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Study of Flavonoids of *Sechium edule* (Jacq) Swartz (Cucurbitaceae) Different Edible Organs by Liquid Chromatography Photodiode Array Mass Spectrometry

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A liquid chromatography−mass spectrometry (LC-MS)-based method was developed for the characterization of flavonoids from *Sechium edule* (Jacq) Swartz (Cucurbitaceae) edible organs, a plant cultivated since pre-Colombian times in Mexico where the fruit is called chayote. Chayote is used for human consumption in many countries; in addition to the fruits, stems, leaves and the tuberous part of the roots are also eaten. Eight flavonoids, including three *C*-glycosyl and five *O*-glycosyl flavones, were detected, characterized by nuclear magnetic resonance spectroscopic data, and quantified in roots, leaves, stems, and fruits of the plant by LC-photodiode array-MS. The aglycone moieties are represented by apigenin and luteolin, while the sugar units are glucose, apiose, and rhamnose. The results indicated that the highest total amount of flavonoids was in the leaves (35.0 mg/10 g of dried part), followed by roots (30.5 mg/10 g), and finally by stems (19.3 mg/10 g).

KEYWORDS: ***Sechium edule*; flavonoids; LC-PDA-MS**

INTRODUCTION

Sechium edule (Jacq) Swartz (Cucurbitaceae) is an herbaceous perennial climbing plant cultivated since pre-Colombian times in Mexico, where the edible fruit is commonly called chayote (*1*). Its fruits as well as stems, tender leaves, and the tuberous part of the adventitious roots have been important elements in the diet of the people living in South America and in other countries such as Australia, Madagascar, China, India, Portugal, and South Italy where the fruit is named "cucuzzeda spinusa". The fruits are much appreciated, in many rural communities of developing countries, as a vegetable and are either just boiled or used in stews and desserts (*2*, *3*). The fruits, and the seeds especially, are rich in several important amino acids such as aspartic acid, glutamic acid, alanine (only in the fruits), proline, serine, tyrosine, threonine, and valine (*4*). Many of these characteristics, including the softness of the fruit flesh, make it particularly suitable for hospital diets and for giving consistency to baby foods, juices, sauces, and pastes (*5*). The medicinal use of *S. edule* has also been documented in the literature. The plant is useful as a complementary treatment for arteriosclerosis and hypertension and as a diuretic and antiinflammatory remedy (*4*, *6*). Previous phytochemical studies on *S. edule* fruits led to the isolation of sterols, nonphenolic alkaloids, and saponins (*7*, *8*), while there are no reports on the flavonoidic composition of the edible organs of the plant.

Flavonoids are polyphenolic compounds with antioxidant properties, and several studies have shown that a high intake of flavonoids is correlated to a decrease in heart diseases, and many biological effects of this class of compounds have been described in *in vivo* and *in vitro* studies. Specific flavonoids are known to have pharmacological activities, particularly antiallergic, antiinflammatory, antiviral, and anticarcinogenic effects. With the recent development of electrospray ionization (ESI) mass spectrometry coupled with liquid chromatography (LC-MS) it has become technically and economically feasible to analyze polar compounds such as flavonoids. LC-photodiode array (PDA)-MS is especially advantageous in that it provides high resolution and rapid compound identification also without the need to isolate individual constituents (*9*).

Because, as in the case of many minor food plants, *S. edule* could be an important export crop from some Latin American countries but little is known about its secondary metabolite composition, the aim of the present study was to promote the conservation and the use of this underutilized and neglected crop, evaluating the profile of flavonoids of roots, leaves, stems, and fruits by LC-PDA-MS. This work, representing the first qualitative and quantitative profile of *S. edule* flavonoid composition in different edible organs of the plant, is intended to draw attention to this species and to contribute to the improvement of the potential value of this crop as food.

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MATERIALS AND METHODS

Chemicals. High-performance liquid chromatography (HPLC) grade acetonitrile (CH_3CN), methanol (MeOH), and acid acetic (AcOH) were purchased from J. T. Baker (Baker Mallinckrodt, Phillipssburg, NJ). HPLC grade water (18 mΩ) was preparated by a Milli-Q^{50} purification sistem (Millipore Corp., Bedford, MA).

