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Essential oil constituents derived from different organs of a relictual conifer *Wollemia nobilis*

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ABSTRACT

The chemical composition of the essential oil of leaves (0.9%, w/v) and twigs (0.33%, w/v) of *Wollemia nobilis* (Araucariaceae) – a remnant species thought to have been extinct for 65 million years – was investigated by GC/MS. The main constituents of both leaf- and twig-derived oil samples were 16-kaurene (61.8% and 38.2%, respectively) and germacrene D (9.9% and 22%). The principal difference was a considerably more pronounced sesquiterpene presence in the twig-oil, amounting to 33.5%, than in its folial counterpart (23.4%). On the contrary, while remaining the dominant group in both oil samples under investigation, diterpenoids were relatively more abundant in leaf-derived oil constituting 65.3%, versus 41.7% detected in twigs. To our knowledge, this is the first report dealing with the essential oil composition of Wollemi pine twigs, as opposed to the leaf-derived volatiles.

1. Introduction

The discovery of Wollemi pine in 1994 made international headlines, hailed as the 'botanical find of the century'. The unique conifer was found in a deep Triassic sandstone gorge in Wollemi National Park within 150 km of Sydney. Interestingly, the aboriginal meaning of the word 'wollemi' translates to 'watch out, look around you' (Hill, 1996). Yet, a rugged Australian wilderness managed to keep its secret for millions of years.

Subsumed, on a morphological basis, to the 200 million year old conifer family Araucariaceae, *Wollemia* constitutes a whole new monotypic genus (Jones et al., 1995). While the evolutionary relationships within the 'monkey puzzle tree' family are poorly known, *Wollemia* was confirmed to be distinct from the related genera *Araucaria* and *Agathis* by Gilmore and Hill (1997) through DNA sequencing of the plastid gene *rbc*L. The sequence data, combined with different ranges of other conifer taxa, imply that *Wollemia* derived prior to its taxonomic counterparts and may be the earliest derived genus in Araucariaceae (Setoguchi et al., 1998). An alternative hypothesis suggests that *Wollemia* be a sister group to *Agathis* with these two forming a clade that is sister to *Araucaria* (Gilmore and Hill, 1997; Stefanovic et al., 1998). Despite the origin of the relictual pine remaining an evolutionary enigma, the preliminary investigation of the chemistry of *W. nobilis* is consistent with that of other araucaroids, with the abundance of diterpenoids in their leaf oils being a seemingly characteristic trait (Brophy et al., 2000).

As surveyed at allozyme, amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) loci, the genetic diversity of Wollemi pine proved to be exceptionally low. It is considered, in fact, the most extreme case known in

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planta accounted for by the combination of such probable contributing factors as small population effects (less than a hundred trees occurring in the wild, in an inaccessible canyon), clonality and below-average genetic variation within the botanical family (Peakall et al., 2003). Thus, each genetic individual seems to play a key role in the future evolution of the monotypic conifer, as the genetic diversity within this species constitutes the full genetic range of the genus. Moreover, microflora and other species associated with the microhabitat provided by the relictual pine are vital and unique elements contributing to the biodiversity of Wollemi National Park, New South Wales and, indeed, the Australian continent. It is noteworthy that *Pestalotiopsis guepini* – a paclitaxel producing fungus, was found to be a representative of the endophytic flora harbored by *W. nobilis* (Strobel et al., 1997).

The aforementioned considerations, combined with the immense popularity of the 'green dinosaur' keenly sought after as a horticultural plant, reinforce the necessity of reducing the threat to the population in the wild from illegal collectors. For this reason, the Australian government has undertaken an unprecedented conservation program, making *W. nobilis* commercially available as a potted plant (NSW Department of Environment and Conservation, 2006). Thus, in early 2006 first Wollemi specimens left 'the land down under', opening a window into an unimaginably ancient past – a great opportunity of studying this extraordinary conifer worldwide.

The present study aims at a detailed analysis of the chemical composition of essential oil samples derived from different plant organs: leaves and twigs. Since the only report on the chemistry of Wollemi pine available to date (Brophy et al., 2000) deals with the analysis of leaves obtained from mature trees indigenous to Australia, we also hope to draw parallels and point out possible discrepancies, as our samples originate from a juvenile Wollemi specimen cultivated ex situ.

2. Experimental

2.1. Plant material

W. nobilis, W.G. Jones, K.D. Hill and J.M. Allan: a juvenile (1-year-old, 30 cm in length) Wollemi pine bearing the registered Wollemi pine™ logo was purchased in 2008 from Arboretum Kalmthout, Belgium – a representative of Wollemi Australia exclusively licensed by the Royal Botanic Gardens Sydney (RBGS) through NSW National Parks and Wildlife Service (NPWS) to propagate and market the Wollemi pine in Australia and internationally. The conifer was additionally authenticated at the Botanical Garden 'De Kruidhof', Buitenpost, the Netherlands. A voucher specimen (gro-ASOK-02) is deposited in our department.

2.2. Isolation procedure

The oil samples were separately isolated from 14.2 g of air-dried and freshly ground (1 mm) leaves as well as 6.3 g of air-dried and coarsely ground twigs by hydrodistillation for 3 h in 300 mL water, according to the determination of the essential oil content in vegetable drugs, using the apparatus described in the *Nederlandse Farmacopee* (Anon., 1966). Xylene (100 μL) was used as the collection liquid, and the oil was stored at −20 °C until analyzed. The oil was diluted 50 times with cyclohexane prior to GC/MS analysis.

2.3. Gas chromatography–mass spectrometry

A Shimadzu GCMS QP5000 system was used (Shimadzu Corporation, Japan) equipped with a GC-17A gas chromatograph, an AOC-20i auto-injector, and GCMS Solution version 1.10 software. The GC conditions were: column, Zebron Capillary GC Column, ZB-5 MS (15 m × 0.25 mm; film thickness 0.1 μm); oven temperature programme, 50–310 °C at 5 °C/min; injector temperature, 260 °C; carrier gas, He; total flow, 59.3 mL/min; split ratio, 21:1; injected volume, 2.0 μL. MS conditions: ionization energy, 70 eV; ion source temperature, 250 °C; interface temperature, 300 °C; scan speed, 4000 scans/s; mass range, 34–600 u.

The identity of the components was assigned by comparison of their retention indices, relative to C_9–C_{29} *n*-alkanes, with mass spectral databases and from literature (Adams, 2001; Joulain and König, 1998; Flavor & Fragrance Library Shimadzu Benelux, 's-Hertogenbosch, the Netherlands, 2003). The percentages of the components were calculated from the GC peak areas, using the normalization method.

3. Results and discussion

Hydrodistillation of the leaves and twigs of *W. nobilis* yielded 0.9%, w/v and 0.3%, w/v oil, respectively. In total 42 components could be identified in the leaves and 40 in twigs, corresponding to 95.9% and 77.8% of the oil derived from the respective organs. To our knowledge, this is the first report dealing with the essential oil composition of Wollemi pine twigs, as opposed to the leaf-derived volatiles.

Comparative analysis of the investigated oil samples, as presented in Table 1, indicates a more pronounced monoterpene content in Wollemi foliage, reaching 7.3%, while the twig-derived oil contains 4.5%. The principal monoterpenoid constituents of both oils are β-pinene (0.8% and 0.5%, for leaves and twigs, respectively) and β-myrcene (0.4% and 0.3%). Sesquiterpene presence is considerably more pronounced in the twig-derived oil sample, amounting to 33.5%, than in its folial counterpart containing