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Phytochemical investigation of Caralluma wissmannii O. Schwart

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ABSTRACT

The chromatographic investigation of Caralluma wissmannii O. Schwart. led to the isolation of two new pregnane glycosides (**5, 6**) and five known compounds (**1-4, 7**). The new pregnane glycosides were identified using spectral means (NMR and MS) as 12-tigiloyl-tayloron 3β-D-thevetopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside **5**, 12-tigiloyl-tayloron 3β-D-thevetopyranosyl-(1→4)-β-D-cymaropyranoside **6**. The known compounds were identified as 3,4-seco-lup-20(29)-en-3-oic acid methyl ester **1**, lupeol **2**, stigmasterol **3**, β-sitosterol **4**, and luteolin 3',4'–di-O-β-D-glucopyranoside **7**. This is the first report on the phytochemical investigation of C. wissmannii.

Keywords: Asclepiadaceae, Caralluma wissmannii, seco-triterpenoid, pregnane glycosides, flavone glycoside.

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INTRODUCTION

The genus Caralluma belongs to the family Asclepiadaceae which comprises about 200 genera and 2500 species [1]. Plants belonging to the genus Caralluma are normally leafless and succulent perennial herbs [2]. Several members of the genus Caralluma are rich in pregnane glycosides or their esters which may have found medicinal uses in the treatment of rheumatism, diabetes, leprosy and as antiseptics as well as disinfectants [3, 4]. Previous studies on plants of genus Caralluma have reported the isolation of several pregnane glycosides or their esters [5-8], of which some showed antitumor activity [5, 9]. This is the first report on the phytochemical investigation of this species.

MATERIALS AND METHODS

General

IR spectra were recorded as thin film cast from $CHCl_3$, or as KBr disc and performed on Mattson 5000 FT-IR spectrometer. ^1H NMR spectra were recorded on a 500 MHz spectrometer (JEOL). Chemical shifts were given in ppm relative to TMS as internal standard.MS spectra were recorded on shimadzu MS-QP 1000 EX, Ionization energy was set at 70 eV.

Chemicals

Analytical and preparative thin layer chromatography were performed on silica gel (Kiesel gel 60, F 254, 0.25 mm and 0.5 mm thickness). Foetal bovine serum was purchased from GIBCO, UK and DMEM and RPMI-1640 media from Sigma-Aldrich chemical CO. St. Louis, USA). All solvents used are of analytical grade and were purchased from Adwic Company, Egypt.

Plant material

The aerial parts of Caralluma wissmannii O. Schwart. were collected from Amran governorate, Yemen in April 2009 and was identified by Dr. Abdulwali A. Alkhulaidi, Plant Ecology and Geography, Agricultural Research Authority, Taiz, Yemen.

Extraction and isolation

Air dried aerial parts of C. wissmannii (740 g) were extracted by maceration with methylene chloride/methanol (3:1) at room temperature for 2 days (3 X 2000 ml). The combined extracts were concentrated to 100 and diluted with water (400 ml) followed by shacking with petroleum ether (PE) and CH_2Cl_2 (3 X 250 ml, each) to give a dark green residue of PE fraction (12.18 g) and CH_2Cl_2 fraction (45.60 g).

The PE fraction was dissolved in hot MeOH (150 ml) and kept at 0^oC for 24 hrs. The solution was then filtered and the filtrate gave upon evaporation defatted PE fraction (8 g). The previous fraction (4 g) was subjected to chromatographic separation on silica gel column using

PE-EtOAc mixture with increasing polarities and fractions 50 ml each was collected. Fraction 16 (0.67 g) eluted with PE-EtOAc (13:7), was separated on TLC using benzene-EtOAc (97:3) as an eluent to give compound **1** (R_f 0.92, 8 mg), and compound **2** (R_f 0.32, 14 mg).

The methylene chloride fraction (10 g) was subjected to chromatographic separation on silica gel column using mixtures of PE-EtOAc followed by mixtures of CH_2Cl_2/MeOH as eluting systems. Fractions of 50 ml each was collected. Fraction 6-7 eluted with PE-EtOAc (7:3, 100 ml) was separated on TLC using PE-EtOAc (79:1) to give compounds **3**(R_f 0.35, 23 mg) and **4** (R_f 0.35, 23 mg). Fractions 46-48 eluted with CH_2Cl_2-MeOH (91:9, 150 ml) was separated on TLC using CH_2Cl_2-MeOH-AcOH (95:4:1) to give compounds **5** (25 mg, R_f 0.30) and **6** (R_f 0.38, 7 mg).

