Characterization and Antioxidant Properties of the Condensed Tannins from Alaska Cedar Inner Bark

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Abstract: The structure and antioxidant activity of condensed tannins isolated from Alaska Cedar inner bark have been investigated. Oligomers of flavan-3-ol were purified by column chromatography (Sephadex LH-20) and analyzed by ¹³C NMR and MALDI-TOF MS spectrometers. Their antioxidant activities were measured using 1,1′-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radicals scavenging, ferric reducing/antioxidant power (FRAP), and β-carotene-linoleic acid model system (β-CLAMS) assays. Results showed that the condensed tannins consists of both homogeneous and heterogeneous oligomers of procyandins (catechin/epicatechin) and prodelphinidins (gallocatechin/epigallocatechin) flavan-3-ol units; and oligomers from trimmers to heptamers with dominant interflavan linkages B-type as it is most common in proanthocyanidins. Condensed tannins showed significant antioxidant activity as the median inhibition capacity IC₅₀ is comparable to the catechin control response. Alaska Cedar inner bark oligomers show high antioxidant capacity, evaluated by both methods based on electron transfer mechanisms and hydrogen atom transfer reactions. This bark may be considered as a new source of natural antioxidants for nutraceutical ingredients.

Keywords: Chamaecyparis nootkatensis; MALDI-TOF; Proanthocyanidins; ABTS; DPPH; FRAP.
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1. Introduction

Alaska Cedar (Chamaecyparis nootkatensis), also known as yellow cedar or Nootka cypress, is an important timber and ecological species of the coastal Pacific Northwest of Canada and the United States. Indigenous peoples have valued and used this tree for centuries as an important material resource. The strong, fine grained wood was used to carve items such as bows, canoe paddles and chests, while the inner bark was highly prized for making fibrous materials such as baskets and clothing items [1, 2]. Because of the durability and commercial value of the heartwood, there have been many chemical studies over the years [3]. Most recently, these studies have concerned terpenes and bioactivity towards arthropods of public health concern [4–7].

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The bark, typically an underutilized forest by-product, has received in contrast relatively little attention. The outer bark extract was shown to have activity against *M. tuberculosis* due to the diterpene (+)-totarol [8]. The inner bark to the best of our knowledge has not been studied. In this paper, we wish to report on the characterization by MALDI-TOF MS and $^{13}$C NMR as well as the antioxidant properties of the purified condensed tannins.

Condensed tannins (also called proanthocyanidins) are oligomers of flavan-3-ol monomer units commonly linked C8→C4 or C6→C4 in what are called B-type interflavan linkages (Figure 1). Some proanthocyanidins also have A-type linkages which additionally have an ether linkage between the C-2 position of an upper unit and the hydroxyl group at either C-5 or C-7 of the lower unit. Structural diversity is additionally added to this family of compounds because of the variability of hydroxylation patterns of the aromatic A and B rings and different stereochemistry at chiral centers at C-2 and C-3 of the C ring. Five distinct families of proanthocyanidins based on the hydroxylation patterns found in their A and B rings are commonly found in nature. These are the procyanidins, prodelphinidins, propelargonidins, profisetinidins, and prorobinetinidins. Perhaps the two more common types of proanthocyanidins are the procyanidins (PC), which are composed of catechin and epicatechin monomer flavan-3-ol units and the prodelphinidins (PD), which are composed of gallocatechin and epigallocatechin monomer flavan-3-ol units. Such oligomers can occur as pure procyanidin chains, pure prodelphinidin chains, and in mixed procyanidin – prodelphinidin oligomers. MALDI-TOF MS has been found to be a highly effective tool for analysis of such polydisperse and heterogeneous proanthocyanidins compounds, especially when combined with $^{13}$C NMR data to give a condensed tannin profile, which includes size and monomer composition of individual oligomer chains [9, 10]. Such structural information is important when considering chemical and biological functions such as antioxidant activity.

Proanthocyanidins are of great interest from the nutritional and medical perspective because of their strong antioxidant capacity and related protective effects on human health. The biological, pharmacological, and medicinal properties of tannins have been related to their free radical scavenging and antioxidant activities. Polyphenol oligomers have shown notable functions, such as anti-allergic, vasodilator, anti-carcinogenic, anti-inflammatory, antibacterial, antiviral, and cardioprotective activities.

