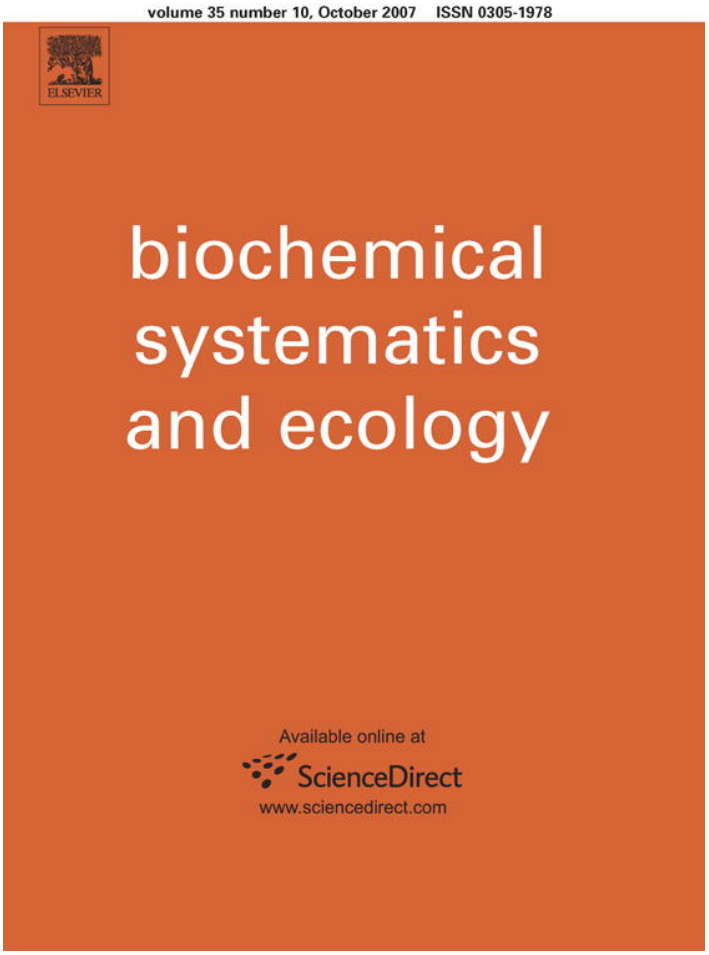

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Flavonoids and volatile constituents from *Achyrocline hyperchlora*

Marta Natacha Liendro [a], María Laura Uriburu [a,*], Carmen Inés Viturro [b], Roberto Ricardo Gil [c], Lázaro Juan Novara [a], Juana Rosa de la Fuente [a]

[a] *Department of Chemistry, Consejo de Investigación, Universidad Nacional de Salta, Avda. Bolivia 5150, 4400 Salta, Argentina*
[b] *Facultad de Ingeniería, Universidad Nacional de Jujuy, Gorriti 237, 4600 San Salvador de Jujuy, Argentina*
[c] *Department of Chemistry, Carnegie Mellon, 4400 Fifth Avenue, Pittsburgh, PA 15213, USA*

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1. Subject and source

Achyrocline hyperchlora Blake (Asteraceae, Inuleae) is one of the eight species of this genus described in South America: *Achyrocline alata* (Kunth) DC., *Achyrocline flaccida* (Weinm) DC., *Achyrocline ramosissima* (Sch. Bip) Rusby, *Achyrocline rupestris* Cabrera, *Achyrocline satureioides* (Lam.) DC., *Achyrocline tomentosa* Rusby and *Achyrocline venosa* Rusby (Giangualani, 1976; Cabrera, 1978; Freire, 1999). Some of these species are used in folk medicine as antispasmodic, febrifuge, tonic, anthelmintic, and antiasthmatic (Mongelli et al., 1996; Hilgert, 2001).

A. hyperchlora is typical on the humid slopes in the mountains of the northern Argentina, at 1000–3000 m above sea level. Aerial parts were collected in April 2001 in Departamento La Caldera, Salta province, Argentina. The plant was identified by Ing. Lázaro J. Novara. A voucher specimen (MCNS 1062) has been deposited in the Herbarium of Facultad de Ciencias Naturales, Universidad Nacional de Salta, Argentina.

2. Previous work

There are no previous phytochemical reports concerning *A. hyperchlora* Blake.