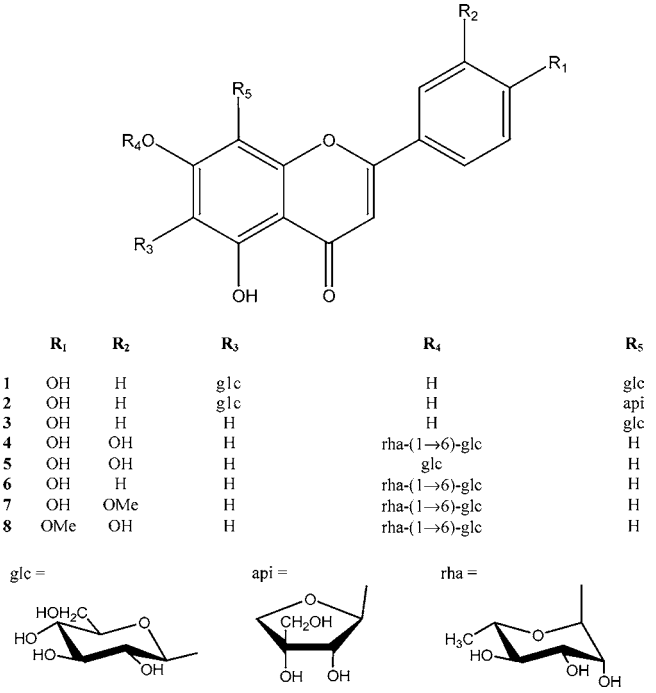
General Experimental Procedures. UV spectra were recorded on a Perkin-Elmer-Lamba 12 spectrophotometer. A Bruker DRX-600 NMR spectrometer, operating at 599.19 MHz for ^{1}H and at 150.86 MHz for ^{13}C using the UXNMR software package, was used for NMR experiments; chemical shifts are expressed in δ (parts per million) referring to the solvent peaks δ_H 3.34 and δ_C 49.0 for CD_3OD and δ_H 2.49 and δ_C 39.5 for DMSO-d_6. Column chromatographies were performed over Sephadex LH-20 (Pharmacia, Uppsala, Sweden); HPLC separations were conducted on a Shimadzu LC-8A (Shimadzu Corp., Kyoto, Japan) series pumping system equipped with a Waters R401 refractive index detector and Shimadzu injector and with a Waters μ-Bondapak C_{18} column (Waters, Milford, MA).

Plant Material. *S. edule* (Jacq) Swartz (Cucurbitaceae) was collected in Monasterace (RC), Italy, in December 2002. A voucher specimen (no. 8636) was deposited in Nuove Acquisizioni at Herbarium Horti Botanici Pisani, Pisa, Italy.

HPLC-PDA-ESI-MS Analyses. HPLC-PDA-ESI-MS analysis were performed using a Surveyor LC pump, a Surveyor Autosampler, coupled with a Surveyor PDA detector and LCQ Advantage ion trap mass spectrometer (Thermo Finnigan, San Jose, CA) equipped with Xcalibur 3.1 software. Analyses were performed using a 250 mm × 4.6 mm i.d., 5 μm, X Terra MS C-18 column (Waters Corp.). The eluent was a mixture of acetonitrile (solvent A) and 0.05% acetic acid (solvent B). The solvent gradient was as follows: 0−2 min from 0 (A) to 15% (A); 2−25 min from 15 (A) to 20% (A); 25−28 min maintained at 20% (A). Elution was perfomed at flow rate of 1.0 mL/min with a splitting system of 2:8 to MS detector (200 μL/min) and PDA detector (800 μL/min), respectively. The total running time was 28 min, and MS data were recorded from 7 to 27 min. The volume of the injection was 20 μL for all of the samples (standards of calibration and extracts of edible organs). All analyses were performed with an ESI interface in the negative ion mode. The ionization conditions were optimized, and the parameters were retained as follows: capillary temperature, 280 °C; capillary voltage, −10.00; tube lens offset, −30.00; sheath gas flow rate, 70; auxiliary gas flow rate, 7; spray voltage, 4.50 kV; scan range of *m*/*z* 100−700 amu. N_2 was used as the sheath and auxiliary gas. PDA data were recorded with a 200−600 nm range with three preferential channels as the detection wavelength: 335 (channel A), 255 (channel B), and 350 nm (channel C).

Extraction, Isolation, and Identification of Flavonoids. One hundred grams of aerial parts of *S. edule*, dried and powered, were submitted to extraction at room temperature with solvents of increasing polarity. The material was defatted with *n*-hexane and after extracted with $CHCl_3$ and MeOH by exhaustive maceration (500 mL of each solvent for three times). The extraction afforded 1.5, 1.6, and 7.5 g, respectively.

