

Supplementary data

The effect of silymarin flavonolignans and their sulfated conjugates on platelet aggregation and blood vessels *ex vivo*

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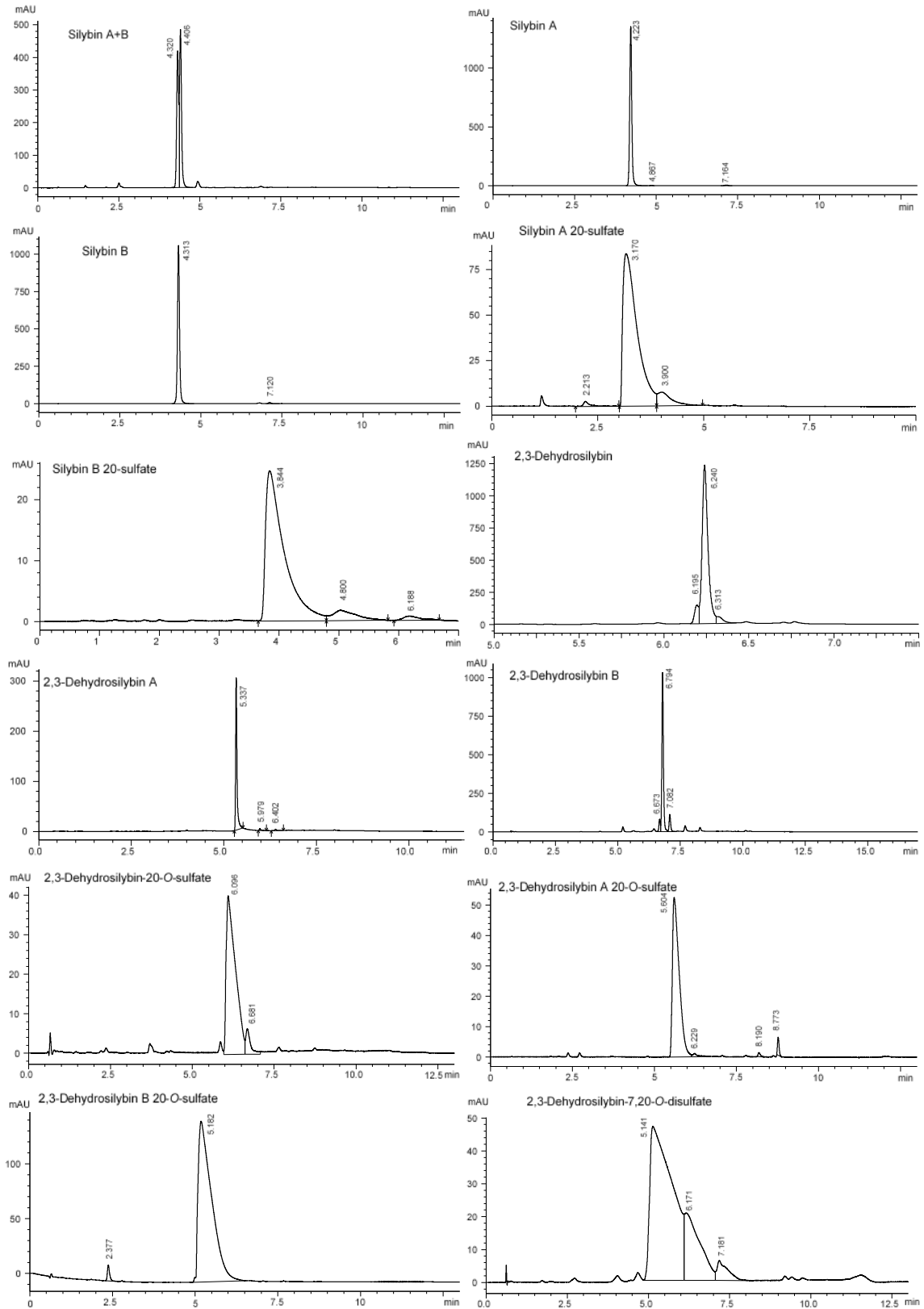
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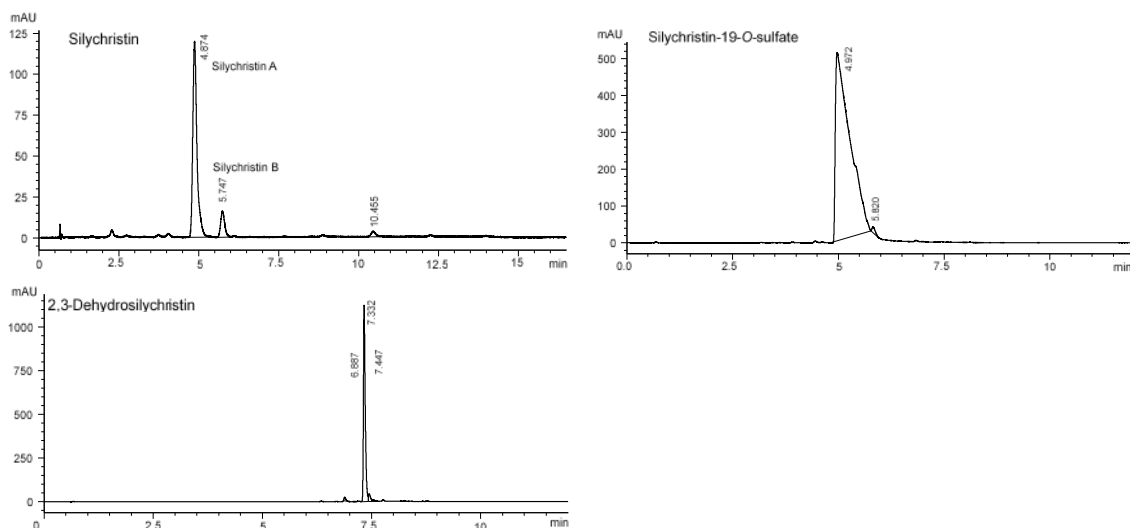


