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Ferns and Lycophytes as new challenges

Phytochemical screening of the *Dicksonia sellowiana* leaves and its structures

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Abstract

Dicksonia sellowiana (Dicksoniaceae) is a tree fern characteristic of the mixed ombrophilous forests of southern Brazil in the Atlantic Forest. Due to its extensive use in the past for making garden pots, this species is at risk of extinction. The objective of this study was to evaluate the phytochemical composition of *D. sellowiana* leaves and their structures and correlate it with their antioxidant potential. Measurements of moisture content, extraction yield, preliminary phytochemical analysis, chemometric analysis by ^{1}H NMR PCA, UPLC-PDA-MS analysis, total polyphenol content, and antioxidant activity were conducted on the leaves and their structures. The phytochemical composition confirmed the presence of polyphenols, including tannins and flavonoids (derived from kaempferol), with higher concentrations in the pinna and lower in the rachis. The pinnule exhibits high diversity and concentration of phytochemical compounds, which justify its antioxidant activity due to the presence of polyphenols. In conclusion, this study highlights that the pinnae and leaves of *D. sellowiana* exhibit a similar and more diverse phytochemical composition compared to the other evaluated structures, showing higher concentrations of polyphenols and antioxidant activity. The results reinforce the preservation of the endangered species and its potential as a resource for pharmacological and nutritional phytochemical compounds.

Key words: chemometric analysis, Dicksoniaceae, flavonol, kaempferol, plant extracts.

Resumo

Dicksonia sellowiana (Dicksoniaceae) é uma samambaia arborescente, característica das florestas ombrófilas mistas do Brasil meridional na Floresta Atlântica. Por ter sido muito utilizada no passado na confecção de vasos para jardinagem, essa espécie está em risco de extinção. Neste estudo se objetivou avaliar a composição fitoquímica das folhas de *D. sellowiana* e suas estruturas, e correlacionar com o seu potencial antioxidante. Foi realizado os teores de umidade, rendimento de extração, análise fitoquímica preliminar, análise quimiomética por PCA de ^{1}H NMR, UPLC-PDA-MS analysis, teores de polifenóis totais e atividade antioxidante da folha e suas estruturas. A composição fitoquímica confirmou a presença de polifenóis, entre eles taninos e flavonoides (derivados de kaempferol), com maior concentração na pínula e menor na raque. A pínula apresenta alta diversidade e concentração de compostos fitoquímicos, o que justifica sua atividade antioxidante devido aos polifenóis. Em conclusão, este estudo evidencia que as pínulas e as folhas de *D. sellowiana* apresentam uma composição fitoquímica semelhante e mais diversificada das demais estruturas avaliadas, se destacando na concentração de polifenóis e atividade antioxidante. Os resultados reforçam a preservação da espécie ameaçada e seu potencial como recurso para compostos fitoquímicos farmacológicos e nutricionais.

Palavras-chave: análise quimiométrica, Dicksoniaceae, flavonol, kaempferol, extratos de planta.

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Introduction

Ferns constitute a group of approximately 10,500 species of vascular plants (PPG I 2016), which reproduce through spores. In humid forests, they find optimal conditions for their development (Tryon & Tryon 1982). Dicksoniaceae is a family of tree ferns, similar in habit to the Cyatheaceae, and distinguished by the marginal position of their spores (Smith *et al*. 2006). This family consists of 35 species, which are classified into three genera (*Calochlaena*, *Dicksonia*, and *Lophosoria*) (PPG I 2016). These species are distributed across tropical and temperate zones worldwide (Korall *et al.* 2006). Among these genera, only *Dicksonia* comprises tree ferns (Smith *et al.* 2006).

Named by James Dickson (1738–1822), the *Dicksonia* (Dicksoniaceae, Cyatheales) has 26 tropical species (PPG I 2016). Showing slow and narrow growth in their basic vascular structure, but which are significantly enlarged by the bundles of roots that extend to the soil to retrieve the water and nutrients needed (Tryon & Tryon 1982), being found in several tropical countries, such as Australia and Brazil. Amongst the various regions, New Guinea boasts the highest diversity of species, with a total of five known species (Churchill et al. 1998). On the other hand, Brazil is home to only one species from this genus, *Dicksonia sellowiana* Hook. (Della & Vasques 2023).

The *Dicksonia sellowiana* species, popularly known as *xaxim*, *xaxim-bugio*, tree ferns, *samambaiaçu*, *samambaiaçu-imperial* (from the Tupi "hamabe+açu" = giant fern), is a tree fern typical to the mixed rain forests of southern Brazil in the Atlantic Forest and Pampa (Della & Vasques 2023), whose presence is greater in areas with a high density of Araucaria [*Araucaria angustifolia* (Bertol.) Kuntze] (Biondi *et al.* 2009). It presents an erect caudice, either simple or branched, as well as bipennate leaves that reach up to 2.4 m, located at the apex of the caudice (Fernandes 2000). This species can reach up to 10 m in height, has a fibrous and thick caudice, which may be completely surrounded by a wide sheath consisting of a tangle of adventitious roots, and presents abundant golden-brown trichomes at the apex (Schmitt *et al.* 2009). As it is cold resistant, this plant presents a slow growth, less than $1m^3$/year (Mielke 2002; Mantovani 2004), with records from the south of Mexico to Uruguay, passing through Central America, Venezuela, Colombia, Bolivia, Paraguay and Brazil (South and Southeast) (Tryon & Tryon 1982; Schmitt *et al.* 2009).

Due to the commercial exploitation of this plant for the manufacture of vases for gardening and floriculture, CONAMA - the Brazilian Council for the Environment - elaborated resolution 278/2001, which vetoes trade, and the species is found in the red book of the flora of Brazil as endangered (Santiago *et al.* 2013) and in Ordinance No. 300 of December 13, 2022 of the Ministry of the Environment, which recognizes the national list of endangered species (Brasil 2022).

A study conducted by Rattmann *et al.* (2011) revealed that the hydroalcoholic extract of *D. sellowiana* leaves exhibited antioxidant activity, providing protection to endothelial cells against hydrogen peroxide (H_2O_2)-induced oxidative stress and inhibiting lipid peroxidation in rats. These antioxidant properties suggest that the traditional use of *D. sellowiana* in the treatment of cardiovascular diseases, asthma, and skin conditions may be associated with its protective effects against oxidative stress and endothelial protection properties.

