Phytochemistry 69 (2008) 1910–1914

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Flavonoids and terpenoids from *Helichrysum forskahlii*

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ARTICLE INFO

Article history:
Received 25 January 2008
Received in revised form 15 March 2008
Available online 14 May 2008

Keywords:
Helichrysum forskahlii
Asteraceae
Flavonoids
Helichrysone A, B and C

ABSTRACT

Three new flavonoids, namely helichrysone A (**1**), helichrysone B (**2**) and helichrysone C (**3**) were isolated from the aerial parts of *Helichrysum forskahlii*, together with 10 known flavonoids, three triterpenes, and one sesquiterpene. The structures of the new flavonoids **1–3** were established by 1D and 2D NMR spectral data. In addition, the antimicrobial activities of the isolated compounds were determined.

1. Introduction

The genus *Helichrysum* (Asteraceae) consists of more than 500 species with a major center of distribution in South Africa (Mabberley, 1997). Several *Helichrysum* species have been used in folk medicine of different countries as diuretics, antiinflammatory and antiallergic (Facino et al., 1990; Cubukcu and Yuksel, 1982). *Helichrysum* species were also reported to relief abdominal pain, heart burn, cough, cold and wounds and to treat female sterility and menstrual pain (Puyvelde et al., 1989). Chemical studies on *Helichrysum* species have been carried out by many investigators and the presence of flavonoids, phloroglucinols, α-pyrones, coumarins and terpenoid compounds has been reported (Bohlmann and Abraham, 1979; Bohlmann and Misra, 1984; Jakupovic et al., 1989, 1990; Randriaminahy et al., 1992; Caffaratti et al., 1994; Matsumoto et al., 1985). In addition, some of these species have been reported to possess antimicrobially active compounds (Tomas-Barberan et al., 1988a, 1988b; Iniesta-Sanmartin et al., 1990). In our search for biologically active compounds from Saudi plants, *Helichrysum forskahlii* (J.F. Gmel.) Hilliard and Burtt, a plant growing in the Southern part of Saudi Arabia (Chaudhary, 2000), was selected since its ethanolic extract showed a strong antimicrobial activity in a preliminary study. Searching the literature on this species revealed the presence of only one report that is related to the Kenyan plant (Jakupovic et al., 1990). The present paper reports on the isolation and characterization of three new flavonoids, helichrysone A (**1**), helichrysone B (**2**) and helichrysone C (**3**) from the aerial parts of *H. forskahlii*. Besides, the plant also yielded 10 flavonoids, 3''-dimethyl-5',6'-pyrano-2',4'-dihydroxychalcone (**4**), a known compound under the name 2,2-dimethyl-8-cinnamoyl-5,7-chromandiol (Backhouse and Robertson, 1939), 6-prenyl-pinocembrin (**5**), glabranin (**6**), cardamomin (7), alpinetin (**8**), desmethylxanthohumol (**9**), 2',4',6'-trihydroxychalcone (**10**), quercetin-3-*O*-methyl ether (**11**), pinocembrin (**12**) and desmethylhelichromanochalcone (**13**), four terpenoids, the sesquiterpene clovandiol (**14**) and the triterpenes oleanolic acid (**15**), oleanolic acid acetate (**16**) and β-amyrin (**17**). Furthermore, the antimicrobial activities of the isolated compounds were also studied.

