Supplementary data to accompany paper:

Antistaphylococcal activity of *Inula helenium* L. essential oil: eudesmane sesquiterpene lactones induce cell membrane damage

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Reduction of the essential oil (catalytic hydrogenation (H₂, Pd/C) and reduction with NaBH₄)

A sample of the essential oil (1 g) was dissolved in absolute ethanol (50 mL) and stirred (room temperature) with 100 mg of 10% Pd/C under hydrogen (atmospheric pressure), until no further consumption of hydrogen was observed. After that, the catalyst was removed using a Puradisc syringe filter (Econofilter, Agilent Technologies: membrane - regenerated cellulose; housing - polypropylene; pore size - 0.45 μm; diameter - 25 mm) and the solvent was evaporated under a gentle stream of nitrogen at room temperature and immediately analyzed (sample IH-H₂/Pd). One portion of IH-H₂/Pd (500 mg) was treated with 60 mL of 5% NaOH in water and extracted 3 times with 10 mL of diethyl-ether. The combined organic phases were washed two times with 25 mL of water, dried over anhydrous magnesium sulphate and the solvent removed under a gentle stream of nitrogen at room temperature and immediately analyzed (sample IH-H₂/Pd-Lactone). The aqueous phase was neutralized with diluted hydrochloric acid (1:5, v/v) and extracted 3 times with 10 mL of diethyl-ether. The combined organic phases were also washed two times with 25 mL of water, dried over anhydrous magnesium sulphate and the solvent removed at room temperature and immediately analyzed (sample IH-H₂/Pd-Acid). A small amount of IH-H₂/Pd-Acid was esterified using a diazomethane solution in diethyl ether (DM). DM was added directly into the GC vial containing 0.5 mL of IH-H₂/Pd-Acid solution in diethyl ether (1:100) until no more N₂ was evolved and in this way we obtained sample IH-H₂/Pd-Ester for GC/MS.

A portion of the essential oil (500 mg) was dissolved in absolute ethanol (10 mL) and stirred (room temperature) with excess NaBH₄ (2.0 g) during 4 hours. After that, the reaction mixture was poured out into 50 mL of cold water and extracted 3 times with 15 mL of diethyl-ether. The combined organic phases were dried over anhydrous magnesium sulphate and the solvent removed as stated above and immediately analyzed (sample IH- NaBH₄).

All samples were, after the bulk of solvent (ether or ethanol) was removed under a stream of N₂, additionally exposed to vacuum at room temperature for a short period to ensure the complete elimination of the solvent (multiple gravimetric measurements).
Preparative medium-pressure liquid chromatography and thin layer chromatography

Preparative medium-pressure liquid chromatography (MPLC) was performed with a pump module C-601 and a pump controller C-610 Work-21 pump (Büchi, Switzerland) and was carried out on pre-packed column cartridges (40x75 mm), Silica-gel 60, particle size distribution 40-63 μm, Büchi. Silica gel 60 on Al plates, layer thickness 0.2 mm (Kieselgel 60 F254, Merck) was used for thin layer chromatography (TLC). The spots on TLC were visualized by UV light (254 nm) and by spraying with 50% (v/v) aqueous H₂SO₄ followed by heating. All the solvents (Aldrich, USA; Merck, Germany; Fluka, Germany) were purified by distillation before use.

A sample of the essential oil (500 mg) and also the sample IH-NaBH₄ (250 mg, essential oil reduced by NaBH₄) were subjected to MPLC (gradient Et₂O : hexane, from pure hexane to pure diethyl ether, 100 mL). The obtained fractions (10 mL) were pooled according to TLC and/or GC/MS analyses.

GC and GC/MS analyses

The GC/MS analyses of all samples (pure oil, transformed oil, MPLC fractions) were repeated three times using a Hewlett-Packard 6890N gas chromatograph. The gas chromatograph was equipped with a fused silica capillary column DB-5MS (5% phenylmethylsiloxane, 30 m × 0.25 mm, film thickness 0.25 μm, Agilent Technologies, USA) and coupled with a 5975B mass selective detector from the same company. The injector and interface were operated at 250 °C and 300 °C, respectively. The oven temperature was raised from 70 °C to 290 °C at a heating rate of 5 °C/min and then isothermally held for 10 min. As a carrier gas helium at 1.0 mL/min was used. The samples, 1 μL of the corresponding solutions in diethyl ether (1 : 100), were injected in a pulsed split mode (the flow was 1.5 mL/min for the first 0.5 min and then set to 1.0 mL/min throughout the remainder of the analysis; split ratio 40 : 1). Mass selective detector was operated at the ionization energy of 70 eV, in the 35–500 amu range with a scanning speed of 0.34 s. GC (FID) analyses were carried out under the same experimental conditions using the same column as described for the GC/MS. The percentage composition was computed from the GC peak areas without the use of correction.
factors. Qualitative analyses of the sample constituents were based on several factors. Firstly, the comparison of the essential oils linear retention indices relative to the retention times of C₈-C₂₅ n-alkanes on the DB-5MS column with those reported in the literature. Secondly, by comparison of their mass spectra with those of authentic standards, as well as those from Wiley 6, NIST02, MassFinder 2.3. Also, a homemade MS library with the spectra corresponding to pure substances and components of known essential oils was used, and finally, wherever possible, the identification was achieved by GC co-injection with an authentic sample. Relative standard deviation (RSD) of repeated measurements (independent sample preparations and GC-MS) was for all substances below 1%. The only exceptions which had higher RSD were minor components such as β-selinene, 4,5-di-epi-aristolochene, β-caryophyllene and β-elemene, where RSD was 6, 7, 10 and 13%, respectively.