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Original article

Pigmented flower extracts of plant species from the Geraniaceae and Lamiaceae families as natural food colourants: anthocyanin composition, thermal and oxidative stability

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Summary This study determined the anthocyanin profile of pigmented flower extracts from some Geraniaceae and Lamiaceae plant species found in South Africa, and their thermal and oxidative stability to assess their potential food application. Anthocyanins in the Geraniaceae (*Pelargonium grandiflorum* Willd. and *Pelargonium* × *hortorum* L.H. Bailey, *Pelargonium zonale* hybrid) were 3,5-diglucosides of delphinidin, petunidin, pelargonidin, peonidin and malvidin and acetyl-acylated malvidin, delphinidin and petunidin. The Lamiaceae species (*Salvia aurea* × *dolomitica* Bae's blue, *Salvia dolomitica* Codd and *Plectranthus zuluensis* T. Cooke) mainly contained rutinosides of pelargonidin, glucosides of petunidin, pelargonidin and *p*-coumaric acid- and malonyl-acylated delphinidin and malvidin. Lamiaceae pigments had higher thermal and oxidative stability. This could be due to aromatic malonyl-acylated anthocyanin self-association and strong intermolecular interactions with phenolic acids and derivatives. Flowers from Lamiaceae and Geraniaceae have potential application as natural food colourants, but temperature and oxidising conditions must be considered depending on the particular species being used.

Keywords Anthocyanins Geraniaceae, lamiaceae, oxidative stability, thermal stability.

Introduction

Although colourants extracted from various natural sources are used extensively in the food industry worldwide (Downham & Collins, 2000), the demand for such nature-derived food colourants remains. Plants from the Geraniaceae and Lamiaceae families have a long history of use as herbal medicine and as aromatic herbs in culinary practice in South Africa (Scott *et al*., 2004). The flowers from these plant families present with different colours and therefore could have potential as sources of natural food colourants.

Anthocyanins are major components of most colourants used in the food industry. These anthocyanins are also known to be affected by several factors such as pH, temperature, oxidation and light which ultimately affect stability of the food colourant. Co-pigment molecules can interact with anthocyanin molecules through several bonding mechanisms, thereby improving their stability by protecting the anthocyanin nucleus from nucleophilic attack by water and oxidising agents and could protect the anthocyanin molecules from light (Renis *et al*., 2008).

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Some work on anthocyanin characterisation going back almost 3 decades indicate that pigmented flowers from the Geraniaceae contain 3,5-diglucoside and acetyl-acylated anthocyanins while pigmented flowers from the Lamiaceae contain aromatic acylated anthocyanins and malonyl anthocyanins (Saito & Harborne, 1992; Mitchell *et al*., 1998; Jordheim *et al*., 2016). However, there seems to be no research on stability of pigmented extracts from these two plant families. There have been some reports on the thermal, hydrogen peroxide (oxidative) and light stability of pigmented extracts from red cabbage and black carrot (Kırca *et al*., 2007; Matsufuji *et al*., 2007; Fenger *et al*., 2019). There have also been some reports on thermal stability of pigmented extracts from *Hibiscus sabdariffa* L. (Maciel *et al*., 2018), *Tulipa gesneriana* L. (tulip) (Sagdic *et al*., 2013) and *Clitoria ternatea* L. (butterfly pea) (Escher *et al*., 2020).

There is increasing demand among consumers for food ingredients from natural sources. This presents an opportunity for innovation in food colourants by studying the flowers of plant species as potential sources of natural food colourants. The objective of this study was therefore to determine the anthocyanin profile of pigmented extracts of a sample of flowers

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from some species of the Geraniaceae and Lamiaceae plant families found in South Africa and their stability with the aim of evaluating their potential as natural food colourants.

Materials and methods

Samples

Flower samples of Geraniaceae family, *Pelargonium grandiflorum* Willd. (large-flowered pelargonium) and *P.* × *hortorum* L.H. Bailey, were procured from Plant Land, Centurion, Gauteng, South Africa. *Pelargonium zonale* (L.) L'Hér. hybrids were procured in Lynnwood Ridge, Pretoria, South Africa.

Flower samples of Lamiaceae family, *Salvia aurea* × *dolomitica* Bae's blue and *Salvia dolomitica* Codd, were procured from the botanical gardens at the Future Africa campus of the University of Pretoria, South Africa. *Plectranthus zuluensis* T. Cooke was procured from the Hatfield Campus, University of Pretoria, South Africa.

Flower samples were air dried at 27°C in an air convection oven for 24–48 h. After drying, the dried flower petals were ground into a fine powder using a hand blender. The flower powder was stored in airtight resealable bags at 4 °C.

Preparation of pigmented extracts

Approximately 0.5 g of dried flower powders were extracted with 150 mL acidified distilled water (1% (v/v) formic acid in water) using magnetic stirring for 24 h. After extraction, the extracts were filtered through a Büchner funnel with filter paper under vacuum and freeze dried. For all degradation kinetics analyses, 10 mg of the freeze-dried extract were re-dissolved in 5 mL distilled water. Yields for each extract were as follows: *P. grandiflorum*: 58.7%, *P.* × *hortorum*: 44.4%, *P. zonale* hybrid: 54.6%, S. *aurea* × *dolomitica*: 52.4%, *S. dolomitica*: 48.4% and *P. zuluensis*: 45.6%.