### Figure 1

Basic proanthocyanidin units in Alaska Cedar inner bark

2. **Materials and Methods**

2.1. **Plant Material**

An Alaska Cedar tree was obtained from the Hungry Mountain area in the Sol Duc drainage of the Olympia National Forest, Washington State (Oregon State University Herbarium voucher specimen #188046).

2.2. **Extraction and Isolation**

The isolation and purification procedure for proanthocyanidin polymers reported by Foo and Karchesy was followed [11], as it is shown in figure 2. Fresh inner bark (1 kg) was extracted at room
temperature with methanol. The methanol extract was filtered and concentrated on a rotary evaporator under reduced pressure to give a crude extract, which was diluted with water and the resulting aqueous solution was successively partitioned with hexane, chloroform, and ethyl acetate. The ethyl acetate fraction gave 20g and the water fraction 108 g of solid material after drying. The ethyl acetate fraction was chromatographed on Sephadex LH-20 to give (+)-catechin. 40 g of the water soluble fraction was applied to a Sephadex LH-20 column and then washed with 50 % aqueous methanol until washings were almost colorless. Catechin-7-O-glucoside and catechin – (4→8) – catechin were isolated from this elution. The condensed tannin fraction was then eluted from the column with 50% aqueous acetone to give 10g of purified condensed tannin material after freeze drying.

Figure 2. Chromatographic purification of Alaska cedar inner bark.

2.3 MALDI-TOF MS and $^{13}$C NMR

MALDI-TOF-MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) was performed using an ABSciEX 4700 tandem time of flight/time-of-flight (TOF/TOF) mass spectrometer run in reflectron positive ion mode. The scan range was from m/z 800-4000. Samples were mixed with 3- indole acrylic acid (t-IAA) matrix and acetonitrile as described by Taylor et al. [12]. Peak assignments for the [M + Na$^+$] adduct ion for each proanthocyanidin oligomer molecular weight and monomer composition was based on the formula given by Monagas et al. [9]. $^{13}$C NMR spectra were obtained on a Bruker model AM 100 MHz with d$_4$-MeOH as the solvent.
Determination of Antioxidant Capacity

2.4. Chemicals

The 1,1′-diphenyl-2-picrylhydrazyl radical (DPPH), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), TPTZ (2,4,6-tripyridyl-s-triazine), linoleic acid, β-carotene, ascorbic acid and catechin were purchased from Aldrich from Sigma Chemical Co.

2.5 DPPH Radical Scavenging Activity

The free radical scavenging activity on the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical was determined according to the method described by Brad-Williams et al. [13]. 50 µL of sample of catechin as standard at different concentrations in methanol (50, 100, 150 and 200 µg/mL) was added to 1950 µL of a methanolic solution of DPPH (6.1×10⁻⁵ M). An equal amount of methanol and DPPH served as control. After incubation by 30 min at room temperature, the decrease in absorbance was measured at 515 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The antioxidant capacity, defined as the concentration of antioxidant necessary to scavenge the initial DPPH free radicals, was calculated using the following equation:

\[
\text{DPPH scavenging effect (\%) = } \left( \frac{A_1 - A_2}{A_1} \right) \times 100
\]

Where \(A_1\) is the absorbance of the reaction control; \(A_2\) is the absorbance in the presence of the sample. Catechin was used as standard. And the EC₅₀ calculated as the median effective concentration.

2.6 ABTS Radical Scavenging Activity

The ABTS antioxidant capacity assay was determined according to the method described by Re et al. [14], and modified by Shu et al. [15]. The blue-green ABTS• radical cation (2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) was generated by reaction of ABTS (7 mM) and potassium persulfate (2.42 mM), after incubation at room temperature in dark for 16 h until reaching a stable oxidative state. On the day of analysis, the ABTS• solution was diluted with 96% ethanol to an absorbance of 0.700 ± 0.05 at 734 nm. 50 µL of extract or catechin standard (20, 40, 50, 60, 80, 100 and 200 µg/mL) dissolved in 80% ethanol was added to 1950 µL of ABTS• solution and mixed thoroughly. The reactive mixture was allowed to stand at room temperature for 6 min and the absorbance was recorded at 734 nm. The results were expressed the same as the DPPH assay described above, with ABTS• inhibition and IC₅₀ value.

