ANALYTICAL ASSAYS OF ARGENTINE HERBAL DRUGS KNOWN AS 'NENCIA'

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Abstract

Botanical, chromatographic and biological assays, were performed on commercial samples known as "nencia" or "pasto amargo" to establish its identity and quality. TLC and HPLC-UV profiles of the MeOH, CH_2Cl_2, hydro-alcoholic and aqueous extracts and the evaluation of the bitterness value of the samples collected from the market were carried out in comparison with authenticated plant material. The analytical assays allowed the identification of the commercial samples as *Gentianella achalensis* (Gilg) T.N. Ho & S.W. Liu. The proposed methodology could be an useful tool in the quality control of the herbal drug and its preparations.

1. Introduction

"Nencia" is an Argentine medicinal plant used for its bitter properties as stomachic, digestive, tonic, for liver disturbances, as febrifuge and as substitute of European Gentian (Ratera and Ratera 1980, Saggese 1959, Toursarkissian 1980). It is sold in the Argentine market as an herbal drug for tea preparations, its hydro-alcoholic extract is used for medicinal purposes and takes part of many aperitif beverages. Up to now the botanical identity of the commercial samples of "nencia" is unknown, most of them are classified as *Gentianella* sp. (Gentianaceae). Many species of Gentianaceae family are known for the same properties as "nencia" (Hieronymus 1882) specially those belonging to *Swertia* genus. In Argentina there are 10 genera of the family and 40 species, mostly from Patagonia and nearby Andes region (Fabris 1953). *Gentianella* is the largest genus of the family with 30 species (Fabris 1983). A few are used with medicinal purposes and are known as "nencia" or "pasto amargo".

Preliminar botanical study allowed us to identify all commercial samples of "nencia" as *Gentianella achalensis* (Gilg) T.N. Ho & S.W. Liu. The aim of the present study was to establish the identity and quality of the herbal drug available in the market through HPLC and TLC profiles and determination of the bitterness value in correlation with authenticated plant material.

2. Materials and methods

2.1. Plant material

Flowering plant material of *Gentianella achalensis* were collected from Las Cortaderas (San Luis) (G_1), and from La Paz (G_2) and Loma Bola (G_3) (Córdoba), the specimens were identified by Professor J.S. Pringle, Royal Botanical Garden, Hamilton, Ontario,

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Canada and Prof. Marta Nájera and Etile Spegazzini from of the LABRAM, Facultad de Ciencias Excatas y Naturales, Universidad Nacional de La Plata, Argentina A voucher specimen has been deposited at the Museo de Botánica y Farmacognosia "Carlos Spegazzini" of the same Institute. *Gentiana lutea* (Gentianaceae) and 6 commercial available samples of "nencia" were collected from different suppliers ($\mathbf{S_1}$-$\mathbf{S_6}$).

2.2. Reference Compounds

Authentic samples of gentiopicroside, sweroside, mangiferin, swertisin, demethylbellidifolin 8-*O*-glucoside, demethylbellidifolin, bellidifolin, isobellidifolin and swerchirin were isolated in our laboratory from MeOH and CH_2Cl_2 extracts of *G. achalensis*. (CC, MPLC, preparative TLC) and identified by usual spectroscopic methods (UV, ^{1}HNMR, ^{13}CNMR, 2D-NMR, EIMS, FAB-MS)

2.2. Preparation of samples for HPLC and TLC analysis

Dried flowering plant material of each sample, was powdered and extracted as follows to carry out the HPLC and TLC analysis:

- 5 g were defatted with petrol and then extracted by maceration during 72 hrs each with 100 ml CH_2Cl_2 and MeOH. The extracts were concentrated to dryness under reduced pressure and the residue was taken up with the corresponding mobile phase.
- 5 g were macerated for 7 days with 100 ml of EtOH 40%, then filtered to a 100 ml volumetric flask and adjusted to volume with EtOH 40%.
- 5 g were extracted with 100 ml of hot water, left standing for 20 min, filtered to a 100 ml volumetric flask and adjusted to volume with water.

