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Analysis of flavonoids from *Cyclanthera pedata* fruits by liquid chromatography/electrospray mass spectrometry

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Abstract

A liquid chromatography/mass spectrometry (LC/MS) based method was developed for the characterization of fruits of *Cyclanthera pedata* Scrabs (Caigua), a Peruvian food and medicinal plant. This method is based on the separation of flavonoid glycosides present in the methanolic extracts from *C. pedata* fruits using high performance liquid chromatography (HPLC) followed by detection with electrospray ionization mass spectrometry (ESI/MS). Chromatographic separation of the analytes of interest was achieved on a Symmetry C-18 column with detection in positive ion mode. Calibration graphs were obtained by determining the area ratio between external standard of each major compound and the internal standard naringine. Due to the sensitivity and the repeatability of the assay, this method is suitable for industrial quality control of raw materials and final products.

Keywords: *Cyclanthera pedata*; Flavonoids; LC/MS; ESI/MS

1. Introduction

The fruits of *Cyclanthera pedata* Scrabs (Caigua), a Peruvian edible plant belonging to the Cucurbitaceae family, are largely used in South America for their anti-inflammatory, hypoglycaemic and hypocholesterolemic properties. Because of this latter activity, supported by a clinical study [1], *C. pedata* have a commercial interest in the phytopharmaceutical market of this geographical area. More recently, this medicinal herb has become more popular and has attracted European market. At the present, no official methods are used for the quality control of the plant and its products; and the authentication of commercial samples of *C. pedata* is generally carried out using classical procedures performed by thin layer chromatography (TLC).

In our previous work, the chemical composition of fruits and seeds of this plant have been investigated; particularly, we reported the isolation and the structure determination of cucurbitacin glycosides from the seeds, triterpenoid saponins and flavone glycosides from the fruits [2–4].

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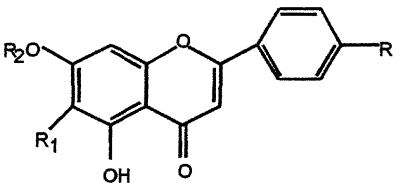
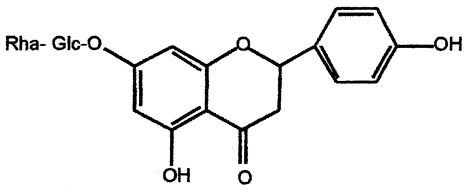
These last metabolites are the major constituents of the fruits and have been isolated for the first time in this plant. For this reason, the flavone glycosides have been selected as "marker compounds" for the chemical evaluation or standardization of *C. pedata* and its products.

Flavonoids are polyphenolic compounds with antioxidant properties [5,6] and several studies have shown that a high intake of flavonoids is correlated to a decrease in heart disease, and other biological effects of this class of compounds have been described in several in vivo and in vitro studies [7–10]. These compounds are largely used for chemotaxonomic surveys of plant genera and families because of their almost ubiquitous presence in vascular plants and of their structural variety. We have previously identified six flavone glycosides isolated from the fruits of *C. pedata*, four of which were reported by us for the first time. In this group of compounds, *O*- and *C*-glycosides, aglycon moieties are represented by chrysine and apigenine, while the sugar units are fucose, glucose and rhamnose (compounds **1**–**6**, see Fig. 1) [4].

Nuclear magnetic resonance spectroscopy (NMR) [11] and mass spectrometry (MS) [12] are among the most powerful techniques for the elucidation of flavonoids structure. During the past decade, electrospray ionisation (ESI) MS has emerged as a highly useful methodology for direct coupling with liquid phase separation techniques such as chromatography [13] and electrophoresis [14]. The utility of high performance liquid chromatography (HPLC) separations was greatly enhanced by mass spectrometric detection, which allowed the confidently identification of the flavones in plant materials [15–17]. Liquid chromatography coupled to mass spectrometry (LC/MS) can give information on sugar and acyl moieties in flavonoids witch not revealed by the UV spectrum without the need to isolate and hydrolyse the compounds [18].