2. Results and discussion

Compound **1** was obtained as orange red crystals, m.p. 120–121 °C and its molecular formula was established as $C_{21}H_{22}O_5$ by HRMS. The IR bands at 3430 (hydroxyl group), 1650 (conjugated carbonyl function) and 1580 (conjugated unsaturation) cm^{-1} and the UV absorption at 238 and 394 nm were suggestive of a chalcone skeleton (Markham, 1982). The *trans*-olefinic proton signals at δ 7.87 (*d*, *J* = 15.5 Hz, H-7) and δ 8.00 (*d*, *J* = 15.5 Hz, H-8), a chelated hydroxyl proton at δ 13.38 (s) and the ^{13}C NMR signal at 192.7 (C-9) (Table 1) suggested that compound **1** was a chalcone with a hydroxyl group at C-2'. The ^{1}H NMR spectrum of **1** also showed signals for a prenyloxyl group at δ 4.56 (2H, *d*, *J* = 7.0 Hz, H-1''), δ 5.43 (1H, *t*, *J* = 7.0 Hz, H-2''), δ 1.74 (3H, *s*, Me-4'') and δ 1.69 (3H, *s*, Me-5''), an aromatic proton at δ 6.25 (1H, *s*, H-3'), a methoxy group at δ 3.81 (3H, *s*, OMe-6') and a phenolic proton at δ 5.28 (1H, *s*, OH-5'). The dimethylallyl unit should be located at C-4' of ring A, as confirmed by the HMBC correlations of H-1'' with C-4' (δ 153.7) and HMBC correlations of H-3' (δ 6.25) and OH-5'

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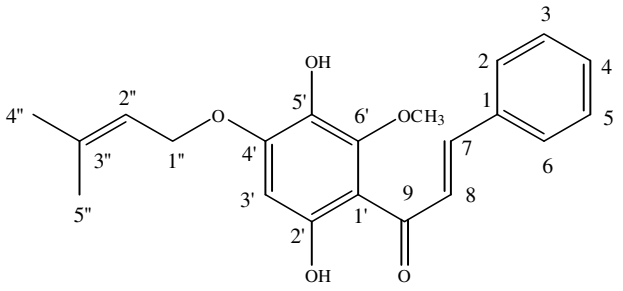
doi:10.1016/j.phytochem.2008.03.025

(δ 5.28) with C-4'. In addition, the ^{1}H NMR spectrum of **1** revealed other five aromatic protons as two asymmetric doublets at δ 7.66 (2H, *d*, *J* = 6.5 Hz, H-2/6) and δ 7.43 (3H, *d*, *J* = 6.5 Hz, H-3/5 and 4) (Table 1). These data indicated that compound **1** is a chalcone with an unsubstituted B ring close to previously isolated compound from *Helichrysum rugulosum* (Bohlmann and Misra, 1984). The new compound was identified as 2',5'-dihydroxy-6'-methoxy-4'-γ,γ-dimethylallyloxychalcone and given the trivial name helichrysone A (**1**).



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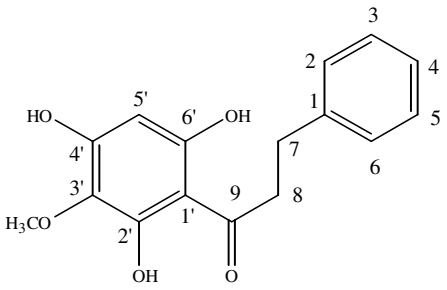

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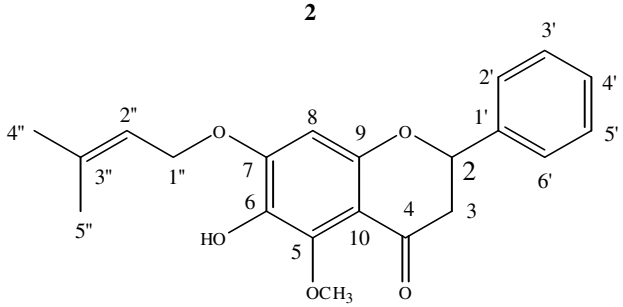

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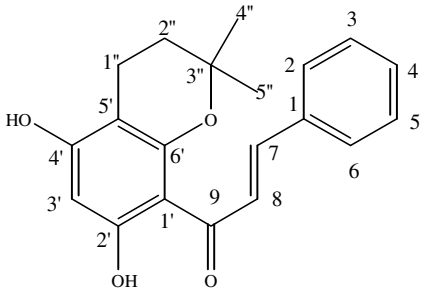

