Supporting Information to:

**The Immunomodulatory Effect of Sambucol on Leishmanial and Malarial Infections**

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**Extraction and Analysis of Sambucol Components**

The following is adapted from Alberte et al., United States Patent Application 20070248700.
Supercritical fluid extraction (SFE)
Dried ground elderberry or flower was added to the extraction solvent, pure carbon dioxide, at a 60:1 ratio. The feedstock was loaded into an SFE extraction vessel, where the pressure was maintained at 60–500 bar and the temperature at 40–80 °C. After 1 hour the extracted, purified essential oil chemical constituents were collected and stored in a light-protective glass bottle at 4 °C.

Gas chromatography–mass spectroscopy (GC-MS) analysis
GC-MS was performed using a Shimadzu GCMS-QP2010 system, which included a high-performance gas chromatograph, a direct coupled GC-MS interface, an electroimpact (E1) ion source with independent temperature control, and a quadrupole mass filter. The system was controlled with GC-MS solution Ver. 2 software for data acquisition and post-run analysis. Separation was carried out on an Agilent J&W DB-5 fused silica capillary column [30 m × 0.25 mm i.d., 0.25 μM film (5% phenyl, 95% dimethylsiloxane) thickness]. The initial temperature was 60 °C; after 2 min the temperature was increased to 120 °C at a rate of 4 °C/min, held for 15 min, increased to 200 °C at rate of 4 °C/min, held for 15 min, and then increased to 240 °C at rate of 4 °C/min and held for 15 min. The total run time was approximately 92 min. The sample injection temperature was 250 °C. One microliter of sample was injected by an autoinjector in split-less mode in 1 min. The carrier gas was helium and the flow rate at 60 KPa was 1.03 mL/min (linear velocity 37.1 cm/min, total flow 35 mL/min). The MS ion source temperature was 230 °C and the GC-MS interface temperature was 250 °C. The MS detector was scanned between $m/z = 50$ and 500 at a scan speed of 1000 AMU/s with an ionizing voltage of 70 eV. Solvent cutoff temperature was 3.5 min {authors: please verify min rather than °C}.

Hydroalcoholic leaching
Elder species ground dry plant material and the extraction solvent, aqueous ethanol, were loaded into an extraction vessel that was heated to about 60–90 °C and stirred for 2 h. The resultant fluid extract was filtered and centrifuged, the filtrate (supernatant) collected, and the residue measured for volume and solid content dry mass after evaporation of the solvent.
Affinity adsorption extraction
A solid affinity polymer adsorbent resin adsorbs the active phenolic acids contained in the hydroalcoholic extract. Amberlite XAD7 HP (Rohm & Hass) is preferably used due to the high affinity for the phenolic acid chemical constituents of elder and related species. The appropriate weight of adsorbent resin beads (5 mg phenolic acids per gram of adsorbent resin) was washed with 4–5 bed volumes (BV) ethanol and 4–5 BV distilled water before and after being loaded onto the column. The phenolic acid–containing aqueous solution was then loaded onto the column at a flow rate of 3–5 BV/hour. Once the column was fully loaded, the column was washed with distilled water at a flow rate of 2–3 BV/h to remove any impurities from the adsorbed phenolic acids. The effluent residue and washing residue were collected, measured for mass content and phenolic acid content, and discarded. Elution of the adsorbed phenolic acids was accomplished in an isocratic fashion with 40% or 80% ethanol/water as an eluting solution at a flow rate of 3–4 BV/h, and the elution curve was recorded for the eluent extract. Elution volumes were collected every 25 min and analyzed using HPLC for solids content and purity.

Process chromatography
A Shimadzu high-performance liquid chromatographic (HPLC) LC-10AVP system equipped with a LC10ADVP pump and an SPD-M 10AVP photo diode array detector was used. The ethanol extraction products were measured on a reversed-phase Jupiter C18 column (250 × 4.6 mm i.d., 5 μ, 300 Å) with a mobile-phase flow rate of 1 mL/min, a column temperature of 25 °C, and a 10-μL injection volume. The mobile phase consisted of acetic acid (A) (5% formic acetic acid v/v) and methanol (B). The gradient was programmed as follows: 5% B (2 min), a linear increase from 5% to 24% B (2–10 min), 24% B (10–15 min), a linear increase from 24% to 35% B (15–30 min), 35% B (30–35 min), a linear increase from 35% to 45% B (35–50 min), 45% B (5 min), a linear increase from 45% to 5% B (55–65 min), 5% B (65–68 min). Stock solutions of reference standards were prepared in methanol and diluted in ethanol to yield final concentrations of 1.0, 0.5, 0.25, 0.1, and 0.05 mg/mL. All solutions were used within 7 days, stored at 4 °C, and brought to room temperature before use. Detection wavelengths were 330 nm for caffeic acid derivatives, 350 nm for flavonoids, 355 nm for aglycones, and 520 nm for anthocyanidins.
**Water solvent leaching and ethanol precipitation**

The solid residue from the hydroalcoholic leaching extraction process and the extraction solvent, distilled water, were loaded into an extraction vessel, heated to 70–90 °C, and stirred for about 2 hours. The slurry was filtered, centrifuged, and evaporated to remove water; anhydrous ethanol was then used to reconstitute the original volume of the solution. The solution was centrifuged and decanted and the supernatant residue discarded. The precipitate product was analyzed for polysaccharides with the colorimetric method using dextran 5000–410,000 molecular weight as reference standards and was analyzed for protein with the Bradford protein analysis method.

**Direct analysis in real time mass spectrometry (DART-MS)**

A JOEL AccuTOF DART LC time-of-flight mass spectrometer (Joel USA, Inc.) was used. This time-of-flight (TOF) mass spectrometer technology does not require any sample preparation and yields masses with accuracies to 0.00001 mass units. For the cationic mode, the DART needle voltage was 3000 V, the heating element was at 250 °C, electrode 1 was at 100 V, electrode 2 was at 250 V, and helium gas flow was 7.45 L/min. For the mass spectrometer, orifice 1 was 10 V, the ring lens was 5 V, and orifice 2 was 3 V. The peak voltage was set to 600 V in order to give resolving power starting at approximately 60 m/z, yet allowing sufficient resolution at greater mass ranges. The micro-channel plate detector (MCP) voltage was set at 2450 V. Calibrations were performed each morning prior to sample introduction using a 0.5 M caffeine solution standard. Calibration tolerances were held to <5 mmu. Samples were introduced into the DART helium plasma with sterile forceps, ensuring that a maximum surface area of the sample was exposed to the helium plasma beam. A sweeping motion was employed, allowing the sample to be exposed repeatedly on the forward and back stroke for approximately 0.5 s/swipe and preventing pyrolysis of the sample. When an appreciable total ion current (TIC) signal was observed, the sample was removed, allowing for baseline/background normalization. For the anionic mode, the DART and AccuTOF MS were switched to negative ion mode. Needle voltage, heating element temperature, electrode voltages, and helium gas flow were as described above. For the mass spectrometer, orifice 1 was −20 V, the ring lens was −13 V, and orifice 2 was −5 V. The peak voltage was 200 V, and the MCP voltage was set at 2450 V. Samples were introduced as described. All data analysis was conducted using the MassCenterMain Suite software provided with the instrument.