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New era of phytochemistry

Mamdouh Abou-Zaid

Agriculture and Agri-Food Canada

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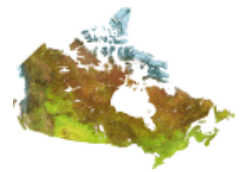


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NEW ERA OF PHYTOCHEMISTRY

Mamdouh Abou-Zaid

Natural Resources Canada,
Canadian Forest Service -
Canadian Wood Fibre Centre,
Sault St. Marie, Ontario



in collaboration with

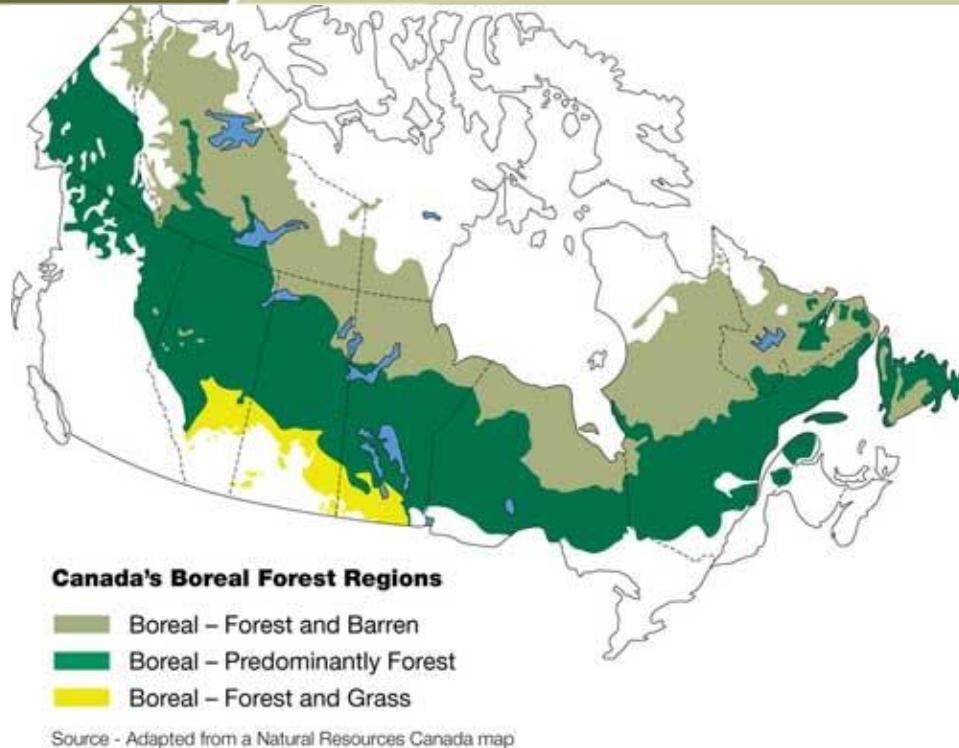
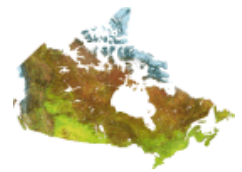
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Western University, Department of Chemical and Biochemical Engineering, Institute for Chemicals and Fuels from Alternative Resources (ICFAR), London, Ontario

Coiffeur Marshal, Ian Scott and Mark Sumarah

Agriculture and Agri-Food Canada, London, Ontario





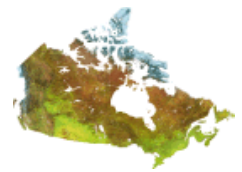
Canada's Boreal Forest Regions

- Boreal – Forest and Barren
- Boreal – Predominantly Forest
- Boreal – Forest and Grass

Source - Adapted from a Natural Resources Canada map

- Canadian boreal forest circles the upper part of the northern hemisphere and covers 31% of Canada
- Boreal forest is primarily an evergreen forest e.g., spruce, pine, fir
- 30% of the world's boreal forest is located in Canada

