

Supplementary Material

Efflux pump inhibition and resistance modulation in *Mycobacterium smegmatis* by *Peucedanum ostruthium* and its coumarins

Katarina Šimunović, Julia Solnier, Fabian Alperth, Olaf Kunert, Sonja Smole Možina, Franz Bucar

Table S1: NMR Spectroscopic Data (700 MHz, CDCl₃) for compound 1, 25 °C, TMS was used as internal standard.

compound 1			compound 1		
position	δ_c , type	δ_H (J in Hz)	position	δ_c , type	δ_H (J in Hz)
2	161.0, C	-	8a	152.6, C	-
3	113.3, CH	6.32, d (9.7)	9	95.1, CH	7.21, s
4	139.0, CH	8.18, d (9.7)	9a	152.6, C	-
4a	107.4, C	-	1'	74.5, CH ₂	4.45, dd (9.8, 7.7) 4.54, dd (9.8, 2.8)
5	148.4, C	-	2'	76.5, CH	3.91, dd (7.7, 2.7)
5a	114.4, C	-	3'	71.7, C	-
6	104.8, CH	6.99, d (2.3)	4'	25.2, CH ₃	1.32, s
7	145.4, CH	7.62, d (2.3)	5'	26.7, CH ₃	1.37, s

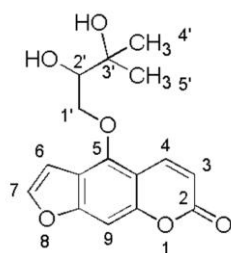


Figure S1. Oxypeucedanin hydrate (1)

Table S2: NMR Spectroscopic Data (700 MHz, CDCl₃) for compound **6**, 25 °C, TMS was used as internal standard.

compound 6			compound 6		
position	δ_c , type	δ_H (J in Hz)	position	δ_c , type	δ_H (J in Hz)
2	161.4, C	-	1'	29.2, CH ₂	3.40, d (7.2)
3	113.2, CH	6.23, d (9.5)	2'	120.6, CH	5.30, m
4	143.5, CH	7.60, d (9.5)	3'	139.9, C	-
4a	112.7, C	-	4'	39.7, CH ₂	2.11, m
5	128.4, CH	7.19, s	5'	26.3, CH ₂	2.12, m
6	124.6, C	-	6'	123.7, CH	5.06, m
7	158.1, C	-	7'	132.2, C	-
8	104.7, CH	6.78, s	8'	25.8, CH ₃	1.68, s
8a	154.4, C	-	9'	17.7, CH ₃	1.60, s
7-OH	-	7.60, s	10'	16.3, CH ₃	1.77, s

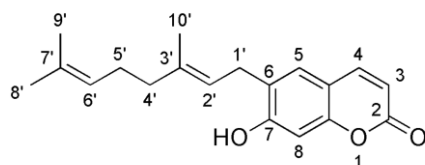


Figure S2. Ostruthin (**6**)

Table S3: Membrane integrity disruption (%) in *M. smegmatis* mc² 155 wild type and $\Delta lfrA$ mutant after treatment with ethanol extract at concentrations of 0.25 and 0.125 x MIC (E 0.25 x MIC and E 0.125 x MIC), hexane extract at 0.25 and 0.125 x MIC (H 0.25 x MIC and H 0.125 x MIC), imperatorin at 0.25 x MIC, and ostruthin at 0.25 x MIC.

Treatment	Membrane integrity disruption (%) in <i>M. smegmatis</i> mc ² 155			
	wild type		$\Delta lfrA$	
E 0.25xMIC	-14.3	± 3.5	-29.9	± 7.4
E 0.125xMIC	-38.2	± 3.6	-23.5	± 6.6
H 0.25xMIC	-11.4	± 6.0	-19.8	± 6.3
H 0.125xMIC	-20.5	± 3.1	-19.9	± 3.3
Imperatorin 0.25xMIC	76.0	± 4.1	86.1	± 2.3
Ostruthin 0.25xMIC	-36.4	± 1.4	-46.9	± 5.3