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Chemical Constituents of *Helichrysum conglobatum* Growing in Egypt

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Chemical Constituents of Egypyian Helichrysum conglobatum

Abstract – Five aromatic compounds, of which two are new glucosides, and six flavonols were isolated and identified for the first time from the flower heads and aerial shoots of *Helichrysum conglobatum* (Asteraceae). Their structures were established on the basis of chemical and spectroscopic methods including UV, MS, 1D- and 2D-NMR. Some fractions and isolates were screened for anti-microbial activities. This is the first report of the isolation of the chemical constituents of this species.
Keywords – *Helichrysum conglobatum*, Asteraceae, flower heads, aerial shoots, aromatic compounds, flavonols, glucosides

Introduction

The genus *Helichrysum* (Asteraceae) is represented in the Egyptian flora by three species, including *H. conglobatum* (VW.) Steud (Boulos, 1995; Täckholm, 1974). Many species of the genus are known for their anti-microbial, anti-inflammatory, and anti-allergic properties. They are also used in migrain, eye therapy, rheumatism, gout, female sterility, and menstrual pain (Cubukcu and Yuksel, 1982; Manitto *et al.*, 1972; Optiz *et al.*, 1971; Van Puyvelde *et al.*, 1989; Watt and Breyer-Brandwijk, 1962). Many of these uses were proved clinically. Antioxidant (Carini *et al.*, 2001; Czinner *et al.*, 1999, 2000, 2001; Skakun and Stepanova, 1988; Terasawa *et al.*, 2001) and cytostatic (De la Puerta *et al.*, 1993) activities were also ascertained.

In this paper we report, for the first time, the isolation and identification of two new aromatic glucosides namely; 2-hydroxy-5-methoxyphenyl-1-*O*-glucoside (**6**) and anisyl alcohol diglucoside (**10**), three known aromatic compounds, six known flavonols, lupeol, β-sitosterol, ursolic acid, β-sitosterol glucoside, and D-glucose. Furthermore, the anti-microbial activities of some fractions and isolates were assessed.

Experimental

Helichrysum conglobatum used in this study was purchased from the local market of Marsa Matrouh, Egypt. The identity of the plant was kindly verified by Prof. Dr. Nabil El-Hadidy, Professor of Plant Taxonomy, Department of Botany, Faculty of Science, Cairo University, Cairo. It was also confirmed by the Royal Botanic Gardens, Kew, Richmond, U.K. A voucher specimen is kept in the Department of Pharmacognosy (AA1), Faculty of Pharmacy, University of Alexandria, Alexandria, Egypt.

General. Mp: uncorrected; ^{1}H- and ^{13}C-NMR spectra: 500, 125 and 300, 75 MHz, samples were dissolved in CD_3OD, DMSO-d_6 and pyridine-d_5. FAB-MS: samples were dissolved in a glycerol matrix and placed on a steel target prior to bombardment with Ar atoms at energy 7-8 kV. TLC and pTLC were carried out on pre-coated plates (silica gel 60F-245, Merck); detection of the spots was achieved by UV lamp before and after exposure to NH_3 and by spraying with anisaldehyde/H_2SO_4 or vanillin/H_2SO_4 followed by heating at 105°C for 5 min. Reference samples were supplied through Pharmacognosy Department, Faculty of Pharmacy, and Organic Chemistry Department, Faculty of Science, University of Alexandria, Egypt.

Extraction and isolation. Air-dried flower heads (0.5 kg) and powdered shoots (1.5 kg) of *H. conglobatum* were exhaustively extracted with 95% EtOH at room temperature. EtOH extracts were concentrated *in vacuo* and the resulting residues were separately partitioned between CH_2Cl_2 and water. Aqueous fractions were extracted successively with EtOAc and *n*-BuOH, while CH_2Cl_2 fractions were concentrated *in vacuo* and partitioned between petroleum ether and 90% CH_3OH. Chromatographically similar fractions were combined together.

Combined petroleum ether extracts were subjected to

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CC and afforded lupeol (450 mg) and β-sitosterol (330 mg). Similarly, the combined 90% MeOH extracts gave ursolic acid (5.25 g) and β-sitosterol glucoside (475 mg).

Combined EtOAc extracts (5.6 g) were subjected to silica gel CC. Elution was started with EtOAc then EtOAc-MeOH mixtures. Fractions 1-4 (100% EtOAc) afforded compound **1**. Fractions 5-8 (100%) were subjected to pTLC, developing solvent, ($CHCl_3$-MeOH-EtOAc) 8:2:1, to give compound **2**. Fractions collected at 2% MeOH yielded compound **4**, while the mother liquor was subjected to pTLC, for double development ($CHCl_3$-MeOH, 8.5:1.5) to give compound **3**. Fractions collected at 7% MeOH were combined and yielded compound **7**, while the mother liquor was subjected to pTLC, developing solvent $CHCl_3$-MeOH-EtOAc, 8:2:1 to give compounds **5** and **6**.