Physical and chemical studies were conducted to describe the composition and activities of *D. sellowiana* extracts. In one of these studies, the crude extract and its fractions showed no toxicity in the evaluated models, revealing potential for combating lipid oxidation (Oliveira *et al*. 2015). In another research, different methods of secondary metabolite extraction were investigated, and the techniques of decoction and turbolysis with hydroalcoholic solvent demonstrated superior results in terms of total phenolic compound content and consequently exhibited better antioxidant activities (Oliveira *et al.* 2016). Furthermore, a thermal analysis of the crude extract revealed its highly polar composition, with no release of byproducts during heating (Malucelli *et al.* 2018).

Plants produce a wide variety of organic compounds that are related to different functions in plants (Zuiter 2014). Many studies have shown that the chemical composition of the different parts of the plant differs (Boscher *et al*. 1995; Naidoo *et al.* 2013; Matarese *et al.* 2014), which is related to the biosynthetic potential of the cells of each plant tissue (Riffault *et al.* 2014). Considering the relevance of research on species at risk of extinction and their chemical

composition, the aim of this study was to evaluate the phytochemical composition of different leaves structures of *D. sellowiana* and correlate them to their antioxidant potential.

Material and Methods

Plant material and preparation of *Dicksonia sellowiana* ethanol extract by turbolysis

The plant material consists of *Dicksonia sellowiana* leaves, which were collected in April 2016 by V. B. Oliveira in the municipality of Inácio Martins, Paraná, Brazil, whose GPS location is 25°29'35.7"S, 51°12'00.0"WO. The leaves were dried in a closed system oven, at a constant temperature of 50 °C, for an approximate period of 36 hours. After drying, the material was divided into two parts, the first in the form of whole leaves and the second part separated according to their structures (rachis, pinna rachis and pinnule). Subsequently, these materials were crushed in a knife mill with a hammer and stored. The leaves were compared for species authentication by the Curator Osmar dos Santos Ribas with the specimen registered at the Botanical Museum of Curitiba under number 358323.

For the extractions, an Ultra-Turrax® turbolysis apparatus, model T-50 Basic, was used. The extractions were performed using 10 g of *D. sellowiana* powder and 150 mL of 96 ºGL ethanol solvent for a period of 10 minutes at 4,000 rpm at room temperature. After this period, the extracts were filtered and concentrated in a rotary evaporator, and subsequently dried in a water bath. The material was stored in an airtight bottle, and, in preparing the samples, part of this extract was previously dried in an oven at 50 ºC for 30 minutes to eliminate possible water residues.

Because it is an endangered species, this study was authorized by IBAMA to access the genetic heritage through Authorization No. 023/2010, for scientific research purposes, meeting the requirements described in Resolution No. 35, of April 27, 2011, which provides for the regularization of access to genetic heritage (Brasil 2011).

Moisture content and total solids

To determine the moisture content of the leaves and their structures, the gravimetric method was applied, using 2 g of each sample, weighed in filters previously dried for 30 minutes. The samples were kept in an oven for 5 hours at 110 ºC for desiccation. Finally, we calculated the percentage of water in the sample in relation to the dry drug (Brasil 2019).

To obtain the total solids content of the plant extracts, 10 mL of the plant extracts were placed in a previously weighed petri dish, which were taken to dry in an oven. The result was presented in weight of dry extract in relation to the plant sample, being performed in triplicate.

In order to verify which structure of the leaves of *D. sellowiana* has the highest solids content and its relationship with the extraction process, we verified the fresh material yield (leaves was considered 100%), as well as the dry material yield (leaves was considered 100%) and the dry extract yield.

Phytochemical analysis

With the aim of establishing a chemical profile of the phytochemical compounds present in *D. sellowiana* extracts, which aids in identification, classification, and authenticity, the following assays were conducted: thin-layer chromatographic analysis, aiming to qualitatively identify the groups of secondary metabolites present; categorization and verification of the chemical similarity of the extract composition through the features of ^{1}H NMR spectra; UPLC-PDA-MS analysis, which determined the masses of the chromatographic peaks in the species' extracts, enabling the proposal of corresponding chemical structures.

Thin layer chromatography (TLC)

The TLC analysis was performed on silica gel 60 plates (Merck), where two micro liters (2 µl) of each extract were deposited. Preliminary phytochemical analysis by TLC aimed to identify potential phytochemicals present in the species and the following phytochemical groups were analyzed: alkaloids, coumarins, steroids and terpenes, flavonoids and tannins. The results were expressed as + or – indicating the presence or absence of phytochemicals in different extracts and performed in triplicate (Souza *et al*. 2014).

Features of ^{1}H NMR spectra of extracts

For the ^{1}H Nuclear Magnetic Resonance (NMR) analysis, a Brucker® model DPX 200 MHz spectrophotometer was used, operating at 4.7 Tesla, observing the ^{1}H nuclei at 200.12 MHz,

each spectrum consisting of 2,048 scans. The spectra were automatically Fourier transformed using an exponential window with a line broadening value of 0.5 Hz, phase and baseline corrected within the automation program. The ^{1}H NMR chemical shifts in the spectra were referenced to TSP-d_4 at δ 0.00.

^{1}H NMR spectra were automatically transformed to ASCII files using AMIX (Analysis of MIXtures software v. 3.9.12., BrukerBiospin). Regions between δ 4.60 and δ 5.10 were removed prior to the multivariate statistical analyses, thus eliminating any variability in the suppression of the water sample. The residual proton signals corresponding to methanol– d_4 (δ 3.365–3.285) and TSP– d_4 (δ0.00) were also removed in this step. The software itself develops a matrix for the application of multivariate methods by PCA.

