CIÊNCIAS AGRÁRIAS, ALIMENTARES E VETERINÁRIAS
AGRICULTURAL SCIENCES, FOOD AND VETERINARY
CIENCIAS AGRÍCOLAS, ALIMENTOS Y VETERINARIA

millenium 79

Millenium, 2(8), 79-87.

en

CARATERIZAÇÃO QUÍMICA, PROPRIEDADES ANTIOXIDANTES E PERFIL FENÓLICO DE CASCA DE PINHEIRO (Pinus pinaster Aiton subsp. atlantica)

CHEMICAL CHARACTERIZATION OF PINE BARK (Pinus pinaster Aiton subsp. atlantica), ANTIOXIDANT PROPERTIES AND PHENOLIC PROFILE OF ITS EXTRACTS

CARACTERIZACIÓN QUÍMICA, PROPIEDADES ANTIOXIDANTES Y PERFIL FENÓLICO DE LA CORTEZA DE PINO (Pinus pinaster Aiton subsp. atlantica)

Catarina da Silva Vieito[1]
Preciosa Pires[2]
Élia Fernandes[2]
Manuela Vaz Velho[2]

1 Polytechnic Institute of Viana do Castelo, Viana do Castelo, Portugal
[2] Polytechnic Institute of Viana do Castelo, School of Technology and Management, Viana do Castelo, Portugal

Catarina da Silva Vieito - cvieito@ipvc.pt | Preciosa Pires - ppires@estg.ipvc.pt | Élia Fernandes - eliaf@estg.ipvc.pt | Manuela Vaz Velho - mvazvelho@estg.ipvc.pt

Corresponding Author
Manuela Vaz Velho
Instituto Politécnico de Viana do Castelo
Rua Escola Industrial e Comercial de Nun'Álvares, n.º 34
4900-347 Viana do Castelo
mvazvelho@estg.ipvc.pt

RECEIVED: 29th May 2018
ACCEPTED: 04th January 2019

Vieito, C. S., Pires, P., Fernandes, E. & Velho, M. V. (2019). Chemical characterization of pine bark (Pinus pinaster Aiton subsp. atlantica), antioxidant properties and phenolic profile of its extracts. *Millenium*, 2(8), 79-87.
DOI: https://doi.org/10.29352/mill0208.07.00197

RESUMO

Introdução: A casca de pinheiro é um resíduo agroindustrial proveniente da indústria madeireira e representa uma fonte de compostos fenólicos. Estes compostos têm várias propriedades benéficas sendo elas antioxidantes, antimicrobianas, anti-inflamatórias, cardiovasculares, entre outras.
Objetivos: O objetivo deste trabalho foi estudar a composição química da casca de *Pinus pinaster* Aiton subsp. *atlantica* e o perfil fenólico dos seus extratos aquosos, etanólicos e hidroetanólicos.
Métodos: Analisou-se o teor de humidade, cinzas, proteínas, gordura total e hidratos de carbono. A casca foi extraída com água, etanol ou uma mistura de ambos num aparelho de Soxhlet e determinou-se o rendimento das extrações, o teor de compostos fenólicos totais (CFT), atividade antioxidante e o perfil fenólico por RP HPLC UV nos extratos com o CFT mais elevado.
Resultados: Os resultados obtidos para a composição química foram: 63,43 de hidratos de carbono, 2,81 de gordura total, 1,60 de proteínas e 1,75 de cinzas, calculados em % m/m de casca seca. O rendimento das extrações foi superior para os solventes etanol e mistura hidroetanólica (17,08 e 17,55% m/m de casca seca, respetivamente). O CFT e a atividade antioxidante foram superiores no extrato hidroetanólico (73,48 mg EAG/g e 108,74 mg EAA/g de casca seca, respetivamente). Na análise do perfil fenólico do extrato hidroetanólico identificou-se o ácido gálico, taxifolin, ácido ferrúlico e quercetina a 280 nm e a catequina no extrato etanólico a 320 nm.
Conclusões: A casca de *P. pinaster* Aiton subsp. *atlantica* é maioritariamente constituída por hidratos de carbono e é rica em extrativos hidroetanólicos e etanólicos, tendo estes elevada atividade antioxidante. O extrato etanólico apresenta concentração de catequina mais elevada comparativamente ao extrato hidroetanólico.

Palavras-chave: casca de pinheiro; propriedades antioxidantes; compostos fenólicos; RP-HPLC-UV.