The remaining aqueous layer was extracted by n-butanol (3 x 250 ml). The combined n-butanol extracts were concentrated to 300 ml, mixed with diethyl ether (100 ml) then filtered. Upon evaporation of n-BuOH filtrate, a yellowish residue (3.53 g) was produced. This fraction was subjected to chromatography on a silica gel column using CH_2Cl_2-MeOH mixtures as solvent system with increasing polarity and fractions of 50 ml each were collected. Fractions 47-51eluted with CH_2Cl_2-MeOH (3:1, 250 ml) were concentrated and separated on preparative to give by TLC (silica gel, EtOAc-MeOH-H_2O, 75:14:1) compound **7** (R_f 0.77, 8 mg).

3,4-Seco-lup-20(29)-en-3oic acid methyl ester (1). White amorphous solid, EI-MS; m/z (rel. int.): 456 (0.5) $[M]^+$, 455 (7) [M-H], 416 (8), 396 (14), 369 (14), 297 (15), 279 (10), 256 (6.2), 205 (5), 189 (10), 121 (25), 95 (65), 68 (55), 57 (75); ^{1}H NMR ($CDCl_3$): δ 2.38 (1H, m, H- 19), 0.78 (3H, d, J= 7 Hz, Me-23), 0.77 (3H, d, J= 7 Hz, Me-24), 0.80 (3H, s, Me-25), 1.03 (3H, s, Me-26), 0.93 (3H, s, Me-27), 0.79 (3H, s, Me-28), 4.57 (1H, br d, H-29a), 4.68 (1H, br d, H-29b), 1.67 (3H, br s, Me-30), 3.65 (3H, s, MeO).

Lupeol (2). White amorphous solid, EI-MS; m/z (rel. int.) 426 (13) $[M]^+$, 411 [M- Me]$^+$ (4.7), 369 (12), 218 (28.7), 204 (10.7), 149 (2), 123 (25), 105 (7), 57 (7), 55 (75); ^{1}H NMR ($CDCl_3$): δ 3.19 (1H, m, H-3) 2.34 (1H, m, H- 19), 0.93 (3H, s, Me-23), 0.75 (3H, s, Me-24), 0.82 (3H, s, Me-25), 1.03 (3H, s, Me-26), 0.96 (3H, s, Me-27), 0.78 (3H, s, Me-28), 4.55 (1H, br d, H-29), 4.67 (1H, br d, H'-29), 1.67 (3H, br s, Me-30).

12 - tigiloyl - tayloron 3β – D – thevetopyranosyl - (1→4) – β – D – cymaropyranosyl - (1→4) – β – D - cymaropyranoside (5). White amorphous powder, IR, ν_{max} cm^{-1}: 3450 (OH), 1720 (C=O), 1651, 1459 (C=C), 1385, 1265, 1083; ^{1}H-NMR ($CDCl_3$, 500 MHz): see Table 1; ^{13}C-NMR ($CDCl_3$, 125 MHz): see Table 1; EI-MS, m/z (rel. int.): 910 (25) [M^+], 744 (25), 611 (20),319 (15), 273 (20), 145 (35),87 (100), 55 (100).

12-Tigiloyl-tayloron 3β-D-thevetopyranosyl-(1→4)-β-D-cymaropyranoside (6). White amorphous, IR, ν_{max}, $CHCl_3$, cm^{-1}: 3420 (OH), 2920, 1715 (C=O), 1652, 1459, 1380, 1270, 1083;^{1}H-NMR ($CDCl_3$, 500 MHz): 5.37 (1H, br s, H-6), 4.63 (1H, dd, J= 5.5, 10.9 Hz, H-12), 1.78 (3H, s, H-18), 1.11 (3H, s, H-19), 2.13 (3H, s, H-21); cymarose: 4.85 (1H, d, J= 9.2 Hz, H-1), 3.22 (1H, dd, J= 9.2, 4 Hz, H-4), 3.84 (1H, m, H-5), 1.22 (3H, d, J= 6.1 Hz), 3.44 (3H, s, MeO); thevetose: 4.34 (1H, d, J= 7.6 Hz, H-1), 3.19 (1H, t, J= 9.2 Hz, H-4), 1.31 (3H, d, J= 6.1 Hz, H-6),

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3.65 (3H, s, MeO); tigloyl: 6.75 (1H, dd, J= 7.3, 1.5 Hz, H-3), 1.77 (1H, d, J= 7.3 Hz, H-4), 1.76 (1H, s, H-5); EI-MS, m/z (rel. int.): 476 (1.0%) [M^+- Thev ring], 435 (2.5%), 364 (3.0%), 337 (3.5%), 278 (13.0%), 256 (4.0%), 197 (4.5%), 167 (13.5%), 148 (28.5%) [Thev ring – H], 83 (17.0%), 68 (25.0%), 58.0 (51.0%), 44.0 (100.0%), 42.0 (71.5%).

