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Diversity of Secondary Metabolites of Araucaria Seed (*Araucaria angustifolia* (Bert.) O. Ktze) Residues

Diversidade dos Metabólitos Secundários dos Resíduos da Semente de Araucária (*Araucaria angustifolia* (Bert.) O. Ktze)

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Abstract

The seed of *Araucaria angustifolia* (Bert.) O. Ktze is known as *pinhão* and is considered a high nutritional value food. In the *pinhão* consumption, its seed coat is discarded, generating a residue that presents a slow decomposition process. The *pinhão* seed coat is composed of three tissue layers: inner layer (endotesta), intermediate layer (mesotesta) and outer layer (exotesta). Studies have explored the use of extracts obtained from this forest residue, since the occurrence of metabolites can lead to characterization and isolation, providing wide applications. Considering the importance of new studies in the identification of the most diverse classes of extractives, this work aimed to carry out the phytochemical prospection of the layers of *pinhão* seed coat. Healthy and injury-free seeds were peeled, separating the three layers of seed coat. For the phytochemical analysis, the hydrophilic extract (methanol) obtained from each layer in the extraction cycle was used. Transverse and longitudinal sections of the outer and middle layers were used for light and epifluorescent microscopy. It was possible to detect the presence of different classes of metabolites in the three layers of the seed coat. In all the layers it was possible to find condensed tannins, flavonoids and triterpenoids. Catechins and Resins were only found in the outer layer. Resinous channels were observed in the outer longitudinal layer. This study allowed to register the diversity of secondary metabolites existing in the layers of the seed coat, suggesting possible uses for them.

Keywords: *Pinhão.* Seed Coat. Antioxidants. Phytochemistry. Fluorescence Microscopy.

Resumo

A semente da Araucaria angustifolia (Bert.) O. Ktze é conhecida como pinhão e é considerada um alimento de alto valor nutricional. No consumo do pinhão, seu tegumento é descartado, gerando um resíduo que apresenta um processo lento de decomposição. Esse material é composto por três camadas de tecido: camada interna (endotesta), camada intermediária (mesotesta) e camada externa (exotesta). Estudos têm explorado o uso de extratos obtidos desse resíduo florestal, uma vez que a ocorrência de metabólitos pode levar à sua caracterização e isolamento proporcionando amplas aplicações. Considerando a importância de novos estudos na identificação das mais diversas classes de extrativos, este trabalho teve como objetivo realizar a prospecção fitoquímica das camadas do tegumento do pinhão. Sementes saudáveis e livre de injúrias foram descascadas, separando as três camadas do tegumento. Para a análise fitoquímica, foi utilizado o extrato hidrofílico (metanol) obtido de cada camada no ciclo de extração. Seções transversais e longitudinais das camadas externa e intermediária foram usadas para microscopia de luz e epifluorescência. Foi possível detectar a presença de diferentes classes de metabólitos nas três camadas do tegumento. Em todas as camadas foi possível encontrar taninos condensados, flavonóides e triterpenóides. Catequinas e resinas só foram encontradas na camada externa. Canais resiníferos foram observados na camada externa longitudinal. Este estudo permitiu registrar a diversidade de metabólitos secundários existentes nas camadas do tegumento, sugerindo possíveis usos para os mesmos.

Palavras-chave: *Pinhão. Casca de Semente. Antioxidantes. Fitoquímica. Microscopia de Fluorescência.*

1 Introduction

Araucaria angustifolia (Bert.) O. Ktze is a conifer species that occurs naturally in the Atlantic Forest Biome; being found in the southeast and south regions of the country (FLORA DO BRASIL, 2020).

Its seeds are known as *pinhão* (PERALTA *et al.*, 2016) and are considered a high nutritional value food (; CONAB, 2019; EMBRAPA, 2017). *Pinhão* is part of the Minimum Price Guarantee Policy for Sociobiodiversity Products developed by the National Supply Company (CONAB) which guarantees a minimum price for the main extractive products that help in the Brazilian biomes conservation. This instrument represents a great opportunity to value the standing forest, contributing to the conservation, preservation and sustainable use of its natural resources (CONAB, 2020).