ABSTRACT

Introduction: Pine bark is an agroindustrial residue from the timber industry and represents a source of phenolic compounds. These compounds have several beneficial properties being antioxidants, antimicrobial, anti-inflammatory, cardiovascular, among others.
Objetives: The aim of this work was to study the chemical composition of the bark from *Pinus pinaster* Aiton subsp. *atlantica* and the phenolic profile of its aqueous, ethanolic and hydroethanolic extracts.
Methods: The moisture content, ash, protein, crude fat and carbohydrates were analysed. The bark was extracted with water, ethanol or a mixture of both in a Soxhlet apparatus and the extraction yield, total phenolic content (TPC), antioxidant activity and phenolic profile by RP-HPLC-UV, in the extracts with higher TPC, were determined.
Results: The results obtained for chemical composition were: 63.43 of carbohydrates, 2.81 of crude fat, 1.60 of proteins and 1.75 of ash, calculated in % w/w of dry bark. The extraction yield was greater for the ethanolic and the hydroethanolic extracts (17.08 and 17.55% w/w dry bark, respectively). The TPC and antioxidant activity were higher in the hydroethanolic extract (73.48 mg GAE/g and 108.74 mg AAE/g dry bark, respectively). Regarding the phenolic profile of the hydroethanolic extract, gallic acid, taxifolin, ferulic acid and quercetin were identified at 280 nm, and catechin was identified in the ethanolic extract at 320 nm.
Conclusion: *P. pinaster* Aiton subsp. *atlantica* bark is mainly constituted by carbohydrates and it is rich in hydroethanolic and ethanolic extractives, being that these have high antioxidant activity. The ethanolic extract presents higher catechin amount when compared to the hydroethanolic extract.

Keywords: pine bark; antioxidant properties; phenolic compounds; RP-HPLC-UV.

RESUMEN

Introducción: La corteza de pino es un residuo agroindustrial proveniente de la industria maderera y representa una fuente de compuestos fenólicos. Estos compuestos tienen varias propiedades benéficas siendo ellas antioxidantes, antimicrobianas, antiinflamatorias, cardiovasculares, entre otras.
Objetivos: El objetivo de este trabajo fue estudiar la composición química de la corteza de *Pinus pinaster* Aiton subsp. *atlantica* y el perfil fenólico de sus extractos acuosos, etanólicos e hidroetanólicos.
Métodos: Se analizó el contenido de humedad, cenizas, proteínas, grasa total y carbohidratos. La corteza se extrajo con agua, etanol o una mezcla de ambos en un aparato de Soxhlet y se determinaron el rendimiento de la extracción, el contenido de compuestos fenólicos totales (CFT), la actividad antioxidante y el perfil fenólico mediante RP-HPLC-UV, en los extractos con CFT más alto.
Resultados: Los resultados obtenidos para la composición química fueron: 63,43 de carbohidratos, 2,81 de grasa total, 1,60 de proteínas y 1,75 de ceniza, calculados en % m/m de corteza seca. El rendimiento de extracción fue mayor para los extractos etanólicos e hidroetanólicos (17,08 y 17,55% m/m de corteza seca, respectivamente). El CFT y la actividad antioxidante fueron

mayores en el extracto hidroetanólico (73,48 mg de EAG/g y 108,74 mg de EAA/g de corteza seca, respectivamente). Con respecto al perfil fenólico del extracto hidroetanólico, se identificaron ácido gálico, taxifolin, ácido ferúlico y quercetina a 280 nm, y se identificó la catequina en el extracto etanólico a 320 nm.
Conclusións: La corteza de *P. pinaster* Aiton subsp. *atlantica* está constituida principalmente por carbohidratos y es rica en extractos hidroetanólicos y etanólicos, ya que estos tienen una alta actividad antioxidante. El extracto etanólico presenta una mayor cantidad de catequina en comparación con el extracto hidroetanólico.

Palabras Clave: corteza de pino; propiedades antioxidantes; compuestos fenólicos; RP-HPLC-UV.

