

Supporting Information

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Essential Oil Constituents from the Leaves of *Anoectochilus setaceus*, *Codonopsis javanica* and *Aristolochia kwangsiensis* from Vietnam

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S.1. Preparation of the Plant Samples

Prior to hydrodistillation, leaf samples were air-dried (18⁰C) under laboratory shade for two weeks to reduce the moisture contents. In addition, sediments and other unwanted materials were separated from the samples. Afterwards, samples were pulverized to coarse powder to enlarge the surface area bearing oils.

S.2. Hydrodistillation of the Oils

In this process 300 g of air-dried and pulverized leaves *A. setaceus*, *C. javanica* and *A. kwangsiensis* were separately introduced into a 5 L flask and distilled water (5 L) was added until it covers the sample completely. Hydrodistillation was carried out with a Clevenger-type distillation unit designed according to the specification as previously described [1]. The distillation time was 3 h and conducted at normal pressure. The volatile oils distilled over water and were collected separately into clean weighed sample bottles. The oils were kept under refrigeration (4⁰C) until the moment of analyses. Each distillation was done in triplicate.

S.3. Gas Chromatography (GC) Analysis of Essential Oils

The GC analysis of essential oils was carried out using an Agilent Technologies HP 6890 Plus GC which was equipped with a flame ionization detector and HP-5MS column. The dimension of the column is 30 m x 0.25 mm (film thickness 0.25 μ m). The GC operating parameters based on temperature programming were as follows: a column oven- 40⁰C, injection pot-250⁰C while the temperature was 260⁰C. Time programming: 40⁰C for 2 min, temperature and then raise to 220⁰C (and held isothermally for 10 min) at 4 ⁰C/min. The carrier gas used was H₂ at a flow rate of 1 mL/min. The split ratio was 10:1 while 1.0 μ L of the essential oil was injected into the GC at inlet pressure was 6.1 kPa. Each analysis was performed in triplicate. Retention indices (RI) value of each component was determined relative to the retention times of a homologous *n*-alkane series (C₆-C₃₂), under the same operating conditions, with linear interpolation on the HP-5MS column as described previously [2].

S.4. Gas Chromatography-Mass Spectrometry (GC/MS) Analysis of Essential Oils

GC/MS was performed on HP 5973 MSD mass spectrometer with HP 6890N Plus GC system fitted with a fused silica capillary HP-5 MS column (30 m x 0.25 mm, film thickness 0.25 μ m). The conditions were the same as described above for GC with He (1 mL/min) as carrier gas. The MS conditions were as follows: ionization voltage 70 eV; emission current 40 mA; acquisitions scan mass range of 50-550 amu at a sampling rate of 1.0 scan/s as described previously [2].

S.5. Identification of the Constituents

The spectra were scanned from *m/z* 50 to 550. Most constituents were identified by gas chromatography by comparison of their retention indices with those in the literature or with those of available authentic compounds. The retention indices were determined in relation to a homologous series of *n*-alkanes (C₈-C₂₄) obtained under the same operating conditions. Further identification was made by comparison of their mass spectra with those stored in the library [3], peak enrichment on co-injection with authentic compounds where possible as described previously [2].

References

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- [3] National Institute of Science and Technology. (2011). Chemistry Web Book. Data from NIST Standard Reference Database 69.