The methanol extract (7.5 g) of *S. edule* was partitioned betwen 24 mL of *n*-BuOH and 24 mL of H_2O, to afford a *n*-BuOH soluble portion. The *n*-BuOH residue (2.2 g) was applied to a Sephadex LH-20 column (100 g), using MeOH as the eluent at a flow rate of 1 mL/min; 63 fractions of 8 mL were collected and grouped into eight major fractions (A−H) by thin-layer chromatography analysis on silica 60 F_{254} gel-coated glass sheets developed with *n*-BuOH−AcOH−H_2O 60:15:25. Fractions G and H were characterized as pure compounds **4** (48.5 mg) and **5** (43.0 mg), respectively (**Figure 1**). Fractions C (120 mg), D (100 mg), E (120 mg), and F (85 mg) were separately purified by RP-HPLC on a 300 mm × 7.8 mm i.d., C_{18} μ-Bondapak column at a flow rate of 2.0 mL/min with MeOH−H_2O (2:3) for fraction C, MeOH−H_2O (2:3) for fraction D, MeOH−H_2O (1:1) for fraction E, and MeOH−H_2O (2:3) for fraction F to afford pure compound **2** (25.0 mg, t_R = 12 min) from fraction C, compound **3** (8.3 mg, t_R = 18 min) from fraction D, compounds **1** (4.5 mg, t_R = 6.0 min) and **6** (8.5 mg, t_R = 13 min) from fraction E, and compounds **7** (1.0 mg, t_R = 22 min) and **8** (3.0 mg, t_R = 25 min) from fraction F.



	R_1	R_2	R_3	R_4	R_5
1	OH	H	glc	H	glc
2	OH	H	glc	H	api
3	OH	H	H	H	glc
4	OH	OH	H	rha-(1→6)-glc	H
5	OH	OH	H	glc	H
6	OH	H	H	rha-(1→6)-glc	H
7	OH	OMe	H	rha-(1→6)-glc	H
8	OMe	OH	H	rha-(1→6)-glc	H

Figure 1. Flavonoids isolated from *S. edule*'s aerial parts.

Vicenin-2 (**1**) was obtained as a yellow amorphous powder. ESI-MS, *m*/*z* 593 [M − H]$^-$. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 4.75 (1H, d, J = 7.8 Hz, H-1_{glcI}), 4.90 (1H, d, J = 8.0 Hz, H-1_{glcII}), 6.55 (1H, s, H-3), 6.91 (2H, d, J = 8.5 Hz, H-3′ and H-5′), 7.95 (2H, d, J = 8.5 Hz, H-2′ and H-6′). ^{13}C NMR (DMSO-d_6, 600 MHz): δ 61.0 (C-6_{glcII}), 61.1 (C-6_{glcI}), 69.9 (C-4_{glcII}), 70.0 (C-4_{glcI}), 71.3 (C-2_{glcI}), 71.5 (C-2_{glcII}), 73.5 (C-1_{glcI}), 74.4 (C-1_{glcII}), 78.7 (C-3_{glcI}), 78.9 (C-3_{glcII}), 81.4 (C-5_{glcI}), 82.0 (C-5_{glcII}), 103.0 (C-3), 104.6 (C-8), 104.8 (C-10), 109.0 (C-6), 116.3 (C-3′ and C-5′), 122.0 (C-1′), 129.1 (C-2′ and C-6′), 155.5 (C-9), 159.6 (C-5), 161.4 (C-4′), 162.7 (C-7), 164.1 (C-2), 182.5 (C-4) (*10*).

Apigenin 6-*C*-β-D-glucopyranosyl-8-*C*-β-D-apiofuranoside (**2**) was obtained as a yellow amorphous powder. ESI-MS, *m*/*z* 563 [M − H]$^-$. ^{1}H NMR (CD_3OD, 600 MHz): δ 3.58 (2H, br s, H-5_{apio}), 4.29 (1H, dd, J = 9.0, 7.5 Hz, H-2_{glc}), 4.98 (1H, d, J = 7.5 Hz, H-1_{glc}), 5.05 (1H, d, J = 1.8 Hz, H-1_{apio}), 6.58 (1H, s, H-3), 6.95 (2H, d, J = 8.0 Hz, H-3′ and H-5′), 7.98 (2H, d, J = 8.0 Hz, H-2′ and H-6′). ^{13}C NMR (CD_3OD, 600 MHz): δ 62.4 (C-6_{glc}), 65.2 (C-5_{apio}), 70.8 (C-2_{apio}), 71.2 (C-4_{glc}), 72.1 (C-4_{apio}), 72.2 (C-2_{glc}), 75.4 (C-1_{glc}), 77.3 (C-1_{apio}), 80.0 (C-3_{glc}), 80.1 (C-3_{apio}), 82.4 (C-5_{glc}), 103.0 (C-3), 105.0 (C-8), 105.3 (C-10), 109.5 (C-6), 116.5 (C-3′ and C-5′), 123.0 (C-1′), 130.1 (C-2′ and C-6′), 157.9 (C-9), 161.7 (C-4′), 162.1 (C-5), 164.0 (C-2), 165.0 (C-7), 182.0 (C-4) (*11*).

Vitexin (**3**) was obtained as a yellow amorphous powder. ESI-MS, *m*/*z* 431 [M − H]$^-$. ^{1}H NMR (CD_3OD, 600 MHz): δ 5.06 (1H, d, J = 7.8 Hz, H-1_{glc}), 3.78 (1H, dd, J = 12.0, 5.0 Hz, H-$6a_{glc}$), 3.98 (1H, dd, J = 12.0, 3.5 Hz, H-$6b_{glc}$), 6.30 (1H, s, H-6), 6.60 (1H, s, H-3), 6.94 (2H, d, J = 8.0 Hz, H-3′ and H-5′), 7.98 (2H, d, J = 8.0 Hz, H-2′ and H-6′) (*12*).