INTRODUCTION

As a consequence of the growing world population, natural resources availability is of current concern. In this sense, the use of materials, such as biomass residues from forests and agriculture, formerly considered waste, appears as a new world tendency. The Portuguese pine sector represents an important component of the total forest economic value (around 17%), being the third most important species after eucalyptus and cork oak (Seabra, Dias, Braga, & de Sousa, 2012). Pine bark is an abundant residue of the wood industry, since it represents 10–20% of the pine tree trunk (Braga et al., 2008). Currently, more than half of the bark is incinerated or landfilled and the remainder is mainly used as a cheap source of energy in saw/pulp mills being that both destinations can lead to environmental problems (Jablonsky et al., 2017). It was observed that bark contains a large fraction of extractives, with some important phytochemical constituents, and lignin, which can be utilized as a renewable source following the worldwide tendency of recovering, recycling and upgrading wastes (Braga et al., 2008). Also, it has low price and long-term stability that together make the usage of this residue highly attractive (Seabra et al., 2012). *P. pinaster* bark extracts have been reported to have several bioactivities including antioxidants, cardiovascular benefits, and anti-diabetic effects (Aspé & Fernandez, 2011; Chupin et al., 2015). The most prominent feature of *P. pinaster* is that it can grow on poor soil that provides minimal nourishment (Tümen, Akkol, Taştan, Süntar, & Kurtca, 2018). Due to large availability of pine bark on a global scale, there is an increasing interest in its use (Ronda, Della Zassa, Biasin, Martin-Lara, & Canu, 2017).
A lot of attention has been recently focused on the addition of polyphenols to foods and biological systems, due to their well-known abilities to scavenge free radicals, i.e. antioxidant power (Pinelo, Rubilar, Sineiro, & Núñez, 2004). They have received considerable attention in the fields of nutrition, health, and medicine owing to their physiological and biological activities, namely antibacterial, antiviral, anticarcinogenic, anti-inflammatory and cardiovascular system diseases' prevention (Seabra et al., 2012). The objective of extracting phenolic compounds from their plant sources is to release these compounds from the vacuolar structures where they are found, either by rupturing plant tissue or by a diffusion process. Usually, a high extraction yield is required for an efficient process, although it will not necessarily ensure a high concentration of bioactive components. Since some of these are very sensitive to oxygen and heat, more care should be taken to prevent their oxidation and thermal degradation. Therefore, the extraction yield and the bioactive components' characteristics should also be considered when an extraction method is selected (Aspé & Fernandez, 2011). Also, when the main goal is to apply these bioactive components in foods or nutraceutical products, the extraction solvent must be suitable for human consumption.
Therefore, the aim of the present study was to assess the chemical composition of bark from *Pinus pinaster* Aiton subsp. *atlantica* and evaluate the antioxidant activity of the aqueous, hydroethanolic and ethanolic extracts. Furthermore, its chromatographic profile by reverse phase high-performance liquid chromatography (RP-HPLC) was studied.

1. METHODS

1.1 Sample preparation

Pine bark (*P. pinaster* Aiton subsp. *atlantica*) was collected in Minho region, Northwest of Portugal, from trees aged 15 years. The inner bark was separated from the outer bark and the latter cut into pieces was oven dried to reach equilibrium humidity at 40 °C for 72 hours. The dried outer bark was ground by using a mixer (Termomix TM31, Vorwerk, Germany) for 20 s and sieved at an amplitude of 0.2 for 1 min to select the particles with a diameter between 200 and 850 µm. All analyses and extractions, except for moisture, were performed on outer dried pine bark. The inner bark was not analysed until the date of this article.

1.2 Reagents

Ethanol 96% was purchased from Aga (Prior Velho, Portugal). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and gallic acid monohydrate were purchased from Sigma-Aldrich (Steinheim, Germany). Folin-Ciocalteu reagent, ascorbic, boric and phosphoric acid were purchased from Merck (Darmstact, Germany). Sodium carbonate anhydrous, potassium sulfate, Kjeldahl catalyst, hydrogen peroxide and sodium hydroxide were purchased from Panreac (Barcelona, Spain). Methanol was purchased from Jt Baker

Vieito, C. S., Pires, P., Fernandes, E. & Velho, M. V. (2019). Chemical characterization of pine bark (Pinus pinaster Aiton subsp. atlantica), antioxidant properties and phenolic profile of its extracts. *Millenium*, 2(8), 79-87.
DOI: https://doi.org/10.29352/mill0208.07.00197

(Deventer, Holland). Petroleum ether and sulfuric acid were purchased from Fisher Scientific (Loughborough, UK). The reagents were of analytical grade, except the ones used for HPLC analysis that were HPLC grade.

1.3 Chemical composition

Pine bark was analysed for moisture, ash, proteins, fat and carbohydrates contents using the AOAC procedures (AOAC, 1995). The moisture was determined by drying in an oven at 103 °C until constant weight (AOAC 930.04); the ash content was determined by incineration at 550 °C (AOAC 930.05); the crude protein content (N x 6.25) of the samples was estimated by Kjeldahl method (AOAC 978.04); the crude fat was determined by extracting a known weight of ground sample with petroleum ether, using a Soxhlet apparatus (AOAC 920.39) and the total carbohydrates were determined by the 3,5-dinitrosalicylic acid reaction with reducing sugars present in the sample after hydrolysis with hydrochloric acid and neutralization (Miller, 1959).

1.4 Extraction of pine bark and evaluation of its antioxidant properties

Different solvent types were tested with Soxhlet extraction. A total of 12.5 g of ground pine bark was put into a cartridge inside a Soxhlet apparatus. Then, 220 mL of solvent (water, hydroethanolic mixture (1+1) or ethanol) was added to the flask and refluxed over four hours as the minimal indicating time for the official AOAC method for crude fat. The extract was collected and completed to 250 mL with the respective solvent and named as PW, PWE and PE, corresponding to the aqueous, hydroethanolic and ethanolic extracts, respectively.

