Supporting Information

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Chemical Composition and *in vitro* Antioxidant Activities of *Mutellina purpurea* Thell. Flowers Essential Oil

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Essential oil by hydrodistillation

The fresh plant material (50 g) was placed in the round-bottomed flask and 500 mL distilled water was added. Hydrodistillation was performed simultaneously for 3 h by use of the Clevenger apparatus. The obtained oil was dried over anhydrous sodium sulphate and stored in 4 ^{o}C before the GC analysis.

GC analysis of essential oil

GC/MS

The gas chromatograph Varian 450-GC with the type triple qadrupol Varian 320-MS was used. The analytes were separated on a 30 m x 0.25 mm VF-5ms capillary column coated with a 0.25 μm film of 5% phenyl methylpolysiloxane, and were inserted directly into the ion source of the MS. The split injection 1:100 was used for the samples. The column oven temperature was programmed at 4 ^{o}C/min from an initial temperature of 50 ^{o}C (held for 1 min) to 250 ^{o}C which was held for 10 min. The injection temperature was 250 ^{o}C and injection volume was 1 μL. Helium (99.999%) was used as carrier gas at a flow rate of 0.5 mL/min. The ionising electron energy was 70 eV and the mass range scanned was 40-1000 m/z with 0.8 sec/scan. Manifold temp. was 45 °C, transfer line temp. was 289.5 °C and the ion source temp. was 271.2 °C.

GC/FID

GC Varian 3800 (Varian, USA) equipped with a CP-8410 auto-injector and a 30 m x 0.25 mm DB-5 column (J&W Scientific, USA), film thickness 0.25 μm, carrier gas Helium 0.5 mL/min, injector and detector FID temperatures were, 260 °C; split ratio 1:100; inject volume 5 μL. A temperature gradient was applied (50°C for 1 minute, then incremented by 4 °C/min to 250 °C, 250°C for 10 minutes).

Headspace analysis

The HT3 Headspace Sampler (Teledyne Tekmar) was used to analyze the volatile fraction of the *M. purpurea* flowers. The fresh flower was immediately introduced into a 22-mL headspace vial. The parameters for the HT3 Headspace auto sampler were as follows: G.C. cycle time - 71.00 min., valve oven temp. and transfer line temp. – 200 °C, flow rate - 50 mL/min., platen/sample temp. – 150 °C, sample equilibration time - 10.00 min., mixing time - 1.00 min., mixer stabilize time - 0.50 min., pressurize - 10 PSIG, pressurize time - 2.00 min. pressurize equilibration time - 0.20 min., loop fill pressure - 5 PSIG, loop fill time - 2.00 min., inject time - 1.00 min. The loop volume used on the HT3 (injection volume into the GC) was 0.25 mL.

Qualitative analysis

The qualitative analysis was carried out on the basis of MS spectra, which were compared with the spectra of the NIST library (2002) and with data available in the literature [1]. The identity of the compounds was confirmed by their retention indices [2], taken from the literature, [1] and our own data for standards (α-pinene, *p*-cymene, limonene, γ-terpinene,

linalool, (*E*)-caryophyllene, caryophyllene oxide; Fluka, Sigma-Aldrich Chemie GmbH, Germany).

Quantitative analysis

Essential oil was diluted 100 times using n-hexane to achieve 1 ml volume, then 100 μL dodecane C12 as internal standards (1 mg/mL in toluene) was added into the diluted oil. Such prepared sample was subjected to GC–MS and GC-FID determinations. The quantitative analysis of essential oil obtained in Clevenger- apparatus, was performed on the basis of calibration curves plotted to find the dependence between the ratio of peak area for the analyte to the area for internal standard ($A_{analyte}$:$A_{i.s.}$) vs. the analyte concentration ($C_{analyte}$), for *p*-cymene, γ-terpinene, linalool, (*E*)-caryophyllene, caryophyllene oxide, in appropriate concentration range [3]. The contents of the analysed substances were read from achieved calibration curves, the data for which originated from peak areas for *M. purpurea* oil components and internal standard peak areas from GC separation. The final result took into account all dilutions during the whole analytical procedure. For comparative purposes, the percentage of components of the *M. purpurea* essential oil obtained in Clevenger apparatus as well as static headspace technique was presented, assuming that the sum of peak areas for all identified constituents was 100%.

Antioxidant assay

ABTS radical cation decolorization assay was carried out, using an ABTS•+ decolorization assay [4]. ABTS was generated by oxidation of ABTS with potassium persulfate. ABTS was dissolved in deionized water to 7 mM concentration, and potassium persulfate added to a concentration of 2.45 mM. The reaction mixture was left to stand at room temperature for 24 h in the dark before the use. The ABTS solution was diluted with ethanol to an absorbance of 0.7 +/- 0.02 at 745 nm. The wavelength was estimated on the basis of maximum absorbance obtained for the complex of ABTS and essential oil solution. After addition of 3.0 ml of diluted ABTS•+ solution to several dilutions of essential oil, the absorbance was read exactly 1 min after initial mixing. All determinations were performed in triplicate.

Statistical analysis

All determinations were conducted in triplicate, and all results were calculated as mean ± standard deviation (SD). Statistical analysis was performed by use of the Statistica version 7 software.

References

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