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Profiling and seasonal variation of chemical constituents from *Pseudotsuga menziesii* wood

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ABSTRACT

The composition of sapwood, transition zone and heartwood extracts from Pseudotsuga menziesii was studied using LC/ESI–MS/MS. Based on retention times, and MS fragmentation patterns, 40 phenolic compounds, 11 terpenes and 7 fatty and organic acids were identified or accordingly characterized. The flavonoids taxifolin-*O*-hexoside and the corresponding aglycon taxifolin, dihydrokaempferol, pinocembrin, pinobanksin, quercetin and flavan-3-ols, catechin and epicatechin were the main compounds detected in wood collected in the fall. Taxifolin derivatives were identified as monomers, dimers and flavonolignans. Comparative metabolite profiling of the three zones showed differences in metabolite distribution. Flavonoids were distributed in the 3 zones. Lignans, flavonolignans, phenolic acid derivatives and terpenes accumulated mainly in the transition zone while tannins were detected only in sapwood. Fatty and organic acids were present in sapwood and the transition zone. Multiple Reaction Monitoring (MRM) was used for the quantification of main metabolites of extracts in order to observe seasonal variations in the wood. The relative quantity of 35 selected compounds in each zone was discussed. The lowest metabolite content was usually observed in winter whereas flavonoids were found in high quantities in heartwood either in spring or autumn.

1. Introduction

The genus *Pseudotsuga* belongs to the Pinaceae family and comprises eight to twelve species. Two of them are distributed in western North America (Little, 1979) whereas the other ones are found in Mexico and eastern Asia (Lavender et al., 2014).

Pseudotsuga menziesii (Mirb.) Franco, called Douglas-fir or Oregon pine, is one of the commonest trees in western North America (Lavender et al., 2014). It was introduced in Europe in the nineteenth century and the origins of the first Douglas-fir were poorly documented. In France, this species was introduced before 1850, but its plantation was developed after the second World War as part of reforestation projects (Ponette et al., 2001). In 2014, 2285 million m^3 of Douglas-fir were harvested and 774,000 m^3 were transformed into boards. Douglas-fir is fast becoming the major resinous species replacing maritime pine and spruce. Douglas fir is exceptionally versatile because it is both strong and visually pleasing. It is widely available in structural (decking, subflooring, beams, stringers, posts and timber), appearance, remanufacturing and industrial grades.

Douglas-fir is the most commonly softwood species used as lumber in the Limousin region (Central France) because of its mechanical properties and natural durability (qualified as intermediary) (Scheffer et al., 1998).

Natural durability corresponds to the ability of wood to resist biological degradation; this resistance is due to the presence of specialized metabolites which display antioxidant, antifungal and insecticide activities either through direct toxicity or synergistic interactions (Dellus et al., 1997; Schultz and Nicholas, 2000). Douglas-fir wood is composed of polymers (lignin, cellulose, hemicelluloses, pectins) and a large pool of secondary metabolites. Polymer interactions as well as specialized metabolite distribution within the wood are responsible for its good mechanical, physical and biological properties (Hillis, 1987; Taylor et al., 2002).

The content of extractives in *Pseudotsuga menziesii* wood is estimated around 6–12% (Taylor et al., 2008). They are mainly constituted of polyphenols, terpenoids, steroids, fatty acids (Dellus et al., 1997; Erdtman et al., 1968; Foster et al., 1980; Hirose and Sakai, 1973; Rogers et al., 1974; Sakai and Hirose, 1973). Oligomeric proanthocyanidins and taxifolin (dihydroquercetin) are the most abundant molecules in Douglas-fir wood of along with flavonoids and phenolic polymers

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(Dellus et al., 1997). Flavonols, dihydroflavonols, lignans and a biflavonoid were isolated from sapwood and heartwood (Dellus et al., 1997). The phytochemical composition of the transition zone has not been studied. Other parts of Douglas-fir such as needles, roots and bark have been well studied for their chemical composition (Ferreira et al., 2016; Foo et al., 1992; Foo and Karchesy, 1989; Graça and Pereira, 2000; Laver and Fang, 1989; Loveland and Laver, 1972a). The potential of Douglas-fir as raw material in the production of biofuel or for access to chemical products has been demonstrated (Oleson and Schwartz, 2016; Pan et al., 2013). In a previous study, metabolite profiling of Douglas-fir was performed by GC/MS in developing xylem. This study has demonstrated the influence of environmental variation (Robinson et al., 2007). The bark is mainly used as source of extractives and in particular of polyphenols (Oleson and Schwartz, 2016).

A research project was designed to study the distribution of specialized metabolites during heartwood formation, which would result in genetic improvements of natural durability of Pseudotsuga menziesii in the Limousin part (North-East) of the Région Nouvelle Aquitaine. The objective of this work was to establish the seasonal variations of extractives (or chemical markers) present in each zone of the wood.

Considering that Douglas fir is of economic interest for industry, this study aims at bringing new parameters for i) the valorisation of sapwood as a source of extractives, ii) finding the most appropriate season for harvest in order to improve durability. A number of applications use only heartwood, which has first to be separated from sapwood.

