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Pigment analysis of flowers of *Clivia gardenii*

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December 2011

A report prepared for

New Zealand Clivia Club

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SPTS No. 6312

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Executive summary

Pigment analysis of flowers of *Clivia gardenii*

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The pigment profiles in flower tissue from three accessions of *Clivia gardenii* were analysed for the New Zealand Clivia Club as part of a study on flower colour in this species. Samples from flowers of accession 8310 (sourced from Japan), 'Wriggal' (sourced from South Australia), and a longstanding New Zealand accession were included as three replicate samples for analysis.

Flower colour and pigment profile did not vary widely among the three different accessions. Flower colour was predominantly orange-red, with a small yellow sector and green tips. The orange colour was due primarily to the presence of a single pelargonidin-based anthocyanin, comprising 90% of the total anthocyanin profile. Chemical identification of the pelargonidin-based anthocyanin would require further chemical analysis. Five other anthocyanins were detected but only at comparatively low concentrations. Anthocyanin concentration varied from 0.4 to 0.7 mg/gDW. The highest concentration was in the New Zealand accession and the lowest in 'Wriggall'; this correlates with the observed darker orange flower colour in the New Zealand accession.

Carotenoid pigments were also present, predominantly lutein and β-carotene. Petal colour is due to a combination of the two pigment groups and the carotenoids will have contributed to the overall colour, but their exact distribution in the petal was not examined. Carotenoid concentration varied in the orange-red petals from 260 to 386 µg/gDW and the highest concentration was in 'Wriggall'. Much higher carotenoid concentrations were detected in the green tips. Both chlorophyll and carotenoid pigments are present in the petal tips.

Pigment concentrations were determined for the anthocyanins, carotenoids and chlorophyll pigments in petal tissue. Mean total concentrations are reported for *C. gardenii,* as well as individual values for the different accessions. These data are useful as a baseline and for a general comparison but wider comparisons must be made with care, as within-accession variation was not considered and flower maturity for the different samples was not specifically determined.

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1 Introduction

Clivia is a genus of monocot flowering plants native to southern Africa. They are from the family Amaryllidaceae, subfamily Amaryllidoideae. Common names include *Kaffir lily* and *bush lily*. They are herbaceous evergreen plants, with green, strap-like leaves. Flowers are bell-shaped and form on a tall inflorescence, and they can be orange, reddish orange, bronze orange, peach, greenish yellow or yellow in colour. They are grown around the world as flowering garden plants.

The information presented in this report is part of a study for the New Zealand Clivia Club on the pigments in flowers of *Clivia gardenii*. Flowers of three different accessions of *C. gardenii* were sent for inclusion in the study. They were accession 8310 – originally sourced from Japan; 'Wriggall' – sourced from a Mr Wriggall in South Australia; and a long-standing New Zealand accession. The aim was to determine the total anthocyanin and carotenoid concentration present in the petal tissue and, as much as HPLC analysis would allow, identify the individual pigments present. No further analysis or interpretation was required.

2 Methods

Flower samples of three accessions (8310, 'Wriggall' and a longstanding NZ) were supplied by the New Zealand Clivia Club in August 2011. Photographs were also supplied. Petal colour was measured using a Minolta chromameter. Flower tissue samples were taken and freeze-dried. Petals were cut at a point above the ovary and then separated into orange tube and green tips. It was this tissue, both the tube and tips, that was used for the pigment analysis. Flavonoid and carotenoid pigments were extracted from the dried tissue using standard methods and quantified by spectrophotometry. The main pigment groups – anthocyanins (coloured flavonoids), other flavonoids, chlorophyll and carotenoids – were analysed separately. Both carotenoid and anthocyanin extracts were subsequently run on an HPLC with a diode array detector to characterise pigment profiles. The three accessions were treated as separate replicates of flowers of *C. gardenii*. Variability in flower colour within an accession was not considered.

