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Sesquiterpene Lactones from *Achillea fragrantissima*

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Abstract

In continuation of our chemical investigation on some medicinal plants of the genus *Achillea*, chromatographic investigation of the methylene chloride/methanol (1:1) extract of the air-dried aerial parts of *Achillea fragrantissima* (family Asteraceae) afforded seven sesquiterpene compounds (**1**-**7**). Herein, we report the carbon data for the metabolites **1, 2, 6** and **7** for the first time. Also, revise the structure of the published compound **2**. Structures were elucidated by 1D and 2D spectroscopic analyses.

Kew Words: Achillea fragrantissima; Compositae; Sesequiteterpene Lactones

1. Introduction

Sinai Peninsula is one of the important centres of medicinal plants in the Arabian deserts [1]. The distribution, utilization in folk medicine, and active constituents of medicinal plants in Sinai have attracted the attention of many ecologists, taxonomists and phytochemists [2-7]. Geographically, environmental unique ecosystem giving rise to great diversity in landforms, rock units, water resources, and aridity conditions, as well as, very cold winters, hot summers and relatively low precipitation and high evaporation [8]. The genus, *Achillea*, belongs to Asteraceae (Compositae), contains around 130 flowering species [9]. *Achillea* species have been used in folk medicine and sold in herbal shops [10]. Herbal teas prepared from some *Achillea* species are traditionally used for abdominal pain and flatulence in different countries [10]. An infusion of the dry or fresh flowering herb is used by the Bedouin for the treatment of coughs, aromatic bitter stomachic and anthelmintic and It is also used as a tea bag for kidney inflammation as well as a carminative [11, 12]. *Achillea fragrantissima*, locally named in Egypt as Qaysum, It is globally distributed in Egypt, Libya, Palestine, Syria, Saudi arabia and Iraq [8,13,14]. Many phytochemical and pharmacological studies have been performed to reveal the importance of *A. fragrantissima* [9]. Essential oils, flavonoids, terpenoids and glucosides were investigated from *A. fragrantissima* [15-26].

2. Experimental

2.1. General experimental procedures

The following instruments were used to obtain physical data: FAB-MS and HR-FAB-MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JEOL JNM-ECA600 (600 MHz) spectrometers; ^{13}C-NMR spectra, JEOL JNM-ECA600 (125 MHz) spectrometers with tetramethylsilane as an internal standard; HPLC detector, Shimadzu RID-10A refractive index detector; and HPLC columns YMC-Pack ODS-A (YMC, Inc., 250 x 4.6 mm i.d.) and (250 x 20 mm i.d.) were used for analytical and preparative purposes, respectively. The following experimental materials were used for chromatography: normal-phase silica gel column chromatography, silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150–350 mesh); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100–200 mesh); TLC, pre-coated TLC plates with Silica gel 60F_{254} (Merck, 0.25 mm) (ordinary phase) and silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reversed phase); and detection was achieved by spraying with 10% H_2SO_4 followed by heating.

2.2. Plant material

Achillea fragrantissima plants were collected in June 2019, from North Sinai, Egypt and aerial parts air-dried. A voucher specimen has been deposited in the Herbarium of St. Katherine protectorate, Egypt (voucher ID SK-164). Collection was takes place under the permission of Saint Katherine protectorate for

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scientific purposes through officially letter obtained from the National Research Centre**.**