1.4.1 Extraction yield

Extraction yield (% w/w) is a measure of the solvent efficiency to extract specific components from the original material, defined as the amount of solid extract recovered in dry mass compared with the initial amount of dry bark. The extraction yield was calculated measuring an exact sample volume (V_1=25 mL) from the total extract (V_2=250 mL) and the volume was reduced at 35 °C until it was obtained a dry solid residue. Finally, it was dried at 103 °C until constant weight.

$$Extraction\ yield\ (\%\ w/w) = \frac{w_2 - w_1}{w_{sample} \times \left(\frac{V_1}{V_2}\right)} \times 100$$

Where w_1 is the empty recipient weight, w_2 is the recipient weight plus the dried extract weight and w_{sample} is the sample amount weighed to perform the extraction.

1.4.2 Total phenolic content

Total phenolic content was estimated by Folin-Ciocalteu colorimetric assay according to the procedure previously described by Lafka, Sinanoglou, and Lazos (2007) based on Gutfinger (1981) and the results were expressed as mg of gallic acid equivalents (GAE) per g of dry sample.

1.4.3 Antioxidant activity

The antioxidant activity in the extracts was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method used by Deng, Penner, and Zhao (2011) based on Brand-Williams, Cuvelier, and Berset (1995). A volume of 2 mL of diluted extract stock solution (in methanol) was mixed with 6 mL of DPPH in methanol and allowed to stand at room temperature, in the dark, for 30 minutes prior to measuring the solution absorbance at 517 nm. The control was a DPPH solution containing absolute methanol instead of the sample. The antioxidant activity was based on the measurement of the reducing ability of pine extracts towards the radical DPPH. The results were obtained as mg of ascorbic acid equivalent (AAE) per g of dry bark. The standard curve was prepared with ascorbic acid at 0, 1, 2.5, 5, 7.5, 10, 20, 30, 35 and 40 mg/L.

1.5 Polyphenols identification (RP-HPLC-UV analysis)

Sample preparation

A tenfold dilution (1/10) in methanol of the extract was performed. The solution was homogenized and filtered with disposable 0.2 µm syringe filters (Whatman, UK) prior to injection.

LC conditions

Qualitative identification of polyphenols in pine bark extracts was carried out using an HPLC method developed in-house. The system used a reverse phase column, Betasil C18 (4.6 x 250 mm, 5 µm particle size, Thermo Scientific, USA). The mobile phase comprised 1% phosphoric acid in water, methanol and water. Gradient elution was as follows: 0-2 min, 80/0/20; 2-5 min, 65/15/20; 5-10 min, 50/30/20; 10-15 min, 45/35/20; 15-25 min, 30/50/20; 25-30 min, 20/60/20; 30-35 min, 0/80/20; 35-45 min, 0/90/10; 45-65 min, 0/100/0; 65-70 min 30/50/20. The flow rate was 1.0 mL/min. The wavelength for UV detection was set at 280 and 320 nm.

The experiments were performed on a Hewlett Packard Series 1100 HPLC system (Agilent Technologies, Waldbronn, Germany), equipped with a G1322A degasser, G1311A quaternary pump, G1314A UV-Vis detector and a G1328A manual injector. The software used was HP ChemSation for LC Rev. A.06.03 [509].

1.6 Statistical analysis

All the assays were carried out in triplicate. The results were expressed as mean values and standard deviation (SD). A correlation between the total phenolic content and antioxidant activity values was calculated and it was obtained a correlation coefficient (R^2). The results were analysed using one-way analysis of variance (ANOVA) followed by Tukey's HSD test (p<0.05). This treatment was carried out using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp, New York, USA).

2. RESULTS AND DISCUSSION

2.1 Chemical composition

There are little recent papers about the chemical composition of pine bark, namely the maritime pine grown in Portugal. Also, the composition of bark depends on the age of the tree, the location and the growing conditions, among other factors, as observed by Vázquez, Antorrena, and Parajó (1987). In this work the two parts of pine bark (outer and inner) were divided to be studied in separate. However, this work only presents the results for the outer pine bark fraction (Table 1). The moisture content of the pine bark studied was 12.82% w/w. The dry bark composition was: carbohydrates 63.43% w/w, fat 2.81% w/w, proteins 1.60% w/w and ash 1.75 % w/w. Vázquez et al. (1987) extracted dry *P. pinaster* bark with organic apolar solvents obtaining yields of 0.9% w/w with ether and 2.5% w/w with hexane and benzene extraction, which is similar to the fat content determined in this work (2.81% w/w). Other authors (Fradinho et al., 2002; Vázquez et al., 1987) studied dry maritime pine bark and obtained lower values for ash, namely 0.5 and 0.8% w/w, respectively. In this work the remaining value calculated by difference (30.41% w/w; Table 1) includes other components such as cellulose, lignin and suberin, among others.