Table 1:NMR spectral data of compound 5.

No.	^{13}C-NMR	^{1}H-NMR	No.	^{13}C-NMR	^{1}H-NMR
1	38.8	1.05, m; 1.84, m	**Cymarose-1**		
2	29.0	1.58 (m), 1.90 (m)	1	96.1	4.83 (dd, 1.5, 9.9)
3	77.9	3.54 (br m)	2	35.6	2.03,m ; 1.53, m
4	38.8	2.22 (m); 2.36 (m)	3	77.1	3.78 (br q, 2.3)
5	140.5	---	4	82.6	3.24 (dd, 3.0, 9.9)
6	117.8	5.34 (brd)	5	68.4	3.83 (dq, 6.0, 9.0)
7	34.3	2.16 (2H, m)	6	18.2	1.19 (d, 6.1)
8	74.3	---	MeO	58.1	3.41 (s)
9	43.7	1.46 (m)	**Cymarose-2**		
10	37.2	--	1	99.7	4.72 (dd, 1.5, 9.9)
11	24.1	1.78 (m), 1.88 (m)	2	35.2	2.1, m; 1.6, m
12	72.6	4.62 (dd, 5.3, 10.7)	3	76.9	3.75 (br q, 2.3)
13	58.4	----	4	82.6	3.19 (dd, 3.0, 9.9)
14	88.0	----	5	68.6	3.88 (dq, 9.0, 6.0)
15	33.4	1.95 (2H, m)	6	18.5	1.25 (d, 6.1)
16	31.9	2.76 (m), 1.83 (m)	MeO	58.0	3.39 (s)
17	91.4	---	**Thevetose**		
18	9.4	1.38 (s)	1	104.4	4.29 (d, 9.2)
19	18.6	1.09 (s)	2	74.7	3.46 (t, 8.4)
20	209.4	---	3	85.6	3.08 (t, 9.1)
21	27.55	2.11 (s)	4	74.7	3.14 (t, 8.4)
Tigloyl			5	71.7	3.34 (dq, 6.2, 9.0)
1	166.8	--	6	17.9	1.29 (d, 6.1)
2	128.6	--	MeO	60.8	3.62 (s)
3	137.9	6.72 (dd, 7.3, 1.5)			
4	14.5	1.77 (d, 7.3)			
5	12.0	1.76 (s)			

Luteolin 3',4'–di-O-β-D-glucopyranoside (7). Yellow amorphous powder. ^{1}H-NMR (DMSO): δ 6.77 (1H, s, H-3), 6.16 (1H, d, J= 1.5 Hz, H-6), 6.5 (1H, d, J = 1.5 Hz, H-8), 12.9 (1H, s, OH-5), 7.75 (1H, d, J= 2.3 Hz, H-2'), 6.94 (1H, d, J= 8.4 Hz, H-5'), 7.62 (1H, dd, J= 8.4, 2.3 Hz, H-6'), 5.09 (1H, d, J= 8.3 Hz, H-1''), 5.06 (1H, d, J= 5.35 Hz,H-1''').

In vitro cytotoxic screening

Cell lines. Erlich ascites carcinoma (EAC), Huh-7 human hepatoma and A-495 human lung cancer cell lines were obtained from National Cancer Institute, Cairo, Egypt. The cells were grown in suspension culture according to methods described by Bennett et al.,(1991) for EAC and Skehan et al. (1990) for HU-7 and A-495 cell lines. Test samples were prepared initially at concentration 1 mg/ml in DMSO.