This study reports the first LC/MS analysis of flavonoid compounds from *C. pedata* by ESI. Protonated molecular ions of flavonoids were observed, and the product ions from this cations were further analysed by MS/MS. The aglycon moiety, the sugar units and the glycosidic linkages of the flavonoids can





I.S. Naringine

Flavonoid glycosides from *Cyclanthera pedata*

	R	R1	R2	M.W.
1	H	Fuc	Glc	562
2	H	Fuc	H	400
3	OH	Fuc	H	416
4	H	H	Glc-Rha-	562
5	H	Glc	H	416
6	OH	Glc	H	432
7	OCH_3	Glc	H	447
8	H	H	Glc-Glc-	578
9	H	Fuc-Ma	H	487
10	H	Fuc-Ma	H	487
11	H	Fuc-Ma	H	487

Fig. 1. Strutcures of flavonoid glycosides from *Cyclanthera pedata* fruits. Glc: glucose, Fuc: fucose, Rha: rhamnose, Ma: malonyl.

be determinate from collision induced dissociation (CID) of pseudomolecular ions.

2. Materials and methods

2.1. Materials

Naringine, used as internal standard, was purchased from Sigma (St. Louis, MO, USA).

Standards of pure compounds **1**–**6** were isolated in our previous experimental study [4], and their structures were elucidated by NMR.

HPLC grade methanol (MeOH), acetonitrile (ACN) and trifluoroacetic acid (TFA) were purchased from J.T. Baker (Baker Mallinckrodt, Phillipsburg, NJ, USA). HPLC grade water (18 mΩ) was prepared using a Millipore Milli-Q purification system (Millipore Corp., Bedford, MA, USA).

2.2. Preparation of flavonoid standards

Stock solutions of the flavonoid standards (1 mg/ml), were prepared by dissolving each compound in MeOH. Four different solutions, containing respectively 5, 25, 50 and 125 $\mu g\,ml^{-1}$ of each flavonoid (external standards) and 40 $\mu g\,ml^{-1}$ of naringine, were prepared in MeOH and used for method development.

2.3. Preparation of plant samples

The fruits of *C. pedata* used in this study were supplied by IPIFA, Istituto Peruano Investigaciones Fitoterapica Andina, and were collected in Perù, 1997. They were air dried and stored at room temperature.

A voucher sample is deposited at the Department of Pharmaceutical Science, University of Salerno, Italy (n. 5).

Capsules preparations of *C. pedata* fruits were purchased from Natural World, Lima, Perù. Powdered dried extracts from *C. pedata* fruits were supplied from Laboratori Biokyma, Anghiari Ar, Italy).

A sample of 5 g of powder from air dried fruits, without the seeds, containing internal standard (10 mg) was extracted with 25 ml of MeOH, using sonication for 60 min at room temperature, and then was kept at room temperature in the dark for a night. The extract was filtered and diluted 1:10 with MeOH, and 20 μl of the sample were injected in to the analytical system. In order to establish if qualitative differences in flavonoid profile could be observed when the extraction was carried out for a longer period, *C. pedata* fruits were also extracted in MeOH for 7 days at room temperature.

Capsules contents and dried extracts were extracted according the procedure described above.

2.4. Electrospray mass spectrometry (ESI/MS)

ESI/MS in the positive ion mode was performed by using a Finnigan LCQ Deca ion trap instrument from Thermo Finnigan (San José, CA, USA) equipped with Xcalibur software. Samples were dissolved in MeOH and infused in the ESI source by using a syringe pump; the flow rate was 3 $\mu l\,ml^{-1}$. The capillary voltage was at 5 V, the spray voltage was at 5 kV and the tube lens offset was at 35 V. The capillary temperature was 220 °C. Data were acquired in MS1 and MS/MS scanning mode.