Table 1 – Chemical composition values of dry pine bark from *P. pinaster* Aiton subsp. *atlantica*.

Parameter	Amount (% w/w)
Ash	1.75 ± 0.03
Proteins	1.60 ± 0.04
Fat	2.81 ± 0.002
Carbohydrates	63.43 ± 3.95
Other components	30.41

Pine bark moisture = 12.82 ± 0.01 % w/w.

2.2 Extraction of pine bark and evaluation of its antioxidant properties

In papers published to date different solvent systems and extraction techniques have been used for the extraction of polyphenols from pine bark. The yield, total phenolic content and antioxidant activity of the extracts is highly dependent on these factors. Water, ethanol, methanol, aqueous alkaline solutions, acetone, dichloromethane, are some of the solvents commonly used to extract phenolic compounds from pine bark (Aspé & Fernandez, 2011; Ćurković-Perica, Hrenović, Kugler, Goić-Barišić, & Tkalec, 2015; Fradinho et al., 2002).

In Table 2 are presented results of extraction yield, total phenolic content and antioxidant properties of *P. pinaster* Aiton subsp. *atlantica* bark extracts. PWE and PE extracts did not show significant differences regarding extraction yield, with 17.55 and 17.08% w/w dry bark being obtained, respectively (p<0.05). Fradinho et al. (2002) have reported a similar value, namely 13.5% w/w dry bark for the successive Soxhlet extractions with ethanol and water in *P. pinaster* bark grown in Portugal. However, the PWE extract showed a higher total phenolic content (73.48±1.84 mg GAE/g dry bark) and therefore, higher antioxidant activity (108.74±2.02 mg AAE/g dry bark, Table 2, R^2=0.983, p<0.05). Water extracts presented the lowest values for all the parameters analysed. Pinelo et al. (2004) observed the same behaviour when extracting *P. pinaster* sawdust with water, denoting that water was not a good solvent for extracting phenolics. However, mixing water and ethanol can improve the rate of extraction by causing the raw material to bloat, enabling the solvent to easily enter the solid particles (Gertenbach, 2002).

Pine bark extracts showed an expressive antioxidant capacity when compared with red fruits. In a recent study (Seraglio et al., 2018), it was found that in *Myrtaceae* fruits, the highest level DPPH value is 50.66 mg AAE/g dry fruit, while in this study the hydroethanolic extract is 108.74 mg AAE/g dry pine bark.

Vieito, C. S., Pires, P., Fernandes, E. & Velho, M. V. (2019). Chemical characterization of pine bark (Pinus pinaster Aiton subsp. atlantica), antioxidant properties and phenolic profile of its extracts. *Millenium*, 2(8), 79-87.
DOI: https://doi.org/10.29352/mill0208.07.00197

Table 2 – Extraction yield, total phenolic content and antioxidant activity of *P. pinaster* Aiton subsp. *atlantica* bark extracts.

Sample	Extraction yield (% w/w dry bark)	Total phenolic content (mg GAE/g dry bark)	Antioxidant activity (mg AAE/g dry bark)
PW	$7.84^{a} \pm 0.56$	$50.09^{a} \pm 4.70$	$82.24^{a} \pm 4.65$
PWE	$17.55^{b} \pm 0.16$	$73.48^{b} \pm 1.84$	$108.74^{b} \pm 2.02$
PE	$17.08^{b} \pm 0.23$	$63.38^{c} \pm 1.26$	$95.58^{c} \pm 0.55$

Note: Means (n=3) with different uppercase letters in the same column are significantly different ($p<0.05$). GAE – Gallic acid equivalent, AAE – ascorbic acid equivalent.

2.3 Polyphenols identification (RP-HPLC-UV analysis)

Polyphenols are among the most widespread class of metabolites in nature, and their distribution is almost ubiquitous (Pereira, Valentão, Pereira, & Andrade, 2009). Biogenetically, phenolic compounds proceed of two metabolic pathways: the shikimic acid pathway where, mainly, phenylpropanoids are formed and the acetic acid pathway in which the main products are the simple phenols (Belščak-Cvitanović, Durgo, Huđek, Bačun-Družina, & Komes, 2018). Flavonoids and stilbenes are the majority of natural-occurring phenolics (Pereira et al., 2009).

Chromatography is needed to obtain more detailed information on polyphenol profiles than is provided by spectrophotometric methods (Sáyago-Ayerdi, Mercado-Mercado, Ramos-Romero, Torres, & Pérez-Jiménez, 2016).

