

# Supplementary Files

The anti-serotonin effect of parthenolide derivatives and standardised extract from the leaves of *Stizolophus balsamita*

Joanna Nawrot<sup>1</sup>, Marta Napierała<sup>2</sup>, Kinga Kaczerowska-Pietrzak<sup>1</sup>, Ewa Florek<sup>2</sup>, Justyna Gornowicz-Porowska<sup>1</sup>, Ewa Pelant<sup>1</sup> and Gerard Nowak<sup>1,\*</sup>

<sup>1</sup>Department of Medicinal and Cosmetic Natural Products, Poznan University of Medicinal Sciences, Poznan, Poland

<sup>2</sup>Laboratory of Environmental Research, Department of Toxicology, Poznan University of Medicinal Sciences, Poznan, Poland

Correspondence: gnowak@ump.edu.pl Phone, Fax: 48 61 8470628

Figure S1. TLC of the purified CH<sub>2</sub>Cl<sub>2</sub> extract and germacranolides isolated from the *Stizolophus balsamita* leaves.

Figure S2. HPLC chromatograms of stizolin (S), izospiciformin (I) and stizolicin (Sc)

Figure S3. Concentrations of serotonin (mg/mL) in the stizolin sample and the control sample.

Figure S4. Concentrations of serotonin (mg/mL) in the izospiciformin sample and the control sample.

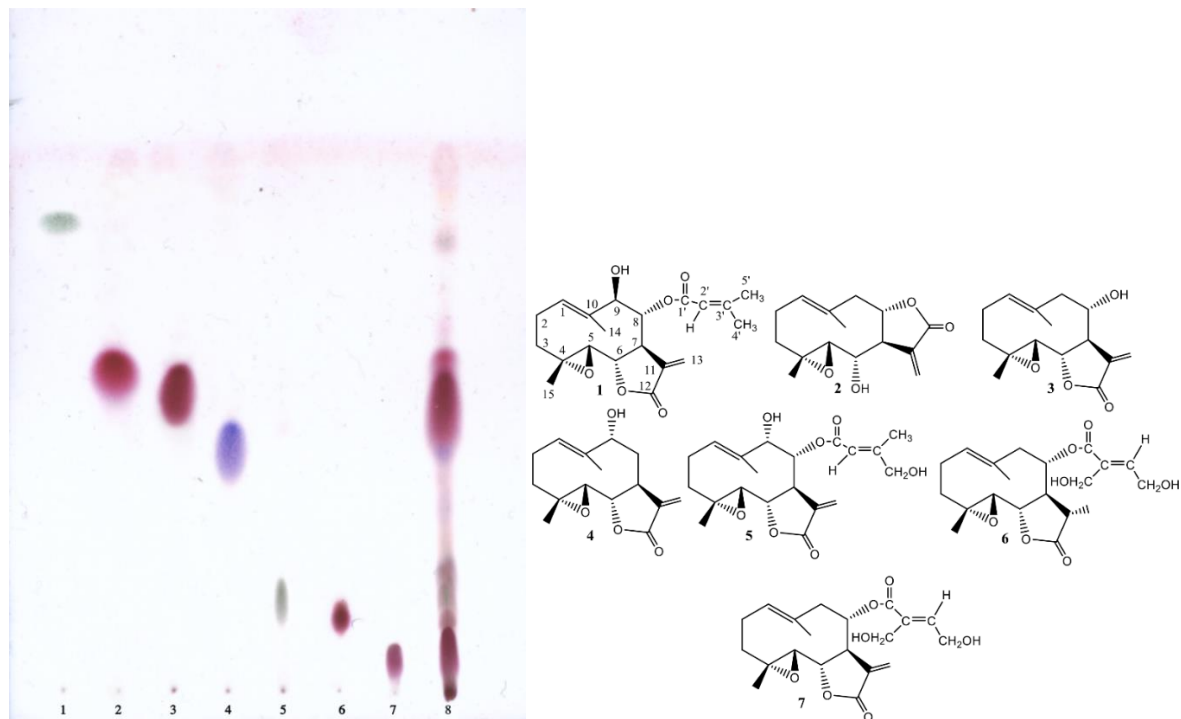
Figure S5. Concentration of serotonin (mg/mL) in the stizolicin sample and the control sample.

