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HOMOISOFLAVANONES FROM *SCILLA* (*LILIACEAE*)

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ABSTRACT

Phytochemical investigation of the ether fraction of the methanolic extract of *Scilla peruviana*, *S. villosa* and *S. numedica*, led to the isolation of three homoisoflavanones **1**, **2**, and **3**. They were identified according to their chemical and spectral data as 5,7-dihydroxy-6-methoxy-3-(4-methoxybenzyl) chroman-4-one, 5,7-dihydroxy-6-methoxy-3-(4-hydroxybenzyl) chroman-4-one, and 5,7-dihydroxy-6-methoxy-3-(4-hydroxy benzylidene) chroman-4-one (autumnalin) respectively The isolated compounds showed anti-bacterial activity against Gram positive and Gram negative bacteria as well as anti-fungal activity. In addition, the ether extracts of *S. peruviana* and *S. villosa* showed anti-inflammatory activity.

INTRODUCTION

The genus *Scilla* belongs to family *Liliaceae*, a family of 250 genera and 3500 species. It consists of about 90 species, and is represented in Libya by four species, *S. villosa*, *S. obtusifolia*, *S. numedica* and *S. peruviana*[1]. The genus *Scilla* is reputed for its cardiac glycosides[2], triterpenoids[3], stelbenoids and homoisoflavanones[4-6]. Homoisoflavanones are characterized by the presence of additional carbon atoms (C-11) between rings B and C. Biosynthetically, they arise by formation intermediate chalcone, cyclization to chromanone and modification giving the parent compound[5]. So far reports of homoisoflavanones are restricted to the genera *Scilla*, *Muscaria* and *Eucomis*[6-20]. Homoisoflavanones have been shown to have anti-inflammatory, anti-histaminic, anti-mutagenic and angioprotective properties[18,21] as well as potent phosphodiestrase inhibitors[22]. Previous phytochemical studies of *Scilla Peruviana* indicated the presence of lanosterol oligosaccharides, peruvianosides A, B, and C and scillasaponins A, B, and C[3,23-26] but nothing was reported about homoisoflavanones from the Libyian *Scilla* species. Therefore, it was found of interest to carry out this study.

EXPERIMENTAL

Plant material:

The bulbs of *Scilla peruviana*, *Scilla villosa* and *Scilla numedica* were collected at the flowering stage from the wild plants growing at the Mediterranean Costal strip near Benghazi, Libya in April, 2001. The plant material was identified by Dr. A. El-Gadi, Dept. of Botany, Faculty of Science, Al-Fateh University, Libya.

General experimental procedure:

CC silica gel Merk, 70-30 mesh. TLC silica gel 60 F_{254} precoated plates (E-Merk, Germany). Agar (Microbiologic, nutrient agar. 20 g/L, PH= 7.0 + 0.2, Germany). UV spectra: UV-visible spectrophotometer (UV-1601 PC. Schimadzu, Japan). IR spectra: Nicolet, Mx-1 FT-IR spectrophotometer, USA. ^{1}H-, ^{13}C-, APT- and COSY NMR spectra: XL-200 (200 MHz) spectrometer. EI-Ms: Finning MAT-96 instument equipped with a MICROVIP data system.

Extraction and isolation:

The fresh bulbs *Scilla peruviana* (1kg) was extracted with methanol till exhaustion. The methanolic extract was evaporated to dryness in a rotary evaporator at 40°C. The residue was suspended in distilled water and successively extracted with petroleum ether, ether and ethyl acetate. All the extracts gave positive test for flavonoids. The ether extract (10 g) was loaded on a silica gel packed column (5×100 cm, 200 g) then gradiently eluted with $CHCl_3$ containing increasing propotions of MeOH. Fractions of 100 ml were collected, concentrated and monitored by TLC on scilia gel G plates using $CHCl_3$–MeOH (9.5:0.5 and 9:1) as solvents systems. Similar fractions were pooled together and concentrated to give three fractions A, B, and C. Each fraction was purified on a another column of silica gel using the same eluting system to affored pure compounds **1** (2 g), **2** (1.5 g), and **3** (0.7 g).

The fresh bulbs of *S. villosa* (100 g) and *S. numedica* (100 g) were extracted separately with methanol till exhaustion. The extract, in each case was fractionated following the previously mentioned procedure and was used for comparison with that of *Scilla peruviana* and the isolated compounds.