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Compound **2** was isolated as a white amorphous powder, m.p. 128 °C and its molecular formula was deduced from the HRMS *m*/*z* 288.09976 (Calc. 288.09912) as $C_{16}H_{16}O_5$. The IR spectrum of compound **2** contained absorption bands due to hydrogen-bonded OH (3400 cm^{-1}), conjugated carbonyl (1640 cm^{-1}) and aromatic band at 1600 cm^{-1}. The ^{1}H NMR spectrum of compound **2** exhibited signals due to one methoxyl at δ 3.75 (3H, *s*, OMe-3'), one aromatic singlet at δ 5.90 (1H, *s*, H-5'), five other aromatic protons appeared as two multiples, one accounting for four protons resonating at δ 7.26 (4H, *m*, H-2-6) and another at δ 7.18 (1H, *m*, H-4) and two aliphatic triplets resonating at δ 2.98 (2H, *t*, *J* = 8.0 Hz, H-7) and δ 3.35 (2H, *t*, *J* = 8.0 Hz, H-8). This information with the ^{13}C NMR data (Table 2) indicated that compound **2** must be a dihydrochalcone derivative (Tanaka et al., 1982). The exact position of the different substituents in compound **2** was ascertained through HMBC correlations. Thus, the aromatic singlet at δ 5.90 (H-5') showed a three bond correlation with C-3' (δ 130.0) and C-1' (δ 105.2) and two bond correlation with C-6' (δ 157.9) and C-4' (δ 158.7), while the methoxy group at δ 3.75 observed a three bond correlation with C-3' (δ 130.0) confirming the position of the methoxy group at C-3' and the aromatic singlet at δ 5.90 at C-5'. The remaining positions on ring A, C-2', C-4' and C-6' must be occupied with hydroxyl groups. This conclusion was further supported by the EIMS fragment at *m*/*z* 183. Based on the above spectral evidence, compound **2** was identified as 3'-methoxy-2',4',6'-trihydroxydihydrochalcone, a new compound, and was named helichrysone B.

Table 1
^{1}H and ^{13}C NMR assignments and HMBC correlations of Helichrysone A (**1**) in $CDCl_3$

C	δ_C[a]	δ_H[b]	Cross-peaks (δ_C) in HMBC spectrum
1	135.3 C	–	–
2/6	128.5 2CH	7.66 *d* (6.5)	C-1, C-4, C-7
3/5	129.0 2CH	7.43 *d* (6.5)	C-1, C-2/6
4	130.3 CH	7.43	C-1, C-2/6
7	143.2 CH	7.87 *d* (15.5)	C-1, C-2/6, C-8, C-9
8	126.4 CH	8.00 *d* (15.5)	C-1, C-7, C-9
9	192.7 C	–	–
1'	108.6 C	–	–
2'	159.9 C	–	–
3'	96.9 CH	6.25 *s*	C-1', C-2', C-4', C-5'
4'	153.7 C	–	–
5'	131.9 C	–	–
6'	146.9 C	–	–
1''	66.1 CH_2	4.56 *d* (7.0)	C-2'', C-3'', C-4'
2''	118.2 CH	5.43 *t* (7.0)	C-1'', C-4'', C-5''
3''	139.1 C	–	–
4''	25.8 CH_3	1.74 *s*	C-2'', C-3'', C-5''
5''	18.3 CH_3	1.69 *s*	C-2'', C-3'', C-4''
2'-OH	–	13.38 *s*	C-1', C-2', C-3'
5'-OH	–	5.28 *s*	C-4', C-5', C-6'
6'-OMe	61.7 OCH_3	3.81 *s*	C-6'

[a] δ (ppm) 125 MHz; multiplicities were determined from DEPT experiment; *J* values (Hz) in parentheses.
[b] δ (ppm) 500 MHz.