2.5. Liquid chromatography/electrospray mass spectrometry (LC/ESI/MS)

The extract was analysed by LC/ESI/MS "on-line" using the same instrument described above equipped with a Spectra System HPLC (Thermo Finnigan, San José, CA, USA). Individual flavonoids were separated on a Symmetry C18 column (150 mm × 2.1 mm, 5 μm) (Waters Corporation, Milford, MA, USA) at a flow rate of 0.3 $ml\,min^{-1}$; solvent A was 0.05% trifluoroacetic acid and solvent B was 0.05% (v/v) trifluoroacetic acid in acetonitrile. After a 5 min hold at 10% solvent B, elution was performed by a linear gradient from 10 to 40% solvent B in 40 min and from 40 to 65% solvent B in 15 min. The eluate was directly injected into the electrospray ion source and the MS1 and MS/MS spectra were acquired and interpreted using the software provided by the manufacturer.

Neutral loss scan mass spectrometric analyses were performed with a triple quadrupole instrument API2000 (Applied Biosystems, Foster City, CA, USA) equipped with a Series 200 HPLC system (Perkin-Elmer, Norwalk, CT, USA). Chromatographic conditions were the same as described above for the LC/ESI/MS experiment. The instrument was used in the tandem MS mode (neutral loss scan).

2.6. Calibration, quantification and statistical analysis

Standard curves for each of the five flavonoid standards were prepared over a concentration range of 5–125 $\mu g\,ml^{-1}$ with four different concentration levels (5, 25, 50, and 125 $\mu g\,ml^{-1}$) and triplicate injections at each level. Peak area ratios between the area of each flavonoid standard and those of naringine, used as internal standard, were calculated and plotted against the corresponding standard concentration using weighed linear regression to generate standard curves.

3. Results and discussion

3.1. ESI/MS and ESI/MS/MS analysis of flavonoid standards

With the aim of obtaining mass spectrometric-based information focused to evaluate the presence of different classes of flavonoids, *O*- or *C*- and mono or di-glycosides, direct flow injection experiments of flavonoid standards were initially performed. $[M + H]^+$ and $[M + Na]^+$ ions were observed when the data were acquired in MS1 scanning mode (*m*/*z* range: 150–700) (see Table 1). In Table 1, the data obtained from MS/MS spectra of compounds **1**–**11** are also shown.

The fragmentation pattern observed for flavonoid *C*-glycosides was in agreement with the results obtained from Li et al. [19] with FAB MS and from Waridel et al. [20] using atmospheric pressure chemical ionisation–ion trap–mass spectrometry (APCI–IT–MS).

The daughter ion spectrum of the pseudomolecular ion at *m*/*z* 563 (Compound **1**), 7-*O*-β-D-glucopyranosyl-6-*C*-fucopyranosylchrysine ($C_{27}H_{30}O_{13}$), showed an intense ion at *m*/*z* 545 corresponding to the loss of water and a minor fragment at *m*/*z* 523 due to the loss of two water molecules, both of them connected with the fragmentation of the C-linked pentose sugar unit; another characteristic fragment was observed at *m*/*z* 489 corresponding to the ion [Glc–A–$CH_2CHOHCH{=}OH]^+$, where A corresponds to the protonated aglycone moiety. The loss of the O-linked hexose sugar unit instead produced an intense ion at *m*/*z* 401 and another ion at *m*/*z* 383 due to a previous loss of water.

In order to obtain more information about the fragmentation of the C-linked pentose sugar unit the ion at *m*/*z* 401 was subjected to a further MS^3 fragmentation experiment. The resulting spectra showed two ions at *m*/*z* 383 and 365 due to the loss of one and two water molecules, respectively and a characteristic ion at *m*/*z* 321 derived from the Retro-Diels–Alder reaction (loss of acetaldehyde) following the loss of two molecules of water. Another ion occurred at *m*/*z* 297 derived from the rearrangement reaction that produce the ion $[A–CH{=}CH–OH_2]^+$. The relative intensity of each fragment ion confirmed the C-6 linkage of the sugar moiety excluding the C-8 linkage hypothesis [19].

The MS/MS analyses of compounds **2**, 6-*C*-fucopyranosylchrysine ($C_{21}H_{20}O_8$), and **3**, 6-*C*-fucopyranosyl apigenin ($C_{21}H_{20}O_9$), showed the same fragmentation pattern of the C-linked pentose sugar unit previously seen. Differences in the intensity of the ion fragments are summarized in Table 1.

