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4th Workshop and 5th Management Committee Meeting

INTRODUCTION

Barks, one of the major wastes from wood-consuming industries, show a large diversity in chemical composition and its components are viewed with a renewed interest as valuable source of chemicals and bioactive compounds.

Plant constituents, namely polyphenols, have been largely studied because these secondary metabolites are generally involved in defense against ultraviolet radiation or aggression by pathogens. [1] There is a growing interest in the potential health benefits of dietary plant polyphenols as antioxidant.[2] Epidemiological studies and associated meta-analyses strongly suggest that long term consumption of diets rich in plant polyphenols offer protection against development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases.[2]

Knowing the chemical constituents of barks is helpful in the discovery of novel therapeutic agents as well as new sources of economic materials like new chemicals, with relevance in a biorefinary context.[2]

MATERIALS & METHODS

The methanolic extracts, free of lipophilic extractives, of bark samples from *Pseudotsuga menziesii*, *Betula pendula*, *Quercus cerris*, *Quercus suber* and *Quercus variabilis* were obtained after a first extraction with dichloromethane in a Soxhlet apparatus during 6 h for removal of lipophilic compounds and than an extraction with methanol during 16h. The extracts were dried under vacuum and at room temperature.

Samples of each bark methanolic extract were first prepared as stock-solutions with a concentration of 0.5 $mgml^{-1}$ and stored in ambar flasks to protect them from possible light degradation. Samples were dissolved in an ultrasound system (5 minutes) and then filtered through 0.45 μm filters.

The samples (5 μl were injected) were analyzed in a ThermoScientific Accella Autosampler, Accella Pump 600 and Accella PDA detector, using methanol (solvent A) and water (solvent B), both acidified with 0.1% formic acid as mobile phases, and with the chosen elution program at a flow rate of 0.5 ml min^{-1} (Program: 30% A, 5 min gradient until 35% A, 10 min gradient until 45% A, 10 min gradient until 70% A, 5 min gradient until 100% A, isocratic for 10 min, with an Accucore RP-C18 column.

RESULTS & DISCUSSION

Tree bark methanolic extracts are complex mixtures of phenolics, namely phenols, flavonoids and tannins. In order to analyze these complex extracts by HPLC-DAD they were submitted to a pre-treatment consisting of a liquid-liquid extraction with cyclohexane, dichloromethane and ethyl acetate. Phenolic compounds were identified using standard substances.

In all methanol bark extracts were identified as minor compounds catechin, epicatechin, quercetin, ellagic and gallic acids.

Syringic acid and vanilliun were identified as major compounds only in *Q. cerris* bark.

In *P. menziesii* methanol bark extract eriodyctiol and naringenin were identified as major compounds, as well as a non-identified structure (Figure 1).

Conipheryl alcohol was identified in *P. menziesii* and in *Quercus* tree species.

In both *B. pendula* and *Q. suber* the major compounds were not identified.

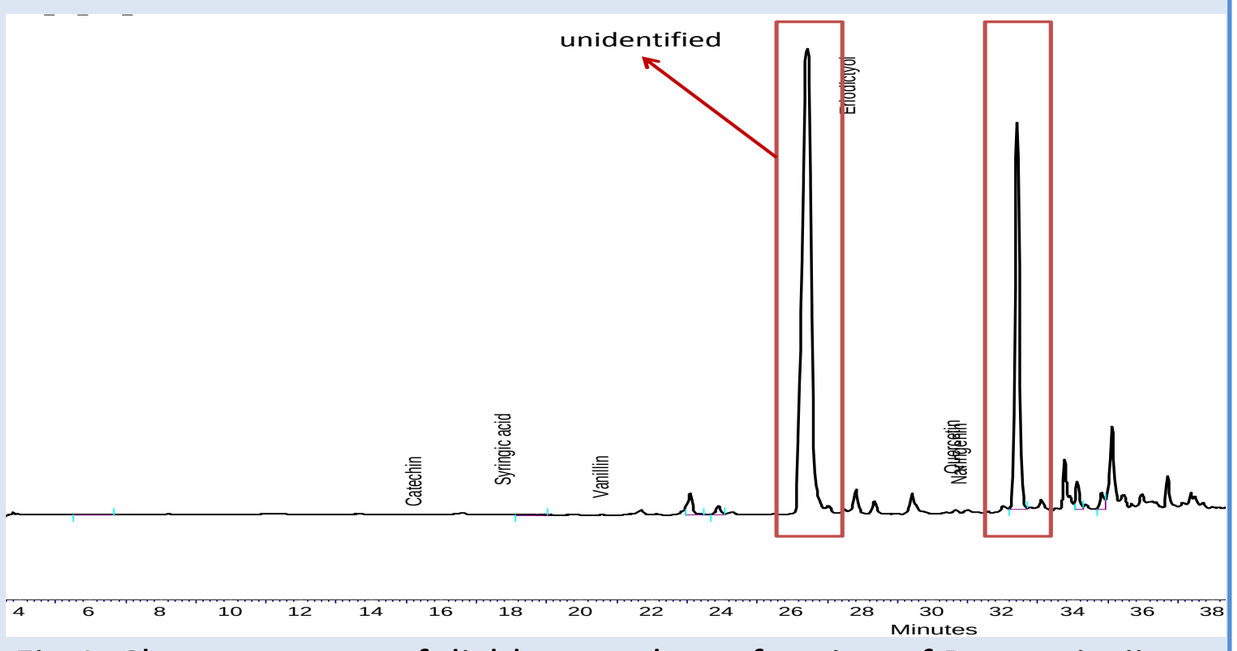

Fig.1. Chromatogram of dichloromethane fraction of P. menziesii bark extract

Conclusions

- An HPLC method (mobile phases, elution gradient and column) was proposed for the identification of the polar analytes present in the methanol extracts of barks from *P.menziesii, B. pendula, Q. suber, Q. cerris* and *Q. variabilis* tree species.
- This preliminary study allowed the identification of several phenolic compounds as catechin, epichatecin, vanillin, quercetin, naringenin, conipheryl alcohol, allagic, syringic and gallic acids.

Bibliographic support

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[2] Pandey, K.B., Rizvi, S.I. 2009. Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev. 2: 270–278.

Acknowledgments

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