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DP2-DP6 Oligomeric Proanthocyanidin Enrichment and Microencapsulation Preserve Antioxidant Function in Maritime Pine Bark Extract

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Abstract: Maritime pine bark extracts contain proanthocyanidins across a broad polymerization range, so total proanthocyanidin content alone does not define the material delivered by a formulation. We prepared a BCLC maritime pine bark microcapsule enriched in DP2-DP6 oligomers using aqueous-ethanol extraction, resin fractionation, sequential membrane separation and composite-wall microencapsulation. Optimized powders contained 70.2-74.8% total proanthocyanidins; DP2-DP6 represented 58.6-62.4%, catechin plus epicatechin 5.1-6.2%, and DP $\geq$ 10 material 7.3-9.4%. Water activity was 0.31-0.34. The reference formulation retained 93.2% of total proanthocyanidins after 30 days at 40°C/75% RH and 85.1% after 90 days; DP2-DP6 retention at 90 days was 83.6%. Simulated digestion left 78.6% of DP2-DP6 after the intestinal phase. DPPH and ABTS scavenging were 76.8% and 82.1%, and ORAC reached 4,120 μ mol TE/g. In H₂O₂-challenged HaCaT keratinocytes, ROS fell to 60.7% of the model value at 100 μ g/mL while viability remained above 93%. Human studies of standardized French maritime pine bark extracts have also reported higher plasma antioxidant capacity and lower reactive-oxygen-metabolite measures. The data identify polymerization-degree control and post-processing retention as practical variables for an oligomer-rich antioxidant ingredient.

Keywords: maritime pine bark; oligomeric proanthocyanidins; DP2-DP6; oxidative stress; microencapsulation; antioxidant capacity.

Introduction

French maritime pine bark extract has been studied for several decades as a source of procyanidin-rich polyphenols, but the clinical literature is broader than any single chemical or redox endpoint. A recent review assembled 39 randomized, double-blind, placebo-controlled trials involving 2,009 participants and showed how widely dose, population and outcome selection vary across the field [1]. Within that larger record, a smaller group of studies is directly relevant to systemic redox measurements. Devaraj et al. gave 150 mg/day to 25 healthy adults for six weeks and reported an increase in plasma oxygen radical absorbance capacity (ORAC) [2]. In healthy smokers, Belcaro et al. used 50 mg/day for eight weeks and observed lower reactive oxygen metabolite values together with higher biological antioxidant potential than in controls [3]. These findings provide useful human context, but they also make a formulation point: a biological response is difficult to interpret unless the material being administered is chemically defined and remains reasonably consistent through storage and digestion.

That requirement is especially relevant to pine bark because it is not a single-compound material. Procyanidin-rich extracts contain phenolic acids, catechin, epicatechin, oligomeric proanthocyanidins and larger condensed tannins. Their antioxidant properties have been described experimentally [4], while reviews of standardized French maritime pine bark extract have emphasized the diversity of its constituent classes and pharmacological settings [5], [6]. The balance among those fractions is not fixed. Comparative work on Pinus bark extracts has shown that procyanidin composition and antioxidant behavior vary with botanical source and extraction conditions [7]. A total proanthocyanidin assay can therefore be useful for quality control without fully describing the molecular distribution that reaches a finished ingredient.

Degree of polymerization is one reason that distribution matters. Procyanidins of different chain lengths do not share identical gastrointestinal behavior, and the wider absorption, distribution, metabolism and excretion literature indicates that polymer size influences both direct absorption and the metabolites formed after ingestion [8]. Human pharmacokinetic work with standardized maritime pine bark extract has detected parent compounds and metabolites in plasma after oral dosing [9]. In a related ex vivo experiment, plasma collected after supplementation reduced NF- κ B activation and MMP-9 secretion under the conditions tested [10]. Taken together, those observations support tracking the oligomer profile rather than assuming that equal total-proanthocyanidin values necessarily represent equal exposure.

