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Scion's core funded experiments on $scCO_2$ wood extracts (proof of concept)

August 2016

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Introduction

The focus of this project is to *extract* and identify *high value chemicals found naturally in trees using* green technologies at a proof of concept level.

A recent demonstration of such an approach was carried out by supercritical fluid extraction of phenolic and flavanone compounds found in Eucalyptus [1]. An example of a compound that was extracted in high selectivity is Eriodictyol which is listed on Sigma-Aldrich at a cost of $491 per 10mg [2].

There is an increasing market for phytochemicals to be extracted, concentrated and sold to the pharmaceutical and nutraceutical markets. ENZO Nutraceuticals Ltd is a New Zealand company that is selling an anti-inflammatory and antioxidant product from the extraction of bark from *Pinus radiata* trees. Plant compounds found in Enzogenol contain polyphenols, proyanidins, bioflavonoids and organic acids [3].

Traditional extraction techniques involve the use of harmful chlorinated solvents, such as dichloromethane and an extremely large amount of solvent is required compared to the amount of extractive isolated. The use of Scion's proprietary method of green wood dewatering using supercritical carbon dioxide ($scCO_2$) offers a number of advantages to obtain wood extracts.CO_2 can be used throughout a widespread range of temperatures and pressures, which aids selective extraction of groups of molecules.

A metabolomics platform has already been developed at Scion for analysing dewatered sap extracts, which has been extended to analyse the $scCO_2$ extracts from trees.

Initial experiments to carry out $scCO_2$ extractions were undertaken on two species of interest: *Douglas fir* and *Eucalyptus nitens* and four tissue types: leaves/needles, bark, sapwood and heartwood.

Sample collection

Stands of the two species of interest, *Douglas fir* and *Eucalyptus nitens* were found on Long Mile road, Rotorua. Leaves/needles and bark were taken by sampling from all over the tree and 10 mm core samples were taken of the sapwood and heartwood.

$scCO_2$ extraction and Analysis

The extractions were undertaken in supercritical carbon dioxide ($scCO_2$) at one set of conditions. The extracts were then analysed without separation using Gas Chromatography–Mass Spectrometry (GC-MS) and Nuclear Magnetic Resonance spectroscopy (NMR) at Scion to identify different substances within the extracts.

$scCO_2$ fluid as an extraction solvent behaves in way that is similar in solvation power to hexane. $scCO_2$ can have co-solvents added (entrainers) to modify this to remove more polar substances. The $scCO_2$ has advantages over traditional solvent extraction it is non-flammable, recyclable with minimal effort and can be modified easily (co-solvents). Low temperature extraction conditions reduce the chance of thermal modification to extracts and allows extracts to be collected into small volumes resulting in little end-of-life solvents to dispose of. One potential drawback is the capital outlay of equipment capable of carrying out extractions.

Results

The top three compounds that were able to be easily identified by GC-MS libraries in each tree species and tissue type are listed below. The amount reported is an estimate based on the mass fraction of compound in µg of compound per g of sample loaded into the extraction vessel. Prices were found from the Sigma-Aldrich [2] website of the high purity chemical unless otherwise stated.

Douglas fir

Bark:

- Dehydroabietic acid 110 µg/g (98% purity value = $0.84/g)
- Tetracosanoic (Lignoceric) acid 82 µg/g (99% purity value = $230/g)
- Isopimaric acid 65 µg/g (98% purity = $25,840/g)

Heartwood:

- Phenylhexanoic acid 530 µg/g (98% purity value = $94.30/g)
- Todomatuic acid 483 µg/g (no commercial supplier found)
    - Defence compound against insect and fungal attack.
- 1-Norbornanecarboxylic acid 343 µg/g (not specified = $1242.0/g [4])

Needles: (Pre-extract weight not recorded)

- Maltol (99% purity value = $0.21/g)
- 9-Octadecenal (not specified = $5.92/g [5])
- Palmitic acid (F16:0) (99% purity value = $4.08/g)

Sapwood:

- Palmitic acid (F16:0) 161 µg/g (99% purity value = $4.08/g)
- Stearic acid (F18:0) 112 µg/g (95% purity value = $0.17/g)
- Isopimaric acid 74 µg/g (98% purity = $25,840/g)

Eucalyptus nitens:
Bark:

- Palmitic acid (F16:0) 36 µg/g (99% purity value = $4.08/g)
- Stearic acid (F18:0) 24 µg/g (95% purity value = $0.17/g)
- Octacosane (C28) 21 µg/g (99% purity value = $2.52/g [6])

Heartwood:

- Palmitic acid (F16:0) 53 µg/g (99% purity value = $4.08/g)
- Stearic acid (F18:0) 33 µg/g (95% purity value = $0.17/g)
- Octacosane (C28) 28 µg/g (99% purity value = $2.52/g [6])

Leaves: (Pre-extract weight not recorded)

- Eucalyptol (99% purity value = $0.48/mL)
- Cannabinol methyl derivative (no commercial supplier found)
    - Medical uses.
- 3-Ethylphenol (95% purity value = $4.57/mL)

Sapwood:

- Palmitic acid (F16:0) 51 µg/g (99% purity value = $7.69/g)
- Stearic acid (F18:0) 29 µg/g (95% purity value = $0.17/g)
- Heptacosane (C27) 29 µg/g (98% purity value = $294.50/g)

These chemical have been found in trees using traditional solvent extractions and by $scCO_2$. Solvent extraction of terpenoids such as Tetracosanoic (Lignoceric) acid, Dehydroabietic acid and other chemicals such as Stearic acid, Phenylhexanoic acid 1-Norbornanecarboxylic acid, 9-Octadecenal and Maltol has been carried out either with dichloromethane or hexane Soxhlet extraction [7,8]. Isopimaric acid and Palmitic acid have been obtained by fresh resin dissolved in ethanol [9]. The same acids along with Heptacosane, Octacosane Stearic acid have been extracted from *E. Globus* or with dichloromethane soxhlet and by $scCO_2$ with and without ethanol as a solvent [10]. Todomatuic acid has been extracted by Soxhlet with petroleum ether for 24 hours [11] and 3-Ethylphenol extracted similarly but with acetone [12]. Eucalyptol has been extracted by subcritical-water extraction [13].

Conclusions

- Leaf samples had different compounds to the other tissues types.
- Bark is similar to sapwood and heartwood which are also very similar to each other.
- Other compounds of interest are more likely to be extracted by modifying the extraction conditions, as well as increasing the extraction efficacy.

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