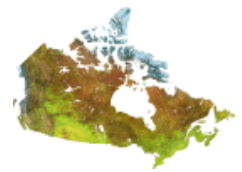




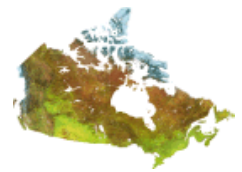
Canadian Forests Contain Rich Resources of Bioactive Natural Products

- ✦ The boreal forest is a natural factory working on the production of enormous numbers of **organic substances with a variety of roles and bioactivities**
- ✦ Each species has a unique phytochemical profile with characteristic classes of substances, a phenomenon known as **phytochemical diversity and redundancy**
- ✦ The biosyntheses and metabolic sequences are interconnected



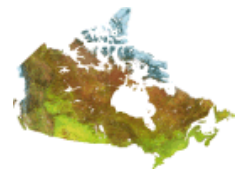


- ✦ When producing bioenergy, **selected chemical species can be of much greater value than energy alone.**
- ✦ Utilizing thermal cracking technologies (e.g. pyrolysis), provides a **novel means to access more readily sources of these biochemical compounds**
- ✦ **New structures** can be created by the depolymerization of lignin, cellulose and hemicellulose.



- ✦ Special opportunities are created by selected species:
 - ex. Canada yew to produce **taxol**,
 - tomato to produce **lycopenes**,
 - grape waste to produce **resveratrol**,
 - and ginseng to produce **ginsenosides**.

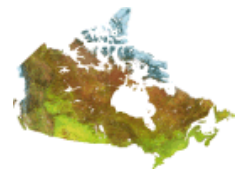
- ✦ Our objective is to assist pyrolysis, bioproducts, pharmaceutical, bioenergy and biobased industries in **developing a broad range of raw materials** from forest residues.



Natural product chemistry

- ✦ Over the years the **Canadian Wood Fibre Centre** is working towards:
 - Baseline data systems of the biochemical profiles of native flora (trees, shrubs and herbs)
 - Correlation of biochemical characteristics with the molecular and genetic variations of native flora
 - Impacts associated with geographical, seasonal and environmental changes

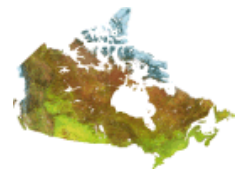




Ultimately...

- ✦ The goal is to help support and maintain both the natural products initiatives and forest industries in Canada by providing overall **natural products expertise and resource tools for the identification and development of value-added products**

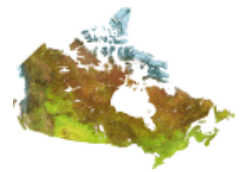




CASE STUDY:

Preparation of Taxanes

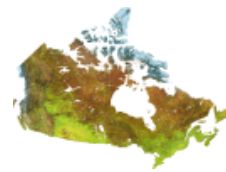




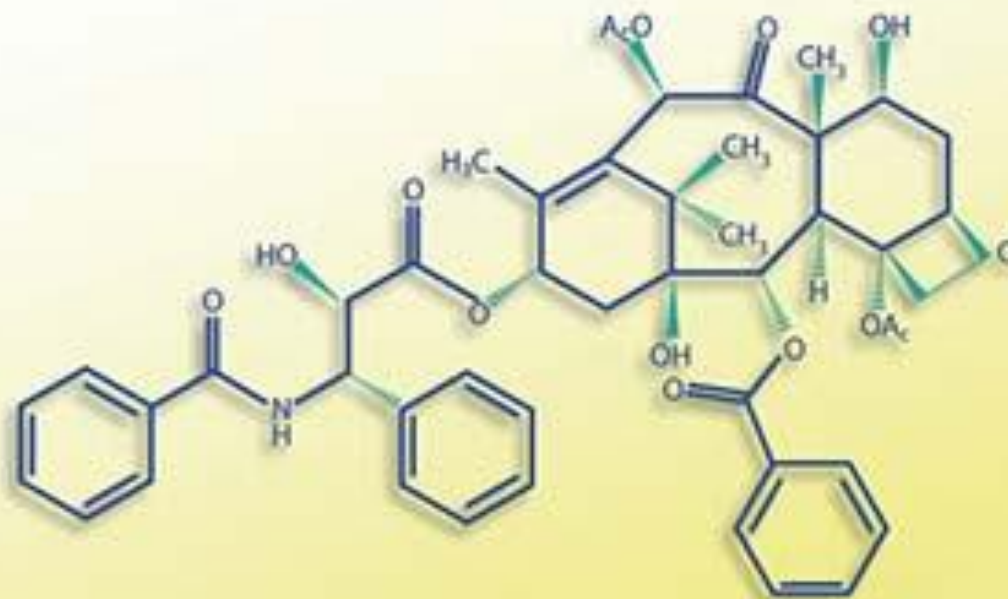
Preparation of Taxanes

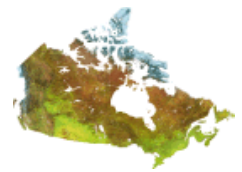
- ✦ **Canada Yew (*Taxus canadensis*)** is a native evergreen shrub found in most parts of Ontario
- ✦ Needles, bark and roots (the biomass) contain varying amounts of the anticancer compound, **Paclitaxel®**



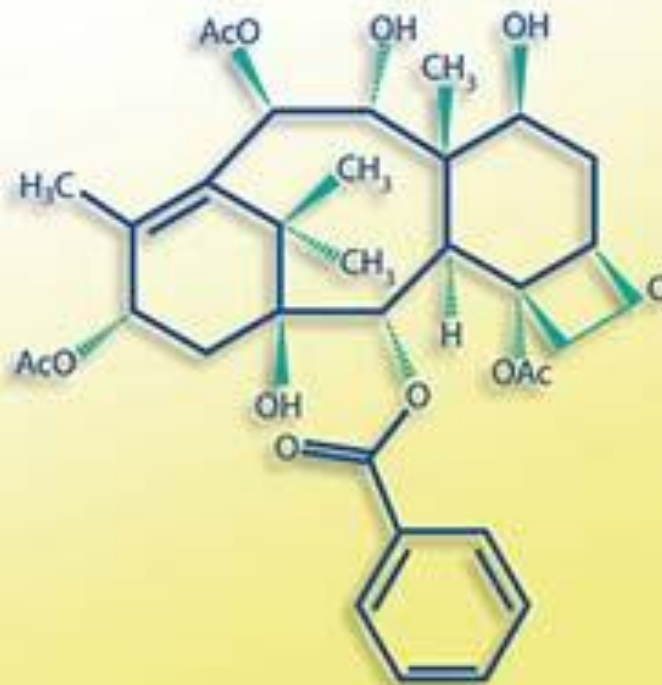


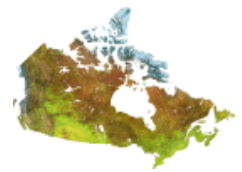
Paclitaxel





13-acetyl-9- dihydrobaccatin III (9DHB)

