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Biopharmaceuticals Hiding in the Woods

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ABSTRACT – Suggestions are provided of how efficient industrial bioprocessing can be accomplished, independently of highly expensive molecular biology approaches, in order to recover natural products of medicinal value from wild plant species.

INTRODUCTION – Medicinally useful plants and traditional medicines had their origins in prehistory. Subsequent sharing of traditional knowledge by aboriginal peoples enabled scientists to identify and investigate the molecular basis of a substantial number of plant species known to have potent human health effects. The rudiments of pharmacognosy gradually evolved, over several centuries, into the fields of natural products chemistry, biopharmacy, anti-cancer screening programs and related pursuits. In recent decades, advances in molecular biology complemented by innovations in microbial and plant cell culture methods have demonstrated, with an eclectic few plant species, that considerable commercial potential exists for production of biological compounds of pharmaceutical value and/or for advancement of medical science.

There are > 320,000 plant species (> 80,000 perennial woody species) on Earth, not to mention even greater numbers of morphological and biochemical variants of them, as well as the species/tissue specific endosymbionts and pathovars dwelling within and displaying their own unique, sometimes valuable biochemical competencies. All plant species provide vitamins, digestive fibres and other generally ubiquitous substances important for human health. In addition, innumerable low molecular weight organics ('secondary' metabolites), proteins, oligo/polysaccharides and polyphenolics having potent agonist, antagonist or toxic effects have already been reported, but immense opportunity remains in this field of plant 'molecular biology' (*sensu* traditional). It is probable that most, if not all, plants once thoroughly investigated will be found to contain additional compounds of value to medical science The endogenous concentration of the compound of interest within the plant tissue vis-à-vis processing costs and economic demand are considerations.

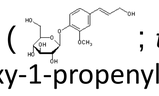
PROBLEM STATEMENT – This field of natural products organic chemistry research remains in a relatively unappreciated and incomplete state, particularly in relation to biopharmaceuticals. A great many plant species have been *qualitatively* investigated in relation to biopharmaceutical potential; however, very few have yet been investigated systematically and thoroughly using *quantitative* physiological methods, in order to understand how compounds of interest are variably distributed between tissues, how levels vary seasonally, and how best to implement commercial extraction and purification processes.

PRE-COMMERCIAL METHODS – Pre-commercial characterization of any plant species for its biopharmaceutical potential can be accomplished using the 10 activities listed below. Although straightforward, each activity nevertheless requires considerable knowledge, technical expertise and scientific equipment.

1. Establish unequivocally through chemical characterization research that a molecule of interest actually exists within a particular plant species;
2. Having identified the plant species, determine which organ(s) and which tissue(s) contains the molecule at successive developmental stages in the plant's life cycle;
3. Understand the morphogenesis of the plant species and how the organ(s) and tissue(s) of interest can vary;
4. Identify the preferred field procedure for harvesting and transporting the desired organ(s);
5. Identify optimal conditions for storage of the harvested organ(s) such that the molecule of interest is not metabolized or otherwise lost within the target tissue;
6. Devise an efficient method for processing the harvested organ(s) in order to obtain the target tissue(s) in isolation, separated as completely as possible from other, adjoining, tissues;
7. Determine conditions for storage of the target tissue(s) such that the molecule of interest is not metabolized or otherwise lost.
8. Use quantitative methodology to optimize (time *vs*. cost *vs*. safety *vs*. percentage recovery) extraction and processing of the molecule from the target tissue(s);
9. Discover in field-grown plants how the molecule's levels vary seasonally, and identify the optimum time of year to harvest;
10. Learn how varied extrinsic factors (e.g., light intensity and spectrum, soil fertility, soil water potential, temperature) influence the molecule's levels in relation to the harvest date of the plant, and use this knowledge to distinguish levels of production in field-grown plants.

IP ASPECTS – Important trade secrets are likely to emerge from the above-listed pre-commercial investigations. Patenting of a process for production of a molecule of interest involves many considerations but, in general, a patented production process will include:

1. Storage-to-extraction processing of the tissue(s), including reduction of tissue size, environmental control, and transport of tissue particles;
2. Extraction of substances including the molecule of interest from the tissue;
3. Separation of non-extracted residual tissue from extracted matter;
4. Removal of the extraction solvent or gas to provide dry crude extract;
5. Chromatographic or other fractionation of the crude extract to obtain the desired fraction;
6. Purification/crystallisation/storage of each molecule of interest;
7. Recovery/recycling of extraction solvents, gases, and other materials.

AN EXAMPLE – *E*-coniferin ( ; *trans*-coniferyl alcohol β-D-glucoside; β-D-glucopyranoside 4-(3-hydroxy-1-propenyl)-2-methoxyphenyl; (2*R*,3*S*,4*S*,5*R*,6*S*)-2-(hydroxymethyl)-6-[4-[(*E*)-3-hydroxyprop-1-enyl]-2-methoxyphenoxy]oxane-3,4,5-triol; molar mass = 342.35) occurs in some plants and has potential pharmaceutical value[1]. Processing of a conifer tree species for either *E*-coniferin or the enzyme catalyzing its biosynthesis provides a simple but informative example of how important it can be to give attention to the 10 pre-commercial aspects identified here, before one decides for or against implementing a commercial operation. An entrepreneur lacking the data shown in Figure 1 would likely become frustrated by the vicissitudes intrinsic to nature and might well abandon a perfectly fine opportunity, whereas one having knowledge of the tree and how its production of *E*-coniferin varies – developmentally, morphologically and phenologically – would likely succeed at the endeavour.