2.3. Chromatographic conditions for HPLC analysis

The HPLC-UV analysis was performed in a Varian Version 4.0 with a 9065 photodiode array detection system. Analysis were carried out using a Phenomenex IB-SIL RP 18 column (250 x 4,6 mm; 5 μ m), with a mobile phase gradient A: CH_3CN (0.05%TFA); B: H_2O (0.05% TFA) (5:95 to 65:35) over 50 min. The flow rate was 1.0 ml/min, the UV trace was observed at 254 nm and UV spectra were recorded between 190 and 600 nm. Injection volume: 20 μl.

2.4. Chromatographic conditions for TLC analysis

TLC was performed on Silica gel HF_{254} precoated plates using the following mobile phases A: C_6H_6:EtOAc (95:5), B: $CHCl_3$:MeOH (95:5), C: EtOAc: MeOH: H_2O (100:17:10); and in Cellulose plates using D: HOAc 15% as mobile phase. Detection: UV 366 and 254 nm light and 50% ethanolic sulfuric acid followed by heating at 105 °C for 3-5 min.

2.5. Determination of bitterness value

0.2 g of each commercial ($\mathbf{S_1}$-$\mathbf{S_6}$) and authentic sample of *G. achalensis* ($\mathbf{G_1}$-$\mathbf{G_3}$) and one of *G. lutea* (commercial origin) were heated with 45 ml of tap water under reflux for 1 h. After cooling, each sample was filtered and diluted to 50 ml with water. 5 ml of the filtrate was diluted with water in a 100 ml measuring flask. This was used as Standard solution (St).

The methodology used is as directed for the Determination of Bitterness Value in Quality Control Methods for Medicinal Plant Materials *WHO*, 1992 and 1980. The test was performed by at least 3 people in 4 different days. Solutions were recently prepared for each determination. The bitterness value was calculated as follows:

$$\text{Bitterness value in units /g} = \frac{2000 \times c}{a \times b}$$

a= quantity of material in mg/ml of the standard solution (St) of the plant material sample.
b= volume of the St in ml of the dilution of threshold bitter concentration.
c= quantity of quinine hydrochloride *R* in mg per 10 ml of the dilution of threshold bitter concentration.

3. Results

The HPLC and TLC profiles were performed for CH_2Cl_2, MeOH, EtOH 40% and aqueous extracts of each sample. The main compounds were identified in the chromatograms by their R*t*., Rt_r, UV spectrum and R*f* respectively (Hostettmann and Hostettmann, 1989; Lacaille-Dubois *et al*, 1996; Nadinic *et al*, 1994 a, b, 1995, 1996) and in comparison with authentic samples. Reference compounds used for HPLC and TLC were previously isolated by conventional chromatographic methods from the MeOH and CH_2Cl_2 extracts (CC, MPLC, preparative TLC) and identified by usual spectroscopic methods (UV, ^{1}HNMR, ^{13}CNMR, EIMS, FAB-MS) (Nadinic *et al*, 1997 and unpublished results) and/ or in comparison with authentic samples.

HPLC-UV with photodiode array detection of the CH_2Cl_2, MeOH, EtOH 40% and aqueous extracts of the authenticated (**G_1-G_3**) and commercial samples of "nencia" (**S_1-S_6**) allowed the identification of the main compounds of each extract. Mangiferin (**1**), gentiopicroside (**2**), sweroside (**3**), swertisin (**4**), 8-glucosildemethylbellidifolin (**5**), demethylbellidifolin (**6**), bellidifolin (**7**) and isobellidifolin (**8**) in MeOH, EtOH 40% and aqueous extracts (Fig 1, 3 and 4 respectively) and demethylbellidifolin (**6**), bellidifolin (**7**), isobellidifolin (**8**) and swerchirin (**9**) in CH_2Cl_2 extract (Figure 2).

TLC analysis in the different chromatographic systems matched very well with the HPLC results, showing the correspondent main components of each extract. (Table 1).

A bitterness value between 1800 and 2700 was found for all samples **G_1-G_3** and **S_1- S_6** using the proposed WHO assay (1990 and 1980). Gentiana sample was used as reference in the determination. The bitterness value of 250-270 found for Gentiana was in accordance with the published monographic data (Table 2).

