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TANNINS EXTRACTION FROM BARK FOR INDUSTRIAL APPLICATIONS – LABORATORY STUDY

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INTRODUCTION

FPInnovations was approached to investigate the extraction of tannin from tree bark in British Columbia (BC). FPInnovations has been working on bark extraction over the last few years and proposed to focus this study on Western Hemlock which showed high tannin content in previous work. The extraction method developed by FPInnovations uses chemicals and elaborate equipment that requires the work to be conducted under strongly controlled conditions, such as a chemical laboratory. This report aimed to find a simpler extraction protocol that could potentially be used by coastal First Nations communities or other parties interested in extracting tannin from bark at a relatively small scale.

Objectives of this contract were:

- Conduct laboratory testing to compare different methods for the extraction of tannin from western hemlock bark
- Analyze the extracts and compare the extraction yields and tannin concentrations
- Report results from previous work on tannin quantification from BC tree species
- Present a summary of identified potential applications for tannin extracts

MATERIAL AND METHODS

Bark extraction

Western hemlock logs were collected in the forest by FPInnovations during the fall of 2016 in Mabel Lake, Bamfield, and Rock Creek. Logs were manually debarked using a drawknife. Bark was then ground using a hammer mill with a 3/16”” screen, dried at room temperature for five days, and stored at -20°C prior to extraction. Material was extracted by maceration using a mixture of ethanol/water (70:30) or water only, with two passes of two hours’ duration. The ethanol/water extraction was done at 50°C and the water extraction at 25°C, 50°C, 70°C, and 90°C using heating plates. Extractions were done using approximately 100g of dried bark for 1L of solvent in 2L Erlenmeyer flasks. Agitation during extraction was provided with a blade mixer set at 250 rpm. Liquid extract was then filtered on Whatman paper grade 4 (25 µm) to remove small bark particles. Every extract was separated into two portions to investigate two drying techniques. The first portion was evaporated at reduced pressure using a rotary evaporator and freeze-dried. The second portion was boiled under agitation in a beaker using a heating/stirrer plate to greatly reduce the volume and then dried overnight in an oven set at 60°C. A 24-hour extraction in water at 50°C was also investigated. Only one pass of extraction was performed. This extract was subsequently freeze-dried. The rest of the process did not differ from the one described above. Extraction yields were calculated with the following formula: $Yield(\%) = 100 \times \frac{Mass\ of\ dry\ extract\ collected}{Mass\ of\ dry\ bark\ used}$

Tannin concentration determination

Total tannin content was measured using the methylcellulose precipitation assay (Sarneckis *et al.* 2006). Samples were dissolved at 0.5 mg/mL in 50% ethanol. For each extract undergoing analysis, a control and an assay must be performed. For the control, 100 µL of the sample was put into Eppendorf vials with 200 µL of a saturated solution of ammonium sulfate and 700 µL of H_2O. For the assay, 100 µL of the sample was put into vials with 300 µL of a 0.04% methylcellulose aqueous solution and slightly agitated. After 2 to 3 min, 200 µL of ammonium sulfate solution and 400 µL of H_2O were added. The control and assay vials were then vortexed, set aside for 10 min and centrifuged at 10,000 rpm for 5 min. The supernatant's absorbance was recorded at 280 nm. Absorbance value of the control, minus the one of the assay, indicated the quantity of tannin precipitated by the methylcellulose. Extracts were analyzed in triplicate. A calibration curve (R^2 = 0.9965) was performed using tannic acid (0-400 µg/mL), and results were expressed in equivalent mg tannic acid/g of extract. Concentrations in mg tannic acid/g of bark were calculated using the extraction yields.

Total phenol concentration determination

Total phenols content was estimated by the Folin-Ciocalteu assay. Extracts were prepared at 0.1 mg/mL in distilled water containing up to 1% of ethanol to facilitate dissolution, and 0.1 mL of these solutions were transferred into screw-top reaction vials with 3.9 mL of distilled water and 0.25 mL of Folin-Denis reagent. The vials were then vortexed, and after 4 min 0.75 mL of an aqueous 20% Na_2CO_3 solution were added. Finally, the vials were vortexed and heated for 25 min in a 40°C water bath. Absorbance was recorded at 765 nm after letting the vials cool down for 5 min at room temperature. Extracts were analyzed in triplicate. A calibration curve (R^2 = 0.9993) was performed using gallic acid (0 – 200 µg/mL) and results were expressed in equivalent mg gallic acid/g of extract.

RESULTS

Bark from British Columbia species as a source of tannin

In past projects, FPInnovations worked with many wood species from Eastern and Western Canada. Extracts from the bark of the species already tested for tannin concentration used an alcohol/water maceration (Table 1). Three species stand out from the rest: western hemlock, western larch and black spruce. These species showed similar tannin concentration and could be potential candidates for tannin extraction.