Luteolin 7-*O*-rutinoside (**4**) was obtained as a yellow amorphous powder. ESI-MS, *m*/*z* 593 [M − H]$^-$. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 1.06 (3H, d, J = 6.0 Hz, H-6_{rha}), 4.54 (1H, d, J = 1.8 Hz, H-1_{rha}), 5.07 (1H, d, J = 7.5 Hz, H-1_{glc}), 6.44 (1H, d, J = 1.8 Hz, H-6), 6.73 (1H, s, H-3), 6.74 (1H, d, J = 1.8 Hz, H-8), 6.90 (1H, d, J = 8.0 Hz, H-5′), 7.41 (1H, d, J = 1.8 Hz, H-2′), 7.43 (1H, dd, J = 8.0, 1.8 Hz, H-6′). ^{13}C NMR (DMSO-d_6, 600 MHz): δ 17.8 (C-6_{rha}), 66.1 (C-6_{glc}), 68.4 (C-5_{rha}), 69.6 (C-4_{glc}), 70.3 (C-3_{rha}), 70.8 (C-2_{rha}), 72.1 (C-4_{rha}), 73.2 (C-2_{glc}), 75.6 (C-5_{glc}), 76.3 (C-3_{glc}), 94.8 (C-8), 99.9 (C-1_{rha}), 100.0 (C-1_{glc}), 100.5 (C-6), 103.2 (C-3), 105.5 (C-10), 113.6 (C-2′), 116.1 (C-5′), 119.3 (C-6′), 121.3 (C-1′), 145.8 (C-3′), 150.1 (C-4′), 157.0 (C-9), 161.3 (C-5), 162.9 (C-2), 164.6 (C-7), 181.9 (C-4) (*13*).

Table 1. Components Identified in the Edible Organs of *S. edule* by LC-PDA-MS

peak	compound	t_R (min)	$[M - H]^-$ (*m*/*z*)	MS/MS fragments (*m*/*z*)	λ_{max}
A	vicenin- 2 (**1**)	9.03	593	575.0, 503.1, 473.0, 383.2, 353.2	230, 270, 335
B	unidentified	9.37	579	518.9, 489.0, 459.0, 399.1	240, 260, 345
C	apigenin 6-*C*-β-D-glucopyranosyl-8-*C*-β-D-apiofuranoside (**2**)	11.34	563	545.2, 503.1, 473.1, 443.1, 383.1, 353.1	235, 270, 330
D	unidentified	15.73	533	515.1, 473.1, 443.1, 383.2, 353.1	270, 290, 315
E	vitexin (**3**)	16.36	431	341.0, 311.0	235, 265, 335
F	luteolin 7-*O*-rutinoside (**4**)	16.85	593	285.1	255, 340
G	luteolin 7 *O*-β-D-glucoside (**5**)	19.05	447	285.1	255, 350
H	apigenin 7-*O*-rutinoside (**6**)	23.20	577	269.1	240, 265, 335
I	chrysoeriol 7-*O*-rutinoside (**7**)	24.44	607	299.0	250, 354
L	diosmetin 7-*O*-rutinoside (**8**)	26.22	607	299.0, 284.1	250, 340