In the *pinhão* consumption, its seed coat is discarded, generating a residue that presents a slow decomposition process (PERALTA *et al.*, 2016). The *pinhão* seed coat is composed of three tissue layers: inner layer (endotest), intermediate layer (mesotesta) and outer layer (exotesta) (SAMPAIO *et al.*, 2016). Research has been carried out using extractives obtained from this forest residue (DE FREITAS, *et al.,* 2018; DE SOUZA, 2020; SANTOS *et al.*, 2018).

Extractives are considered secondary metabolites (ROFFAEL, 2016), as they are not part of the cell wall structure. They are chemical substances present in the most different biomasses that can be extracted using different solvents (MORAIS *et al.*, 2010). According to the solvent used, it is possible to obtain certain classes of extractives, non-polar or polar (LIMA *et al.*, 2007). The preliminary phytochemical analysis aims to characterize and identify the secondary metabolites in the different types of extracts obtained from all parts of the plant (TORO *et al.*, 2017; SILVA *et al.*, 2018).

Research using extracts obtained from the most diverse biomass sources can lead to the characterization and isolation of metabolites providing wide applications. In this context, the present study aimed to perform the phytochemical prospection of the layers of the *pinhão* seed coat.

2 Material and Methods

2.1 Material

In this study, seeds from 15 individuals located in the region of Vale dos Eucaliptos, Alagoa (Minas Gerais) were used. Healthy and injury-free seeds were chosen. The seed coats were peeled and separated into layers: external (exotesta), intermediate (mesotesta) and internal (endotesta); and, packed in plastic bags stored in the Wood Chemistry Laboratory of the Federal Rural University of Rio de Janeiro.

2.2 Preparation of extracts

Each layer of the seed coat was ground in a Wiley mill (Model SL 31) in the Wood Chemistry Laboratory at Forest Institute - UFRRJ at a room temperature of 25ºC. Each sample was subjected to an extraction cycle according to the methodology described by Abreu *et al.* (2006). Each extraction occurred as a triplicate.

2.3 Phytochemical approach

Analyses were performed from the hydrophilic extract (methanol) obtained in the extraction cycle. The methodology used to detect extractive groups (phenols and tannins; anthocyanins, anthocyanidins and flavonoids; flavonols, flavanones and xanthones; steroids and triterpenoids; saponins and resins) was proposed by Mattos (2009), which is based on color change or formation of precipitates after the addition of certain reagents. All analyzes were performed in triplicate.

2.4 Anatomical approach

For the fluorescence test, the cuts were made with the help of a Ranvier microtome. The samples from the outer layer (exotesta) of the *pinhão* were cooked for 5 minutes in a Becker containing distilled water at +/-100 ºC. Transverse and longitudinal sections of the outer (exotesta) and intermediate (mesotesta) layers were observed in an Olympus optical microscope coupled to a digital image analysis system (Imaging Software cellf) used for light and epifluorescent microscopy. A blue excitation cube at 450-480nm and a FITC filter (U-MWB2) were used, and the autofluorescence was verified. The inner layer (endotest) measures only 86 µm (SAMPAIO *et al*., 2019) so it was not possible to make anatomical sections of the inner layer with Ranvier microtome.

3 Results and Discussion

Table 1 demonstrates the diversity of secondary metabolites found in the three layers of the seed coat after the phytochemical analysis.

Table 1 - Secondary metabolites present in the hydrophilic extract of each layer of the *pinhão* seed coat

Constituents	Exotesta	Mesotesta	Endotesta
Condensed tannins	+	+	+
Hydrolysable tannins	-	-	-
Anthocyanins and Anthocyanidins	-	-	-
Chalcones and Aurones	-	-	-
Flavanonols and Flavones	+	+	+
Flavonols, Flavanones and Xanthones	+	+	+
Leucoanthocyanidins	-	-	-
Catechins	+	-	-
Steroids	-	-	-
Triterpenoids	+	+	+
Saponins	-	-	-
Resins	+	-	-

Caption: +: presence; -: absence. There was no disagreement in the analyzed repetitions.
Source: Resource data.

The seed coat layers are a material rich in polyphenols, indicating the presence of condensed tannins and flavonoids (flavanonols, flavanones, flavonols, flavanones and xanthones), and present triterpenoids. In a complementary study, Sampaio *et al.* (2019) found that the extractive content was higher in the inner layer; presenting a value of 27.5%, while the outer layer presented a value of 11.9%; this is due to the fact that the inner layer is composed of parenchyma cells while the inner layer is composed of fiber-tracheids.