Table 2
^{1}H and ^{13}C NMR assignments and HMBC correlations of Helichrysone B (**2**) in CD_3OD

C	δ_C[a]	δ_H[b]	Cross-peaks (δ_C) in HMBC spectrum
1	143.1 C	–	–
2/6	129.4* 2CH	7.26 *m*	C-1, C-4, C-7
3/5	129.5* 2CH	7.26 *m*	C-1, C-4
4	126.9 CH	7.18 *m*	C-2/6, C-3/5
7	32.1 CH_2	2.98 *t* (8.0)	C-1, C-2/6, C-8, C-9
8	46.9 CH_2	3.35 *t* (8.0)	C-1, C-7, C-9
9	206.4 C	–	–
1'	105.2 C	–	–
2'	161.2 C	–	–
3'	130.0 C	–	–
4'	158.7 C	–	–
5'	95.6 CH	5.90 *s*	C-1', C-3', C-4', C-6'
6'	157.9 C	–	–
3'-OMe	61.1 OCH_3	3.75 *s*	C-3'

[a] δ (ppm) 125 MHz; multiplicities were determined from DEPT experiment; *J* values (Hz) in parentheses.
[b] δ (ppm) 500 MHz.
* Interchangeable carbon signals.

Compound **3** was obtained as yellow gummy residue, $[\alpha]_D$ +5° (*c*; 0.06 in $CHCl_3$) and its molecular formula was established as $C_{21}H_{22}O_5$. The IR spectrum showed absorption bands for conjugated carbonyl (1680 cm^{-1}) and a hydroxyl group (3380 cm^{-1}). The UV spectrum exhibited absorptions at 242, 281 and 340 nm suggesting a flavanone moiety (Markham, 1982). This was further confirmed by the ^{13}C NMR and DEPT spectral data, which disclosed two methyl, two methylene, one methoxyl, one oxymethine, one olefinic carbon, four aromatic methine (two of them accounting for four methine) and eight quaternary carbons (Table 3). The ^1H NMR spectrum revealed the presence of one methoxyl at δ 3.88 (3H, *s*, OMe-5), one aromatic singlet at δ 6.31 (1H, *s*, H-8), five other aromatic protons resonating at δ 7.31–7.41 (5H, *m*, H-2'–6'), and three protons representing one methylene at δ 2.72 (1H, *dd*, *J* = 17.0, 2.5 Hz, H-3a) and δ 2.95 (1H, *dd*, *J* = 17.0, 13.5 Hz, H-3b) and one oxymethine at δ 5.33 (1H, *dd*, *J* = 13.5, 2.5 Hz, H-2). In addition, the ^1H NMR spectrum of compound **3** showed signals for a prenyloxyl group at δ 4.53 (2H, *d*, *J* = 7.0 Hz, H-1''), δ 5.43 (1H, *dd*, *J* = 7.0, 1.5 Hz, H-2''), δ 1.73 (3H, *s*, Me-4'') and δ 1.67 (3H, *s*, Me-5''). The positions of the various substituents at the flavanone skeleton were determined by HMBC experiment. The aromatic proton at δ 6.31 (H-8) showed three bond correlations with C-6 (δ 134.1) and C-10 (δ 108.5), and two bond correlations with C-9 (157.0) and C-7 (δ 153.2). These and three bond correlation between H-1'' (δ 4.53) and C-7 (δ 153.2) confirmed the attachment of prenyloxyl group at C-7. In addition, the proton of the hydroxyl function exhibited a two bond correlation with δ 134.1 (C-6) and three bond correlations with δ 153.2 (C-7) and δ 146.1 (C-5) confirming the position of the hydroxyl group at C-6 since the protons of the methoxyl group correlated via three bond correlation with C-5 (δ 146.1). Other important HMBC correlations are shown in Table 3. Thus, compound **3**, a new natural product, was identified as 6-hydroxy-5-methoxy-7-γ,γ-dimethylallyloxyflavanone and given the trivial name helichrysone C.

The previously reported compound, 2,2-dimethyl-8-cinnamoyl-5,7-chromandiol (**4**) (Backhouse and Robertson, 1939), which is better named as (3''-dimethyl-5',6'-pyrano-2',4'-dihydroxychalcone), was also isolated from *H. forskahlii* as a new natural product and its spectral data (1D and 2D NMR) are reported here for the first time (Table 4).