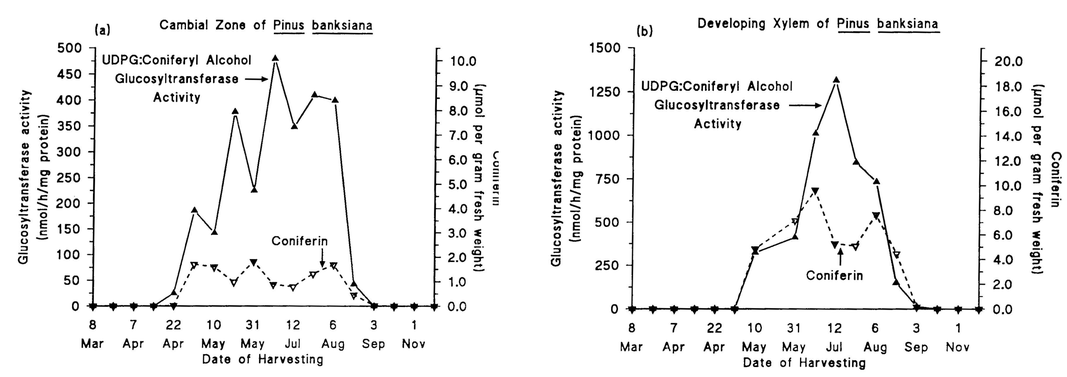


Figure 1. Seasonal variation of *E*-coniferin and UDPG:coniferyl alcohol glucosyltransferase (the enzyme catalyzing coniferin biosynthesis) in the trunk of *Pinus banksiana,* a Canadian conifer that grows May – August and remains dormant September – April[2]. a), Cambial tissue obtained by bark removal and scraping a 30 µm wide zone of tissue from the exposed inner bark face; b) Developing xylem, a 200 µm wide zone of living differentiating cells scraped from the surface of mature wood following bark peeling.

In addition to cambial zone and developing xylem tissues as indicated in Figure 1, *E*-coniferin occurs at trace concentrations in pine needles during the summer months. However, no *E*-coniferin is to be found in bark, wood or other tissues of the tree during any month of the year. The total weights of cambial zone and developing xylem in a tree of height 10 m and diameter 0.2 m early in the growing season are about 10 and 1000 g, respectively, and during that season several hundred grams of *E*-coniferin can readily be obtained after those tissues have been isolated. In contrast, no *E*-coniferin is present in the cambial region when the tree is dormant. A trunk of average diameter 20 cm has a volume of ~30 dm^3 and green weight of ~200 kg. If, instead of isolating specific trunk tissues enriched in *E*-coniferin, an entire tree trunk is harvested and processed, the cost of processing the biomass and obtaining *E*-coniferin is considerably more, and the percentage recovery of *E*-coniferin is relatively low.

DISCUSSION – The plant kingdom is Earth's most abundant biological resource, and accompanying its multi-species diversity are a huge number of biomolecules having great potential for medical science and healthcare. Because a rudimentary state of knowledge about bioprocessing of wild plants is persisting (due mainly to the current preoccupation by government, industry and academia with DNA, RNA and protein), efficiencies of harvesting, storing, and processing of plant tissues for their useful chemicals remain less than optimized. Few of the many already chemically characterized biopharmaceutical compounds are yet being economically produced from plants, and it is becoming increasingly difficult to find personnel having expertise in traditional fields of plant science, such as morphological plant taxonomy, plant physiology, biochemistry, chemotaxonomy and pharmacognosy, in order to broaden and deepen understanding of biopharmaceutical diversity and increase appreciation of its potential to advance scientific knowledge and help humanity.

Chemical characterizations of biomolecules generally have been done by organic chemists who, though competent in the realm of chemical characterization, not uncommonly lack knowledge of plant morphology and phenology. Conversely, students of plant science rarely have the abilities and critical insight of organic chemists. Widening the long-standing void separating the two disciplines, perceptions of what biology students need to know have undergone a paradigm shift since about 1980. Education in analytical organic chemistry remains solid, but biology education now skims over many of what were once considered to be the essentials, placing emphasis instead on molecular genetic aspects and, quite mistakenly, presenting biochemical pathways as *fait accompli*. From a P = G x E perspective, when investigations are aimed at achieving practical biochemical outcomes, e.g., production of biopharmaceutically useful compounds, the current molecular biology bias fosters assumptions about the competencies of organisms (including tissues and cells) in relation to metabolic change, hence biosynthetic capability, particularly as they are influenced by E (i.e., the physico-chemical environment affecting gene expression within the living cell). Bioprocessing options in addition to those involving molecular biology and laboratory cultures not only exist but may be the better choice, at least until such time as all biophysical and biochemical aspects of gene regulation, expression, pre- and post-translational processing are known. Ironically, major industrial opportunities have disappeared from sight because of this paradigm shift. Timing and awareness in the affairs of humanity tend to be critical considerations. Is the world at the beginning of the 21st century ready to embrace the non-genetically engineered biopharmaceuticals lurking in the woods?

Literature cited

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2. Savidge, RA , Förster, H (1998) Seasonal activity of uridine-5'-diphsophoglucose:coniferyl alcohol glucosyltransferase in relation to cambial growth and dormancy in conifers. *Can. J. Bot.* 76:486-493.