2.3. Extraction and isolation

Aerial parts (1.8 kg) of *A. fragrantissima* were powdered and extracted with CH_2Cl_2-MeOH (1:1) at room temperature. The extract was concentrated *in vacuo* to obtain a residue of 122 g. The residue was fractionated on a silica gel column (6 x 120 cm) eluting with *n*-hexane (3000 ml) followed by a gradient of *n*-hexane-CH_2Cl_2 up to 100 % CH_2Cl_2 and CH_2Cl_2-MeOH up to 50 % MeOH (3000 ml each of the solvent mixture). The *n*-hexane- CH_2Cl_2 (1:2) fraction (200 mg) was chromatographed on a Sephadex LH-20 column (3 x 90 cm) eluted with *n*-hexane-CH_2Cl_2-methanol 7:4:0.25 (3 L). Fractions were obtained and combined into two main portions: A (80 mg), and B (100 mg). Sub-fraction A was re-purified by reversed-phase HPLC using MeOH/H_2O (65–30% 2500 mL) to afford compound **4** (22 mg). The 100 % CH_2Cl_2 fraction (300 mg) was chromatographed on a Sephadex LH-20 column (3 x 90 cm) eluted with *n*-hexane-CH_2Cl_2-methanol 7:4:0.5 (3000 mL). Fractions were obtained and combined into two main portions: A (120 mg) and B (130 mg). Sub-fraction A was re-purified by reversed-phase HPLC using MeOH/H_2O (45–55%, 2500 ml) to afford **1** (23 mg), **6** (40 mg) and **7** (16 mg). Sub-fraction B was re-purified via reversed-phase HPLC using MeOH/H_2O (40–60%, 1.5 L) to afford **2** (22 mg), **3** (16 mg), **5** (12 mg).

3. Results and Discussion

As a part of a continuing search for biologically active metabolites from Egyptian medicinal plants belonging to the Compositae family, a CH_2Cl_2:MeOH (1:1) solvent extract of aerial tissue of *A. fragrantissima* was subjected to normal and reverse phase chromatography to yield seven metabolites (Fig. 1).

Compound **1** was isolated as colorless oil, and showed a molecular ion peak at [M+1] at *m/z* 307 in the FAB mass spectrum, in accord with the molecular formula $C_{17}H_{22}O_5$. An interesting peak appeared at *m/z* 247 (20%) was due to elimination of a CH_3COOH molecule from the $[M]^+$, suggesting the presence of one acetoxy group (1735 cm^{-1}). Two strong peaks appeared at *m/z* 229 (45%) and 201 (43%) resulted from the elimination of water molecule and CO group respectively. The structure of compound **1** was determined from careful investigation of the 1D and 2D NMR measurements. The ^{1}H-NMR spectrum showed a multiplet signals at δ_H 2.16 and 2.46, H-2, which showed clear correlations in ^{1}H-^{1}H COSY spectrum with a doublet of doublets signal at δ_H 5.10 (J = 6.2, 10.32 Hz, H-3) and with a doublet signal at δ_H 4.42 (J = 10.32 Hz, H-1). No H-7 signal was observed and H-6 was appeared as a doublet signal at relatively down filed at δ_H 5.42 (J = 10.32 Hz), showed strong correlation with a doublet signal at δ_H 4.75 (J = 10.98 Hz, H-5), in the ^{1}H-^{1}H COSY spectrum. The appearance of the two doublets at δ_H 4.42 and 4.75 (H-1, H-5) and the presence of the homoallylic coupling between H-6 and H-13, indicating that compound **1** was a glaucolide.

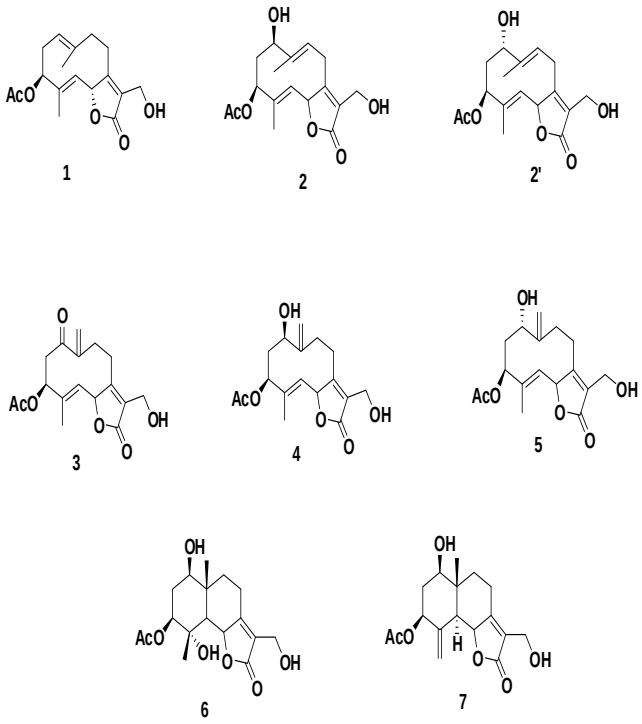
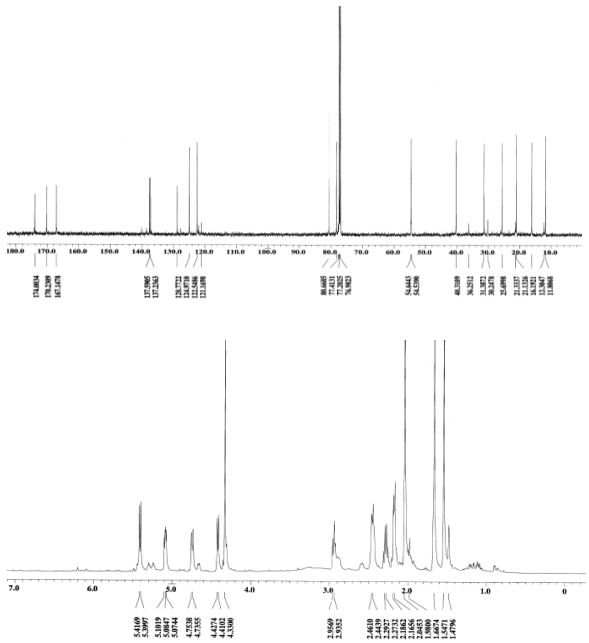


