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CONSTITUENTS OF *Helichrysum stoechas* variety *olonnense*

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The isolation of 18 phenolic compounds (α-pyrones, phloroglucinols, phenolic acids, flavonoids, and coumarin) from the aerial parts of Helichrysum stoechas *var.* olonnense *is reported.*

Key words: *Helichrysum stoechas* var. *olonnense*, *Asteraceae*, phenolic compounds, phloroglucinols, flavonol glycosides.

Helichrysum stoechas variety *olonnense* Jordan and Fourreau (*Asteraceae*) is endemic to the french Atlantic coast, where it is commonly known as "Immortelle des Sables d'Olonne" [1]. To our knowledge, there is no previous report on the phytochemical or biological properties of this plant.

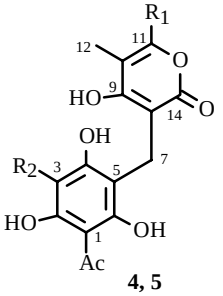
Nonetheless, *Helichrysum* species growing in various Mediterranean areas as *H. italica*, *H. stoechas*, *H. picardii*, *H. arenaria*, or *H. decumbens* are known to contain essentials oils, sterols, a-pyrones, acetophenones, phloroglucinols, or flavonoids.

The species *H. stoechas* is used as a diuretic, digestive and, expectorant [2]. The essential oil is used in perfumery [3].

The present study is a complete investigation of the phenols of *H. stoechas* variety *olonnense*.

The aerial parts of *Helichrysum stoechas* variety *olonnense* were collected in the dunes of the domain forest of Olonne in the vicinity of Les Sables d'Olonne, Vendée, France, on July 2000.

A voucher specimen (LAV 001) has been deposited in the herbarium of the Musee Botanique de la ville d'Angers (ANG), France.



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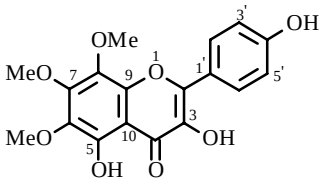
4: R_1 = ethyl, R_2 = geranyl;
5: R_1 = methyl, R_2 = geranyl



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The dried and powdered capitula (400.0 g) were successively percolated at room temperature with *n*-hexane, $CHCl_3$, EtOAc, and MeOH. The hexane extract (10.5 g) was fractionated by successive chromatography on silica gel G columns with *n*-hexane or petroleum ether with increasing amounts of EtOAc as the eluent. Six compounds (**1–6**) were isolated in this manner and purified by preparative TLC (silica gel, $CHCl_3$–MeOH 99:1). The $CHCl_3$ extract (3.0 g) was chromatographed on silica gel with a mixture of hexane–EtOAc–MeOH of increasing polarity to give coumpounds (**6–9**). The MeOH residue was solubilized in H_2O–MeOH 80:20 and successively extracted by EtOAC and *n*-butanol. The EtOAc extract (0.8 g) and the butanol extract (26 g) were fractionated by chromatography on Sephadex LH-20 with MeOH–$CHCl_3$ 30:70 and then repeated

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chromatographies on silica gel G columns using EtOAc with increasing amounts of MeOH as the eluent. The fractions were finally purified by preparative TLC on silica using different solvent systems: EtOAc–butanone–formyl acid–H_2O (50:50:0.5:1); EtOAc–formyl acid–H_2O (88:6:6) or EtOAc–MeOH–formyl acid–H_2O (90:10:0.5:1) to give four flavonoids (**10–13**), four phenolic acids (**14–17**), and one coumarin (**18**).

The air dried and powdered stems and leaves (800 g) were extracted and chromatographed as described above. From the hexane extract (25 g) and the $CHCl_3$ extract (9 g), were identified six compounds (**1–6**) The EtOAC residue (9 g) and the butanol one (8.7 g) finally yielded three coumpounds (**15–17**).