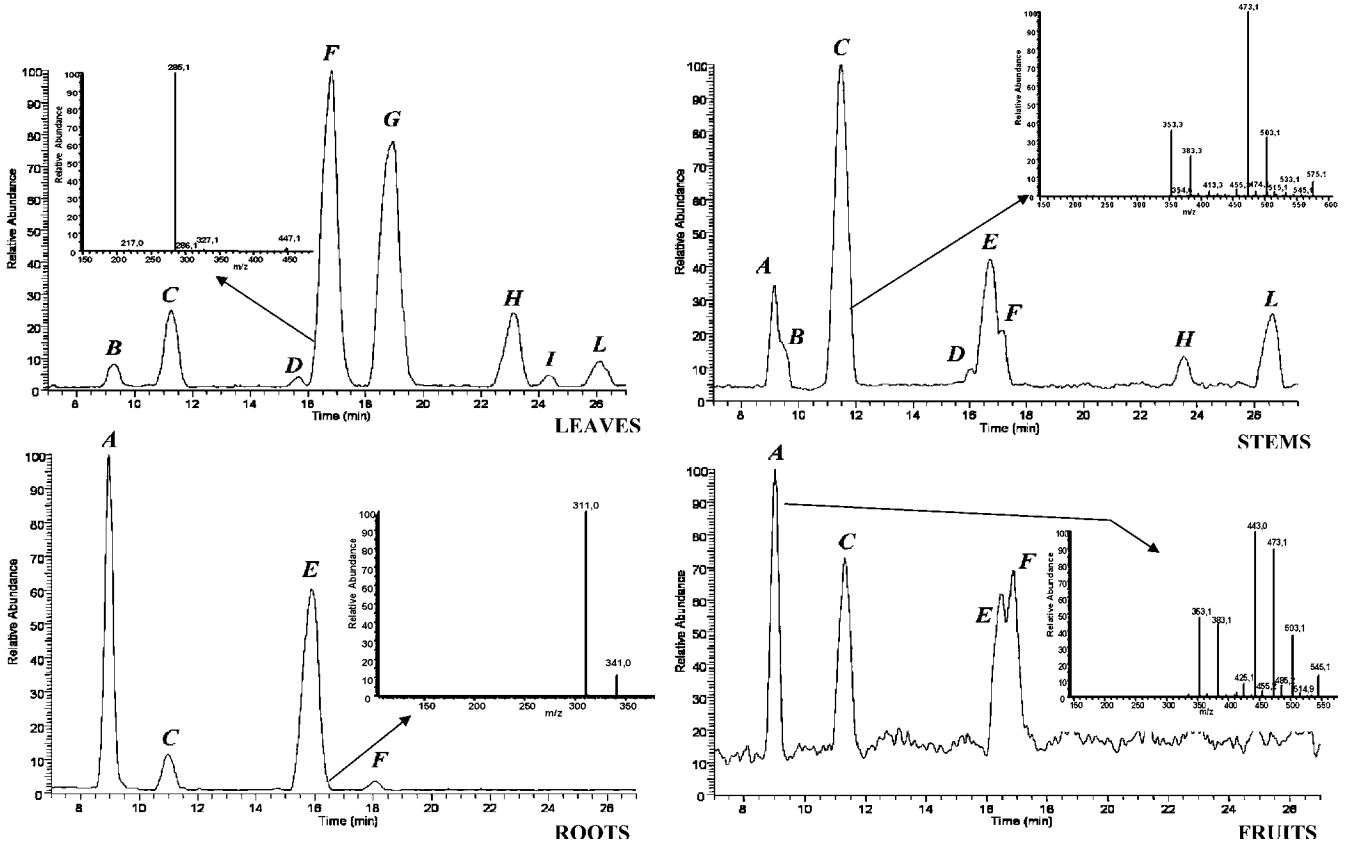


Figure 2. Profile LC-ESI-MS of methanolic extract of each edible organ of *S. edule*.

Luteolin 7-*O*-β-D-glucopyranoside (**5**) was obtained as a yellow amorphous powder. ESI-MS, *m*/*z* 447 $[M - H]^-$. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 5.07 (1H, d, $J = 7.5$ Hz, H-1$_{glc}$), 6.44 (1H, d, $J = 2.0$ Hz, H-6), 6.74 (1H, s, H-3), 6.78 (1H, d, $J = 2.0$ Hz, H-8), 6.89 (1H, d, $J = 8.0$ Hz, H-5′), 7.41 (1H, d, $J = 1.8$ Hz, H-2′), 7.47 (1H, dd, $J = 8.0$, 1.8 Hz, H-6′). ^{13}C NMR (DMSO-d_6, 600 MHz): δ 60.6 (C-6$_{glc}$), 69.5 (C-4$_{glc}$), 73.0 (C-2$_{glc}$), 76.4 (C-3$_{glc}$), 77.1 (C-5$_{glc}$), 94.7 (C-8), 99.5 (C-6), 99.9 (C-1$_{glc}$), 103.0 (C-3), 105.3 (C-10), 113.5 (C-2′), 115.9 (C-5′), 119.0 (C-6′), 121.2 (C-1′), 145.7 (C-3′), 150.0 (C-4′), 156.9 (C-9), 161.0 (C-5), 162.9 (C-2), 164.4 (C-7), 181.8 (C-4) (*14*).

Apigenin 7-*O*-rutinoside (**6**) was obtained as a yellow amorphous powder. ESI-MS, *m*/*z* 577 $[M - H]^-$. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 1.07 (3H, d, $J = 6.0$ Hz, H-6$_{rha}$), 4.52 (1H, d, $J = 1.8$ Hz, H-1$_{rha}$), 5.10 (1H, d, $J = 7.5$ Hz, H-1$_{glc}$), 6.42 (1H, d, $J = 2.0$ Hz, H-6), 6.74 (1H, d, $J = 2.0$ Hz, H-8), 6.77 (1H, s, H-3), 6.93 (2H, d, $J = 8.0$ Hz, H-3′ and H-5′), 7.90 (2H, d, $J = 8.0$ Hz, H-2′ and H-6′) (*13*).