The tandem mass spectrum of compound **4**, 7-*O*-β-D-glucopyranosyl-(1 → 4)-α-L-rhamnopyranosylchrysine ($C_{27}H_{30}O_{13}$), presents the characteristic fragmentation of flavonoid *O*-diglycosides [21].

The most intense ion observed in the full MS spectrum was the ion at *m*/*z* 255, corresponding to the protonated aglycone moiety. Other two peaks, observed at *m*/*z* 401 and 417, were due to the loss of the external glucose unit according to the Y and Z series of fragmentation in polysaccharide chains [22].

Compounds **5**, 6-*C*-glucopyranosylchrysine ($C_{21}H_{20}O_9$), and **6**, isovitexin ($C_{21}H_{20}O_{10}$), showed fragmentation patterns characteristic for the C-6-linked hexose [19].

Compound **7** was not observed in the overnight extract of *Cyclanthera pedata* fruits and was isolated only from the 7 days extract. It was identified as a methoxyl derivative of compound **6**, glucopyranosil apigenin ($C_{22}H_{22}O_{10}$). It was not an artefact because of it is present also in an Ethanolic extract, obtained in 7 days. The MS/MS spectrum obtained from the fragmentation of the pseudomolecular ion at *m*/*z* 447 showed the presence of a most abundant ion at *m*/*z* 415 due to the loss of one molecule of methanol. The other ions derived from the typical fragmentation of *C*-6-glucosyl-flavonoids.

Table 1
Molecular ions and fragment ions of the flavonoids, acquired by FIA (compounds **1**–**7**), using ES–MS and by SIM (compounds **8**–**11**), using LC/ESMS.

Compound	Full MS (intensity)[a]	MS/MS (intensity)[a]	MS/MS/MS
1	585 $[M + Na]^+$ (100) 563 $[M + H]^+$ (80)	563 $[M + H]^+$ parent ion (10) 545 $[M + H - w]^+$ (100) 523 $[M + H - 2w]^+$ (16.3) 489 $[M + H - 74]^+$ (31.8) 401 $[M + H - 162]^+$ (51.3) 383 $[M + H - 162 - w]^+$ (23.5)	401 $[M + H - 162]^+$ parent ion (20) 383 $[M + H - 162 - w]^+$ (70) 365 $[M + H - 162 - 2w]^+$ (100) 321 $[M + H - 162 - 44 - 2w]^+$ (50) 297 $[M + H - 162 - 104]^+$ (20)
2	423 $[M + Na]^+$ (100) 401 $[M + H]^+$ (60)	401 $[M + H]^+$ parent ion (10) 383 $[M + H - w]^+$ (100) 365 $[M + H - 2w]^+$ (60) 321 $[M + H - 44 - 2w]^+$ (51) 297 $[M + H - 104]^+$ (18.5)	
3	439 $[M + Na]^+$ (100) 417 $[M + H]^+$ (56)	417 $[M + H]^+$ parent ion (15) 399 $[M + H - w]^+$ (100) 381 $[M + H - 2w]^+$ (32) 337 $[M + H - 44 - 2w]^+$ (21) 313 $[M + H - 104]^+$ (16)	
4	585 $[M + Na]^+$ (70) 563 $[M + H]^+$ (100) 255 $[M + H - 162 - 146]^+$ (20)	563 $[M + H]^+$ parent ion (30) 417 $[M + H - 146]^+$ (60) 401 $[M + H - 162]^+$ (20) 255 $[M + H - 162 - 146]^+$ (100)	
5	439 $[M + Na]^+$ (50) 417 $[M + H]^+$ (100) 297 $[M + H - 120]^+$ (50)	417 $[M + H]^+$ parent ion (0) 399 $[M + H - w]^+$ (100) 351 $[M + H - 2w - 30]^+$ (58) 321 $[M + H - 2w - 60]^+$ (25) 297 $[M + H - 120]^+$ (41)	
6	455 $[M + Na]^+$ (50) 433 $[M + H]^+$ (100) 313 $[M + H - 120]^+$ (50)	433 $[M + H]^+$ parent ion (0) 415 $[M + H - w]^+$ (100) 397 $[M + H - 2w]^+$ (15) 367 $[M + H - 2w - 30]^+$ (58) 337 $[M + H - 2w - 60]^+$ (25) 313 $[M + H - 120]^+$ (41)	
7	447 $[M + H]^+$ (100)	447 $[M + H]^+$ parent ion (30) 415 $[M + H - 32]^+$ (100) 397 $[M + H - w - 32]^+$ (20) 379 $[M + H - 2w - 32]^+$ (22) 321 $[M + H - 2w - 60]^+$ (15) 295 $[M + H - 32 - 120]^+$ (51)	
8	579 $[M + H]^+$ (100)	579 $[M + H]^+$ parent ion (5) 417 $[M + H - 162]^+$ (100) 255 $[M + H - 162 - 162]^+$ (50)	
9–**11**	487 $[M + H]^+$ (100)	487 $[M + H]^+$ parent ion 469 $[M + H - w]^+$ 451 $[M + H - 2w]^+$ 401 $[M + H - 86]^+$ 383 $[M + H - 104]^+$ 365 $[M + H - w - 104]^+$ 347 $[M + H - 2w - 104]^+$	