Upon standing, *n*-BuOH extract of flower heads (3.5 g) afforded dark yellow crystals designated as compound **8**. The mother liquor was filtered and subjected to silica gel CC. Elution was started with EtOAc then EtOAc-MeOH mixtures. Fractions collected at 5% MeOH were subjected to pTLC ($CHCl_3$-MeOH-EtOAc, 8:2:1) and afforded compounds **9** and **10**. Fractions collected at 20% MeOH yielded D-glucose (45 mg), mp 155-157°C. The identity of D-glucose was confirmed by comparison with authentic sample and by performing glucose GOD-PAP test (LabKit) (Dingeon, 1975; Lott, 1975).

n-BuOH extract of shoots (4 g) was subjected to silica gel CC. Elution was started with EtOAc then EtOAc-MeOH mixtures. Fractions collected at 5% MeOH yielded further amounts of compounds **9** (19 mg) and **10** (24 mg). Fractions collected at 10% MeOH were subjected to PTLC ($CHCl_3$-MeOH, 8:2) to give compound **11**.

Compound **1** (110 mg): yellow crystals (MeOH), mp 298-300°C, R_f 0.52 ($CHCl_3$-MeOH, 9:1). UV λ_{max} nm (MeOH): 372.5, 254.3, (+NaOMe): 426.2, 324.7, 275.3, (+$AlCl_3$): 432.8, 267.5, (+$AlCl_3$+HCl): 418.2, 346.7, 262.7, (+NaOAc): 390.8, 271.2. EI-MS *m/z* (% rel. int.): 302 (M^+, $C_{15}H_{10}O_7$, 100); 301 (14.4); 285 (8.4); 284 (10.8); 273 (14.1); 256 (14.1); 222 (8.7); 209 (14.3); 193 (14.1); 178 (10.9); 154 (25.1); 153 (A_1^++1, 19.7); 137 (B_2^+, 35.7), 126 (35.6). ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm: 6.33 (1H, d, *J* = 1.9Hz, H-6), 6.55(1H, d, *J* = 1.9Hz), 7.83(1H, d, *J* = 2.1Hz, H-2), 7.03(1H, d, *J* = 8.4Hz, H-5'), 7.69(1H, dd, *J* = 2.1Hz, H-6'). ^{13}C-NMR (75 MHz, DMSO-d_6) δ ppm: 147.1 (C-2), 136.1 (C-3), 176.2 (C-4), 161.1 (C-5), 98.6 (C-6), 164.0 (C-7), 93.7 (C-8), 156.5 (C-9), 103.3 (C-10), 122.3 (C-1'), 115.4 (C-2'), 145.4 (C-3'), 148.1 (C-4'), 115.9 (C-5'), 120.3 (C-6').

Compound **2** (25 mg): pale yellow deposit, mp 132-134°C, R_f 0.27 ($CHCl_3$-MeOH, 8:2). UV λ_{max} nm (MeOH): 340 (sh.), 290, 257.5, (+$AlCl_3$): 321.5 (sh.), 288, (+$AlCl_3$+HCl): 340 (sh.), 291, 258. EI-MS *m/z* (% rel. int.): 182 (M^+, $C_9H_{10}O_4$, 10); 155 (12.2); 153 (M^+-C_2H_5, 14); 138 (M^+-CO_2, 14.3); 137 (M^+-OC_2H_5, 9.6); 112 (18.5); 111 ($[C_6H_7O_2]^+$, 26.6); 110 ($[C_6H_6O_2]^+$, 9.9); 109 ($[C_6H_5O_2]^+$, 14.9); 84 (100). ^{1}H-NMR data (300 MHz, DMSO-d_6) δ ppm: 7.25 (1H, d, *J* = 1.8Hz, H-2), 6.67(1H, d, *J* = 8.1Hz, H-5), 7.19 (1H,dd, *J* = 1.9, 8.1Hz, H-6), 3.74(1H, *J* = 6.9Hz, OCH_2), 1.11 (3H,t, *J* = 6.9Hz, CH_3), ^{13}C-NMR data (75 MHz, DMSO-d_6) δ ppm: 13.0(C-1), 117.1(C-2), 144.1(C-3), 147.3(C-4), 114.5 (C-5), 121.5(C-6), 171.4(C=O), 69.4(OCH_2-), 20.8(CH_3).

