SUPPLEMENTARY MATERIAL

Comparative analysis of essential oil composition from flower and leaf of *Magnolia kwangsiensis* Figlar & Noot.

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ABSTRACT

The essential oils from *Magnolia kwangsiensis* Figlar & Noot. were obtained using hydrodistillation, and analyzed by GC and GC–MS. A total of 31, 27 and 26 constituents were identified in the oils from male flower, female flower and leaf of *M. kwangsiensis*, and they comprised 99.2, 98.5 and 96.2% of the oils, respectively. Monoterpene hydrocarbons predominated in the oils and accounted for 48.3% of male flower oil, 54.0% of female flower oil, and 44.6% of leaf oil. The compositions of flower oils were quite similar but with different content, and were different from those of leaf oil.

Keywords: Composition, Essential oil, Flower, *Magnolia kwangsiensis* Figlar & Noot., Leaf

Background

Magnolia kwangsiensis Figlar & Noot. (*M. kwangsiensis*), also known as *Kmeria septentrionalis* Dandy, the member of Magnoliaceae, was recognized in 1931 (Dandy, 1931). It is an evergreen tree, and has leathery leaves and unisexual flowers in the dioecious individuals (Fu & Jin, 1992). The white tepals are usually three, but vary from two to six, both in male and female flowers (Figure 1 B and C). The earliest flowers were regarded as small to moderate in size with undifferentiated perianth, stamens lacking a well-differentiated filament, and a gynoecium composed of one or more unilocular ovaries (Zanis, Soltis, Qiu, Zimmer, & Soltis, 2003). It has trouble with reproduction due to pollination relying on few shared insects, except for phenological mismatch between the blooming of the male and female. And its testas with oil make seeds decompose easily in damp woodlands and sprout with difficulty. What's worse, it has been destroyed by cutting with the straight good wood. Therefore, the resources of *M. kwangsiensis*

significantly dwindled. Until 1999, *M. kwangsiensis* was listed as first-grade State Protection plant in China (under state protection category I). The plant is an endemic genus to China, only locates in Guangxi, Guizhou and Yunnan Province. At present, studies on *M. kwangsiensis* are mainly involved in the aspects of pollen wall structure(Xi, Zhang, Lin, & Zeng, 2000; Xu & Kirchoff, 2008), seed storage and germination test (Lai, Huang, Pan, Qin, Shi, & Liu, 2007). However, researches about extractives and essential oils of *M. kwangsiensis* are very scarce.

Essential oils, complex mixtures of volatile compounds, are produced by living organisms and isolated by physical means (pressing and distillation) from the part of plant with known taxonomic origin(Baser & Buchbauer, 2009). Plant essential oils are valuable natural products and frequently used as antimicrobial, antioxidant, and anticancer agents and for their cosmetic, and food applications(Bakkali, Averbeck, Averbeck, & Idaomar, 2008; Casiglia, Jemai, Riccobono, Bruno, Scandolera, & Senatore, 2014; Jerkovic, Mastelic, & Milos, 2001; Khadhri, El Mokni, Almeida, Nogueira, & Araujo, 2014; Li, Yang, Zhong, & Yu, 2015). This has attracted more and more attention of many researchers to study more plants essential oils from chemical components(Jing, Lei, Li, Xie, Xi, Guan, et al., 2014; Karami & Dehghan-Mashtani, 2015; Russo, Cardile, Graziano, Formisano, Rigano, Canzoneri, et al., 2014; Tuttolomondo, Dugo, Ruberto, Leto, Napoli, Cicero, et al., 2015; Vekiari, Protopapadakis, Papadopoulou, Papanicolaou, Panou, & Vamvakias, 2002). Studies on testa oil from *M. kwangsiensis* indicated they are excellent sources of aroma constituents(Huang, Zhou, Lai, Li, & LIU, 2010). Researches on other Magnoliaceae reported that essential oils contained medicinal active compounds (Chen, Wang, & Liu, 2002; Hao, Yu, & Tian, 2000). Whereas, so far, the volatile components in the flower and leaf of *M. kwangsiensis* have hardly been studied.

Experimental

Samples

Fresh flower and leaf of *M. kwangsiensis* were collected from Wuming (Guangxi, China) in the fourth week of May and April, 2014, respectively. The plant was identified by two plant experts (Prof. Jia-Ye Lai and Zai-Liu Li, Forestry College of Guangxi University). The voucher specimen has been deposited at the herbarium of Forestry College of Guangxi University (GXU. FC.2013111601) . The fresh samples were kept at 4°C and analyzed within 24 hours.

Extraction of the essential oil

The essential oils were extracted by hydrodistillation for 6~8 hours. The volatile compounds containing the water-soluble fraction were allowed to settle for 1 hour. The oil layer was separated and stored in sealed glass tubes under refrigeration at 4°C prior to chemical analysis. The yields of essential oils were determined by the volumetric method (v/w) and expressed as a mean value of triplicates from three independent experiments.

Essential oil analysis

The essential oils from flower and leaf of *M. kwangsiensis* were analyzed by GC-FID and GC-MS. Before injection, essential oils were diluted with ethanol at 1:1 volume ratio, and 0.2µL of dilution of sample was used for each run in a split-mode (1:60).

Analysis of essential oils was carried out by GC performed on GC-2010 (SHIMADZU, Japan), equipped with a RTX-1 fused silica capillary column (30m × 0.25mm, film thickness 0.25µm; Restek, USA), and fitted to a flame ionization detector. The injector and detector temperature was set at 250°C and 270°C, respectively. The initial temperature was kept at 60°C for 1 min, then with an increase of 2°C /min to 80°C, and the temperature was gradually increased to 150°C at a rate of 3°C /min, finally ramped at a rate of 6°C /min until 250°C and kept at the temperature for 5 min. The percentage composition was computed from the peak areas, without correction factors.

GC-MS analysis of essential oils was performed on QP5050A (SHIMADZU, Japan), equipped with a DB-1 fused silica capillary column (30m × 0.25mm, film thickness 0.25µm; J&W Scientific, USA). GC parameters were similar to GC-FID, the interface and MS source temperature was set at 250°C and 270°C, respectively. The carrier gas was helium with a column head pressure of 47kPa and flow rate of 1.2mL/min. The mass selective detector was operated in electron-impact ionization mode with a mass scan range from m/z 35 to 500 at 70 eV. The identification of the components was achieved based on their retention indices and their recorded mass spectra fragmentation patterns with the standard mass spectra reported in the literature (Adams, 2007; Cavaleiro, Salgueiro, Miguel, & Proença da Cunha, 2004; Ceccarini, Macchia, Flamini, Cioni, Caponi, & Morelli, 2004; Khadhri, El Mokni, Almeida, Nogueira, & Araujo, 2014) and mass spectra from NIST08 and NIST08S (National Institute of Standards and Technology, US).

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Figures

Figure 1 A) *Magnolia kwangsiensis* Figlar & Noot.. B) Male flower, with three tepals. C) Female flower, with three tepals.