2.7. β-carotene-linoleic acid model system (β-CLAMS) assay

In this assay the oxidative destruction of β-carotene by linoleic acid radicals is related to the decrease in absorbance at 470 nm. 20 mg of linoleic acid and 100 mg of Tween 40 were transferred to a flask with 1 mL of β-carotene solution (0.2 mg/mL) in chloroform. Solvent was vacuum evaporated at 40 °C then, 50 mL of oxygenated distilled water were slowly added to the residue and vigorously agitated to form a stable emulsion. To an aliquot (5 mL) of this emulsion, 0.2 mL of antioxidant solution at concentration of 0, 100, 250, 500 y 1000 mg/L was added, and the absorbance immediately measured at 470 nm (t = 0) against a blank consisting of the emulsion without β-carotene. The samples were then subjected to thermal autoxidation at 50°C for 2 h [16]. The absorbance was monitored taking measurements at 30 min intervals, and the rate of bleaching of β-carotene was calculated by fitting linear regression to data over time [17].
2.8. Ferric-reducing/antioxidant power (FRAP) potential assay

The FRAP method measures the ability of antioxidants to reduce ferric-2,4,6-tripyridyl-s-triazine ($\text{Fe}^{3+}$-TPTZ) to a ferrous form ($\text{Fe}^{2+}$), which absorbs light at 593 nm. The ferro- and ferric-iron ions form complexes with TPTZ reagent and are the main products of this reaction [18]. To prepare the FRAP reagent, a mixture of 0.3 mol/L acetate buffer (pH 3.6), 10 mmol/L TPTZ dissolved in 40 mmol/L hydrochloric acid, and 20 mmol/L ferric chloride (10:1:1 v:v:v) was made. 100 µL sample or standard at concentrations 25, 50, 75 and 100 µg/mL was added to 3 mL of FRAP reagent and incubated at 25°C for 10 min. The absorbance was measured at 593 nm. The blank consisted of methanol into sample. The FRAP level was calculated by plotting a standard curve of absorbance against concentration of ascorbic acid standard solution (100 to 600 µM).

3. Results and Discussion

3.1. Tannin characterization

MALDI-TOF MS and $^{13}$C NMR spectra of the purified condensed tannin showed that it consists of both homogeneous and heterogeneous oligomers of procyanidins (catechin/epicatechin flavan-3-ol units) and prodelphinidins (gallocatechin/epigallocatechin flavan-3-ol units) as shown in figure 1. MALDI-TOF MS showed the existence of oligomer clusters from trimmers to heptamers as shown in Table 1 and Figure 3. Figure 4 shows an expanded view of the tetramer oligomers. Procyanidin monomer units clearly dominate the oligomer composition in these clusters. Each oligomer size, trimmer through heptamer, shows two dominant [M + Na$^+$] adduct peaks. The first is a homogeneous procyanidin oligomer and the second one a 16 Da higher due to one more hydroxyl group on the B ring of a gallocatechin/epigallocatechin flavan-3-ol unit. These second peaks are heterogeneous oligomers, which consist of catechin/epicatechin monomer units plus on gallocatechin/epigallocatechin monomer unit. The rest of the oligomers with higher prodelphinidin content are significantly less abundant. The heptamer ions are barely seen above the S/N ratio.

