Supporting material

Short Communications

Nutritional composition, antioxidant activity and isolation of scopoletin from *Senecio nutans*: Support of new and ancestral uses

Claudio Parra,[a,*] Emilio Soto,[a] Gloria León,[b] Cristian O. Salas,[c] Michael Heinrich,[d] Carlos Echiburú-Chau,[a,e]

[a]*Laboratorio de Química Médica y Productos Naturales, Centro de Investigaciones del Hombre en el Desierto (CIHDE), Arica, Chile;* [b]*Departamento de Química, Facultad de Ciencias, Universidad de Tarapacá, Arica, Chile;* [c]*Departamento de Química Orgánica, Facultad de Química, Pontificia Universidad Católica de Chile, Santiago, Chile;* [d]*Research Cluster 'Biodiversity and Medicines' / Centre for Pharmacognosy and Phytotherapy', UCL School of Pharmacy, London, UK;* [e]*Facultad de Ciencias de la Salud, Universidad de Tarapacá, Arica, Chile*

[*]Corresponding Author; e-mail: cparra@cihde.cl

Experimental

Collection and sample preparation

Fresh aerial parts of *S. nutans* were collected in month of February 2016 from the Chungará Lake zone, (Andean Altiplano, northern Chile) (18°12'55"S; 69°17'40"O) placed approx. 4500 m.a.s.l. The plant was identified by Dr. Gloria Rojas. A voucher specimen N°SGO 165116 was submitted to the herbarium of Chilean National History Natural Museum (MNHN). The collected aerial parts of *S. nutans* were properly cleaned and subjected to drying. All the samples were mashed to fine powder using a mechanical grinder, and finely powdered through mesh size number 80 (≈180 μm) and stored in polyethylene bags at 4 ºC prior to analysis.

Proximate analysis

The powdered plant was analyzed for proximate composition by AOAC methods (AOAC 2005). To determine the moisture, the sample was dried to a constant weight in an oven at 105 ºC. Total ash content was determined by incinerating the samples in a muffle furnace at 550 ºC for 5 h. Total lipids were determined by extracting a known weight of powdered samples with diethyl ether, using a Soxhlet apparatus. Crude protein content was calculated from the total nitrogen content by Kjeldahl procedure using a conversion factor of 6.25. The samples were digested using a DK-6 digester and distilled using a UDK 129 distilling unit (VELP Scientifica, Usmate Velate, Italy). Crude fiber content of the sample was determined by acid/alkaline hydrolysis of fat-free samples. Total carbohydrates were calculated by difference.

Table 1. Nutritional composition content in *S. nutans* from north of Chile.

Moisture (%)	Total Ash (%)	Protein (%)	Fat (%)	Fiber (%)	Carbohydrate (%)
8.70	7.19	8.18	14.31	13.23	57.09

Mineral content analysis

To determine the mineral content in *S. nutans*, a sample was incinerated as previously described, and the residues dissolved in 5 mL of HNO_3 (50%) solution and heated on a hotplate (stirring/hotplate PC-620D, Corning, NY, USA) until digestion was complete.. The concentrations of Na, K, Ca, Mg, Mn, Fe, Cu and Zn were determined using atomic spectrophotometer absorption (AA240, Varian Inc., CA, USA). All measurements were carried out using standard flame operating conditions, as recommended by the manufacturer. Phosphorus was determined using the ammonium molybdate/ammonium vandate method (Chapman and Pratt 1968).

Table 2. Macro and micronutrients content in the *S. nutans* versus *S. biafrae*.

Elements	***S. nutans*** [a] **(mg/100g)**	***S. biafrae*** [a] **(mg/100g)**
K	2130 ± 0.01	536 ± 0.03
Ca	1390 ± 0.02	242 ± 0.02
Mg	290 ± 0.02	392 ± 0.03
P	230 ± 0.10	536 ± 0.03
Na	190 ± 0.01	14.48 ± 0.01
Fe	10.41 ± 0.29	4.16 ± 0.01
Mn	8.48 ± 0.24	--
Cu	1.18 ± 0.03	0.53 ± 0.02
Zn	0.67 ± 0.03	0.67 ± 0.03

[a] Values are means of three determinations.

Preparation of ethanol extract

Dried and powdered sample (1 g) was macerated with ethanol absolute for 72 hours at room temperature. The extracts were filtered through Whatman filter paper (Nº 1) and concentrated on a rotary evaporator under reduced pressure at 40 ºC. The residues were re-dissolved in EtOH to yield a final concentration of 1 mg/mL.

