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Phenolic profile and antioxidant activity of Maritime pine (*Pinus pinaster* subsp. *Atlantica*) bark extract during simulated gastrointestinal tract digestion

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Maritime pine (*Pinus pinaster* subsp. *atlantica*) is native to the western Mediterranean basin, especially southwestern Europe which includes the Iberian Peninsula. It has economic importance due to the use of its wood in the furniture, construction and paper industries. Bark and other parts of the plant are by-products with a variety of potential applications. This is due to the presence of secondary metabolites, especially phenolic compounds and terpenes [1]. In this work, an extract rich in phytochemicals, obtained from Maritime pine bark was selected to be evaluated as an additive to meat cured products. The gastrointestinal environment can modify the compounds present in the extract and lead to new combinations with different bioactivities. Thus, two concentrations of pure extract were submitted to a simulated gastrointestinal tract digestion.

Conditions tested:

Pure extract:
- **3 mg** extract – Equivalent to one product dose.
- **27 mg** extract – Equivalent to a whole product.

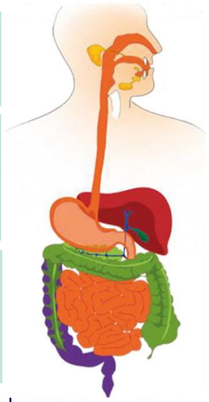
Infogest Protocol [2]:

Oral Phase:
Mix 1:1 with Simulated Salivary Fluid (SSF)
2 min at 37 ºC pH 7, 200 rpm

Gastric Phase:
Mix 1:1 with Simulated Gastric Fluid (SGF)
pepsin (2000 U/mL) and gastric lipase (60 U/mL)
2 h at 37 ºC, pH 3, 130 rpm

Intestinal Phase:
Mix 1:1 with Simulated Intestinal Fluid (SIF)
Pancreatin (100 U/mL) and bile salts (10 nM)
2 h at 37 ºC, pH 3, 45 rpm

At the end of each phase, samples were collected and characterized for their phenolic profile and antioxidant activity.



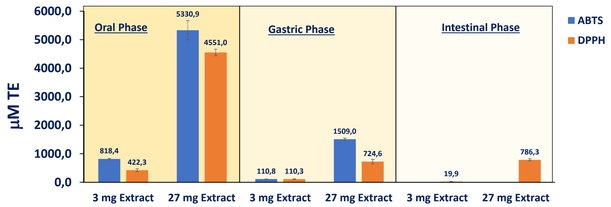


Figure 1. Antioxidant activity of Maritime pine bark extract during simulated gastrointestinal digestion through ABTS and DPPH methods.

Table 1. Phenolic profile obtained through HPLC-DAD-ESI/MS of the 3 and 27 mg of Maritime pine bark extract in the oral and intestinal phases.

3mg Extract							Oral µg/mL	Intestinal µg/mL
Peak	*Rt*	*λmax*	*[M-H]⁻ m/z*	*MS²*	*Tentative Identification*	*Average ± SD*	*Average ± SD*
1	4.24	281	577	559(47),467(18),451(100),425(65),407(49),289(55)	B-type procyanidin dimer	1.269±0.001	-
2	4.86	280	289	245(100),205(35),179(22),125(5)	(+)-Catechin	3.722±0.065	18.63±0.43
3	5.44	281	865	801(31),789(47),779(100),720(65),695(41),577(64),575(35)	B-type procyanidin trimer	2.52±0.04	-
4	5.94	281	1153	865(27),577(56),289(49)	B-type procyanidin tetramers	0.982±0.005	-
5	6.36	280	575	449(50),423(100) 407(30),289(5),287(20),285(10)	A-type procyanidin dimer	1.18±0.004	-
6	6.69	280	865	801(31),789(47),779(100),720(65),695(41),577(64),575(35)	B-type-procyanidin trimer	1.873±0.062	-
7	6.87	280	289	245(100),205(35),179(22),125(5)	(-)-Epicatechin	1.194±0.002	-
8	7.47	280	1153	865(27),577(56),289(49)	B-type procyanidin tetramers	1.714±0.056	-
9	7.93	280	1153	865(27),577(56),289(49)	B-type procyanidin tetramers	1.331±0.003	-
10	10.51	280	1151	981(100),863(89),575(36),289(18)	A-type procyanidin tetramers	1.911±0.062	-
11	11.54	282sh322	465	303(100),285(20)	Taxifolin-7-O-hexoside	0.986±0.01	-
12	12.52	280	575	449(50),423(100) 407(30),289(5),287(20),285(10)	A-type procyanidin dimer	2.022±0.074	-
13	14.41	281	1151	1009(100),863(89),575(36),289(18)	A-type procyanidin tetramers	2.149±0.032	-
14	14.75	281	1151	1009(100),863(89),575(36),289(18)	A-type procyanidin tetramers	18.714±0.462	-
15	15.06	280	1153	865(27),577(56),289(49)	B-type procyanidin tetramers	1.952±0.046	-
					Total Phenolic Compounds	**43.52±0.63**	**18.63±0.43**