Figure S1. HPLC chromatograms of the compounds tested
HPLC gradient method: Chromolith RP-18e (100 × 3 mm) column equipped with Chromolith RP-18e guard cartridge (5 × 4.6 mm), mobile phase A=5% acetonitrile in water, 0.1% HCOOH; mobile phase B= 80% acetonitrile in water, 0.1% HCOOH; gradient: 0-5 min 0-25 % B, 5-8 min 25-60 % B, 8-10 min 60 % B, 10-11 min 60-0 %B, 12 min stop. Flow rate was 1.2 mL/min, temperature 25 °C.

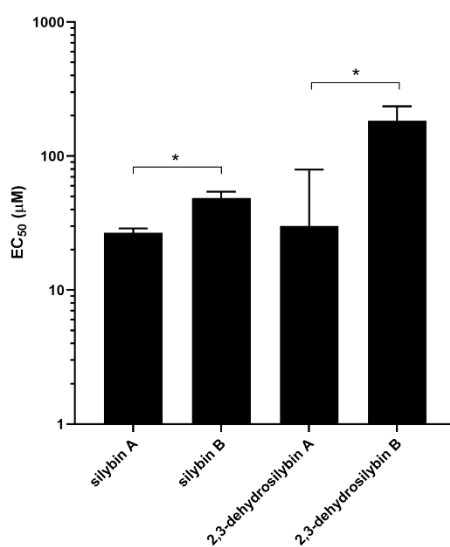


Figure S2. Comparison of *ex vivo* vasorelaxant effects of stereoisomers
Silybin A and 2,3-dehydrosilybin A were compared with respective B stereoisomers on intact rat aortic rings precontracted with phenylephrine. The EC₅₀ values of both diastereoisomers A were significantly lower than the EC₅₀ values of corresponding diastereoisomers B. Number of used rings: 4 (for silybin A and silybin B), 6 (2,3-dehydrosilybin A) and 3 (2,3-dehydrosilybin B). * p < 0.05.

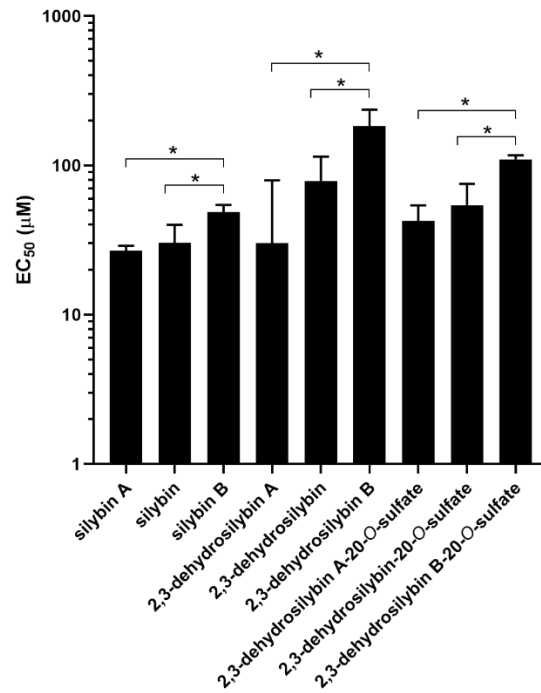


Figure S3. Vasorelaxant effects of equimolar mixture of stereomers (A+B). The effect was studied *ex vivo* on intact rat aortic rings precontracted with phenylephrine. No potentiation or inhibition of vasorelaxant potency was observed. Number of used rings: 6 for 2,3-dehydrosilybin A, 3 for 2,3-dehydrosilybin B and 2,3-dehydrosilybin B-20-O-sulfate, 5 for 2,3-dehydrosilybin A-20-O-sulfate, and 4 for other substances. * $p < 0.05$.