Extracts for UPLC-MS analysis were prepared by extracting 0.5 g of flower powder with 10 mL of 0.5% formic acid in distilled water for 24 h at room temperature using magnetic stirrers. The extracts were filtered through 0.45 μm PTFE filters into amber vials and stored at −20°C until UPLC-MS analysis.

UPLC-MS analysis

The chromatographic analysis was performed as described by Alberts *et al.* (2012). A Waters Acquity UPLC system (Waters Corporation, Milford, MA, USA) incorporating a binary pump, vacuum degasser, autosampler, column oven and Micromass Xevo tandem quadrupole mass spectrometric detector (Manchester, UK) equipped with ESI probe was used. Reversed-phase separation was performed using an Acquity BEH C18 column (2.1 mm × 100 mm, 1.7 m particle size) at a temperature of 50 °C. Mobile phase A was 7.5% formic acid in water and mobile phase B was acetonitrile. The gradient started with 1% B isocratically for 0.5 min followed by a linear increase to 15% at 15 min, 23% at 20 min and 28% at 25 min. Column clean-up at 100% B was then followed for 1 min followed by re-equilibration for 4 min to obtain a total runtime of 30 min. The flow rate was 0.1 mL min^{-1} throughout and injection volumes of 10 L were used. The source conditions were as follows: capillary voltage 2.5 kV and cone voltage 15V, while the desolvation temperature was 500 °C and the desolvation and cone gas (both nitrogen) flows were 1000 and 50 L h^{-1} respectively. Ideain (cyanidin-3-*O*-galactoside) was used as a standard for anthocyanin quantification. The concentration of anthocyanins identified in the pigmented flower extracts was measured based on the individual compound peak area response and calculated using the Ideain standard curve.

Thermal Temperature stability

The thermal stability of flower extracts was determined using a temperature degradation kinetics method described by Selim *et al.* (2008) and Sagdic *et al.* (2013) with modifications. A 0.2% (w/v) solution of the freeze-dried pigmented extracts was prepared in distilled water. A 1 mL volume of the extract was made up to 9 mL with 0.1 M citrate buffer (pH 1), placed in a screw cap test tube and heated in a thermostatically controlled water bath at temperatures of 70, 80°C for 270 min and 90°C for 180 min. Test tubes were cooled down immediately to ambient temperature under running tap water and the change in absorbance at 520 nm was measured every 30 min. The zero-time absorbance values were determined before each experiment. The experiment was done in duplicate.

Hydrogen peroxide (H_2O_2) oxidative stability

Oxidative stability of flower extracts was determined using an oxidative degradation kinetics method described by Özkan *et al.* (2002) with modification. Three different concentrations of H_2O_2 (9.31, 18.61 and 27.92 mM) were used. The pre-determined H_2O_2 amounts were rapidly added to 1 mL of the flower extracts (in citrate-phosphate buffer pH 1) to make up 8 mL of 8.15, 16.28 and 24.43 mM. The change in absorbance at 520 nm was measured every 15 min for 180 min for each concentration of hydrogen peroxide used. The zero-time absorbance values were determined by preparing the samples with the same amount

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of distilled water instead of hydrogen peroxide. The experiment was done in duplicate.

Calculation of degradation kinetics parameters

The degradation rate constant (k) and half-life ($t_{1/2}$) for stability was calculated with the following first-order kinetic equations used by Sagdic *et al.* (2013).

$$-\frac{dC}{dt} = kC \tag{1}$$

Solving eqn (1) gives (3).

$$C = \frac{A_t}{A_0} \tag{2}$$

$$\ln(C) = -kt \tag{3}$$

$$\ln\left(\frac{A_t}{A_0}\right) = -kt \tag{4}$$

$$t_{1/2} = \ln\frac{0.5}{K} \tag{5}$$

Where A_t: Absorbance at 520 nm at t min; A_0: Absorbance at 520 nm at 0 min.

Statistical analysis

Experiments were conducted in triplicates. One-way analysis of variance (ANOVA) was conducted as required using SPSS software. Significant differences between means were determined at $P \leq 0.05$. Significant means were separated using the least significant difference (LSD), Tukey and Scheffe tests at $P \leq 0.05$.

Results and discussion

Identification of anthocyanins in flower extracts of the Geraniaceae and Lamiaceae

Table 1 shows chromatographic and mass spectral data of anthocyanins identified in the pigmented flower extracts. The anthocyanins were all sugar derivatives of delphinidin, cyanidin, petunidin, pelargonidin, peonidin and malvidin and they were identified mainly based on their reported mass spectral characteristics and fragmentation patterns (Cuyckens & Claeys, 2004; Alberts *et al.*, 2012).

