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SHORT COMMUNICATION

A NEW FLAVONE IN MATURE *CATHARANTHUS ROSEUS* PETALS

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The flavonoid spectrum of intact mature fully open *Catharanthus roseus* petals was scanned through HPLC mass spectrometry for identification and characterization of the specific flavonoids existing in these petals. Besides already reported petunidin, malvidin, hirsutidin trihydrates or glycosides, quercetin and kaempferol, the presence of tricin, a flavone, is being reported for the first time. Interestingly enough 540 nm absorbance peak could resolve only flavones whereas 340nm and 266nm absorbance peaks resolved anthocyanins. Its appearance in 540 nm absorbance peak fractions suggests it to be a probable copigment in this flower.

Key words **: *Catharanthus roseus*, flavonoids, tricin.**

Flavonoids are involved in a wide variety of plant functions including pigmentation (Hahlbrock and Scheel 1989, Heller and Forkman 1988). They not only impart colours to the flowers but also participate in plant defense and protection against UV rays (Davies et al. 1993, Dixon 1986). Due to their diversity and species-specific nature, they are also used as an important chemotaxonomic parameter. The present investigation has been carried out in view of characterizing the flavonoid profile of *Catharanthus roseus* petals and finding out their relationship, if any, with the phenomenon of development and senescence. The absorption spectrum of mature *C. roseus* petal extracts exhibits more than one λmax and it was interesting to note that 540nm λmax which is otherwise supposed to represent the anthocyanins, pointed towards presence of certain flavones.

The flowers of *Catharanthus roseus* L.G. Don were tagged at 1.5 cm bud stage (initial, 0) and collected after 3 days of tagging when they were fully open and mature. Flavonoids were extracted following the method of Sharma (1981). The absorption spectra of extracted flavonoids of fresh mature petals were scanned through 200 nm (UV) to 700nm (visible) range of light spectrum on a Shimadzu UV - 160A UV-visible spectrophotometer. For purification and characterization the flavonoids were extracted in 99:1 methanol: HCl (Strack and Wray 1989, Lee and Hong 1992) and subjected to the High Performance Liquid Chromatography. The HPLC system consisted of Shimadzu L6CA equipped with a column of μ Bondapak C_{18} (30cm x 4mm ID x 10μ) (Wilkinson *et al.* 1977, Lee and Hong 1992) maintained at room temperature. The flavonoids were separated with a mobile phase comprising water : acetic acid : methanol (71:10:19 v/v) in an isocratic phase with a flow rate of 1 ml/minute. Five μl sample was injected having 10 μl loop connected to the solvent delivery system. The detection was carried out by Kratos SF 773 detector for visible range (540nm) and Shimadzu SPD2AM for UV range (340nm and 266nm) using a recorder LKB 2220 with integrator. The fractions of various peaks at 540, 340 and 266nm were collected for further characterization by mass spectrometry. The Electro Spray Ionization Mass Spectrometry analysis was performed on a Finnigan Mat TSQ 7000 Mass Spectrometer filled with TSQ 7000 ESI probe and ICIS Data system. The samples were introduced into the injection loop (5 μl) of a Rheodyne Injector. The sheath gas (nitrogen) pressure was 60 psi and the capillary

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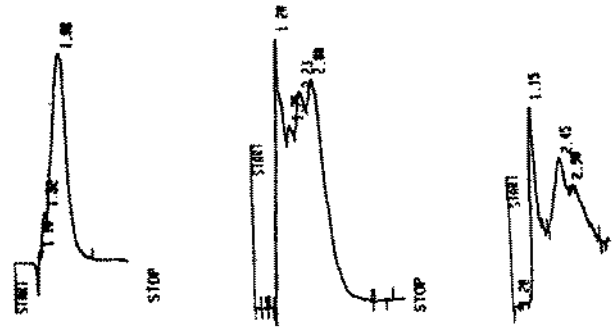
temperature was set at 250°C. The spray needle was held at 4.5 KV. In order to induce fragmentation a 10KV CID voltage was also applied. The mobile phase used was methanol : water (50 : 50) with 0.5% acetic acid.