All the isolated compounds were tested for antimicrobial activity against certain microorganisms (see Section 3). Compound **6** (glabranin) was found to be the most active component of *H. forskahlii* with MIC values of 3 and 6 μg/ml against *Bacillus subtilis* and *Staphylococcus aureus*, respectively. This is followed by compound **9** (desmethylxanthohumol) which exhibited activity especially against *B. subtilis* (MIC 50 μg/ml). In addition, compounds **1** and **12** (pinocembrin) exhibited weak activity against both microorganisms with MIC values of 100 μg/ml. On the other hand, compounds **4, 5, 8** and **13–17** were completely inactive against the tested microorganisms.

Table 3
^1H and ^{13}C NMR assignments and HMBC correlations of Helichrysone C (**3**) in $CDCl_3$

C	δ_C[a]	δ_H[b]	Cross-peaks (δ_C) in HMBC spectrum
2	79.4 CH	5.33 *dd* (13.5, 2.5)	C-1', C-2'/6', C-4
3	45.6 CH_2	2.72 *dd* (17.0, 2.5)	C-4, C-10
		2.95 *dd* (17.0, 13.5)	C-1', C-2, C-4
4	189.5 C	–	–
5	146.1 C	–	–
6	134.1 C	–	–
7	153.2 C	–	–
8	97.1 CH	6.31 *s*	C-6, C-7, C-9, C-10
9	157.0 C	–	–
10	108.5 C	–	–
1'	138.8 C	–	–
2'/6'	126.1 2CH	7.31-7.41 *m*	C-1', C-2'/6', C-3'/5', C-4'
3'/5'	128.8 2CH	7.31-7.41 *m*	C-1', C-2'/6', C-3'/5', C-4'
4'	128.7 CH	7.31-7.41 *m*	C-2'/6', C-3'/5'
1''	66.1 CH_2	4.53 *d* (7.0)	C-2'', C-3'', C-7
2''	118.3 CH	5.43 *dd* (7.0, 1.5)	C-4'', C-5''
3''	139.5 C	–	–
4''	25.8 CH_3	1.73 *s*	C-2'', C-3'', C-5''
5''	18.3 CH_3	1.67 *s*	C-2'', C-3'', C-4''
5-OMe	61.6 OCH_3	3.88 *s*	C-5
6-OH	–	5.39 *s*	C-5, C-6, C-7

[a] δ (ppm) 125 MHz; multiplicities were determined from DEPT experiment; *J* values (Hz) in parentheses.
[b] δ (ppm) 500 MHz.

Table 4
^1H and ^{13}C NMR assignments and HMBC correlations of 3''-dimethyl-5',6'-pyrano-2',4'-dihydroxychalcone (**4**) in $CDCl_3$

C	δ_C[a]	δ_H[b]	Cross-peaks (δ_C) in HMBC spectrum
1	136.1 C	–	–
2/6	128.5 2CH	7.62 *br d* (6.8)	C-4, C-7
3/5	129.2 2CH	7.42 *m*	C-1, C-2/6
4	130.2 CH	7.42 *m*	C-2/6
7	141.9 CH	7.75 *d* (15.6)	C-2/6, C-8, C-9
8	128.4 CH	8.05 *d* (15.6)	C-1, C-9
9	193.4 C	–	–
1'	107.1 C	–	–
2'	165.9 C	–	–
3'	95.9 CH	5.91 *s*	C-1', C-2', C-4', C-5'
4'	161.1 C	–	–
5'	100.3 C	–	–
6'	157.5 C	–	–
1''	16.8 CH_2	2.64 *t* (6.8)	C-4', C-5', C-6', C-2'', C-3''
2''	31.9 CH_2	1.86 *t* (6.8)	C-5', C-1'', C-3'', C-4''/5''
3''	76.5 C	–	–
4'', 5''	27.3 $2CH_3$	1.47 *s*	C-2'', C-3'', C-4''/5''
2'-OH	–	14.0 *s*	C-1', C-2', C-3'

[a] δ (ppm) 125 MHz; multiplicities were determined from DEPT experiment; *J* values (Hz) in parentheses.
[b] δ (ppm) 500 MHz.

