Anti-inflammatory compounds and a new sesquiterpene lactone from *Centaurea gabrieljanae* Greuter

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Abstract

The traditional use of *Centaurea* spp. for anti-inflammatory purposes is widespread among the people in Turkiye. For this, the methanol extract of *Centaurea gabrieljanae* and sub-fractions of the methanol extract were tested for anti-inflammatory activity using 5-LOX, while their antioxidant activities, total phenol, and total flavonoid contents were also examined. The ethyl acetate fraction exhibited potent anti-inflammatory activity (IC_{50}= 3.864±0.9 µg/ml), from which five known compounds (astragalin, picein, *p*-hydroxy benzoic acid, 3,4-dimethoxy-cinnamic acid, 4-hydroxybenzoic acid, 4-*O*-*β*-glucopyranoside) and a new sesquiterpene lactone named Pterochlorin were obtained. Pterochlorin showed very potent anti-inflammatory activity with a value of IC_{50} 12.71±0.7 µg/ml compared to standard indomethacin. Similarly, astragalin was found to be strong (IC_{50}= 18.23 µg/ml). In addition, 4-hydroxybenzoic acid 4-*O*-*β*-glucopyranoside was isolated for the first time in *Centaurea* species, and its anti-inflammatory activity was tested. This study may be a guide for the discovery of a new anti-inflammatory drug derived from natural sources.

Keywords: *Centaurea gabrieljanae*; Anti-inflammatory; Sesquiterpene lactone; flavonoid

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Astragalin: kaempferol 3-*O*-glucoside: yellow powder: $C_{21}H_{20}O_{11}$: (Yekta et al. 2008) ^{1}H-NMR (MeOD, 400 MHz, δ, ppm, *J*/Hz): 6.18 (d, *J*= 2.0 Hz, 1H, H6), 6.37 (d, *J*= 1.9 Hz, 1H, H8), 8.07 (d, *J*= 8.9 Hz, 1H, H2′), 6.9 (d, *J*= 8.9 Hz, 1H, H3′), 6.9 (d, *J*= 8.9 Hz, 1H, H5′), 8.07 (d, *J*= 8.9 Hz, 1H, H6′), 5.21 (d, *J*= 7.4 Hz, 1H, H1″), 3.33-3.47 (m, 3H, overlapped H2″, H3″ and H4″), 3.22 (ddd, *J*= 9.6, 5.3, 2.3 Hz, 1H, H5″), 3.71 (dd, *J*= 11.8, 2.3 Hz, 1H, H6″a), 3.55 (q, *J*=11.9, 5.4 Hz, 1H, H6″b). ^{13}C NMR (100 MHz, MeOD, δ, ppm): 157.39 (C2), 134.00 (C3), 177.8 (C4), 161.53 (C5), 99.33 (C6), 167.15 (C7), 93.94 (C8), 157.31 (C9), 103.61 (C10), 121.41 (C1′), 130.83 (C2′), 114.69 (C3′), 160.24 (C4′), 114.69 (C5′), 130.83 (C6′), 102.97 (C1″), 74.30 (C2″), 76.66 (C3″), 69.92 (C4″), 76.99 (C5″), 61.20 (C6″).

***p*-Hydroxy benzoic acid:** $C_7H_6O_3$**:** colorless crystal **(**Chen et al. 2010): ^{1}H-NMR (MeOD, 400 MHz, δ, ppm, *J*/Hz): 7.89 (d, *J*=8.1 Hz, 2H, overlapped H6 and H2), 6.82 (d, *J*= 8.1 Hz, 2H, overlapped H5 and H3). ^{13}C NMR (100 MHz, MeOD, δ, ppm): 131.55 (C2), 114.51 (C3), 161.56 (C4), 114.51 (C5), 131.55 (C6).