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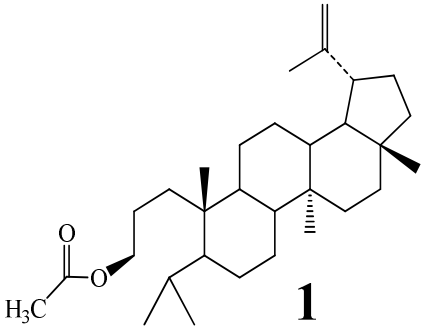
RESULTS AND DISCUSSION

The investigation of the PE, CH_2Cl_2 and n-BuOH fractions of the aerial parts of C. wissmannii afforded two triterpenoids (**1** and **2**), two steroids (**3** and **4**), a flavonoid glycoside (**7**), and two new pregnane glycosides (**5** and **6)**.



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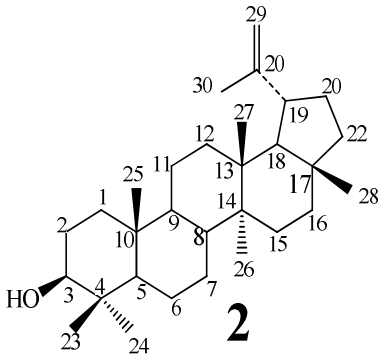

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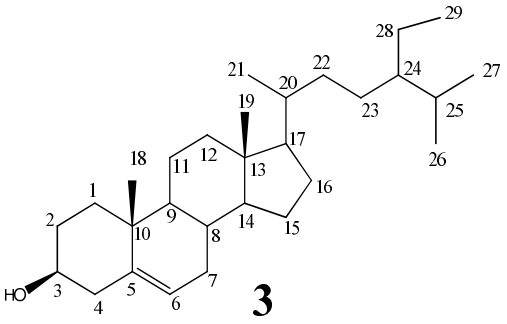

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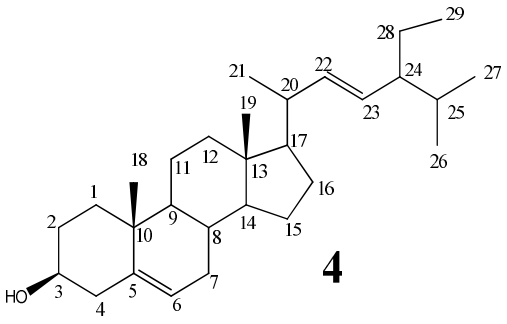

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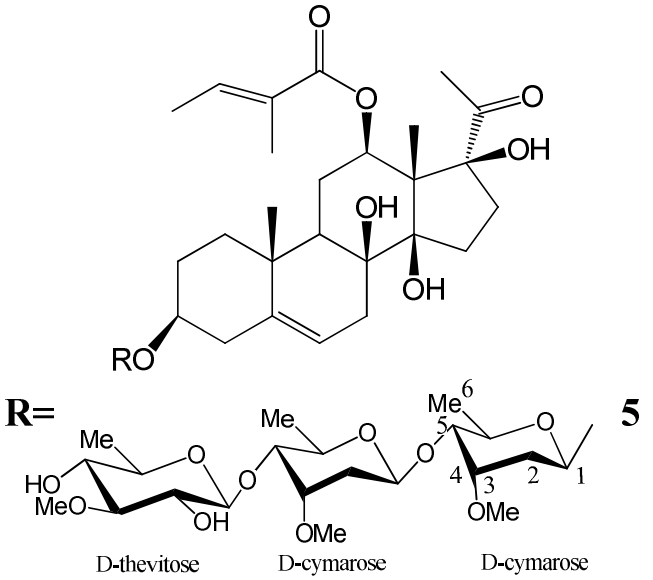

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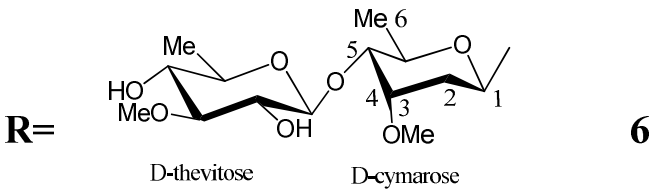

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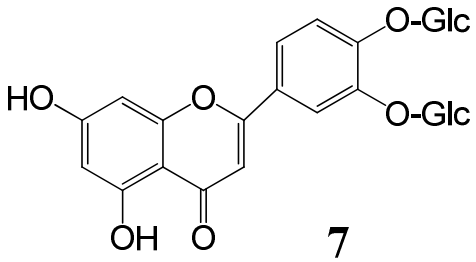