Anti-microbial activities:

The anti-bacterial and anti-fungal activity was assessed using agar diffusion method [27]. The strains used were *Staphylococcus aureus* NTCC 6538 (Gram positive), *Escherichia coli* NTCC 10536 (Gram negative) and *Candida albicans* GDH 2346 (fungus). Nutrient agar plates were seeded using 0.1 ml of diluted organisms (a plate for each strain). Cylindrical plugs were removed from agar plates using a sterile cork borer and 50 µl of the tested compounds (20 mg/ 500 µl) as well as of standard antibiotics and blank solvent (Dimethyl formamide) were added to each well in the plates which were kept in the incubator at 37°C for 24 hours. The diameters of the inhibition zones were measured in mm. Table (4) and Chart (1) reveals the results of this test.

Anti-inflammatory activity:

Anti-inflammatory activity was assessed by formalin-induced paw oedema test [28] using male Fisher rats (150-200 gm). Animals were divided into eight groups, each consisted of five rats. The first group (control) received normal saline, the second group received diclofenac sodium (10 mg/kg i.p.). Groups 3-5, each group, received one of the tested three compounds (300 mg/kg i.p.). The remaining groups (6-8) received one of the ether extracts of the studied plants (300 mg/kg i.p.). One hour after drug administration, oedema was induced by subplantar

injection of 0.2 ml of 2% formalin in the left hand paw. The right paw was injected with 0.2 ml saline. Ten hours after formalin injection rats were killed by spinal dislocation. The right and left paws were cut at the tibiotarsal articulation and weighted. The percentage increase in weight of the formalin injected left paw in comparison with the saline injected right one of each rat was used as an indicator of the inflammation produced, and was calculated as follows:

percentage increase in weight = L-R / R × 100

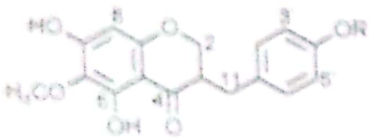
where L is the weight of left paw and R is the weight of right paw.

The results are cited in Table (5).

Characterization of the isolated compounds:

Compound **1** occurred as yellow needles (MeOH). It gave a yellow color after spraying with NaOH reagent. R_f 0.78 [CH_2Cl_2 – MeOH (9.5:0.5)]. UV λ_{max} (MeOH) 339 and 293 nm, +$AlCl_3$ 381 and 314 nm, +$AlCl_3$/Hcl 382 and 314 nm. IRν_{max} (KBr disc) 3450 (OH), 3057, 2985, 1631 (hydrogen bonded CO), 1443, 1377, 1305, and 1251 cm^{-1}. ^{1}H-NMR spectral data (200 MHz, DMSO-*d6*), Table (1) and ^{13}C-NMR data (95 MHz, DMSO-*d6*), Table (2).

Compound **2** occurred as a dark yellow powder. It gave a yellowish- orange color with NaOH spray reagent. R_f 0.45 [CH_2Cl_2–MeOH (9.5:0.5)]. UV λ_{max} (MeOH) 320 and 293 nm, +$AlCl_3$ 390 and 314 nm, +$AlCl_3$/Hcl 389 and 313 nm. IRν_{max} (KBr disc) 3400 (OH), 3004, 2935, 1633 (hydrogen bonded CO), 1443, 1378, 1313, and 1158 cm^{-1}. ^{1}H-NMR spectral data (200 MHz, DMSO-*d6*) Table (1) and ^{13}C-NMR spectral data (95 MHz, DMSO-*d6*) Table (2).



1R = CH_3
2 R = H

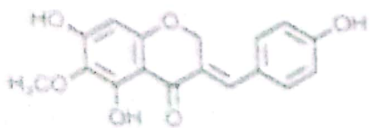


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Table (1): ^{1}H-NMR spectral data of the isolated compounds.

Proton at C-atom	Compound 1	Compound 2	Compound 3
H-2	4.15, dd (11, 8), 4.33, dd (11, 4.4)	4.14, dd (11, 8.2), 4.33, dd (11,4)	4.12, d (5.2)
H-3	5.10, (m)	5.09, (m)	—
H-8	6.18, (s)	5.98 (s)	6.01 (s)
H-11	2.65, dd (13, 8.6), 3.02 (m)	2.59, dd (12.8,8.8), 2.99 (m)	5.40 (s)
H-2'&H-6'	7.15, d (8.4)	7.08, d (8.2)	7.35, d (8.6)
H-3'&H-5'	6.86, d (8.4)	6.74, d (8.2)	6.90, d (8.6)
6-OCH$_3$	3.63 (s)	3.63 (s)	3.65 (s)
4'-OCH$_3$	3.72	—	—