EXPERIMENTAL

Compound 1, white powder, $C_{17}H_{20}O_6$, Mass spectrum (EI, 70 eV) *m/z*: 320 $[M]^+$, 291, 207, ^{1}H-NMR (270 ^{1}H-NMR (270 MHz, $CDCl_3$, δ, ppm, J/Hz): 11.20 (OH), 3.54 (2H, s, H-7), 2.57 (4H, t, J = 7, CH_2-CH_3), 1.96 (6H, s, CH_3), 1.20 (6H, t, J = 7, CH_2-CH_3). Identified as helipyrone [4].

Compound 2, white powder, $C_{15}H_{16}O_6$, ^{1}H-NMR (270 MHz, $CDCl_3$, δ, ppm, J/Hz): 11.16 (OH), 3.54 (2H, s H-7), 2.25 (6H, s, CH_3), 1.95 (6H, s, CH_3). Identified as bisnorhelipyrone [4].

Compound 3, white powder, $C_{16}H_{18}O_6$, ^{1}H-NMR (270 MHz, $CDCl_3$, δ, ppm, J/Hz): 11.20 (OH), 3.54 (2H, s H-7), 2.58 (2H, t, J = 7.6, CH_2-CH_3), 2.24 (3H, s, CH_3), 1.95 (6H, s, CH_3), 1.25 (3H, t, J = 7.4, CH_2-CH_3). Identified as norhelypyrone [4].

Compound 4, gum, $C_{27}H_{34}O_7$, Mass spectrum (EI, 70 eV) *m/z* 470 $[M]^+$, 347, 304; ^{1}H-NMR (270 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.15 (1H, t, J = 7, H-16), 5.13 (1H, t, J = 7, H-20), 3.61 (2H, s, H-7), 3.44 (2H, d, J = 7, H-15), 2.67 (3H, s, 25-CH_3), 2.56 (2H, d, J = 7.3, H-13a, CH_2-CH_3), 2.28 (2H, d, J = 7, H-18), 2.18 (2H, d, J = 7, H-19), 1.93 (3H, s, 12-CH_3), 1.79 (3H, s, 22-CH_3), 1.71 (3H, s, 23-CH_3), 1.64 (3H, s, 24-CH_3), 1.18 (3H, t, J = 7.5, H-13, CH_2-CH_3), $_{13}$C-NMR (75 MHz, $CDCl_3$, δ, ppm): 105.6 (C-1), 161.1 (C-2), 106.5 (C-3), 161.2 (C-4), 109.0 (C-5), 161.1 (C-6), 17.8 (C-7), 108.3 (C-8), 159.7 (C-9), 102.0 (C-10), 160.1 (C-11), 9.4 (C-12), 24.7 (C-13) 11.5 (C-13a), 169.3 (C-14), 21.7 (C-15), 123.4 (C-16), 132.9 (C-17), 32.0 (C-18), 25.8 (C-19), 122.3 (C-20), 140.3 (C-21), 17.6 (C-22), 23.4 (C-23), 25.7 (C-24), 32.4 (C-25), 204.2 (C=O). Identified as phloroglucinol derivative already described in *H. decumbens* [5].

Compound 5 gum, $C_{26}H_{32}O_7$, Mass spectrum (EI, 70 eV) *m/z*: 456 $[M]^+$, 315, 304, ^{1}H-NMR (270 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.14 (1H, t, J = 7, H-16), 5.13 (1H, t, J = 7, H-20), 3.61 (2H, s, H-7), 3.44 (2H, d, J = 7, H-15), 2.66 (3H, s, 25-CH_3), 2.26 (2H, d, J = 6.8, H-18), 2.23 (3H, s, 13-CH_3), 2.18 (2H, d, J = 7, H-19), 1.94 (3H, s, 12-CH_3), 1.78 (3H, s, 22-CH_3), 1.71 (3H, s, 23-CH_3), 1.63 (3H, s, 24-CH_3). Identified as phloroglucinol derivative isolated also from *H. decumbens* [5].