Considering the results obtained in the 3.2 section, as the hydroethanolic and ethanolic extracts showed better antioxidant properties, they were studied by RP-HPLC-UV. Figure 1 shows the base-peak chromatogram of phytochemical compounds in pine bark hydroethanolic extract obtained by reverse phase with the corresponding retention times. Tentative assignment of these phytochemical compounds was obtained by comparing with the retention time of pure standards (Table 3). For each of the five phytochemicals presented in Table 3, a second chromatogram was obtained after addition of a known amount to the original pine bark extract. In the chromatogram with added standard it could be observed which peak increased its intensity. This methodology helps to clarify the assignment as for example, with compounds assigned with similar RT, taxifolin (22.9 min) and ferulic acid (23.6 min).

The HPLC-UV phytochemical profile recorded at 280 nm includes, by elution order: gallic acid, taxifolin, ferulic acid and quercetin. Catechin was detected at 320 nm and exhibits an intense signal in the ethanolic extract (Figure 2), but not in the hydroethanolic one. Braga et al. (2008) also observed an higher content of catechin in the ethanolic extract, however at 280 nm.

Although the pine bark water or ethanolic extracts have not been completely elucidated, the main constituents described in the literature for a commercialized standardized extract, Pycnogenol, indicates that it contains catechin, epicatechin and taxifolin (Packer, Rimbach, & Virgili, 1999).

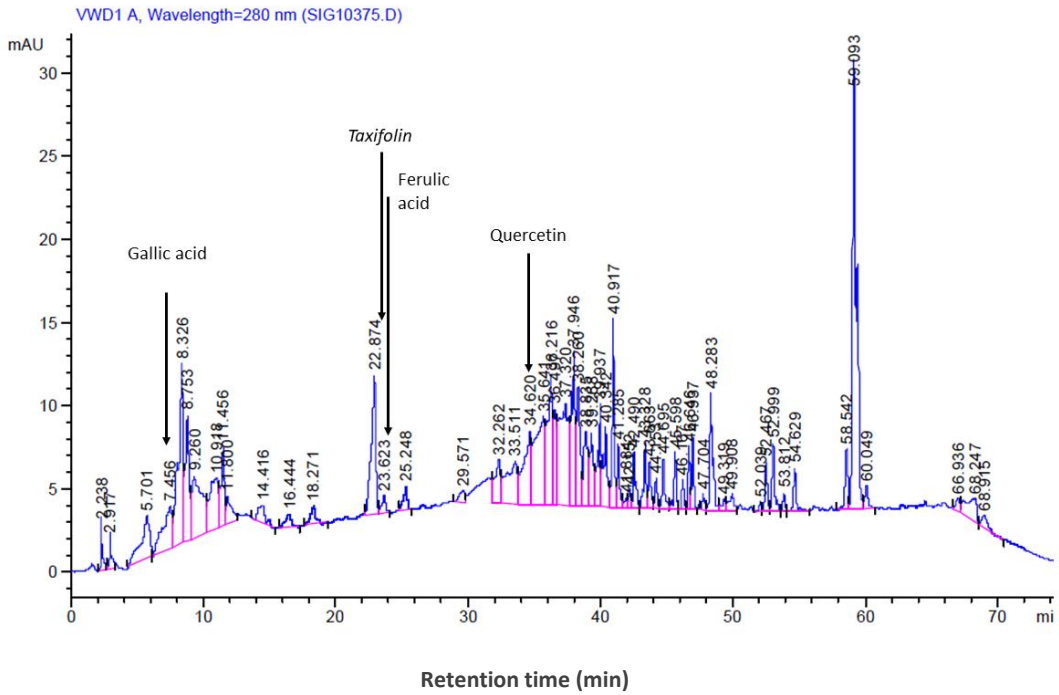
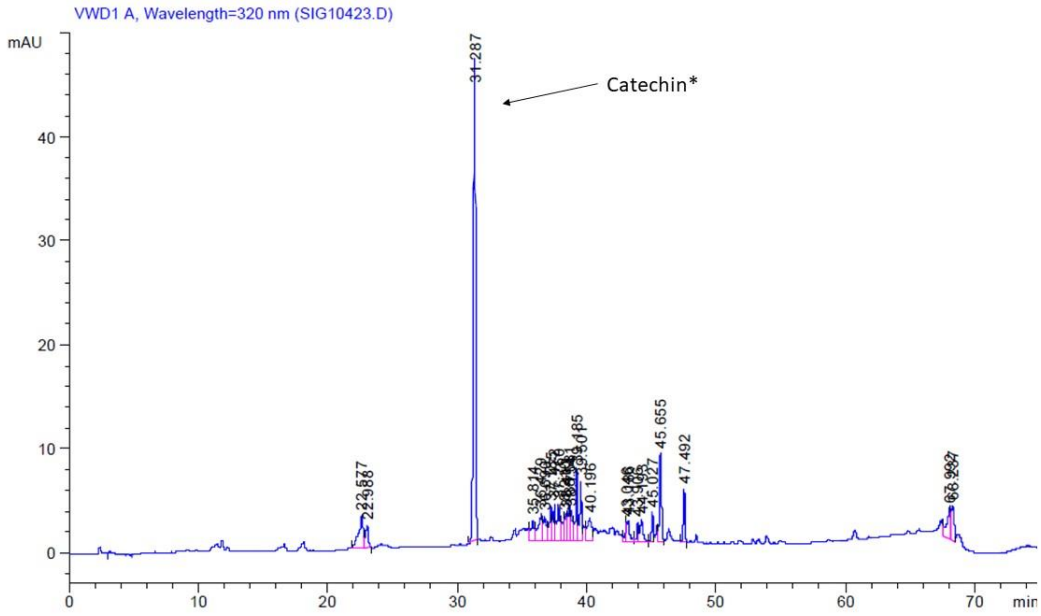