Compound **3** occurred as a yellowish-brown amorphous substance. It gave a deep yellow color with NaOH spray reagent. R_f 0.44 [CH_2Cl_2- MeOH (9.5:0.5)]. UV λ_{max}(MeOH) 364, 296, and 221 nm, +$AlCl_3$ 398 and 317 nm, +$AlCl_3$/Hcl 395 and 316 nm. IRν_{max} (KBr disc) 3450, 2950, 1660 (hydrogen bonded CO), 1590, 1500, 1430, 1160, and 1012 cm^{-1}. ^{1}H-NMR spectral data (200 MHz, DMSO-*d6*) Table (1) and ^{13}C-NMR data (95 MHz, DMSO-*d6*) Table (2).

Table (2): ^{13}C-NMR spectral data of the isolated compounds.

Carbons	Compound 1	Compound 2	Compound 3
2	69.8	69.3	67.6
3	45.6	45.8	132.1
4	198.3	198.5	185.1
5	154.7	154.8	153.8
6	128.6	128.6	126.5
7	159.4	159.3	160.1
8	96.2	96.2	96.5
9	160.3	160.3	160.6
10	101.6	101.5	102.1
11	31.1	31.3	137.2
1'	130.2	128.4	125.1
2'	130.4	130.3	133.2
3'	114.1	115.6	116.2
4'	158.3	156.4	159.9
5'	114.1	115.6	116.2
6'	130.4	130.3	133.2
OCH$_3$ at 6	60.6	60.6	60.7
OCH$_3$ at 4'	55.0		

Table (3): Comparison of the ether extracts of the studied plants.

Compound	*S. villosa*	*S. Peruviana*	*S. numedica*
1	+	++	-
2	+	+++	+
3	++	+++	+

Table (4): Anti-microbial activities of the isolated compounds in comparison with other antibiotics.

Tested compounds	Inhibition zones in mm		
	Staph. Aureus	*E. coli*	*Cand. Albicans*
Compound 1	23	20	13
Compound 2	25	18	15
Compound 3	26	21	13
Gentamycin 30	16	12	10
Kanamycin 30	19	15	—
Augmentin	—	8	—
Ceftriaxone 75	10	14	11
Ofloxacin 10	30	19	13
Pefloxacin 5	18	18	—
Clotrimazol	—	—	18

Chart (1): Anti-microbial activities of the isolated compounds in comparison with other standards antibiotics.