Fig. 1. Isolated compounds from *A. fragrantissima*

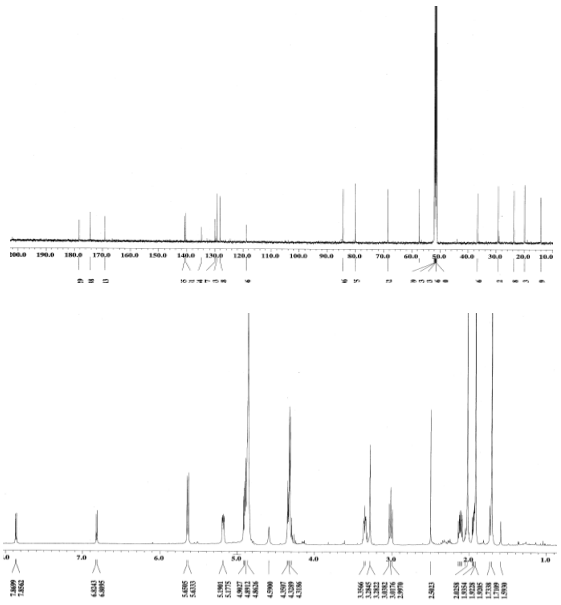
Furthermore, The ^{1}H-NMR spectrum revealed the presence of four singlet signals at δ_H 4.33, 1.55, 1.67 and 2.4 for H-13, H-14, H-15 and the methyl of acetate respectively. The molecular formula of $C_{17}H_{22}O_5$ was also confirmed by ^{13}C-NMR and DEPT analysis. The ^{13}C-NMR spectrum exhibited seventeen carbon signals were classified by a DEPT as follows: six quaternary carbon signals at δ_C 137.59, 167.15, 137.24, 170.23, 128.77 and 174.00 for C-4, C-7, C-10, C-11, C-12 and the carbonyl of the acetate group; four methine carbon signals at δ_C 124.96, 78.22, 122.54, 80.67, for C-1, C-3, C-5 and C-6; four methylene carbon signals at at δ_C 31.39, 26.10, 40.32, 54.64 for C-2, C-8, C-9 and C-13 and three methyl carbon signals at at δ_C 16.20, 12.81, 21.49 for C-14, C-15 and methyl of acetate. All ^{1}H and ^{13}C-NMR resonances were assigned using HMQC, and HMBC measurements of **1**. The connectivity of the partial