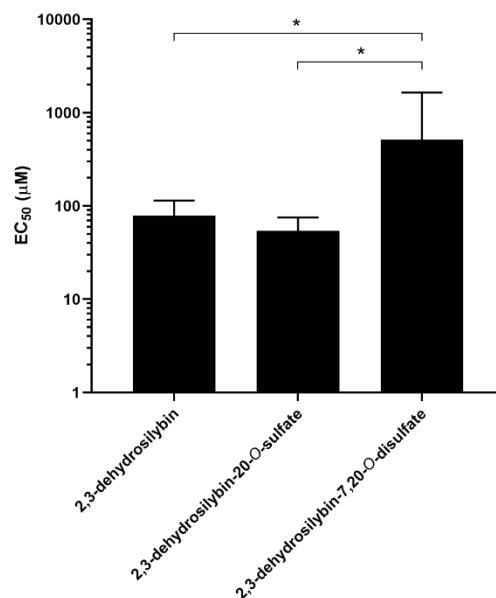


Figure S4. Comparison of *ex vivo* vasorelaxant effects of 2,3-dehydrosilybin and its conjugates. The effect of 2,3-dehydrosilybin was compared with that of 2,3-dehydrosilybin-20-O-sulfate and 2,3-dehydrosilybin-7,20-O-disulfate on intact rat aortic rings precontracted with phenylephrine. $N = 4$; * $p < 0.05$.

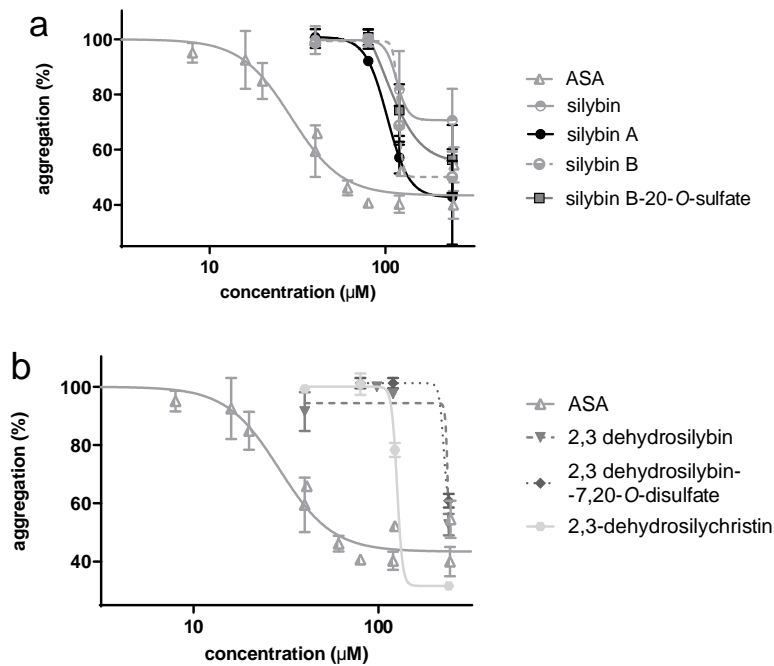


Figure S5. Effect of flavonolignans on platelet aggregation induced by collagen in whole blood. (a): Concentration-dependent curves of the most active silybins, (b): Concentration-dependent curves of the most active 2,3-dehydrosilybins and 2,3-dehydrosilychristin. ASA = acetylsalicylic acid.

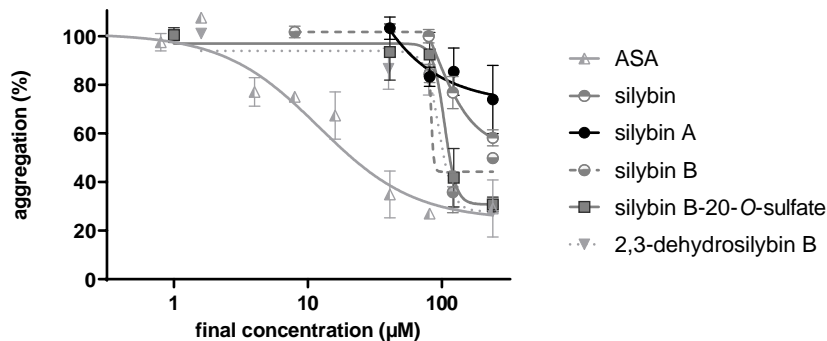


Figure S6. Effect of flavonolignans on whole blood platelet aggregation induced by arachidonic acid. Concentration-dependent curves of selected active substances; ASA = acetylsalicylic acid.