3. Present study

In this work we describe the isolation and chemical characterization of flavonoids from *A. hyperchlora* and its essential oil composition.

The characteristic strong odor of this species in its natural habitat is noteworthy.

* Corresponding author. Tel./fax: +54 387 4255363.
E-mail address: luriburu@unsa.edu.ar (M.L. Uriburu).

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3.1. Extraction, isolation and identification of flavonoids

Ground air-dried aerial parts of *A. hyperchlora* (630 g) were macerated with MeOH for 1 week at room temperature. After filtration and evaporation of the MeOH at reduced pressure, the extract was suspended in MeOH/H_2O (9:1 v/v) and successively extracted with *n*-hexane and CH_2Cl_2.

The CH_2Cl_2 extract (6.4 g) was chromatographed over silica gel 60, 70–230 mesh using mixtures (100 mL) of hexane, C_6H_6, EtOAc, Me_2CO, and MeOH of increasing polarity (5%). Fr. EtOAc–C_6H_6 (5:5) was purified by PTLC ($CHCl_3$–MeOH, 10:0.3) to yield galangin 3-OMe ether (**1**, 15 mg) (Mabry et al., 1970), 5,7-dihydroxy-3,6,8-trimethoxyflavone (**2**, 6 mg) (Horie et al., 1995), and 5,7-dihydroxy-3,6-dimethoxyflavone (**3**, 5 mg) (Buschi et al., 1980; Biekofsky et al., 1991). Fr. EtOAc–C_6H_6 (5.5:4.5) was also purified by PTLC ($CHCl_3$–MeOH, 10:0.5) to give calycopterin (5,4′-dihydroxy-3,6,7,8-tetramethoxyflavone, **4**, 6 mg) (Rodriguez et al., 1972), and 5,7,8-trihydroxy-3,6-dimethoxyflavone (**5**, 4 mg). Fr. EtOAc–C_6H_6 (6.5:3.5 and 7:3) were combined and separated by Sephadex LH-20 ($CHCl_3$–MeOH, 5:5), and further purification by PTLC ($CHCl_3$–MeOH, 10:1.0) to afford 5,7,8-trihydroxy-3-methoxyflavone (**6**, 5 mg) (Wagner et al., 1971). Fr. EtOAc–acetone (9.5:0.5) was fractionated by Sephadex LH-20 ($CHCl_3$–MeOH, 5:5) to yield quercetin (**7**, 8.5 mg) (Mabry et al., 1970), and 3-methoxyquercetin (**8**, 4 mg) (Voirin and Jay, 1974).

Compounds **1**–**8** were identified by UV and/or NMR spectroscopy.

The location of the OH group at position C-7 in flavonoids **2** and **3** could not be unambiguously determined by UV spectroscopy, since upon addition of NaOAc only a small bathochromic shift was observed. Consequently, these phenolic compounds were also examined by 2D NMR spectroscopy, especially on the basis of the one-bond and the long-range ^1H, ^{13}C correlations observed in the HSQC and HMBC experiments, respectively.

The ^1H NMR spectrum in $CDCl_3$ of **2** (5,7-dihydroxy-3,6,8-trimethoxyflavone) exhibited signals for three methoxy groups at δ 3.88 (OMe-3), 3.99 (OMe-8) and 4.05 (OMe-6). The HMBC spectrum showed correlations between OH-5 (δ 12.54)/C-5 (δ 147.9), C-6 (δ 130.4), C-10 (δ 104.9), and OH-7 (δ 6.38)/C-6 (δ 130.4), C-7 (δ 148.8), C-8 (δ 127.0) that unambiguously confirmed the structure.

The ^1H NMR spectrum in $CDCl_3$ of **3** (5,7-dihydroxy-3,6-dimethoxyflavone) showed signals for two methoxy groups at δ 3.88 (OMe-3) and 4.05 (OMe-6). In the HMBC spectrum, correlations from OH-5 (δ 12.86) to C-5 (δ 151.4), C-6 (δ 129.8), C-10 (δ 106.2); from OH-7 (δ 6.50) to C-6 (δ 129.8), C-7 (δ 154.8), C-8 (δ 93.1); and from H-8 (δ 6.57) to C-6 (δ 129.8), C-7 (δ 154.8), C-9 (δ 152.3), C-10 (δ 106.2), unambiguously proved the structure.