Figure S6. Concentrations of serotonin (mg/mL) in the methanol extract from the leaves of *S. balsamita* sample and the control sample.

Table S1. <sup>1</sup>H NMR (600 MHz) spectroscopic data of compounds 2, 3 and 7.

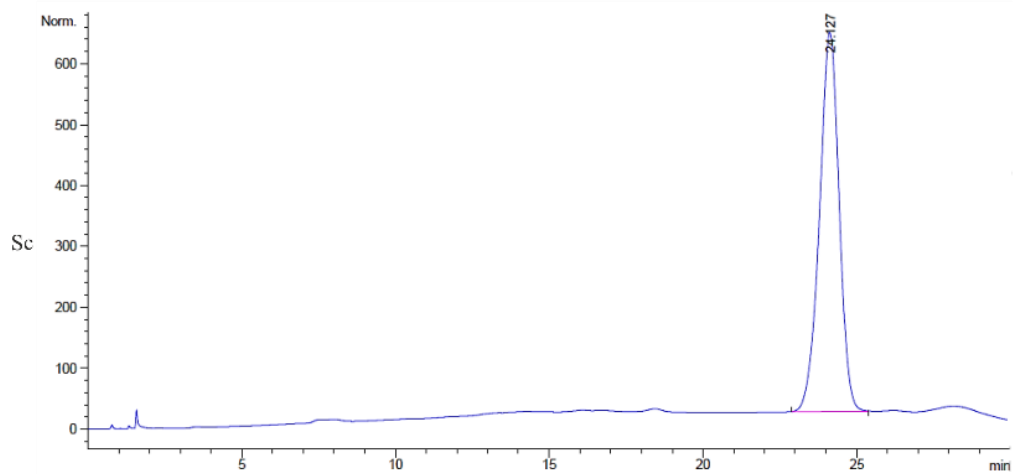
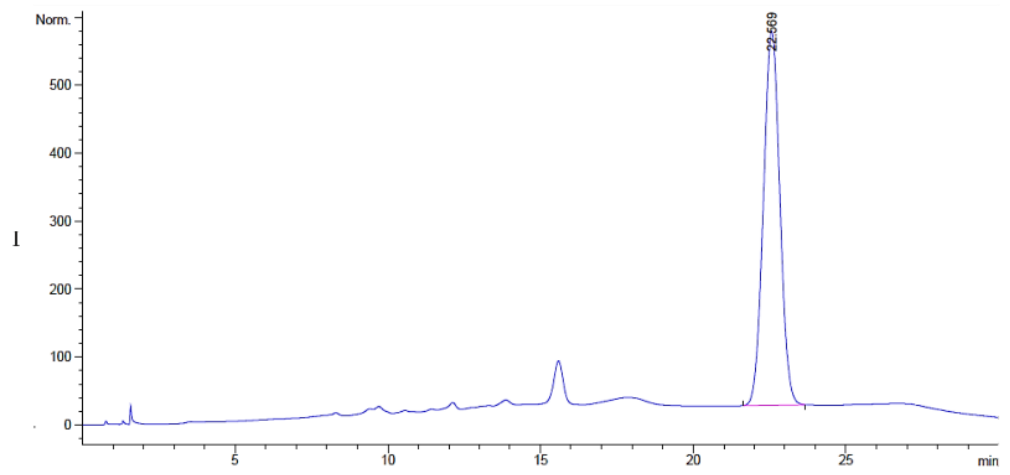
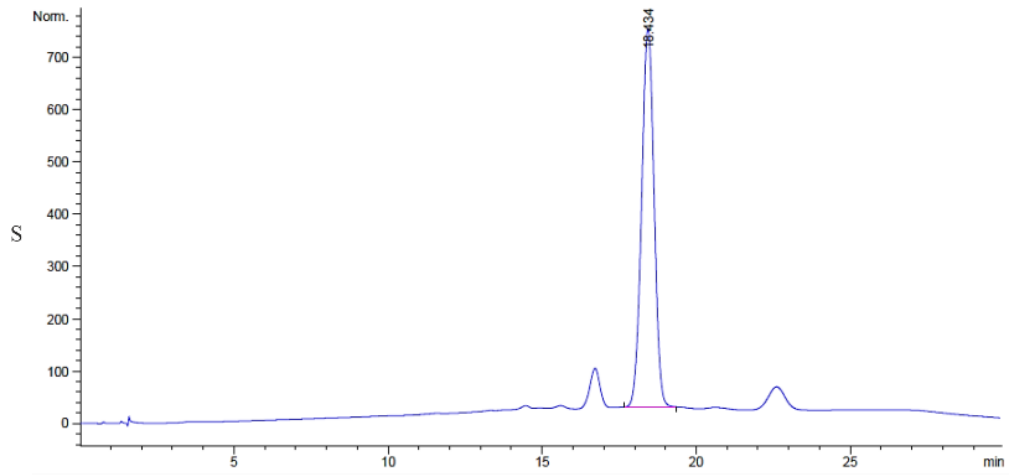
Table S2. <sup>13</sup>C NMR (150 MHz) spectroscopic data of compounds 2, 3 and 7.