Delphinidin derivatives

All the nine delphinidin derivatives identified produced a main fragment at m/z 303 which corresponds to the delphinidin aglycone. Peaks 5 and 10 were identified as delphinidin-3-*O*-glucoside and delphinidin-3-*O*-rhamnoside due to loss of glucose (−162 amu) and rhamnose (−146) moieties, respectively, from the anthocyanin. Peaks 1, 3 and 21 were identified as delphinidin-3,5-diglucoside, delphinidin-3-*O*-rutinoside and its isomer due to loss of diglucoside (−324 amu) and rutinoside (−308 amu), respectively, from the anthocyanin. Peak 13 was identified as delphinidin-3-*O*-(6-acetyl)-5-*O*-diglucoside based on loss of a glucose moiety (−162 amu) from the anthocyanin to produce the fragment at m/z 507 and loss of an acetyldiglucoside (−336 amu) to produce the delphinidin aglycone (m/z 303). Peak 16 was identified as delphinidin-3-*O*-robinobiosido-5-*O*-rhamnoside based on loss of the trisaccharide unit of robinose and rhamnose (−454 amu) from the anthocyanin to produce the delphinidin aglycone. Peaks 26 and 30 were identified as isomers of delphinidin-3-(6-p-coumaroylglucoside)-5-(4,6-dimalonyl) glucoside based on loss of a 642 amu fragment [*p*-coumaroylglucoside (−308 amu) + dimalonylglucoside (−334 amu)] from the anthocyanin to produce the delphinidin aglycone. The 637 amu fragment is produced via loss of the *p*-coumaroylglucoside (−308 amu) moiety.

Petunidin derivatives

All three petunidin derivatives identified produced a main fragment at m/z 317.07 corresponding to the aglycone. Peaks 6 and 8 were identified as petunidin-3,5-*O*-diglucoside and petunidin-3-*O*-rutinoside based on the loss of a diglucoside (−324 amu) and rutinoside (−308 amu) from the petunidin aglycone. Peak 19 was identified as petunidin-3-*O*-(6-acetyl)-5-*O*-diglucoside based on the loss of an acetyldiglucoside (−366 amu) to produce the m/z 317.065, a glucose (−162 amu) moiety to produce a m/z 521.13 fragment and loss of an acetylglucoside (−204 amu) to produce a m/z 479.12 fragment.

Cyanidin derivatives

All three of the cyanidin derivatives identified produced a main fragment at m/z 287.06 which corresponds to the cyanidin aglycone. Peak 4 was identified as cyanidin-3,5-*O*-diglucoside based on the loss of a diglucoside (−324 amu) to yield the cyanidin aglycone. Peaks 23 and 25 were identified as cyanidin-3-*O*-rutinoside and its isomer, cyanidin-3-(glycosyl) rhamnoside, respectively, based on the loss of a rutinose (−308 amu) and a rhamnose (−146 amu) moiety resulting in the m/z 449.11 fragment.

Peonidin derivatives

All three peonidin derivatives identified produced a main fragment at m/z 301.07 which corresponds to the

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Table 1 Retention time (T_R), UV-visible absorption maxima (λmax) and mass spectral characteristics of anthocyanins found in pigmented flower extracts from the Geraniaceae and Lamiaceae