These [M + Na$^+$] molecular ions indicate that the dominant interflavan linkage is the B-type, which is the most common in proanthocyanidins. Close examination of the ion peaks in Figure 4 shows that there are a series of small ion peaks at 2 mass units lower than the main ion peaks for each oligomer. For example, m/z 1175 vs. m/z 1177 and so forth. These might represent a series of oligomers in which there is an A-type interflavan ether linkage in which there would be two less hydrogen atoms present in the oligomer [9, 15]. This would need to be confirmed by isolation of individual oligomers and $^{13}$C NMR analysis. At any rate, the predominant linkage is indicated to be the B-type by both MALDI and NMR. The $^{13}$C NMR spectrum of the condensed tannin is summarized in Table 2 and is consistent with a mixed procyanidin-prodelphinidin proanthocyanidin with B-type linkages when compared to other reported procyanidins, prodelphinidins and mixed oligomers where procyanidins are dominant [15, 19–22]. Typical signals from the characteristic hydroxylation patterns of both procyanidins and prodelphinidins are observed, but those of procyanidins are dominant. Signals for the phloroglucinol A-ring are observed at 154.2-157.6 ppm for the oxygen bearing carbons C-5, C-7 and C-8a of both types. However, characteristic procyanidin B-ring signals are seen at 115.6-116.4 ppm (C-2’, C-5’), 119 ppm (C-6’) and 144.8-145.2 (C-3’, C-4’). Prodelphinidin B-ring signals for C-3’ and C-5’ are observed at 146 ppm, and a signal for C-6’ is seen at 107 ppm. Other signals overlap are shown in Table 2 and agree with literature values reported for both PC and PD proanthocyanidins. The PC/PD ratio of proanthocyanidins has been determined from the relative ratio of the peak areas at 145 ppm and 146 ppm when the amount of prodelphinidin has not been too low. While these signals are used for structural determination, it is difficult to accurately measure the relative peak areas of the 146 ppm signal when procyanidins are present in a much higher concentration than the prodelphinidins since these signals are completely resolved. However, the ratio of the relative heights of the 116/107 ppm signals has been used in this type of situation to estimate the procyanidin/prodelphinidin ratio by Ku and Mun [20], where they found a 94% procyanidin/ 6% prodelphinidin ratio for Pinus radiata bark tannins. In the case of Alaska cedar condensed tannin this
method estimates 86% procyanidin/ 14% prodelphinidin. The rest of the signals in Table 2 are in full agreement with reported values for flavan-3-ol signals with a B-type interflavan linkage.

Table 1. MALDI-TOF MS of condensed tannins from Alaska Cedar inner bark.

<table>
<thead>
<tr>
<th>Oligomer</th>
<th>Number of PC units(^a)</th>
<th>Number of PD units(^b)</th>
<th>Calculated(^c) [M+Na(^+)]</th>
<th>Observed(^d) [M+Na(^+)]</th>
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<tr>
<td></td>
<td>2</td>
<td>1</td>
<td>905.2</td>
<td>905</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>921.2</td>
<td>921</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>3</td>
<td>937.2</td>
<td>937</td>
</tr>
<tr>
<td>Tetramer</td>
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<td>0</td>
<td>1177.3</td>
<td>1177</td>
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<td>1241</td>
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<td>Pentamer</td>
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<td>1465</td>
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<td>1529</td>
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<td>5</td>
<td>1545.3</td>
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<tr>
<td>Hexamer</td>
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<td>0</td>
<td>1753.4</td>
<td>1753</td>
</tr>
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<td>1</td>
<td>1769.4</td>
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<td></td>
<td>6</td>
<td>1</td>
<td>2057.4</td>
<td>2057</td>
</tr>
</tbody>
</table>

\(^a\) procyanidin (catechin/epicatechin),
\(^b\) prodelphinidin (gallocatechin/epigallocatechin),
\(^c\) calculation based on Monagas et al. [9]
\(^d\) remaining oligomer ions not shown in hexamer and heptamer ion clusters were not significantly above the signal to noise ratio.
Figure 3. MALDI-TOF MS of condensed tannins from Alaska Cedar inner bark.

Table 2. $^{13}$C NMR data ($\delta$ ppm) of condensed tannins from Alaska Cedar inner bark (d$_4$-MeOH).