Picein: 4-*O*-beta-glucopyranosylacetophenone: $C_{14}H_{18}O_7$: yellow amorphous: (Strack et al. 1989) ^{1}H-NMR (MeOD, 400 MHz, δ, ppm, *J*/Hz): 8.0 (d, *J*=8.9 Hz, 2H, overlapped H-2 and H-6), 7.19 (d, *J*=8.9 Hz, 2H, overlapped H3 and H5), 2.58 (s, 3H, H8 (OCH_3)), 5.05 (d, *J*=7.6 Hz, 1H, H1′), 3.55-3.39 (m, 4H, overlapped H2′, H3′, H4′ and H5′), 3.72 (dd, *J*= 12.1, 5.6 Hz, 1H, H6′a), 3.92 (dd, *J*= 12.0, 2.2 Hz, 1H, H6′b). ^{13}C NMR (MeOD, 100 MHz, δ, ppm): 131.26 (C1), 130.24 (C2), 115.85 (C3), 161.65 (C4), 115.85 (C5), 130.24 (C6), 178 (C7), 25.0 (C8), 100.18 (C1′), 73.39 (C2′), 76.53 (C3′), 69.87 (C4′), 76.90 (C5′), 61.04 (C6′).

4-Hydroxybenzoic acid 4-O-*β*-glucopyranoside: $C_{13}H_{16}O_8$: yellow amorphous powder: ^{1}H-NMR (400 MHz, MeOD, δ, ppm, *J*/Hz): 8.0 (d, *J*=8.9 Hz, 2H, H6 and H2), 7.18 (d, *J*=8.9 Hz, 2H, H5 and H3), 5.1 (d, *J*=7.5 Hz, 1H, H1′), 4.0 (dd, *J*=11.9, 1.9 Hz, 1H, H6′a), 3.8 (dd, *J*=12.0, 2.0 Hz, 1H, H6′b), 3.58-2.20 (m, 4H, H5′, H4′, H3′, H2′). ^{13}C NMR (100 MHz, MeOD, δ, ppm): 124.5 (C1), 131.6 (C2, C6), 115.1 (C3, C5), 160.2 (C4), 167 (C7), 100.5 (C1′), 74.2 (C2′), 76.8 (C3′), 69.5 (C4′), 77.8 (C5′), 60.8 (C6′) (Tabata et al. 1989)

3,4-Dimethoxy-cinnamic acid: $C_{11}H_{12}O_4$: White amorphous powder: ^{1}H-NMR (MeOD, 400 MHz, δ, ppm, *J*/Hz): 7.0 (d, *J*=1.7 Hz, 1H, H2), 6.63 (d, *J*=8.7 Hz, 1H, H5), 6.9 (dd, *J*=8.7, 1.7 Hz, 1H, H6), 7.5 (d, *J*=15.7 Hz, 1H, H7), 6.25 (d, *J*=15.7 Hz, 1H, H8). 3.91 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, MeOD, δ, ppm): 126.6 (C1), 118.7 (C2), 149.4 (C3),

151.7 (C4), 116.5 (C5), 124.4 (C6), 145.8 (C7), 115.01 (C8), 178 (C9), 56 (OCH_3), 56.5 (OCH_3) (Hu et al. 2012).

Pterochlorin: $C_{21}H_{25}ClO_7$: White oil: (8-acetoxy-3,6,9-trimethylidene-2-oxo-3a,4,5,6a,7,8,9a,9b-octahydroazuleno[4,5-b]furan-4-yl) 3-chloro-2-hydroxy-2-methylpropanoate.

Pterochlorin HRMS (ESI (+)) $C_{21}H_{26}ClO_7$ $[M-H]^+$: 425.8793914; Found: 112.98588 (55), 160.84244 (25), 173.04584 (100), 190.92883 (16), 248.96091 (16), 340.09631 (15), 399.12250 (30), 425.14624 (15). (ESI (-)) $C_{21}H_{24}ClO_7$ $[M-H]^-$: 423.8635114; Found: 121.06818 (2), 157.03523 (100), 179.01707 (9), 256.95978 (8), 301.14096 (1), 359.14651 (2), 423.19257 (1).

