

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

Natural Product Communications
Volume 15(5): 1–7
© The Author(s) 2020
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/1934578X20922637
journals.sagepub.com/home/npx



Christopher Adaku^{1,2} , Irene Skaar³, Robert Byamukama¹, Monica Jordheim³ , and Øyvind M. Andersen³

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 ($IC_{50} \pm SD = 13.1 \pm 0.8$ μ g/mL) against quercetin ($IC_{50} \pm SD = 4.5 \pm 0.4$ μ g/mL) compared to the individual anthocyanins ($IC_{50} \pm SD = 13.8 \pm 0.5$ to 16.4 ± 0.7 μ g/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

¹Department of Chemistry, Makerere University, Uganda

²Department of Chemistry, Mbarara University of Science and Technology, Uganda

³Department of Chemistry, University of Bergen, Norway

Corresponding Author:

Christopher Adaku, Department of Chemistry, Mbarara University of Science and Technology, P.O. Box 1410, Mbarara, Uganda.

Email: cadaku@must.ac.ug



Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (<https://creativecommons.org/licenses/by-nc/4.0/>) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (<https://us.sagepub.com/en-us/nam/open-access-at-sage>).

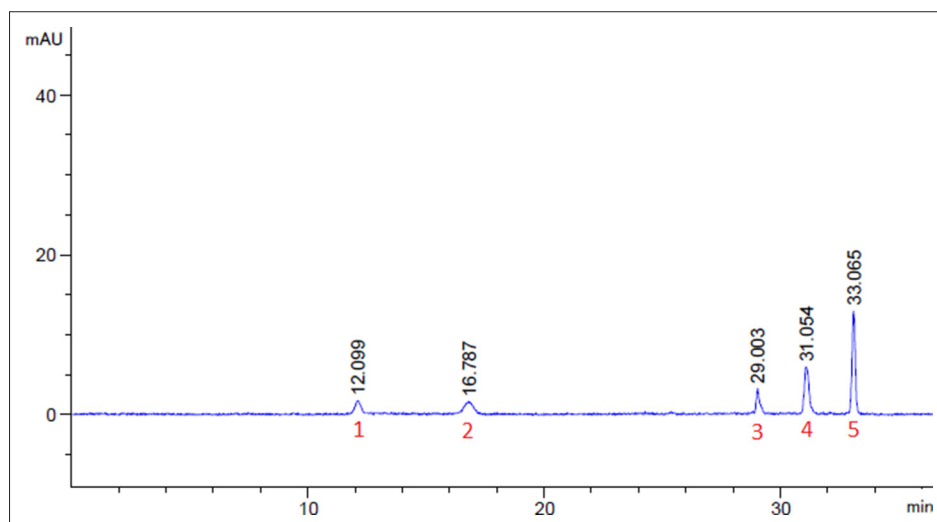


Figure 1. HPLC profile of *Cordyline terminalis* extract.

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 $\lambda_{\max}$ at 514 to 523 nm and $A_{440}/A_{\text{vis-max}}$ ratio of 17% to 18% (Table 1), in accordance with 3,5-substituted cyanidin or peonidin derivatives.¹⁷ The presence of local $\lambda_{\text{acyl-max}}$ 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 $A_{\text{UV-max}}/A_{\text{vis-max}}$ of 0.70, 0.77, and 0.81 for pigments **3**, **4**, and **5**, respectively (Table 1), indicate monoacylation.¹⁷

The downfield region of ^1H 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 ^1H NMR signals were assigned to *p*-coumaroyl acyl moiety as follows: δ 7.38 (d, 8.6 Hz; H-2', 6'), δ 6.85 (d, 8.6 Hz; H-3', 5'), δ 6.29 (d, 15.9 Hz; H- α^1), δ 7.47 (d, 15.9 Hz; H- β^1). The coupling constants of H- α^1 and H- β^1 (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 ^1H 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)	$\lambda_{\text{vis-max}}$ (nm)	$\lambda_{\text{UV-max}}$ (nm)	$A_{440}/A_{\text{vis-max}}$ (%)	$A_{\text{UV-max}}/A_{\text{vis-max}}$
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. ^1H (600.13 MHz) and ^{13}C (150.92 MHz) NMR Spectral Data for Compounds **1-5**.

	^1H δ (ppm) <i>J</i> (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.

