Materials and Methods

Isolation of the Essential Oils

After the collection of the plant material, the inflorescences were separated from the branches and divided into two parts. The first part was subjected to hydrodistillation for 3 h, using a modified Clevenger-type apparatus. The oils (Acfa1EO to Acfa5EO) were obtained using n-pentane as a collecting solvent and subsequently dried over anhydrous sodium sulphate and stored under N₂ atmosphere in amber vials at 4°C until they were analyzed. Based on the estimated obtained volume of each essential oil, the corresponding amount of capillary GC grade pentane was added to afford an appropriate concentration of 10 µl/ml.

Preparation of absolute ethanol extract (absolute cassie)

The remaining quantity (second part) of the fresh flowers of all samples was extracted with petroleum ether (PE). The PE residues were subsequently extracted with absolute ethanol and the samples Acfa1abs to Acfa5abs were obtained with highly aromatic odour.

Gas chromatography analysis

Gas chromatography (GC) analysis was carried out using a SRI 8610C GC-FID system, equipped with DB-5 capillary column (30 m x 0.32 mm; film thickness 0.25 µm) and connected to a FID detector. The injector and detector temperature was programmed at 280 °C. The carrier gas was He, at flow rate of 1.2 mL/min. The thermal program was 60-280 °C at a rate of 3 °C/min. Two replicates of each oil sample were processed in the same way.

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Gas chromatography – Mass spectrometry (GC-MS) analysis

Analyses of the oils were performed using a Hewlett Packard 5973-6890 GC-MS system operating in the EI mode at 70eV, equipped with a split/splitless injector (200 °C). The transfer line temperature was 250 °C. Helium was used as carrier gas (1 mL/min) and the capillary column used was HP-5MS (30 m x 0.25 mm; film thickness 0.25 μm). The temperature program was the same with that used for the GC analysis and the split ratio 1:10. The injected volume was 1μL. Acquisition mass ranges between 40-400 amu.

Identification of Components

The identification of the compounds was based on comparison of their retention indices (RI), their retention times (RT) and mass spectra with those obtained from authentic samples (purchased from the Sigma-Aldrich Group) and/or the NIST/NBS, Wiley libraries and the literature [S1].

References