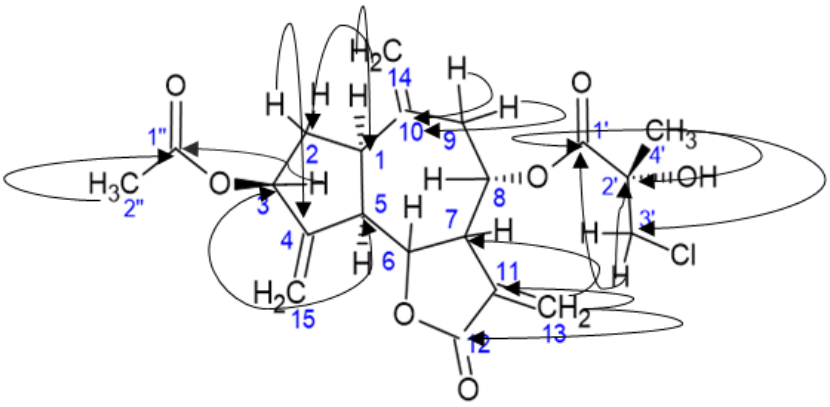

Figure S1. HMBC correlation of new sesquiterpene lactone

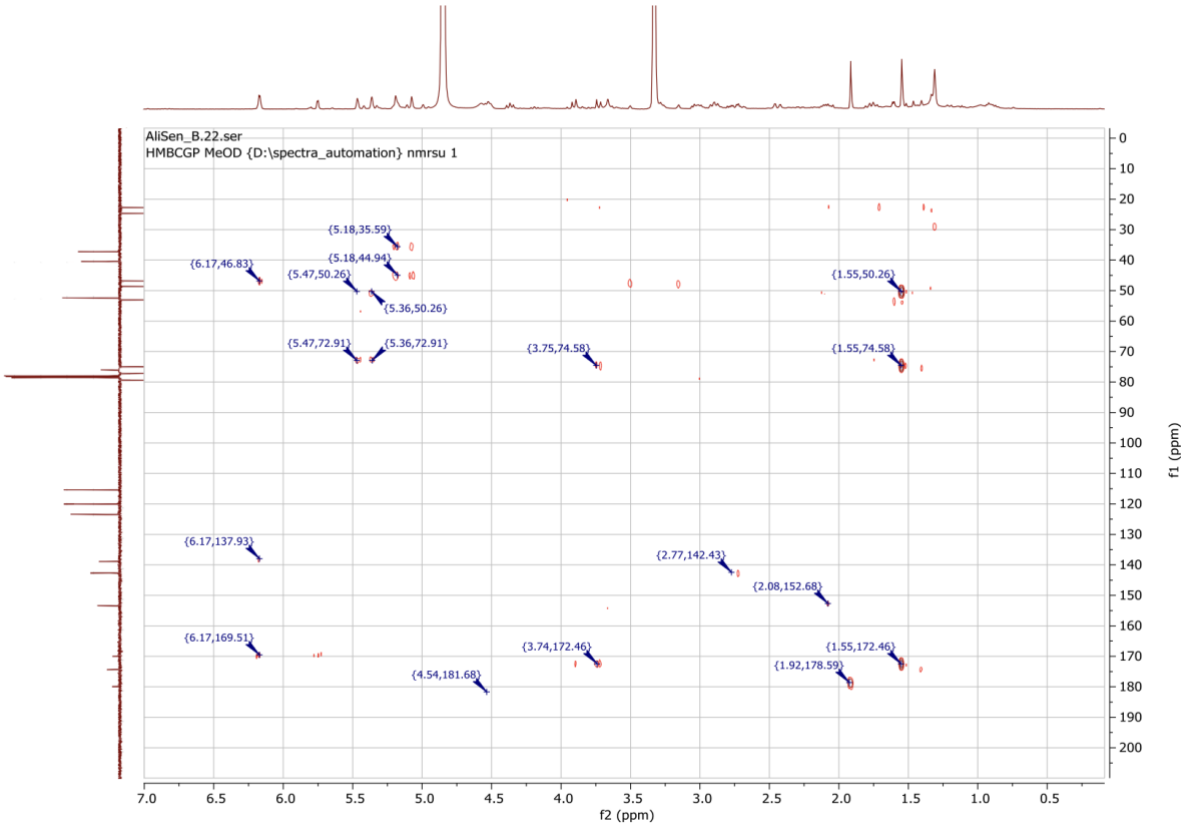

Figure S2. HMBC spectrum of pterochlorin in MeOD

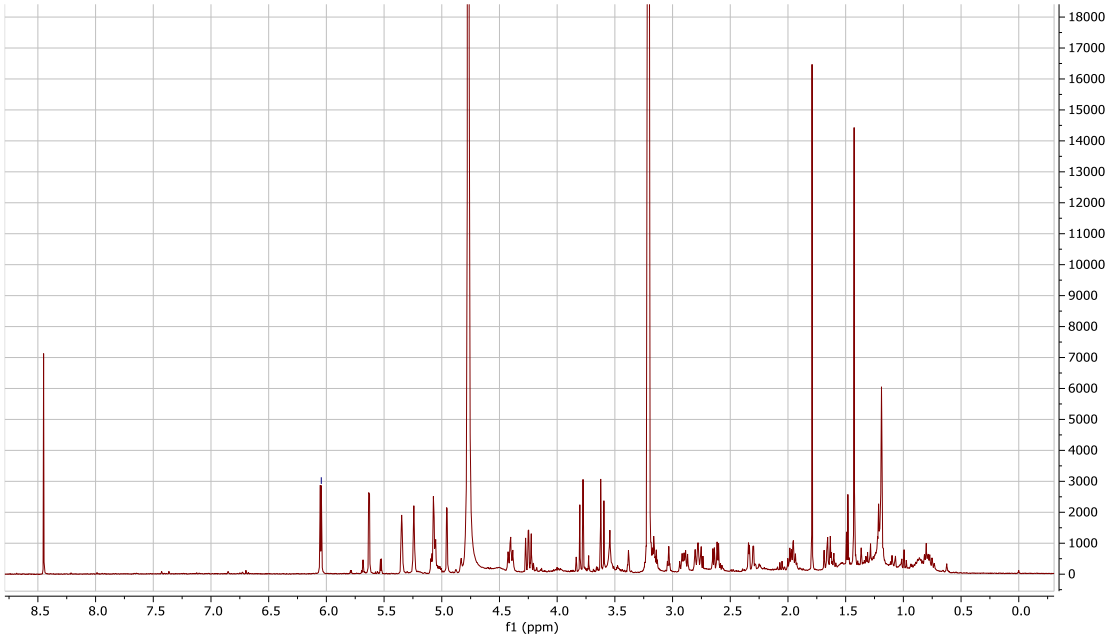

Figure S3. ^{1}H-NMR (400 MHz) spectrum of pterochlorin in MeOD

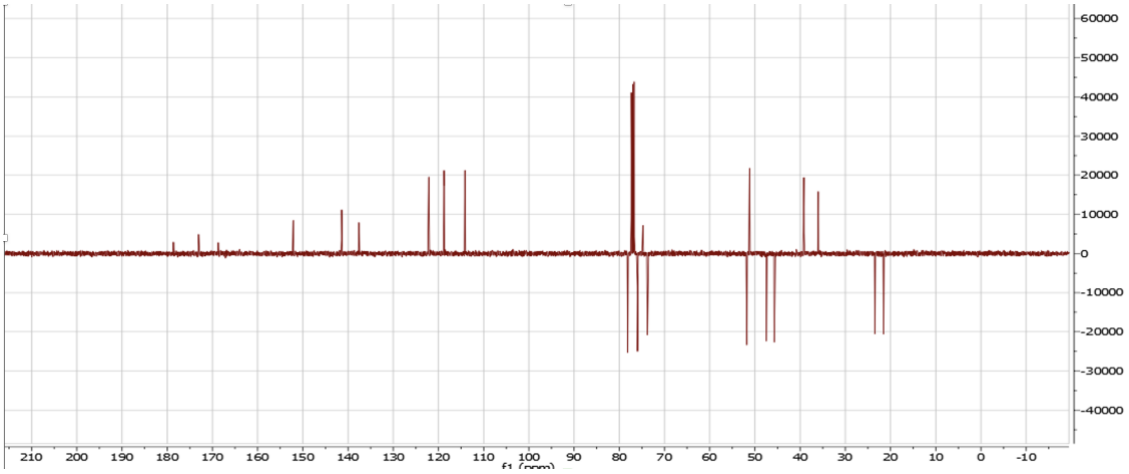