3. Experimental

3.1. General

Mp uncorr.; UV spectra were recorded on a Hewlett-Packard HP-845 UV-VIS spectrophotometer; FTIR spectra were obtained on a Nicolet Impact 410 spectrophotometer; Specific rotation measurements were recorded on a Perkin-Elmer 242 MC polarimeter; NMR spectra were acquired in CD_3OD or DMSO on a Bruker Avance DRX-500 instrument at 500 (^1H) and 125 (^{13}C) MHz using the residual solvent signal as internal standard. Standard Bruker pulse programs were used for APT, DEPT, and 2D NMR: COSY, HMQC and HMBC spectra. HRFABMS were obtained on a Bruker Bioapex-FTMS with electrospray ionization; EIMS were measured using an E.I. Finnigan model 4600 quadrupole system or a Shimadzu QP500 GC/mass spectrometer; TLC: silica gel 60 F254 (Merck) plates; solvents: different concentration of MeOH–$CHCl_3$ and H_2O–MeOH; CC:silica gel 60/230–400 mesh (EM Science); RP C-18 silica gel. Centrifugal preparative TLC (CPTLC; using Chromatotron®, Harrison Research Inc. model 7924): 1–4 mm silica gel P_{254} disc. The isolated compounds were visualized under short- and long-wave UV light, followed by spraying with *p*-anisaldehyde reagent.

3.2. Plant material

Helichrysum forskahlii (J.F. Gmel.) Hilliard and Burtt was collected in March 1999 from Abha, Saudi Arabia and identified by Dr. Sultanul Abidin, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. A voucher specimen (#14052) was deposited at the herbarium of the College of Pharmacy, KSU.

3.3. Extraction and isolation

The air-dried aerial parts (20.0 kg) of *H. forskahlii* were percolated at room temperature with 95% EtOH to yield a dark brown residue (195 g). This extract was dissolved in 60% aqueous ethanol and successively extracted with hexane (70 g), chloroform (50 g) and EtOAc (4 g) and the aqueous ethanol fraction (20 g). The hexane fraction (70 g) was partitioned between hexane and 10% aqueous MeCN to afford the hexane fraction (15 g) and aqueous MeCN (21 g). All these extracts (hexane, aqueous MeCN, $CHCl_3$, EtOAc and aqueous EtOH) were tested for antimicrobial activity. Only aqueous MeCN and $CHCl_3$ extracts showed antimicrobial activities against *B. subtilis*, *S. aureus* and *Mycobacterium smegmatis* using the agar dilution assay (Mitscher et al., 1972). Furthermore, these two extracts were separately subjected to bioautography (Hamburger and Cordell, 1987) on Silica gel plates [5 × 10 cm; solvent: Petroleum ether–EtOAc (4:1)] for aqueous MeCN fraction; $CHCl_3$–MeOH (19:1) for $CHCl_3$ fraction, using *B. subtilis* as the test organism. Three zones with R_f values at 0.12, 0.40 and 0.54 were observed for aqueous MeCN after 24 h of incubation, while three zones with R_f 0.15, 0.41 and 0.51 were observed for $CHCl_3$ fraction.

The aqueous acetonitrile fraction (19 g) was subjected to flash chromatography on silica gel (600 g) using $CHCl_3$ and MeOH (0–2%) to give five pooled fractions. Fraction 1 (2.3 g) was crystallized from $CHCl_3$–hexane to afford **1** as orange red crystals (350 mg). Fraction 2 (2.1 g) was crystallized from $CHCl_3$ to give 6-prenyl-pinocembrin (**5**, 150 mg). Fraction 3 (400 mg) was separated by CPTLC (1 mm silica gel disc) using 1% EtOAc–CH_2Cl_2 to afford oleanolic acid acetate (**16**, 40 mg). Fraction 4 (1.3 g) was rechromatographed on silica gel column, using 1% EtOAc–$CHCl_3$ to give glabranin (**6**, 35 mg). Fraction 5 (420 mg) was subjected to CPTLC (1 mm silica gel disc) using 1% EtOAc–$CHCl_3$ to get β-amyrin (**17**, 25 mg).