**Figure 1.** TLC of the purified  $\text{CH}_2\text{Cl}_2$  extract and germacranolides isolated from the *Stizolophus balsamita* leaves. Adsorbent: silica gel; mobile phase: n-hexane –  $(\text{CH}_3)_2\text{CO}$  2:1; 1—balsamin; 2—izospiciformin; 3—stizolin 4—9a-hydroxyparthenolide; 5—8 $\alpha$ -E-(4'-hydroxy)-seneciyoxyloxy-9 $\alpha$ -hydroxyparthenolide; 6—11 $\beta$ ,13-dihydrostizolicin; 7—stizolicin; 8—purified  $\text{CH}_2\text{Cl}_2$  extract of *S. balsamita* leaves.

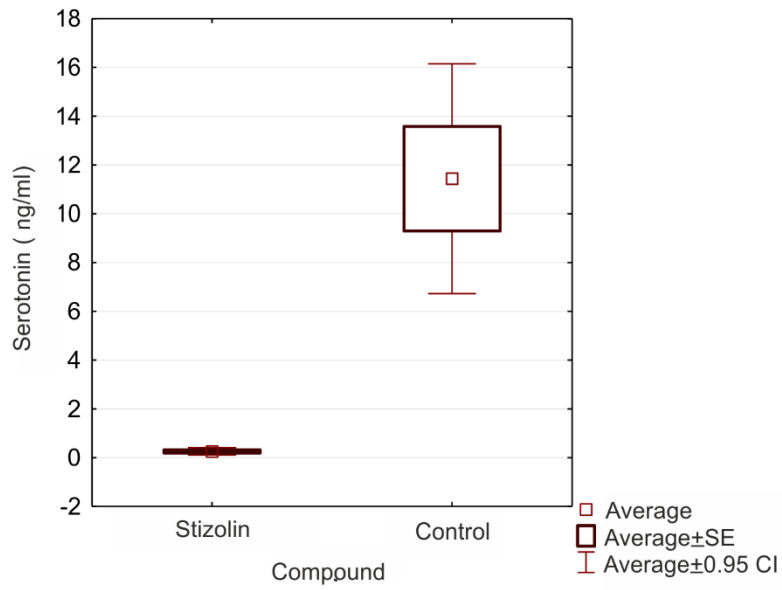


The purple color of the spots of 4,5-epoxygermacranolides indicates the presence of an OH group at C-6 and a substituent at C-8 or the substituent only at C-8 (compounds 2, 3, 6 and 7). The violet color of the spot indicates the presence of a hydroxyl group at C-9 and the absence of this group at C-8 (compound 4). Finally, the green color of the spot of parthenolide derivatives shows the presence of two substituents.