<table>
<thead>
<tr>
<th>C number (as Fig. 1)</th>
<th>$\delta$ ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-2</td>
<td>76.1 cis, 82.6 trans</td>
</tr>
<tr>
<td>C-3</td>
<td>67.4 t, 72 ext</td>
</tr>
<tr>
<td>C-4</td>
<td>30 – 31 t, 38 – 39.5 ext</td>
</tr>
<tr>
<td>C-4a</td>
<td>100 – 102</td>
</tr>
<tr>
<td>C-5, 7, 8a</td>
<td>154.2 – 157.6</td>
</tr>
<tr>
<td>C-6,8 (unsubstituted)</td>
<td>96.5 – 98.2</td>
</tr>
<tr>
<td>C-6,8 (substituted), C-2’, 6’ (PD)</td>
<td>106.4–107.1</td>
</tr>
<tr>
<td>C-1’, 4’ (PD)</td>
<td>131.2 – 131.6</td>
</tr>
<tr>
<td>C-2’(PC), 5’(PC)</td>
<td>115.6–116.4</td>
</tr>
<tr>
<td>C-3’, 4’(PC),</td>
<td>144.8–145.2</td>
</tr>
<tr>
<td>C-3’, 5’(PD)</td>
<td>146</td>
</tr>
<tr>
<td>C-6’(PC)</td>
<td>119</td>
</tr>
</tbody>
</table>

$t$ = terminal unit of oligomer,
$ext$ = extending unit of oligomer,
PC = procyanidin unit,
PD = prodelphinidin unit

A-type linkages are assigned to a signal at 102-104 ppm because of the doubly linked ketal nature of C-2 in the flavan-3-ol unit and are readily apparent in procyanidins with an abundance of A-type linkages such as those from Dock (*Rumex obtusifolius*) [23] and American Cranberry (*Vaccinium macrocarpon* Ait) [24], where the MALDI-TOF MS also show major ion peaks corresponding to these A-linked oligomers. This signal was not readily apparent in the Alaska cedar tannin $^{13}$C-NMR spectrum, but it is consistent with the relatively low amount of A-type linkage indicated in the MALDI spectra and might also be obscured by the C-4a signal. Confirmation of this linkage in the Alaska cedar tannin needs isolation of more pure isomers and analysis to confirm.
Figure 4. MALDI-TOF MS of condensed tannins from Alaska Cedar inner bark. Expanded view of tetramer [M+ Na⁺] peaks.

C= catechin/epicatechin flavan-3-ol monomer units
G= gallocatechin/epigallocatechin flavan-3-ol monomer units

3.2 Antioxidant assays

Several methods have been developed to determine the antioxidant potential of extracts and plant products. The trolox equivalent antioxidant capacity (TEAC) using ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) as an oxidant, the ferric reducing antioxidant power (FRAP), the DPPH (2,2’-diphenyl-1-picrylhydrazyl) and the β-carotene-linoleic acid model system (β-CLAMS) assays. Depending upon the reactions involved, these tests can roughly be classified into two types: assays based on electron transfer (ET) and assays based on hydrogen atom transfer (HAT) reactions [25].

ET-based assays measure the capacity of an antioxidant in the reduction of an oxidant, which changes color when reduced. The degree of color change is correlated with the antioxidant concentration of the samples. These tests include the total phenolic content using the Folin-Ciocalteu reagent (FCR), the ABTS, the ferric ion reducing antioxidant power (FRAP), and the DPPH assays.

The majority of HAT-based assays imply a competitive reaction scheme, in which antioxidant and substrate compete for thermally generated peroxyl radicals through the decomposition of azo compounds. These tests include the β-carotene-linoleic acid model system (β-CLAMS) assay, the inhibition of induced low-density lipoprotein autoxidation, and others [26]. To measure the antioxidant potential of condensed tannin from Alaska cedar inner bark, we have chosen the ABTS, FRAP and DPPH methods, which utilize the same single ET- mechanism, and the β-CLAMS assay, based on HAT reactions.

Our results show that condensed tannins from Alaska cedar inner bark are able to stabilize DPPH and ABTS radicals, otherwise they can act as metal-reducing agents, as it is indicated by the FRAP reaction. The percentages of DPPH radicals inhibition at different concentrations are shown in Figure 5. The corresponding percentages of ABTS radicals inhibition are shown in Figure 6. A positive dose-response relationship was found in the radical scavenging activity, i.e., the antioxidant activity improved at increasing concentrations of condensed tannins. The median inhibition
concentration, IC\textsubscript{50} values (the concentration with scavenging activity of 50%), are shown in Table 3. A lower value of IC\textsubscript{50} indicates greater antioxidant capacity. The results for DPPH (100.5 ± 0.5 µg/mL) were similar to the catechin standard (97.0 ± 3.4 µg/mL). Results of IC\textsubscript{50} for Alaska cedar inner bark tannins were about 10% higher (less effective) than those reported for Delonix regia bark (90.0 ± 2.0 µg/mL) [19], as well for Acacia confusa stem bark (87.85 ± 0.52 µg/mL), and root bark (89.03 ± 0.50 µg/mL) [27].