Figure 1 – Chromatogram of the pine bark hydroethanolic extract recorded at 280 nm, tenfold dilution in methanol.



Retention time (min)

Figure 2 – Chromatogram of the pine bark ethanolic extract recorded at 320 nm, tenfold dilution in methanol.

Table 3 – Tentative assignment of phytochemical compounds detected by HPLC-UV in pine bark hydroethanolic extracts.

Tentative assignment	Retention time (min)	Wavelength (nm)
Gallic acid	7.5	280
Taxifolin	22.9	280
Ferulic acid	23.6	280
Quercetin	34.6	280
Catechin	31.3	320

CONCLUSIONS

The bark of *P. pinaster* Aiton subsp. *atlantica* is composed of carbohydrates (63.43% w/w), fat (2.81% w/w), proteins (1.60% w/w), ash (1.75% w/w) and other components (30.41% w/w). Pine bark is rich in hydroethanol and ethanol extractable phytochemicals, namely 73.48 and 63.38 mg GAE/g dry bark, respectively. The pine bark extracts have high levels of antioxidant properties (82-109 mg AAE/g dry pine bark), higher than some red fruits known to be rich in antioxidant properties. The ethanolic extract presents higher concentration of catechin compared to the hydroethanolic extract. Further studies should be developed to fully confirm the chemical composition of *P. pinaster* Aiton subsp. *atlantica* bark extracts.

ACKNOWLEDGEMENTS

The accomplishment of this work was possible due to the SUDOE INTERREG IVB REDVALUE SOE1/P1/E0123 research grant.

REFERENCES

AOAC. (1995). Official Methods of Analysis of AOAC INTERNATIONAL (16th ed., Vol. II): The Scientific Association Dedicated to Analytical Excellence.

Vieito, C. S., Pires, P., Fernandes, E. & Velho, M. V. (2019). Chemical characterization of pine bark (Pinus pinaster Aiton subsp. atlantica), antioxidant properties and phenolic profile of its extracts. *Millenium*, 2(8), 79-87.
DOI: https://doi.org/10.29352/mill0208.07.00197

Aspé, E., & Fernandez, K. (2011). The effect of different extraction techniques on extraction yield, total phenolic, and anti-radical capacity of extracts from Pinus radiata Bark. Industrial Crops and Products, 34(1), 838-844. doi: 10.1016/j.indcrop.2011.02.002

Belščak-Cvitanović, A., Durgo, K., Huđek, A., Bačun-Družina, V., & Komes, D. (2018). Overview of polyphenols and their properties. In C. M. Galanakis (Ed.), Polyphenols: Properties, Recovery, and Applications (pp. 3-44). Cambridge, United Kingdom: Woodhead Publishing.

Braga, M. E. M., Santos, R. M. S., Seabra, I. J., Facanali, R., Marques, M. O. M., & de Sousa, H. C. (2008). Fractioned SFE of antioxidants from maritime pine bark. The Journal of Supercritical Fluids, 47(1), 37-48. doi: 10.1016/j.supflu.2008.05.005

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology, 28(1), 25-30. doi: https://doi.org/10.1016/S0023-6438(95)80008-5

Chupin, L., Maunu, S. L., Reynaud, S., Pizzi, A., Charrier, B., & Charrier-El Bouhtoury, F. (2015). Microwave assisted extraction of maritime pine (Pinus pinaster) bark: Impact of particle size and characterization. Industrial Crops and Products, 65, 142-149. doi: 10.1016/j.indcrop.2014.11.052

Ćurković-Perica, M., Hrenović, J., Kugler, N., Goić-Barišić, I., & Tkalec, M. (2015). Antibacterial Activity of Pinus pinaster Bark Extract and its Components Against Multidrug-resistant Clinical Isolates of Acinetobacter baumannii. Croatica Chemica Acta, 88(2), 133-137. doi: 10.5562/cca2548