moieties and the position of the acetyl group were established by the HMBC spectrum of **1**. The correlation in the HMBC experiments, between the resonances of H_3-14 with C-1, C-9, C-10 and H_3-15 with C-3, C-4, C-5, resulted in the two methyls directly bonded to C-10 and C-4 respectively. Clear correlation was observed between H-3 with the carbonyl of the acetate led to the disposition of the acetate group on C-3. The correlations between H-6 with C-4, C-5, C-7, C-11 and C-12, supported the lactonization $C_{6,12}$. Moreover, the HMBC experiments showed the following correlations: H-1 with C-9, C-14; H-2 with C-1; H-5 with C-3, C-6; H-8 with C-6, C-7, C-9, C-12. These couplings established the C-C bonds from C-1 to C-12. The relative stereochemistry of **1** was assigned on the basis of the study of chemical shifts, values of coupling constants ($J_{5,6}$ = 10.3 Hz, $J_{3,2}$ = 6.2 Hz, $J_{3,2'}$= 10.3 Hz) and by comparing with the published data [27]. Therefore, compound **1** was assigned to 13-hydroxy-3*β*-acetoxygermacra-1(10)*E*,4*E*,7(11)-trien-12,6*α*-olide.



^{1}H-NMR and ^{13}C-NMR Spectrum of Compound **1**

Compound **2** was isolated as colourless oil. Its FABMS spectrum exhibited a significant molecular ion peak [M+H]+ at *m/z* 323, consistent with the formula molecular for $C_{17}H_{22}O_6$. The fragment ions at *m/z* 263 and 245 were due to elimination of HOAc and H_2O molecules respectively, suggesting that compound **2** contains acetoxy and hydroxyl group. The results of **2** clearly showed that again glaucolide was present. This was supported by the ^{13}C-NMR spectra (Table 1). A comparison of the ^{1}H and ^{13}C-NMR data with those of **1** (Table 1), suggests that the structures of **1** and **2** are very close and the new hydroxyl group in **2** is at C-1 and the double bond between 9/10 instead of 1/10 in compound **1**. The ^{13}C-NMR spectroscopic data revealed the presence of 17 carbon atoms classified by DEPT experiments as follows: four oxygenated carbons at δ_C 68.69 (C-19), 80.02 (C-3), 84.45 (C-6), 51.91 (C-13); two oleifinic methine carbos at δ_C 129.42 (C-5), 128.02 (C-9); two methylene carbons at δ_C 36.62 (C-2), 29.20 (C-8); four quaternary carbons at δ_C 140.42 (C-7), 142.42 (C-10), 169.25 (C-11), 178.29 (C-12), 174.44 (OAc). The remaining carbons are listed in Table 1. The connectivities of the moieties, positions of hydroxyl group, location of the double bond between 9/10 and the lactonization were established by the HMBC spectrum. The most important correlations were found between H-14 (δ_H 1.71) with C-1(δ_C 68.69), C-9 (129.02), C-10 (142.42); H-15 (δ_H 1.92) with C-3 (δ_C 80.02), C-4 (142.86); H-13 (δ_H 4.35) with C-11(δ_C 129.25), C-12 (178.29); and H-3 (δ_H 4.90) with OAc (δ_C 174.44).

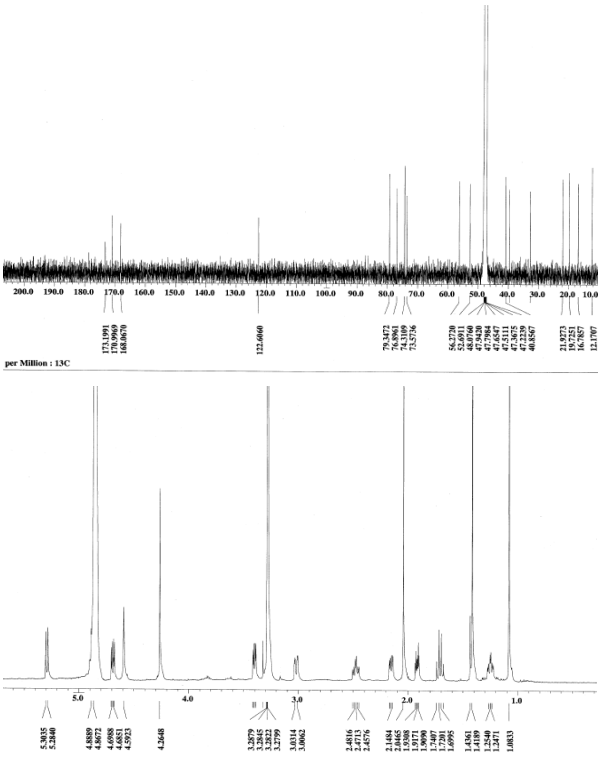


^{1}H-NMR and ^{13}C-NMR Spectrum of Compound **2**

The relative stereochemistry of **2** was established from chemical shift, coupling constants and comparison with published data. H-1 appeared as a doublet at (δ_H 4.65, *J*= 10.32 Hz), while H-2 appeared as doublet doublet doublet at (δ_H 2.12, *J*= 6, 10.4, 13 Hz), indicating the β-configuration of the hydroxyl group at C-1, in agreement with the published data of