Compound **3** (8 mg): yellow crystals (MeOH), mp 194-196°C, R_f 0.6 (EtOAc-MeOH-water, 30:5:4). UV λ_{max} nm (MeOH): 368.9, 274.4, (+NaOMe): 414.2, 276, (+$AlCl_3$): 417.7, 275.9, (+$AlCl_3$+HCl): 417.8, 351.2, 276.1, (+NaOAc): 385.1, 274.7. EI-MS *m/z* (% rel. int.): 448 (M^+, $C_{21}H_{20}O_{11}$, 10.7); 287 ($[C_{15}H_{11}O_6]^+$, 11.6); 285 (15.2); 223 (10.7); 221 (12.5); 211 (10.7); 180 (12.5); 155 (11.6); 152 (A_1^+, 11.6); 129 (19.6); 123 (13.4); 121 (B_2^+, 13.4); 116 (19.6); 83(100). ^{1}H-NMR (300 MHz, CD_3OD) δ ppm: 6.14(1H, d, *J* = 2.1Hz, H-6), 6.32(1H, d, *J* = 2.1Hz, H-8), 8.04(2H, d, *J* = 9.0Hz, H-6', H-2'), 6.87(2H, d, *J* = 9.0Hz, H-3', H-4'), 5.15(1H, d, *J* = 7.4, H-1").

Compound **4** (300 mg): yellow crystals (MeOH), mp 223-225°C, R_f 0.58 (EtOAc-MeOH-water, 30:5:4). UV λ_{max} nm (MeOH): 369.5, 270 (sh.), 252, (+NaOMe): 418.5, 326, 277, (+$AlCl_3$): 427, 352.5, 263.5, (+$AlCl_3$+HCl): 427.5, 354.5, 261, (+NaOAc): 383, 312.5, 272. FAB-MS *m/z* (% rel. int.): 463 ([M-1]$^+$, $C_{21}H_{20}O_{12}$, 9.7); 459 (8.5); 367 (14.7); 301 ($[C_{15}H_9O_7]^+$, 7.9); 276 (10.6); 275 (100); 273 (16.5); 257 (11.2); 243 (8.8). ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm: 6.33(1H, d, *J* = 2.0Hz,H-6), 6.63(1H, d, *J* = 2.0Hz, H-8), 8.12(1H, d, *J* = 2.0Hz, H-2'), 7.11(1H, d, *J* = 8.6Hz, H-5'), 8.01(1H, dd, *J* = 2.0, 8.6Hz, H-6), 4.92(1H,d, *J* = 7.0Hz, H-1). ^{13}C-NMR (125 MHz, pyridine-d_6) δ ppm: 146.0 (C-2), 136.2 (C-3), 176.3 (C-4), 161.4 (C-5), 98.2 (C-6), 165.1 (C-7), 93.5 (C-8), 157.0 (C-9), 103.1 (C-10), 122.8 (C-1'), 116.9 (C-2'), 146.5 (C-3'), 150.0 (C-4'), 116.0 (C-5'), 123.9 (C-6'), 103.4 (C-1"), 74.1 (C-2"), 76.2 (C-3"), 70.2 (C-4"), 77.0 (C-5"), 61.9 (C-6").

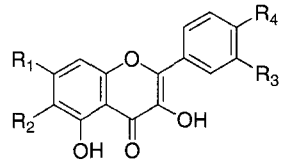
Compound **5** (6 mg): yellow crystals (MeOH), mp 214-216°C, R_f 0.49 ($CHCl_3$-MeOH-EtOAc, 8:2:1). UV λ_{max} nm (MeOH): 363.8, 293.3 (sh.), 266.4, (+NaOMe): 414.3, 312.9, 286.2, (+$AlCl_3$): 415 (sh.), 367.8, 269.7, (+$AlCl_3$+HCl): 415.1 (sh.), 369.9, 271.2, (+NaOAc): 377.2, 295.1 (sh.), 268.2. EI-MS *m/z* (% rel. int.): 478 (M^+, $C_{22}H_{22}O_{12}$, 6.2); 315 ($[C_{16}H_{11}O_7]^+$, 7.8); 268 (8.9); 213 (13.7); 196 (11.5); 152 (A_1^+, 22.9); 151 (B_2^+, 15.6); 138 (32.5); 113 (41.8). ^{1}H-NMR (300 MHz, CD_3OD) δ ppm: 5.89(1H, d, *J* = 1.9Hz,H-6), 6.02(1H, d, *J* = 1.9Hz, H-8), 7.75(1H, d, *J* =

2.1Hz, H-2"), 6.74(1H, d, J = 8.5Hz, H-5'), 7.49(1H, dd, J = 2.1, 8.5Hz, H-6'), 3.82(3H, s, OCH$_3$-3), 5.06(1H, d, J = 7.4Hz, H-1"). ^{13}C-NMR (75 MHz, CD_3OD) δ ppm: 97.2 (C-6), 92.6 (C-8), 54.8 (OCH$_3$-3') .112.1 (C-2'), 114.8 (C-5'), 122.2 (C-6'), 101.2 (C-1"), 74.1 (C-2"), 75.9 (C-3"), 69. 6(C-4"), 76.6 (C-5"), 60.9 (C-6"). Quaternary carbons were not detected.