Figure S4. APT (13C-NMR) (100 MHz) spectrum of pterochlorin in MeOD

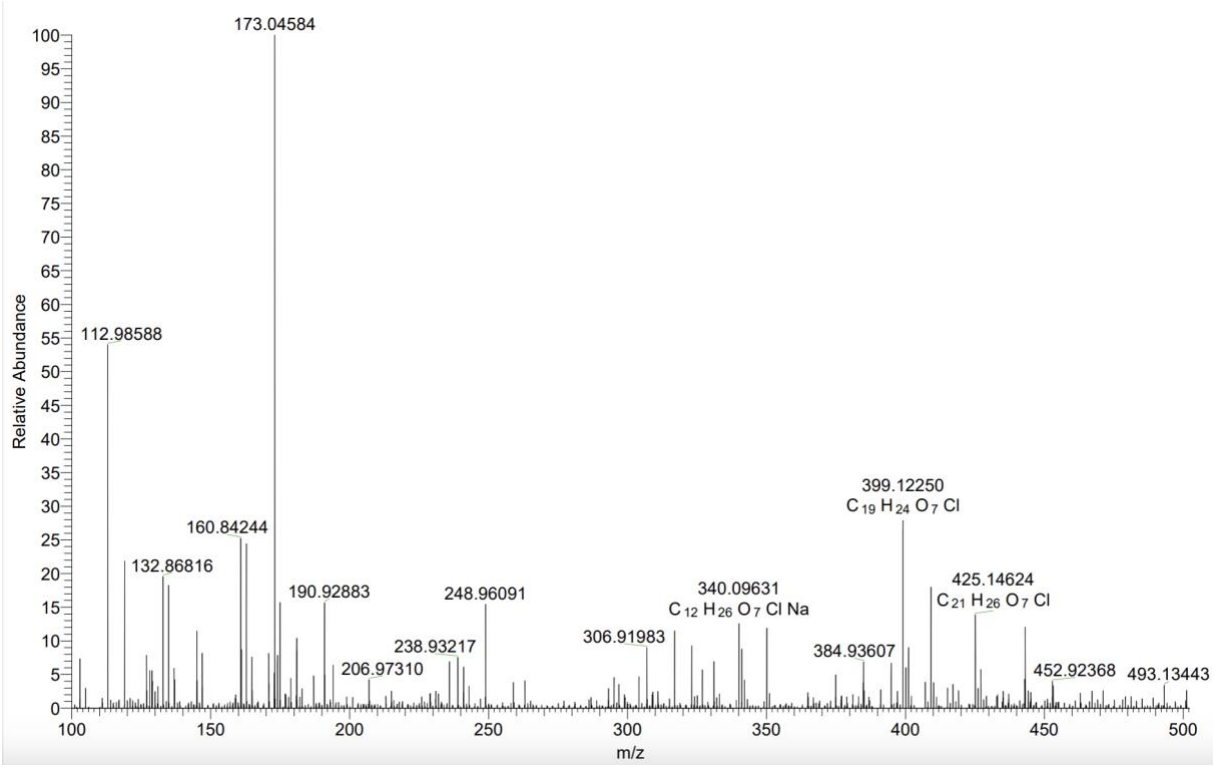

Figure S5. HR-ESİ(+)MS spectrum of pterochlorin

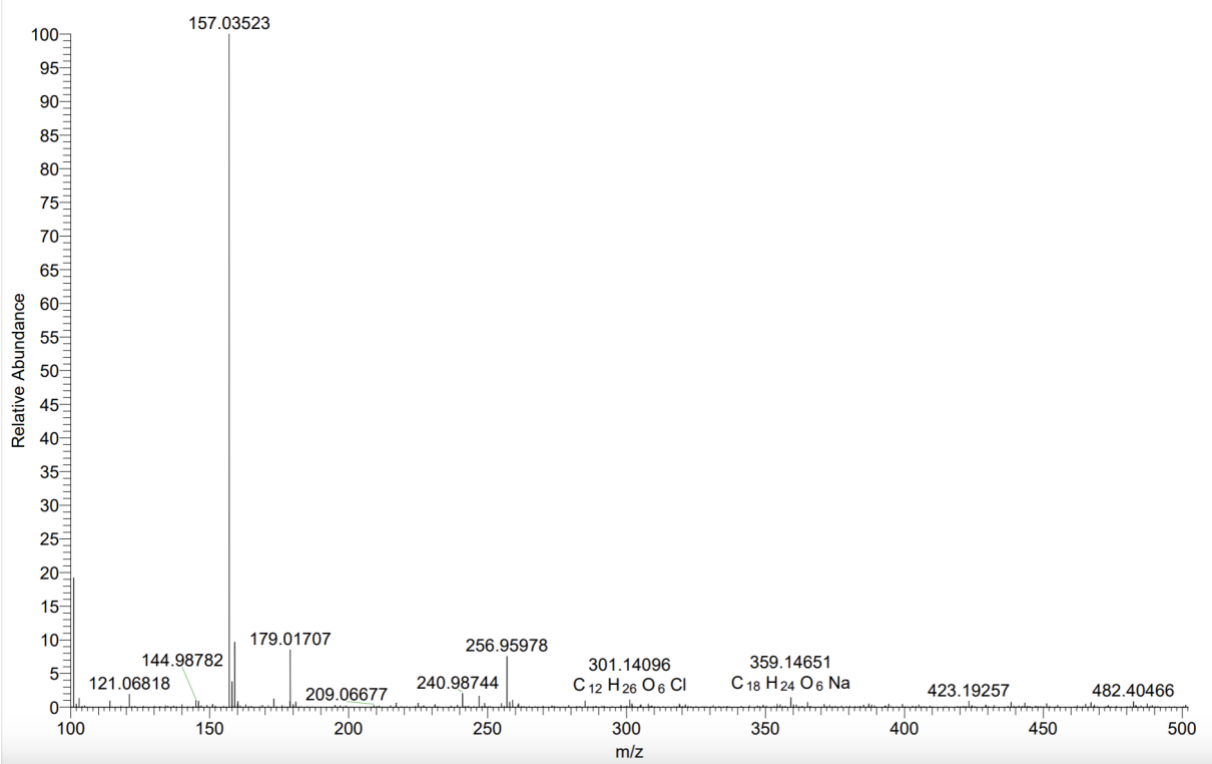

Figure S6. HR-ESİ(-)MS spectrum of pterochlorin

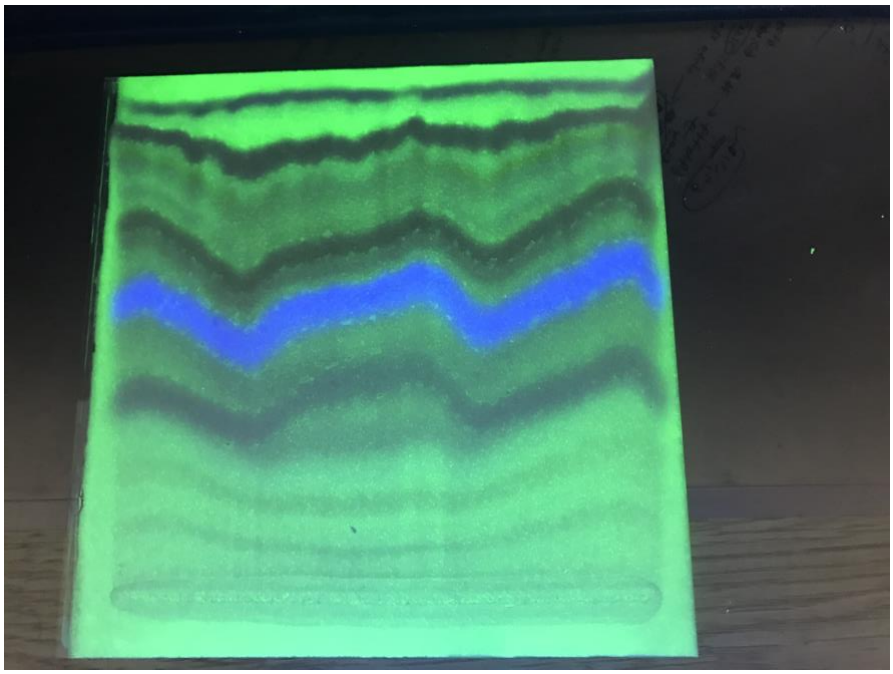

