Supporting Information

Defined Structure-Activity Relationships of Boswellic Acids Determine Modulation of Ca²⁺ Mobilization and Aggregation of Human Platelets by *Boswellia serrata* Extracts

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Preparation of *Boswellia serrata* extracts and neutral and acidic fractions

*Boswellia serrata* gum resin was obtained Gerhard Eggebrecht Vegetabilien und Harze, denomination: “Gummi Olibanum indisch No. 1; Origin: India”. The material was correctly assigned as authentic material in Ref. [1] by comparison with voucher specimens obtained from the Royal Botanical Garden, Kew Gardens, England, UK, donated by Mrs. J. Steele.

The gum resin (200 g) was cooled in a freezer (- 20°C, 1-2 h) and powdered in an electrical mixer. The powder was filled into an extraction thimble, covered with a wad, and extracted with 1.5 L diethyl ether in a Soxhlet apparatus overnight. The solvent was removed under vacuum at 40°C, and a crude yellow and fragrant solidus material was obtained (designated: *B. serrata* extract; yield approximately 60%).
The subsequent discontinuous extraction was implemented in a separation funnel. The crude extract was dissolved again in ca. 250 mL diethyl ether and extracted with 100 mL of 5% (m/m) KOH solution. The alkaline aqueous solution was then extracted with diethyl ether again (3 x 30 mL). The combined organic phases were washed with saturated NaCl, dried (MgSO$_4$), and evaporated in vacuo, yielding the neutral fraction (NF, fragrant and yellow-orange oil; yield approximately 35%). Then, the alkaline aqueous phase was acidified to pH 2-3 (conc. HCl) and extracted with diethyl ether (3 x 50 mL). After combination of the organic phases, it was washed with brine, dried (MgSO$_4$), and evaporated to dryness in vacuo. A less fragrant, yellowish solid material was obtained, designated the acidic fraction (AF) containing the boswellic acids and other triterpene acids; yield approximately 25%.

Analytical HPLC of *Boswellia serrata* extracts and quantification of boswellic acids

The analytical HPLC device consisted of an S 7121 reagent organizer, an S 1122 solvent delivery system, an S 8111 low-pressure gradient mixer, and an S 3210 UV/VIS diode array detector (all components from Sykam). A Jetstream 2 Series column oven (Techlab) was used for temperature control. Samples were injected by an S 5111 valve bracket injector (Rheodyne) with a 20-μL loop (100 μL were injected for quantitative determinations, overfilling) and a 250-μL syringe (Hamilton). Solvent reservoirs were degassed with helium (He) and kept constant under He pressure during analysis. Injected samples (20 μL) were adjusted to 5 mg/mL for the entire extract and the AF, and 1.25 mg/mL for the NF. Every sample was injected twice unless stated otherwise. After each injection, the injector was purged with 500 μL MeOH and the injection syringe was purged twice with 250 μL MeOH. The chromatographic data was recorded for 1D and 2D chromatograms with Chromstar DAD software, Version 6.3 (SCPA). Detection was carried out at 210 nm. Peak Integration was manually implemented by comparison of the baseline with blank runs. Minor inhomogeneities at peak edges were separated by the perpendicular drop function of the integration software.
The analytical HPLC device, as described above, was equipped with a guard column (GROM Saphir 110 C18, 5 μm, 20 x 4 mm I.D.; GROM) and two separation columns YMC-Pack Pro C18 RS, 5 μm, 250 x 4.6 mm I.D.; YMC-Europe) in series connected; flow rate = 0.85 mL/min; T = 45°C. The mobile phase consisted of MeOH (A) and H2O plus 0.1% TFA (B); gradient profile: Isocratic elution with 85% A for 2 min, then a linear gradient to 100% A in 13 min, followed by an isocratic elution with 100% A for additional 45 min and an isocratic period with 85% A for 15 min to equilibrate the columns again (total time: 75 min each run). Data recording was carried out as described above. Note that with only one YMC column, a similar resolution can be obtained if the flow rate is set to 0.43 mL/min and the gradient delay to 1 min (isocratic), followed by a gradient to 100% MeOH in 6.5 min while keeping all other parameters constant (see also [2]). However, the method with two columns in series connected has been giving slightly better resolution values. For quantitation experiments, the method of external calibration has been applied (peak area plotted against concentration in mg/mL). Routinely, each analyte was dissolved in MeOH due to its corresponding linearity range. Normally, a complete master mix containing all target analytes of interest in one solution was prepared. Samples were dissolved in 8 mL MeOH in a 10-mL volumetric flask and sonicated (approximately 15-30 min). At last, the final volume was adjusted to 10 mL with the additional MeOH. From the stem solution, five to six standards with decreasing concentrations levels of a factor of two were generated (10 mL volumetric flasks or by calibration qualified automatic pipette systems). Prior to injections, each solution was filtered (0.2 μm syringe filter). The expected peak area was determined by preliminary tests. The analyte concentration range was adjusted to the expected value in the pure resin sample.
Fig. S1 HPLC analysis of A) *B. serrata* extract, B) the AF of the *B. serrata* extract, and C) the NF of the *B. serrata* extract under UV detection at 210 nm.
Table S1 Calculated contents of boswellic acids (BAs) in *B. serrata* extracts investigated in this study. Extracts of *B. serrata* gum resin were prepared by extraction using diethyl ether and analysis of the AF by HPLC (see Fig. S1) as described.

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>Calculated content in <em>B. serrata</em> extract [g/g %]</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>β-BA</td>
<td>5.05</td>
</tr>
<tr>
<td>4</td>
<td>Aβ-BA</td>
<td>4.01</td>
</tr>
<tr>
<td>5</td>
<td>KBA</td>
<td>0.92</td>
</tr>
<tr>
<td>6</td>
<td>AKBA</td>
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</table>

References