Table 1. Tannin concentration in various bark extract from Western species studied by FPInnovations

Common name	Scientific name	Tannin concentration in extract (mg TAE/g)[a]	Tannin extraction efficacy (mg TAE/g of bark extracted)
Western Hemlock	*Tsuga heterophyla*	246.5	41.2
Western Larch	*Larix occidentalis*	292.1	45.3
Lodgepole Pine	*Pinus contorta*	116.3	23.6
Aspen	*Populus tremuloides*	65.3	11.5
Paper Birch	*Betula papyrifera*	59.6	9.4
Black Spruce	*Picea mariana*	221.6	44.3

[a] concentration shown in mg tannic acid equivalent per gram

Note: Black spruce bark was collected in Québec, but we should expect similar concentration in BC trees and also in other western spruce species such as Sitka, White, and Engelmann spruce. Paper birch was also collected in Québec, but concentration should be similar in BC.

When considering application towards skin tanning, many other parameters probably need to be accounted for, such as extract colour. It is also possible that some specific tannin could be more desirable than others, or that other compounds found in the bark extract could have positive or negative effects. For example, some bark extracts studied by FPInnovations have shown both antimicrobial activity against pathogens and strong antioxidant activity. Interestingly, the western hemlock extract was also one of the best candidates.

A preliminary trial is currently under way with the Vancouver-based company 7Leagues to investigate the application of bark extracts for fish skin tanning. Three samples of bark and three of extracts (hemlock, pine, and aspen) were sent to 7Leagues in January 2020. Results will give an indication about the suitability of the bark extract for this application.

Tannin applications

A review of tannin industrial applications was published in 2019 in the open access *Biomolecules* journal (Pizzi, 2019). The main applications are listed in Table 2 in order of economic importance.

Table 2. Industrial applications of tannin

Application	**Details**
Leather tanning	Impregnation of skin with tannin solutions
Wood adhesives	Tannin based adhesives for particleboard and plywood to replace synthetic adhesives
Beverage	Increase the tannin concentration in wines and beer
Foam	Fireproof and insulation foam
Mining	Separation of germanium from cooper ore and fluorite from calcite
Wastewater treatment	Absorption of heavy metals and flocculant agent

It is important to keep in mind that the extract product from bark will not be 100% tannin. The presence of the other compounds could make some application unsuitable. The regulatory constraint might be particularly difficult for food and beverage applications. Moreover, it is believed that the tannin can be used as is for the leather and beverage applications only. The other uses require preparation of a more developed product, involving different chemicals such as urea, formaldehyde, isocyanates, or furfuryl alcohol.

Simplification of the tannin extraction process

The usual method used by FPInnovations for bark extraction involves ethanol as a solvent and a freeze-dryer to dry the extract into a concentrated powder. Ethanol is a toxic and dangerous chemical to work with and requires proper facilities and equipment. In addition, a freeze-dryer is an expensive piece of equipment requiring a long drying time. In order to develop a simpler process, more suitable to small-scale operations, some modifications of the extraction method were investigated. First, the ethanol was removed, and bark was extracted using water as the sole solvent. Different temperatures were tested to find the optimal conditions. To facilitate the drying step, liquid extract was simply boiled to remove most of the water. When the volume was low, samples were transferred to an oven set at 60°C, and dry powder was collected. All extracts were then compared to the usual ethanol extraction and drying method for their yield and tannin content. All results are presented in Table 3.

Table 3. Extraction yields and tannin content of western hemlock extracted under different conditions

Extraction	Drying method	Extraction yield (%)	Tannin concentration in extract (mg TAE/g)[a]	Tannin extraction efficacy (mg TAE/g of bark extracted)
Ethanol control -50°C	Freeze-drying	16.6	333.2	55.3
Water-25°C	Freeze-drying	4.5	336.3	15.1
Water-25°C	Evaporation	4.1	413.9	16.9
Water-50°C	Freeze-drying	7.4	354.9	26.3
Water-50°C	Evaporation	5.2	472.8	24.6
Water-70°C	Freeze-drying	15.7	332.6	51.2
Water-70°C	Evaporation	10.8	448.4	48.4
Water-90°C	Freeze-drying	14.4	234.3	33.7
Water-90°C	Evaporation	17.9	300.5	53.8
Water 24hrs-50°C	Freeze-drying	7.9	340.5	26.9

[a] concentration shown in mg tannic acid equivalent per gram

As expected, the yields were lower when using no ethanol and extracting at the same temperature (16.6% against 7.4%). However, when the extraction temperature was increased, the yield improved significantly. Extraction at 90°C looks to be the more promising condition, with a yield similar to the control method and an identical tannin content. The tannin concentration of the water extracts was in the same range as the ethanol extract, which means that the extracts are quite similar. Nevertheless, when taking into account the extraction yields, the results show that the ethanol extraction method is still more efficient to extract as much tannin as possible from the bark. The two drying techniques showed similar yield, without a large unexpected difference. The slightly lower yields observed when evaporating the extracts (except for the 90°C extractions) are probably due to the loss of a portion of extract against the walls of the glassware when boiling away the water before final drying in the oven. The new drying method does not seem to be problematic for the tannin concentration either. The initial hypothesis was that, when submitted to high temperature during boiling, some degradation could happen, leading to a reduced tannin content. This does not seem to be the case here. The thermal degradation of tannins starts at 196°C (Lisperger et al., 2016). Nonetheless, the quantification assay used measured the total tannin concentration. There could potentially be a difference in the tannin present on a molecular level, such as the presence of tannin with smaller molecular weight after boiling. The same hypothesis can be made for the extracts produced at 70°C and 90°C. A 24-hour investigatory extraction was performed in water at 50°C. The yield and tannin concentration were similar to the 2x2 hours extraction at the same temperature. The benefit of the 24-hour extraction is that there is only one pass of extraction for the same yield, so only half the amount of liquid to dry to collect the same quantity of extract. However, longer extraction time involves higher production cost, mainly due to the energy to heat the extraction for 24 hours. This energy cost increase for extraction could potentially be balanced by the reduction of energy needed for water evaporation and extract drying, but we do not have data to support this case, and it would probably be closely related of the volume of extraction.