UPLC-PDA-MS analysis

In order to carry out the ultra-performance liquid chromatography (UPLC) analysis, carried out in an Acquity-UPLC™ system (Waters, MA, USA), a liquid-liquid partition was performed with solvents of increasing polarity until obtaining the ethyl acetate fraction of the leaves and its structures. The system consists of a binary system and a photodiode array detector (PDA). The 3mg/ml sample was prepared in H_2O–MeOH (1:1 v/v) and the analysis was performed on a 1.7 µm (2.1 × 50 mm) reversed phase BEH-C18 column. The binary solvent was composed of (A) H_2O and (B) acetonitrile. The linear solvent gradient was: initial (B) at 0–40% in 20 minutes. The column was heated to 60 °C and the samples kept at room temperature (22 °C). The injection volume was of 2 µl and the compounds were detected at λ = 210–400. Electrospray ionization (ESI) was used for mass spectrometry, triple quadrupole (Quattro-LC, Waters), operating in atmospheric pressure ionization (API). The positive ionization mode was used to detect compounds, at m/z 100–2,000, with energies of 2.6 kV on the capillary and 70 V on the cone. Nitrogen was used as a nebulizer and desolvation gas at 850 l/h.

In vitro assays

Phenolic compounds are commonly found in edible and non-edible plants, and have been reported to have multiple biological effects, including antioxidant activity (Beara *et al.* 2015). The evaluation of the antioxidant activity by the DPPH method and the dosage of polyphenols by the Folin-Ciocalteau method were carried out with the purpose of verifying which leaves structure had the highest levels of phenolic compounds, and, consequently, greater antioxidant activity.

Total phenolic content

The total phenolic content in ethanol extracts of *Dicksonia sellowiana* leaves, rachis, pinna rachis, and pinnules was estimated using the Folin-Ciocalteu method at 760 nm (Singleton *et al*. 1999). Total phenolic content were expressed in terms of gallic acid equivalents (mg GAE.g^{-1}extract) and performed in triplicate. A Shimadzu® UV/VIS spectrophotometer, UV-1800, was used for absorbance measurements.

Free radical scavenging activity - DPPH (1,1-diphenyl-2-picrylhydrazyl)

The measurement of DPPH radical scavenging activity of ethanol extracts at different concentrations was performed according to the methodology established by Mensor *et al*. (2001). In this method, the reaction mixture consisted of 2.5 mLof the sample at different concentrations (20 to 200 mg/mL) and 1.0 mL of the 0.03 mM solution of DPPH in methanol. After incubation for 30 minutes, at room temperature, the absorbance was read at 518 nm. The antioxidant activity was expressed as EC50 (µg.mL^{-1} ± SD), which is the concentration that produces half the maximum effect.

Results and Discussion

Analysis of moisture content and extraction yield of leaves and their structures

By obtaining total solids, it is possible to quantitatively interpret the total presence of matter other than water in a sample, whether in the form of dissolved, colloidal or suspended substances. In relation to the results obtained in Table 1, in the fresh material, the rachis presents the greatest mass followed by the pinnule, a result that is reversed after the plant material is dried. This inversion of values is due to the size of the rachis in relation to the other structures, in addition to its water content, which, after drying, shows a predominance of fibers that support the leaves.

The dry extract yield was higher for pinnule (10.13%) compared to the other extracts, being higher than the leaves yield (7.09%) (Tab. 1). This value is justified by the fact that the yields of

Table 1 – Relation between the average weight of *Dicksonia sellowiana* leaves and their structures and their extraction yield.

	% of in nature plant structure in relation to the frond	% of dry plant structure in relation to the frond	g/100 mL of dry extract
Fronds	100	100	7,09
Rachis	52,39	39,06	3,99
Pinna rachis	8,56	9,16	4,21
Pinnule	39,05	51,78	10,13

rachis and pinna rachis were lower, as we have two plant organs that not only are more fibrous but also present greater stiffness. Other works demonstrate that more fibrous materials such as root, stem and bark have lower yields than leaves (Abdulkadir *et al*. 2016; Rezende *et al*. 2021).

Phytochemical analysis and thin-layer chromatography of the plant extract and fraction

Chromatographic techniques are essential in the separation of chemical constituents, which helps in the identification and quantification of the components present in plant extracts (Kowalska & Sajewicz 2022). In the phytochemical analysis carried out by TLC, the groups of secondary metabolites- flavonoids, tannins, steroids, triterpenes and coumarins- were identified in the leaves, which varied according to the structure of the leaves (Tab. 2).

In this analysis, it was possible to identify that the leaves and the pinnule have similar characteristics related to secondary metabolism, whereas the rachis was the one that differed the in metabolites characteristics. The presence of flavonoids in the *D. sellowiana* species had already been identified by Rattmann *et al*. (2011) and Oliveira *et al*. (2015). The presence of polyphenols, flavonoids, tannins and steroids had also been evidenced in a study, which demonstrated that the species has a higher concentration of tannins than flavonoids (Oliveira *et al.* 2016).

The identification of these metabolite groups is similar to those found in the leaves of another Tree fern species, *Cyathea atrovirens*, where the same groups were identified, except for coumarins (Zuchetto *et al.* 2018). In a review study conducted by Chaparro-Hernández *et al*. (2022), it was verified the presence of these classes of metabolites in leaves of species of the *Cyathea*, a genus of tree ferns closely related to the *Dicksonia* genus. The polarity of the compounds identified in this analysis corroborates with the findings of the study conducted by Malucelli *et al.* (2018), which demonstrated that the extract of the species exhibits a highly polar composition.

Screening by ^{1}H NMR of the crude leaves extracts and their structures

A variety of analytical techniques have been used in order to identify compounds present in complex mixtures, among the analytical techniques

Table 2 – Phytochemical analysis by TLC of crude extracts of *Dicksonia sellowiana* leaves and their respective structures.

	CE. Fronds	CE. Pinnule	CE. Pinna rachis	CE. Rachis
Flavonoids	+	+	+	-
Tannins	+	+	+	+
Steroids and Triterpenes	+	+	+	+
Alkaloids	-	-	-	-
Coumarins	+	+	-	-

Note that: + = positive and - = negative; CE = crude extract.

used, NMR spectroscopy has been highlighted in the elucidation of components of plant secondary metabolism (Combarieu *et al*. 2015).

In the NMR analysis of the crude extracts of the leaves organs of *D. sellowiana*, an accumulation of signals in the region between 3.5 to 4.5 ppm is observed, typical signals of the sugar region. In the region between 0.5 to 2 ppm there is also an accumulation of signals in the pinna rachis and in the pinnule, which are characteristic of methyl, methine and methylene hydrogens referring to fatty acids (Fig. 1a).