Compound **5** is reported for the first time and it was identified as 5,7,8-trihydroxy-3,6-dimethoxyflavone by its spectral data: UV λ_{max}^{MEOH} nm (log ε): 283 (3.63), 337 (3.08); $\lambda_{max}^{MEOH+AlCl_3}$ nm: 309, 367; $\lambda_{max}^{MEOH+AlCl_3+HCl}$ nm: 304, 346; $\lambda_{max}^{MEOH+NaOMe}$ nm: decomposes; $\lambda_{max}^{MEOH+NaOAc}$ nm: decomposes; IR ν_{max}^{KBr} cm^{-1}: 3437 (OH), 1637 (C=O), 1446, 1408, 1171, 1078, 1032; EIMS *m/z* (rel. int.): 330 $[M]^+$ (69), 315 $[M-Me]^+$ (100), 198 $[A_1]^+$ (6), 183 $[A_1-Me]^+$ (28), 131 $[B_1-H]^+$ (35), 105 $[B_2]^+$ (50), 77 $[C_6H_5]^+$ (47); HREIMS *m/z* 330.0734 $[M]^+$ (calcd. for $C_{17}H_{14}O_7$ 330.0735); ^1H NMR (acetone-d_6, 500 MHz), δ, multiplicity: 12.32 (1H, s, 5-OH), 8.98 and 7.94 (2-OH, brs, *ortho* position) 8.16–8.14 (2H, m, H-2′ and H-6′), 7.58–7.57 (3H, m, H-3′, H-4′ and H-5′), 3.90 (6H, s, 3-OMe and 6-OMe); ^1H NMR ($CDCl_3$, 200 MHz) 12.25 (1H, s, 5-OH), 8.13–8.08 (2H, m, H-2′ and H-6′), 7.52–7.51 (3H, m, H-3′, H-4′ and H-5′), 3.87 (3H, s, 3-OMe), and 4.07 (3H, s, 6-OMe); ^{13}C NMR from the ^1H,^{13}C correlations in the HSQC and HMBC experiments (acetone-d_6, 500 MHz and 125 MHz), δ: 156.8 (C-2), 146.5 (C-5), 140.0 (C-3), 132.3 (C-6), 131.8 (C-1′), 129.2 (C-2′ and C-6′), 129.2 (C-3′, C-4′ and C-5′), 105.5 (C-10), 60.4 (OMe C-3 and C-6).

Compound **6**, reported several times in the genus *Achyrocline* (Wagner et al., 1971; Norbedo et al., 1984; Broussalis et al., 1988), was identified as 5,7,8-trihydroxy-3-methoxyflavone by NMR spectroscopy. Its ^1H NMR spectrum in acetone-d_6, showed only one methoxy group signal at δ 3.89 (OMe-3), the aromatic protons at δ 6.34 (1H, s, H-6), δ 8.16–8.14 (2H, m, H-2′ and H-6′), and δ 7.58–7.56 (3H, m, H-3′, H-4′ and H-5′), three hydroxyl groups (exchangeable with D_2O), OH-5 at δ 12.12 and δ 8.06 and 9.28 (*ortho* position). Although this compound was reported several times in the literature, we did not find NMR spectral data. The HMBC spectrum strongly support the proposed structure by the correlations from OH-5 to C-5 (δ 154.2), C-6 (δ 98.3), C-10 (δ 104.8), and from the aromatic proton H-6 to C-7 (δ 153.4), C-8 (δ 124.9), C-10 (δ 104.8). The position of the methoxy group was also confirmed by the correlation from δ_H 3.88 (δ_C 59.6) to C-3 (δ 139.5) in the HMBC spectrum.