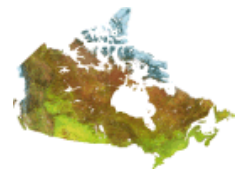




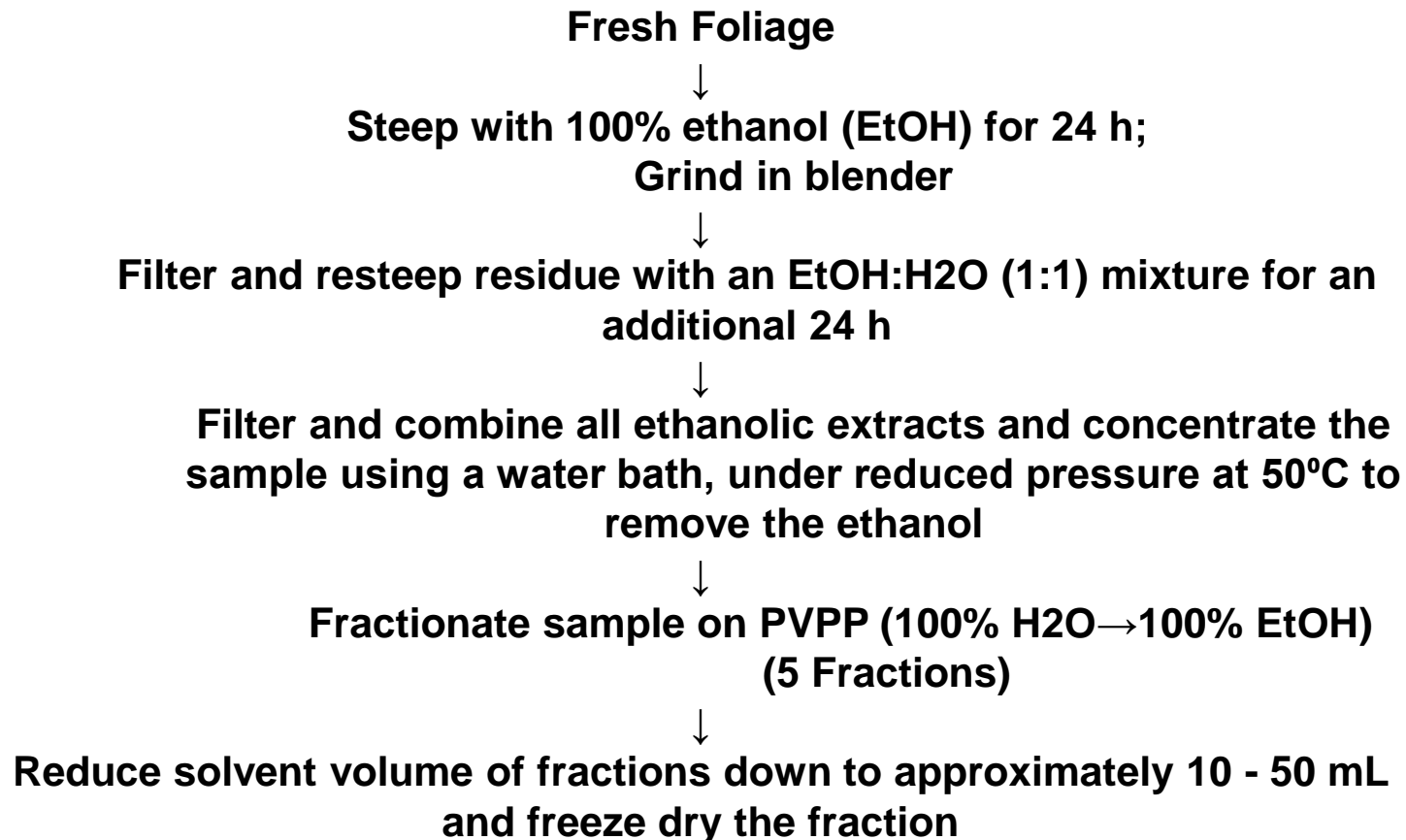
Preparation of Taxanes

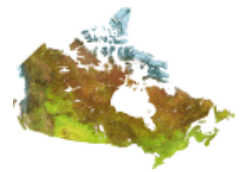
- ✦ Current methods for the production of commercial **Taxol®** and taxane compounds are **complex, time consuming and costly**
- ✦ Based on solvent extraction from raw material





SOLVENT EXTRACTION PROCEDURE:



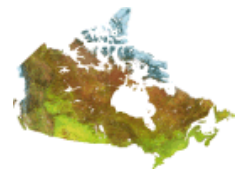


Solvent Extraction:

Challenging!