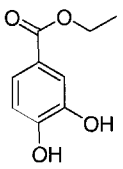
Compound **6** (9 mg): pale yellow sticky deposit, R_f 0.47 ($CHCl_3$-MeOH-EtOAc, 8:2:1). UV λ_{max} nm (MeOH): 270.7, 222.8 (sh.), 207.8. EI-MS *m/z* (% rel. int.): 302 (M^+, $C_{13}H_{18}O_8$, 16.7); 301 (14.8); 180 ($[C_6H_{12}O_6]^+$, 22.2); 163 ($[C_6H_{11}O_5]^+$, 14.8); 141 ($[C_7H_9O_3]^+$, 45.4); 127 ($[C_6H_7O_3]^+$, 16.7); 126 ($[C_6H_6O_3]^+$, 14.8); 125 ($[C_7H_9O_2]^+$, 16.7); 124 ($[C_7H_8O_2]^+$, 17.6); 123 ($[C_7H_7O_2]^+$, 27.8); 111 ($[C_6H_7O_2]^+$, 42.6); 110 ($[C_6H_6O_2]^+$, 28.7); 77 (100). Table 1 shows ^{1}H-NMR data (300 MHz, CD_3OD) of **6**. Table 2 shows ^{13}C-NMR data (75 MHz, CD_3OD) of **6**.

Compound **7** (261 mg): yellow crystals (MeOH), mp 228-230°C, R_f 0.55 (EtOAc-MeOH-water, 30:5:4). UV λ_{max} nm (MeOH): 365.5, 270 (sh.), 255, (+NaOMe): 414.5, 270, (+$AlCl_3$): 436.5, 271, (+$AlCl_3$+HCl): 424, 357.1, 267.5, (+NaOAc): 377, 328.6, 270 (sh.), 256. FAB-MS *m/z* (% rel. int.): 465 ($[M+1]^+$, $C_{21}H_{20}O_{12}$, 34.9); 369 (6.3); 317 (8.3); 303 ($[C_{15}H_{11}O_7]^+$, 100); 302 ($[C_{15}H_{10}O_7]^+$, 25); 277 (28.1); 275 ($[C_{14}H_{11}O_6]^+$, 9.4); 259 (8.3); 245 (5.2). ^{1}H-NMR (500 MHz, CD_3OD) δ ppm: 6.45(1H, d, J = 2.1Hz, H-6), 6.74(1H, d, J = 2,1Hz, H-8), 7.75(1H, d, J = 2.1Hz, H-2'), 6.88(1H, d, J = 8.5Hz, 5'), 7.65(1H, dd, J = 2.1, 8.5Hz, H-6'), 5.04(1H, d, J = 7.4Hz, H-1"). ^{13}C-NMR (125 MHz, CD_3OD) δ ppm: 149.0 (C-2), 137.6 (C-3), 177.5 (C-4), 162.1 (C-5), 100.2 (C-6), 164.4 (C-7), 95.6 (C-8), 157.7 (C-9), 106.3 (C-10), 123.9 (C-1'), 116.3 (C-2'), 146.2 (C-3'), 148.8 (C-4'), 116.1 (C-5'), 121.9 (C-6'), 101.0 (C-1"), 74.7 (C-2"), 77.9 (C-3"), 71.3 (C-4"), 78.4 (C-5"), 62.5 (C-6").

Compound **8** (570 mg): dark yellow crystals (MeOH), mp 235-237°C, R_f 0.32 (EtOAc-MeOH-water-gl. acetic acid, 30:5:4:4 drops). UV λ_{max} nm (MeOH): 364.4, 272.6 (sh.), 257.5, (+NaOMe): 398, 271.4, (+NaOMe after 5 min.): 420, 292 (degradation), (+$AlCl_3$): 453, 281, (+$AlCl_3$+HCl): 393.2, 269.9, (+NaOAc): 380.3, 264.5. FAB-MS *m/z* (% rel. int.): 481 ($[M+1]^+$, $C_{21}H_{20}O_{13}$, 2.4); 461 (3.8); 460 (7.9). ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm: 7.08(1H, s, H-8), 12.38(1H, s, OH-5), 7.87(1H,d, J = 2.1Hz, H-2'), 7.06(1H, d, J = 8.6Hz, H-5'), 7.69(1H, dd, J = 2.1, 8.5Hz, H-6'), 5.16(1H,d, J = 7.1Hz, H-1"). ^{13}C-NMR (75 MHz, DMSO-d_6) δ ppm: 144.1 (C-2), 134.4 (C-3), 174.9 (C-4), 146.3 (C-5), 128.4 (C-6), 150.4 (C-7), 92.3 (C-8), 146.9 (C-9), 103.9 (C-10), 120.8 (C-1'), 114.2 (C-2'), 143.9 (C-3'), 146.6 (C-4'), 114.4 (C-5'), 118.8 (C-6'), 99.7 (C-1"), 72.0 (C-2"), 74.6 (C-3"), 68.5 (C-4"), 76.1 (C-5"), 59.4 (C-6").