The same concern appears repeatedly in broader reviews of maritime pine bark preparations. D'Andrea discussed the mixed procyanidin composition and the wide range of reported biological applications [11], while Li et al. reviewed nutraceutical, pharmacological and toxicological considerations for pine bark extracts [12]. Iravani and Zolfaghari likewise noted that extraction, composition and intended use can differ substantially among preparations [13]. Analytical endpoints also need to be interpreted at the correct level. ORAC, for example, is a standardized chemical assay for antioxidant capacity and is valuable for comparing preparations under controlled conditions, but it is not itself a clinical outcome [14]. Reviews of pine bark phenolic extracts used in food and nutraceutical settings further underline the effect of extraction and processing on the final phenolic profile [15]. These distinctions are important when an ingredient is designed around a selected oligomer range rather than around total polyphenols alone.

The BCLC process was developed with that narrower objective. Resin-gradient fractionation is used first to reduce small phenolic acids and monomeric catechin/epicatechin. A 3-10 kDa membrane step removes part of the highly polymerized material, followed by a 300-500 Da separation intended to concentrate the DP2-DP6 fraction. Composite-wall microencapsulation is then applied to the enriched material. The practical question is not simply whether the starting extract contains proanthocyanidins, but whether this selected distribution can be reproduced and whether it survives the stresses that occur after processing. Storage at elevated temperature and humidity, followed by simulated gastric and intestinal exposure, therefore forms part of the formulation assessment rather than being treated as a separate stability exercise.

In the present study, composition, storage retention and gastrointestinal-phase retention were examined together with chemical antioxidant assays and an H₂O₂-challenged HaCaT keratinocyte

model. DPPH, ABTS and ORAC were used as complementary in-vitro measurements, while intracellular ROS and cell viability provided a cellular redox readout. Published human studies were used to place these formulation data in context, not to attribute the outcomes of other standardized pine bark products to the BCLC preparation. The study was designed to determine whether enrichment of DP2-DP6 oligomers, followed by microencapsulation, produces a chemically narrower material that retains its profile and antioxidant function sufficiently well to justify formulation-specific human evaluation.

Materials and Methods

Fractionation and microencapsulation

Dried maritime pine bark was extracted with 45-65% aqueous ethanol at 45-65°C. The clarified extract was concentrated at low temperature under reduced pressure. A nonionic styrene-divinylbenzene macroporous resin was then used for staged elution. The 20-35% ethanol fraction was discarded to reduce small phenolic acids and monomeric flavanols; the 45-65% ethanol fraction was collected for DP2-DP6 enrichment. The collected material passed through a 3-10 kDa membrane to remove high-molecular-weight condensed tannins and was then concentrated with a 300-500 Da membrane. The enriched liquid was microencapsulated with the composite wall system and dried by spray- or freeze-drying.

Compositional analysis

Total proanthocyanidins were quantified by the DMAC method with proanthocyanidin B2 as reference. Catechin and epicatechin were quantified by HPLC. Polymerization-degree distribution was characterized by normal-phase HPLC with fluorescence detection. Water activity was measured at 25°C, and particle-size distribution was measured by laser diffraction.

Stability and gastrointestinal simulation

Samples were stored at 40°C and 75% relative humidity through 90 days. Simulated digestion consisted of 60 min in gastric fluid followed by 120 min in intestinal fluid. DP2-DP6 and total proanthocyanidin retention were measured at the specified time points.

Antioxidant and cell-redox evaluation

DPPH and ABTS radical-scavenging assays and ORAC were used for in-vitro antioxidant characterization; ORAC was expressed as μmol Trolox equivalents per gram using the established assay framework [14]. Human HaCaT keratinocytes were exposed to H_2O_2 . BCLC extract was tested at 25, 50 and 100 $\mu\text{g/mL}$, with cell viability and intracellular ROS as endpoints.

Human evidence review

PubMed and journal sources were searched through August 2026 using combinations of 'French maritime pine bark', 'Pinus pinaster', 'Pycnogenol', 'oxidative stress', 'antioxidant capacity', 'randomized', and 'placebo'. Randomized or controlled human studies with objective redox biomarkers were prioritized.

Results

Polymerization-degree distribution

The three optimized BCLC preparations contained 70.2-74.8% total proanthocyanidins. DP2-DP6 accounted for 58.6-62.4% of total proanthocyanidins; combined catechin and epicatechin were 5.1-6.2%, and $\text{DP} \geq 10$ fractions were 7.3-9.4%. The ordinary extract contained 34.6% DP2-DP6, 15.4% monomers and 21.8% $\text{DP} \geq 10$ material. The optimized preparations differed from the broad comparator at both ends of the polymerization distribution.