Deng, Q., Penner, M. H., & Zhao, Y. Y. (2011). Chemical composition of dietary fiber and polyphenols of five different varieties of wine grape pomace skins. Food Research International, 44(9), 2712-2720. doi: 10.1016/j.foodres.2011.05.026

Fradinho, D. M., Neto, C. P., Evtuguin, D., Jorge, F. C., Irle, M. A., Gil, M. H., & Pedrosa de Jesus, J. (2002). Chemical characterisation of bark and of alkaline bark extracts from maritime pine grown in Portugal. Industrial Crops and Products, 16(1), 23-32. doi: https://doi.org/10.1016/S0926-6690(02)00004-3

Gertenbach, D. D. (2002). Solid-liquid extraction technologies for manufacturing nutraceuticals. In J. Shi, G. Mazza, & M. L. Maguer (Eds.), Functional Foods: Biochemical and Processing Aspects. Boca Raton, Florida, USA: CRC Press.

Gutfinger, T. (1981). Polyphenols in olive oils. Journal of the American Oil Chemists Society, 58(11), 966-968. doi: 10.1007/bf02659771

Jablonsky, M., Nosalova, J., Sladkova, A., Haz, A., Kreps, F., Valka, J., . . . Surina, I. (2017). Valorisation of softwood bark through extraction of utilizable chemicals. A review. Biotechnology Advances, 35(6), 726-750. doi: https://doi.org/10.1016/j.biotechadv.2017.07.007

Lafka, T. I., Sinanoglou, V., & Lazos, E. S. (2007). On the extraction and antioxidant activity of phenolic compounds from winery wastes. Food Chemistry, 104(3), 1206-1214. doi: 10.1016/j.foodchem.2007.01.068

Miller, G. L. (1959). Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. Analytical Chemistry, 31(3), 426-428. doi: 10.1021/ac60147a030

Packer, L., Rimbach, G., & Virgili, F. (1999). Antioxidant activity and biologic properties of a procyanidin-rich extract from pine (Pinus maritima) bark, pycnogenol. Free Radical Biology and Medicine, 27(5-6), 704-724. doi: 10.1016/s0891-5849(99)00090-8

Pereira, D., Valentão, P., Pereira, J., & Andrade, P. (2009). Phenolics: From Chemistry to Biology. Molecules, 14(6), 2202-2211. doi: 10.3390/molecules14062202

Pinelo, M., Rubilar, M., Sineiro, J., & Núñez, M. J. (2004). Extraction of antioxidant phenolics from almond hulls (Prunus amygdalus) and pine sawdust (Pinus pinaster). Food Chemistry, 85(2), 267-273. doi: 10.1016/j.foodchem.2003.06.020

Ronda, A., Della Zassa, M., Biasin, A., Martin-Lara, M. A., & Canu, P. (2017). Experimental investigation on the smouldering of pine bark. Fuel, 193, 81-94. doi: https://doi.org/10.1016/j.fuel.2016.12.028

Sáyago-Ayerdi, S. G., Mercado-Mercado, G., Ramos-Romero, S., Torres, J. L., & Pérez-Jiménez, J. (2016). Analysis and Characterization of Polyphenol Extracts. In J. Cuevas Valenzuela, J. R. Vergara-Salinas, & J. R. Perez-Correa (Eds.), Advances in technologies for producing food-relevant polyphenols (pp. 27). Boca Raton, Florida: CRC Press.

Seabra, I. J., Dias, A. M. A., Braga, M. E. M., & de Sousa, H. C. (2012). High pressure solvent extraction of maritime pine bark: Study of fractionation, solvent flow rate and solvent composition. The Journal of Supercritical Fluids, 62, 135-148. doi: 10.1016/j.supflu.2011.10.016

Seraglio, S. K. T., Schulz, M., Nehring, P., Della Betta, F., Valese, A. C., Daguer, H., . . . Costa, A. C. O. (2018). Nutritional and bioactive potential of Myrtaceae fruits during ripening. Food Chemistry, 239, 649-656. doi: https://doi.org/10.1016/j.foodchem.2017.06.118

Tümen, İ., Akkol, E. K., Taştan, H., Süntar, I., & Kurtca, M. (2018). Research on the antioxidant, wound healing, and anti-inflammatory activities and the phytochemical composition of maritime pine (Pinus pinaster Ait). Journal of Ethnopharmacology, 211, 235-246. doi: https://doi.org/10.1016/j.jep.2017.09.009

Vázquez, G., Antorrena, G., & Parajó, J. C. (1987). Studies on the utilization of Pinus pinaster bark - Part 1: Chemical constituents. Wood Science and Technology, 21(1), 65-74. doi: 10.1007/bf00349718