Supporting Information

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Chemical Composition of Vegetative Parts and Flowers Essential Oils of Wild *Anvillea garcinii* Grown in Saudi Arabia

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S1: Experimental

Chemicals

Analytical-grade acetone (Sigma-Aldrich, Germany) was used for the dilution of oil samples. Pure volatile compounds such as limonene, *α*-phellandrene, *α*-pinene, *β*-pinene, etc. as well as some essential oil fractions enriched with 1,8-cineole, linalool, *p*-cymene, terpenene-4-ol and *α*-terpinolene were available in our laboratory and were used for co-injection analysis.

Isolation of essential oils

The flowers and leaves along with the thin stems from freshly obtained *A. garcinii* plant material were separated and chopped into small pieces. The chopped fresh flowers (439.0 g) and leaves with thin stems (643.0 g) were separately subjected to hydro-distillation performed on a conventional Clevenger-type apparatus for 4 h to afford light-yellowish oils with a pleasant odor. The oils obtained after the distillation were dried over anhydrous sodium sulfate and stored at 4°C until further use. The yield of the volatile oils derived from the flowers and leaves were 0.05% and 0.04% (v/w), respectively, based on the fresh weight.

GC–FID and GC–MS analyses

The essential oils were analyzed using a GC–MS and GC–FID equipped with two columns, one of which was polar (DB-Wax), and the other was nonpolar (HP-5MS). GC–MS was performed on an Agilent single-quadrupole mass spectrometer with an inert mass selective detector (MSD-5975C detector, Agilent Technologies, USA) coupled directly to an Agilent 7890A gas chromatograph equipped with a split–splitless injector, a quickswap assembly, an Agilent model 7693 autosampler and a HP-5MS fused silica capillary column (5% phenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm i.d., film thickness 0.25 μm, Agilent Technologies, USA). Supplementary analyses were performed on a DB-Wax fused silica capillary column (polyethylene glycol, 30 m × 0.25 mm i.d., film thickness 0.25 μm, Agilent Technologies, USA). The HP-5MS column was operated using an injector temperature of 250°C and the following oven temperature profile: an isothermal hold at 50°C for 4 min, followed by a ramp of 4°C/min to 220°C, an isothermal hold for 2 min, a second ramp to 280°C at 20°C/min and finally an isothermal hold for 15 min. Conversely, the DB-Wax column was operated using an injector temperature of 250°C and the following oven temperature profile: an isothermal hold at 40°C for 4 min, followed by a ramp of 4°C/min to 220°C and an isothermal hold for 10 min.

Approximately 0.2 μl of each sample diluted in acetone (5% solution in acetone) was injected using the split injection mode; the split flow ratio was 10:1. The helium carrier gas was flowed at 1 ml/min. The GC–TIC profiles and mass spectra were obtained using the ChemStation data analysis software, version E-02.00.493 (Agilent). All mass spectra were acquired in the EI mode (scan range of *m*/*z* 45–600 and ionization energy of 70 eV). The temperatures of the electronic-impact ion source and the MS quadrupole were 230°C and 150°C, respectively. The MSD transfer line was maintained at 280°C for both polar and non-polar analysis. The GC analysis was performed on the same instrument, an Agilent GC-7890A dual-channel gas chromatograph (Agilent Technologies, USA) equipped with flame ionization detection (GC-FID) using both polar (DB-Wax) and non-polar (HP-5MS) columns under the same experimental conditions as described above for GC-MS. A splitter ("Y" tube) was used in order to get dual detector (MS and FID) response with the same injection and time. The FID detector temperature was maintained at 300°C for both polar and nonpolar analyses. The relative composition of the oil components was calculated on the basis of the GC–FID peak areas measured using the HP-5 MS column without the use of a correction for the response factors. The results are reported in Table 1 according to their elution order on the HP-5MS column.

Retention indices

A mixture of a continuous series of straight-chain hydrocarbons, C8-C31 (C8-C20, 04070, Sigma-Aldrich, USA and C20-C31, S23747, AccuStandard, USA) was injected onto both polar (DB-Wax) and nonpolar (HP-5MS) columns under the same conditions previously described for the oil samples to obtain the linear retention indices (LRIs) (also referred to as linear temperature programmed retention indices [LTPRI]) of the oil constituents provided in Table 1. The LRIs were computed using van den Dool and Kratz's equation [1].