3 Results

The three accessions provided for analysis had similar flower colours, an orange-red tube with green tips. Flower colour did vary somewhat: accession 8310 showed a graded change in the upper petal from orange to yellow to green in the tips; 'Wriggal' was similar but with a paler orange colour; longstanding NZ was a brighter orange and did not show the yellow band. The accessions are shown in Figure 1 and their colour properties are described in Table 1.

8310

'Wriggall'

Longstanding NZ selection

Figure 1: *Clivia gardenii* accessions analysed as part of a study on flower colour in selected lines for the New Zealand Clivia Club.

Table 1: Flower colour characteristics seen in the selected *C. gardenii* accessions. Colour parameters (lightness (L), chroma (C) and hue angle (H^o)) were measured with a Minolta CR-400 chromameter. Lightness represents the proportion of total incident light that is reflected, chroma is a measure of colour intensity (in relative intensity units), and hue angle relates colour

to a position on a colour circle or wheel, with red purple at 0°, yellow at 90°, blue/green at 180°, and blue at 270°. .

		Chromameter colour readings		
Accession	Observed colour	L	C	H°
8310	Orange flower green tips Yellow band	63.4	43.4	54.16
'Wriggal'	Orange flower green tips Yellow band but paler orange	60.8	33.5	54.5
Longstanding NZ Selection	Orange flower green tips Less yellow brighter orange	61.2	30.3	52.9

The colour parameters presented in Table 1 are from a single chromameter reading and are only indicative. They do correlate with observations that the colour intensity (C) is greater in accession 8310, while hue (H) angle in the longstanding NZ accession shows a small shift to red and away from yellow.

Pigment concentration was an important factor influencing colour. Total pigment concentrations are summarised in Table 2. Petal tissue was split into the orange region and the green tips. Chlorophyll is the major pigment in the green areas and there seemed value in keeping the petal colour sectors separate and using spectrophotometric methods to estimate chlorophyll concentration.

The highest anthocyanin concentration was in the longstanding NZ accession (0.7 mg/gDW), then 8310 and 'Wriggal' with the lowest concentration. This pattern is consistent with the observed colours. Mean anthocyanin concentration (0.58 mg/gDW – calculated across all three accessions) was lower than is seen in other flowers such as petunia. This is potentially linked with the thickness of the petal tissue. The petals were reasonably thick in width; as anthocyanin is only produced in the epidermis, the apparent concentration would appear diluted in comparison with flowers that have thinner petal tissue.

The concentration of other flavonoids was also measured: concentrations were in the same order and of the same magnitude as the anthocyanins (Table 2). This is somewhat unusual as 'other' flavonoids are generally much greater in concentration than the anthocyanins. Reasons for this are not known. The other flavonoids are generally regarded as colourless but can act as co-pigments and influence the intensity and hue of colour in plant tissue. The 'other' flavonoids had spectra indicative of the flavone class of flavonoids. Anthocyanin and flavonoid concentrations were not determined in the green tips. No anthocyanin colouration was observed in the green sector and therefore only trace (if any) levels are likely to have been present. Other flavonoids are likely to be present in the green tissue but were not measured.

Table 2: Summary of the total anthocyanin, flavonoid (mg/gDW), carotenoid and chlorophyll concentrations (µg/gDW) detected in *Clivia gardenii* petal tissue samples from three different clivia accessions. Petal tissue was split into orange petal and green tips. Data presented are the values for the individual accessions and the mean ± SEM (n=3), determined from HPLC data. Chlorophyll concentration was estimated from spectrophotometric measurements. Data for individual accessions is a single measurement. Anthocyanin concentration was determined as Cyanidin 3-O-glucoside equivalents. Carotenoid concentration was determined as β-carotene equivalents.