When analyzing the NMR spectra, differences among the ^{1}H spectra are observed. These changes in signals correspond to the different metabolites present in the different extracts (in the leaves and in its structures). Chemometrics applied to ^{1}H NMR data for all extracts allowed verifying the separation between samples. The dispersion of the ^{1}H points in the NMR spectra is demonstrated in Figure 1b, while the PCA score graph (Fig. 1c) displays 92.4% of the original variability information. PC1 describes 79.1% and PC2 describes 13.3% of the total variability.

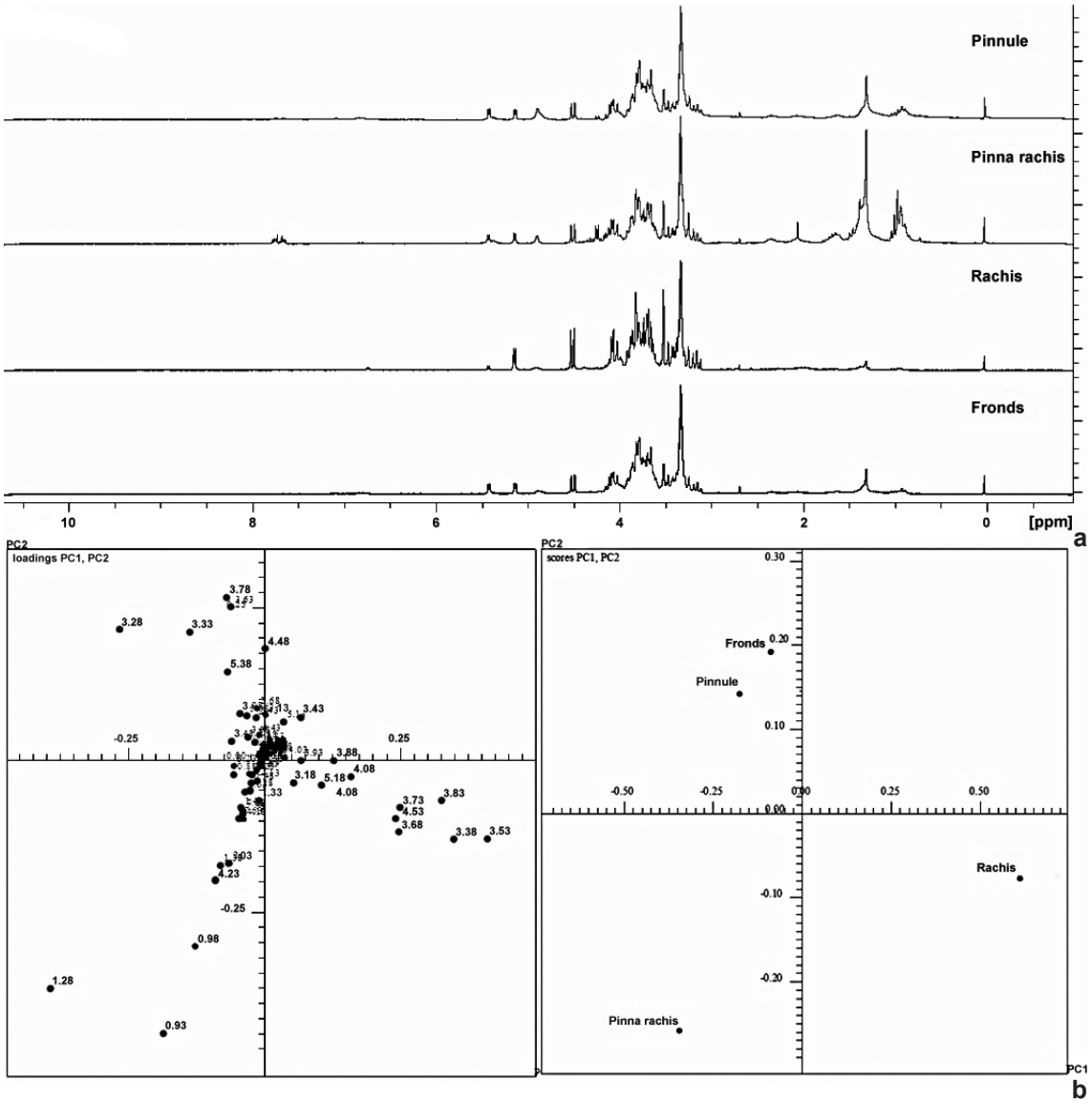

Figure 1 – a. ^{1}H NMR spectrum of the crude extract of leaves and their respective parts. b. dispersion of the ^{1}H points in the NMR spectra. c. chemometric analysis by PCA of the ^{1}H NMR spectra.

The data obtained by PCA analysis reveal that the crude extract of the pinnule shares more similarities with the crude extract of the leaves, and that the other extracts differ from each other and from the other samples (Fig. 1c). PCA analysis is an exploratory data technique that detects natural patterns of clustering to explore and visualize the dataset, aiming to assess the variations among the samples (Combarieu *et al.* 2015; Boffo *et al.* 2022). The results obtained in this analysis are consistent with the findings of the phytochemical analysis, which revealed a similarity in the identified phytochemical groups. Additionally, the results of total phenolic content and antioxidant activity also confirmed this trend, with the leaf and pinnule extracts showing closer results compared to the other samples.

There is no literature on this specific analysis conducted with other species of tree ferns, but other studies conducted with olive oil and plant extracts highlight the importance of this analysis (Rohman *et al.* 2020; Ray *et al.* 2022). The ease of sample preparation, quick analysis, and easily interpretable results make this method a valuable tool for detecting fraud through substitution or dilution of plant extracts compared to other methods (Stoyanova & Brown 2001; Ray *et al.* 2022). It should be noted that the aim of this study was to compare the chemical profile of the leaf and its structures and assess the similarity between them, which can be observed through chemical shift and peak integration values (Boffo *et al.* 2022). These values are used to create a dataset that can be employed in multivariate analyses such as hierarchical cluster analysis (HCA) and PCA. These chemometric methods serve to group the most similar samples and provide information among similar ones (Bhatia *et al*. 2013).

UPLC-PDA-MS analysis

The analysis carried out by UPLC-PDA-MS of the ethyl acetate fractions of the leaves and its structures revealed that there is a diversity of compounds in the fractions, as can be seen in the chromatograms in Figure 2.