Storage and digestive stability

The reference BCLC formulation retained 93.2% of total proanthocyanidins at 30 days, 89.4% at 60 days and 85.1% at 90 days under 40°C/75% RH. DP2-DP6 retention was 83.6% at 90 days. After simulated digestion, DP2-DP6 retention was 86.8% after the gastric phase and 78.6% after the intestinal phase; total proanthocyanidin retention after the intestinal phase was 80.4%. Each value was higher than the corresponding value for the ordinary extract and single-wall comparator.

Antioxidant performance

The reference optimized material showed DPPH scavenging of 76.8±2.5%, ABTS scavenging of 82.1±2.6%, and ORAC of 4,120±135 µmol TE/g. The two other optimized preparations followed the same direction. In H₂O₂-stressed HaCaT cells, ROS was 100.0±3.5% in the model and fell to 78.4±2.8%, 68.5±2.6% and 60.7±2.4% at 25, 50 and 100 µg/mL. Cell viability remained 93.5-94.1% across those concentrations.

Table 1. Polymerization-degree and powder-quality profile

Sample	Total PACs (%)	DP2-DP6 (%)	Catechin+epicatechin (%)	DP≥10 (%)	aw	D90 (µm)
Ordinary extract comparator	67.8±2.3	34.6±1.5	15.4±0.7	21.8±1.1	0.48±0.02	232±10
No low-MW elution comparator	72.6±2.1	50.8±1.7	13.6±0.6	10.9±0.7	0.39±0.01	178±8
No graded resin collection comparator	70.4±1.9	38.9±1.5	6.4±0.4	20.7±1.0	0.37±0.01	166±7
No polymer-removal comparator	75.8±2.0	42.2±1.6	5.8±0.3	23.5±1.2	0.36±0.01	171±8
BCLC F1	74.8±2.1	62.4±1.8	5.1±0.4	7.3±0.6	0.31±0.01	142±6
BCLC F2	70.2±1.9	58.6±1.7	6.2±0.4	8.8±0.7	0.33±0.01	153±7
BCLC F3	72.6±2.0	60.1±1.6	5.8±0.3	9.4±0.7	0.34±0.01	165±8

Table 2. Human studies providing redox-related clinical context

Study	Design / population	Exposure	Relevant finding
Devaraj et al., 2002 [2]	Human supplementation; 25 healthy adults	150 mg/day; 6 weeks	Plasma ORAC increased during supplementation; lipid-profile changes also reported
Belcaro et al., 2013 [3]	Controlled study; 155 healthy smokers	50 mg/day; 8 weeks	d-ROM decreased and biological antioxidant potential increased versus control
Weichmann & Rohdewald, 2024 [1]	Review of randomized double-blind placebo-controlled trials	39 trials; 2,009 participants	Documents breadth of controlled human evidence and heterogeneity of clinical contexts