Figure S7. Image of the pTLC chromatogram of the combined fraction (CGIII/8-12/15-23) at UV 254 nm

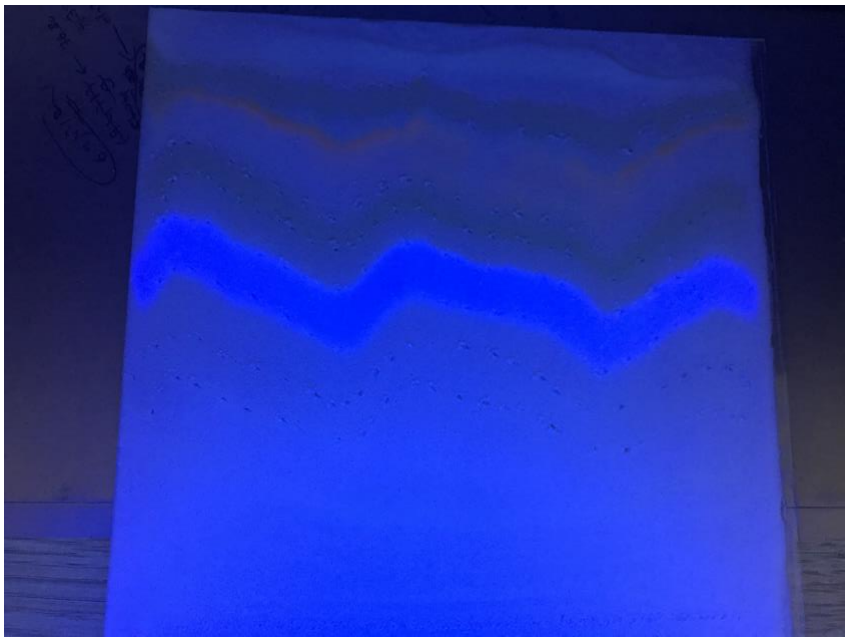

Figure S8. Image of the pTLC chromatogram of the combined fraction (CGIII/8-12/15-23) at UV 366 nm

Table S1. Anti-inflammatory activities of *C. gabrieljanae* methanol extract and its fractions

Extract/Fractions*	**Anti-inflammatory activity (5-LOX)**
	IC_{50} (µg/ml)
CGM	63.33 ± 1.3^{e}
CGH	96.24 ± 2.1^{f}
CGC	17.41 ± 1.1^{b}
CGEA	3.86 ± 0.9^{a}
CGAM	48.78 ± 0.7^{d}
Indomethacin	22.39 ± 0.2^{c}

*Abbreviations: CGM represents abbreviation for methanol extract of *C. gabrieljanae*, CGAM, CGEA, CGC, CGH represent abbreviations for aqueous methanol fractions, ethyl acetate, chloroform, and *n*-hexane respectively. Each value is presented as mean ± SD (n=3). Values in the table are accompanied by different superscripts in the same column. These superscripts indicate significant differences ($p<0.001$) between values.