Tannins can be classified as hydrolysable and condensed (procyanidins) (FRAGA-CORRAL, 2020). Condensed tannins are the main compounds that have medicinal properties attributed to tannins (BULE et al., 2020; FRAGA-CORRAL *et al*., 2020) and represent the main source of commercial tannins (FRAGA-CORRAL *et al*., 2020). They have a large application in industry (FRAGA-CORRAL *et al*., 2020); from leather manufacturing (SHIRMOHAMMADLI et al., 2018), adhesives (SANTIAGO *et al.*, 2018; FREITAS; LENZ, 2019), polymers (SZCZUREK *et al.*, 2014) even pharmaceuticals and cosmetics (MOTA *et al.*, 2014; SILVA *et al.*, 2014).

Flavonoids are secondary metabolites often synthesized in response to stimuli from plant-environment interactions (ALSEEKH *et al.*, 2020). They have functions in plants, and their function of protection against ultraviolet rays, insects, fungi, viruses and bacteria can be highlighted (DOS SANTOS;

RODRIGUES, 2017).

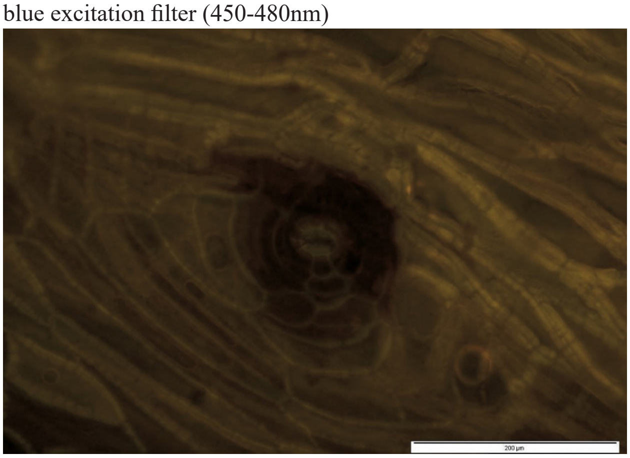
Due to structural diversity, the triterpenoids in plants have broad bioactivity: insecticide, anti-inflammatory, antimicrobial, antiviral, antioxidant, hepatoprotective and antidiabetic, which have important pharmaceutical and industrial applications (SANDEEP, 2020).

The exotesta also presented Catechins and Resins (Table 1). Catechins may be associated with the cellular tissue protection mechanism by UV exposure (REZENDE *et al.*, 2016) and Resins are powerful healing agents (CASTRO; MACHADO, 2006); the exotesta, being an outer layer, is more susceptible to deterioration by biological and abiotic factors, such factors can contribute to the production of defense substances.

Catechins are potent antioxidants, free radical scavengers, metal chelators and peroxidation inhibitors (SCHMITZ *et al.*, 2005), which prevent further oxidative damage to the plant cell. Catechins may form polymeric proanthocyanidins, Koehnlein *et al.* (2012) and Branco *et al.* (2016) reported the presence of this compound in the phenolic extract obtained from *pinhão*.

Resins act as protection against bacteria, insects and fungi (SILVA *et al.*, 2005). In the outer layer in the longitudinal section, it was possible to observe through epifluorescent microscopy the presence of resinous channels (Figure 1). The resinous channels are commonly found in conifer species, although the *Araucaria angustifolia* wood itself does not present such structure (SIEGLOCH; MARCHIORI, 2015).

Figure 1 - Photomicrograph of the outer layer (exotesta) in longitudinal section of the *in natura pinhão* seed coat under the blue excitation filter (450-480nm)



Source: Authors.

Anthocyanin and anthocyanidin metabolites; chalcones and aurones; leucoanthocyanidins; saponins and steroids were not found in any layer of the integument (Table 1).

4 Conclusions

The phytochemical prospection made it possible to identify different secondary metabolites in the three layers of the *pinhão* seed coat with potential for studies. The outer layer (exotesta) showed a greater diversity of secondary metabolites compared to the other layers. Considering the classes of secondary metabolites, the intermediate (mesotesta) and internal (endotest) layers of the *pinhão* seed coat showed no distinction between them. This study can suggest possible uses of the metabolites found in the *pinhão* seed coat.

