.8 ± 0.5
Anthocyanin 4	6.2 ± 0.4	16.4 ± 0.7
Anthocyanin 5	9.8 ± 0.2	15.0 ± 0.8
Anthocyanins combined		11.1 ± 0.8
Quercetin		4.5 ± 0.4

the results (Table 3), the cyanidin- and peonidin-based anthocyanins identified in the flowers of *C. terminalis* make a similar contribution to the radical scavenging activity. The small differences in quantity seem to have negligible effect on the antioxidant property. Overall, the combined anthocyanins shows a better radical scavenging activity probably due to the synergistic effect.

Therefore, the presence of anthocyanin phytochemicals and the appreciable antioxidant activity of the extract of the purple flowers of *C. terminalis* may provide a scientific rationale for the efficacy of the extract as anti-asthma drug. Thus, the pharmaceutical potential of flowers of *C. terminalis* may further be investigated to explore the full potential of *C. terminalis* flowers for pharmaceutical and functional food benefits.

Experimental

Plant Material

Fresh purple flowers of *C. terminalis* were collected from the University of Kisubi lawns in Entebbe (Uganda). The plant was authenticated from the Botany Department at Makerere University where a voucher specimen (Adaku 02/2011) was deposited in the herbarium.

Extraction and Isolation

The fresh flowers (520 g) were extracted for 6 hours with 0.8 L of MeOH/TFA (0.5% v/v), in sealed Erlenmeyer flask in a freezer. The filtered extract was concentrated under reduced pressure at 28°C, partitioned against ethyl acetate (3 times), and applied to an Amberlite XAD-7 column. The anthocyanins adsorbed on the column were washed with water till neutral conditions were achieved and then eluted with MeOH/TFA (0.5% v/v). The individual anthocyanins in the concentrated eluate were separated by Sephadex LH-20 column chromatography using H_2O-MeOH-TFA (79.5:20:0.5, v/v/v) as the eluent. The Sephadex LH-20 fractions with similar qualitative anthocyanin contents (revealed by analytical HPLC) were combined and purified by preparative HPLC.

Spectroscopy

The UV-Vis absorption spectra were recorded on-line during the HPLC analysis, and the spectral measurements were made over the wavelength range 200 to 600 nm in steps of 2 nm. The NMR experiments were carried out at 600.13 and 150.92 MHz for ^{1}H and ^{13}C, respectively, on a Bruker Biospin Ultrashield Plus AV-600 MHz instrument equipped with a TCI ^{1}H-^{13}C/^{15}N CryoProbe head, at 298 K. The deuteriomethyl ^{13}C signal and the residual ^{1}H signal of the solvent, CF_3COOD-CD_3OD (95:5; v/v), were used as secondary references (δ 49.0 and δ 3.4 ppm from TMS for ^{1}H and ^{13}C, respectively).[28] The NMR experiments ^{1}H, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC, ^{1}H-^{1}H DQF-COSY, and ^{1}H-^{1}H TOCSY were recorded. High-resolution LC-MS (ESI+/TOF) spectra were recorded using a JMS-T100LC with an AccuTOF mass separator. A Develosil C18 (100 mm × 2.0 mm, 3.0 μm particle size) column was used for separation.

HPLC Analysis

The analytical HPLC system was Agilent 1100 Series, equipped with a HP 1050 diode-array detector (Hewlett-Packard), a 20 μL loop, Micro Auto-sampler, and a 200 × 4.6 mm i.d., 5 μm ODS Hypersil column (Supelco, Bellefonte, PA, USA). Two solvents: A, water (0.5% TFA) and B, acetonitrile (0.5% TFA) were used, following the elution profile according to Byamukama et al.[29] Anthocyanins isolated from blackcurrant (*Ribes nigrum*)[30] and Fuchsia spp.[31] were used as reference compounds. The preparative HPLC system consisted of a Gilson 321 pump equipped with an Ultimate 3000 Variable Wavelength Detector, a 25 × 2.2 cm (10 μm) Econosphere C18 column (Grace, USA). Solvents: A, water (0.5% TFA) and B, acetonitrile (0.5% TFA) were used, following the elution profile according to Andersen et al.[32]

Quantitative Determination

HPLC method was used to determine the amounts of the individual anthocyanins.[31] A sample of fresh flowers of *C. terminalis* (1.2 g) was extracted for 4 hours in 10 mL of methanol containing 0.5% TFA (v/v) in a refrigerator. The extraction

was repeated twice to exhaust the pigments. The quantitative amounts of anthocyanins **1-5** were determined from a HPLC calibration curve based on cyanidin 3-glucoside isolated from wild Norwegian blackberries, without taking into account the variation of molar absorption coefficients for individual pigments. Each of the concentration points of the calibration curve was based on average data from three parallel injections. The relative quantitative amounts of **1-5** (Table 3) were based on peak areas in analytical HPLC profile.

Antioxidant Assay

The free radical scavenging activity of the flower extract and the individual anthocyanins was evaluated against DPPH as described by Wangensteen et al.[33] Briefly, solutions of DPPH in methanol (4 mg/mL) and the test compound in DMSO (10 mg/mL) were prepared. UV/Vis absorbance at 517 nm for an aliquot of the DPPH solution (2.95 mL) was measured. The aliquot was mixed with the test compound solution (0.05 mL) and the UV/Vis absorbance at 517 nm was measured for 5 minutes. The procedure was repeated for the test compound serial dilutions of 5, 2.5, 1.3, and 0.7 mg/mL. Quercetin was used as a positive control. All aliquots were analyzed in triplicate and results given as averages ± standard deviation.

Cyanidin 3,5-di-*O*-β-Glucopyranoside (1)

UV/Vis λ_{max}: Table 1.

^{1}H NMR and ^{13}C NMR: Table 2.

LC-MS (ESI+/TOF): m/z $[M]^+$ calcd for $C_{27}H_{31}O_{16}$: 611.1612; found: 611.1619.

Peonidin 3,5-di-*O*-β-Glucopyranoside (2)

UV/Vis λ_{max}: Table 1.

^{1}H NMR and ^{13}C NMR: Table 2.

LC-MS (ESI+/TOF): m/z $[M]^+$ calcd for $C_{28}H_{33}O_{16}$: 625.1769; found: 625.1770.

Cyanidin 3-*O*-β-(6''-*O*-*E*-*p*-Caffeoylglucopyranoside)-5-*O*-β-Glucopyranoside (3)

UV/Vis λ_{max}: Table 1.

^{1}H NMR and ^{13}C NMR: Table 2.

LC-MS (ESI+/TOF): m/z $[M]^+$ calcd for $C_{37}H_{39}O_{19}$: 787.2080; found: 787.2145.

Cyanidin 3-*O*-(6''-*E*-*p*-Coumaroylglucopyranoside)-5-*O*-β-Glucopyranoside (4)

UV/Vis λ_{max}: Table 1.

^{1}H NMR and ^{13}C NMR: Table 2.

LC-MS (ESI+/TOF): m/z $[M]^+$ calcd for $C_{36}H_{37}O_{18}$: 757.1980; found: 757.2033.

Peonidin 3-*O*-β-(6''-*E*-*p*-Coumaroylglucopyranoside)-5-*O*-β-Glucopyranoside (5)

UV/Vis λ_{max}: Table 1.

^{1}H NMR and ^{13}C NMR: Table 2.

LC-MS (ESI+/TOF): m/z $[M]^+$ calcd for $C_{37}H_{39}O_{18}$: 771.2136; found: 771.2155.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material

Supplemental material for this article is available online.

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