Cultivar	Sample no a	Anthocyanin conc. (mg/gDW)	Flavonoid conc. (mg/gDW)	Carotenoid conc. (µg/gDW)	Chlorophyll conc. (µg/gDW)
Orange Petal					
8130	1	0.58	0.75	264.7	26.2
'Wriggal'	1	0.43	0.42	386.8	21.9
Longstanding NZ	1	0.73	0.79	195.6	36.4
Mean	3	0.58 ± 0.09	0.65 ± 0.12	282.4 ± 55.9	28.2 ± 4.3
Green tips					
8130		nd	nd	1013	2088.3
'Wriggal'		nd	nd	764	711.9
Longstanding NZ		nd	nd	803	1117.7
Mean		nd	nd	860.0 ± 77.3	1305.6 ± 408.3

nd = not detemined

The highest carotenoid concentration was detected in the 'Wriggall' accession (386 µg/gDW), then 8310 and the longstanding NZ accession. Carotenoid concentration for 'Wriggal' was nearly double that of the NZ accession although any conclusions are tempered by the fact these data are derived from one extraction for each accession. Chlorophyll concentration was minimal in the orange sector but much greater in the green tips, again with some differences between accessions that were consistent with the tissue colours observed. Carotenoid concentration was higher in the green tips than the orange sector. This was expected given the accessory pigment role for carotenoids in photosynthetic tissue. The stage of development of the flowers may account for some differences in pigment profiles and concentrations. A higher chlorophyll concentration may simply be due to a lack of chlorophyll breakdown in flowers collected at an earlier maturity stage.

Flowers from all three accessions had one major anthocyanin present. Peak 4 made up 90% of the anthocyanin in all three clivia accessions. A spectral maxima at 502 nm indicates that it is a pelargonidin-based anthocyanin but its exact identity could not be determined from these studies. Six anthocyanins were detected in total; the other five are also likely to be pelargonidin-based but contributed only minimally to the anthocyanin profile (Tables 3 & 4). The predominant accumulation of this anthocyanin is show in the HPLC chromatograms presented in Figure 2.

Table 3: Retention times and spectral maxima for anthocyanin pigments detected at 530nm in extracts from clivia flower tissue. .

Peak	Retention time (min)	Spectral maxima	Identity
1	5.4	516	Pelargonidin based anthocyanin
2	6.6	502	Pelargonidin 3-*O*-glucoside
3	6.9	505	Pelargonidin based anthocyanin
4	7.1	502	Pelargonidin based anthocyanin
5	8.4	508	Pelargonidin based anthocyanin
6	12.9	513	Pelargonidin based anthocyanin

Peak identification was based on the spectral maxima and retention time relative to other known anthocyanins. Pelargonidin 3-*O*-glucoside was identified from a known standard.

Table 4: Summary of the relative proportion (% of total amount of anthocyanin) that individual anthocyanins contributed to the anthocyanin profile in petal tissue of *Clivia gardenii*. Mean value is calculated from the individual values for each accession

	Relative contribution (%) of individual anthocyanins to overall profile					
	Pelargonidin-based anthocyanin	Pelargonidin 3-*O*-glucoside	Pelargonidin-based anthocyanin	Pelargonidin-based anthocyanin	Pelargonidin-based anthocyanin	Pelargonidin-based anthocyanin
Peaks	1	2	3	4	5	6
Accession						
8310	3.0	1.4	3.3	92.2		1.7
'Wriggall'	3.4	1.3	3.9	89.9		1.5
Longstanding NZ	2.4	1.8	5.7	87.2	1.3	1.5
Mean	2.9 ± 0.3	1.5 ± 0.2	4.3 ± 0.7	89.8 ± 1.5	0.4 ± 0.4	1.6 ± 0.1