4. Discussion

HPLC/UV and TLC profiles of the CH_2Cl_2, MeOH, EtOH 40% and aqueous extracts of the commercial samples (**S_1- S_6**) matched very well with the HPLC/UV and TLC profiles of the same extracts prepared with authenticated plant material (**G_1-G_3**). Little quantitative differences were detected in the HPLC analysis.

The results of the bitterness determination of the aqueous extracts of the samples also showed some differences, but this may be due to the different proportions of the bitter compounds, as shown in HPLC profiles. All the samples were almost 10 times more bitter than gentian in the same condition. The analytical methology could be proposed for identification and quality assurance of the commercial herbal drugs known as "nencia".

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Table 1 - R*f* values for main compounds identified in *G. achalensis* and commercial samples of "nencia"

Main Compounds	**TLC Chromatographic Systems**			
	A	**B**	**C**	**D**
Mangiferin (**1**)			0.42	0.45
Gentiopicroside (**2**)			0.39	
Sweroside (**3**)			0.37	
Swertisin (**4**)			0.56	0.39
Demethylbellidifolin-8-glu (**5**)			0.52	0.37
Demethylbellidifolin (**6**)	0.06	0.34	0.88	0.12
Bellidifolin (**7**)	0.36	0.82		
Isobellidifolin (**8**)	0.29	0.77		
Swerchirin (**9**)	0.86	0.96		

Table 2 - Bitterness value of *G. achalensis* ($\mathbf{G_1}$-$\mathbf{G_3}$) and commercial samples of "nencia" ($\mathbf{S_1}$- $\mathbf{S_6}$) in comparison with *G. lutea*. Cs= Concentration of the sample

Cs (mg/ml)	Gentianella Samples									G. lutea
	$\mathbf{G_1}$	$\mathbf{G_2}$	$\mathbf{G_3}$	$\mathbf{S_1}$	$\mathbf{S_2}$	$\mathbf{S_3}$	$\mathbf{S_4}$	$\mathbf{S_5}$	$\mathbf{S_6}$	
0.002	-	-	-	-	-	-	-	-	-	-
0.004	-	+	+	+	+	-	+	+	-	-
0.006	+	+	+	+	+	+	+	+	+	-
0.03										-
0.06										+
Bitterness Value	1800	2700	2700	2700	2700	1800	2700	2700	1800	270

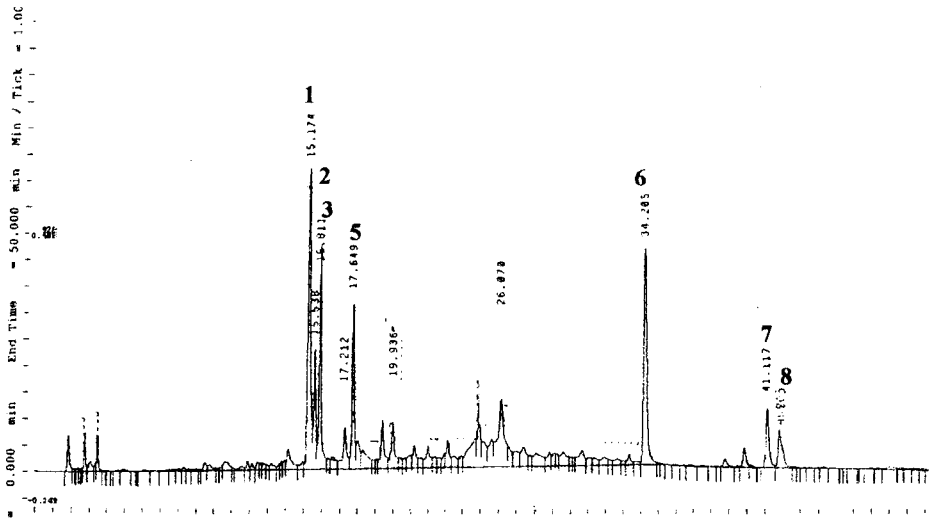

Figure 1 - HPLC analysis of the methanolic extract of *G. achalensis*; peak numbers correspond to compound number given in Table 1. For chromatographic protocol see Materials and methods section.