Peak no.	T_R (min)	λ_{max} (nm)	Molecular formula [M^+]	[M^+] (m/z)	MS/MS fragments (% intensity)	Proposed compound
1	8.33	277, 520	$C_{27}H_{31}O_{17}$	627.15	303.05 (100), 465.10 (12)	Delphinidin-3,5-*O*-diglucoside
3	9.55	278, 518	$C_{27}H_{31}O_{16}$	611.16	303.05 (100), 465.10 (21)	Delphinidin-3-*O*-rutinoside
4	10.00	276, 516	$C_{27}H_{31}O_{16}$	611.16	287.06 (100), 449.11 (17)	Cyanidin-3,5-*O*-diglucoside
5	10.10	276, 525	$C_{21}H_{21}O_{12}$	465.10	303.05 (100)	Delphinidin-3-*O*-glucoside
6	10.99	279, 524	$C_{28}H_{33}O_{17}$	641.17	641.173 (100), 317.066 (93), 479.118 (21)	Petunidin-3,5-*O*-diglucoside
7	11.37	267, 500	$C_{27}H_{31}O_{15}$	595.17	271.06 (100)	Pelargonidin-3,5-*O*-diglucoside
8	12.05	281, 524	$C_{28}H_{33}O_{16}$	625.18	317.066 (100), 479.121 (14)	Petunidin-3-*O*-rutinoside
9	12.78	284, 525	$C_{28}H_{33}O_{16}$	625.18	301.070 (100), 463.122 (22)	Peonidin-3,5-*O*-diglucoside
10	13.03	276, 524	$C_{21}H_{21}O_{11}$	449.11	303.049 (100)	Delphinidin-3-*O*-rhamnoside
11	13.44	242, 500	$C_{21}H_{21}O_{10}$	433.12	271.06 (100)	Pelargonidin-3-*O*-glucoside
12	13.49	274, 524	$C_{29}H_{35}O_{17}$	655.19	331.080 (100), 493.134 (22)	Malvidin-3,5-*O*-diglucoside
13	13.71	245, 529	$C_{29}H_{33}O_{18}$	669.17	303.049 (100), 507.116 (20)	Delphinidin-3-*O*-(6-acetyl)-5-*O*-diglucoside)
14	14.04	271, 525	$C_{27}H_{31}O_{17}$	627.16	319.0453 (100), 303.05 (37)	Delphinidin-3,7-diglucoside
15	14.53	244, 500	$C_{28}H_{31}O_{16}$	623.16	271.06 (100), 461.11 (23)	Pelargonidin glucoside derivative
16	15.17	273, 522	$C_{33}H_{41}O_{20}$	757.22	303.050 (100)	Delphinidin-3-*O*-robinobiosido-5-*O*-rhamnoside
17	15.67	237, 500	$C_{29}H_{33}O_{17}$	653.17	301.07 (100), 491.11 (14)	Peonidin glucoside derivative
19	16.39	275, 524	$C_{30}H_{35}O_{18}$	683.18	317.065 (100), 521.127 (25), 479,1232 (5)	Petunidin-3-*O*-(6-acetyl)-5-*O*-diglucoside)
20	17.04	275, 500	$C_{30}H_{35}O_{17}$	667.19	301.07 (100)	Peonidin 3-glucoside-5-(6-acetylglucoside)
21	17.14	267, 351, 514	$C_{27}H_{31}O_{16}$	611.16	303.048 (21), 465.102 (15)	Delphinidin 3-*O*-rutinoside isomer
23	18.14	266, 525	$C_{27}H_{31}O_{15}$	595.16	287.054 (100), 449.108 (8)	Cyanidin-3-*O*-rutinoside
24	18.83	275, 527	$C_{31}H_{37}O_{18}$	697.20	331.081 (100), 535.146 (23), 493.136 (9)	Malvidin-3-*O*-(6-*O*-acetyl)-5-*O*-diglucoside
25	19.59	245, 532	$C_{27}H_{31}O_{15}$	595.17	287.054 (100), 449.105 (7)	Cyanidin-3-(glycosyl) rhamnoside
26	19.02	278, 531	$C_{42}H_{41}O_{25}$	945.20	303.05 (100), 637.104 (17)	Delphinidin-3-(6-*p*-coumaroylglucoside)-5-(4,6-dimalonylglucoside) isomer
27	19.60	245, 500	$C_{41}H_{43}O_{22}$	887.23	331.08 (100), 579.14 (15)	Malvidin-3-(6-*p*-coumaroylglucoside)-5-(4-malonylglucoside) isomer
28	19.94	245, 500	$C_{38}H_{41}O_{19}$	801.22	331.08 (100), 639.17 (15)	Malvidin 3-(6-coumaroylglucoside) 5-glucoside
30	20.44	276, 531	$C_{42}H_{41}O_{25}$	945.19	303.049 (100), 637.104 (26), 611.14 (6)	Dimalonylawobanin (delphinidin-3-(6-*p*-coumaroylglucoside)-5-(4,6-dimalonylglucoside)
31	20.56	268, 531	$C_{27}H_{31}O_{14}$	579.17	271.061 (100), 433.11 (23)	Pelargonidin-3-*O*-rutinoside
32	20.72	245, 500	$C_{41}H_{43}O_{22}$	887.23	331.08 (100), 579.14 (15)	Malvidin-3-(6-*p*-coumaroylglucoside)-5-(4-malonylglucoside)

peonidin aglycone. Peaks 9 and 17 was identified as peonidin-3,5-*O*-diglucoside and a peonidin glucoside derivative, respectively, based on the loss of a diglucoside (−324 amu) moiety to produce the m/z 301 fragment (peak 9) and loss of a glucoside (−162 amu) resulting in the m/z 491.11 fragment (peak 17). Peak 20 was identified as peonidin 3-glucoside-5-(6-acetylglucoside) based on the loss of an acetyldiglucoside (−366 amu) to produce the m/z 301 fragment.

Malvidin derivatives

All five malvidin derivatives identified produced a main fragment at m/z 331.08 corresponding to the malvidin aglycone. Peak 12 was identified as malvidin-3,5-*O*-diglucoside based on the loss of a diglucoside (−324 amu) moiety from the malvidin aglycone. The loss of a glucoside (−162 amu) resulted in the m/z 493.13 fragment. Peak 24 was identified as malvidin-3-*O*-(6-*O*-acetyl)-5-*O*-diglucoside based on loss of an acetyldiglucoside (−366 amu) to produce the m/z 331 fragment. The loss of an acetylglucoside (−204 amu) resulted in a fragment at m/z 493.14 while the m/z 535.15 fragment is due to the loss of a glucoside (−162 amu) moiety. Peaks 27 and 32 were identified as malvidin-3-(6-*p*-coumaroylglucoside)-5-(4-malonylglucoside) and its isomer. For both compounds, loss of a *p*-coumaroyl-malonyldiglucoside (−556 amu) moiety from the malvidin molecule produced the m/z 331.08 fragment and the loss of coumaroylglucoside (−308 amu) resulting in the m/z 579.14 *m/z* fragment. Peak 28 was identified as malvidin 3-(6-coumaroylglucoside) 5-glucoside based on the loss of a coumaroyldiglucoside (−470 amu) to produce