In order to analyse flavonoids occurring in flowers of *C. roseus* we used Cyano column C_8 and C_{18} with mobile system water : acetic acid : methanol. The resolution of the peaks was better in μ Bondapak C_{18} column. The analyses of the peaks were carried out at wavelengths ranging from 260nm to 540nm with 20nm difference as per absorption spectra. On the basis of absorption peaks in the spectra (Fig.1) and the resolution observed in HPLC, separation was carried out in the visible range at 540nm and in UV at 340 and 266nm. At each wavelength peak more than one retention time was observed indicating better separation at higher retention time. For further tentative identification and characterization, due to the absence of reference compound ESI-MS was carried out in HPLC fractions collected with varying retention times at peak exhibiting wavelengths. In intact mature flower petals, 540nm λmax fractions with retention time 1.98 min. showed the base peak of m/z 161, the characteristic backbone of a flavone and the intense peak of m/z 322 indicating tricin. The minor peaks of m/z 321 and 304 represent kaempferol dihydrate and quercetin respectively. Although most of the favonoids exhibit a λmax at around 340nm, yet marked separation of a flavone (Tricin) has been observed at 540nm (anthocyanin - specific λmax). The interconversion between kaempferol, quercetin and tricin is probably through methylation/hydroxylation, as can be predicted through differences in molecular masses. (Table 1)



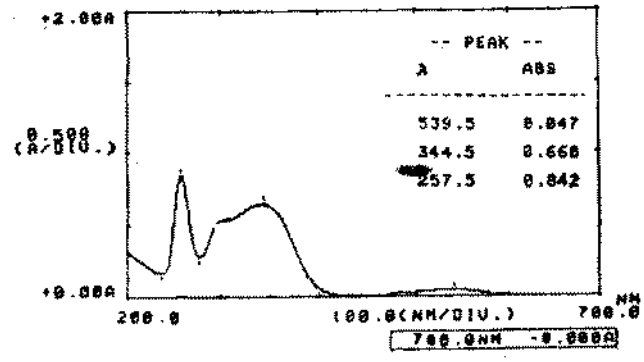


Fig. 1. Absorption spectrum and high performance liquid chromatograms of intact mature *C. roseus* petals

Fractions with λmax 340nm and retention time of 1.75 min. showed the base peak of m/z 197, characteristically representing the backbone of anthocyanin [C_6 - C_3 - C_6O]. The intense peak of m/z 429 is the molecular ion representing hirsutidin trihydrate. The other peaks of m/z 333 and 317 represent malvidin

Table 1. Mass spectra of flavonoids of mature *C. roseus* petals

λ max, nm	Retention Time	m/z of major Fragments	
	min	M^+	Fragments m/z
540	1.98	322	321, 304, 279, **161**, 125
340	1.75	429	333, 317, 279, **197**, 145
340	2.23	415	393, 333, 317, 257, **197**, 159, 145
340	2.88	485	429, 333, 317, 295, 257, 235, **197**, 145
266	2.45	431	333, 317, 295, 235, **197**, 145

Base peak is printed in bold.

and petunidin respectively. The fractions collected at higher retention time, *i.e.* 2.23 min. exhibited a similar base peak of m/z 197 representing anthocyanins but major molecular ion peak varied being m/z 415 corresponding to malvidin pentahydrate. The m/z 333 and 317 peaks were retained.

Another fraction collected at 340nm but with retention time 2.88 min. showed a molecular ion peak of m/z 485 indicating malvidin with a possible pentose glycone moiety, besides the earlier mentioned peaks of m/z 333, 317 and also of 419 representing petunidin, malvidin and hirsutidin trihydrate, respectively. Fractions collected at λmax 266 nm with retention time of 2.45 min. exhibited similar base peak (m/z 197) representing anthocyanin, the molecular ion peak of m/z 431 representing hirsutidin trihydrate and other peaks of m/z 333 and 317 representing petunidin and malvidin. This indicates malvidin and petunidin to be fairly stable compounds of *C. roseus* petals. However, the rest of the flavonoids can be identified by their characteristic absorption maxima and appearance at different retention time fractions of HPLC. ESI-MS has been helpful in characterization by estimating the stability and size of molecular masses. A further confirmation through NMR cannot be overlooked, yet the reproducibility of data makes it reliable.

Forsyth and Simmonds (1957) could characterize kaempferol, quercetin, petunidin, malvidin and hirsutidin trihydrate besides an unknown flavone in *C. roseus*. Tricin has been identified for the first time in addition to the already reported flavones besides the presence of arabinose (a pentose glycone) as the glycone moiety of the given flavonoid. It has been observed that if these compounds are stored for a longer period at room temperature the flavonoids degrade and attach with other secondary products to make more complex compounds. The current study reports the presence of an additional flavonoid compound, which may be of interest as a copigment to anthocyanins being available at λmax 540 nm; its possible functions in this flower are yet to be ascertained.

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