Table S2. ABTS radical scavenging activity, total phenolic and total flavonoid amounts of *C. gabrieljanae* extract, and fractions

Extracts/ Standards	**ABTS radical scavenging activity**	**TPA (mg GAE/g extract)***	**TFA (mg QE/g extract)***
	IC_{50} (µg/ml)		
CGM	26.76±1.0[d]	187.6±3.7[b]	170.5±0.6[b]
CGH	166.7±3.7[g]	133.4±0.9[e]	50.1±0.2[e]
CGC	86.79±2.5[e]	150.3±0.6[d]	143.7±1.5[c]
CGEA	15.94±0.9[a]	266.4±1.5[a]	205.3±2.3[a]
CGAM	108.8±1.5[f]	153.2±2.3[c]	123.1±1.5[d]
AA**	14.5±0.32[b]		
Trolox**	13.00±0.21[a]		
BHA**	17.06±0.58[c]		
BHT**	26.82±1.12[d]		

* The total phenolic and flavonoid contents are expressed as gallic acid (GAE) and quercetin equivalent (QE), respectively. **AA: ascorbic acid, BHA: butylhydroxyanisole, BHT: butylhydroxytoluene. The values with different letter superscripts in the same column indicate significant differences, with a p-value of less than 0.001. Each value in the table is represented as mean ± standard deviation (SD) with a sample size of 3 (n=3).

Table S3. ^{1}H-NMR (400 MHz, MeOD), ^{13}C-NMR (100 MHz, MeOD) and HMBC Data of new sesquiterpene lactone

No	**δH ppm, J Hz**	**HMBC**	**δC ppm**
1	2.90 (t, *J*=10.5 Hz, 1H)		45.09
2	1.77 (q, *J*=11.8 Hz, 1H)	4C	35.6

	2.08 (dt, *J*=13.5, 6.9Hz, 1H)		
3	4.52 (m, 1H)	1´´C	72.79
4	-		152.75
5	2.75 (dd, *J*=15.1, 5.2 Hz, 1H)		50.91
6	4.37 (dd, *J*=10.8, 8.8 Hz, 1H)		78.73
7	3.01 (dt, *J*=9.0, 7.1 Hz, 1H)		46.76
8	5.19 (m, overlapped, 1H)		75.26
9	2.75 overlapped	10C	38.70
	2.44 (dd, *J*=14.8, 3.0 Hz, 1H)		
10	-		142
11	-		138
12	-		169.1
13	6.17 (d, *J*=3.5 Hz, 1H)	7C, 11C, 12C	121.06
	5.75 (d, *J*=2.9 Hz, 1H)		
14	5.19 (s, overlapped, 1H)	2C, 1C	117.19
	5.07 (d, *J*=2.0 Hz, 1H)		
15	5.47 (s, 1H)	3C, 5C	111.77
	5.36 (s, 1H)		
1´	-		172.3
2´	-		74.68
3´	3.73 (d, *J*=11.3 Hz, 1H)	2´C, 1´C	50.35
	3.90 (d, *J*=11.1 Hz, 1H)		

4´	1.55 (s, 1H)	1´C, 2´C, 3´C	22.74
1´´	-		178
2´´	1.91 (s, 1H)	1´´C	22.30

Table S4. Anti-inflammatory activities of compounds obtained from the ethyl acetate fraction of *C. gabrieljanae*

Compounds/Standard	**Anti-inflammatory activity (5-LOX)** IC_{50} **(µg/ml)**
Astragalin	18.23 ± 1.1^{b}
Picein	113.6 ± 0.8^{g}
p-hydroxy benzoic acid	67.71 ± 0.9^{e}
3,4-Dimethoxy-cinnamic acid	47.9 ± 1.2^{d}
4-Hydroxybenzoic acid 4-*O*-*β*-glucopyranoside	83.94 ± 1.5^{f}
Pterochlorin	12.71 ± 0.7^{a}
Indomethacin	22.39 ± 0.2^{c}