7

Compound **1** was isolated from the PE fraction as a white amorphous solid. The MS spectrum of **1** showed a molecular ion peak [M^+] at m/z 456 in accordance with molecular formula $C_{31}H_{52}O_2$. The ^{1}H NMR spectrum revealed the presences of six methyl signals in the up-field region (δ_H, 0.77, 0.78, 0.79, 0.80, 0.93, 1.03), two olefinic protons as broad singlets at δ_H 4.57 and 4.68 ppm and methyl singlet of a MeO group at δ_H 3.65, in addition to the absence of signal corresponding to H-3. This future is in accordance with structure of triterpenoid of a pentacyclic lupine structure [12, 13]. The ^{1}H NMR spectrum revealed the presence of a downfield olefinic methyl singlet at δ_H 1.67 (H-30) and a signal at δ_H 2.39 of an allylic proton (H-19). Signals of methyl groups of H-23 and H-24 appeared as doublets (J= 7 Hz) at δ_H 0.78, 0.77, respectively. Signals of the four tertiary methyl groups as singlets at δ_H 0.80, 1.03, 0.93, 0.79, were assigned for methyl groups of Me-25, -26, -27, and -28, respectively. From the aforementioned discussion and by comparison with the reported data [13], compound **1** was

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identified as 3,4-seco-lup-20(29)-en-3-oic acid methyl ester which, previously isolated from C. buchardii [13].

Compound **2** was isolated from the PE fraction as white needle crystals, m.p. 211-213^{o}C. The MS spectrum showed a molecular ion peak [M^{+}] at m/z 426 in accordance with molecular formula of $C_{30}H_{50}O$. The ^{1}H NMR spectrum of **2** showed signals similar to that of compound **1** except the presence of proton signal of H-3 which appeared at δ_H 3.18, indicating a probable triterpene structure. By comparing its spectral data with data in literature [12], compound **2** was found to be lupeol, which was isolated previously from C. buchardii [13].

Compounds **3** and **4** were identified as β-sitosterol and stigmasterol by comparing their IR data with those of authentic samples and by TLC**.**

Two new pregnane glycosides (**5** and **6**) were isolated from the CH_2Cl_2 fraction of the aerial parts of C. wissmannii through repeated column chromatography on Si gel columns. Both compounds showed positive reactions with Libermann–Buchard and Keller–Kiliani reagents, indicating the presence of a steroidal skeleton with 2-deoxysugar moiety. Spectroscopic analysis demonstrated that both glycosides had a pregnane skeleton with an acyl group at C-12 position and a straight sugar chain consisting of two or three sugar units connected to C-3 position of the aglycone.

Compound **5** was isolated as a white amorphous powder and had a molecular formula $C_{47}H_{74}O_{17}$ based on EI-MS (m/z= 910 [M^{+}] and NMR data (HMQC spectrum)**.** The IR spectrum showed a diagnostic absorption bands at 3450 (-OH), 1720 (ester, C=O) cm^{-1}. Compound **5** showed NMR features characteristic for a pregnane moiety [6-8]. The aglycone was identified as tayloron by comparison of its spectroscopic data to those in the literature [14, 15]. The ^{13}C-NMR spectrum of **5** showed the presence of signals for three tertiary methyl groups (δ_C = 27.55, 9.4 and 18.6), four olefinic carbons [δ_C = 117.8, 140.5, (H-6 and H-5, respectively) and 128.6, 137.9 (H-2 □ and H-3□, respectively)] and five oxy carbons at δ_C 77.9, 74.3, 72.6, 88.0, 91.4 assigned for C-3, C-8, C12, C-14 and C-17, respectively. The ^{1}H- and ^{13}C- NMR spectrum of **5** showed the presence of tigloyl moiety (see Table 1). This finding was further confirmed by comparing the spectral data of **1** with those reported in literature [16, 17]. The identification of tigloyl moiety was established from the ^{1}H-NMR of **5**, which showed the presence of signals at δ_H 1.77 (d, J=7.3 Hz, H-4), 1.76 (s, H-5) and 6.72 (dd, J=7.3, 1.5 Hz, H-3) due to two methyl groups and one olefinic signals, respectively and further confirmed from the ^{13}C-NMR and HMQC spectra of the corresponding carbon signals. From the analysis of ^{1}H-^{1}H COSY, HMQC and HMBC spectra, the structure of the pregnane moiety as tayloron was confirmed. The downfiled of H-12 (δ_H 4.62) and C-12 (δ_C 72.6) confirmed the acylation of OH at C-12, which further confirmed from the long range correlation between carbonyl group of tigloyl moiety and H-12 in the HMBC spectrum. From the aforementioned data, the aglycone of **5** could be determined as 12-tigloyltayloron. The ^{1}H- and ^{13}C-NMR spectra of **5** showed the presence 21 signals ascribable to a saccharide portion made up of three sugar units. The NMR (^{1}H NMR, ^{13}C-NMR, HMQC, and HMBC) spectral data of compound **5** showed that it contained three