Chrysoeriol 7-*O*-rutinoside (**7**) was obtained as a yellow amorphous powder. ESI-MS, *m*/*z* 607 $[M - H]^-$. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 1.10 (3H, d, $J = 6.0$ Hz, H-6$_{rha}$), 3.86 (3H, s, 3′-OCH$_3$), 4.58 (1H, d, $J = 1.8$ Hz, H-1$_{rha}$), 5.07 (1H, d, $J = 7.5$ Hz, H-1$_{glc}$), 6.42 (1H, d, $J = 1.8$ Hz, H-6), 6.72 (1H, s, H-3), 6.78 (1H, d, $J = 1.8$ Hz, H-8), 6.91 (1H, d, $J = 8.0$ Hz, H-5′), 7.48 (1H, dd, $J = 8.0$, 1.8 Hz, H-6′), 7.58 (1H, d, $J = 1.8$ Hz, H-2′) (*15*).

Diosmetin 7-*O*-rutinoside (**8**) was obtained as a yellow amorphous powder. ESI-MS, *m*/*z* 607 $[M - H]^-$. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 1.07 (3H, d, $J = 6.0$ Hz, H-6$_{rha}$), 3.88 (3H, s, 4′-OCH$_3$), 4.60 (1H, d, $J = 1.8$ Hz, H-1$_{rha}$), 5.05 (1H, d, $J = 7.5$ Hz, H-1$_{glc}$), 6.40 (1H, d, $J = 1.8$ Hz, H-6), 6.72 (1H, s, H-3), 6.74 (1H, d, $J = 1.8$ Hz, H-8), 7.06 (1H, d, $J = 8.0$ Hz, H-5′), 7.42 (1H, d, $J = 2.0$ Hz, H-2′), 7.50 (1H, dd, $J = 8.0$, 2.0 Hz, H-6′) (*16*).

Sample Preparation for HPLC-PDA-ESI-MS Study. A representative quantity (10 g) of all edible organs of the plant (leaves, roots, stems, and fruits) was dried, powdered, and extracted at room temperature with solvents at increasing polarity. Each dried part was defatted with *n*-hexane (4 × 100 mL) and successively extracted for 48 h with $CHCl_3$, and MeOH, by exhaustive maceration (4 × 100 mL of each solvent). The yield of methanol extracts of each edible organ was 0.881 g from leaves, 0.559 g from roots, 2.4 g from fruits, and 0.907 g from stems, respectively.

Solutions (3 mg/mL) of each methanol residue of edible organs were prepared dissolving the extract in the same solvent, and 20 μL of each solution was injected for analysis. Triplicate injections were made for

Table 2. Quantitative Amount (mg/10 g of Dried Material) of Flavonoids by LC-ESI-MS[a]

peak	leaves	stems	roots	fruits
A (**1**)	ND	0.6	14.72	trace
B	trace	trace	ND	ND
C (**2**)	5.58	13.29	0.58	trace
D	trace	trace	ND	ND
E (**3**)	ND	3.02	15.11	trace
F (**4**)	14.10	trace	0.09	trace
G (**5**)	13.51	ND	ND	ND
H (**6**)	1.76	1.14	ND	ND
I (**7**)	trace	ND	ND	ND
L (**8**)	0.05	1.24	ND	ND
flavonoid content	35.00	19.29	30.50	ND

[a] ND, not detected.

each extract. To detect the flavonoid composition of the plant, the sample preparation and HPLC analytical condition were adjusted to avoid any detectable degradation of these compounds during the performance.

Calibration, Quantification, and Statistical Analysis. Vicenin-2 and luteolin 7-*O*-β-D-glucopyranoside were selected as external standards of calibration for *C*-glycoside and *O*-glycoside flavonoids, respectively. Standard curve calibrations were prepared over a concentration range of 5−125 μg/mL with four different concentration levels (5, 25, 50, and 125 μg/mL) for vicenin-2 and luteolin 7-*O*-β-D-glucopyranoside. Triplicate injections were made for each level, and a weighed linear regression was generated for both external standards of calibration. The curve of calibration with external standard was obtained using concentration (μg/mL) with respect to area obtained from integration of the MS base peak extracting the value of area of each standard for $[M - H]^-$. The relation between variables was analyzed using linear simple correlation. For the linear regression of the external standards, R^2 was 0.9913 for vicenin-2 and 0.9890 for luteolin 7-*O*-β-D-glucopyranoside. For the quantification of the compounds, a GraphPad Software Prism 3.0 was used. The amount of the compounds was finally expressed as mg/10 g of dried edible organ of plant.