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Table 2 Concentration of anthocyanins (μg IE/g) in pigmented extracts from flowers of some plant species from the Geraniaceae and Lamiaceae families

	Geraniaceae			Lamiaceae		
Compound	***P. grandiflorum***	***P. × hortorum***	***P. zonale* hybrid**	***S. aurea × dolomitica***	***S. dolomitica***	***P. zuluensis***
Delphinidin-3,5-diglucoside	6693 ± 1003	ND	ND	153 ± 12	75 ± 6	ND
Delphinidin-3,7-diglucoside	479 ± 22	ND	ND	ND	ND	ND
Pelargonidin glucoside derivative	ND	620 ± 62	120 ± 45	ND	ND	ND
Pelargonidin-3-*O*-glucoside	ND	ND	294 ± 12	ND	ND	ND
Vincanin A (delphinidin-3-robinobiosido-5-rhamnoside)	50 ± 6	ND	ND	ND	ND	ND
Peonidin glucoside derivative	ND	146 ± 0	88 ± 0	ND	ND	ND
Delphinidin-3-*O*-rutinoside	859 ± 45	ND	ND	ND	ND	ND
Delphinidin 3-neohesperidoside	238 ± 20	ND	ND	ND	ND	ND
Delphinidin3-*O*-(6-acetyl)-5-*O*-diglucoside	2426 ± 465	ND	ND	46 ± 2	ND	ND
Dimalonylawobanin (delphinidin-3-(6-*p*-coumaroylglucoside)-5-(4,6-dimalonylglucoside))	ND	ND	ND	22 ± 0	ND	ND
Dimalonylawobanin isomer (delphinidin-3-(6-*p*-coumaroylglucoside)-5-(4,6-dimalonylglucoside))	ND	ND	ND	67 ± 4	ND	ND
Malvidin-3-(6-*p*-coumaroylglucoside)-5-(4-malonylglucoside)	ND	ND	ND	ND	ND	142 ± 0
Malvidin-3-(6-coumaroylglucoside) 5-glucoside/isomer	ND	ND	ND	ND	ND	187 ± 45
Pelargonidin-3-rutinoside	ND	ND	ND	35 ± 6	56 ± 2	ND
Cyanidin-3,5-diglucoside	107 ± 2	473 ± 0	ND	ND	ND	ND
Delphinidin-3-*O*-rhamnoside	622 ± 83	ND	ND	ND	ND	ND
Cyanidin-3-rutinoside	192 ± 4	ND	ND	14 ± 2		ND
Petunidin-3,5-diglucoside	2488 ± 110	ND	ND	19 ± 1	ND	ND
Pelargonidin-3,5-diglucoside	ND	ND	6349 ± 0	ND	ND	ND
Petunidin-3-*O*-rutinoside	120 ± 2	ND	ND	ND	ND	ND
Petunidin-3-*O*-(6-acetyl)-5-*O*-diglucoside)	2656 ± 150	84 ± 0	ND	ND	ND	ND
Malvidin-3-*O*-(6-*O*-acetyl)-5-*O*-diglucoside	3889 ± 76	730 ± 0	ND	ND	ND	ND
Peonidin-3, 5-*O*-diglucoside	124 ± 0	3750 ± 0	2061 ± 0	ND	ND	ND
Malvidin-3,5-*O*-diglucoside	1899 ± 1	1803 ± 0	396 ± 0	ND	ND	ND
Total anthocyanins	19745	7606	8912	334	131	329

IE, Ideain (cyanidin-3-galactoside) equivalents; ND, Not detected.

the m/z 331.08 fragment and the loss of a glucose (−162 amu) molecule resulting in the m/z 639.17 fragment.

Pelargonidin derivatives

All four pelargonidin derivatives identified produced a main fragment at m/z 271.06 which corresponds to the pelargonidin aglycone. Peak 11 was identified as pelargonidin-3-*O*-glucoside based on the loss of a glucose (−162 amu) moiety to produce the aglycone fragment (m/z 271.06). Peak 7 was identified as pelargonidin-3,5-*O*-diglucoside based on the loss of a diglucoside (−324 amu) from the anthocyanin to produce the aglycone. Peak 15 was identified as a pelargonidin glucoside derivative based on the loss of a glucose (−162 amu) molecule resulting in the m/z 461.11 fragment. Peak 31 was identified as pelargonidin-3-*O*-rutinoside based on the loss of a rutinose (−308 amu) from the anthocyanin resulting in the aglycone fragment (m/z 271.06) and the loss of a rhamnose (−146 amu) resulting in the m/z 433.11 fragment.

Quantification of anthocyanins in pigmented extracts of flowers from Geraniaceae and Lamiaceae plant species

Table 2 shows the anthocyanin content (μg IE/g) of the pigmented extracts from flowers of plant species from the two families. More anthocyanins could be identified and in higher concentrations in pigmented flower extracts from the Geraniaceae than from the Lamiaceae. The total anthocyanin content was therefore higher in pigmented extracts from the Geraniaceae compared to the Lamiaceae. The main anthocyanins in flowers from the Geraniaceae were 3,5-diglucosides of delphinidin, petunidin, pelargonidin, peonidin and malvidin and acetyl-acylated malvidin, delphinidin and petunidin. For the Lamiaceae, the main anthocyanins identified in their flowers were

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rutinosides of pelargonidin, glucosides of petunidin, pelargonidin and *p*-coumaric acid- and malonyl-acylated delphinidin and malvidin.