27mg Extract							Oral µg/mL	Intestinal µg/mL
Peak	*Rt*	*λmax*	*[M-H]⁻ m/z*	*MS²*	*Tentative Identification*	*Average ± SD*	*Average ± SD*
1	4.24	281	577	559(47),467(18),451(100),425(65),407(49),289(55)	B-type procyanidin dimer	5.31±0.04	-
2	4.86	280	289	245(100),205(35),179(22),125(5)	(+)-Catechin	17.25±0.36	10.98±0.41
3	5.44	281	865	801(31),789(47),779(100),720(65),695(41),577(64),575(35)	B-type procyanidin trimer	12.58±0.28	-
4	5.94	281	1153	865(27),577(56),289(49)	B-type procyanidin tetramers	2.48±0.05	-
5	6.36	280	575	449(50),423(100) 407(30),289(5),287(20),285(10)	A-type procyanidin dimer	3.1±0.04	-
6	6.69	280	865	801(31),789(47),779(100),720(65),695(41),577(64),575(35)	B-type-procyanidin trimer	9.99±0.54	5.23±0.06
7	6.87	280	289	245(100),205(35),179(22),125(5)	(-)-Epicatechin	2.15±0.04	-
8	7.47	280	1153	865(27),577(56),289(49)	B-type procyanidin tetramers	3.44±0.04	-
9	7.93	280	1153	865(27),577(56),289(49)	B-type procyanidin tetramers	4.8±0.03	-
10	10.51	280	1151	981(100),863(89),575(36),289(18)	A-type procyanidin tetramers	10.11±0.41	10.87±0.58
11	11.54	282sh322	465	303(100),285(20)	Taxifolin-7-O-hexoside	3.33±0.16	11.09±0.47
12	12.52	280	575	449(50),423(100) 407(30),289(5),287(20),285(10)	A-type procyanidin dimer	13.46±0.62	-
13	14.41	281	1151	1009(100),863(89),575(36),289(18)	A-type procyanidin tetramers	9.17±0.41	12.58±0.39
14	14.75	281	1151	1009(100),863(89),575(36),289(18)	A-type procyanidin tetramers	120.82±6.43	-
15	15.06	280	1153	865(27),577(56),289(49)	B-type procyanidin tetramers	10.35±0.37	-
					Total Phenolic Compounds	**228.35±9.83**	**50.74±1.92**

- Whole product concentration showed higher antioxidant activity than the one product dose concentration.
- Antioxidant activity was reduced throughout gastrointestinal digestion for the lowest concentration, while, at the highest concentration there were no differences between the gastric and intestinal phases.
- ABTS method was not able to quantify the antioxidant activity of the intestinal phase due to interference from bile salts.
- The extract showed to be rich in condensed tannins.
- Only catechin was detected at the end of the digestion of 3 mg of extract.
- Bioaccessibility of catechin increased when using 3 mg of extract while the opposite was observed for 27 mg of extract.
- At 27 mg of extract, the number of detected phenolics decreased through the digestion process. However, for some of the detected phenolic compounds, their bioaccessibility increased.

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