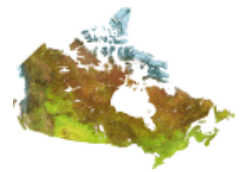
**There is the need for alternative
extraction procedures**





**Can pyrolysis processes be
an alternative to solvent
extraction for the
segregation and extraction
of valuable biochemicals?**

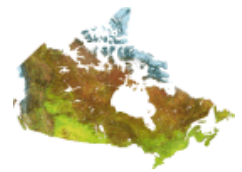




Preparation of Taxanes

- ✦ **Pyrolysis** involves the destructive distillation of the biomass (needles, bark, roots or combinations thereof) to produce a bio-oil
- ✦ **Bio-oil** contains a mixture of taxanes along with phenolic compounds

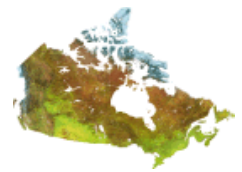




Preparation of Taxanes

- ✦ Isolation of the mixture of taxanes from the bio-oil is carried out by mixing the taxane laden bio-oil with a **chromatographic resin** to form a bio-oil /resin mixture
- ✦ **Elution with a solvent mixture yields individual taxanes in purified form**

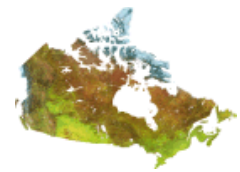




- ✦ **Pyrolysis eliminates the use of an initial liquid-phase fractionation procedure**, such as liquid-liquid partitioning or solvent extraction of the bio-oil composition, before one or more of the taxanes are isolated by chromatographic separation

(WO/2007/045093) PREPARATION OF TAXANES, Pub.No.: WO/2007/045093 **International Application No.:** PCT/CA2006/001717 **Publication Date:** 26.04.2007 **International Filing Date:** 20.10.2006 **IPC:** *C07D 305/14* (2006.01), *C07B 63/00* (2006.01)



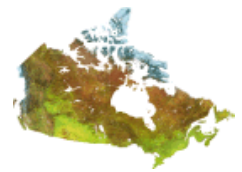


Comparison between Solvent Extraction and Fast Pyrolysis

Compound	Organic Solvent Extraction	Pyrolysis
Taxol (Bark)	~0.01 %	NA
Taxol (Clippings and needles)	~0.003 to 0.015 %	~0.031 to 0.049 %
DBH (Clippings and needles)	~0.04 %	~0.08 to 0.12 %
DAB (Clippings and needles)	~0.06 %	~0.46 to 0.53 %
Total Taxanes (Clippings and needles)	~0.25 %	~ 5 to 7 %

➤ Pyrolysis resulted in significantly **higher yields of taxanes** than obtained using standard extraction procedures !!!





Instruments

- ✦ Gas Chromatography - MS/MS
- ✦ ORBITRAP LC-MS
- ✦ HPLC-DAD
- ✦ UPLC-DAD
- ✦ Nuclear Magnetic Resonance





THIS ELECTRICAL
KEPT CLEAR FOR

Wavelength (nm)	Absorbance	Transmittance (%)
200	0.15	85
220	0.10	90
240	0.08	92
260	0.06	94
280	0.04	96
300	0.03	97
320	0.02	98
340	0.01	99
360	0.01	99
380	0.01	99
400	0.01	99

Model: UV-1601PC
Manufacturer: Shimadzu
Serial Number: 10000000000000000000
Date of Purchase: 1/1/2000
Warranty: 3 years

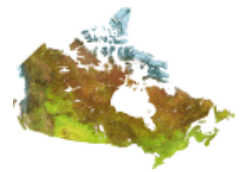




Mix-RX
20

Small Parts
Enclosed

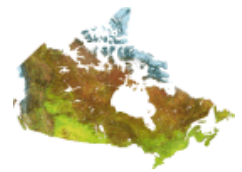
C7) BENCH COVERS
TEFLON LID
CHROMATO.
GRAPHY PAPER
ETC



Natural Products Chemical Library:

- ✦ There are close to **1,800** samples of **crude plant extracts, fractions and purified compounds** which were collected from trees, shrubs and herbs from Northern Ontario and also across Canada
- ✦ Many of these are **novel “natural” products** that possess antioxidant properties





In Conclusion...

- ✦ Trees and waste products from the forest industries serve as a reservoir for valuable raw materials for the natural products industries, both existing (traditional pharmaceutical) and novel (natural health products and dietary supplement, including antioxidant and antimicrobial compounds)
- ✦ Bioproducts holds promising future for forest industry. We develop methods for the identification and characterization of phytochemicals and other value added bioproducts and for their efficient extraction.