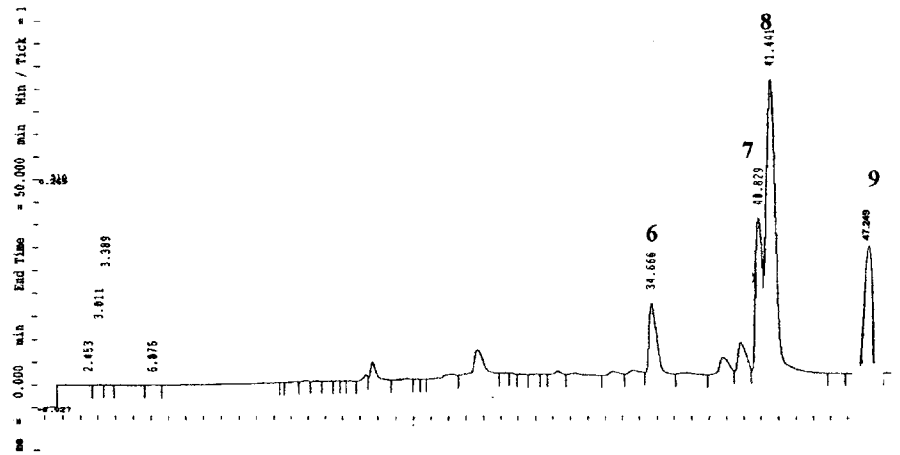

Figure 2 - HPLC analysis of dichloromethane extract of *G. achalensis*; peak numbers correspond to compound number given in Table 1. For chromatographic protocol see Materials and methods section.

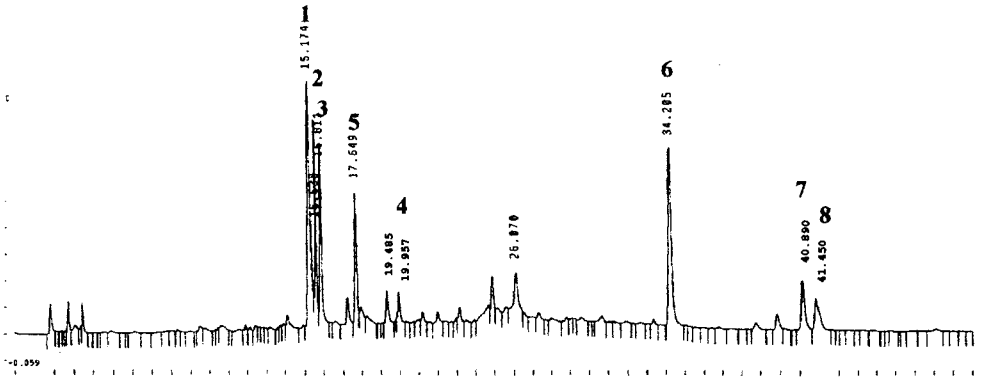

Figure 3 - HPLC analysis of hydroalcoholic extract of *G. achalensis*; peak numbers correspond to compound number given in Table 1. For chromatographic protocol see Materials and methods section.

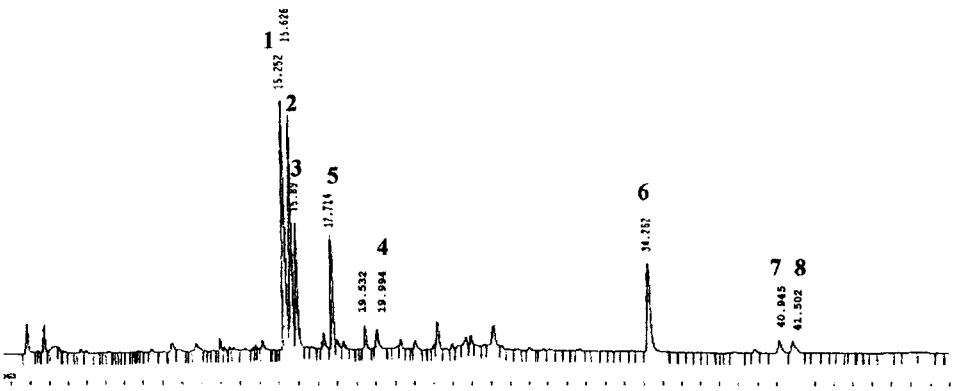

Figure 4 - HPLC analysis of aqueous extract of *G. achalensis*; peak numbers correspond to compound number given in Table 1. For chromatographic protocol see Materials and methods section.