On the other hand, the $CHCl_3$ fraction (18 g) was chromatographed on a silica gel column (600 g) using $CHCl_3$ and MeOH (0–20%) to afford nine pooled fractions. Fraction 1 (400 mg) was crystallized from $CHCl_3$ to give additional amount of **5** (50 mg), and the mother liquor was recrystallized from $CHCl_3$–hexane to give also additional of **1** (150 mg). Fraction 2 (600 mg) was separated by CPTLC (2 mm silica gel disc) using 1% absolute EtOH in CH_2Cl_2 to yield desmethylxanthohumol (**9**, 90 mg). Fraction 3 (2 g) was chromatographed over silica gel using 1% MeCN–$CHCl_3$ to afford cardamomin (**7**, 150 mg). Fraction 4 (1 g) was separated by CPTLC (4 mm silica gel disc) using 0.5% MeOH–$CHCl_3$ to yield three sub-fractions A–C. Sub-fraction C (300 mg) was rechromatgraphed on reverse phase column using 30% H_2O–MeOH to get **2** as white amorphous powder (37 mg). Fraction 5 (80 mg) was subjected to repeated Chromatotron developments using $CHCl_3$ to give **3** as a yellow gummy residue (20 mg). Fraction 6 (1 g) was rechromatographed by CPTLC (4 mm silica gel disc) using petroleum ether and acetone (0–20%) to afford two sub-fractions A–B. Sub-fraction B (200 mg) was subjected to reverse phase column chromatography on C-18 using 20% H_2O–MeCN to give further three sub-fractions (i–iii). Sub-fraction i (34 mg) was separated by CPTLC (1 mm silica gel disc) using $CHCl_3$ as a solvent to give pinocembrin (**12**, 24 mg). Sub-fraction ii (27 mg) was purified by CPTLC (1 mm silica gel disc) using 1% EtOAc–$CHCl_3$–acetic acid as eluent to afford **4** as yellow amorphous powder (3 mg). Sub-fraction iii (11 mg) was also subjected to CPTLC (1 mm silica gel disc) using 1% EtOAc–$CHCl_3$–acetic acid as to yield oleanolic acid (**15**, 6 mg). Fraction 7 (150 mg) was crystallized from MeOH–$CHCl_3$ to afford alpinetin (**8**, 34 mg), and the mother liquor was subjected to repeated cc over silica gel using 1% MeOH–CH_2Cl_2 to give desmethylhelichromanochalcone (**13**, 16 mg). Fraction 8 (1.5 g) was subjected to cc over silica gel (60 g) using $CHCl_3$ to afford three sub-fractions A–C. Sub-fraction B (200 mg) was purified by CPTLC (2 mm silica gel disc) using $CHCl_3$ to give 2',4',6'-trihydroxychalcone (**10**, 30 mg). Sub-fraction C (500 mg) was rechromatographed by CPTLC (4 mm silica gel disc) using 25% acetone–hexane–NH_3 to get clovandiol (**14**, 12 mg).

In addition, EtOAc fraction (4 g) was subjected to cc over silica gel (100 g) using $CHCl_3$ and MeOH (0–20%) to afford three sub-fractions A–C. Sub-fraction A (160 mg) was purified by CPTLC (1 mm silica gel disc) using 15% MeOH–$CHCl_3$–NH_3 to give quercetin-3-*O*-methyl ether (**11**, 13 mg).

3.4. Helichrysone A (*1*)

Orange red crystals, m.p. 120–121 °C; UV λ_{max} ($CHCl_3$) nm (log ε): 238 (2.96), 324 (3.32), 394 (2.88); IR (KBr) υ_{max} cm^{-1}: 3430, 3010, 2980, 1650, 1580, 1510, 1495, 1420, 1370, 1340, 1280, 1180, 1100, 1055, 1010, 1000, 940 and 850; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$), see Table 1; EIMS *m*/*z* (rel. int.%) 354 $[M]^+$ (4.2), 286 (22.4), 285 (25.8), 182 (39.8), 181 (53.8), 77 (13.4), 69 (81.4) and 41 (100); HRMS: obsd. *m*/*z* 354.1474451; (calc. for $[C_{21}H_{22}O_5]$ 354.1467162).