Work on the identification of the anthocyanins of the flower species of these two plant families is limited and most research has focused on other phenolic compounds or compounds in the essential oils from the leaves (Kwee & Niemeyer, 2011; Zhu *et al.*, 2014; Jordheim *et al.*, 2016; Marchioni *et al.*, 2020) and other aerial parts (Katanić Stanković *et al.*, 2020) of these plants. The findings from this study on anthocyanin identification generally agree with the limited reports available on anthocyanins in flowers of the Geraniaceae and Lamiaceae (Mitchell *et al.*, 1998; Jordheim *et al.*, 2016).

Mitchell *et al.* (1998) identified 3, 5-diglucoside and 3-glucoside-5-(6-acetyl) glucoside of delphinidin, malvidin and petunidin in a wide variety of zonal *Pelargonium* flowers. They also reported that the salmon pink *Pelargoniums* consist almost entirely of pelargonidin derivatives, which agrees with the salmon pink *P. zonale* hybrid used in this study. The red-coloured *P.* × *hortorum* mainly contained peonidin, malvidin, pelargonidin and cyanidin derivatives, respectively, and the pigments in red-coloured *Pelargoniums* consisted mainly of pelargonidin and peonidin derivatives.

Our identification of mainly delphinidin glucosides in the blue *Salvia* species studied in this research is in agreement with Saito & Harborne (1992) who reported that blue flower species from the Lamiaceae generally contain delphinidin glucosides as major pigment. They also identified the malonyl-acylated anthocyanins and aromatic anthocyanins acylated with *p*-coumaric and caffeic acids in various species of flowers in the Lamiaceae as found in the current study.

The anthocyanins identified in the dark purple *Plectranthus zuluensis* (Lamiaceae) used in this study exclusively contained *p*-coumaroylglucoside and malonylglucoside-acylated malvidin derivatives. In contrast, Jordheim *et al.* (2016) reported high concentrations of coumaroylglucoside, caffeoylglucoside and malonlylglucoside of peonidin in the cauline hairs of the leaves of the dark purple *Plectranthus ciliates* E. Mey.

Degradation kinetics

Thermal Temperature and oxidative (hydrogen peroxide) stability of pigmented flower extracts of some plant species from the Geraniaceae and Lamiaceae families

For food applications, thermal and oxidative stability of pigments are important parameters to consider. The thermal and oxidative (hydrogen peroxide concentration) degradation kinetics parameters of the pigmented flower extracts from the Geraniaceae and Lamiaceae are shown in Tables 3 and 4 respectively. The thermal and oxidative degradation of the pigments showed first-order reaction kinetics. The general trend was that all the pigmented flower extracts showed an increase in degradation rate constant accompanied with a decrease in half-life ($t_{½}$) with increasing temperature (Table 3) and increasing H_2O_2 concentration (Table 4). This is an indication that the pigments became less stable with increasing temperature and increasing H_2O_2 concentration.

Table 3 Thermal degradation kinetics parameters [degradation rate constant (k) and half-life ($t_{1/2}$)] of pigmented flower extracts from some plant species of the Geraniaceae and Lamiaceae

Sample	Temperature (°C)	$-k \times 10^3$ (min^{-1})	$t_{1/2}$ (min)
Geraniaceae			
Pelargonium.	70	$3.90^{o} \pm 0.30$	$4.86^{d} \pm 0.08$
grandiflorum	80	$7.00^{q} \pm 0.10$	$4.27^{b} \pm 0.01$
(purple)	90	$11.15^{r} \pm 0.25$	$3.80^{a} \pm 0.02$
Pelargonium ×	70	$0.85^{ec} \pm 0.20$	$6.39^{no} \pm 0.18$
hortorum (scarlet)	80	$1.50^{ji} \pm 0.10$	$5.81^{ij} \pm 0.07$
	90	$5.50^{pn} \pm 0.30$	$4.51^{ce} \pm 0.05$
Pelargonium	70	$0.70^{ceg} \pm 0.00$	$6.57^{onlq} \pm 0.00$
zonale hybrid	80	$1.30^{ijk} \pm 0.10$	$5.96^{jihp} \pm 0.08$
(pink)	90	$2.80^{npm} \pm 0.00$	$5.18^{ecfk} \pm 0.00$
		Mean = 3.86	**Mean = 5.26**
Lamiaceae			
Salvia aurea ×	70	$0.45^{ab} \pm 0.05$	$7.02^{rl} \pm 0.11$
dolomitica	80	$0.95^{fd} \pm 0.15$	$6.28^{mh} \pm 0.16$
(dark purple)	90	$1.75^{lh} \pm 0.65$	$5.73^{gf} \pm 0.39$
Salvia	70	$0.60^{bg} \pm 0.20$	$6.78^{qr} \pm 0.35$
dolomitica	80	$0.75^{dk} \pm 0.45$	$6.73^{pm} \pm 0.69$
(light purple)	90	$1.10^{hm} \pm 0.30$	$6.16^{kg} \pm 0.28$
Plectranthus	70	$1.05^{gec} \pm 0.45$	$6.27^{lo} \pm 0.46$
zuluensis	80	$1.65^{kji} \pm 0.55$	$5.77^{hj} \pm 0.35$
(Dark purple)	90	$2.70^{mnp} \pm 0.10$	$5.22^{fe} \pm 0.04$
		Mean = 1.22	Mean = 6.22