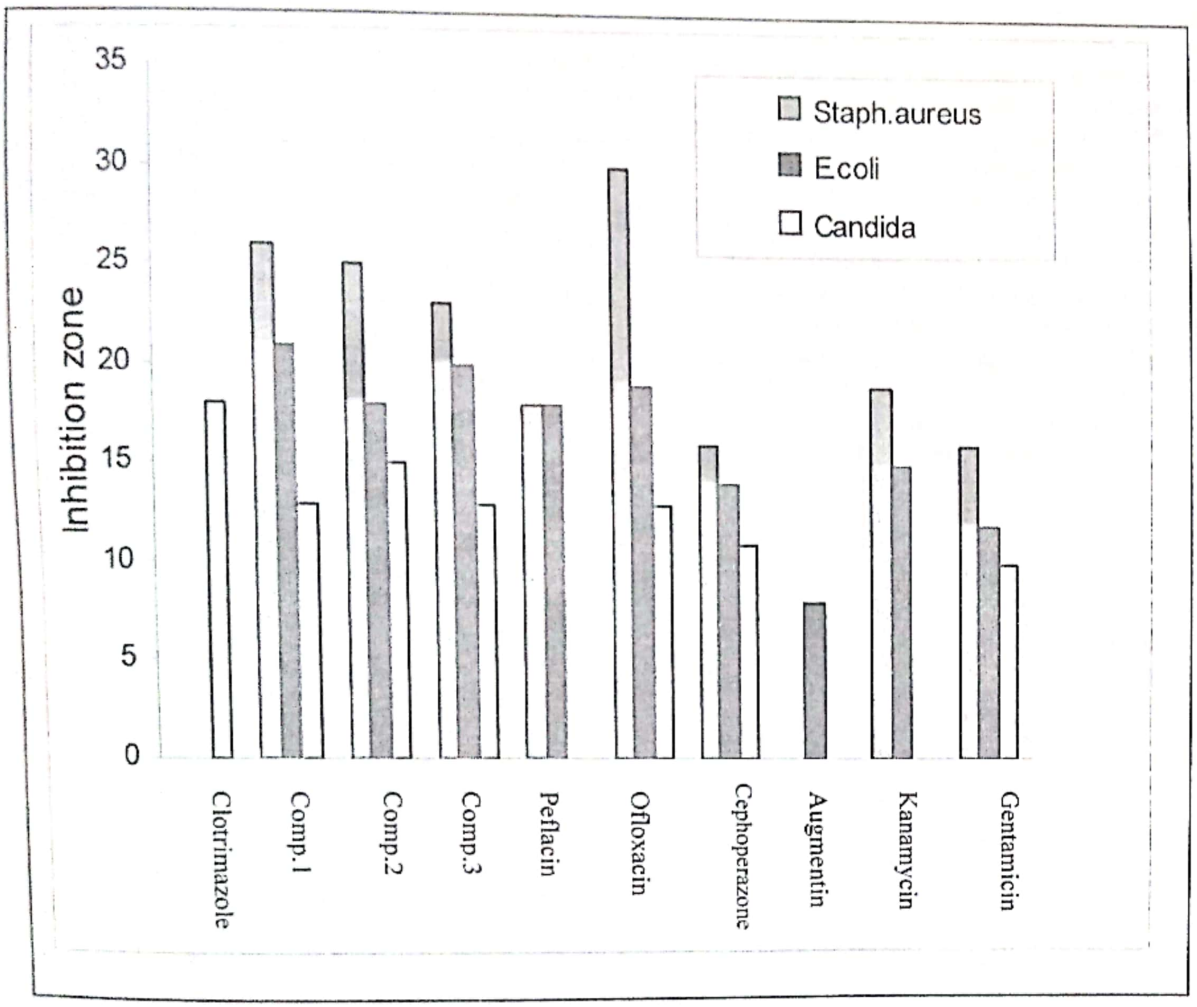

Table (5): Effect of injection of the tested materials in a model of formalin-induced rat Paw oedema.

Test groups	Saline injected paw (gm)	Formalin injected paw (gm)	% increase in weight
Control (saline)	1.25 ± 0.115	1.76 ± 0.11	41.30 ± 3.72
Diclofenac sodium	1.11 ± 0.93	1.37 ± 0.11	23.40 ± 2.10*
Compound 1	1.18 ± 0.55	1.67 ± 0.12	40.48 ± 3.80
Compound 2	1.21 ± 0.51	1.67 ± 0.13	39.94 ± 2.50
Compound 3	1.20 ± 0.43	1.67 ± 0.19	39.73 ± 3.70
Ether extract of *S. peruviana*	1.23 ± 0.13	1.58 ± 0.15	28.30 ± 3.10*
Ether extract of *S. villosa*	1.27 ± 0.12	1.61 ± 0.21	25.40 ± 5.56*
Ether extract of *S. numedica*	1.23 ± 0.098	1.68 ± 0.19	37.28 ± 4.30

Data are expressed as a means ± S.D n (number of rats in each groups) = 5

* Significantly different from control group at $P < 0.05$ using student "t" test for unpaired data.

DISCUSSION

Compounds **1**, **2**, and **3** gave a yellow color with NaOH indicating their flavonoidal nature. The negative EI-MS of compound **3** showed a molecular ion peak $(M-1)^-$ at *m/z* 313 indicating that its molecular weight is 314 which is consistent with the molecular formula $C_{17}H_{14}O_5$. The analysis of both ^{13}C- and DEPT-NMR suggesting the presence of 17 carbon atoms, one OCH_3 group, one CH_2, six CH and nine quaternally carbons atoms which could be assigned to methoxylated 3-benzylidenechroman-4-one system (homoisoflavone). The 3 proton singlet at δ 3.65 (s) in the ^1H- and the signal at δ 60.7 in the ^{13}C-NMR were assigned for the OCH_3 group. H-8 appeared as a singlet at δ 6.01 indicating that positions 6 and 7 were substituted. The appearance of H-2 as a doublet at 4.12 ppm (J=5.2 Hz) and H-11 as a singlet at 5.4 ppm confirm the results. The two coupled doublets, each integrated for two protons were assigned for H-2'/H-6' and H-3'/H-5' of ring B. These data were almost identical to those reported for 5, 7- dihydroxy-6-methoxy-3-(4-hydroxybenzylidene) chroman-4-one (autumnalin) isolated from the bulbs of *Eucomis autumnalis* and *Colchicum doerfleri*[25,26].