[a] The intensity is determined relative to the maximum mass peak.

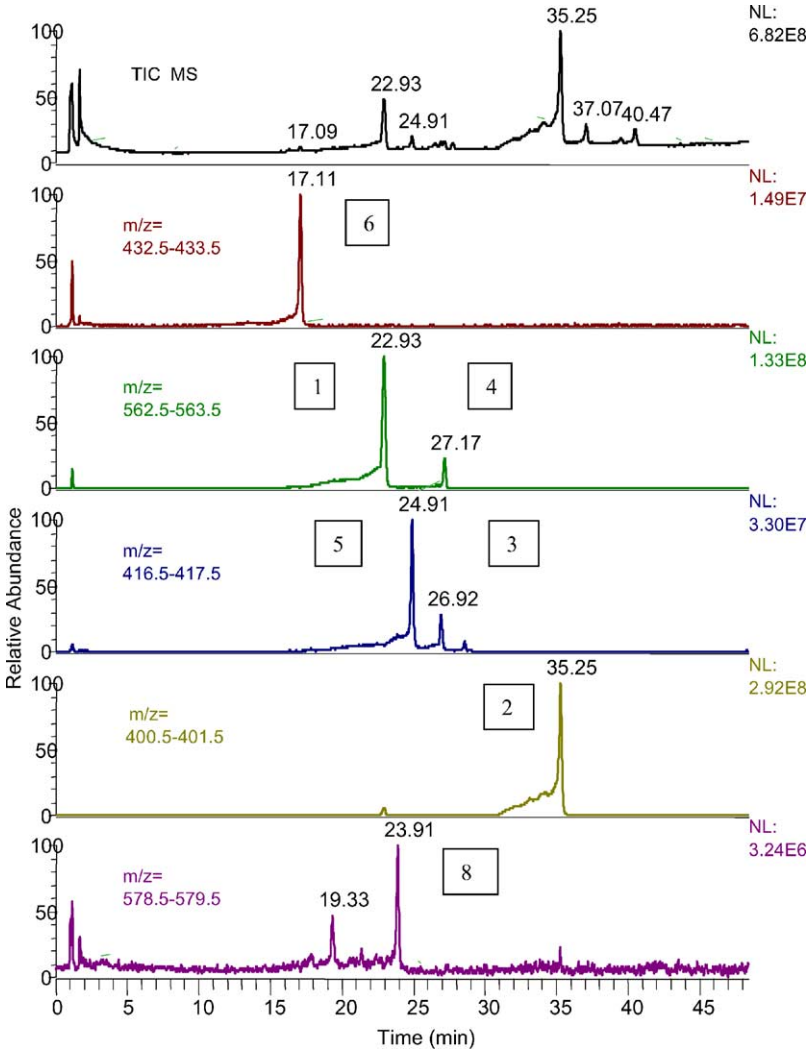


Fig. 2. LC/ESMS analysis of *Cyclanthera pedata* fruits extract for the separation and identification of seven flavonoid glycosides.