Determination of total polyphenols content

The total polyphenol content was determined by the Folin-Ciocalteu method (Singleton

and Rossi 1965). Briefly, an aliquot (50 μL) of ethanolic extract was mixed with 1 mL Folin-Ciocalteu reagent (1:1) and allowed to stand for 5 min at room temperature, followed by the addition of 1 mL of 20% (w/v) sodium carbonate. The mixture was made up to 8 mL with distilled water and allowed to stand for a further 30 min at room temperature. Absorbance was measured at 760 nm using an UV-VIS spectrophotometer (GENESYS 10S UV-Vis, Thermo Fisher Scientific, MA, USA). The total polyphenol content was calculated from the calibration curve, and the results were expressed as mg gallic acid equivalent per gram of dry weight (mg GAE/g DW).

Determination of total flavonoid content

The total flavonoid content in the samples was determined by the aluminum chloride colorimetric method (Simirgiotis *et al*. 2013). Briefly, 1 mL of the ethanolic extract was diluted with 3 mL of distilled water and then 1 mL of 10% $NaNO_2$ solution and allowed to stand for 6 min at room temperature. 2 mL of 10% $AlCl_3 \cdot 6H_2O$ solution was added and the mixture was allowed to stand for 6 min. Then, 1 mL of 1 M NaOH solution and 2 mL of distilled water were added to a final volume of 10 mL. The mixture was allowed to stand for 15 min, and absorbance was measured at 415 nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg quercetin equivalent per gram of dry weight (mg QE/g DW).

Ferric reducing antioxidant power (FRAP) assay

A modified method of Benzie & Strain. (F and J 1996) was adopted for the FRAP assay. FRAP reagent was prepared daily by mixing 25 ml acetate buffer (300 mmol/L, pH 3.6), 2.5 ml TPTZ solution (10 mmol/L) in hydrochloric acid (40 mmol/L) and 2.5 ml of $FeCl_3 \cdot 6H_2O$ solution (20 mmol/L) and then incubated for 1 h at 37 °C before using. For determination of the antioxidant activity, 1.5 mL of freshly prepared FRAP was mixed with 100 μL of distilled water and 100 μL of the ethanolic extract. The

reaction mixture was allowed to stand for 30 min at room temperature and the absorbance was measured at 593 nm. Standard curve was prepared using Trolox as standard and the result were expressed as µmol Trolox equivalent per 100 g of dry weight (µmol TE/100 g DW).

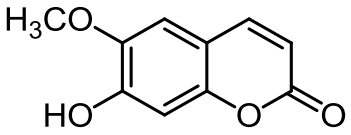
ABTS radical scanvenging activity

The free radical-scavenging activity was determined by ABTS radical cation decolorization assay (Re *et al*. 1999). Briefly, $ABTS^{\bullet+}$ solution (7 µM) was reacted with potassium persulfate (2.45 µM) and kept for overnight in the dark at room temperature before use. For the antioxidant assay with ethanolic extract, the concentration of the $ABTS^{\bullet+}$ solution was diluted with ethanol for an initial absorbance of about 0.70 ± 0.02 at 734 nm. The decolorization of the $ABTS^{\bullet+}$ solution was measured with the addition of 10 µL of the extract to 200 µL $ABTS^{\bullet+}$ solution and incubated at room temperature for 5 min, and the absorbance at 734 nm was measured immediately. A calibration curve was prepared with different concentrations of Trolox and the result was expressed as µmol Trolox equivalents per 100 g of dry weight (µmol TE/100g DW).

Table 3. Phenolic compounds, total flavonoids and antioxidant activities in *nutans* versus *A. caudatus* and *C. quinoa.*

Scientific Name	Total Phenolic Content (GAE/g DW)	Total Flavonoid Content (QE/g DW)	TEAC (µmol TE/g DW)	
			FRAP	ABTS
S. nutans	20.58 ± 0.59	14.84 ± 0.07	27.65 ± 0.06	13.01 ± 0.08
A. caudatus	0.3 ± 0.00	N.D	--	3.7 ± 0.10
C. quinoa	1.3 ± 0.00	N.D	--	8.3 ± 0.10

