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Short communication

Aerial parts of *Callitris* species as a rich source of deoxypodophyllotoxin

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ABSTRACT

Podophyllotoxin is a naturally occurring plant lignan with potent anticancer applications. Its supply from *Podophyllum hexandrum*, its current natural source, is becoming ever scarcer and alternative sources are required. Deoxypodophyllotoxin, one precursor of podophyllotoxin, could be an interesting commercial alternative. In such a context, *Callitris* species could represent an attractive source of this compound. However, to date, only two *Callitris* species have been analyzed for their content in lignans. In this work, we were able to detect high amounts of deoxypodophyllotoxin in leaves of *C. endlicheri*, *C. rhomboidea*, *C. preissii* and *C. drummondii*. The highest content (5.8 mg/g DW) was measured in young leaves of *C. endlicheri*. Moreover, important variation was observed among the four species and the different tested organs but no significant variation over the two different years studied. Regarding our results, aerial parts of *Callitris* species should be considered as a promising raw source for the large scale production of deoxypodophyllotoxin.

1. Introduction

Podophyllotoxin (Fig. 1A) is a well-known plant lignan serving as exclusive starting compound for the semi-synthesis of potent topoisomerase II inhibitors (Etoposide, Teniposide and Etopophos) used as leading anticancer drugs (Koulman et al., 2004). These semi-synthetic drugs are widely used, alone or in combination with other drugs, to treat lung, testicular, pancreatic and stomach cancers as well as myeloid leukaemia (Ekstrom et al., 1998; Holm et al., 1998). Podophyllotoxin is also known to inhibit the *Herpes simplex* type I virus replication (Hammonds et al., 1996). Other podophyllotoxin derivatives display pronounced anti-HIV properties (Chen et al., 2007).

Podophyllotoxin is extracted from rhizomes of *Podophyllum hexandrum*, a perennial herb coming from the Himalayas, which accumulate huge amounts of this compound (around 4% of DW) (Bedir et al., 2002). However, due to both overcollection and a lack of cultivation, this species is now endangered (Koulman et al., 2004). Consequently, since a full synthetic approach for podophyllotoxin is not a commercially viable solution because of its complex structure, the question of podophyllotoxin supply generate an active search for alternative natural sources (Gordaliza et al., 2004).

Deoxypodophyllotoxin (Fig. 1A), the plant precursor of podophyllotoxin, has also demonstrated a wide range of relevant activities against a number of cancer cell lines (Ikeda et al., 1998; Kim et al., 2002; Masuda et al., 2002; Muto et al., 2008; Xu et al., 2010). Deoxypodophyllotoxin could represent a promising alternative source for the large-scale production of the semi-synthetic derivatives of podophyllotoxin in pharmaceutical industry. Indeed its bioconversion into podophyllotoxin by *Linum album* cell suspension (Federolf et al., 2007) or into epipodophyllotoxin by using *Penicillium* species or *Aspergillus niger* cultures (Kondo et al., 1989) and by the human cytochrome P450 3A4 (Vasilev et al., 2006; Julsing et al., 2008).