3.5. Helichrysone B (*2*)

White amorphous powder, m.p. 128 °C; UV λ_{max} (MeOH) nm (log ε): 210 (3.69), 287 (3.60), 340 (2.90); IR (KBr) υ_{max} cm^{-1}: 3400, 3030, 2960, 1640, 1600, 1500, 1450, 1390, 1370, 1310, 1260, 1220, 1190, 1150, 1080, 1070, 990, 975 and 830; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR(125 MHz, CD_3OD), see Table 2; EIMS *m*/*z* (rel. int.%) 288 $[M]^+$ (17.4), 183 (35.6), 91 (37.8), 45 (23.3), 44 (100) and 43 (47.2); HRMS: obsd. *m*/*z* 288.0997686; (calc. for $[C_{16}H_{16}O_5]$ 288.0991244).

3.6. Helichrysone C (*3*)

Yellow gummy residue, $[\alpha]_D$ +5° (*c*; 0.06 in $CHCl_3$); UV λ_{max} ($CHCl_3$) nm (log ε): 242 (4.29), 281 (4.27), 340 (3.84); IR (KBr) υ_{max} cm^{-1}: 3380, 3020, 2980, 2940, 1680, 1620, 1490, 1450, 1425, 1380, 1280, 1240, 1175, 980 and 760; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$), see Table 3; HRMS: obsd. *m*/*z* 354.1467831; (calc. for $[C_{21}H_{22}O_5]$ 354.1467162).

3.7. 3''-Dimethyl-5',6'-pyrano-2',4'-dihydroxychalcone (*4*)

Yellow amorphous powder, IR (KBr) υ_{max} cm^{-1}: 3300, 1620, 1580, 1510, 1470, 1400, 1340, 1230, 1160 and 1100; ^1H NMR (500 MHz, $CDCl_3$): 7.62 (2H, br *d*, *J* = 6.8 Hz, H-2/6), 7.42 (2H, *m*, H-3/5), 7.42 (1H, *m*, H-4), 7.75 (1H, *d*, *J* = 15.6 Hz, H-7), 8.05 (1H, *d*, *J* = 15.6 Hz, H-8), 5.91 (1H, *s*, H-3'), 14.0 (1H, *s*, 2'-OH), 2.64 (2H, *t*, *J* = 6.8 Hz, H-1''), 1.86 (2H, *t*, *J* = 6.8 Hz, H-2'') and 1.47 (6H, *s*, 4''-Me/5''-Me) and ^{13}C NMR (125 MHz, $CDCl_3$): 136.1 (C-1), 128.5 (C-2/6), 129.2 (C-3/5), 130.2 (C-4), 141.9 (C-7), 128.4 (C-8), 193.4 (C-9), 107.1 (C-1′), 165.9 (C-2′), 95.9 (C-3'), 161.1 (C-4'), 100.3 (C-5'), 157.5 (C-6'), 16.8 (C-1''), 31.9 (C-2''), 76.5 (C-3'') and 27.3 (C-4''/C-5''); EIMS *m*/*z* (rel. int.%) 324 $[M]^+$ (35.8), 267 (24), 247 (37.9), 191 (51.2), 165 (100) and 69 (30); HRMS: obsd. *m*/*z* 324.126782; (calc. for $[C_{20}H_{20}O_4]$ 324.126245).

3.8. Antimicrobial activity

The preliminary antimicrobial activity of the extracts/fractions were determined by using agar dilution technique (Mitscher et al., 1972) and the MIC values of the active compounds were determined by twofold serial broth microdilution assay (Hufford et al., 1975). The test organisms used were *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 15441, *Mycobacterium smegmatis* ATCC 35797

and *Candida albicans* ATCC 10231. Chloramphenicol and nystatin were used as positive controls, and DMSO as a negative control.

Acknowledgements

The authors thank Mr. Mohammad Mukhair and Mr. Mustafa Omar for their technical assistance. The authors also thank Dr. John Hill, Kent Mass Spectrometry, Dartford, UK, for recording the HRMS.

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