3.2. Liquid chromatography/electrospray mass spectrometry analysis of flavonoids

Positive ion electrospray LC/MS analysis, total ion current (TIC) profile, and reconstructed ion chromatograms of *C. pedata* fruits extract are shown in Fig. 2. Flavonoids were identified comparing retention times and *m*/*z* values in the total ion current chromatogram to those of the selected standards. Reconstructed ion chromatograms were obtained for each value of *m*/*z* observed for standard compounds in order to improve the separation and the identification of the single flavonoids; this step is very important to reach a higher selectivity for the quantitative analysis. Using LC/MS experiments, we have identified a large group of flavonoid compounds in the extracts without time-consuming pre-purification steps or optimisation of chromatographic procedures.

LC/MS analysis of the extract from the plant revealed the presence of several other compounds not previously detected in this species, and not reported in Fig. 1. In particular peaks at *m*/*z* 649, 579 and 487 were observed and the reconstructed ion chromatograms showed the presence of one peak at *m*/*z*

649, two compounds at different retention times for the ion with *m*/*z* 579 and three compounds in the last part of the chromatogram with a *m*/*z* 487. These evidences suggest us to use more sophisticated mass spectrometric analyses to achieve a partial characterization of these compounds, identified in the fruits of this plant for the first time.

3.3. LC/ESI/MS/MS product ion scan: identification of new compounds

In order to characterize these unexpected compounds and to reveal the presence of other correlated molecules in the extract, simultaneous experiments of LC/ESI/MS in selected ion monitoring (SIM) mode and LC/ESI/MS/MS, selecting the same ions as precursors, were performed.

The *m*/*z* values selected were the following: *m*/*z* 563, chosen in order to have a differentiation between the two *O*- and *C*-glycosides flavonoids showing the same molecular weight and *m*/*z* 579, 487 and 649 chosen because of their presence in the total ion current of the LC/MS experiment.

The chromatographic profile obtained from the LC/ESI/MS SIM experiment for the ion at *m*/*z* 563 showed the same profile of the reconstructed LC/MS chromatogram obtained for this ion in the previous experiment. In particular, it shows two peaks at retention time of 22.96 and 27.42 min that were attributed respectively to compounds **1** and **4**, based on the LC/MS/MS data and on the comparison with the MS/MS spectra originated from the standards.

Analysis of the chromatogram obtained by LC/ESI/MS/MS for the ion at *m*/*z* 579 provides structural information on the two flavonoid compounds not previously detected. The tandem mass spectrum produced by the compound at retention time of 19.53 min showed a major ion fragment at *m*/*z* 401, probably corresponding to the loss of a sugar moiety and a subsequent fragmentation, similarly to a *C*-glycoside flavonoid. Nevertheless, this result was insufficient for a complete structural characterization of this compound. On the other hand, the compound at retention time 24.03 min produced an unambiguous tandem mass spectrum, with two major ions, due to the sequential loss of two molecules of hexose sugar O-linked to a flavonoid aglycone. For this reason, we have hypothised that this compound has the structure of an *O*-diglucopyranosilchrysine. The structure and the spectral data are reported in Fig. 1 and in Table 1, respectively.

The chromatographic profile obtained from the LC/ESI/MS SIM experiment for the ion at *m*/*z* 487 revealed the presence of three peaks at different retention times (see Fig. 3). Analysis of the tandem mass spectra of the three different species, in the simultaneous LC/MS/MS experiment, showed the same fragment ions at different intensities. In particular, the compound at retention time 37.56 min shows an initial loss of one, two or three molecules of water and a subsequent loss of malonic acid corresponding to the loss of a 86 Da fragment. From this evidence we thought that the three compounds are the malonyl derivatives of the fucosyl chriysine, each of them substituted with the malonyl unit in one of the three possible free hydroxyl position of the sugar moiety.

The chromatogram obtained by the LC/ESI/MS SIM experiment for the ion at *m*/*z* 649 gave a single peak at retention time of 26.63 min; the corresponding MS/MS spectrum showed a fragment ion at *m*/*z* 487, corresponding to the loss of an hexose unit, followed by the same fragmentation pattern of malonyl-derivatives previously described. In this case the hypothesized structure differs from the one described above by the presence of an additional glucose unit, like compound **1** differs from compound **2**.