Based on the analysis of the chromatograms and their respective mass spectra, it was possible to identify seven substances through the masses obtained and their fragmentation, four of which were derived from the flavonoid Kaempferol. In the identification of derivatives of this flavonoid, the loss of sugar units led to the formation of the fragment referring to the deprotonated aglycone (m/z 285). For the sugar molecules, the masses were taken into account, as in substance 3, which presented the deprotonated molecular ion [M-H]- in m/z 431, and the loss of a rhamnose unit led to the formation of the fragment of deprotonated aglycone (m/ z 285).

For the identification of substance 2 with m/z 863, comparison with the literature was used, taking into account the high molecular mass and mass fragments (Foo *et al*. 2000).

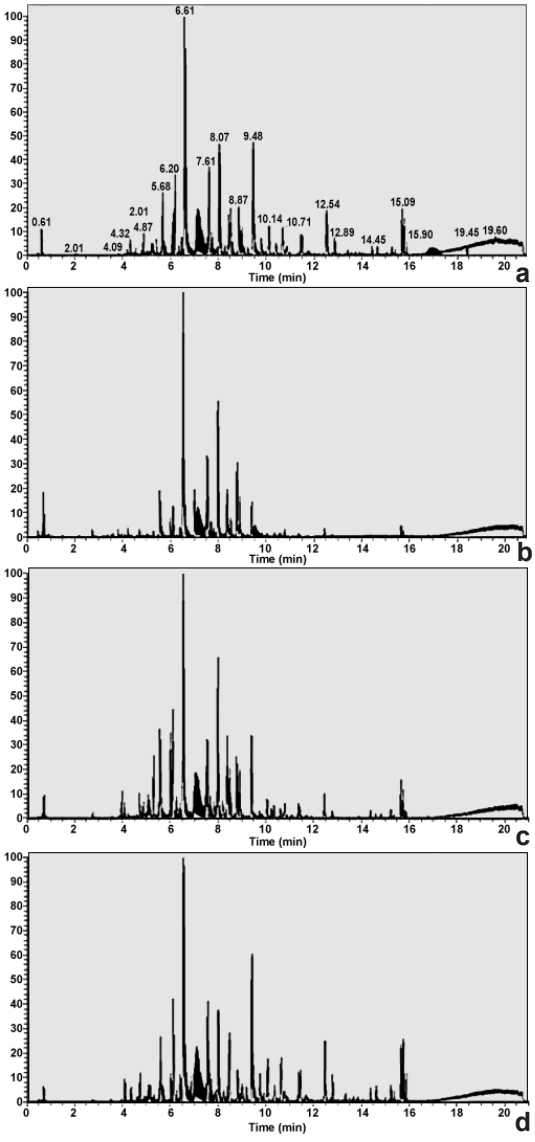

Figure 2 – a-d. Chromatograms of the ethyl acetate fraction of *Dicksonia sellowiana* leaves and their structures – a. leaves; b. rachis; c. pinna rachis; d. pinnule.

Moreover, in the identification of substance 4 (Quercetin-3-O-glucoside-7-O-rhamnoside), it presented [M-H]- in m/z 609, the loss of a rhamnose unit [M-146+H]+ which led to the formation of the fragment (m/z 463), and the loss of a glucose unit [M-162+H]+ which led to the formation of the fragment of deprotonated quercetin (m/z 301).

Table 3 shows the seven identified substances as well as their masses, fragments, retention time and intensity in the chromatogram, and, in Figure 3, we have the chemical structures of these proposed substances.

The study by Rattmann *et al*. (2011) had already identified the presence of flavonoids derived from quercetin and Kaempferol, results that corroborate the analyzes carried out in this study, where these compounds are found in greater concentration in the pinnule, not being detected in the rachis, a result similar to that found in the analysis by TLC.

Liquid chromatography coupled with mass spectrometry (LC-MS) has become increasingly important in the rapid separation and analysis of natural products (Karioti *et al*. 2014). Several studies have used this resource in an attempt to identify the phenolic compounds present in the ethyl acetate extract and fractions (González-Gómez *et al*. 2010; Karioti *et al*. 2014; Abu-Reidah *et al*. 2015).

Polyphenol determination and antioxidant activity of crude extracts from leaves and their structures

From the results found (Tab. 4), it is possible to verify that the part of the leaves that obtained the best antioxidant activity and the highest levels of phenolic compounds was the pinnule extract, followed by the leaves extract. These results corroborate those obtained in the UPLC-PDA-MS analysis, where the presence of flavonoids was identified with greater intensity than in pinna rachis and rachis, directly influencing this biological activity.

When analyzing the results, it is observed that the rachis is the one that presents less expressive results, from the extraction yield to the amount of total phenolics as well as the antioxidant activity. These results corroborate with other studies that defend that materials such as rachis, stem, roots and bark are rich in fiber and water, presenting lower extraction yield and lower concentration and variety of secondary metabolites (Alves *et al*. 2020).

There is a scarcity of chemical and biological studies on fern species belonging to the Dicksoniaceae family in the literature. Previous studies conducted on *D. sellowiana* had identified the levels of total phenolics and antioxidant activity in the species leaves using the DPPH method (Oliveira *et al.* 2015, 2016). However, these analyses were performed on crude extracts and their fractions, not on specific leaf structures. The results of this study are similar to those found by Oliveira *et al*. (2016), where crude extracts exhibited a significant amount of total phenolics and antioxidant activity. However, in this present study, it becomes evident that these activities are attributed to the pinnule structure present in the leaf.

In a study conducted on the leaves of *Cyathea atrovirens*, a tree fern species, which evaluated the levels of total phenolics and antioxidant activity using the same method, the results were comparable (Zuchetto *et al.* 2018), with a slight advantage for *D. sellowiana*. The crude extract of *C. atrovirens* showed a phenolic content of 185.99 mg GAE.g^{-1} and an antioxidant activity with an EC50 of 48.82 µg.mL^{-1}, while the results of the leaf extracts in the present study were 207 mg GAE.g^{-1} and 42.89 µg.mL^{-1}, respectively.