Values are mean ± standard deviation.
For each plant family, mean values with different superscript letters in a column differ significantly ($P \leq 0.05$).

Similar trends have been reported from thermal degradation kinetics studies of red Roselle (*Hibiscus sabdariffa*) anthocyanin extracts (Mourtzinos *et al.*, 2008; Maciel *et al.*, 2018) and corn poppy (*Papaver rhoeas* L.), red tulip (*Tulipa* sp.), rose (*Rosa* sp.) anthocyanin extracts (Bayram *et al.*, 2015) and various types of tulip (*Tulipa spp.*) (Sagdic *et al.*, 2013) and from oxidative degradation kinetics studies of red radish extracts (Matsufuji *et al.*, 2007). These studies also reported decreases in thermal and oxidative degradation rate constants with increase in temperature and concentration of oxidising agent.

High temperatures may promote breakdown of anthocyanins via hydrolysis of the sugar residue of

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Table 4 Oxidative (H_2O_2) degradation kinetics parameters [degradation rate constant (k) and half-life ($t_{1/2}$)] of pigmented flower extracts from some plant species of the Geraniaceae and Lamiaceae

Sample	H_2O_2 (mM)	$-k \times 10^3$ (min^{-1})	$t_{1/2}$ (min)
Geraniaceae			
Pelargonium.	8.15	$4.70^c \pm 0.1$	$4.67^b \pm 0.02$
grandiflorum (purple)	16.28	$4.80^d \pm 0.00$	$4.65^a \pm 0.00$
	24.43	$5.95^d \pm 0.35$	$4.43^a \pm 0.06$
Pelargonium ×	8.15	$0.65^a \pm 0.05$	$6.58^d \pm 0.14$
hortorum (scarlet)	16.28	$1.20^b \pm 0.10$	$6.04^c \pm 0.08$
	24.43	$1.50^b \pm 0.20$	$5.82^c \pm 0.13$
Pelargonium	8.15	$1.00^a \pm 0.30$	$6.26^d \pm 0.31$
zonale hybrid (pink)	16.28	$1.25^b \pm 0.05$	$5.99^c \pm 0.04$
	24.43	$1.15^b \pm 0.25$	$6.10^c \pm 0.22$
		Mean = 2.47	**Mean = 5.62**
Lamiaceae			
Salvia	8.15	$1.10^a \pm 1.00$	$6.24^d \pm 0.49$
aurea × *dolomitica*	16.28	$1.40^b \pm 0.00$	$5.88^c \pm 0.00$
(dark purple)	24.43	$1.25^b \pm 0.25$	$6.01^c \pm 0.20$
Salvia dolomitica	8.15	$0.75^a \pm 0.05$	$6.50^d \pm 0.07$
(light purple)	16.28	$0.75^b \pm 0.15$	$6.52^c \pm 0.02$
	24.43	$0.95^b \pm 0.05$	$6.27^c \pm 0.05$
Plectranthus	8.15	$0.40^a \pm 0.00$	$7.13^d \pm 0.00$
zuluensis	16.28	$1.90^b \pm 0.10$	$5.57^c \pm 0.05$
(purple)	24.43	$1.25^b \pm 0.25$	$6.01^c \pm 0.20$
		Mean = 1.08	Mean = 6.24

Values are mean ± standard deviation.
For each plant family, mean values with different superscript letters in a column differ significantly ($P \leq 0.05$).

anthocyanins followed by isomerisation and opening of the ring system resulting in the formation of chalcones which could ultimately be cleaved into various degradation products (Patras *et al.*, 2010). Oxidising agents such as hydrogen peroxide and its radicals could degrade anthocyanins by adding to the C ring of anthocyanins resulting in opening of the structure and release of usually phenolic acids as by-products (Satake & Yanase, 2018). These mechanisms could account for the observed decreased stability of the flower pigments with increasing temperature and increasing H_2O_2 concentration.

In this study, the pigmented flower extracts from the plant species from the Lamiaceae had lower thermal degradation rate constant (Mean $k = 1.2 \times 10^3$ min^{-1}) and longer half-life (Mean $t_{½} = 6.22$ min) than the pigmented extracts from the Geraniaceae (Mean $k = 3.86 \times 10^3$ min^{-1}; Mean $t_{½} = 5.26$ min) (Table 3). Thermal degradation rate constants have also been reported for several tulip flower species with mean k range of −3.23-4.38 × 10^3 min^{-1} at temperatures of 70, 80 and 90 °C (Sagdic *et al.*, 2013); poppy flower extract (mean $k = -0.63 \times 10^3$ min^{-1}), rose flower extract (mean $k = -4.29 \times 10^3$ min^{-1}) and roselle extract (mean $k = -5.21 \times 10^3$ min^{-1}) at the same temperature ranges (Bayram *et al.*, 2015).