In order to confirm these structures we carried out a study focused on fragmentation reactions of the malonate moiety. Using a triple quadrupole instrument, these fragmentations allow the specific detection of a malonate containing class of molecules by neutral loss scanning; as a matter of fact when a group is lost as a neutral fragment, neutral loss scanning provides specific detection. In fact, in a neutral loss scan experiment, both mass analysers are scanned simultaneously in a synchronized fashion but with an offset to lower *m*/*z* values applied to Q3, which is equivalent to the neutral loss to be detected.

Since CID causes the loss of two molecules of water and a neutral acid malonic unit from $[M+H]^+$ ions of malonate containing compounds, we performed an LC/ESI/MS/MS experiment scanning for neutral loss of 140 Da. The three peaks revealed were located at the end of the chromatogram at the same retention time already observed in the previous experiment; this

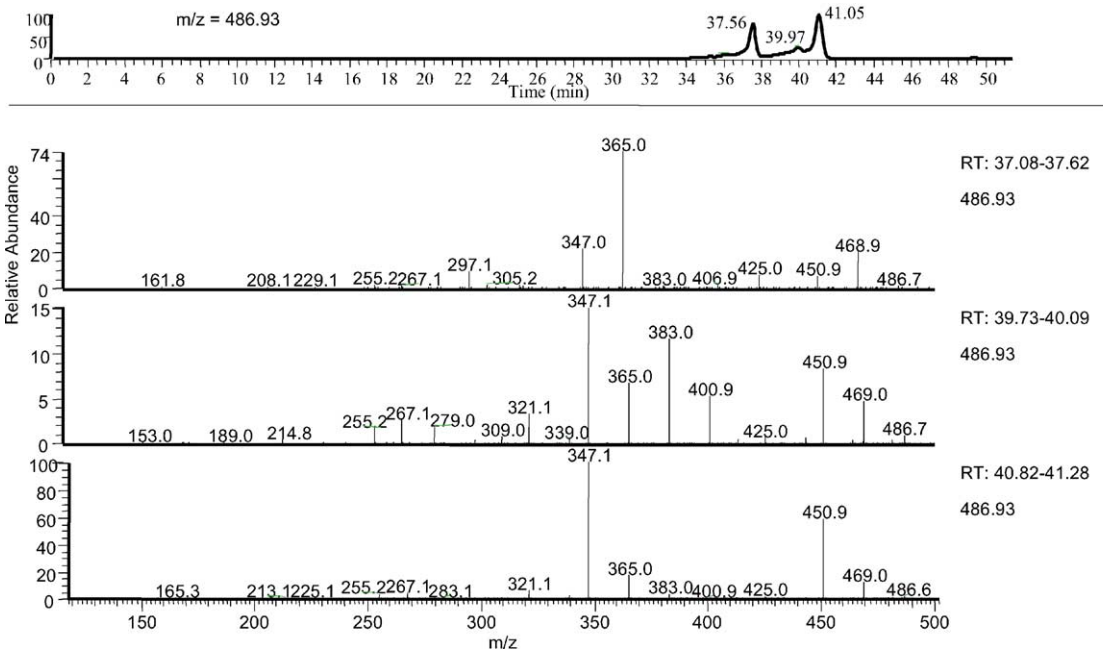


Fig. 3. LC/ES MS/MS SIM profile of malonyl derivatives of fucosyl chrysine.

allowed us to rule out the presence of other similar malonyl derivatives. The absence of the peak corresponding to the ion at *m*/*z* 649 is easily explained, because this compound gives an initial loss of a charged fragment of 162 Da not detectable with neutral loss scanning.

3.4. Quantitative analisys of flavonoids in cyclanthera pedata fruits

The calibration graphs, obtained by plotting area ratio between external and internal standard versus the known concentration of each compound, were linear in the range of 5–125 $\mu g\,ml^{-1}$ for all six flavonoids. Five aliquots of crude extract of *C. pedata* fruits were analysed in order to quantify the content of flavonoids. The method proved to be specific for the flavonoids and the internal standard, since no interfering compounds could be seen in the TIC profile at the elution positions of the flavonoid compounds. The flavanone, naringin, was selected as a suitable internal standard for the present calibration, since no interfering peaks were seen in any extract sample, thus confirming the advantage in the use of this compound. It is worth noting that the internal standard was introduced into both samples and calibration standards before the extraction, improving the precision and accuracy of the quantitative analysis.