compound **2'** which mistakenly drawn in the previously published paper [28]**.** Compound **2** was assigned as 13-O- desacetyl- 1β-hydroxy-afraglaucolide.

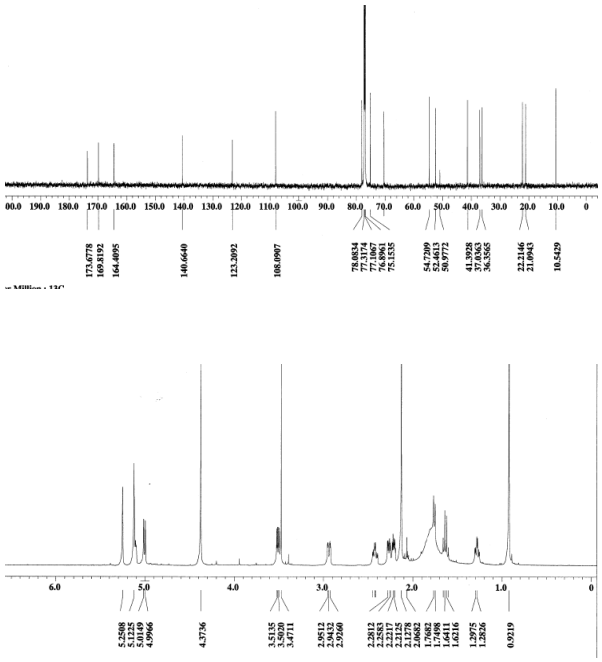
Complete structural information was obtained from ^{1}H-NMR, ^{13}C-NMR, ^{1}H-^{1}H COSY, HMQC, HMMC and MS spectra of compound **6.** Its FABMS showed a molecular ion peak at $[M+H]^+$ and $[M+Na]^+$ at *m/z* 341 and 363, establishing the elemental composition $C_{17}H_{25}O_7$ and $C_{17}H_{24}O_7Na$, confirming the molecular formula of **6** is $C_{17}H_{24}O_7$. Two fragments were appeared at *m/z* 323 and 281 resulting from loss of water and acetic acid molecules, respectively. The upfiled shift of the methyl signals and of H-5 in the ^{1}H-NMR spectra, indicating the presence of eudesmanolides. The ^{13}C-NMR and DEPT experiments revealed the presence of 17 carbon signals classified



^{1}H-NMR and ^{13}C-NMR Spectrum of Compound **6**

as: five oxygenated carbon signals at δ_C 74.31 (C-1), 76.90 (C-3), 73.57 (C-4), 79.35 (C-6), 52.69 (C-13); three methylene carbon signals at δ_C 33.00 (C-2), 41.00 (C-8), 25.87 (C-9); three methyl carbon signals at δ_C 16.13 (C-14), 20.73 (C-15), 23.70 (CH_3 of OAc). The remaining carbons are listed in Table 1. Again, the connectivities of the moieties, location of the hydroxyl groups and the lactonization were established from HMBC spectrum, which showed correlations between: H-13 (δ_H 4.30) with C-7 (δ_C 168.07), C-11 (122.60), C-12 (173.20); H-14 (δ_H 1.10) with C-1 (δ_C 74.31), C-5 (56.27), C-9 (41.00). From above data, compound **6** was assigned as 3β-acetoxy-1β, 4α, 13-trihydroxy eudesm-7(11)-en-6α,12-olide [24].