Compound 6 yellow crystals, mp 158°C, $C_{18}H_{16}O_7$, UV spectrum (MeOH, λ_{max}, nm): 276, 324, 368, ^{1}H-NMR (270 MHz, $CDCl_3$, δ, ppm, J/Hz): 11.46 (OH), 8.27 (2H, m, H-2′, H-6′), 7.53 (3H, m, H-3, H-4′, H-5′), 4.13, 3.97, 3.95 (9H, 3 s attributed respective to 7-OMe, 8-OMe, 6-OMe). Identified as 3,5-dihydroxy 6,7,8-trimethoxyflavone [6].

Compound 7, amorphous powder, $C_{18}H_{16}O_8$, Mass spectrum (EI 70 eV) *m/z:* 360 $[M]^+$, 345, 330, 317, UV spectrum (MeOH, λ_{max}, nm): 276, 344, 382, ^{1}H-NMR (270 MHz, $CDCl_3$, δ, ppm, J/Hz): 11.52 (s br, OH), 8.20 (2H, d, J = 8 Hz, H-2′, H-6′), 7.01 (2H, d, J = 8 Hz, H-3′, H-5′), 6.63 (s br, OH), 4.12 (3H, s, H-7), 3.98 (3H, s, H-8), 3.96 (3H, s, H-6). ^{13}C-NMR (75 MHz, $CDCl_3$, δ, ppm): 146.8 (C-2), 134.4 (C-3), 174.7 (C=O), 146.8 (C-5), 134.4 (C-6), 152.0 (C-7), 132.4 (C-8), 144.0 (C-9), 105.0 (C-10), 122.4 (C-1′), 128.7 (C-2′), 114.7 (C-3′), 156.6 (C-4′), 128.7 (C-5′), 114.7 (C-6′), 61.8 (C-8), 61.5 (C-6), 60.6 (C-7). Identified as 3,5,4′-trihydroxy-6,7,8-trimethoxyflavone [7].

Compound 8, gum, $C_8H_8O_2$, ^{1}H-NMR (270 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.91 (2H, dd, J = 8.8, 2.4, H-2, H-6), 6.88 (2H, dd, J = 9.7, 2.2, H-3, H-5), 2.47 (3H, s, CH_3). Identified as 4-hydroxyacetophenone.

Compound 9, ^{13}C-NMR (75 MHz, $CDCl_3$, δ, ppm, J/Hz): 199.2 (C=O), 162.8 (C-4), 132.0 (C-1), 131.3 (C-2, C-6), 116.9 (C-3, C-5), 101.3 (C-1′), 77.9 (C-5′), 77.6 (C-3′), 74.5 (C-4′), 70.9 (C-4′), 62.1(C-6′) [8]. Identified as picein (glycoside of 4-hydroxyacetophenone).

Compound 10, $C_{15}H_{10}O_7$, Mass spectrum (EI 70 eV) *m/z:* 302 $[M]^+$, 271, 248, UV spectrum (MeOH, λ_{max}, nm): 255, 293, 368. Characterized as quercetin [9].

Compound 11, $C_{21}H_{19}O_{12}$, Mass spectrum ESI- *m/z:* 463 [M-H], 927 $[M-H]^-$, UV spectrum (MeOH, λ_{max}, nm): 260, 356, ^{1}H-NMR (270 MHz, $C_{25}H_{24}O_{12}$, δ, ppm, J/Hz): 7.79 (1H, d, J = 2.1, H-2′), 7.67 (1H, dd, J = 8.5, 2.1, H-6′), 6.94 (1H, d, J = 8.4, H-5′), 6.46 (1H, d, J = 2.1, H-8), 6.22 (1H, d, J = 2.1, H-6), 5.31 (1H, d, J = 7.5, H-1″glu), 3.67–3.78 (2H, m, H-6″glu), 3.37–3.57 (4H, m, H-2″, H-3″, H-4″, H-5″glu). Identified as isoquercitrin (quercetin-3-O-β-D-glucoside) [9, 10].