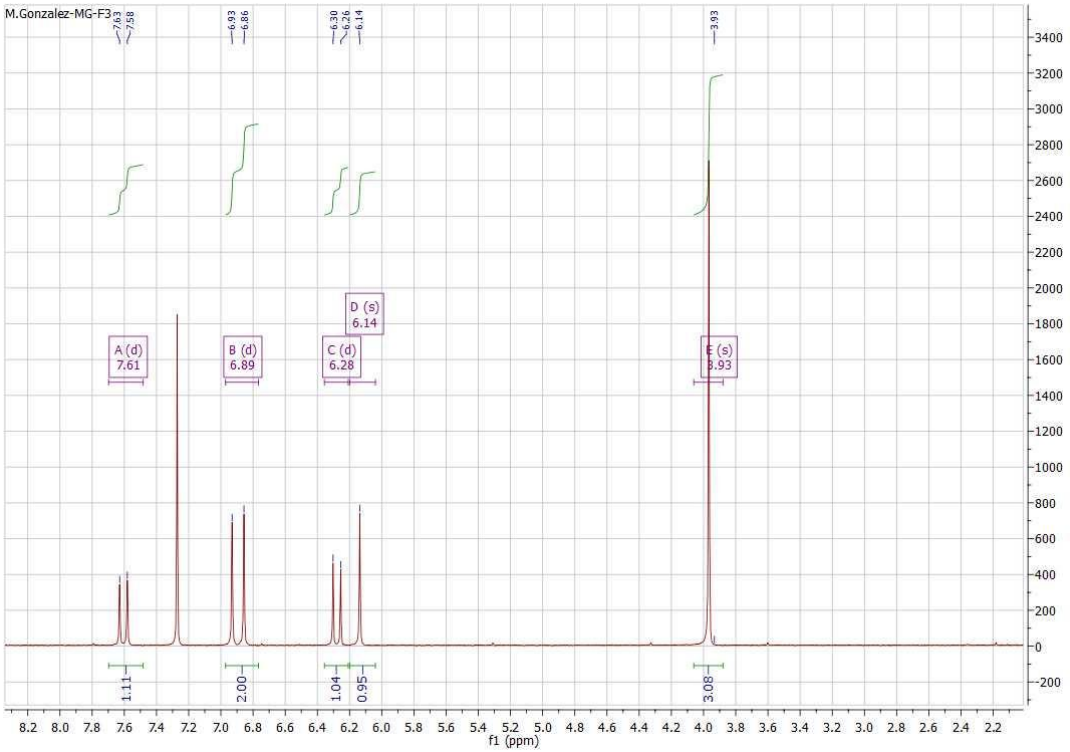
7-hydroxy-6-methoxy-2*H*-chromen-2-one (scopoletin)