Material	R_1	R_2	R_3	R_4
1	OH	H	OH	OH
3	O-glc	H	H	OH
4	OH	H	O-glc	OH
5	O-glc	H	OCH_3	OH
7	O-glc	H	OH	OH
8	O-glc	OH	OH	OH



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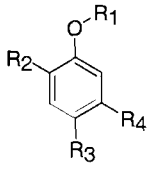
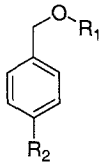


6. R_1= Glc, R_2 = OH, R_3 = H, R_4 = OH
11. R_1= Glc, R_2 = OCH_3, R_3 = CH_2OH, R_4 = H



9. R_1= Glc, R_2 = H
10. R_1= Glc-Glc, R_2 = OCH_3

Compound **9** (16 mg): colorless oily deposit, R_f 0.52 ($CHCl_3$-MeOH-EtOAc, 8:2:1). UV λ_{max} nm (MeOH): 262.5. EI-MS *m/z* (% rel. int.): 270 (M^+, $C_{13}H_{18}O_6$, 0.9); 213 (0.8); 199 (0.9); 180 ($[C_6H_{12}O_6]^+$, 2); 179 ($[C_6H_{11}O_6]^+$, 3.5); 178 (14.3); 164 ($[C_6H_{12}O_5]^+$, 1.6); 163 ($[C_6H_{10}O_5]^+$, 2.6); 107 ($[C_7H_8O]^+$, 5.4); 91 ($[C_7H_8]^+$, 7.3); 84 (100). Table 1 shows ^{1}H-NMR data (300 MHz, DMSO-d_6) of **9**. Table 2 shows ^{13}C-NMR data (75 MHz, DMSO-d_6) of **9**.

Compound **10** (18 mg): colorless oily deposit, R_f 0.42 ($CHCl_3$-MeOH, 8:2), 0.49 ($CHCl_3$-MeOH-EtOAc, 8:2:1) and 0.76 (EtOAc-MeOH-water, 30:5:4). UV λ_{max} nm (MeOH): 273.75. EI-MS *m/z* (% rel. int.): 462 (M^+, $C_{20}H_{30}O_{12}$, 0.7); 432 (M^+-OCH_2, 0.8); 336 (0.7); 324 ($[C_{12}H_{20}O_{10}]^+$, 0.7); 323 (0.8); 322 (0.9); 315 (0.7); 284 ($[C_{14}H_{20}O_6]^+$, 1.3); 253 ($[C_{13}H_{17}O_5]^+$, 1.6); 208 (2.2); 192 (2.8); 180 ($[C_6H_{12}O_6]^+$, 4.1); 179 ($[C_6H_{11}O_6]^+$, 18.4); 178 ($[C_6H_{10}O_6]^+$, 100); 164 ($[C_6H_{12}O_5]^+$, 3.5); 163 ($[C_6H_{10}O_5]^+$, 4.7); 138 ($[C_8H_{10}O_2]^+$, 5.9); 137 ($[C_8H_9O_2]^+$, 7.8). Table 1 shows ^{1}H-NMR data (300 MHz, DMSO-d_6) of **10**. Table 2 shows ^{13}C-NMR data (75 MHz, DMSO-d_6) of **10**.

Compound **11** (75 mg): white crystals, mp 119-121°C, R_f 0.14 ($CHCl_3$-MeOH, 8.5:1.5), 0.26 ($CHCl_3$-MeOH, 8:2), 0.27 ($CHCl_3$-MeOH-EtOAc, 8:2:1) and 0.43 (EtOAc-MeOH-

Table 1. ^{1}H-NMR data of compounds **6, 10, and 11**

Proton	**6** (CD_3OD)	**10** (DMSO-d_6)	**11** (DMSO-d_6)
2	7.58 (1H, d, *J*=2.2 Hz)	7.29 (H, d, *J*=8.7 Hz)	6.68 (1H, d, *J*=2.7 Hz)
3	–	6.88 (H, d, *J*=8.7 Hz)	–
5	6.52 (1H, d, *J*=8.5 Hz)	6.88 (H, d, *J*=8.7 Hz)	6.66 (1H, d, *J*=8.7 Hz)
6	7.41 (1H, dd, *J*=2.2, 8.5 Hz)	7.29 (H, d, *J*=8.7 Hz)	6.45 (1H, dd, *J*=2.7, 8.6 Hz)
OCH_3	3.69 (3H, s)	3.77 (3H, s)	3.69 (3H, s)
CH_2O	–	4.72 (1H, d, *J*=11.6 Hz)	3.48 (2H, s)
	–	4.48 (1H, d, *J*=11.6 Hz)	–
Glucose			
1′	4.6 (1H, d, *J*=7.26 Hz)	4.18 (2H, d, *J*=7.77 Hz)	4.66 (1H, d, *J*=7.5 Hz)
2′-6′	3.3-3.7 (6H, m)	3.02-3.80 (6H, m)	3.10-3.70 (6H, m)
Glucose			
1″	–	4.18 (2H, d, *J*=7.77 Hz)	–
2″-6″	–	3.02-3.80 (6H, m)	–