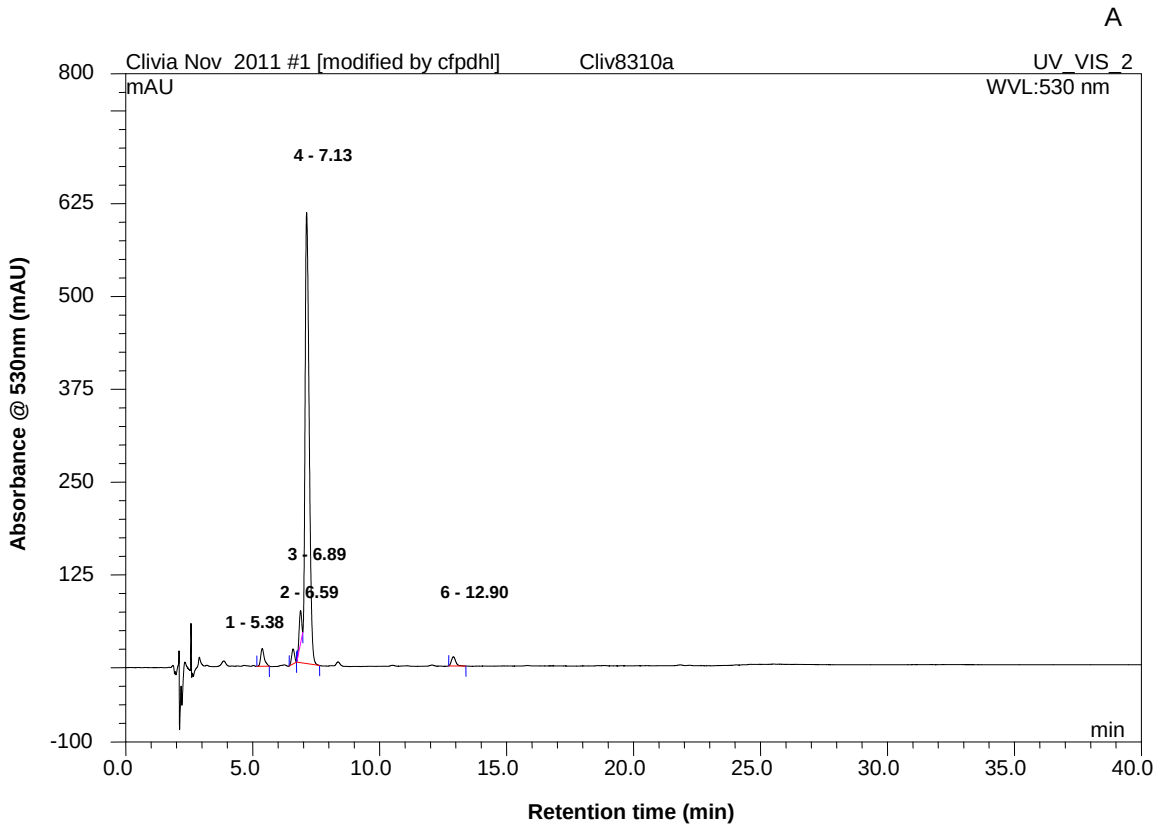