RESULTS AND DISCUSSION

HPLC-PDA-ESI-MS Analyses. To evaluate the phytochemical profile of flavonoids present in the extracts of roots, stems, leaves, and fruits of *S. edule*, LC-PDA-MS analyses were carried out using the on-line method. Analyses of *n*-hexane, $CHCl_3$, and MeOH extracts of each edible organs showed that flavonoids were present only in the methanolic residue. The LC-ESI-MS base peak chromatograms of the methanolic extract of each edible organ are shown in **Figure 1**. The retention time (t_R), $[M - H]^-$, MS/MS fragments, and UV λ_{max} value of the individual peak are listed in **Table 1**. All compounds had similar UV spectra with their absorption maxima (λ_{max}) between 250−270 and 315−355 nm. These UV spectra are typical of flavone derivatives (*17*).

In the ESI-MS spectra of peaks A−E, the pattern observed is in agreement with those of *C*-glycoside derivatives (*18*). In fact, from a qualitative viewpoint, the characteristic fragment ions of the *C*-glycoside moiety, obtained by selecting the $[M - H]^-$ as a precursor ion, were $[M - H - 60]^-$, $[M - H - 90]^-$, $[M - H - 120]^-$, $[M - H - 150]^-$, and $[M - H - 180]^-$. The daughter ion spectra of the ESI-MS/MS of peaks F−L showed an intense ion at *m*/*z* 285 corresponding to luteolin aglycone for peaks F and G, at *m*/*z* 269 according with the presence of an apigenin skeleton for peak H, and at *m*/*z* 299 corresponding to a methylated luteolin derivative for peaks I and L, respectively (*19*). Besides, the loss of *O*-linked sugar in these compounds corresponded to one hexose unit (162 amu) for peak G and one hexose and one deoxyhexose (162 +146 amu) moieties for peaks F and H−L.

Isolation and Structure Determination of Compounds 1−8. To confirm the hypothesized structures from the analytical investigation, the isolation procedure of methanolic extract of the aerial parts of *S. edule* was undertaken and eight compounds were purified (see **Figure 2**) and characterized as vicenin-2 (**1**), apigenin 6-*C*-β-D-glucopyranosyl-8-*C*-β-D-apiofuranoside (**2**), vitexin (**3**), luteolin 7-*O*-rutinoside (**4**), luteolin 7-*O*-β-D-glucopyranoside (**5**), apigenin 7-*O*-rutinoside (**6**), chrysoeriol 7-*O*-rutinoside (**7**), and diosmetin 7-*O*-rutinoside (**8**). The structures of the isolated compounds were established by ^1H and ^{13}C NMR data and confirmed by comparison with those reported in the literature (*10*−*16*). Compound **2** was previously

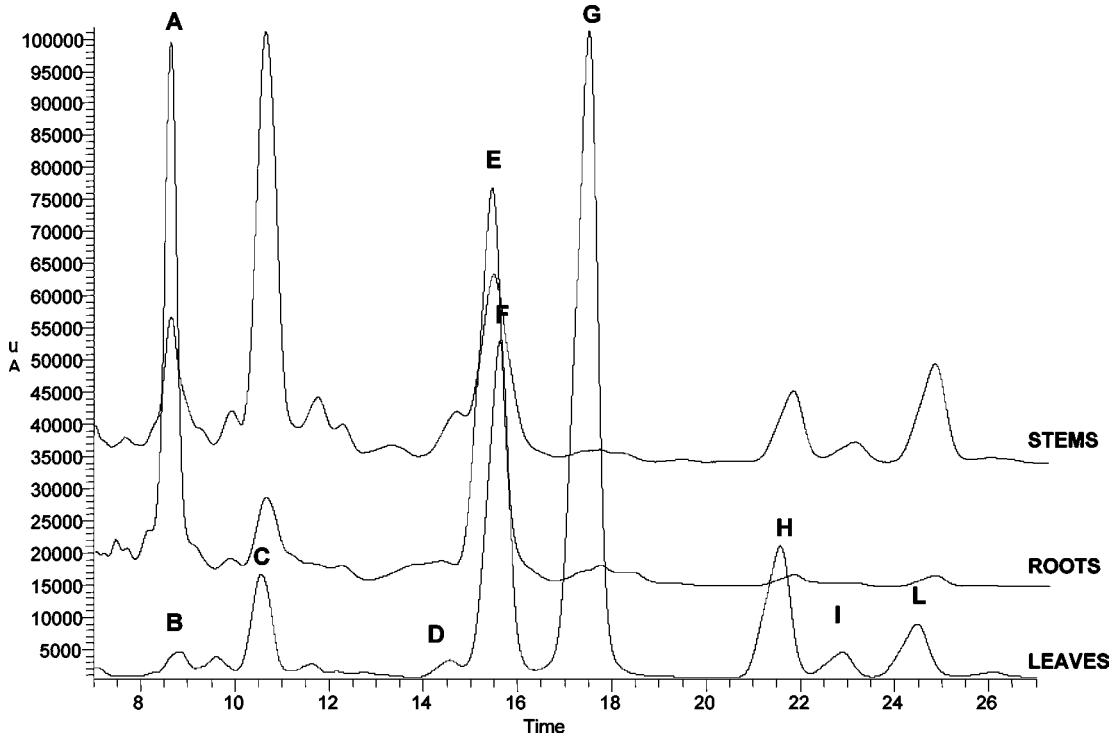


Figure 3. Qualitative HPLC comparison between flavone glycosides of leaves, roots, and stems monitored at 250 nm on the basis of their UV absorbance.