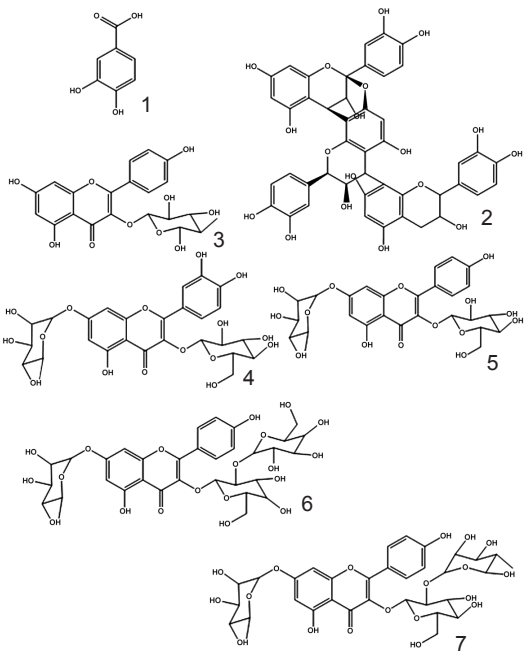

Figure 3 – Substances identified by mass spectrometry of the leaves and structures of *Dicksonia sellowiana.*

Table 3 – Chemical composition of the ethyl acetate fraction of *Dicksonia sellowiana* leaves and their respective structures.

Peak	RT. (min.)	MS¹ $[M-H]^-$	Relevant negative fragments	Molecular formula	MS¹ $[M + Na]^+$	Identification proposal	Fronds	Rachis	Pinna rachis	Pinnule
1	2,76	153	-	$C_7H_6O_4$	-	3,4-Dihydroxybenzoic acid				
2	6,61	863	711, 693, 573, 451, 411, 299	$C_{47}H_{42}O_{18}$	-	Trimer-flavan-3-ol tipo A				
3	10,71	431	285	$C_{21}H_{20}O_{10}$	-	Kaempferol-3-O-Rhamnoside				
4	11,44	609	463, 301	$C_{27}H_{30}O_{16}$	633	Quercetin-3-O-glucoside-7-O-rhamnoside				
5	12,54	593	447, 285	$C_{27}H_{30}O_{15}$	617	Kaempferol-3-O-glucoside-7-O-rhamnoside				
6	15,24	755	609, 593, 469, 323, 285	$C_{33}H_{40}O_{20}$	779	Kaempferol-3-O-rhamnosyl(1->2)glucoside-7-O-rhamnoside				
7	15,69	739	593, 453, 285	$C_{33}H_{40}O_{19}$	763	Kaempferol-3-O-glucoside-2´´-rhamnoside-7-O-rhamnoside				

= Absence of chromatographic peak

= Average chromatographic peak intensity

= Higher chromatographic peak intensity

Malaysia is home to a wide variety of ferns, many of which have been used as traditional medicines for treating various diseases or for general healthcare. A comparative study of the biological activities of the leaves of fifteen ferns used in ethnomedicine in Malaysia showed that, in general, these ferns exhibit good concentrations of total phenolics (Lai & Lim 2011). Among them, *Cyathea latebrosa*, a tree fern species, stood out with the highest concentration of total phenolics in the study. When compared to the total phenolic content of *D. sellowiana* in the present study, it would fall among the ferns with a very high concentration of total phenolics.

In another study (Nurhasnawati *et al*. 2019) that compared the levels of total phenolics and antioxidant activity of five fern species used in the East Kalimantan region of Indonesia as food, ornamentals, and traditional medicine, a high concentration of total phenolics was also observed, with four species exceeding 240 mg, a value similar to that found in the pinnae extract of *D. sellowiana*. In the antioxidant evaluation using the DPPH method, the extracts of *D. sellowiana* showed higher potential compared to four of the species evaluated in the study. The studies conducted by Lai & Lim (2011) and Nurhasnawati *et al*. (2019), which evaluated a total of 20 fern species, demonstrate that ferns possess a variety of phytochemical compounds with polar characteristics such as tannins and flavonoids, findings that are consistent with those of *D. sellowiana.*

Studies have shown the potential use of polyphenols (Oyenihi & Smith 2019; Nani *et al*. 2021), with antioxidant and anti-inflammatory activities, in the treatment of cancer, in diets, in treating obesity, among others. In this way, it becomes essential to research these compounds in plant extracts as possible markers of biological activities, as well as to study antioxidant activity, taking into account that, according to studies, oxidative stress is one of the main causes of tissue damage, being associated with various chronic disorders, due to increased generation of cellular free radicals, which causes an imbalance in the more oxidative intracellular environment (Pizzino *et al*. 2017).

The tests carried out with the *Dicksonia sellowiana* leaves and its structures corroborate the identification of the part responsible for the biological potential of the species. In all tests, the part of the leaves that stood out the most was the pinnule, showing superior yield in the extraction process, a greater variety of flavonoids identified, higher levels of total phenolics and greater antioxidant potential. In the PCA analysis of the crude extracts by NMR, the pinnule was the extract that most resembled the crude extract of the leaves.

Through the results obtained by different analytical techniques, it is possible to verify that the pinnule is the part of the leaves with the best results obtained in this study and probably the most interesting in the prospect of a future pharmacological use. As the results between leaves and pinna show more similarities, and the PCA analysis of ^{1}H NMR confirms this fact, the use of the whole leaves in pharmacological assays is reinforced by the chemical and biological similarity between these samples, also contributing to the conservation of the species, since no specific structure of the species obtained results as significant as the leaf extract.

The results obtained in this study emphasize the promising pharmacological potential of the species *D. sellowiana*, highlighting the crucial importance of preserving these ferns for biodiversity maintenance and environmental sustainability. By conserving endangered species and implementing effective propagation strategies, we will ensure that future generations can benefit

Table 4 – Total phenolics and antioxidant activity of *Dicksonia sellowiana* leaves and their respective structures.

	TPC (mg GAE.g^{-1} ± SD)	**DPPH (EC50 µg.mL^{-1} ± SD)**
Fronds	207,13 ± 0,346	42,89 ± 1,492
Rachis	51,86 ± 0,127	156,28 ± 0,884
Pinna rachis	174,05 ± 0,433	59,61 ± 0,227
Pinnule	217,84 ± 0,321	40,65 ± 0,346

Note: TPC = total phenolic content; GAE = gallic acid equivalentes; SD = standard deviation.

from the medicinal properties of this species, while preserving its natural habitat and contributing to the balance of our ecosystem.