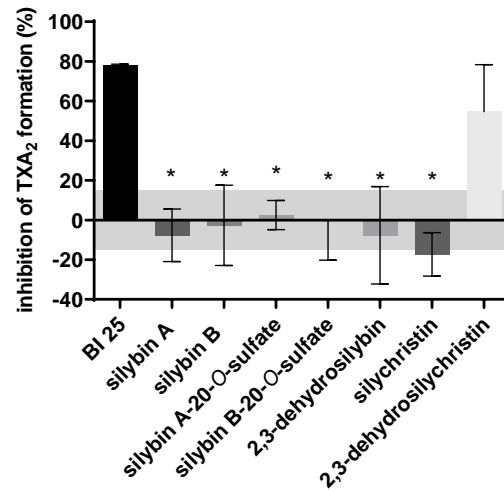


Figure S7. Comparison of tested flavonolignans and 1-benzylimidazole on thromboxane A₂ synthase activity. Compounds were tested at 100 μ M while 1-benzylimidazole (BI 25) at 25 μ M. Data are expressed as mean \pm SD. * $p < 0.01$ vs. 1-benzylimidazole. Grey area means the error of the method.

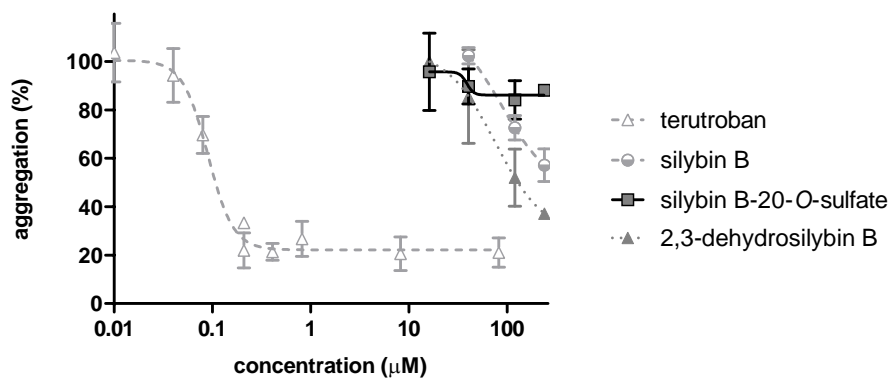


Figure S8. Effect of tested compounds on aggregation induced by the thromboxane analogue U-46619. The effect is presented as dose-dependent curves; terutroban is a standard antagonist of thromboxane receptors.



Table S1. Effect of flavonolignans on collagen induced platelet aggregation at 240 μ M.

silybin	**																		
silybin A	ns	ns																	
silybin B	ns	ns	ns																
silybin A-20-O-sulfate	**	ns	**	ns															
silybin B-20-O-sulfate	ns	ns	ns	ns	ns														
2,3-dehydrosilybin	ns	ns	ns	ns	ns	ns													
2,3-dehydrosilybin-20-O-sulfate	***	ns	***	**	ns	*	*												
2,3-dehydrosilybin-7,20-O-disulfate	ns	ns	ns	ns	ns	ns	ns	ns											
2,3-dehydrosilybin A	***	ns	***	**	ns	*	*	ns	ns										
2,3-dehydrosilybin B	***	ns	**	*	ns	ns	ns	ns	ns	ns									
2,3-dehydrosilybin A-20-O-sulfate	***	ns	***	***	ns	***	***	ns	**	ns	ns								
2,3-dehydrosilybin B-20-O-sulfate	***	ns	***	***	ns	**	**	ns	ns	ns	ns	ns							
silychristin	***	ns	***	**	ns	*	*	ns	ns	ns	ns	ns	ns						
silychristin-19-O-sulfate	***	ns	***	***	ns	**	**	ns	*	ns	ns	ns	ns	ns					
2,3-dehydrosilychristin	ns	**	ns	ns	***	ns	ns	***	ns	***	***	***	***	***	***				
DMSO	***	*	***	***	ns	***	***	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	***
	acetyl salicylic acid (ASA)	silybin	silybin A	silybin B	silybin A-20-O-sulfate	silybin B-20-O-sulfate	2,3-dehydrosilybin	2,3-dehydrosilybin-20-O-sulfate	2,3-dehydrosilybin-7,20-O-disulfate	2,3-dehydrosilybin A	2,3-dehydrosilybin B	2,3-dehydrosilybin A-20-O-sulfate	2,3-dehydrosilybin B-20-O-sulfate	silychristin	silychristin-19-O-sulfate	2,3-dehydrosilychristin			

ns $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