isolated only from *Xanthosoma violaceum* (*11*). The compounds corresponding to peaks B and D were not purified from the plant material, and they remained unidentified. From the UV and LC-MS/MS spectra, we can hypothesize that they are *C*-glycosyl flavones. In particular, the MS/MS fragmentation of peak D is typical of an apigenin *C*-glycoside. A reference methanol solution of pure compounds **1**−**8** (0.1 mg/mL, each) was prepared, and each solution (20 μL) was subjected to LC-PDA-MS in order to determine the retention times (t_R), UV, and MS data of the isolated compounds and to compare with the peaks present in the chromatograms of edible organ methanol extracts. In this way, peak A was identified as compound **1**, peak C as **2**, peak E as **3**, peak F as **4**, peak G as **5**, peak H as **6**, peak I as **7**, and peak L as **8**.

Comparative Study of the Composition and Content of Flavonoids of the Different Edible Organs. Each methanol fraction of edible organs was analyzed by LC-PDA-MS under gradient conditions. The LC-PDA-MS base peak chromatograms are reported in **Figure 2**. Results obtained from quantitative analysis are listed in **Table 2**. Leaves and steams are the edible organs richest in the number of flavonoids. Both *C*- and *O*-glycosides are present; the *O*-glycosides are the major components particularly in the leaves, while in the stems they are *C*-glycosides. The roots of the plant contained only *C*-glycosides, taking into account that luteolin 7-rutinoside (peak F) is the minor component. In the fruits, the flavonoids are present in small amounts. Compounds **5** and **7** (peaks G and I, respectively) were found only in the leaves, while compounds **1** and **3** (peaks A and E, respectively), present in all edible organs, were absent (**Figure 3**).

Our results from the quantitative analysis showed that the highest total amount of flavonoids was in the leaves of the plant (35.0 mg/10 g of dried part), followed by roots (30.5 mg/10 g), and finally by stems (19.3 mg/10 g). The roots contained the highest content of vicenin-2 (**1**). The content of apigenin 6-*C*-β-D-glucopyranosyl-8-*C*-β-D-apiofuranoside (**2**) in the stems was highest, followed by that of the leaves and finally roots. From a qualitative point of view, vitexin (**3**) is the major constituent of the roots, while in the stems it is the second component of the flavonoid mixture. The highest amount of luteolin 7-*O*-rutinoside (**4**) and luteolin 7 *O*-β-D-glucopyranoside (**5**) was observed in the leaves. Apigenin 7-*O*-rutinoside (**6**), present in traces in stems, was quantified in the leaves. Finally, diosmetin 7-*O*-rutinoside (**8**) is more present in the stems with respect to leaves, where it is the minor quantified constituent.

In this study, the qualitative and quantitative analysis of the flavonoid content of different edible organs of *S. edule* was determined by LC-PDA-MS using external standards. In total, eight flavonoids, including three *C*-glycosyl flavones and five *O*-glycosyl flavones, were detected, characterized, and quantified in these plant extracts. LC-MS techniques showed better performances, in terms of both sensitivity and specificity, and provide two independent parameters (retention time and mass spectra), to identify the flavonoid constituents in *S. edule* plant crude extracts. Performing MS/MS analyses during the chromatographic run allows the discrimination of *O*-glycosylation and *C*-glycosylation of flavonoids, as already reported in the literature (*20*). The developed LC-MS/MS analytical method seems to provide an accurate profile of flavonoid-related compounds present in the extracts, and, at the same time, it results in a target compounds approach.

Our result showed that the aerial part and roots of *S. edule* are more rich in flavonoidic constituents than the fruits, which are in any case important for the high nutritional value, due to the presence of amino acids and sugars (*4*). Flavonoids are increasingly recognized as playing potential roles in health including, but not limited to, their roles as antioxidants. The increasing importance of functional ingredients in food provides health-promoting phytochemicals in crop plants by crop husbandry, plant breeding, and genetic engineering. Assuming that the recent epidemiological and experimental studies are correct in suggesting that higher intakes of flavonoids from food are associated with a reduced risk of cancer, heart disease, and stroke, the immediate challenge is how to increase the level of these beneficial phytochemicals in major food plants. The high flavonoidic content of *S. edule* leaves and roots should promote the consumption of these plant organs as foods and could have a role in the prevention of human diseases especially in developing countries where economical resources are low.

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