References

ABREU, H.S. *et al.* Métodos de análise em química da madeira. *Flor. Amb.*, v.20, 2006.

ALSEEKH, S. *et al.* The style and substance of plant flavonoid decoration; towards defining both structure and function. *Phytochem*., v.174, p.112347, 2020. doi: 10.1016/j.phytochem.2020.112347.

CASTRO, M.M.; MACHADO, S.R. Células e tecidos secretores. In: APPEZZATO, B.G.; GUERREIRO, S.M.C. *Anatomia vegetal*. Viçosa: UFV, 2006.

BULE, M. et al. Tannins (hydrolysable tannins, condensed tannins, phlorotannins, flavono-ellagitannins). In: *Recent Advances in Natural Products Analysis*. Publisher: Elsevier, 2020.

BRANCO, C. S. *et al.* Chemical constituints and biological activies of *Araucaria angustifolia* (Bertol.) O. Ktze: a review. *J. Organic Inorganic Chem.*, v.2, n. 1, p.1-10, 2016. doi: 10.21767/2472-1123.100008.

CONAB – Companhia Nacional de Abastecimento. Bol. Soc., v.3, n.2, p.1-56, 2019.

DE FREITAS, T.B. *et al.* Antioxidants extraction from Pinhão (*Araucaria angustifolia* (Bertol.) Kuntze) coats and application to zein films. *Food Packaging Shelf Life*, v.15, p.28-34, 2018. doi:10.1016/j.fpsl.2017.10.006

DE SOUZA, K.C. *et al*. Filmes isolados de proteína de soja incorporados com extrato de pinhão (*Araucaria angustifolia* (Bertol.) Kuntze) para potencial uso como embalagem ativa de óleo comestível. *Food Bioprocess Technol*, v.13, p.998-1008, 2020. doi:10.1007/s11947-020-02454-5

DOS SANTOS, D.S.; RODRIGUES, M.M.F. Atividades farmacológicas dos flavonoides: um estudo de revisão. *Est. Cient.*, v.7, n.3, p.29-35, 2017. doi: 10.18468/estcien.2017v7n3.p29-35.

EMBRAPA FLORESTAS - *Araucária: particularidades, propagação e manejo de plantios.* Brasília, DF: Embrapa, 2017.

FRAGA-CORRAL, M. *et al.* Technological application of tannin-based extracts. *Molec.*, v.25, n.3, p.614-641, 2020. doi: 10.3390/molecules25030614

FREITAS, A.; LENZ, D.M. Produção de painéis de madeira com resíduos de MDF e MDP da indústria moveleira e resina à base de tanino de Acácia Negra. *Engevista*, v. 21, n.1, p.141-153, 2019.

IGANCI, J.R.V.; DORNELES, M.P. *Araucariaceae in Flora do Brasil 2020*. Available on: http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB33971

KOEHNLEIN, E.A. *et al.* Antioxidant activities and phenolic compounds of raw and cooked Brazilian pinhão (*Araucaria angustifolia*) seeds. *Africa J. Food. Sci*., v. 6, p. 512-518, 2012. doi:10.5897/AJFS12.128

LIMA, S.R. *et al.* Estudo dos constituintes macromoleculares, extrativos voláteis e compostos fenólicos da madeira de candeia *Moquinia polymorpha* (LESS.) DC. *Ciênc. Florestal*, v.17, p.145-155, 2007. doi:10.5902/198050981946

MATTOS, F.J.A. *Introdução à fitoquímica experimental*. Fortaleza: UFC, 2009.

MORAIS, J.P.S.; ROSA, M.F.; MARCONCINI, J.M. *Procedimentos para análise lignocelulósica*. Campina Grande: Embrapa Algodão, 2010.