Table 2. ^{13}C-NMR data of compounds **6, 10,** and **11**

Carbon	**6** (CD_3OD)	**10** (DMSO-d_6)	**11** (DMSO-d_6)
1	*	138.0	141.2
2	117.9	128.2	102.0
3	*	112.7	150.6
4	*	158.1	147.7
5	117.7	112.7	114.8
6	126.1	128.2	107.5
OCH_3	50.1	53.8	54.8
CH_2O	–	68.0	69.1
Glucose			
1′	102.9	100.8	101.3
2′	73.1	72.4	72.7
3′	75.9	75.8	76.2
4′	69.5	69.0	69.3
5′	76.4	75.9	76.5
6′	60.7	68.6	60.1
Glucose			
1″	–	102.0	–
2″	–	72.4	–
3″	–	75.8	–
4″	–	69.0	–
5″	–	75.9	–
6″	–	60.0	–

*The quaternary carbons were not detected.

water, 30:5:4). UV λ_{max} nm (MeOH): 286.25. EI-MS *m/z* (% rel. int.): 316 (M^+, $C_{14}H_{20}O_8$, 6.2); 239 (2.8); 225 (5.1); 211 (3.9); 179 ($[C_6H_{11}O_6]^+$, 9.3); 164 ($[C_6H_{12}O_5]^+$, 7.7); 163 ($[C_6H_{11}O_5]^+$, 5.7); 155 ($[C_8H_{11}O_3]^+$, 14.1); 141 (34); 140 ($[C_7H_8O_3]^+$, 15.1); 139 ($[C_7H_7O_3]^+$, 10.1); 138 ($[C_8H_{10}O_2]^+$, 13.7); 126 ($[C_6H_6O_3]^+$, 18.8); 125 ($[C_7H_9O_2]^+$, 31.8); 124 ($[C_7H_8O_2]^+$, 11.7); 123 (12.7); 112 (41.9); 111 ($[C_6H_7O_2]^+$, 28.5); 110 ($[C_6H_6O_2]^+$, 14.5); 84 (100). Table 1 shows ^{1}H-NMR data (300 MHz, DMSO-d_6) of **11**. Table 2 shows ^{13}C-NMR data (75 MHz, DMSO-d_6) of **11**.

Results and Discussion

Compounds **1**, **3**, **4**, **5**, **7,** and **8** were found to be 5-hydroxyflavonol derivatives. Acid hydrolysis of compounds **3**, **4**, **5**, **7,** and **8** afforded only glucose, as the sugar part. Compound **1** was found to be quercetin (Agrawal, 1989; Grande *et al.*, 1985; Harborne *et al.*, 1975; Pouchet and Behnke, 1993). Acid hydrolysis of compounds **4** and **7** resulted in the same hydrolytic products; glucose and quercetin. UV spectra of compound **4** in $AlCl_3$ and $AlCl_3$/HCl showed the absence of free 3'-OH, while that in NaOMe indicated the absence of free 7-OH. Accordingly compounds **4** and **7** were identified as quercetin-3'-*O*-glucoside and quercetin-7-*O*-glucoside, respectively. (Benayache *et al.*, 1991; Mabry *et al.*, 1970; Markham *et al.*, 1978, 1985; Wagner *et al.*, 1976).

UV spectral data of compounds **3** and **5** showed the presence of free 3-, 5- and 4′-hydroxyl groups. Their MS showed the appearance of the molecular ion peaks at *m/z* 448 ($C_{21}H_{20}O_{11}$) and 478 ($C_{22}H_{22}O_{12}$) corresponding to an additional OCH_3 in compound 5 appeared as singlet at ä 3.82 with its corresponding carbon observed at δ 54.75 in NMR spectra. Acid hydrolysis of both compounds gave kaempferol and isorhamnetin, respectively. Thus, compounds **3** and **5** were identified as kaempferol-7-*O*-glucoside and isorhamnetin-7-*O*-glucoside, respectively (Kaouadji, 1990; Liu *et al.*, 1994; Ross, 1984; Singh and Pandey, 1986).