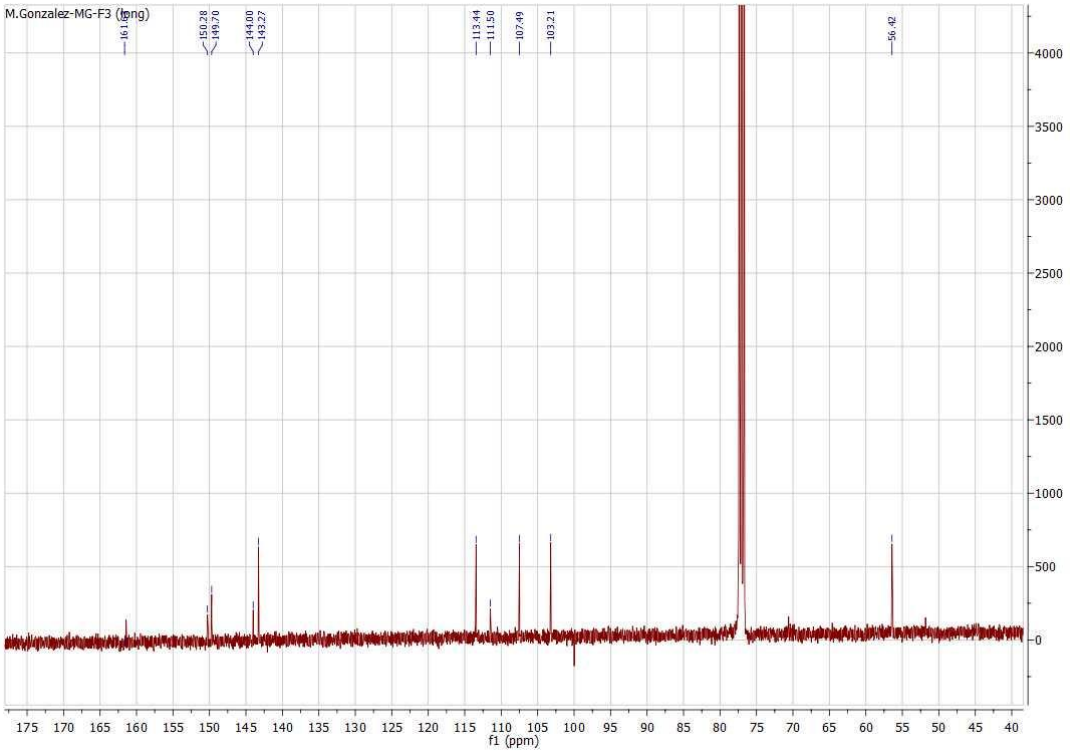
Scopoletin

The scopoletin was isolated from *S. nutans*, according to the method described by Islam *at al*.; NMR spectra was recorded in $CDCl_3$ on a Bruker Avance 400 Digital. Chemical shifts of ^{1}H and ^{13}C NMR spectra are reported in ppm downfield (δ) from Me_4Si. HRMS-ESI-MS experiments were carried out using a Thermo Scientific Exactive Plus Orbitrap spectrometer with a constant nebulizer temperature of 250 °C. The experiments were carried out in positive ion mode, with a scan range of m/z 300.00–1510.40 with a resolution of 140.000. The samples were infused directly into the ESI source, via a syringe pump, at low rates of 5 µL min^{-1}, through the instrument's injection valve. ^{1}H NMR (400 MHz, COSY): δ 7.61 (d, *J* = 9.5 Hz, 1H, H-4), 6.93 (s, 1H, H-8), 6.86 (s, 1H, H-5), 6.28 (d, *J* = 9.5 Hz, 1H, H-3), 6.14 (s, 1H, H-10), 3.93 (s, 3H, H-9). ^{13}C NMR (101 MHz, HSQC): δ 161.63 (C-2), 150.28 (C-8a), 149.70 (C-7), 144.00 (C-6), 143.27 (C-4), 113.44 (C-3), 111.50 (C-4a), 107.49 (C-5), 103.21 (C-8), 56.42 (C-9). HRMS calcd for $C_{10}H_8O_4$ $[M+1]^+$ 193.0423, found 193.0486.

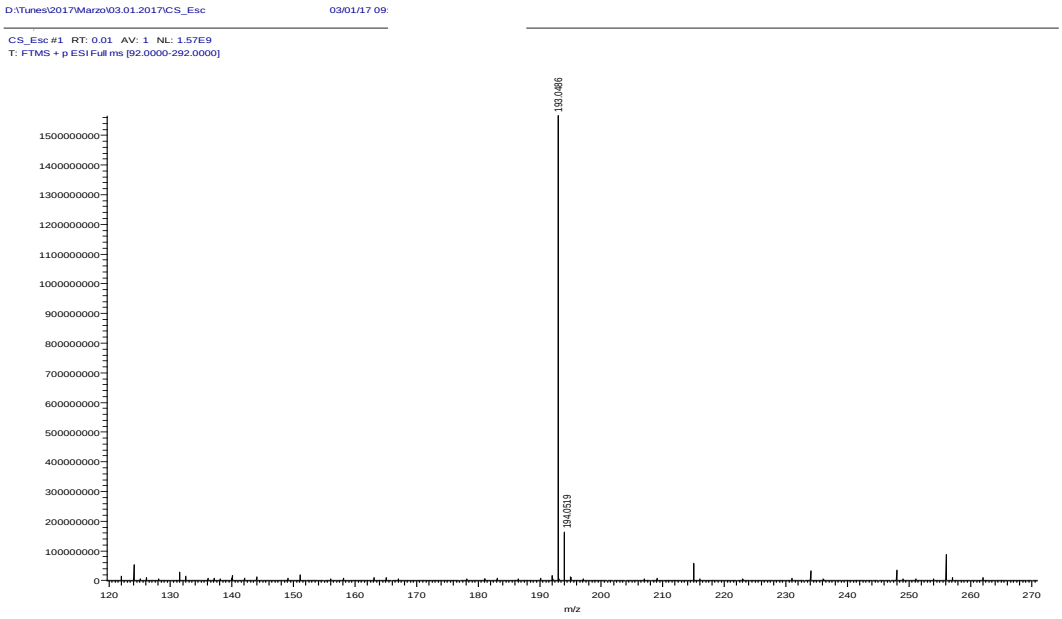
^{1}H NMR spectra of scopoletin



^{13}C NMR spectra of scopoletin



Mass spectra of scopoletin



Reference

AOAC. 2005. Official methods of analysis ,18th ed. Gaithersburg, MD: AOAC International.

Chapman H, Pratt F. 1968. Ammonium molybdate-Ammonium vandate method for determination of phosphorus: Methods of Analysis for Soils, Plants and Water. California Univ Public Division of Agriculture and Science, phosphorus.169-170.

F BIF, J SJ. 1996. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. Analytical Biochemistry.239:70-76.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine.26:1231-1237.

Simirgiotis MJ, Ramirez J, Hirschmann G, Kennelly E. 2013. Bioactive coumarins and HPLC-PDA-ESI-ToF-MS metabolic profiling of edible queule fruits (*Gomortega keule*), an endangered endemic Chilean species. Food Res Int.54:532-543.

Singleton V, Rossi JA. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture.16:144-158.