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Data availability statement

In accordance with Open Science communication practices, the authors inform that all data are available within the manuscript.

References

Abdulkadir AR, Zawawi DD, & Jahan MS (2016) Proximate and phytochemical screening of different parts of *Moringa oleifera*. Russian Agricultural Sciences 42: 34-36. DOI: 10.3103/S106836741601002X.

Abu-Reidah IM, Ali-Shtayeh MS, Jamous RM, Arráez-Román D & Segura-Carretero A (2015) HPLC-DAD-ESI-MS/MS screening of bioactive components from *Rhus coriaria* L. (Sumac) fruits. Food Chemistry 166: 179-191.

Alves JJL, Dias MI, Barreira JCM, Barros L, Resende O, Aguiar ACR & Ferreira ICFR (2020) Phenolic profile of *Croton urucurana* Baill. leaves, stems and bark: pairwise influence of drying temperature and extraction solvent. Molecules 25: 2020-2032.

Beara I, Zivkovic J, Lesjak M, Ristic J, Savikin K, Maksimovic Z & Jankovic (2015) Phenolic profile and anti-inflammatory activity of three *Veronica* species. Industrial Crops and Products 63: 276-280.

Bhatia A, Bharti SK, Tewari SK, Sidhu OP & Roy R (2013) Metabolic profiling for studying chemotype variations in *Withania somnifera* (L.) Dunal fruits using GC-MS and NMR spectroscopy. Phytochemistry 93: 105-115.

Biondi D, Leal L, Martini A & Natal CM (2009) Caracterização dendrométrica de *Dicksonia sellowiana* Hook. em povoamento de *Araucaria angustifolia* (Bertol.) Kuntze. Cerne 15: 453-459.

Boffo EF, Melo KS, Shiromoto MO, Silva AD, Vieira PC & Ambrozin ARP (2022) Chemometrics applied to 1H NMR and UV-Vis spectroscopy as a way to evaluate solid-liquid extraction of leaves of artichoke. Food Chemistry 377: 131979.

Boscher J, Auger J, Mandon N & Ferary S (1995) Qualitative and quantitative comparison of volatile sulphides and flavour precursors in different organs of some wild and cultivated garlics. Biochemical Systematics and Ecology 23: 787-791.

Brasil (2011) Ministério do Meio Ambiente. Resolução n° 35, de 27 de abril de 2011. MMA, Brasília. Pp. 1-4.

Brasil (2019) Agência Nacional de Vigilância Sanitária. Farmacopeia Brasileira. Vol. 2. Ministério da Saúde, Brasília. Pp. 119-120.

Brasil (2022) Ministério da Saúde. Gabinete do Ministro. Portaria nº 300, de 13 de dezembro de 2022. Ministério da Saúde, Brasília.

Chaparro-Hernández I, Rodríguez-Ramírez J, Barriada-Bernal LG & Méndez-Lagunas L (2022) Tree ferns (Cyatheaceae) as a source of phenolic compounds - a review. Journal of Herbal Medicine 35: 100587.

Churchill H, Tryon R & Barrington DS (1998) Development of the sorus in tree ferns: Dicksoniaceae. Canadian Journal of Botany 76: 1245-1252.

Combarieu E, Martinelli EM, Pace R & Sardone N (2015) Metabolomics study of Saw palmetto extracts based on 1H NMR spectroscopy. Fitoterapia 102: 56-60.

CONAMA - Conselho Nacional do Meio Ambiente (2001) Resolução CONAMA 278, de 24 de maio de 2001. Diário Oficial da União de 18 de julho de 2001, Brasília. Pp. 1-2.

Della AP & Vasques DT (2023) Dicksoniaceae in Flora e Funga do Brasil. Jardim Botânico do Rio de Janeiro. Available at <https://floradobrasil.jbrj.gov.br/FB90947>. Access on 24 October 2023.

Fernandes I 2000. Taxonomia dos representantes de Dicksoniaceae no Brasil. Pesquisas Botânica 50: 5-26.

Foo LY, Lu Y Howell AB & Vorsa N (2000) A-type proanthocyanidin trimers from cranberry that inhibit adherence of uropathogenic P-fimbriated Escherichia coli. Journal of Natural Products 63: 1225-1228.

González-Gómez D, Lozano M, Fernández-León MF, Bernalte MJ, Ayuso MC & Rodríguez AB (2010) Sweet cherry phytochemicals: Identification and characterization by HPLC-DAD/ESI-MS in six sweet-cherry cultivars grown in Valle del Jerte (Spain). Journal of Food Composition and Analysis 23: 533-539.

Karioti A, Chiarabini L, Alachkar A, Chehna MF, Vincieri FF & Bilia AR (2014) HPLC-DAD and HPLC-ESI-MS analyses of *Tiliae flos* and its preparations. Journal of Pharmaceutical and Biomedical Analysis 100: 205-214,

Korall P, Pryer KM, Metzgar JS, Schneider H & Conant DS (2006) Tree ferns: monophyletic groups and their relationships as revealed by four protein-coding plastid loci. Molecular Phylogenetics and Evolution 39: 830-845.

Kowalska T & Sajewicz M (2022) Thin-Layer Chromatography (TLC) in the screening of botanicals-its versatile potential and selected applications. Molecules 27: 6607.

Lai HY & Lim YY (2011) Evaluation of antioxidant activities of the methanolic extracts of selected ferns in Malaysia. International Journal of Environmental Science and Development 2: 442-447.

Mantovani M (2004) Caracterização de populações naturais de Xaxim [*Dicksonia sellowiana* (Presl.) Hooker], em diferentes condições edafo-climáticas no estado de Santa Catarina. Dissertação de Mestrado. Universidade Federal de Santa Catarina, Florianópolis. 107p.

Santiago ACP, Mynssen CM, Maurenza D, Penedo TSA & Sfair JC (2013) Dicksoniaceae. *In*: Martinelli G & Moraes MA (eds.) Livro vermelho da flora do Brasil. Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, Rio de Janeiro. Pp. 475-476.

Malucelli LC, Massulo T, Magalhães WLE, Stofella NCF, Vasconcelos EC, Carvalho Filho MAS & Murakami FS (2018) Thermal and chemical characterization of *Dicksonia sellowiana* extract by means of thermal analysis. Revista Brasileira de Farmacognosia 28: 626-630.