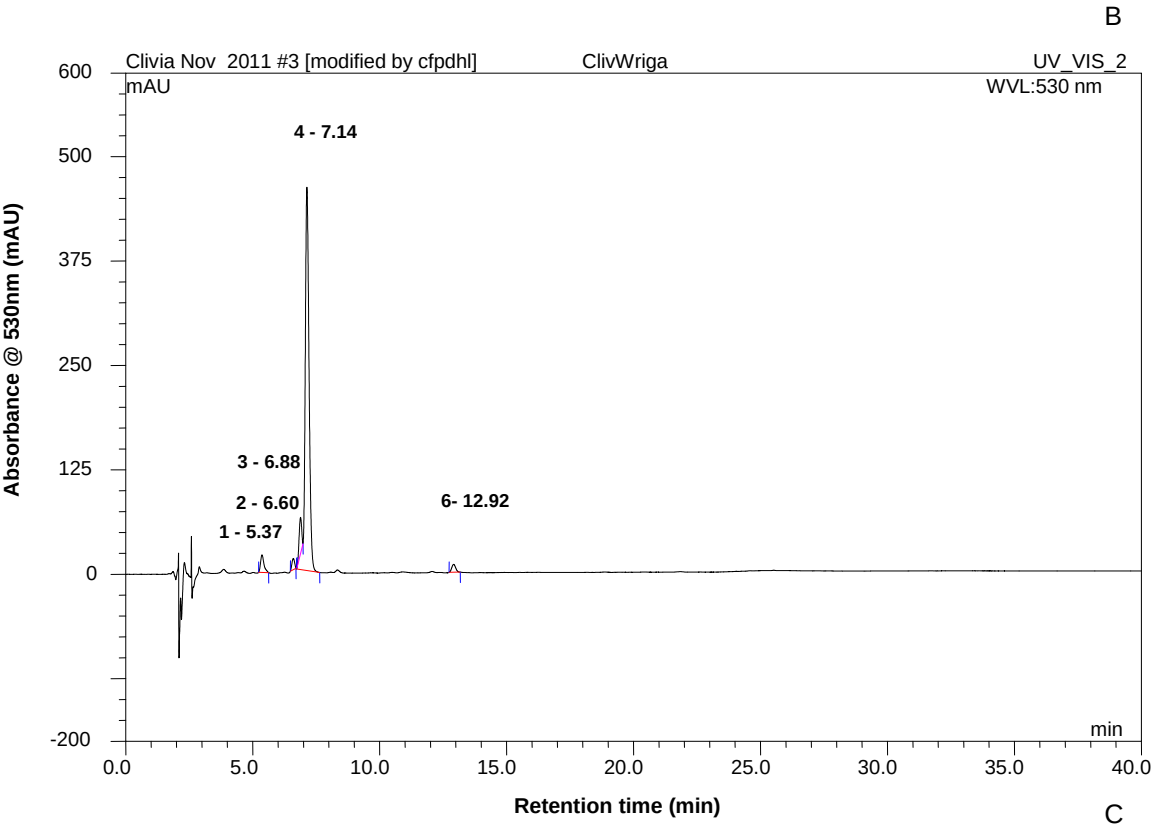