^{13}C-NMR and DEPT spectra of compound **2** displayd signal for one CH_3, 2 CH_2, 6 CH and 8 quaternary carbons. Its positive EI-MS displayed molecular ion peack $(M+1)^+$ at *m/z* 317 and negative EI-Ms $(M-1)^-$ at *m/z* 315 indicating that its molecular weight is 316 and consistent with the molecular formula $C_{17}H_{16}O_6$. The previous data suggested 3-benzyl chroman-4 one (homoisoflavanone) with 3 OH substitutions and one OCH_3. Its ^1H-NMR spectral data (Table 1) indicated that ring B is substituted at position 4' as its protons appeared as two coupled doublets, each integrated for 2 protons. The ^1H-NMR singlet at δ 5.98 assigned for H-8 indicated that positions 6 and 7 are oxygenated. The methoxy group is located at position 6 as it appeared downfield shifted at δ 60.6 (orthodioxygenated). Therefore, compound **2** was identified as 5, 7- dihydroxy-6-methoxy-3-(4-hydroxybenzyl) chroman-4-one. This was confirmed by comparison with the data of the same compound isolated from *Eucomis autumnalis*[26] and *Scilla draconontana*[16].

EI- Ms of compound **1** displayed $(M+1)^+$ and $(M-1)^-$ at *m/z* 331 and 329 respectively indicating that its molecular weight is 330 (14 mass units higher than compound **2**). ^{13}C-NMR and ^1H-NMR of compound **1** is almost identical to that of compound 2 except for the appearance of extra methoxy signal. This methoxy group was assigned to position 4' as indicated by the downfield shift of C-4' at δ 158.3 instead of 156.4 in compound **2** and this was confirmed by the increase of molecular weight by 14 amu than that of compound **2**. So, compound **1** is considered to be the methoxy derivatives of compound **2** and was identified as 5,7-dihydroxy-6-methoxy-3-(4-methoxybenzyl) chroman-4-one. This was confirmed by comparison with the data of the same compound isolated from *Scilla draconontana*[16].

Comparative TLC investigation of the ether extacts of the three *Scilla* species revealed highest percentages of the three isolated homoisoflavanones in *Scilla peruviana*. Compound **1** was absent in *Scilla numedica* (Table 3).

Anti-microbial activities:

The isolated compounds showed anti-bacterial activity comparable to antibiotics used in this study except ofloxacin, which was slightly more potent than the tested compounds.

In addition, all compounds showed variable anti-fungal activity, and compound **2** has the most potent anti-fungal activity compared with compounds **1** and **3**.

In conclusion the isolated compounds have potent anti-bacterial activity against both Gram positive and Gram negative microorganisms. In addition compound **2** has a strong anti-fungal activity.

Anti-inflammatory activities:

According to the percentage increase in paw weight, the ether extract of *S. peruviana and S. villosa* had anti-inflammatory activity (28.3% and 25.4% respectively) comparable with that of diclofenac sodium (23.4%). It is recommended to follow up the investigation of the anti-inflammatory activity of the ether extracts of these plants in other models of inflammation.

Acknowledgment

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هوموأيزوفلافانونات من نباتات السيلا (العائلة الزنبقية)

منى جودة زغلول – حسنى عبد الفتاح عبد الرحمن – أحمد محمد زغلول

قسم العقاقير – كلية الصيدلة – جامعة المنصورة – المنصورة – مصر

أدت الدراسة الكيميائية لخلاصة الاثير لنباتات سيلا بيروفيانا ، وسيلا فيلوزا ، وسيلا نيوميديكا الى فصل ثلاثة من الهومو أيزوفلافانونات ١ ، ٢ ، ٣ . وقد تم التعرف عليهم بواسطة خواصهم الطبيعية والطيفية ، وهم ٧،٥-ثنائى هيدروكسى-٦-ميزوكسى-٣-(٤-ميزوكسى بتريل)كرومان-٤-أون ، ٧،٥-ثنائى هيدروكسى-٦-ميزوكسى-٣-(٤-هيدروكسى بتريل) كرومان-٤-أون ، ٧،٥-ثنائى هيدروكسى-٦-ميزوكسى-٣-(٤-هيدروكسى بتريليدين) كرومان-٤-أون . وقد أظهرت المركبات المفصولة قدرة على مقاومةنشاط البكتريا موجبة جرام والبكتريا سالبة جرام ، وكذلك قدرة على مقاومة نشاط الفطريات . بالإضافة الى ذلك فقد أظهرت خلاصة الاثير لنباتى سيلا بيروفيانا وسيلا فيلوزا نشاط مضاد للالتهاب .