anomeric carbon signals at δ_C 96.1, 99.7, and 104.4, correlating with anomeric protons at δ_H 4.83 (dd, J = 1.5, 9.9 Hz), 4.72 (dd, J = 1.5, 9.9 Hz), and 4.29 (d, J = 9.2 Hz), respectively, confirming the presence of three sugar units in compound **5** (see Table 1). These data together with the observation of signals for three methyl groups (δ_H 1.19, 1.25, 1.29), three methoxyl groups (δ_H 3.39. 3.41, 3.62) and two aliphatic methylene groups (δ_C 35.61, 35.18) indicated their nature as a 3-O-methyl-6-deoxyhexopyranose and two 3-O-methyl-2,6-dideoxyhexopyranose units. This was further confirmed by comparing their chemical shift values with those reported for sugar units in pregnane glycosides previously isolated from family Asclepiadaceae [6, 16]. The β-configuration of the anomeric protons was indicated from their large $J_{H1,H2}$ coupling constant (8-10 Hz) [18] (see Table 1). Thus, the sugar units were identified as cymarose and thevetose. The long-range correlation observed in the HMBC spectrum of **5** between H-1_{cym-1} (δ_H 4.83) and C-3 (δ_C77.9) of the aglycone indicated that cymarose-1 is the inner sugar moiety, while the long-range correlation between H-1_{cym-2} and C-4_{cym-1} and between H-1_{thev} and C-4_{cym-2} confirmed the identification of sugar portion as β-D-thevetopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside. From the aforementioned data, the structure of compound **5** was established as 12-tigiloyl-tayloron 3β-D-thevetopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside.

Compound **6** was isolated as a white amorphous powder and had a molecular formula $C_{40}H_{62}O_{14}$ based on EI-MS and NMR data. The NMR data of **6** were identical with those of **5**, with less of one sugar unit corresponding to cymarose. This was confirmed from the presence of only two anomeric protons at δ_H 4.85 (dd, J = 1.5, 9.2 Hz, H-1_{cym}), 4.34 (d, J = 8.0 Hz, H-1_{thev}), two proton singlets at δ_H3.44 (3H, s, MeO_{cym}) and 3.65 (3H, s, MeO_{thev}) and two proton doublets at δ_H 1.22 (3H, d, J= 6.1 Hz, H-6_{cym}) and 1.31 (3H, d, J= 6.1 Hz, H-6_{thev}). The NMR data of the aglycone part was identical with of **5** and identified as 12-tigloyltayloron. From the previous data, compound **6** was identified as 12-tigiloyl-tayloron 3β-D-thevetopyranosyl-(1→4) -β-D-cymaropyranoside.

Compound **7** was isolated as yellow amorphous powder with R_f 0.78, (Si gel, EtOAc-MeOH-H_2O, 76: 13: 1). The ^{1}H-NMR spectrum revealed the presence of a flavone skeleton with two meta-doublet signals at δ 6.16 and 6.5 (J= 1.5 Hz, H-6, H-8), one singlet at δ 6.77 ascribable to H-3, and an ABX system of ring B [δ 6.94 (d, J= 8.4 Hz, H-5'), 7.62 (dd, J = 8.4, 2.3 Hz, H-6') 7.75 (d, J = 2.3 Hz, H-2')]. These data were found consistent with the data of luteolin aglycon [19]. The ^{1}H-NMR spectrum revealed the presence of two sugar residues, with two anomeric proton signals appeared at δ_H 5.09 ppm and 5.06 ppm. The H-NMR data indicated the presence of two glucopyranose moieties. On comparing the data of **7** with those reported in literature, copmpound **7** could be identified as luteolin 3',4'-di-O-β-D-glucopyranoside, previously isolated from C. negevensis [20].

The CH_2Cl_2 fraction did not show any cytotoxic activity up to 500 µg/ml against Huh-7 and A-49 cell lines and weak activity against EAC at 50 µg/ml (37.5% inhibition).

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