The results for ABTS were different to DPPH assays, in this case the inhibition by condensed tannins from Alaska cedar inner bark (IC\textsubscript{50} = 138.5 ± 2.5 µg/mL) was significantly inferior than the catechin standard (IC\textsubscript{50} = 69.5 ± 1.5 µg/mL). The difference between ABTS and DPPH assays might be due to color interferences, the more color present in a sample, the smaller the absorbance decrease and lower the corresponding antioxidant activity measured [28].

Table 3. Antioxidant activity of condensed tannin from Alaska Cedar inner bark using the DPPH and ABTS radicals scavenging assays

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC\textsubscript{50} DPPH (µg/mL)</th>
<th>IC\textsubscript{50} ABTS (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condensed tannins from Alaska Cedar inner bark</td>
<td>100.5 ± 0.5</td>
<td>138.5 ± 2.5</td>
</tr>
<tr>
<td>Catechin</td>
<td>97.0 ± 3.4</td>
<td>69.5 ± 1.5</td>
</tr>
</tbody>
</table>

LSD \( P < 0.05, n = 3 \)

The antioxidant activity of condensed tannins measured by the FRAP assay, expressed in µM of ascorbic acid equivalents/g dried tannin, is shown in Figure 7. A higher absorbance corresponds to a higher ferric reducing power. In this assay, the higher activity shown by condensed tannins (522.4 ± 1.4 µM) was similar to the catechin standard (469.7 ± 4.2 µM). In the FRAP assay, the reducing ability of extracts is evident through the conversion of ions Fe\textsuperscript{3+} to Fe\textsuperscript{2+}. This reaction is nonspecific and under the assay conditions, any reaction having lower redox potential than the ferric-ferrous half reaction, will contribute to the ferrous ion formation. The change in absorbance is therefore directly related to the total reducing power of the electron-donating antioxidants present in the samples of the reaction mixture [29]. An oligomeric phenolic fraction, consisting of proanthocyanidins trimmers and tetramers from Quercus sideroxyla bark presented similar results [30]. The reported outcome was 499 µM ascorbic acid equivalents per gram of sample at 100 µg/mL.

In the β-carotene–linoleic acid model system (β-CLAMS) assay, one of the hydrogen atoms from the linoleic acid methylene groups is withdrawn, leaving the free radical of the acid ready to attack β-carotene molecules. They lose their double bond and eventually the characteristic orange color degrades. This oxidative destruction of β-carotene by linoleic acid radicals is related to the decrease in absorbance at 470 nm. The decrease in absorbance of β-carotene in the presence of
different concentrations of condensed tannins from Alaska cedar inner bark, with the oxidation of β-carotene and linoleic acid is shown in Figure 8.

The antioxidant capacity observed was 12.8% at 100 mg/L, 43.2% at 250 mg/L, 54.7% at 500 mg/L, and 64.6% at 1000 mg/L of tannin concentrations. The higher relative increase was observed at tannin concentrations between 100 and 250 mg/L, even superior to the 250 – 1000 mg/L range. The time needed by the blank to reduce the absorbance by a 50% factor was 29 min. However, when adding tannin (100 mg/L) it takes 42 min and even more up to 90 min at higher concentration (250 mg/L) as shown in Figure 8.

The Alaska Cedar inner bark shows significant antioxidant capacity, evaluated by both methods based on single ET- mechanism and HAT reactions. This bark may be considered as a new source of natural antioxidants for nutraceutical products.

![Figure 7](image-url). Antioxidant activity of condensed tannins from Alaska Cedar inner bark by the FRAP assay.

![Figure 8](image-url). Antioxidant activity of condensed tannins from Alaska Cedar inner bark by the β-carotene bleaching assay.

References