Matarese F, Cuzzola A, Scalabrelli G & D'Onofrio C (2014) Expression of terpene synthase genes associated with the formation of volatiles in different organs of Vitis vinífera. Phytochemistry 105: 12-24.

Mensor LL, Menezes FS, Leitão GG, Reis AS, Santos TC, Coube CS & Leitão SG (2001) Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytotherapy Research 15: 127-130.

Mielke EJC (2002) Análise da cadeia produtiva e comercialização do xaxim *Dicksonia sellowiana*, no estado do Paraná. Dissertação de Mestrado. Universidade Federal do Paraná, Curitiba. 90p.

Naidoo D, van Vuuren SF, van Zyl RL & de Wet H (2013) Plants traditionally used individually and in combination to treat sexually transmitted infections in northern Maputaland, South Africa: antimicrobial activity and cytotoxicity. Journal of ethnopharmacology 149: 656-667.

Nani A, Murtaza B, Sayed Khan A, Khan NA, & Hichami A (2021) Antioxidant and anti-inflammatory potential of polyphenols contained in mediterranean diet in obesity: molecular mechanisms. Molecules 26: 985.

Nurhasnawati H, Sindu R, Sapri S, Supriningrum R, Kuspradini H & Arung ET (2019) Antioxidant activity, total phenolic and flavonoid content of several indigenous species of ferns in East Kalimantan, Indonesia. Biodiversitas 20: 576-580.

Oliveira VB, Zuchetto M, Paula CS, Verdam MCS, Campos R, Duarte AFS, Miguel MD & Miguel OG (2015) Avaliação do potencial antioxidante frente à oxidação lipídica e da toxicidade preliminar do extrato e frações obtidas das leaveses de *Dicksonia sellowiana* (Presl.) Hook. Revista Brasileira de Plantas Medicinais 17: 614-621.

Oliveira VB, Zuchetto M, Oliveira CF, Paula CS, Duarte AFS, Miguel MD & Miguel OG (2016) Efeito de diferentes técnicas extrativas no rendimento, atividade antioxidante, doseamentos totais e no perfil por clae-dad de *Dicksonia sellowiana* (presl.). Hook, Dicksoniaceae. Revista Brasileira de Plantas Medicinais 18: 230-239.

Oyenihi AB & Smith C (2019) Are polyphenol antioxidants at the root of medicinal plant anti-cancer success? Journal of ethnopharmacology 229: 54-72.

Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D & Bitto A (2017) Oxidative stress: harms and benefits for human health. Oxidative Medicine and Cellular Longevity 2017: 8416763.

PPG I (2016) A community-derived classification for extant lycophytes and ferns. Journal of Systematics and Evolution 54: 563-603.

Rattmann YD, Mendéz-Sánchez SC, Furian AF, Paludo KS, Souza LM, Dartora N, Oliveira MS, Costa EMS, Miguel OG, Sassaki GL, Iacomini M, Mello CF, Franco CRC, Silva-Santos JE, Cadena SMSC, Marques MCA & Santos ARS (2011) Standardized extract of *Dicksonia sellowiana* Presl. Hook (Dicksoniaceae) decreases oxidative damage in cultured endothelial cells and in rats. Journal of Ethnopharmacology 133: 999-1007.

Ray C, James AG & Michael CG (2022) A new method for olive oil screening using multivariate analysis of proton NMR spectra. Molecules 27: 213.

Rezende YRRS, Nogueira JP, Silva TOM, Barros RGC, Oliveira CS, Cunha GC, Gualberto NC, Rajan M & Narain N (2021) Enzymatic and ultrasonic-assisted pretreatment in the extraction of bioactive compounds from Monguba (*Pachira aquatic* Aubl) leaf, bark and seed. Food Research International 140: 109869.

Riffault L, Destandau E, Pasquier L, André P & Elfakir C (2014) Phytochemical analysis of *Rosa hybrida* cv. "Jardin de Granville" by HPTLC, HPLC-DAD and HPLC-ESI-HRMS: polyphenolic fingerprints of six plant organs. Phytochemistry 99: 127-134.

Rohman A, Theresia W, Anjar W & Sugeng R (2020) The authentication of Java turmeric (*Curcuma xanthorrhiza*) using thin layer chromatography and ^{1}H-NMR based-metabolite fingerprinting coupled with multivariate analysis. Molecules 25: 3928.

Schmitt JL, Schneider PH & Windisch PG (2009) Crescimento do cáudice e fenologia de *Dicksonia sellowiana* Hook. (Dicksoniaceae) no sul do Brasil. Acta Botanica Brasilica 23: 283-291.

Singleton VL, Orthofer R & Lamuela-Raventós RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagente. Methods in Enzymology 299: 152-178.

Smith AR, Pryer KM, Schuettpelz E, Korall P, Schneider H & Wolf PG (2006) A classification for extant ferns. Taxon 55: 705-731.

Souza AM, Armstrong L, Merino FJZ, Cogo LL, Monteiro CLB, Duarte MR, Miguel OG & Miguel MD (2014) *In vitro* effects of *Eugenia pyriformis* Cambess. Myrtaceae: antimicrobial activity and synergistic interactions with Vancomycin and Fluconazole. African Journal of Pharmacy and Pharmacology 8: 862-867.

Stoyanova R & Brown TR (2001) NMR spectral quantitation by principal component analysis. NMR in Biomedicine 14: 271-277.

Tryon RM & Tryon AF (1982) Ferns and allied plants. With special reference to Tropical America. Springer-Verlag, New York. Pp. 138-155.

Zuchetto M, Oliveira CSP, Rodrigues AA, Merino FJZ, Oliveira VB, Kulik JD, Krause MS, Ocampos FMM, Kerber VA, Miguel OG & Miguel MD (2018) Isolamento de flavonoide, avaliação do potencial antioxidante e toxicidade preliminar do extrato e frações obtidas da espécie *Cyathea atrovirens* (Cyatheaceae). Revista Brasileira de Plantas Medicinais 20: 1-9.

Zuiter AS (2014) Proanthocyanidin: chemistry and biology: from phenolic compounds to Proanthocyanidins. Reference Module in Chemistry, Molecular Sciences and Chemical Engineering. doi: 10.1016/b978-0-12-409547-2.11046-7

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