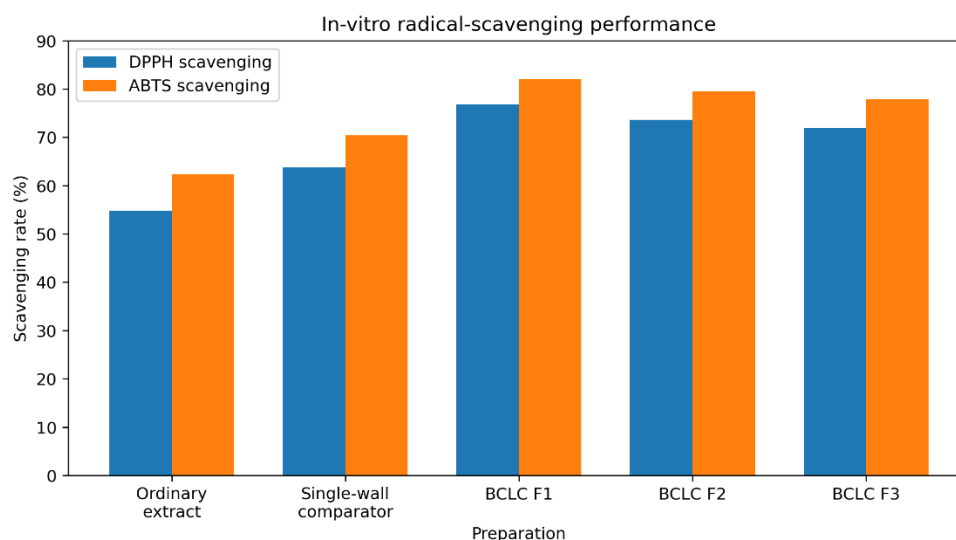


Figure 1. DPPH and ABTS radical-scavenging performance of selected preparations. Values are mean percentages reported under the defined experimental conditions

Discussion

Fractionation and encapsulation affected different properties in this experiment. The ordinary extract contained 34.6% DP2-DP6, 15.4% catechin plus epicatechin and 21.8% DP $\geq$ 10 material. BCLC F1 contained 62.4%, 5.1% and 7.3%, respectively. That shift was created by the separation sequence before storage stability was considered. The subsequent microencapsulation step acted on a narrower molecular distribution, an important distinction because pine-bark procyanidin composition changes with extraction and processing conditions [7], [15].

Chemical antioxidant assays and the HaCaT experiment measured different levels of response, but neither required a loss of cell viability to produce the observed pattern. The reference preparation gave DPPH and ABTS values of 76.8 $\pm$ 2.5% and 82.1 $\pm$ 2.6%, with ORAC of 4,120 $\pm$ 135 μ mol TE/g. In H₂O₂-challenged cells, ROS fell from 100.0 $\pm$ 3.5% to 60.7 $\pm$ 2.4% at 100 μ g/mL while viability stayed within the 93.5-94.1% range. Antioxidant and radical-scavenging properties are well described for procyanidin-rich maritime pine bark extracts [4], and ex vivo human plasma work has identified effects on NF- κ B and MMP-9 after oral intake [10].

The human redox studies used different standardized extracts and are best read study by study. Devaraj et al. gave 150 mg/day to 25 healthy adults for six weeks and reported an increase in plasma ORAC [2]. Belcaro et al. followed 155 healthy smokers receiving 50 mg/day for eight weeks and reported lower d-ROM values together with higher biological antioxidant potential than control [3]. The 2024 review by Weichmann and Rohdewald documents a much wider clinical literature, but also shows substantial variation in dose, population and endpoint across trials [1].

For BCLC, the narrower question is whether a DP2-DP6-defined fraction can be delivered reproducibly. Human pharmacokinetic data show measurable pine-bark-derived compounds and metabolites after oral dosing [9], and the broader procyanidin ADME literature indicates that chain length influences gastrointestinal fate [8]. That makes polymerization-degree distribution a useful exposure descriptor alongside total proanthocyanidins. Reviews of French maritime pine bark extracts reach the same practical issue from different angles: composition, bioavailability and study standardization all affect how results are compared [5], [6], [11]-[13].

Study Considerations and Future Directions

A formulation-specific human study could quantify the administered DP2-DP6 profile and collect plasma and urinary phenolic metabolites at prespecified times. Redox endpoints could include d-ROM, biological antioxidant potential, F2-isoprostanes, oxidized lipoprotein markers and inflammatory-redox indices. The same batches should be assayed before dosing for total proanthocyanidins and DP2-DP6 retention so that biological measurements can be interpreted against the actual ingredient specification.

Conclusion

Resin fractionation shifted BCLC toward DP2-DP6 and away from monomeric and DP $\geq$ 10 material, while composite-wall microencapsulation preserved the selected profile during storage and simulated digestion. The reference preparation retained 85.1% of total proanthocyanidins and 83.6% of DP2-DP6 after 90 days at 40°C/75% RH, and 78.6% of DP2-DP6 remained after the intestinal phase. It also showed strong DPPH, ABTS and ORAC responses and lowered intracellular ROS in H₂O₂-challenged keratinocytes. A direct next comparison is between this measured oligomer distribution and systemic redox responses in BCLC-specific human studies.

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