Compound 12, $C_{30}H_{26}O_{14}$, Mass spectrum ESI- *m/z:* 609 [M-H]$^-$, 463, 301, UV spectrum (MeOH, λ_{max}, nm): 260, 315, 360, ^{1}H-NMR (270 MHz, CD_3OD, δ, ppm, J/Hz): 7.68 (1H, d, J = 1.7, H-2′), 7.66 (1H, dd, J = 8.3, 2, H-6′), 6.90 (1H, d, J = 8.4, H-5′), 6.38 (1H, d, J = 1.9, H-8), 6.21 (1H, d, J = 1.9, H-6), 5.32 (1H, d, J = 7, H-1″glu), 4.29 (1H, m, H-6a″ glu), 4.37 (1H, m, H-6b″ glu); 3.3.38–3.56 (4H, m, H-2″, H-3″, H-4″, H-5″ glu), 7.48 (1H, d, J = 15.7, H-7‴), 7.40 (2H, d, J = 8.5, H-6‴, H-2‴), 6.87 (2H, d, J = 8.8, H-5‴, H-3‴), 6.16 (1H, d, J = 15.7, H-8‴). ^{13}C-NMR (75 MHz, CD_3OD, δ, ppm): 158.8 (C-2), 135.5 (C-3), 179.6 (C=O), 163.2 (C-5), 100.6 (C-6), 167.3 (C-7), 95.3 (C-8), 158.8 (C-9), 105.6 (C-10), 123.4 (C-1′), 115.0 (C-2′), 146.2 (C-3′), 146.9 (C-4′), 116.2 (C-5′), 123.4 (C-6′), 104.2 (C-1″), 76.1 (C-2″), 78.3 (C-3″), 72.0 (C-4″), 75.9 (C-5″), 64.6 (C-6″), 131.5 (C-2‴), 117.6 (C-3‴), 161.5 (C-4‴), 131.6 (C-5‴), 117.6 (C-6‴), 140.2 (C-7‴), 115.0 (C-8‴), 169.3 (C=O). Identified as quercetin 3-O-[6-*trans*-*p*-coumaroyl]-*β*-D-glucoside-helichrysoside [11].

Compound 13, yellow crystals, $C_{21}H_{19}O_{12}$, mp 231°C, Mass spectrum ESI- *m/z:* 479 [M-H]$^-$, 959 [2M-H]$^-$, UV spectrum (MeOH, λ_{max}, nm): 259, 273, 361, ^{1}H-NMR (270 MHz, $C_{25}H_{24}O_{12}$, DMSO-d_6, δ, ppm, J/Hz): 9.37 (OH), 7.69 (1H, d, J = 2.1, H-2′), 7.53 (1H, d d, J = 8.5, 2.1, H-6′), 6.91 (1H, s, H-8), 6.89 (1H, d, J = 8.5, H-5′), 5.01 (1H, s, H-1″glu), 3.16–3.84 (unresolved, m, others sugar protons), ^{13}C-NMR (75 MHz, DMSO-d_6, δ, ppm): 147.7 (C-2), 135.8 (C-3), 176.3 (C=O), 145.5 (C-5), 129.8 (C-6), 151.7 (C-7), 93.7 (C-8), 148.3 (C-9), 105.3 (C-10), 122.2 (C-1′), 115.6 (C-2¢), 145.2 (C-3′), 147.9 (C-4′), 115.7 (C-5′), 120.1 (C-6′), 101.1 (C-1″), 73.3 (C-2″), 75.9 (C-3″), 70.8 (C-4″), 77.8 (C-5″), 60.8 (C-6″). Identified as quercetagetin 7-O-*β*-D-glucoside [12].

Compoumd 14, ^{1}H-NMR (270 MHz, CD_3OD, δ, ppm, J/Hz): 7.58 (1H, d, J = 16, H-7), 7.15 (1H, s, H-2), 7 (1H, dd, J = 8.3, 2, H-6), 6.86 (1H, d, J = 8.3, H-5), 6.30 (1H, d, J = 16, H-8). Identical to caffeic acid [13].