The ^{1}H-NMR and ^{13}C-NMR spectral data of compound **7**, indicated that the structure of compound **7** is very close to compound **6**, except the presence of a double bond between 4/15 instead of methyl and



^{1}H-NMR and ^{13}C-NMR Spectrum of Compound **7**

hydroxyl groups at C-4 in compound **6**. ^{13}C-NMR spectral data revealed the presence of 17 carbon atoms while their multiplicities (by DEPT analysis) confirmed the number of atoms of the formula. The carbons were assigned as: two methyls, five methylene, four methine and six quaternary carbon atoms. Moreover, all proton and carbon signals were determined by ^{1}H-^{1}H COSY, HMQC and HMBC. The position of the hydroxyl groups, acetoxy group, location of the double bond and lactonization were established by HMBC spectrum. Strong correlations were observed between H-14 (δ_H 0.92) with C-1 δ_C (75.14), C-5 (52.46), C-9 (36.35), C-10 (41.39); H-5 (δ_H 1.77) with C-1 (δ_C 75.14), C-3 (70.41), C-4 (140.06), C-6 (78.08), C-10 (41.31), C-14 (10.54), C-

15 (108.09); H-13 (δ_H 14.37) with C-7 (δ_C 164.41), C-11 (123.21), C-12 (173.68); H-15a,b (δ_H 5.12, 5.25) with C-3 (δ_C 70.41) and C-5 (52.46). From all above data, compound **7** assigned as 13-O desacetyl-eudesma-afraglaucolide [28].

The structures of the known compounds **3**, **4** and **5** could be easily deduced from their NMR data and comparison with the published compounds [24, 28]. A plausible biosynthesis pathway of the isolated compounds was suggested.

Table 1. ^{13}C- NMR data of compounds (**1, 2, 6, 7**) at 125 MHz ($CDCl_3$).

δ_C	1	2	6	7
C-1	124.96, d	68.69, d	74.31, d	75.14, d
C-2	31.39, t	36.62, t	33.00, t	37.03, t
C-3	78.22, d	80.02, d	76.90, d	70.41, d
C-4	137.59, s	142.86, s	73.57, s	140.06, s
C-5	122.54, d	129.42, d	56.27, d	52.46, d
C-6	80.67, d	84.45, d,	79.34, d	78.08, d
C-7	167.15, s	169.25, s	168.07, s	164.41, s
C-8	26.10, t	29.20, t	41.00, t	22.21, t
C-9	40.32, t	128.02, t	25.87, t	36.35, t
C-10	137.24, s	142.42, s	40.00, s	41.39, s
C-11	128.77, s	140.42, s	122.60, s	123.21, s
C-12	174.00, s	178.29, s	173.20, s	173.68, s
C-13	54.64, t	51.91, t	52.89, t	54.71, t
C-14	12.81, q	14.06, q	16.13, q	10.54, q
C-15	12.81, q	19.81, q	20.73, q	108.09, t
OAc	21.49, q	23.58, q	23.70, q	21.09, q
	170.23, s	174.44, s	171. 00, s	169.82, s

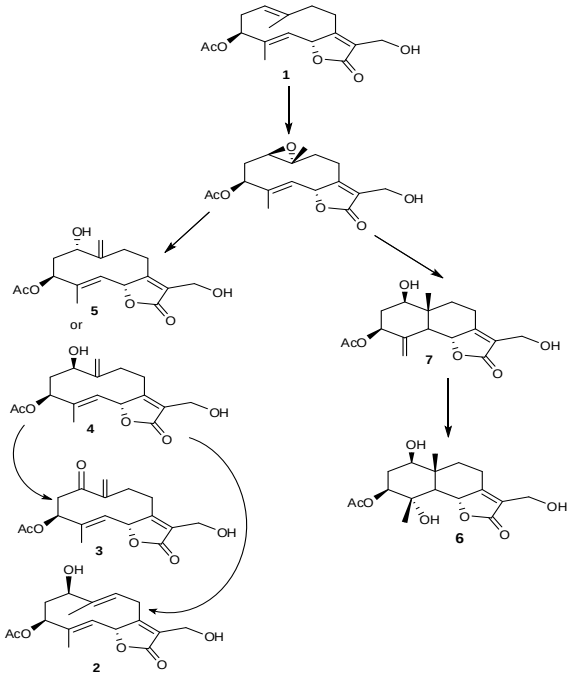


Fig. 2: Suggested Biosynthesis pathway of compounds (**1-7**)

4. Conflicts of Interest

The authors declare no conflict of interest.

5. Acknowledgments

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