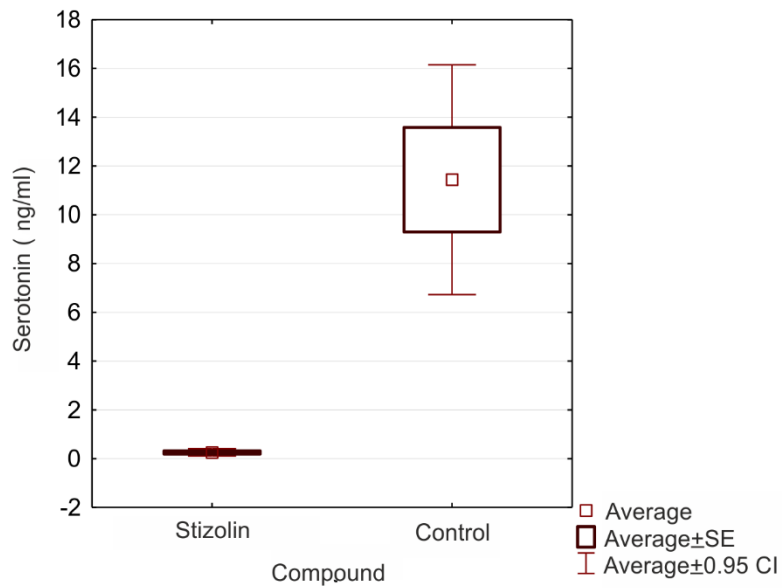
**Figure 2.** The HPLC chromatograms of stizolin (S), izospiciformin (I) and stizolicin (Sc).



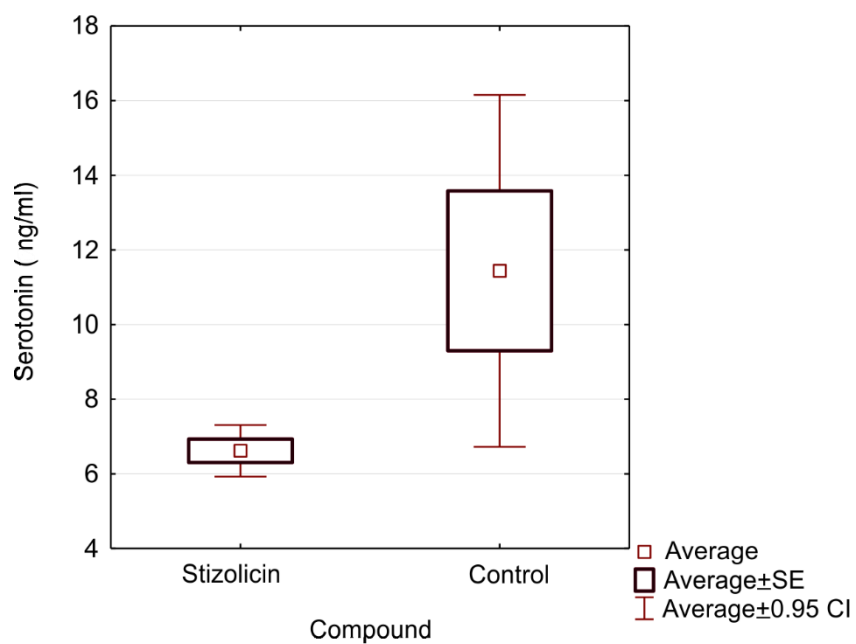
**Figure 3.** Concentrations of serotonin (mg/mL) in the stizolin sample and the control sample.