Quantitative results for flavonoid glycosides are shown in Table 2, the concentrations are expressed as milligram of each compound present in 1 g of dried fruit. Quantitative data are not reported for compound **4**, occurring as a minor constituent. Quantitative data showed that compounds **1** and **2** are the most abundant; this evidence is particularly important because the same compounds are also structurally characteristic for this species.

Five samples from commercial products, capsules containing powered dried fruits and powdered dried fruits, were analysed and quantified for flavonoid content using the same procedure.

Quantitative analyses results showed that the relative quantities of each flavonoid compound are in agreement with results obtained from plant samples, and, in particular, compounds **1** and **2** appear to be the most abundant (Table 3). This finding demonstrated a good quality for the commercial products analysed.

Table 2
Quantitation of flavonoid glycosides by LC/MS for Peruvian samples of *Cyclanthera pedata* fruits

	Compound **1** (mg/g of fruit)	Compound **2** (mg/g of fruit)	Compound **3** (mg/g of fruit)	Compound **4** (mg/g of fruit)	Compound **5** (mg/g of fruit)	Compound **6** (mg/g of fruit)
Mean ($n = 5$)	1.84	2.62	0.36	Minor	0.66	1.02
S.D.	0.18	0.18	0.23		0.09	0.11
r^2	0.9996	0.9984	0.9984	0.99	0.9982	0.9952

Table 3
Quantitation of flavonoid glycosides by LC/MS for commercial samples of *Cyclanthera pedata* fruits ($n = 5$)

Flavonoids	Capsules (mg/g of fruit)	Powdered dried fruits (mg/g of fruit)
1	2.92 ± 0.16	1.42 ± 0.06
2	1.76 ± 0.04	2.41 ± 0.06
3	0.70 ± 0.08	0.44 ± 0.06
4	Minor	Minor
5	0.59 ± 0.05	0.61± 0.06
6	1.41 ± 0.11	1.25 ± 0.2
8	Minor	Minor
9	Minor	Minor
10	Minor	Minor
11	Minor	Minor
Total	7.38 ± 0.11	6.14 ± 0.26

Note: For compound **7** are not reported data, not founding this compound with the standard extraction procedure (see *Section 2*).

4. Conclusions

In conclusion, we quantified the flavonoidic content of *C. pedata* extracts by LC/MS using external and internal standard. Five other flavonoids, three malonyl derivatives of 6-*C*-fucosil-chrysine, a 6-*C*-gentobiosil-chrysine and a glucopyranosil acacetin, were also detected in these plant extracts. Four of them are new compounds, but occurred in *C. pedata* as minor constituents. With this analytical approach we have been able to reveal the presence of flavonoid glycoside malonates in *Cyclanthera* species.

LC/MS techniques showed good performances both in terms of sensitivity and specificity and provide two independent parameters, i.e. retention time and mass information for the identification of the flavonoid constituents in a complex mixture such as a plant crude extract.

To perform MS/MS analyses during the chromatographic run allows the discrimination of the *O*-glycosylation and *C*-glycosylation of flavonoids, as already reported in the analysis of one crude extract containing daidzein and genistein glycosides [23].

The developed LC/MS/MS analytical method seems to provide an accurate picture of flavonoid-related compounds present in fruit extracts and, at the same time, it results in a target-compounds approach.

The neutral loss scanning experiment reported in this paper did not give additional information about some other malonyl derivatives of flavonoids glycosides; but could be a useful tool for a rapid and sensitive screening of natural products conjugate, such as malonyl or acetyl derivatives, in a crude plant extract.

The developed method is straightforward and convenient, requiring no expensive and time-consuming sample preparation procedures. As such it will be a good tool for industrial quality control in order to quantify marker compounds in both raw materials and in final products.

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