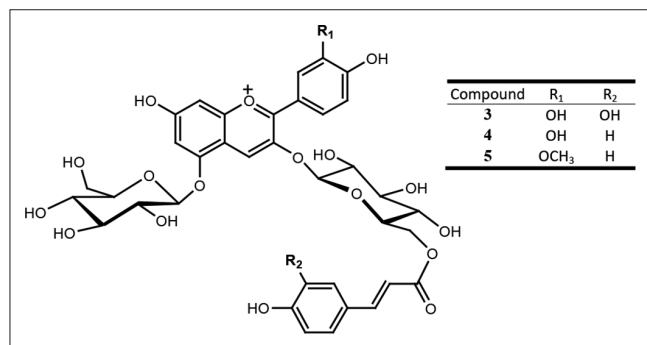


Figure 2. Structures of cyanidin 3-*O*-β-(6''-*O*-*E*-*p*-caffeoylglucopyranoside)-5-*O*-β-glucopyranoside (**3**), cyanidin 3-*O*-(6''-*E*-*p*-coumaroylglucopyranoside)-5-*O*-β-glucopyranoside (**4**), and peonidin 3-*O*-β-(6''-*E*-*p*-coumaroylglucopyranoside)-5-*O*-β-glucopyranoside (**5**).

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 ¹³C 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⁺) 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 C₃₇H₃₉O₁₈, 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 ¹H, 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 C₃₆H₃₇O₁₈, confirming the identification of cyanidin 3-*O*-(6''-*E*-*p*-coumaroylglucopyranoside)-5-*O*-β-glucopyranoside (Figure 3).

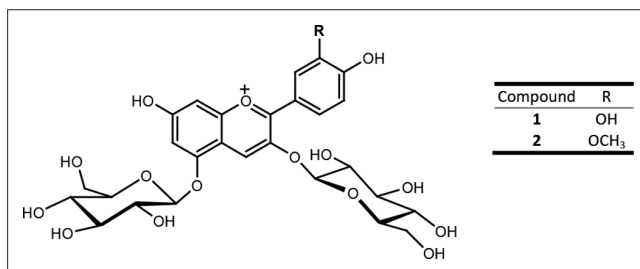


Figure 3. Structures of cyanidin 3,5-di-*O*-β-glucopyranoside (**1**) and peonidin 3,5-di-*O*-β-glucopyranoside (**2**).

oylglucopyranoside)-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 $\pm$ SD (μ g/mL)
Anthocyanin 1	2.6 $\pm$ 0.2	15.8 $\pm$ 0.7
Anthocyanin 2	2.8 $\pm$ 0.3	17.5 $\pm$ 0.8
Anthocyanin 3	3.2 $\pm$ 0.2	13.8 $\pm$ 0.5
Anthocyanin 4	6.2 $\pm$ 0.4	16.4 $\pm$ 0.7
Anthocyanin 5	9.8 $\pm$ 0.2	15.0 $\pm$ 0.8
Anthocyanins combined		11.1 $\pm$ 0.8
Quercetin		4.5 $\pm$ 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 H₂O-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 ¹H and ¹³C, respectively, on a Bruker Biospin Ultrashield Plus AV-600 MHz instrument equipped with a TCI ¹H-¹³C/¹⁵N CryoProbe head, at 298 K. The deuteriomethyl ¹³C signal and the residual ¹H signal of the solvent, CF₃COOD-CD₃OD (95:5; v/v), were used as secondary references (δ 49.0 and δ 3.4 ppm from TMS for ¹H and ¹³C, respectively).²⁸ The NMR experiments ¹H, ¹H-¹³C HSQC, ¹H-¹³C HMBC, ¹H-¹H DQF-COSY, and ¹H-¹H 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 $\times$ 2.0 mm, 3.0 μ m 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 μ L loop, Micro Auto-sampler, and a 200 $\times$ 4.6 mm i.d., 5 μ m 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 $\times$ 2.2 cm (10 μ m) 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 Wangenstein 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 $\pm$ standard deviation.

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

UV/Vis $\lambda_{\max}$: Table 1.

¹H NMR and ¹³C NMR: Table 2.

LC-MS (ESI+/TOF): m/z [M]⁺ calcd for C₂₇H₃₁O₁₆: 611.1612; found: 611.1619.

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

UV/Vis $\lambda_{\max}$: Table 1.

¹H NMR and ¹³C NMR: Table 2.