MOTA, G.S.T. *et al.* Biodiversity hotspots for conservation priorities. *Nat,* v.403, p.853-858, 2000. doi: 10.1038/35002501

SANTOS, C.H.K. *et al.* Systematic study on the extraction of antioxidants from pinhão (*Araucaria angustifolia* (bertol.) Kuntze) coat. *Food. Chem.*, v.61, p.16-223, 2018. doi:10.1016/j.foodchem.2018.04.057

MOTA, G. *et al.* Antioxidant activity of cosmetic formulations based on novel extracts from seeds of Brazilian Araucaria (Bertoll) Kuntze. *J. Cosm., Dermato. Sci. and Appl.*, v.4, p.190-202, 2014. doi:10.4236/jcdsa.2014.43027

ONEM, E. et al. Optimization of tannin isolation from acorn and application in leather processing. *Ind. Crop. Prod.*, v.53, p.16–22, 2014. doi: 10.1016/j.indcrop.2013.12.014

PERALTA, R.M. *et al.* Biological activities and chemical constituents of *Araucaria angustifolia*: An effort to recover a species threatened by extinction. *Tr. Food. Sci. Tech.*, v.54, p.85-93, 2016. doi: 10.1016/j.tifs.2016.05.013

PIZZI, A.; MITTAL, K.L. *Handbook of adhesive technology.* New York: Marcel Dekker, 2003.

REZENDE, F.M. *et al. Vias de síntese de metabólitos secundários em plantas.* São Paulo: Instituto de Biociências da Universidade de São Paulo, 2016.

ROFFAEL, E. Significance of wood extractives for wood bonding. *App. Microbio. Biotech.*, v.100, n.4, p.1589-1596, 2016. doi: 10.1007/s00253-015-7207-8

SANTIAGO, S. B. *et al.* Colagem de madeira de eucalipto com adesivos naturais. *Rev. Matéria*, v.23, n.3, 2018. doi:10.1590/S1517-707620180003.0485

SAMPAIO, D. A. *et al.* Perfil lignoídico del tegumento de semillas de *Araucaria angustifolia*. *Bosq*., v.37, n.3, p.549-555, 2016. doi:10.4067/S0717-92002016000300012

SAMPAIO, D.A.; GARCIA, R.A.; LIMA, H.R.P. Anatomical and physicochemical characterization of the *Araucaria angustifolia* Seed Coat. *Flor. Amb.*, v.26, n.2, e20170867, 2019. doi:10.1590/2179-8087.086717

SANDEEP, S.G. Triterpenoids: structural diversity, biosynthetic pathway, and bioactivity. *Stud. Nat. Prod. Chem.,* v.67, p.411-461, 2020. doi:10.1016/B978-0-12-819483-6.00012-6.

SCHMITZ, W. *et al.* O chá verde e suas ações como quimioprotetor. *Semina*, v.26, n.2, p.119-130, 2005.

SHIRMOHAMMADLI, Y.; EFHAMISISI, D.; PIZZI, A. Tannins as a sustainable raw material for green chemistry: a review. *Ind. Crops Prod.*, v.126, p.316-332, 2018. doi:10.1016/j.indcrop.2018.10.034

SIEGLOCH, A.M.; MARCHIORI, J.N.C. Anatomia da madeira de treze espécies de coníferas. Ciênc. Mad., v.3, n.6, p.149-165, 2015. doi:10.12953/2177-6830/rcm.v6n3p149-165

SILVA, L.M.; ALQUINI, Y.; CAVALLET, V.J. Inter-relações entre a anatomia vegetal e a produção vegetal. *Acta Bot. Bras.,* v.19, n.1, p.183-194. 2005. doi:10.1590/S0102-33062005000100018

SILVA, S.M. *et al.* Inhibition of salivary and pancreatic α-amylases by a pinhão coat (*Araucaria angustifolia*) extract rich in condensed tannin. *Food. Res. Intern*., v.56, p.18, 2014. doi:10.1016/j.foodres.2013.12.004 -

SILVA, F.A.; BIZERRA, A.M.C.; FERNANDES, P.R.D. Testes fitoquímicos em extratos orgânicos de *bixa orellana* L (urucum). *Holos*, v.2, p.484-498, 2018. doi:10.15628/holos.2018.6929

SZCZUREK, A. *et al.* A new method for preparing tannin-based foams. *Ind. Crop. Prod*., v.54, p.40-53, 2014. doi:10.1016/j.indcrop.2014.01.012

TORO, D.M. *et al.* Análisis preliminar de los metabolitos secundários de polvos mixtos de hojas de plantas medicinales. *Rev. Cubana Plantas Med.*, v.22, n.1, 2017.