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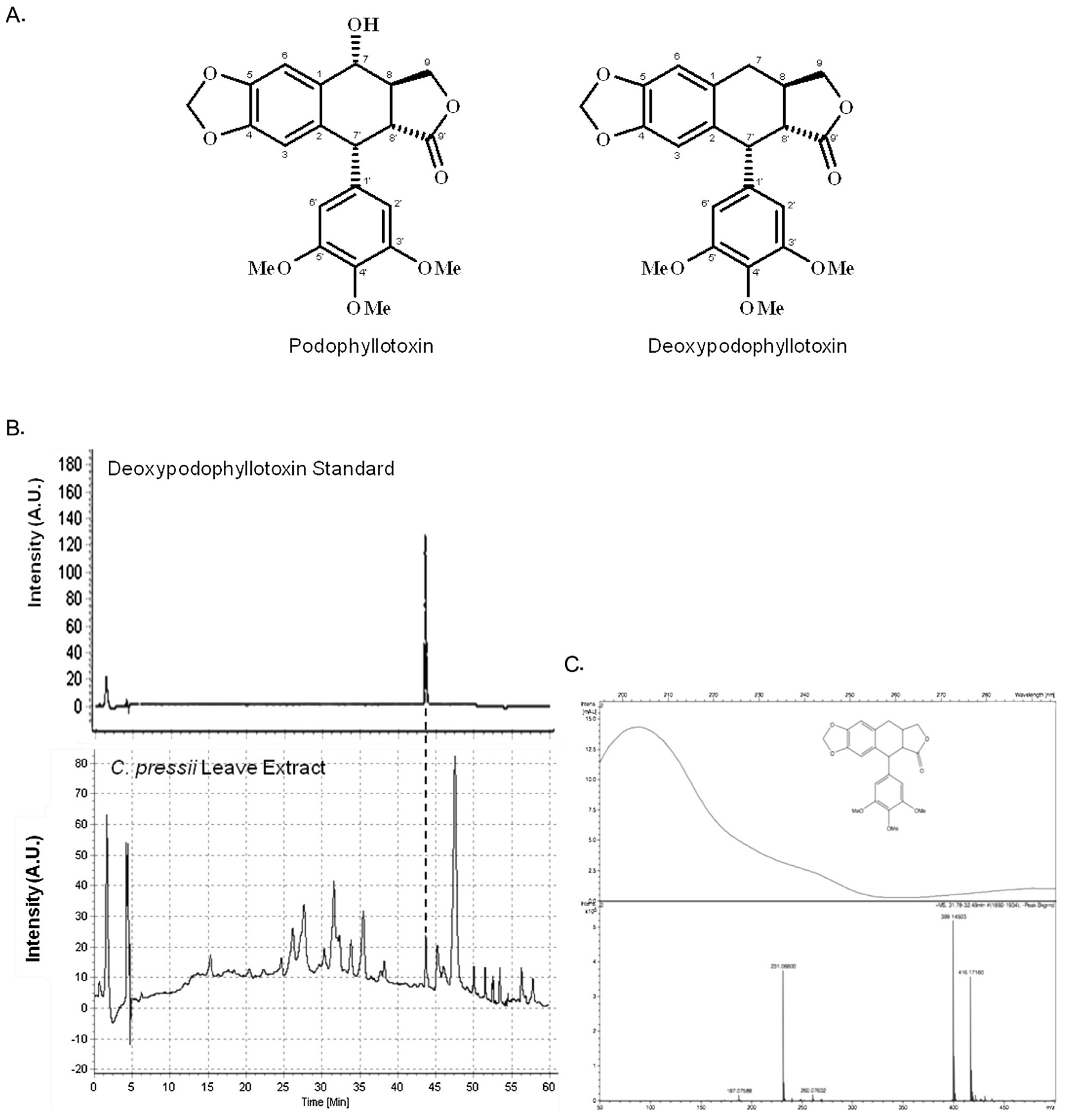

Fig. 1. (A) Chemical structures of podophyllotoxin and deoxypodophyllotoxin; (B) Typical HPLC (at 280 nm) chromatograms of ethanol extract of leaves of *Callitris pressii*; (C) MS spectra of deoxypodophyllotoxin in ethanol extract of leaves of *Callitris pressii*.

The occurrence of podophyllotoxin and deoxypodophyllotoxin has been described in a large range of plant species and primarily among the cupressaceae family (Table A.1). *Juniperus* species could represent an attractive source of podophyllotoxin and its direct precursor deoxypodophyllotoxin (Cantrell et al., 2013; Gawde et al., 2009a, 2009b; Renouard et al., 2011; Zheljazkov et al., 2013). Another interesting cupressaceae member is the *Callitris* genus comprising endemic trees of Australia or New Caledonia. These trees are very tolerant to a wide variety of climate changes (temperature and drought) (Brodribb et al., 2010) and to pest attack such as termites (Watanabe et al., 2005). This could be related to the occurrence of deoxypodophyllotoxin, described in *C. drummondii* (Van Uden et al., 1990) and *C. columellaris* (Aynehchi, 1971) aerial parts (Table A.1), known to have pronounced insectical activity (Gao et al., 2004). Interestingly, the *Callitris* genus is the second most important genus in the native Australian forest industry after *Eucalyptus* and represents the sole significant softwood timber commercially harvested from native forest. Moreover, the leaves of *Callitris* constitute a by-product for the timber industry and could therefore be an attractive renewable source for the extraction of anti-cancer drugs. However, only few studies have focused on the occurrence of deoxypodophyllotoxin derivatives in *Callitris* species (Kier et al., 1963; Aynehchi, 1971; Van Uden et al., 1990). The aim of the present study was thus to evaluate the deoxypodophyllotoxin production potential of aerial parts of 4 important *Callitris* species (*C. drummondii*, *C. rhomboidea*, *C. endlicheri* and *C. preissii*) among