Table 1
Chemical composition of the essential oil from *A. hyperchlora*

	RI[a]	%
Isopropyl tiglate	973	0.40
1-Octen-3-ol (isomer)	973	1.60
1-Octen-3-ol	978	0.35
6-Methyl-5-hepten-2-one	985	7.25
3-*E*-hexenyl acetate	1007	0.20
1,8-Cineole	1033	4.85
2-Phenylacetaldehyde	1043	1.50
Dihydrotagetone	1054	1.35
cis-Linalool oxide	1088	1,40
Linalool	1098	0.50
Nonanal	1098	1.05
trans-Thujone	1114	1.40
cis-Limonene oxide	1134	0.85
cis-Verbenol	1144	0.25
trans-Pinocamphone	1160	0.50
Borneol	1165	0.85
neo-Menthol	1165	1.60
Terpinen-4-ol	1177	2.50
α-Terpineol	1189	0.35
Neral	1240	0.20
Butyrophenone	1251	8.65
Isobornyl acetate	1285	0.30
Eugenol	1350	4.25
α-Copaene	1376	0.15
(*E*)-caryophyllene	1418	1.55
Aromadendrene	1439	0.55
cis-β-Farnesene	1443	1.60
Drima-7,9(11)-diene	1469	0.15
Viridiflorene	1491	1.25
α-Selinene	1494	6.45
β-Bisabolene	1509	0.35
γ-Cadinene	1513	0.35
trans-Cadina-1(2),4-diene	1532	1.05
Spathulenol	1576	1.70
β-Copaen-4-α-ol	1584	0.90
cis-Isolongifalanone	1606	0.45
γ-Eudesmol	1630	4.60
Cubenol	1642	2.90
α-Eudesmol	1652	4.60
Acorenone	1685	0.60
Nootkatone	1800	3.55
Phytol	1949	1.05
Monoterpene hydrocarbons		1.05
Oxygenated monterpenes		17.45
Sesquiterpene hydrocarbons		17.30
Oxygenated sesquiterpenes		11.95
Others		22.50
Total identified compounds		70.25

Compounds are listed in order of elution on HP5-capillary column.
[a] GC retention indices relative to a homologous series of *n*-alkanes.

3.2. Extraction of the essential oil

The volatile oil was extracted from 300 g of aerial fresh flowering parts. The extraction was carried out by steam distillation in a Clevenger-type apparatus for 3 h for a yield of 0.09% v/w.

The gas chromatographic analysis of the essential oil was carried out in a KONIK KNK 3000 G GC/FID instrument equipped with a flame ionization detector and in a Hewlett Packard Gas Chromatograph 6890 linked to Hewlett Packard 5972 mass spectrometer system. The GC/FID instrument was equipped with an HP5 capillary column (30 m × 0.25 mm, film thickness 0.25 μm). The column temperature program was 60 °C for 5 min and then heated to 230 °C at a rate of 3 °C/min. The carrier gas was hydrogen at a flow-rate of 1 mL/min. The GC/MS instrument was equipped with an HP-5/MS (5% phenyl) capillary column (30 m × 0.25 mm, film thickness 0.25 μm) and it was operated in EI mode. The column temperature program was the same as for the GC/FID instrument. Helium was used as a carrier gas.

The GC/MS analysis was scanned over the 40–400 *m/z* range with an ionizing voltage of 70 eV.

The identification of the volatile constituents was determined by a combination of mass spectrometry and Kovats retention indices using co-chromatographed standard hydrocarbons. The retention indices (relative to C7-C24 *n*-alkanes), the mass spectra, and the retention times of each individual compound were compared with those of authentic samples and with data available in the literature (Adams, 1995). The chemical composition of the essential oil is shown in Table 1.

4. Chemotaxonomic significance

Flavonoids, especially lacking substitution at the B ring, have been shown to be characteristic of the tribe Inuleae, distinguishing members from other Asteraceae tribes. The chemical composition of *A. hyperchlora* is in agreement with that of other members of the genus *Achyrocline* regarding to the presence of flavonoids, especially those lacking substitution on the B ring (Ferraro et al., 1981, 1985; Norbedo et al., 1982, 1984; Schmeda Hirschmann, 1984; Bauer et al., 1987; Martino et al., 1988; Broussalis et al., 1988).

The essential oil from *A. hyperchlora* (0.09% mL/100 g) showed some important differences in relation to other species of *Achyrocline* such as *A. satureoides*, *A. alata*, and *A. tomentosa*. Labuckas et al. (1999) described (*E*)-caryophyllene as the major compound (39–48%) in this species. However, in *A. hyperchlora* we found that (*E*)-caryophyllene was present in small concentration (1.55%), being the ketones butyrophenone (8.65%) and 6-methyl-5-hepten-2-one (7.25%) the main constituents (Table 1).

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