Figure 2: HPLC chromatograms for anthocyanin extracts from petal tissue of flowers of *Clivia gardenii*. Flowers from three different clivia accessions were analaysed: 8310 (A), 'Wriggall' (B) and longstanding New Zealand (C). Peak identities are listed in Table 3.



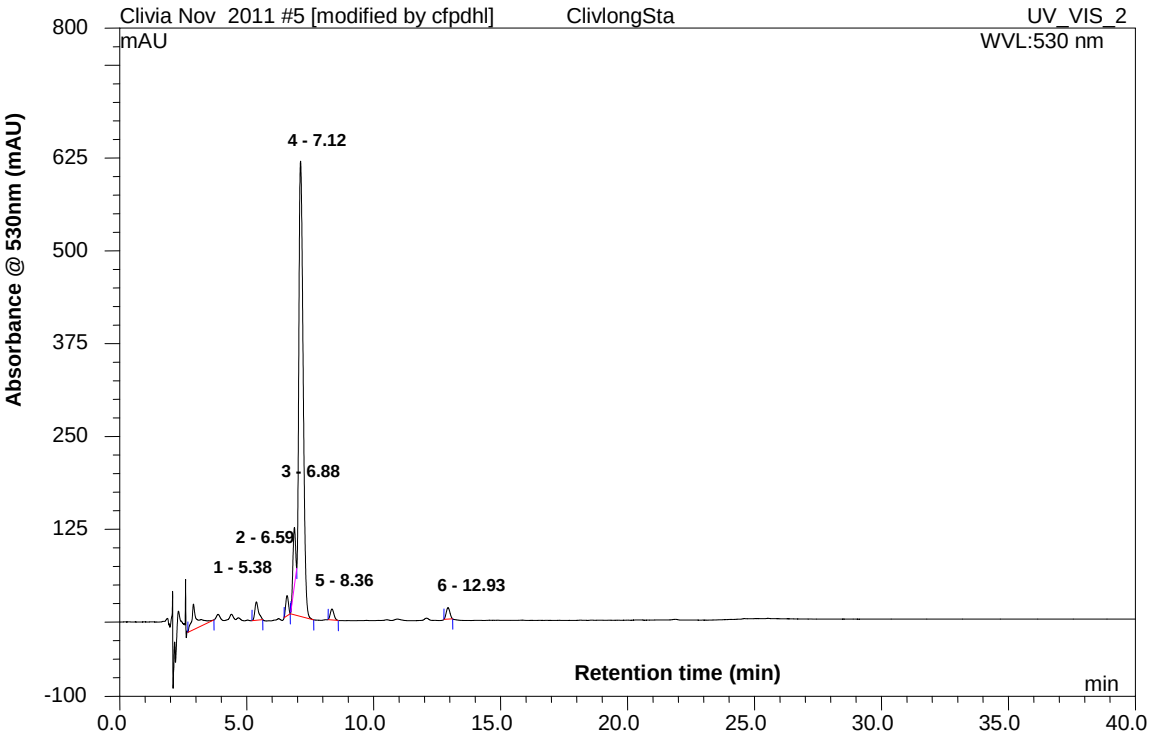


Figure 2 cont'd: HPLC chromatograms for anthocyanin extracts from petal tissue of flowers of *Clivia gardenii*. Flowers from three different clivia accessions were analaysed: 8310 (A), Wriggall (B) and longstanding New Zealand (C). Peak identities are listed in Table 3.

Flowers from all three accessions had a greater range of carotenoid pigments present (Table 5 & 6). Lutein and β-carotene were the major carotenoids present, accounting for 70% of the total carotenoids, and although there was some variation among samples it appears there may be a shift from lutein to β-carotene as the flowers mature. Ten carotenoids were detected, with two identified, four identified tentatively and four unknowns. The two later carotenoids were likely to be esterified but this was not known definitively. The carotenoid profile across the three accessions was relatively consistent. some differences were observed in the minor carotenoid components (Figure 3).

Table 5: Retention times and spectral maxima for carotenoid peaks detected at 450 nm from extracts of clivia petal tissue.

Peak	Retention time (min)	Spectral maxima	Identity
1	9.8	437/467	Violaxanthin?
2	11.2	434/463	Neoxanthin ?
3	14.6	443/471	Lutein
4	20.8	423/449	unknown
5	22.0	444/472	α-carotene?
6	22.6	444/472	unknown
7	23.9	450/475	β-carotene
8	25.3	445/468	cis-β-carotene ?
9	29.3	445/473	Unknown esterified carotenoid
10	30.2	445/473	Unknown esterified carotenoid

Peak identification was based on the spectral maxima and retention time relative to other known compounds. Lutein and β-carotene were based on known standards. Other identifications were tentative or recognised as unknown