LC-MS (ESI+/TOF): m/z [M]⁺ calcd for C₂₈H₃₃O₁₆: 625.1769; found: 625.1770.

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

UV/Vis $\lambda_{\max}$: Table 1.

¹H NMR and ¹³C NMR: Table 2.

LC-MS (ESI+/TOF): m/z [M]⁺ calcd for C₃₇H₃₉O₁₉: 787.2080; found: 787.2145.

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

UV/Vis $\lambda_{\max}$: Table 1.

¹H NMR and ¹³C NMR: Table 2.

LC-MS (ESI+/TOF): m/z [M]⁺ calcd for C₃₆H₃₇O₁₈: 757.1980; found: 757.2033.

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

UV/Vis $\lambda_{\max}$: Table 1.

¹H NMR and ¹³C NMR: Table 2.

LC-MS (ESI+/TOF): m/z [M]⁺ calcd for C₃₇H₃₉O₁₈: 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.

ORCID ID

Christopher Adaku  <https://orcid.org/0000-0003-3640-4958>

Monica Jordheim  <https://orcid.org/0000-0002-6804-921X>

Supplemental Material

Supplemental material for this article is available online.

References

1. Global Asthma Network (GAN). The Global Asthma Report 2014. Auckland, New Zealand; 2014.
2. Li X-M, Brown L. Efficacy and mechanisms of action of traditional Chinese medicines for treating asthma and allergy. *J Allergy Clin Immunol*. 2009;123(2):297-308. doi:10.1016/j.jaci.2008.12.026
3. Watson JP, Lewis RA. Is asthma treatment affordable in developing countries? *Thorax*. 1997;52(7):605-607. doi:10.1136/thx.52.7.605
4. Kotwani A. Availability, price and affordability of asthma medicines in five Indian states. *Int J Tuberc Lung Dis*. 2009;13(5):574-579.
5. Crapo JD, Day BJ. Modulation of nitric oxide responses in asthma by extracellular antioxidants. *J Allergy Clin Immunol*. 1999;104(4 Pt 1):743-746. doi:10.1016/S0091-6749(99)70282-4
6. Crapo JD. Oxidative stress as an initiator of cytokine release and cell damage. *Eur Respir J*. 2003;44:4s-6. doi:10.1183/09031936.03.00000203a
7. Broadhurst CL, Badmaev V. *The whole family guide to natural asthma relief: comprehensive drug-free solutions for the treatment and prevention of asthma and allergies*. Penguin Putnam Inc; 2002:128.
8. Wang H, Cao G, Prior RL. Oxygen radical absorbing capacity of anthocyanins. *J Agric Food Chem*. 1997;45(2):304-309. doi:10.1021/jf960421t
9. Tsuda T, Horio F, Osawa T. The role of anthocyanins as an antioxidant under oxidative stress in rats. *Biofactors*. 2000;13(1-4):133-139. doi:10.1002/biof.5520130122
10. Rossi A, Serraino I, Dugo P, et al. Protective effects of anthocyanins from blackberry in a rat model of acute lung inflammation. *Free Radic Res*. 2003;37(8):891-900. doi:10.1080/1071576031000112690