As follow-up, the extracts were analyzed for their total phenol content. This assay measures all the phenolic compounds present, including the tannin, but also other extractives like phenolic acids, flavonoids, lignans, or stilbenoids that have previously been found in the bark extracts. Results are presented in Table 4. The trends are similar to those observed previously for tannin concentration. The extracts have quite similar chemical composition, the classic ethanol method is the more efficient to extract phenolics, and the drying method was not found to impact the chemical composition. The best condition for water extraction appears to be at 70°C. When increasing the temperature further, phenolic concentration was found to be lower.

Table 4. Phenolic content of western hemlock bark extracted under different conditions

Extraction	**Drying method**	**Phenol concentration in extract (mg GAE/g)[a]**	**Phenol extraction efficacy (mg GAE/g of bark extracted)**
Ethanol control-50°C	Freeze-drying	569.8	94.6
Water-25°C	Freeze-drying	425.7	19.2
Water-25°C	Evaporation	503.9	20.7
Water-50°C	Freeze-drying	465.4	34.4
Water-50°C	Evaporation	472.4	24.6
Water-70°C	Freeze-drying	527.1	82.7
Water-70°C	Evaporation	546.7	59.0
Water-90°C	Freeze-drying	470.1	67.7
Water-90°C	Evaporation	415.2	74.3
Water 24hrs-50°C	Freeze-drying	519.5	41.0

[a] concentration shown in mg gallic acid equivalent per gram

CONCLUSION

The different extraction trials showed that less extract and tannin are collected from hemlock bark when using water only, instead of a mixture of water and ethanol. However, the extract powders produced have the same tannin concentration. As such, more bark would be needed to generate the same quantity of tannin when using water only, but the extract will be of the same quality as the one made with FPInnovations' laboratory method. Heating the bark water mixture during maceration greatly improved the extraction yields but did not impact the tannin content in the resulting extracts. The drying is not an issue for the extraction yields and tannin concentration of the bark extract. The extract produced with water showed tannin content of approximately 30% to 45%. As previously mentioned, the other compounds might have a positive or negative impact depending on the targeted application. The next step will be to test the bark tannin for the specific application. A trial is currently under way for fish skin tanning.

Having an easy method to quantify the tannin content of the bark extracts would be a good asset for parties interested in doing bark extraction. We tried to develop a kitchen-type protocol based on our laboratory method. The objective was to use easily accessible products and materials and develop a method effortlessly performed by anybody without a chemistry background. The tests were based on the capacity of tannin to precipitate proteins. Experiments were conducted using egg and whey protein and a standard tannin powder. At this point, results are not conclusive, and more time would be needed to develop such a method and make sure that results are reliable.

As additional conclusions, the following recommendations about the different steps of the tannin extraction from bark are presented.

Bark grinding

Bark grinding was not investigated in this report. Bark used was already ground from previous work using a Hammermill. We assume that bark could be ground to adequate particle size with a classic wood chipper. With smaller particle size extraction yield is better, due to larger exposed surface area.

Extraction

Based on results, water can be a suitable solvent for tannin extraction from bark. The best temperature for extraction was 90°C, but 50°C and 70°C can still be considered. Our protocol used two extractions of two hours for the same sample of bark. Based on another bark extraction work, approximately 75% of the total yield can be expected during the first pass. The second pass could be removed in order to reduce the volume of liquid requiring drying. Extraction time was not investigated in detail during this project, but a 24-hour extraction could be a suitable option too. The tested extractions were conducted under agitation using a blade mixer in order to increase extraction yield. If no suitable equipment can be found, a punctual manual homogenization is advised to ensure that all bark pieces are in contact with the water extraction solution.

Filtration

Filtration was not investigated. Simple sieve, tissue, or cheesecloth filtration could be suitable options. Having a method not as fine as filter paper will not be critical, depending on the application targeted for tannin. Some small bark particles could end up in the extract after filtration. This debris will not be soluble when putting the tannin extract into the solution for application. This could be an issue for food and beverage application but probably not for leather tanning.

Extract drying

As discussed above, the results from this study showed that the liquid extract collected after bark maceration can successfully be dried by boiling most of the water first and then drying in an oven. Second-hand equipment such as that used to evaporate maple sap into maple syrup could be one option.

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