UV spectra of compound **8**, in different shift reagents, indicated the presence of free 3-, 5-, 3'- and 4'-hydroxyl groups. FAB-MS showed the molecular ion peak at *m/z* 481 $[M+1]^+$, calculated for $C_{21}H_{20}O_{13}$ corresponding to an additional OH group compared to quercetin glucosides. ^{1}H-NMR confirmed the presence of ring B protons to be at 2', 5' and 6' positions. It also showed a singlet signal at δ 7.08 assigned for H-8. The position of *O*-glucosylation

was confirmed to be at C-7 by comparing the UV spectra before and after hydrolysis. Comparison of the observed data with those previously reported for quercetagetin-7-*O*-glucoside (Liu *et al.*, 1993; Thomas and Mabry, 1968) indicated their similarity.

EI-MS of compound **2** showed a molecular ion peak at *m/z* 182 ($C_9H_{10}O_4$). ^{1}H-NMR indicated the presence of tri-substituted benzene ring structure, and an ethyl group most probably attached to an ester function. HMQC and HMBC confirmed the structure of compound **2** to be the ethyl ester of 3,4-dihydroxybenzoic acid (Pouchet and Behnke, 1993).

EI-MS of compound **9** showed molecular ion peak at *m/z* 270 (M^+, calculated for $C_{13}H_{18}O_6$). ^{13}C-NMR and DEPT spectra showed the following peaks: four signals in the aromatic region corresponding to six non-oxygenated carbons, six signals due to glucose carbons, and one signal at δ 68.35 due to hydroxymethylene group. ^{1}H-NMR spectrum showed a multiplet at δ 7.25-7.45 (5H) indicated a mono-substituted aromatic structure. The methylene protons appeared as two doublets (J = 12.2 Hz), indicating the possible presence of glucosylated -CH_2O group. The anomeric proton doublet was detected at δ 4.22 (1H, J = 7.72 Hz). Compound **9** was subjected to acid hydrolysis and the aglycone was found to be benzyl alcohol. Accordingly, it could be identified as benzyl alcohol glucoside (Coen *et al.*, 1995).

EI-MS of compound **10** indicated the molecular ion peak at *m/z* 462 (M^+, calculated for $C_{20}H_{30}O_{12}$). The following signals were detected in the ^{13}C-NMR and DEPT spectra: four signals in the aromatic region corresponding to six carbons, of which only one is oxygenated; twelve signals due to two glucose moieties and two signals at δ 67.96 and 53.83 due to OCH_2- and OCH_3 groups, respectively. ^{1}H-NMR spectrum showed an aromatic AB system, integrated for four protons, confirming a *p*-substituted benzene ring. The appearance of the methylene protons as two doublets (J = 11.7Hz) at δ 4.34 and δ 4.72 indicated the possible attachement to the sugar part. The singlet at δ 3.63 (3H) indicated a methoxyl group and the two anomeric proton doublets were superimposed at δ 4.18 (2H, J = 8.17 Hz). All previous assignments were supported by HMQC experiments. Compound **10** was subjected to acid hydrolysis and the aglycone was found to be anisyl alcohol. It could be concluded that, compound **10** is anisyl alcohol diglucoside. Comparison of observed data of **10** with those reported data for similar compounds gave further evidence for the elucidated structure (Coen *et al.*, 1995; Itokawa *et al.*, 1982; Pouchet and Behnke, 1993). This is the first report for the isolation of anisyl alcohol diglucoside (**10**) from a natural source.

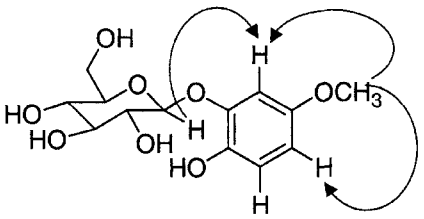


Fig. 1. NOESY relations of material **6**.

Compound **6** was found to be phenolic in nature. EI-MS illustrated a molecular ion peak at *m/z* 302 (M^+, calculated for $C_{13}H_{18}O_8$). ^{13}C-NMR and DEPT spectra revealed the presence of six signals due to glucose and a signal at δ 50.1 most probably due to $O\underline{C}H_3$ substitution. ^{1}H-NMR spectrum showed two doublets (1H each), a doublet of doublet (1H) assigned for the three aromatic protons. A singlet (3H) was observed at δ 3.69, due to a methoxyl group. The location of the methoxyl group as well as the position of glucosylation were detected by extensive study of HMBC and NOESY experiments (Fig. 1). Further evidence was achieved by UV spectral analysis of the aglycone following acid hydrolysis. $AlCl_3$ shift reagent induced a 28 nm bathochromic shift, which was reversed upon addition of HCl, confirming the presence of an *ortho*-dihydroxy system. Accordingly, Compound **6** could be identified as 2-hydroxy-5-methoxyphenyl-1-*O*-glucoside (Asakawa *et al.*, 1985), isolated for the first time from a natural source.