Identification of volatile components

We identified single components by matching their mass spectra with the library entries (WILEY 9th edition, NIST-08 MS library version 2.0 f as well as the Adams and Flavor libraries) of a mass spectra database as well as by comparing their mass spectra and linear retention indices (LRI) with published data obtained using both polar and nonpolar columns [2-7] and the co-injection of authentic standards available in our laboratory.

S2: References

[1] H. Van den Dool and P. D. Kratz (1963). A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography, *J. Chromatogr.* **11**, 463-471.

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[4] NIST, NIST Chemistry WebBook: NIST Standard Reference Database Number 69. Available: http://webbook.nist.gov/chemistry/ [accessed 17October2012].

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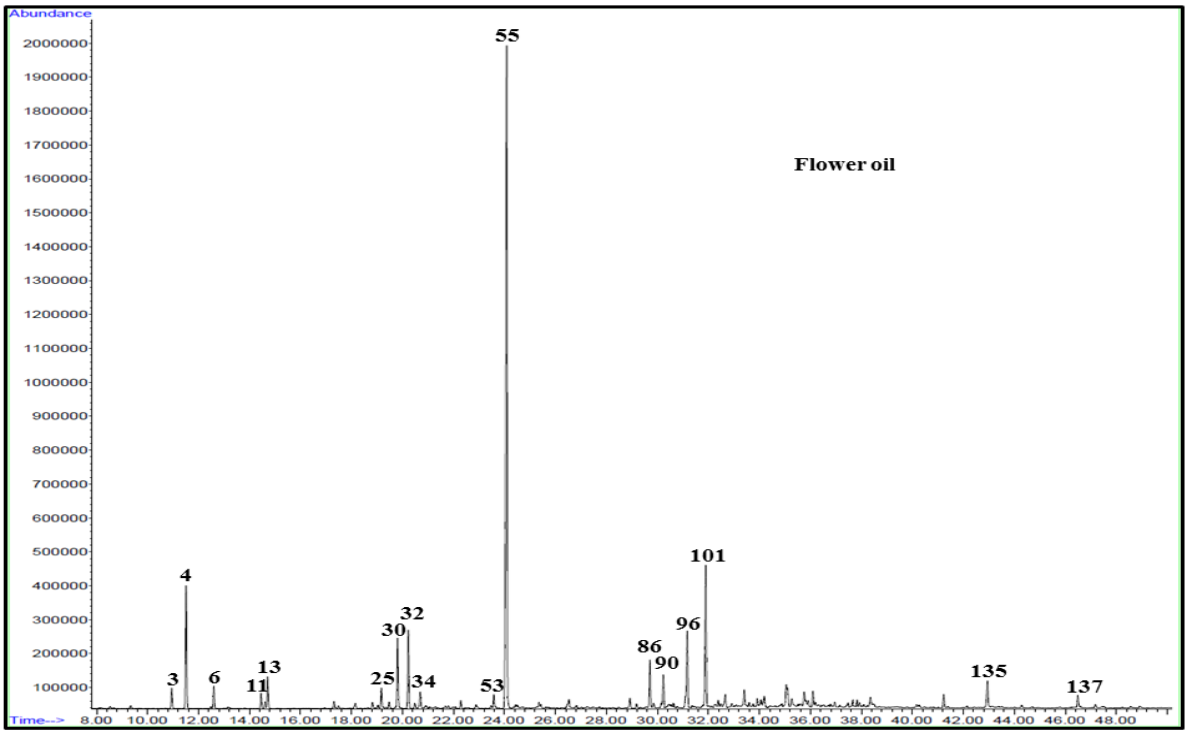
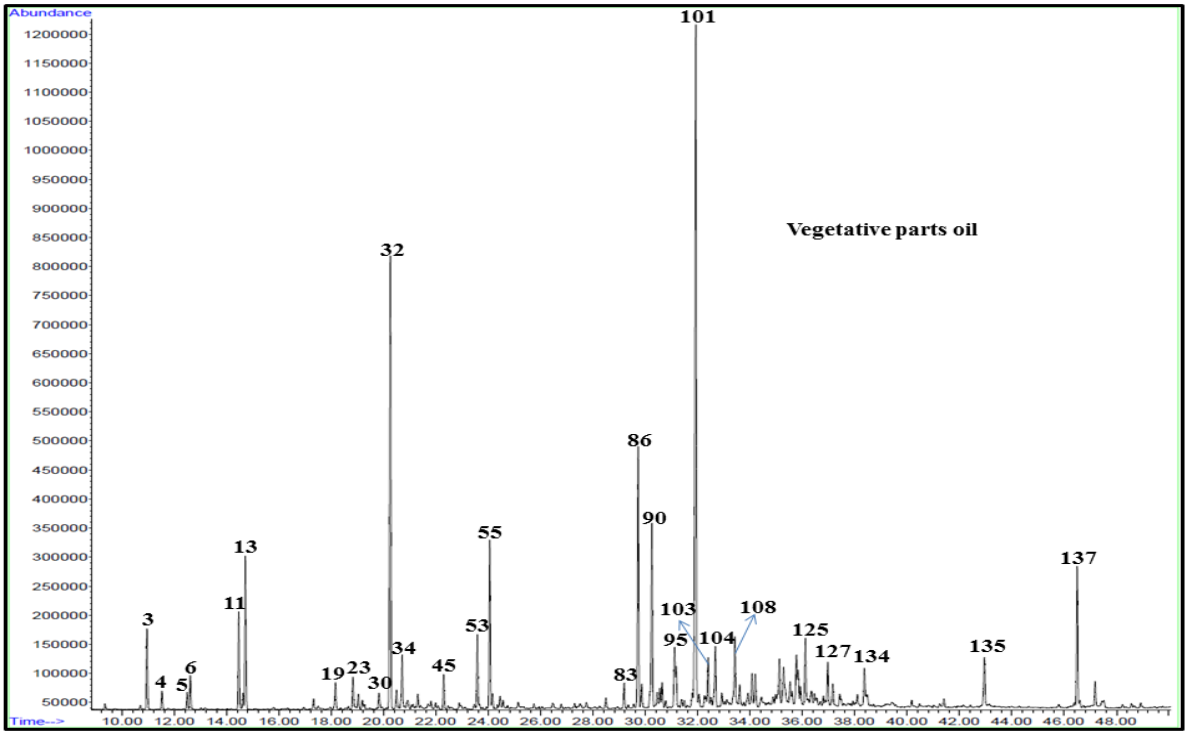
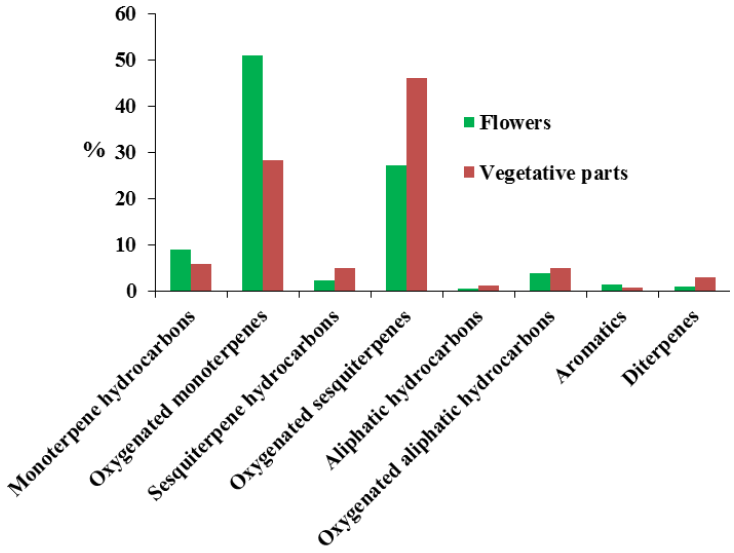
[7] N. W. Davis (1990). Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20M phases, *J. Chrom.* **50**, 1-24.



S3: GC–FID chromatogram of *A. garcinii* flowers essential oil on HP-5MS column. Numbering of identified peaks is given according to the serial number of compounds in Table 1.



S4: GC–FID chromatogram of *A. garcinii* vegetative parts essential oil on HP-5MS column. Numbering of identified peaks is given according to the serial number of compounds in Table 1.



S5: Compound classes found in the oils derived from the flowers and vegetative parts of *A. garcinii*.

S6: Common components of *A. garcinii* essential oils found in different parts of Saudi Arabia and Iran.

Sl. No.	Compounds	Saudi *A. garcinii* oils		Iranian *A. garcinii* oils*	
		Flowers (%)	Leaves and Stems (%)	Flowers (%)	Leaves (%)
1	α-Pinene	0.9	1.7	7.5	16.1
2	Limonene	0.3	0.3	6.5	12.9
3	1,8-Cineole	1.4	2.9	1.8	5.0
4	Bornyl acetate	33.7	3.4	41.5	4.9

*Obtained from reference 20.