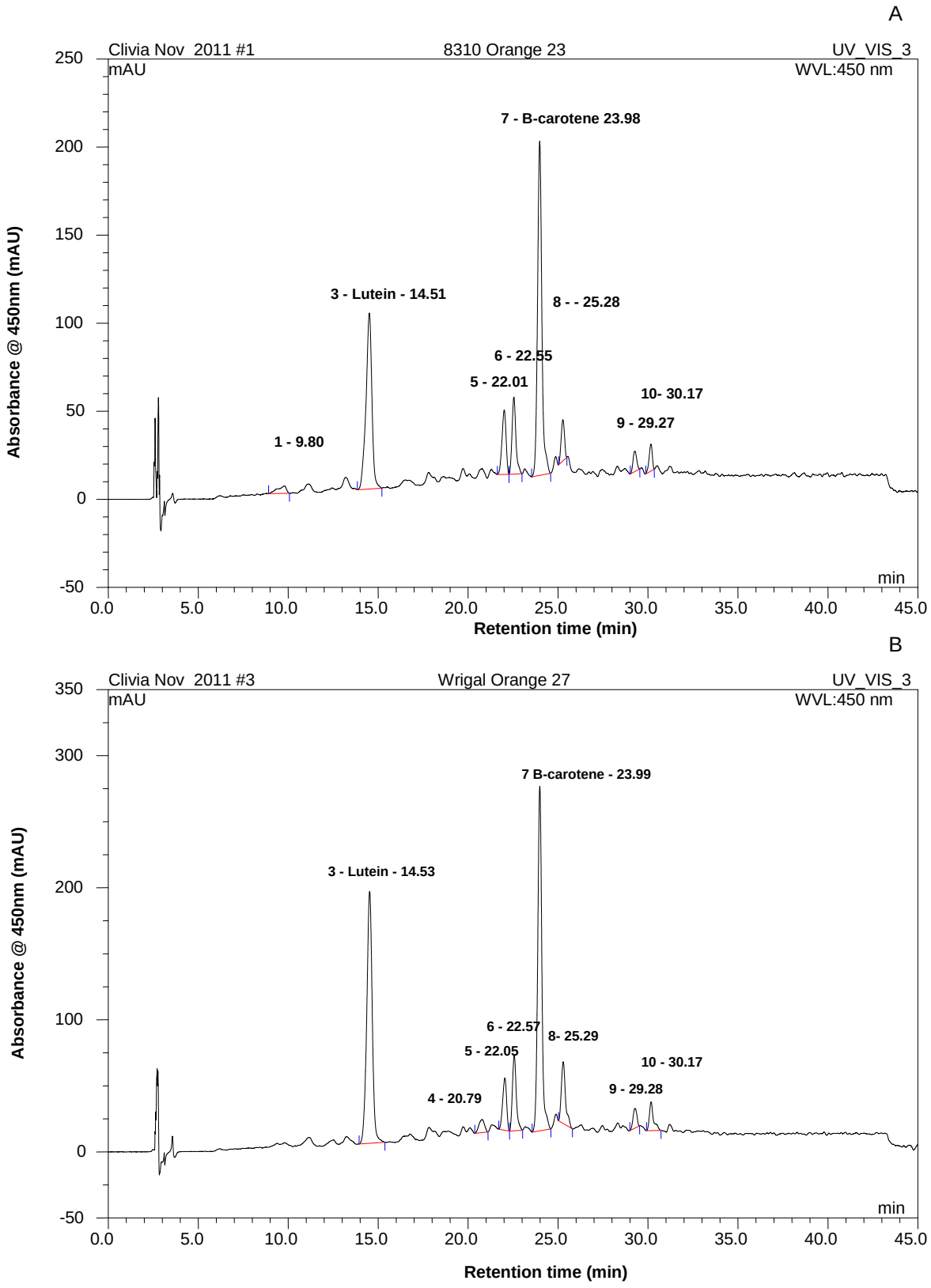


Figure 3: HPLC chromatograms for carotenoid extracts from petal tissue of flowers of *Clivia gardenii*. Flowers from three different clivia accessions were analaysed: 8310 (A), 'Wriggall' (B) and longstanding New Zealand (C). Peak identities are listed in Table 5.