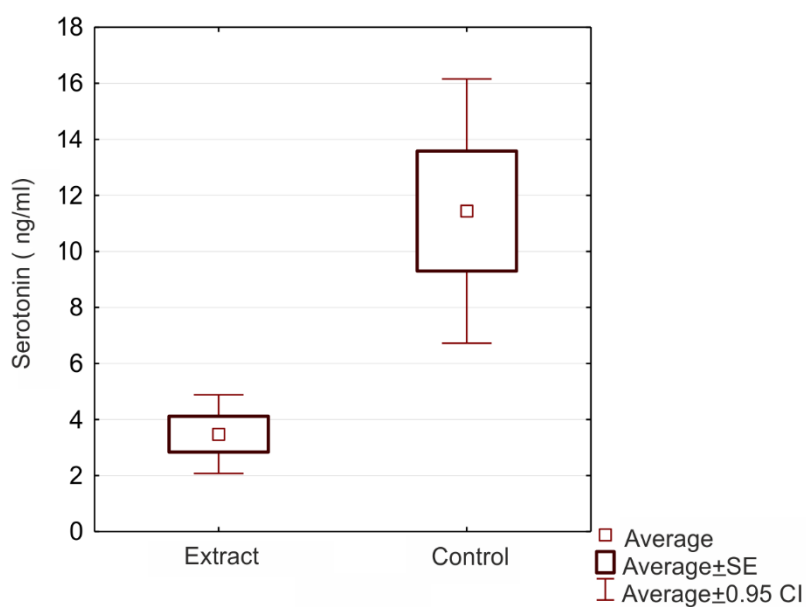
**Figure 4.** Concentrations of serotonin (mg/mL) in the isospiciformin sample and the control sample.



**Figure 5.** Concentrations of serotonin (mg/mL) in the stizolicin sample and the control sample. .



**Figure 6.** Concentrations of serotonin (mg/mL) in the methanol extract from the leaves of *S. balsamita* sample and the control sample.



**Table 1.**  $^1\text{H}$  NMR (600 MHz) spectroscopic data ( $\delta\text{H}$  in ppm, mult;  $J$  in Hz) of: izospiciformin (2), stizolin (3), and stizolicin (7).

Pos.	2 <sup>a</sup>	3 <sup>a</sup>	7 <sup>b</sup>
1	5.36 bs	5.25 bd (12.1)	5.45 bd (10.5)
2a	1.21 m	2.43 m	2.52 dd (5.8; 13.5)
2b	0.98 m	2.23 m	2.25 bd (13.6)
3a	2.13 m	2.15 m	2.13 dd (12.9; 6.0)
3b	1.34 m	1.24 m	1.29 m
5	2.66 d (8.9)	2.73 d (8.1)	2.85 d (9.3)
6	3.44 dd (8.9; 9.6)	3.98 bt (7.4)	4.37 t (9.3)
7	3.00 m	3.09 m	3.59 m
8a	4.01 m	-	4.57 bdd (3.9; 2.7)
8b	-	3.88 m	-

9a	2.84 m	2.57b d (12.5)	2.70 dd (11.8; 11.6)
9b	2.14 m	2.43 d (12.2)	2.47 bd (12.1)
13a	6.46 dd (1.2; 3.0)	6.50 d (3.1)	5.80 d (3.0)
13b	6.28 dd (1.2; 2.6)	6.16 d (2.4)	6.20 d (3.4)
14	1.79 s	1.76 s	1.82 bs
15	1.28 s	1.28s	1.28 bs
3'	-	-	6.94 t (5.9)
4'	-	-	4.40 d (5.9)
5'	-	-	4.28 s

<sup>a</sup> in CDCl<sub>3</sub>; <sup>b</sup> in CD<sub>3</sub>OD; <sup>c</sup> overlapped signals.

**Table 2.** <sup>13</sup>C NMR (150 MHz) spectroscopic.

data of compounds <b>2</b> , <b>3</b> and <b>7</b>			
<b>C</b>	<b>2<sup>a</sup></b>	<b>3<sup>a</sup></b>	<b>7<sup>b</sup></b>
1	126.86	128.26	128.59
2	14.17	24.71	25.30
3	36.18	35.65	37.00
4	64.09	61.45	62.57
5	69.69	66.33	67.63
6	71.06	78.00	82.25
7	49.06	52.21	50.48
8	80.35	71.35	75.07
9	44.45	52.00	47.84
10	134.02	129.78	132.65
11	169.51	134.03	131.06
12	171.15	169.49	171.57
13	128.94	128.55	126.19
14	18.86	18.13	18.40
15	17.53	17.40	17.65
1'	-	-	167.92
2'	-	-	135.28
3'	-	-	147.02
4'	-	-	59.45
5'	-	-	56.78

<sup>a</sup> in CDCl<sub>3</sub>; <sup>b</sup> in CD<sub>3</sub>OD.