The purpose of this study was to establish the distribution of these compounds in each wood zone including sapwood, the transition zone and heartwood and to look for variations of this distribution in relation with the season. Accordingly, specific compounds were detected and quantified in extracts of wood samples collected in autumn, more than twenty-one compounds from sapwood, forty-nine from the transition zone and twenty from heartwood. The developed LC/MS/MS method was able to separate taxofolin stereoisomers along with their aglycon or *O*-glycosylated dimer derivatives. The relative quantification of 35 selected metabolites was performed by MRM (Multiple Reaction Monitoring) on samples collected during all of the four seasons.

2. Materials and methods

2.1. Plant material and wood samples

Douglas fir log slices were collected at Gimel-les-Cascades, Nouvelle Aquitaine region in October 2013 (autumn). A 25 year-old Douglas-fir tree genotype 1039 was cut down (tree diameter 42 cm) and disks were taken at 1.3 m above the soil line. These disks from Douglas-fir woodlots were cooled to −30 °C to block metabolic activity and were then stored 3 weeks at −80 °C. The disk was lyophilized and cut with a microsaw to separate the bark as well as the three wood tissues which were carefull delimited (heartwood diameter 26 cm) (Fig. 1). Each zone was ground with a high performance knife mill Retsch grinder SM 2000.

The same process was used for disks collected in winter (January), spring (April) and summer (July) 2014 at the same site from 25 year-old Douglas-fir trees.

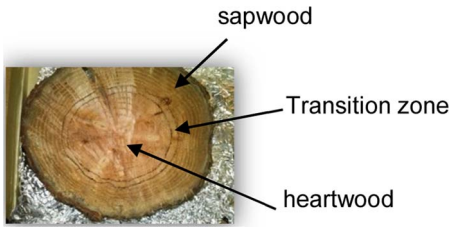

Fig. 1. Picture of the washer with the delimitation of the three wood tissues.

2.2. Materials and reagents

The solvents used for extraction were HPLC grade from Carlo Erba Reagents (Val de Reuil, France) and VWR Chemicals (Fontenay-sous-Bois, France).

The standards taxifolin and quercetin were isolated from *Pseudostuga menziesii*. Their structures were elucidated by spectroscopic methods (IR, MS, 1D and 2D NMR).

Silybin B, (+)-catechin and (−)-epicatechin were purchased from Extrasynthese (Genay, France). Standard purity was up to 95% as determined by HPLC-DAD. Stock solution for each standard (1 mg/mL) was prepared in acetonitrile (ACN).

^{1}H and ^{13}C NMR were recorded on a Bruker DPX spectrometer at 400.13 and 100 MHz respectively. ^{1}H and ^{13}C NMR chemical shifts are given in ppm relative to TMS, with coupling constants (*J*) reported in Hz. Preparative TLC were carried out on precoated silica gel 60 F_{254} plates (Merck) and spots were detected under UV (254 and 366 nm).

Column chromatography was performed on 200–400 mesh silica gel 60H (35–70 µm, Merck) and on Sephadex LH-20® (25–100 µm; Pharmacia Biotech Ltd) colomns. Preparative medium-pressure liquid chromatography (MPLC) was performed with a Buchi pump model (C-605, C-615)

2.3. Extraction procedure

Two distinct extraction procedures were used. Maceration was used for large scale extraction and isolation of standards and an Accelerated Solvent Extractor (ASE) was used for seasonal comparison studies.

Finely ground powder (10 g) was macerated with acetone/H_2O 70/30 (4 × 50 mL) to yield crude extracts (200 mg for sapwood and 540 mg for heartwood) after evaporation of solvent.

Using ASE, powder (5 g) was extracted thrice in 22 mL acetone/H_2O 70/30 in one static cycle (Fig. 2) at 100 °C under constant pressure of 10 bar for 5 min. This process with solvent mixture rather than free water is adapted for extraction of middle polar and hydrophilic compounds, it excluded volatile and apolar compounds (Gironi and Piemonte, 2011).

2.4. Access to standards

A portion (5 g) of the acetone aqueous extract obtained from 280 g sapwood was subjected to Sephadex LH-20 column chromatography eluted with MeOH to give 15 fractions. Fraction F3 (102 mg) was purified by silica gel eluted with CH_2Cl_2/MeOH (98:2 to 90:10 v/v) to furnish dihydrotodomatuic acid (**41**) (2 mg). Fraction F8 (70 mg) was chromatographed on silica gel eluted with CH_2Cl_2/MeOH (90:10, 80:20) gradient to give taxifolin (**15**) (20 mg). Chromatography of

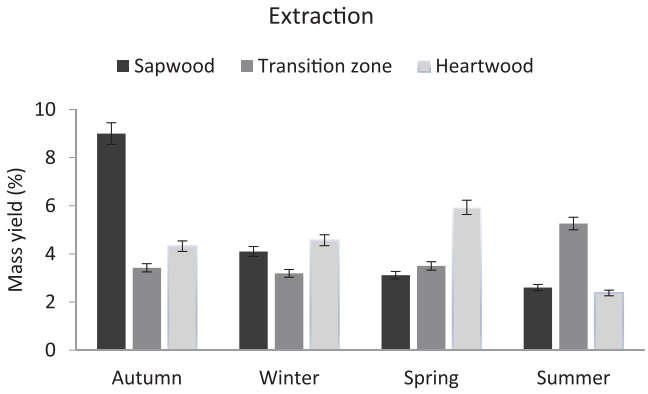

Fig. 2. Mass yield from extraction of three areas of *Pseudotsuga menziesii* wood from samples collected at different seasons. Extraction was performed in triplicate. Results are expressed by mean ± SD.