C

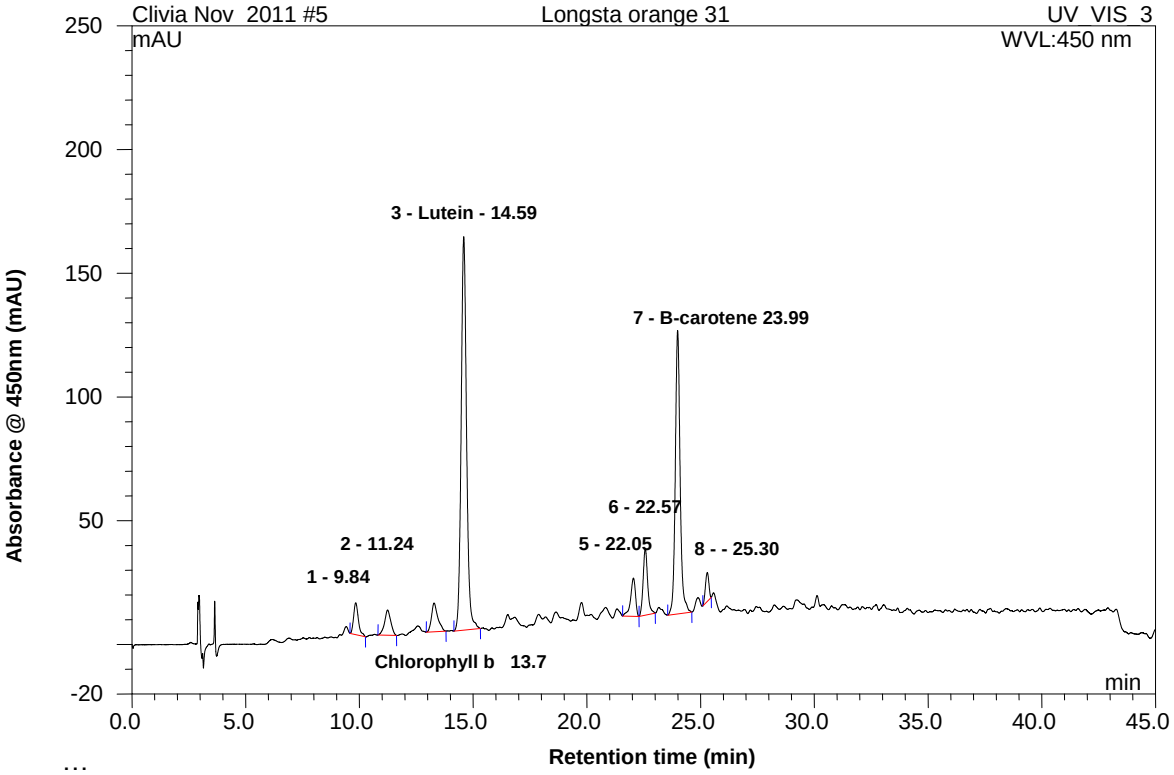


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Figure 3 cont'd: HPLC chromatograms for carotenoid extracts from petal tissue of flowers of *Clivia gardenii*. Flowers from three different clivia accessions were analaysed: 8310 (A), 'Wriggall' (B) and longstanding New Zealand (C). Peak identities are listed in Table 5. In Figure 2C note the presence of chlorophyll b and absence of peaks 9 and 10, indicating that flowers in this sample may have been younger and had not completed changed from green bud to mature flower.

Table 6: Summary of the relative proportion (% of total) that individual carotenoids contributed to the carotenoid profile in petal tissue of *Clivia gardenii*.

		Relative contribution (%) of individual carotenoids to the overall profile									
		Violaxanthin?	Neoxanthin ?	Lutein	unknown	α-carotene?	unknown	β–carotene	cis-β-carotene ?	Unknown esterified carotenoid	Unknown esterified carotenoid
Accession	Peaks	1	2	3	4	5	6	7	8	9	10
8310		2.7	0.0	29.4	0.0	8.1	9.0	40.0	4.6	2.9	3.2
'Wriggall'		0.0	0.0	34.9	2.4	5.7	7.9	36.0	7.0	2.5	3.6
Longstanding NZ		4.3	4.5	45.6	0.0	5.1	7.5	29.8	3.3	0.0	0.0
Mean		2.4	1.5	36.6	0.8	6.3	8.1	35.2	5.0	1.8	2.3

4 Conclusions

Flower colour and pigment profile did not vary widely among three different accessions of *Clivia gardenii*, analysed for pigment content. Flower colour was predominantly orange-red, with a small yellow sector and then green tips. The orange colour was due to the presence of a single pelargonidin-based anthocyanin, comprising 90% of the total anthocyanin profile. Chemical identification of the pelargonidin-based anthocyanin would require further chemical analysis. Underlying the anthocyanin were carotenoid pigments, predominantly lutein and β-carotene. They will have contributed to the overall colour but their exact distribution in the petal was not examined. The anthocyanin pigment did not extend the full length of the petal and there was a small yellow area that was likely to be carotenoid pigment alone before the chlorophyll pigment at the petal tips. Essentially, this same pattern of pigmentation was seen in all three accessions and the main individual pigments were the same in the different accessions as well.

Pigment concentrations were determined for the anthocyanins, carotenoids and chlorophyll pigments. A mean total concentration is reported for *C. gardenii* as well as individual values for the different accessions. These data are useful as a baseline and for general comparison but wider comparisons must be made with care, as within-accession variation was not considered and flower maturity for the different samples was not specifically determined.

This report presents an initial view of the pigment profiles and concentrations in three selected samples of flowers of *Clivia gardenii*, providing a starting point for understanding the basis of flower colour in this species.