EI-MS of compound **11** indicated the molecular ion peak at *m/z* 316 (M^+, calculated for $C_{14}H_{20}O_8$). Thus, it showed a fourteen mass units increase than compound **6**, corresponding to an additional CH_2 group, which observed at δ 3.48 and 69.14 in NMR spectra. ^{1}H-NMR spectra showed the presence of one OCH_3 group and the same aromatic substitution pattern as in compound **6**. NMR spectra illustrated also signals corresponding to glucose moiety. The substitution pattern of the benzene ring was confirmed by HMBC experiment (Fig. 2). The aglycone resulting from acid hydrolysis gave a brown colour with $FeCl_3$, indicating the attachment of the sugar moiety to a phenolic hydroxyl group. Upon comparison of the aglycone with a reference vanillic alcohol sample, by co-chromatography, both samples were found to be identical. Thus, compound **11** could be identified as vanillic alcohol-4-*O*-glucoside (Kobata *et al.*, 1999; Pouchet and Behnke, 1993). All the previous reports for vanillic alcohol glucoside were concerned with using vanillic alcohol as a starting material in glucosylation reactions, cell culture or gene technology experiments. Accordingly, this is the first report

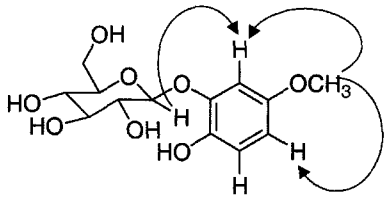


Fig. 2. The most significant ^{1}H, ^{13}C-correlations as observed from HMBC spectrum of material **11**.

for its isolation and identification of compound **11** using different spectroscopic techniques.

Anti-microbial screening. Anti-bacterial and anti-fungal assays were carried out using the agar diffusion technique. 5 mg of each fraction or isolate, accurately weighed, were dissolved in 1 ml DMF except quercetagetin-7-*O*-glucoside, was dissolved in H_2O. The sensitivities were compared by measuring the inhibition zones (IZ) in mm. (Jain and Kar, 1971).

Fractions or isolates showing IZ diameters 10-14 mm were considered weakly active, those with diameters in the range >14 <17 mm were considered moderately active, while highly active fractions or isolates showed inhibition zone diameters >17 mm (Table 3).

The results showed that, EtOAc fractions of flower heads and shoots were active against *K. pneumonia* and all other fractions were totally inactive against the four test organisms. On the other hand, EtOH extract of shoots showed very week anti-fungal activity. Moreover, quercetin showed weak activity against the G-negative organisms, quercetin-3'-*O*-glucoside showed high activity against the same organisms, while quercetin-7-*O*-glucoside was inactive. This may indicate a possible loss of activity due to blocking of 7-OH, which is one of the known acidic centers of flavonoidal structures. Quercetagetin-7-*O*-glucoside was the most potent compound being moderately active against *S. aureus*, and highly active against the remaining test organisms. Finally, the infusion of the plant (5 g/100 ml) showed high activity against *S. aureus* and *K. pneumonia*, but with moderate activity against *C. albicans*. On contrast, a decoction of the same concentration was completely inactive. Accordingly, EtOAc fractions may be considered as possible G-negative antibacterial agents with specifically high activity against *K. pneumonia*. The flavonoidal fraction showed promising activities as well. Furthermore, the use of plant infusion may be favored due to possible loss of active ingredients upon preparing plant decoction, which may be due to decomposition or volatilization.

Table 3. Results of anti-bacterial and anti-fungal screening of different fractions and some isolates of *Helichrysum conglobatum*

Fraction or isolate no.	Inhibition zones (IZ) in mm			
	Bacteria			Fungi
	G-positive	G-negative		
	Staphylococcus aureus	*Escherichia coli*	*Klebsiella pneumonia*	*Candida albicans*
1-4	–	–	–	–
5	–	–	19.0	–
6	–	–	17.0	–
7-11	–	–	–	–
12	–	–	–	7.5
13-14	–	–	–	–
15	–	14.5	11.5	–
16	–	21.5	20.5	–
17	–	–	–	–
18	16.0	20.3	30.5	30.0
19	22.5	–	18.5	16.5
20	-	–	–	–

1,2: Petroleum ether fractions of flower heads and shoots, **3,4:** 90% methanol fractions of flower heads and shoots, **5,6:** Ethyl acetate fractions of flower heads and shoots, **7,8:** *n*-butanol fractions of flower heads and shoots, **9,10:** water fractions of flower heads and shoots, **11,12:** Total alcoholic extraxts of flower heads and shoots, **13:** ursolic acid, **14:** β-sitosterol glucoside, **15:** quercetin, **16:** quercetin-3'-*O*-glucoside, **17:** quercetin-7-*O*-glucoside, **18:** quercetagetin-7-*O*-glucoside, **19,20:** infusion and decoction of the plant (5 g /100 ml), respectively.

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