The oxidative stability kinetics (Table 4) showed a similar trend with the pigmented flower extracts from the Lamiaceae having lower oxidative degradation rate constant (Mean $k = 1.08 \times 10^3$ min^{-1}) and longer half-life (Mean $t_{½} = 6.24$ min) than the pigmented extracts from the Geraniaceae family (Mean $k = 2.47 \times 10^3$ min^{-1}; Mean $t_{½} = 5.62$ min). Özkan *et al.* (2002) reported oxidative degradation rate constants for strawberry anthocyanin extract (mean $k = -2.37$ to -19.46×10^3 min^{-1}), pomegranate anthocyanin extract (mean $k = -1.80 \times 10^3$ min^{-1} to -21.19×10^3 min^{-1}) and sour cherry anthocyanin extract (mean $k = -1.54 \times 10^3$ min^{-1} to -15.25×10^3 min^{-1}) at hydrogen peroxide concentrations of 9.31, 13.96, 23.27 and 27.92 mM and temperatures of 10, 20 and 30 °C. The pigmented flower extracts from plant species from the Lamiaceae studied in the current research were more thermally and oxidatively stable than the pigmented extracts from the Geraniaceae. This might be related to the anthocyanin type (acetyl or aromatic acylated) identified in the pigments and/or the presence of other phenolic compounds such as flavonoids or phenolic acids. The pigmented flower extracts from the Lamiaceae contained *p*-coumaroyl and malonyl-acylated anthocyanins (aromatic acylated), whereas the pigmented flower extracts from the Geraniaceae contained acetyl-acylated anthocyanins. The aromatic residues from the acylated anthocyanins identified in the pigmented flower extracts from the Lamiaceae could protect the flavylium nucleus from degradation through intra-molecular self-association interactions as described by Goto & Kondo (1991). This could partly explain the relative stability of the pigmented flower extracts from the Lamiaceae.

The pigmented flower extracts of the Lamiaceae in this study contained high amounts of rosmarinic acid (96.31 mg CAE/g) and its derivatives sagerinic acid (63.5 mg CAE/g) and salvianolic acid B (31.66 mg CAE/g) while these were not detected in pigmented flower extracts from the Geraniaceae. The results of this study suggest that the self-association interactions of aromatic acylated anthocyanins (Goto & Kondo, 1991) could be accompanied by the strong intermolecular interactions between rosmarinic acid and its derivatives, thereby preventing nucleophilic attack by water and hydrogen peroxide (H_2O_2).

Some studies have reported the role of rosmarinic acid in the stabilisation of anthocyanins. Kean & Reilly (2011) patented a method for stabilisation of red radish anthocyanin extract with rosmarinic acid during storage for use in food beverages and cosmetics where addition of rosmarinic acid resulted in a 36% retention in colour, outperforming all other stabilising agents tested. Zhao *et al.* (2021) also reported that

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rosmarinic acid in an anthocyanin extract consisting of mainly cyanidin-3-*O*-β-glucoside caused a bathochromic shift in maximum absorption wavelength (λ_{max}) and resulted in an improved stability after 7 days of storage.

From the results obtained in this study, it is clear that for use of these pigmented extracts in food applications, the thermal and oxidising conditions must be considered especially for the pigmented flower extracts from the Geraniaceae studied in this research.

Conclusion

Pigmented extracts from flowers of the Geraniaceae and Lamiaceae in this study contain mainly acetyl-acylated and aromatic malonyl-acylated anthocyanins respectively. The pigmented extracts from the Lamiaceae contain high levels of rosmarinic acid and its derivatives, sagerinic acid and salvianolic acid, which are absent in extracts from the Geraniaceae. The pigmented extracts from the Lamiaceae have higher thermal and oxidative stability as shown by lower degradation constants (k) and higher half-lives ($t_{1/2}$) compared to pigments from the Geraniaceae. The observed stability of the pigmented extracts from the Geraniaceae and Lamiaceae at a wide range of temperatures and oxidative environments suggests that they have potential as natural food colourants.

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Author contribution

Anton Venter: Conceptualization (equal); Formal analysis (equal); Investigation (equal); Methodology (equal); Writing – original draft (equal). **Hennie Fisher:** Conceptualization (equal); Supervision (equal); Writing – review & editing (equal). **Gary I. Stafford:** Conceptualization (equal); Investigation (equal); Methodology (equal); Supervision (equal); Writing – review & editing (equal). **Kwaku Gyebi Duodu:** Conceptualization (lead); Investigation (equal); Methodology (equal); Supervision (lead); Writing – review & editing (equal).

Conflict of interest

The authors declare no conflict of interest. Ethics approval was not required for this research.

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Data availability statement

The data for the research reported in this manuscript can be made available upon request from the corresponding author.

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