Compound 15, $[\alpha]_D$ – 44 (c 1.4, MeOH), ^{1}H-NMR (270 MHz, CD_3OD, δ, ppm, J/Hz): 7.69 (1H, d, J = 16.2, H-7′), 7.15 (2H, d, J = 2, H-2′), 7.02 (2H, dd, J = 8.3, H-6′), 6.86 (2H, d, J = 8, H-5′), 6.43 (2H, d, J = 15, H-8′), 5.46 (1H, m, H-5), 4.27 (1H, m, H-3), 3.81 (1H, d, J = 8, H-4). Identified as chlorogenic acid (5-caffeoyl quinic acid) [14].

Compound 16, $C_{25}H_{24}O_{12}$, $[\alpha]_D$ –171 (c 1.4, MeOH), Mass spectrum ESI- *m/z:* 515 [M-H]$^-$, ^{1}H-NMR (270 MHz, CD_3OD, δ, ppm, J/Hz): 7.63 (2H, d, J = 16, H-7′, H-7″), 7.11 (2H, d, J = 2, H-2′, H-2″), 7.01 (2H, dd, J = 8, 2, H-6′, H-6″), 6.82 (2H, d, J = 8.3, H-5′, H-5″), 6.39 (1H, d, J = 16, H-8′), 6.35(1H, d, J = 16, H-8″), 5.47 (1H, m, H-5), 5.44 (1H, m, H-3), 4.02 (1H, dd, J = 6.6, 3, H-4). Identified as 3,5-dicaffeoyl quinic acid [14] accompanied by its methyl ether derivative (compound **17**) $C_{26}H_{26}O_{12}$. Mass spectrum ESI- *m/z:* 529 [M-H] [15].

Compound 18, ^{1}H-NMR (270 MHz, CD_3OD, δ, ppm, J/Hz): 7.94 (1H, d, J = 9.6, H-4), 7.19 (1H, s, H-8), 6.82 (1H, s, H-5), 6.28 (1H, d, J = 9.5, H-3), 3.99 (3H, s, 6-OCH_3). Identical to scopoletin [16].

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REFERENCES

1. G. Bonnier, *Flore complete de France*, Suisse et Belgique, Paris (1934).
2. P. Font Quer, *Les plantes medicinales*. El Dioscorides renovado, 8 edn, Ed. Labor, Barcelona, 783 (1983).
3. L. Peyron and M. Roubaud, *Parf. Cosm. Savons de France*, **1**, 3, 129 (1971).
4. J. Vrkoc, L. Dolejs, and M. Budesinsky, *Phytochemistry*, **14**, 1384 (1975).
5. F. Tomas-Lorente, E. Iniesta-Sanmartin, and F. Tomas-Barberan, *Phytochemistry*, **28**, 6, 1613 (1989).
6. H. A. Candy, W. Wright, and J. Van Die S-Afr., *Chem. Inst*., **XXVIII**, 215 (1975).
7. E. Wollenweber and J. N. Roitman, *Z. Naturforsh*, **46c**, 325 (1991).
8. P. Junior, *Planta Medica*, **3**, 218 (1986).
9. J. Mendez, A. R. Bilia, and I. Morelli, *Pharm. Acta Helv*., **70**, 223 (1995).
10. A. M. Malik, M. P. Yuldashev, A. Obid, T. Ismoil, and L. Ya. Ping, *Chem. Nat. Comp*., **38**, 6, 612 (2002).
11. J-T. Cheng, F-L. Hsu, and H-F. Chen, *Planta Medica*, **59**, 405 (1993).
12. J. Heilmann, E. Muller, and I. Merfort, *Phytochemistry*, **51**, 713 (1999).

13. G. Flaminii, E. Antognoli, and I. Morelli, *Phytochemistry*, **57**, 559 (2001).
14. A. Cheminat, R. Zawatzky, H. Becker, and R. Brouillard, *Phytochemistry*, **27**, 9, 2787 (1988).
15. B. Timmermann, J. Hoffmann, S. Jolad, K. Scham, R. Klenov, and R. Bates, *J. Nat. Prod*., **46**, 3, 365 (1983).
16. J. Vasconcelos, M. Silva, and A. Cavaleiro, *Phytochemistry*, **49**, 5, 1421 (1998).