11. Mi-Ja C, Ha T, Choi H, Lee J, Park Y. Inhibitory effects of anthocyanins isolated from black soybean (*Glycine max* L.) seed coat on degranulation and cytokine generation in RBL-2H3 cells. *J Korean Soc Food Sci Nutr*. 2011;40(12):1662-1667.
12. Watson RR, Zibadi S, Rafatpanah H, et al. Oral administration of the purple passion fruit peel extract reduces wheeze and cough and improves shortness of breath in adults with asthma. *Nutr Res*. 2008;28(3):166-171. doi:10.1016/j.nutres.2008.01.003
13. Qasemzadeh MJ, Sharifi H, Hamedanian M, et al. The Effect of *Viola odorata* Flower Syrup on the Cough of Children With Asthma: A Double-Blind, Randomized Controlled Trial. *J Evid Based Complementary Altern Med*. 2015;20(4):1-5. doi:10.1177/2156587215584862
14. Kähkönen MP, Heinonen M. Antioxidant activity of anthocyanins and their aglycons. *J Agric Food Chem*. 2003;51(3):628-633. doi:10.1021/jf025551i
15. Ehrlich C. Special problems in an ethnobotanical literature search: Cordyline terminalis Kunth (L.), the "Hawaiian ti plant". *J Ethnobiol*. 1989;9(1):51-63.
16. Kent HW. *Treasury of Hawaiian Words in One Hundred and One Categories. The Masonic Public Library of Hawaii*. University of Hawaii press; 1995:218.
17. Hong V, Wrolstad RE. Use of HPLC separation/photodiode array detection for characterization of anthocyanins. *J Agric Food Chem*. 1990;38(3):708-715. doi:10.1021/jf00093a026
18. Harborne JB. Spectral methods of characterizing anthocyanins. *Biochem J*. 1958;70(1):22-28. doi:10.1042/bj0700022
19. Choung MG, Baek IY, Kang ST, et al. Isolation and determination of anthocyanins in seed coats of black soybean (*Glycine max* (L.) Merr.). *J Agric Food Chem*. 2001;49(12):5848-5851. doi:10.1021/jf010550w
20. Fan-Chiang H-J, Wrolstad RE. Anthocyanin pigment composition of blackberries. *J Food Sci*. 2005;70(3):C198-C202. doi:10.1111/j.1365-2621.2005.tb07125.x
21. Kidøy L, Nygård AM, Andersen ØM, Pedersen AT, Aksnes DW, Kiremire BT. Anthocyanins in fruits of *Passiflora edulis* and *P. suberosa*. *Journal of Food Composition and Analysis*. 1997;10(1):49-54. doi:10.1006/jfca.1996.0514
22. Tatsuzawa F, Suzuki Y, Sato M, Kato K. Flower Colors and Pigments in the Cultivars of *Impatiens walleriana* >. *Hort. Res.* 2016;15(2):115-122. doi:10.2503/hrj.15.115
23. He J-J, Liu Y-X, Pan Q-H, Cui X-Y, Duan C-Q. Different anthocyanin profiles of the skin and the pulp of Yan73 (Muscat Hamburg × Alicante Bouschet) grape berries. *Molecules*. 2010;15(3)-. doi:10.3390/molecules15031141
24. Prior RL, Wu X. Anthocyanins: structural characteristics that result in unique metabolic patterns and biological activities. *Free Radic Res*. 2006;40(10):1014-1028. doi:10.1080/10715760600758522
25. Miguel MG. Anthocyanins: antioxidant and/or anti-inflammatory activities. *J Appl Pharm Sci*. 2011;1(6):7-15.
26. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Biol Med*. 1996;20(7):933-956. doi:10.1016/0891-5849(95)02227-9
27. Fukumoto LR, Mazza G. Assessing antioxidant and prooxidant activities of phenolic compounds. *J Agric Food Chem*. 2000;48(8):3597-3604. doi:10.1021/jf000220w
28. Andersen ØM, Fossen T. Characterization of anthocyanins by NMR. In: Wrolstad RE, ed. *Handbook of Food Analytical Chemistry: Pigments, Colorants, Flavors, Texture, and Bioactive Food Components*. . John Wiley and Sons, Inc; 2005:2005. 47-69.
29. Byamukama R, Jordheim M, Kiremire B, Andersen ØM. New Anthocyanins from Stem Bark of Castor, *Ricinus communis*. *Nat Prod Commun*. 2008;3(9):1497-1500. doi:10.1177/1934578X0800300920
30. Frøytløg C, Slimestad R, Andersen ØM. Combination of chromatographic techniques for the preparative isolation of anthocyanins — applied on blackcurrant (*Ribes nigrum*) fruits. *J Chromatogr A*. 1998;825(1):89-95. doi:10.1016/S0021-9673(98)00673-6
31. Jordheim M, Enerstvedt KH, Andersen OM. Identification of cyanidin 3-O-β-(6''-(3-hydroxy-3-methylglutaroyl)glucoside) and other anthocyanins from wild and cultivated blackberries. *J Agric Food Chem*. 2011;59(13):7436-7440. doi:10.1021/jf201522b
32. Andersen ØM, Jordheim M, Byamukama R, et al. Anthocyanins with unusual furanose sugar (apiose) from leaves of *Synadenium grantii* (Euphorbiaceae). *Phytochemistry*. 2010;71(13):1558-1563. doi:10.1016/j.phytochem.2010.05.025
33. Wangensteen H, Samuelsen AB, Malterud KE. Antioxidant activity in extracts from coriander. *Food Chem*. 2004;88(2):293-297. doi:10.1016/j.foodchem.2004.01.047